AISLAMIENTO DE *N*-ACILHOMOSERINLACTONAS DE ALGUNAS BACTERIAS PROCEDENTES DEL MAR CARIBE COLOMBIANO, COMO EVIDENCIA DE LA EXISTENCIA DE CIRCUITOS DE *QUORUM SENSING*

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ABREVIATURAS Y ACRÓNIMOS

ACN	Acetonitrilo
AcOEt	Acetato de etilo
CC	Cromatografía en columna
CC-FR	Cromatografía en columna en fase reversa
CCD	Cromatografía en capa delgada
CCD-FR	Cromatografía en capa delgada en fase reversa
CCDP	Cromatografía en capa delgada preparativa
CG-EM	Cromatografía de gases acoplada a espectrometría de masas
CG-IE-EM	Cromatografía de gases acoplada a espectrometría de masas en modo de ionización por impacto electrónico
CLAE	Cromatografía líquida de alta eficiencia
CLAE-EM	Cromatografía líquida de alta eficiencia acoplada a espectrometría de masas
CLAE-ESI-EM	Cromatografía líquida de alta eficiencia acoplada a espectrometría de masas en modo de ionización por electrospray
cLB	Caldo Luria-Bertani
CM	Caldo marino
EM	Espectrometría de masas
h	horas
H_2O	agua
HSL	homoserinlactona
IE	Ionización por impacto electrónico
IR	Infrarrojo
MeOH	Metanol
min	Minutos
mLB	Medio Luria-Bertani
mLBr	Medio Luria-Bertani para revelar las placas
MM	Medio marino
MMm	Medio marino modificado
Patrón C6-HSL	*N*-hexanoil-DL-homoserinlactona (Fluka)
QS	*Quorum sensing*
RMN	Espectroscopía de resonancia magnética nuclear
UV	Ultravioleta

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RESUMEN

En el ambiente marino las superficies vivas o inertes que no se hallen protegidas son rápidamente colonizadas mediante un proceso conocido como *fouling.* Este proceso se da en varias etapas, las cuales incluyen la adsorción de moléculas orgánicas, el posterior asentamiento de procariotas y de eucariotas unicelulares (*microfouling*), y finalmente el asentamiento de larvas de invertebrados marinos y esporas de algas (*macrofouling*). Este fenómeno es inducido, dado que el espacio en el mar es un factor limitante y limitado. La selección de la superficie a asentarse por parte de los microorganismos depende de muchos factores, tanto bióticos como abióticos. Entre los factores bióticos se destaca la presencia de *biofilm*s bacterianos, pues la presencia de ellos puede estimular o disuadir el asentamiento de determinados organismos. Estos *biofilm*s se pueden entender como comunidades bacterianas sésiles adheridas a la superficie.[1] En el proceso de formación y maduración del *biofilm* influyen muchos factores, pero se destaca el papel fundamental que juega el *quorum sensing*.[2] Hace poco tiempo se planteó que si el fenómeno del *quorum sensing* bacteriano se interrumpe la maduración del *biofim* se afectará evitando (o al menos reduciendo) la llegada del *macrofouling*. No obstante este fenómeno se ha estudiado muy poco a nivel mundial y aún en menor medida a nivel local.

En este trabajo, y como contribución al conocimiento de la comunicación de bacterias marinas se evaluó la presencia de *N*-acilhomoserinlactonas en las siguientes cepas recolectadas en Santa Marta (Colombia): *Ochrobactrum* sp, *Vibrio* sp (23-6PIN), *Vibrio campbellii*, *Vibrio* sp (11-6DEP), *Ochrobactrum pseudogringnonense, Schewanella* sp, *Vibrio harveyi*, *Alteromona* sp. Para la detección e identificación de las HSLs presentes se

[1] Shapiro, J.A. **1998**. Thinking about bacterial populations as multicellular organisms. *Annu. Rev. Microbiol. 52*. 81–104.
[2] Greenberg, E. P. **2003**. Bacterial communication and group behaviour. *The Journal of Clinical Investigation*. *112*, 1288-1290.

hizo uso del biosensor *E. coli* (pSB401), CG-EM y CLAE-EM. Encontrando que todos los aislamientos Gram-negativos poseen circuitos de QS mediados por HSLs, lográndose identificar la *N*-butanoilhomoserinlactona (C4-HSL) y la *N*-hexanoilhomoserinlactona (C6-HSL). Con lo anterior, se hicieron evidentes circuitos de QS mediados por HSLs que no se habían presenciado anteriormente en dos géneros bacterianos (*Ochrobactrum* y *Alteromona*), se encontraron HSLs que no habían sido reportadas para *Shewanella* y HSLs ya reportadas para el género *Vibrio*.

INTRODUCCIÓN

El grupo de Investigación "Estudio y Aprovechamiento de Productos Naturales Marinos y Frutas de Colombia" desde el año 2005 se ha interesado en el fenómeno conocido como *fouling*,[3] el cual hace referencia a la acumulación de material inorgánico, orgánico, microorganismos, plantas y animales sobre superficies sumergidas en el agua, y particularmente en el mar. La importancia de su estudio radica en que a pesar de ser un proceso natural y en algunos casos deseable, se ha convertido en un problema para la industria naval, petrolera, y otras más; ya que las embarcaciones, plataformas y demás piezas que quedan sumergidas se ven afectadas por esta colonización, cambiando sus propiedades físicas y químicas, en algunos casos se genera corrosión, por lo que se hace necesario el uso de técnicas costosas para la limpieza de la superficie colonizada.

La prevención del fenómeno de colonización, que es uno de los objetivos del grupo de investigación "Estudio y Aprovechamiento de Productos Naturales Marinos y Frutas de Colombia", puede abordarse tanto en la etapa de *microfouling* (asentamiento de bacterias, diatomeas y protozoos), como en la etapa de *macrofouling* (asentamiento de macrófitos y epizoitos). De otro lado, y teniendo en cuenta que el proceso del *fouling* se ha caracterizado como un fenómeno sucesional, es de esperarse que la disminución del *microfouling* pueda retrasar o evitar la llegada del *macrofouling* que es la etapa posterior a la consolidación del *microfouling*; por este motivo, estamos interesados en investigar a fondo el asentamiento bacteriano, y la consecuente formación y maduración del *biofilm*, que es una etapa fundamental de la consolidación del *microfouling*. El *biofilm* bacteriano, se desarrolla en una serie de etapas consecutivas que pueden describirse como: a) El asentamiento inicial de

[3] Duque, C.; Puyana, M.; Zea, S.; Osorno, O.; Alvarado, E.; Lecompte, O. **2006**. Valoración de las propiedades anti*fouling* de algunas especies vegetales y marinas (ají e invertebrados marinos) y su posible aplicación en recubrimientos industriales. Universidad Nacional de Colombia. Proyecto financiado por Colciencias. Proyecto número: 20101007015; Contrato Nº 207-05.

células microbianas en la superficie, b) Asociación a dicha superficie, c) Producción de exopolisacáridos para adherirse irreversiblemente, d) Estratificación y maduración del *biofilm*, y e) Dispersión de células individuales de los microorganismos asentados en búsqueda de nuevos nichos colonizables.

La comunicación entre las células bacterianas por medio de señales químicas, juega un papel fundamental en la formación y maduración de este *biofilm*, y en la expresión de otros fenotipos que le permiten adaptarse mejor al entorno. Así, cuando la población bacteriana alcanza un mínimo (*quorum*) se induce la expresión de estos fenotipos específicos, los cuales son inducidos por medio de cascadas de señalización reguladas por efectores químicos, que no se activan en las etapas iniciales del crecimiento bacteriano. Este fenómeno se conoce como *quorum sensing*, cuya definición formal está dada como la expresión genética regulada por las fluctuaciones en la densidad de población, y cuyas moléculas señalizadoras en bacterias Gram-negativas son principalmente las *N*-acilhomoserinlactonas (HSLs), y en bacterias Gram-positivas en su mayoría algunos péptidos; además, se han encontrado moléculas que pueden mediar la comunicación entre ambos tipos de bacterias como el autoinductor–2 (AI-2).[4]

Con lo anterior creemos que se hace importante estudiar la comunicación que se da entre bacterias de origen marino e involucradas en el proceso del *fouling*, pues si la podemos evitar, se podrá impedir, retrazar o debilitar la maduración del *biofilm* y por consiguiente la aparición del *biofouling* se verá disminuida.[5] De ésta manera, el objetivo principal de este trabajo fue determinar si las bacterias que hemos aislado de distintas superficies sumergidas en el mar Caribe Colombiano poseen circuitos de QS, puesto que estas bacterias son las usadas en nuestro ensayo *antifouling* y necesitamos caracterizar el comportamiento de cada aislamiento.

[4] Zavilgelsky, G.B.; Manukhov, I.V. **2001**. Quorum sensing, or how bacteria "talk" to each other. *Molecular Biology. 35*. 224–232.
[5] Dobretsov, S.; Dahms, H. W.; YiLi, H.; Wahl, M.; Qian, P.Y. **2007**. The effect quorum-sensing blockers on the formation of marine microbial communities and larval attachment. *Microbiol. Ecol. 60*, 177–188.

Los resultados alcanzados en este trabajo se presentan de la siguiente manera: En primera instancia se muestra el estado actual del tema, con el fin de realizar la introducción al trabajo y abordar todos los conceptos básicos concernientes al estudio realizado y su aplicación. A continuación se exponen los resultados obtenidos para la determinación de la existencia de circuitos de QS por medio de biosensores, y los resultados obtenidos para la extracción e identificación de *N*-acilhomoserinlactonas en las bacterias marinas Gram-negativas usando diferentes técnicas cromatográficas y espectroscópicas. Los resultados conseguidos con las diferentes técnicas fueron comparados entre ellos con el fin de correlacionar los datos entre sí y plantear conclusiones pertinentes sobre el trabajo.

1. ESTADO ACTUAL DEL TEMA

En ambientes acuáticos, todos los sustratos sin protección artificial o natural son rápidamente colonizados por la biota presente, mediante un proceso conocido como *fouling*. Éste proceso puede definirse como la acumulación de material inorgánico, orgánico, microorganismos, plantas y animales sobre superficies sumergidas en el agua del mar.[6] El proceso del *fouling* se da en tres etapas las cuales incluyen la adsorción de moléculas orgánicas disueltas,[7] colonización por parte de procariotas y eucariotas unicelulares tales como bacterias, diatomeas y protozoos (*microfouling*), y el posterior reclutamiento de larvas de invertebrados y esporas de algas (*macrofouling*) (figura 1).[8,9] Estas etapas se pueden superponer, ocurrir de manera sucesional o de manera paralela, sin embargo en la mayor parte de estudios se ha corroborado que el modelo sucesional es el predominante en el proceso del *fouling*. El asentamiento de larvas o esporas de especies involucradas en el *macrofouling* se ve afectada por un gran número de factores ambientales como la hidrodinámica[10] y las características de la superficie, incluyendo la presencia de *biofilms*.[11]

[6] Yebra, D.M.; Kiil, S.; Dam-Johansen, K. **2004**. Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Prog. Org. Coat. 50,* 75-104.
[7] Davis, A.D.; Targett, N.M.; McConell, O.J.; Young, C.M. **1989**. Epibiosis of marine algae and benthic invertebrates: natural products chemistry and other mechanisms inhibiting settlement and overgrowth. *Bioorg. Mar. Chem. 3*, 85-114.
[8] Whittaker, R.H.; Feeny, P.P. **1971**. Allelochemics: chemicals interactions between species. *Science. 171,* 757.
[9] Aldred, N.; Clare, A.S. **2008**. The adhesive strategies of cyprids and development of barnacle-resistant marine coatings. *Biofouling 24*, 351–363.
[10] Koehl, M.R.A. **2007**. Mini review: hydrodynamics of larval settlement into fouling communities. *Biofouling 23*, 357–368.
[11] Dobretsov, S.; Teplitski, M.; Paul, V. **2009**. Mini-review: quorum sensing in the marine environment and its relationship to biofouling. *Biofouling 25*, 413–427.

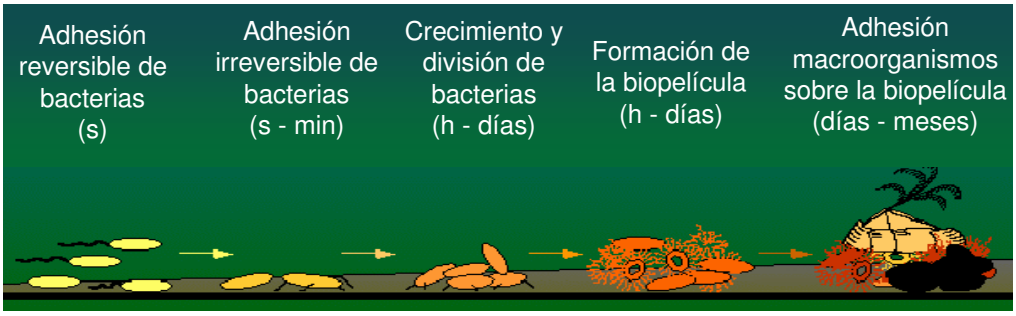

Figura 1. Etapas de la formación del *fouling* marino.[12]

Con lo anterior se hace evidente que la primera etapa del *fouling* es la formación del *biofilm* bacteriano, que se define como un ensamble de células que están asociadas irreversiblemente a una superficie y encerradas dentro de una matriz primaria de polisacáridos. Los *biofilms* se desarrollan sobre superficies abióticas como también sobre superficies bióticas (epibiosis). El *biofilm* se caracteriza por interacciones complejas entre los miembros de la comunidad bacteriana, no obstante en estas interacciones también se pueden dar con el hospedero.[13] La maduración del *biofilm* hace que las bacterias inmersas en esta comunidad sean resistentes a muchos factores ambientales y convierte al *biofilm* en el medio apropiado para que otros organismos se adhieran a las superficies a las que se encuentran unidas las bacterias, promoviendo el desarrollo del *fouling*. Existen diferentes interacciones al interior de la comunidad bacteriana, ellas conducen a la formación de un *biofilm* maduro, dentro de estas interacciones se destacan: la atracción de las bacterias hacia una superficie inducida por mecanismos de quimiotaxis, las propiedades de adhesión de la bacteria que implican secreción del exopolisacárido, la utilización de los apéndices de movimiento que ayudan a superar las fuerzas físicas y la distancia, y la comunicación bacteriana.[14]

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[12] Imagen tomada de http://www.biofilmsonline.com/cgi-bin/biofilmsonline/ed_how_primer.html (13/10/2007).

[13] Wahl, M. **2008**. Ecological lever and interface ecology: epibiosis modulates the interactions between host and environment. *Biofouling 24*, 427–438.

[14] Flemming H.C. **2002**. Biofouling in water systems – cases, causes and countermeasures. *Appl Microbiol Biotechnol, 59,* 629–640.

Hoy en día se están estudiando estas interacciones,[15] encontrando que muchas larvas de invertebrados marinos prefieren asentarse sobre *biofilms* bacterianos que sobre superficies limpias por esta razón la interrupción del *biofilm* bacteriano puede conducir a la reducción del *macrofouling* de las superficies sumergidas.[5] Así mismo se ha demostrado que al impedir la comunicación bacteriana (*quorum sensing*) la fortaleza, resistencia, consistencia, entre otras, del *biofilm* disminuye.[15] Dicha comunicación se conoce como *quorum sensing*, que es la regulación de la expresión genética en función de la densidad de población bacteriana, la cual se censa a través del reconocimiento de moléculas señalizadoras (que son producidas por las mismas bacterias).[16] Por todo lo anterior inhibir la comunicación bacteriana es una buena aproximación para debilitar el medio en el que se forma el *fouling*, este motivo hace importante estudiar cuales son las moléculas que están mediando esta comunicación en un *biofilm* de bacterias marinas.

La mayor parte de los estudios en QS se han enfocado hacia los microorganismos patógenos, ya que se ha comprobado que la expresión de muchos factores de virulencia se regula mediante estos circuitos,[17] para dichos estudios en el modelo *in-vivo* se han utilizado como especímenes otros mamíferos diferentes a los humanos, siendo las ratas los más utilizados. La bacteria patógena Gram-negativa *Pseudomonas aeruginosa,* es una de las más estudiadas, debido a su importancia clínica. Se ha encontrado que ella posee dos sistemas de QS LasR/LasI y RhlR/RhlI, análogos a los sistemas LuxR/LuxI de *Vibrio fischeri,* que le permiten expresar de forma controlada factores de virulencia y pigmentos, entre otros fenotipos, además de generar un *biofilm* estratificado.[18] La relación entre los sistemas de QS con dichos fenotipos se ha comprobado con estudios como el realizado por Wu. *et al*, en el 2001, en donde se hicieron ensayos *in vivo* con ratas, las cuales fueron

[15] Fusetani, N.; Clare, A. S. Antifouling compounds. **2006**. Springer, Germany. pp 1-41.
[16] Kato, N.; Morohoshi, T.; Nozawa, T.; Matsumoto, H.; Ikeda, T. **2006**. Control of Gram-Negative bacterial quorum sensing with cyclodextrin immobilized cellulose ether gel. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*. *56*, 55–59.
[17] You, J.L.; Xue, X.L.; Cao, L.X.; Lu, X.; Wang, J.; Zhang, L.X.; Zhou, S.N. **2007**. Inhibition of *Vibrio* biofilm formation by a marine actinomycete strain A66. *Appl Microbiol Biotechnol. 76*, 1137-1144.
[18] Williams, P.; Camara, M.; Hardman, A.; Swift, S.; Milton, D.; Hope, V.J.; Winzer, K.; Middleton, B.; Pritchard, D.I.; Bycroft, B.W. **2000**. Quorum sensing and the population-dependent control of virulence. *Phil. Trans. R. Soc. Lond. B. 355*, 667-680.

infectadas con *P. aeruginosa wild-type* y *P. aeruginosa lasI rhl* (cepa mutante en los genes *lasI* y *rhlI*, lo que le impide sintetizar HSLs y por lo tanto es incapaz de comunicarse), encontrando que la respuesta inmune de los anticuerpos hacia la infección ejercida por *P. aeruginosa lasI rhlI*, fue rápida y efectiva, comparada con la cepa *P. aeruginosa wild-type* (bacteria nativa), que es resistente a la acción de dichos anticuerpos.[19] De aquí que la comunicación bacteriana dada por la presencia de circuitos de QS le permiten a la comunidad bacteriana defenderse de compuestos antibióticos y de los macrófagos de los mamíferos, haciendo a la comunidad bacteriana del *biofilm* mucho más resistente que las bacterias no asociadas a un *biofilm* o en estado planctónico.

De otro lado también se han estudiado, aunque en menor medida, las interacciones ecológicas entre bacterias, mediadas por sistemas de QS. Así, por ejemplo en ambientes marinos se ha observado que bacterias Gram-positivas, bacterias Gram-negativas y otros microorganismos, se encuentran asociados a invertebrados (plantas, esponjas, macroalgas, o ascidias). Las bacterias que están dentro de dichas comunidades liberan compuestos que permiten la comunicación entre ellas, los cuales a su vez son detectados por los eucariotas a los que se encuentran asociados, e inducen determinadas respuestas en el invertebrado. Así mismo, la expresión de un determinado fenotipo por parte de la bacteria le permite interactuar con su entorno (luminiscencia, maduración del *biofilm,* simbiosis), y lograr una mejor adaptación al medio. Un ejemplo concreto de lo anterior, es la inducción del asentamiento de organismos superiores (larvas de poliquetos y briozoos, zooesporas de algas, etc) sobre un *biofilm* maduro,[20] que se da no solo por las características propias del sustrato sino también por la presencia de las moléculas señalizadoras de QS. Lo anterior permitió comprobar que el *fouling* se encuentra influenciado por la comunicación

[19] Wu, H.; Song, Z.; Givskov, M.; Doring, G.; Worlitzsch, D.; Mathee, K.; Rygaard, J.; Høiby, N. **2001**. *Pseudomonas aeruginosa* mutations in *lasI* and *rhlI* quorum sensing systems result in milder chronic lung infection. *Microbiology.* 147, 1105–1113.
[20] Lowery, C.A.; Dickerson, T.J.; Janda, K.D. **2008.** Interspecies and interkingdom communication mediated by bacterial quorum sensing. *Chem. Soc. Rev. 37*, 1337–1346.

bacteriana (QS). Dentro de los casos observados también se destacan algunas bacterias asociadas a esponjas, las cuales producen HSLs *in-situ* sobre los tejidos de las esponjas. [21]

1.1. *Quorum sensing*

El *quorum sensing* es la regulación de la expresión genética en función de la densidad de población bacteriana, la cual se censa a través del reconocimiento de moléculas señalizadoras (que son producidas por las mismas bacterias).[16] La esencia del QS radica en que las bacterias pueden hacer una labor más efectiva en comunidad que de forma aislada, así, si existe una población mínima (el *quorum*) la comunidad de bacterias expresa fenotipos para adaptarse mejor como conjunto al medio ambiente.[2] El QS permite que las bacterias dentro de una población microbiana realicen actos concertados, comportándose de manera similar a un organismo multicelular.[1] Así, el QS se puede ver como una forma biológica análoga al *quorum* legal de una reunión humana, donde solo cuando existe un número apropiado de miembros se toma una decisión. Dentro de las "actividades" controladas por QS se encuentra la producción de metabolitos secundarios, motilidad, simbiosis, nodulación, transferencia de plásmidos conjugados, maduración del *biofilm* y expresiones de virulencia en numerosos géneros bacterianos. Por tal motivo, se dice que los circuitos de QS regulan de forma global la expresión de diversos procesos fisiológicos.[22]

La comunicación bacteriana depende de una serie de moléculas pequeñas, conocidas anteriormente como autoinductores y en la actualidad como moléculas señalizadoras, las cuales se difunden fuera de las bacterias por difusión pasiva. Cuando la población de las bacterias llega al *quorum*, la concentración de estas moléculas es suficientemente alta para unirse al factor de trascripción de forma estable generando la respuesta genética sincronizada de la población bacteriana. Se han identificado varios tipos de moléculas

[21] Taylor, M.W.; Schupp, P.J.; Baillie, H.J.; Charlton, T.S.; de Nys, R.; Kjelleberg, S.; Steinberg, P.D. **2004**. Evidence for acyl homoserine lactone signal production in bacteria associated with marine sponges. *Applied and environmental microbiology*. 4387–4389.
[22] Joint, I.; Tait, K.; Wheeler, G. **2007.** Bacterial conversations: talking, listening and eavesdropping. An introduction. *Philos Trans R Soc Lond B Biol Sci. 362*, 1115-1117.

señalizadoras (figura 2), siendo las *N*-acilhomoserinlactonas (HSL), que regulan los sistemas de QS en bacterias Gram-negativas, las más estudiadas; también se destacan los péptidos, las principales moléculas señalizadoras de las bacterias Gram-positivas, pero que hasta el momento se encuentran muy poco estudiados.[4] También es importante mencionar que existen moléculas señalizadoras que permiten la comunicación interespecies, y que también le permiten a las bacterias Gram-negativas comunicarse con las bacterias Gram-positivas, algunos de estos compuestos son: furanosil borato diéster (AI-2), γ-butirolactona, derivados de 2-heptil-3-hidroxi-4-quinolona (PQS), y dicetopiperacinas (DKPs: ciclo(Ala-Val) y ciclo(L-Pro-L-Tyr)).

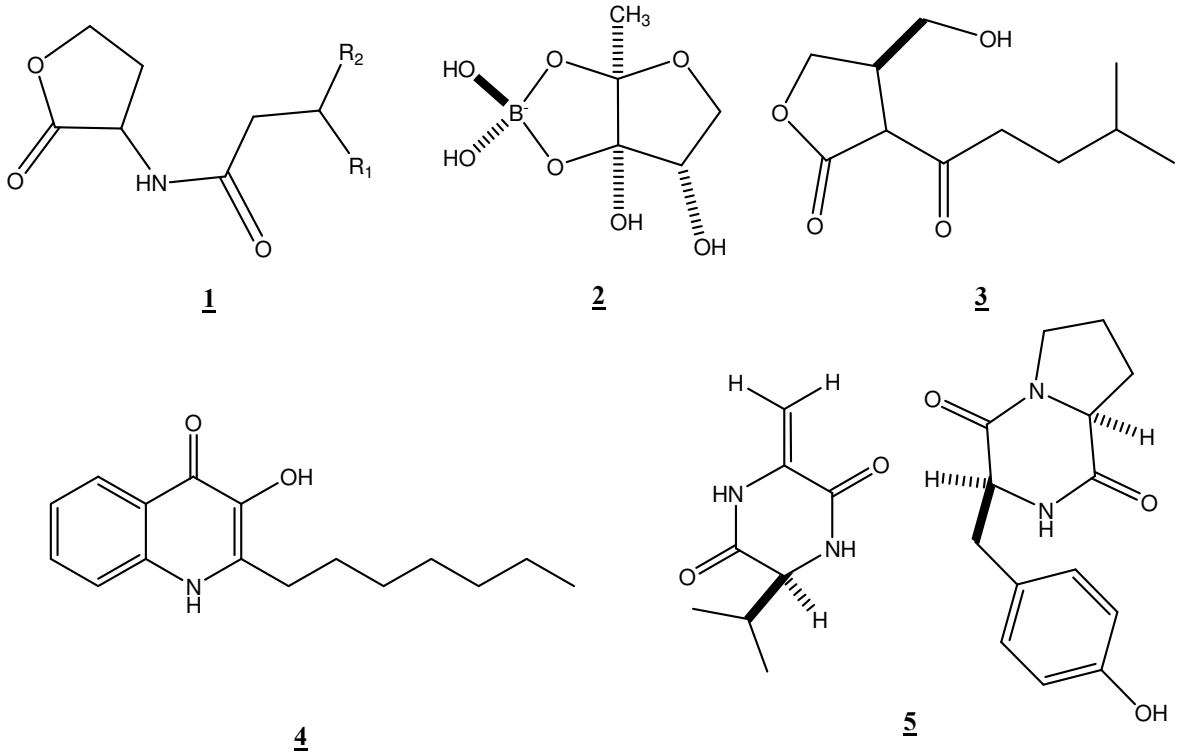

Figura 2. Principales compuestos señalizadores de los sistemas de QS: **1** *N*-acilhomoserinlactonas (HSLs) (R_1 corresponde a la cadena alifática y R_2 son los grupos sustituyentes que puede tener la cadena tales como: grupos oxo, e hidroxilo), **2** AI-2: furanosil borato diéster, **3** γ-butirolactona, **4** PQS: 2-heptil-3-hidroxi-4-quinolona, y **5** dicetopiperacinas DKPs: ciclo(Ala-Val) y ciclo(L-Pro-L-Tyr).[4]

Los circuitos de QS funcionan de la siguiente manera (figura 3): Las moléculas señalizadoras, sintetizadas por la sintasa tipo LuxI, se producen basalmente por las

bacterias; cuando la población bacteriana alcanza la densidad suficiente (*quorum*), las moléculas señalizadoras acumuladas se unen establemente a la proteína tipo LuxR (un factor de trascripción). Este complejo regula tanto la expresión de la sintasa LuxI, generando una retroalimentación por incremento de la producción de las HSL, como la activación de la trascripción de los genes regulados por los promotores tipo *Plux* lo que conlleva a la expresión de determinados fenotipos.[23]

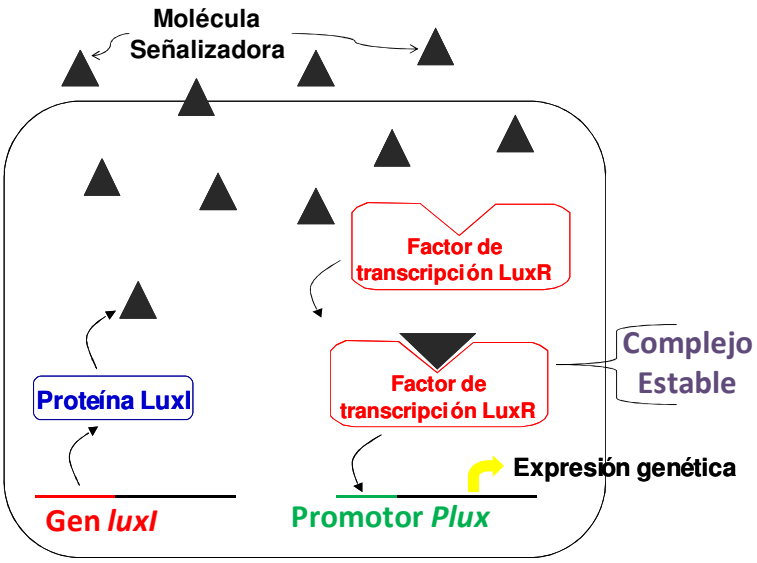

Figura 3. Mecanismo del QS, producción de moléculas señalizadoras y expresión genética.

El primer estudio en donde se evidenció la presencia de circuitos de *quorum sensing* mediados con HSLs, fue el realizado a la bacteria bioluminiscente *Vibrio fischeri* que le proporciona luz a órganos de algunos peces y calamares, que la contienen. El mecanismo por el cual estas bacterias emiten luz radica en la reacción de oxidación de la flavina y de aldehídos grasos, y la reducción de oxígeno molecular por parte de la luciferasa (enzima), el exceso de energía que se genera durante el proceso se libera como "luminiscencia".[24] Hastings, *et al*, en 1977 encontraron las HSLs en el medio de cultivo de *V. fischeri* y mencionaron que se comportaban como "feromonas bacterianas".[24] En estudios

[23] Fuqua, C.; Parsek, M.R.; Greenberg, E.P. **2001**. REGULATION OF GENE EXPRESSION BY CELL-TO-CELL COMMUNICATION: Acyl-Homoserine Lactone Quorum Sensing. *Annu. Rev. Genet. 35*. 39–68.
[24] Hastings, J.W.; Nealson, K.H. **1977**. Bacterial bioluminescence. *Ann. Rev. Microbiol. 31,* 549-595.

posteriores,[25] al silenciar (mutar) el gen que codifica para la proteína sintasa de las HSLs encontraron que no se daba la producción de luminiscencia, razón por la cual se dice que la luminiscencia se encuentra autoinducida por HSLs.

1.2. *N*-acilhomoserinlactonas como señalizadores del *quorum sensing*

Las *N*-acilhomoserinlactonas (HSLs), aisladas de bacterias Gram-negativas,[26] son moléculas señalizadoras y cada célula bacteriana produce un nivel basal de ellas. No obstante, la producción de estas moléculas se incrementa cuando el crecimiento de las bacterias llega a su fase exponencial (figura 4), ya que en éste momento las HSLs producidas y acumuladas se unen de manera estable al factor de transcripción que regula la expresión de la sintasa, de aquí en adelante se da el proceso de transcripción y por consiguiente la expresión genética regulada por QS.

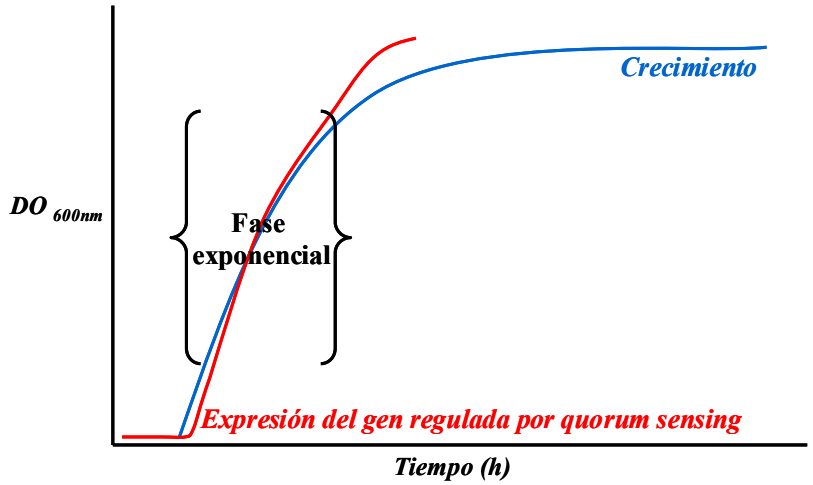

Figura 4. Correlación del crecimiento bacteriano con la autoinducción y aumento de la producción de HSLs.

[25] Hanzelka, B.L.; Stevens, A.M.; Parsek, M.R.; Crone, T.J.; Greenberg, E. P. **1997**. Mutational Analysis of the *Vibrio fischeri* LuxI Polypeptide: Critical regions of an autoinducer synthase. *Journal of bacteriology*, *179*. 4882–4887.
[26] Gobbetti, M.; De Angelis, M.; Di Cagno, R.; Minervini, F.; Limitone. A. **2007**. Cell–cell communication in food related bacteria. *Int. J. Food Microbiol. 120*, 34-45.

Todas las HSLs se caracterizan por tener una unidad de homoserinlactona unida a una cadena acílica normal, la cual suele contener entre 4 y 14 átomos de carbono. Algunas de las cadenas acílicas pueden contener sustituyentes oxo o hidroxi en la posición tres, y también se han aislado algunas de cadena insaturada, no obstante éstas últimas son muy poco comunes. Los precursores más importantes de las HSLs son la acil-ACP (ACP: Proteína acarreadora del grupo acilo) y la *S*-adenosilmetionina (SAM), como se observa en la figura 5. Estos precursores también son usados en el metabolismo (central) de los ácidos grasos y los aminoácidos, respectivamente. La unidad acílica proviene de la biosíntesis de ácidos grasos, que genera ácidos grasos de cadena par, de ahí que todas las HSLs que se han aislado hasta el momento tengan cadenas carbonadas de número par.[23]Así mismo, se conoce que las HSLs aisladas a partir de fuentes naturales tienen una estereoquímica (*S*), ya que se conserva la configuración de la SAM.[27]

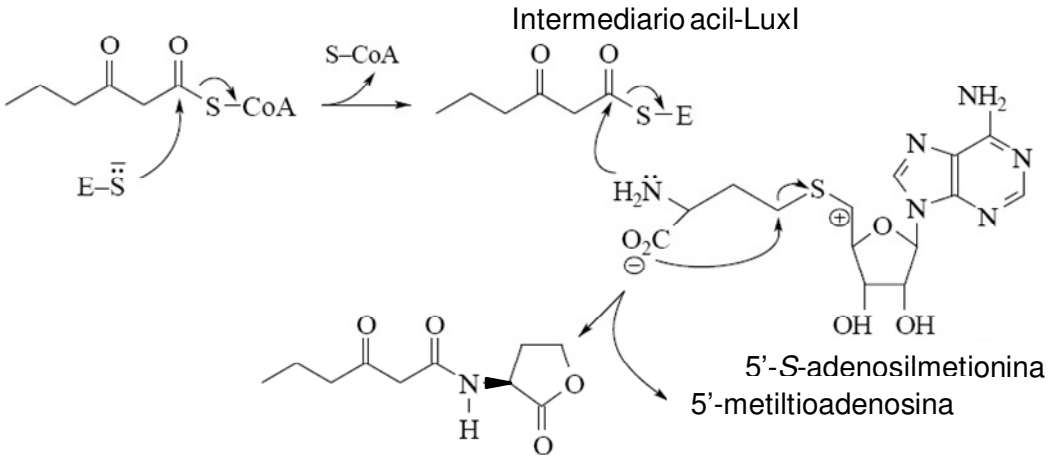

Figura 5. Posible ruta biosintética de la *N*-(3-oxohexanoil)-*L*-homoserinlactona,[4] en donde E (enzima) y S (azufre).

En los estudios realizados sobre bacterias Gram-negativas se ha visto que una especie bacteriana no produce una gran variedad de HSLs, debido a que cada circuito de QS tiene un gen que codifica para una sintasa específica, por lo que ésta solo sintetiza una o a lo

[27] Pomini, A.M.; Araújo, W.L.; Marsaioli, A.J. **2006**. Structural Elucidation and Biological Activity of Acyl-homoserine Lactones from the Phytopathogen *Pantoea ananatis* Serrano 1928. *J. Chem. Ecol. 32*. 1769–1778.

sumo un par de HSLs, las cuales son propias de la expresión de determinado fenotipo.[23] Por ejemplo, dos de las bacterias que producen enfermedades en peces *Aeromonas hydrophila* y *Aeromonas salmonicida*, sintetizan la *N*-butanoil y *N*-hexanoil homoserinlactonas (C4-HSL y C6-HSL), estas HSLs regulan la producción de la proteína sintasa de HSLs en ambas especies, por lo que se encuentran involucradas en la producción de otras homoserinlactonas de longitud de cadena mayor como la *N*-dodecanoil homoserinlactona.[28]

Entre las bacterias marinas se han identificado HSLs de estructura novedosa, de cadena larga (C_{10} a C_{16}) e insaturada, este es el caso de *Mesorhizobium* sp. que produce las HSLs 5-*cis*-3-oxo-C12-homoserinlactona y 5-*cis*-C12-homoserinlactona (Figura 6), las cuales al parecer le sirven a la bacteria como moléculas señalizadoras de la comunicación con el mismo género y con otros géneros.[29] Lo anterior puede estar relacionado con el hecho que los organismos marinos, en especial las esponjas y algunas bacterias de origen marino, tienen rutas metabólicas de síntesis de ácidos grasos diferentes a las presentadas por organismos terrestres, lo cual se ve reflejado en la producción de ácidos grasos con estructuras químicas novedosas (cadenas de número impar de carbonos, presencia de insaturaciones, presencia de halógenos, etc.)[30]

[28] Cataldi, T-R.I.; Bianco, G.; Palazzo L.; Quaranta, V. **2007**. Occurrence of *N*-acyl-L-homoserine lactones in extracts of some Gram-negative bacteria evaluated by gas chromatography–mass spectrometry. *Analytical Biochemistry*. *361*, 226–235.
[29] Krick, A.; Kehraus, S.; Eberl, L.; Riedel, K.; Anke, H.; Kaesler, I.; Graeber, I.; Szewzyk, U.; König, G.M. **2007**. A marine *Mesorhizobium* sp. produces structurally novel long-chain *N*-Acyl-L-homoserine lactones. *Applied and Environmental Microbiology*. 3587–3594.
[30] Berge J.P.; Barnathan G. **2005**. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Adv Biochem Eng Biotechnol 96*, 49.

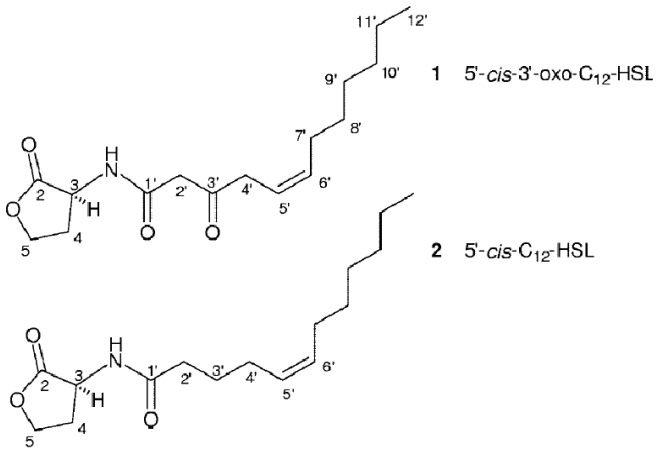

Figura 6. *N*-acilhomoserinlactonas aisladas de *Mesorhizobium* sp.[29]

1.3. Detección de *quorum sensing*

La presencia de circuitos de QS en bacterias nativas (*wild-type*) se pueden evidenciar indirectamente a través de la detección de las HSLs que ellas producen, para esto se usan sistemas de monitoreo biológico, tales como los biosensores, que son cepas bacterianas modificadas (mutadas en el gen que codifica para la sintasa tipo LuxI), incapaces de producir las HSLs pero que contienen proteínas tipo LuxR que se unen a las HSLs exógenas y expresan un fenotipo específico. Estos fenotipos se pueden observar a simple vista a fin de facilitar la detección de HSLs y/o su cuantificación en el laboratorio.

Los biosensores se dividen en dos grandes grupos, los que se construyen a partir de una bacteria blanco a la cual se le inserta un plásmido que contiene todo el circuito de QS de una bacteria conocida, por ejemplo el biosensor *Escherichia coli* pSB401 que expresa el fenotipo de la bioluminiscencia de la bacteria *Vibrio fischeri*; y los biosensores que se construyen a partir de bacterias que naturalmente poseen circuitos de QS, pero a los cuales se les inserta un represor de la producción de HSLs, los ejemplos más conocidos de éste tipo de biosensores y el fenotipo que expresan son los siguientes: el biosensor *Chromobacterium violaceum* CV026 expresa el gen que codifica para la sintasa de la violaceína, el biosensor *Agrobacterium tumefaciens* A136 expresa el gen que codifica para

la sintasa de la β-galactosidasa, el biosensor *Pseudomonas fluorescens* F113 expresa el gen que codifica para la sintasa de la β-glucoronidasa y β-galactosidasa, etc.

Se sabe, que cada proteína receptora (LuxR, LasR, RhiR, etc) responde solamente a unas cuantas HSLs, según el dominio de unión de la proteína. De este modo se sabe que para la detección de HSLs de cadena corta se utilizan biosensores como *Chromobacterium violaceum* CV026 o *Escherichia coli* pSB401, para la detección de HSLs de cadena larga se usa el biosensor *Agrobacterium tumefaciens* A136; cuando la cadena acílica se encuentra sustituida con grupos hidroxilo, se utiliza el biosensor *Pseudomonas fluorescens* F113*,* cuando se quiere detectar HSLs de diferente tipo se suele usar *Agrobacterium tumefaciens* A136 que es considerado de amplio rango de detección.[31]

Los biosensores revelan información a cerca de la presencia de HSLs, sin embargo no proveen información concluyente sobre la identidad de las HSLs que produce la bacteria objeto de estudio. Por lo anterior existen varias alternativas para determinar la identidad de las HSLs, una de ellas es el uso de la CCD utilizando un patrón de HSL y revelando con el biosensor, no obstante la identificación de las HSLs por éste método tiene las limitaciones propias del parámetro cromatográfico Rf, por lo cual se ha hecho necesario utilizar técnicas espectroscópicas que permitan la completa caracterización estructural de las HSLs.

1.4. Aislamiento y caracterización de *N*-acilhomoserinlactonas

Para realizar la extracción de las HSLs se debe realizar un cultivo de la bacteria de interés en el caldo de cultivo apropiado, el cual debe permitir un óptimo crecimiento. También es importante que el tiempo de incubación del cultivo, corresponda con el tiempo en que las bacterias se encuentran en su fase exponencial de crecimiento (figura 4), ya que es el momento en el cual las bacterias se retroalimentan, aumentando la producción de HSLs y la concentración de dichos compuestos en el medio de cultivo. Teniendo en cuenta los

[31] Steindler, L.; Venturi, V. **2007.** Detection of quorum-sensing *N*-acyl homoserine lactone signal molecules by bacterial biosensors. *FEMS Microbiol Lett. 266,* 1–9.

anteriores parámetros se han desarrollado diferentes técnicas de extracción de las HSLs presentes en el medio de cultivo, entre las cuales la técnica más utilizada es la partición líquido-líquido o extracción con solventes (cloroformo, diclorometano, o acetato de etilo)[32], aunque en algunos trabajos se ha utilizado microextracción en fase sólida[33] o la extracción con resinas.[34] Dentro de nuestro grupo de Investigación ya se han realizado estudios con el fin de encontrar las mejores condiciones para realizar la extracción de HSLs de algunas bacterias conocidas como la *P. putida* IsoF, encontrando que la extracción con acetato de etilo acidificado, es la técnica más apropiada en nuestras condiciones.[35]

El aislamiento y la purificación de las HSLs se realiza por medio de técnicas cromatográficas convencionales tales como la cromatografía en capa delgada preparativa (CCDP) o la cromatografía líquida de alta eficiencia (CLAE), ésta última se lleva a cabo sobre columnas RP-18, usando como fase móvil MeOH/H_2O, o ACN/H_2O.[32] La elucidación estructural de las *N*-acilhomoserinlactonas se hace utilizando los métodos espectroscópicos convencionales como lo son la Resonancia Magnética Nuclear (RMN) mono y bidimensional, y la espectrometría de masas (EM). También, aprovechando el hecho de que muchas de las estructuras de las HSLs se han elucidado, se ha optado por hacer comparación de los perfiles cromatográficos obtenidos por CG[28] o CLAE,[32] y de sus espectros de masas. Para esto se emplean patrones comerciales y se comparan con los extractos objeto de estudio. Una ventaja adicional de las técnicas cromatográficas es que permite su cuantificación y detección a concentraciones muy bajas que por RMN no siempre es posible.

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[32] Fekete, A.;Frommberger, M.; Rothballer, M.; Li, Xi.; Englmann, M.; Fekete, J.; Hartmann, A.; Eberl, L.; Schmitt-Kopplin, P. **2007**. Identification of bacterial N-acylhomoserine lactones (HSLs) with a combination of ultra-performance liquid chromatography (UPLC), ultra-high-resolution mass spectrometry, and in-situ biosensors. *Anal Bioanal Chem. 387*, 455–467.

[33] Schupp, P.J.; Charlton, T.S.; Taylor M.W.; Kjelleberg, S.; Steinberg, P.D. **2005**. Use of solid-phase extraction to enable enhanced detection of acyl homoserine lactones (AHLs) in environmental samples. *Anal Bioanal Chem. 383*, 132–137.

[34] Magarvey, N.A.; Keller, J.M.; Bernan, V.; Dworkin, M.; Sherman, D.H. **2004**. Isolation and characterization of novel marine-derived actinomycete taxa rich in bioactive metabolites. *Appl. Environ. Microbiol. 70.* 7520-7529.

[35] Pardo, M. A. Extracción y purificación de 3-oxo-C8HSL y 3-oxo-C10HSL de *Pseudomona putida* IsoF. Trabajo de grado en Química, Universidad Nacional de Colombia, 2008.

1.5. *Quorum sensing* en el ambiente marino

Muchas bacterias aisladas a partir de fuentes terrestres producen moléculas de señalización que se encuentran ampliamente caracterizadas,[36] pero existe mucho menos información acerca de la producción de moléculas señalizadoras de QS por parte de bacterias marinas excepto por *Vibrio* spp, como se mencionó anteriormente las primeras moléculas señalizadoras se encontraron en la bacteria *Vibrio fischeri* y se han realizado múltiples estudios enfocados hacia éste género. Se sabe que las bacterias Gram-negativas son dominantes en el ambiente marino,[37] dentro de éste tipo de bacterias al parecer tanto las bacterias marinas que viven libres y como aquellas que se encuentran asociadas a invertebrados tienen circuitos de QS. Uno de los estudios que se ha realizado utilizando bacterias asociadas a organismos marinos plantónicos es el realizado por Gram, *et al* en 2002, en donde se analizaron 43 bacterias aisladas de *marine snow* y de las algas diatomáceas *Thalassiosira rotula* y *Skeletonema costatum*, las cuales se usaron con el fin de evaluar la producción de HSLs en el laboratorio.[38] Los investigadores usaron los biosensores *A. tumefaciens*, *C. violaceum* CV026 y *E. coli* pSB403, encontrando que los sobrenadantes del 9% de las bacterias aisladas contienen HSLs. Los aislamientos que tienen circuitos de QS coincidieron con α-Proteobacterias (*Roseobacter* spp.) y γ-Proteobacterias (*Marinobacter* spp.). El bioensayo empleando cromatografía en capa delgada y los biosensores *A. tumefaciens*, *C. violaceum* CV026 y *E. coli* pSB403, para los extractos de cultivos de las cepas de *Roseobacter* envejecidos durante 7 días mostraron perfiles concordantes con las moléculas señalizadoras *N*-hexanoilhomoserinlactona (C6-HSL) y *N*-octanoilhomoserinlactona (C8-HSL), que son algunas de las HSLs que se presentan más comúnmente en los circuitos de QS de bacterias Gram-negativas.[38]

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[36] Waters, C.M.; Bassler, B.L. **2005**. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol 21*, 319–346.

[37] Zhang Y, Jiao N, Cottrell MT, Kirchman DL. **2006**. Contribution of major bacterial groups to bacterial biomass production along a salinity gradient in the South China Sea. *Aquat Microb Ecol. 43*, 233–241.

[38] Gram, L.; Grossart, H.P.; Schlingloff, A.; Kirboe, T. **2002**. Possible quorum sensing in marine snow bacteria: production of acylated homoserine lactones by Roseobacter strains isolated from marine snow. *Appl Environ Microbiol 68,* 4111–4116.

Otros estudios realizados con bacterias asociadas a organismos marinos (como por ejemplo algas, moluscos y esponjas) han mostrado que las bacterias asociadas a las esponjas marinas también producen HSLs.[21] Ensayos con los biosensores *A. tumefaciens* y *C. violaceum* CV026, y cromatografía de gases acoplado a espectrometría de masas (CG-EM), demostraron la producción de C6-HSL y *N*-(3-oxo)-hexanoilhomoserinlactona (3-oxo-C6-HSL) por parte de *Vibrio* sp. asociada a la esponja *Cymbastela concentrica*. Los resultados de los experimentos con extractos metanólicos de la esponja, demostraron que los mismos extractos de la esponja purificados contienen HSLs, lo cual sugiere que las bacterias asociadas a la esponja en condiciones naturales producen moléculas señalizadoras.[21] Así mismo, se ha comprobado la producción de HSLs por parte de α- y γ-Proteobacterias, obtenidas de las esponjas marinas *Mycale laxissima* e *Ircinia strobilina*. La presencia de las moléculas señalizadoras se ha detectado tanto en los extractos de las bacterias como en los alrededores de la columna de agua que rodea a las esponjas.[39] Entre las bacterias asociadas a la esponja marina *M. laxissima* se encontró un gran número de cepas productoras de HSLs (*Ruegeria* spp., *Silicobacter* spp., y γ-Proteobacteria), mientras que un bajo número de cepas con circuitos de QS fueron recuperadas del agua de mar circundante. Es más, las cadenas acílicas encontradas en las HSLs, producidas por las bacterias aisladas, por las esponjas y por la columna de agua son diferentes. Todo lo anterior sugiere que las moléculas señalizadoras pueden ser producidas por bacterias planctónicas y por las bacterias que se encuentran asociadas a organismos marinos.

[39] Mohamed, N.M.; Cicirelli, E.M.; Kan, J.; Chen, F.; Fuqua, C.; Hill, R.T. **2008**. Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges. *Environ Microbiol 10,*75–86.

2. METODOLOGÍA

2.1. PROCEDIMIENTOS GENERALES

La cromatografía en capa delgada en fase reversa (CCD-FR) se desarrolló sobre cromatoplacas Sílica Gel 60 RP-18 $F_{254}S$ Merck, (20 x 20 y 0,30 mm de espesor), usando como eluente MeOH/H_20 en proporciones 6:4 y 7:3. El revelado se realizó utilizando una lámpara UV (longitud de onda 254 nm y 366nm) y mLBr inoculado con el biosensor *E. coli* (pSB401). La cromatografía en columna en fase reversa (CC-FR) se realizó sobre la fase estacionaria LiChroprep RP-18 (40-63 μm, Merck), en una columna con longitud de fase estacionaria de 30,0 cm y un diámetro interno de 1,5 cm. Las fracciones recolectadas fueron monitoreadas por CCD-FR. Como patrón se empleo *N*-hexanoil-DL-homoserinlactona (Fluka).

a. Equipos

Se usó agitador orbital marca LAB-LINE instruments modelo 3527, termostatado, a temperatura ambiente o a 37°C, y a 200 rpm. Se usó un microscopio OLYMPUS CH20 modelo CH20BIMF110. La densidad óptica de las bacterias se determinó con un espectrofotómetro Espectronic 20 GENESYS, realizando lecturas a una longitud de onda de 600 nm. La luminiscencia se registró en un digitalizador de geles BIORAD Quantity-One 4.1.0.

Se empleó una centrifuga marca SORVALL instruments OU-PONT modelo RC-5C, a una temperatura de 15°C, 5000 rpm durante 15 minutos. Se usó agitador orbital marca LAB-LINE instruments modelo 3527, termostatado a temperatura ambiente o a 37°C, a 200 rpm.

Los espectros infrarrojos se registraron en un espectrofotómetro PERKIN-ELMER FT-IR PARAGON 500 SERIE 1000.

Los análisis por CGAR-EM se realizaron en un cromatógrafo de gases GC-17A SHIMADZU acoplado a espectrómetro de masas QP-5050A SHIMADZU. La inyección de la muestra se realizó en modo *split* (10:1) dentro de una columna RPX-5 (30 m, 0,32 mm, 0,25 μm). Se inyectó en todos los casos 1 μg de muestra disuelta en 1 μL de acetona. Como gas de arrastre se empleó Helio (grado UAP) a una velocidad de 1,9 mL min^{-1}. Durante los análisis se mantuvo la temperatura del inyector a 200ºC y el detector a 300°C. La temperatura programada para el horno se optimizó manteniéndolo durante 3 minutos a 50°C e incrementando la temperatura 8,3°C min^{-1} hasta alcanzar una temperatura de 300°C. El espectrómetro de masas operó en modo de ionización por impacto electrónico (IE) a 70 eV, el monitoreo de los iones se llevó a cabo en modo SCAN detectando los iones positivos con masas entre 40 y 800 u. También se llevó a cabo el análisis en modo SIM monitoreando los iones *m/z* 102 y 143.

Los análisis por CLAE-EM se realizaron en un cromatógrafo líquido LC-10AD SHIMADZU acoplado a espectrómetro de masas LCMS-2010 EV SHIMADZU. La inyección de la muestra se realizó dentro de una columna RP-18 phenomenex Luna 5 μ 100A de dimensiones150 x 2,0 nm. Se inyectaron 2 μL del patrón C6-HSL, de una disolución de 1 mg en 60 μL de MeOH/H_2O (1:1). En el caso de los extractos y fracciones se inyectaron 5 μL de una disolución de 5 mg en 100 μL de MeOH/H_2O (1:1). Como fase móvil se utilizaron MeOH grado CLAE-EM y H_2O/ácido fórmico 0,1%. Las muestras fueron eluídas con un gradiente lineal, el cual inició con MeOH al 15% hasta llegar al 100% en 15 minutos, el flujo se fijó en 0,3 mL min^{-1}. El espectrómetro de masas operó en modo de ionización por electrospray (ESI) a 1,5 kV, utilizando como gas nebulizador

nitrógeno a un flujo de 1,5 L min^{-1}, se detectaron los iones positivos con masas entre 100 y 300 u.

b. Medios de cultivo

Los medios de cultivo que se utilizaron para el crecimiento de las diferentes bacterias fueron los siguientes:

Para el caldo marino (CM) se usó la siguiente composición: NaCl 2,08%, KCl 0,06%, $MgSO_4$ 0,48%, $MgCl_2$ 0,40%, Rila 0,15%, Tris 0,04%, Peptona 0,30%, levadura 0,15% y Glicerol 0,15%. Se utilizó para el crecimiento de las bacterias marinas objeto de este estudio.

Para el medio marino (MM) se usó la siguiente composición: NaCl 2,08%, KCl 0,06%, $MgSO_4$ 0,48%, $MgCl_2$ 0,40%, Rila 0,15%, Tris 0,04%, Peptona 0,30%, levadura 0,15%, Glicerol 0,15% y agar-agar 1,80%. Se utilizó para el crecimiento de las bacterias marinas objeto de este estudio.

Para el medio marino modificado (MMm) se usó la siguiente composición: NaCl 1,00%, KCl 0,06%, $MgSO_4$ 0,48%, $MgCl_2$ 0,40%, Rila 0,15%, Tris 0,04%, Peptona 0,60%, levadura 0,30%, Glicerol 0,15% y agar-agar 1,80%. Se utilizó para realizar los ensayos de estriado en cruz, ya que en él crecen tanto las bacterias marinas, como el biosensor.

Para el caldo Luria-Bertani (cLB) se usó la siguiente composición: Levadura 0,5%, NaCl 0,5% y Peptona 1,0%. Se utilizó para el crecimiento de las bacterias control.

Para el medio Luria-Bertani (mLB) se usó la siguiente composición: Levadura 0,5%, NaCl 0,5%, Peptona 1,0% y agar-agar 1,2%. Se utilizó para el crecimiento de las bacterias control y realizar ensayos de estriado en cruz de ellas.

Para el medio Luria-Bertani al 0,8% usado como revelador (mLBr) se usó la siguiente composición: Levadura 0,5%, NaCl 0,5%, Peptona 1,0% y agar-agar 0,8%. Se utilizó para el crecimiento del biosensor y se empleó como revelador en la cromatografía en capa delgada.

c. Bacterias de estudio

Los aislamientos bacterianos a estudiar hacen parte de cepario del grupo de investigación "Estudio y Aprovechamiento de Productos Naturales Marinos y Frutas de Colombia" y fueron obtenidos por J. Mora[40] a partir del *biofilm* que recubría las esponjas *Aplysina lacunosa, Aplysina fistularis,* la concha de un bivalvo *Donax* sp., y de discos de phytagel sumergidos en la bahía de Santa Marta (Mar Caribe Colombiano). El biosensor y las bacterias patrón fueron donadas por Dr. Katrin Riedel del Departamento de Microbiología del Instituto de Biología de Plantas de Zúrich, Suiza. Todas las cepas bacterianas usadas en este estudio se encuentran dentro de la Tabla 1 y 2.

TABLA 1: Bacterias utilizadas en el estudio como control o biosensor.

CÓDIGO CEPA	DESCRIPCIÓN
E. coli (pSB401)	Bacteria biosensora que contiene plásmido con sistema LuxI/R, gen reportero: operón *luxCDABE*; Tet^r. La cual produce bioluminiscencia cuando detecta las HSLs C6-HSL, C8-HSL, 3-oxo-C6-HSL y 3-oxo-C8-HSL. Pero no las produce.
P. putida IsoF	Bacteria aislada de raíces de tomate "*Wild Type*" que produce las HSLs C4-HSL, C6-HSL, C8-HSL, C10-HSL, C12-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL, 3-oxo-C10-HSL y 3-oxo-C12-HSL. Usada como control positivo.
P. putida F117	Bacteria mutante de IsoF Km^r, ppuI::npt, que no produce las HSLs. Usada como control negativo. No produce algún fenotipo visible.

[40] Mora, J. Posibles mecanismos químicos y biológicos para el control de la epibiosis en las esponjas *Aplysina insularis* y *Aplysina lacunosa* (Demospongiae, Verongida). **2007**. Tesis M.Sc. Biología, Universidad Nacional de Colombia.

TABLA 2: Bacterias utilizadas en el estudio. Descripción macro, tinción de Gram, morfología, y resultados de las pruebas bioquímicas de las 13 cepas de bacterias marinas.

CÓDIGO CEPA	CARACTERÍSTICAS	GRAM	MORFOLOGÍA	DESCRIPCIÓN MACRO	MAL (- gas)	LAC (- gas)	GLU (- gas)	SAC (- gas)	TSI	CIT (Simmons)	LIA	MR	VP	SIM	NITRATOS
21-6PIN	Bacterias aisladas del *biofilm* que recubría el pinacodermo de la esponja *Aplysina lacunosa.* La esponja fue recolectada en la bahía de Santa Marta (Mar Caribe Colombiano).	-	Bacilos cortos redondeados	Bacteria redondeada, brillante, de color crema, traslúcida.	+	+	+	+	A/A	+	K/K	-	-	m- s- i-	-
23-6PIN		-	Bacilos curvos	Bacteria brillante, cremosa, de color crema y traslúcida.	+	+	+	+	A/A	-	K/K	+	-	m- s- i-	-
6-8PIN		-	Bacilos curvos	Bacteria expansiva, cremosa, de color crema, opaca y traslúcida.	+	+	+	+	A/A	-	K/K	-	-	m+ s- i-	-
11-6DEP	Bacterias aisladas del *biofilm* que recubría la depresión inhalante de la esponja *Aplysina lacunosa.* La esponja fue recolectada en la bahía de Santa Marta (Mar Caribe Colombiano).	-	Bacilos cortos redondeados	Bacteria redonda, de color crema, brillante y traslúcida.	-	+	+	+	A/K	-	K/K	+	-	m- s- i-	-
4-4DEP		-	Bacilos cortos redondeados	Bacteria redonda, brillante, de color blanco y aspecto cremoso.	+	+	+	-	A/A	+	K/A	+	+	m+ s- i-	-
10-6 DEP		-	Bacilos	Bacteria redonda, brillante, elevada, color crema y traslúcida.	-	-	+	+	A/K	-	A/K	+	-	m+ s- i-	-
10-4DEP		-	Bacilos redondeados	Bacteria de color blanco, aspecto cremoso y traslúcida.	+	-	+	+	A/A	-	A/K	-	-	m- s- i-	-
12-AINS-6B	Bacterias aisladas del *biofilm* que recubría el pinacodermo de la esponja	-	Bacilos curvos en cadena	Bacteria redonda, brillante, elevada, color crema, de	-	-	-	-	K/K	-	A/K	-	+	m+ s- i-	-

16-AINS-5	*Aplysina insularis.* La esponja fue recolectada en la bahía de Santa Marta (Mar Caribe Colombiano).	+	Bacilos	aspecto cremoso y traslúcida. Bacteria expansiva, cremosa, de color crema, opaca y traslúcida.	+	+	+	-	A/K	-	K/A	+	-	m- s- i-	-
PHY-11	Bacteria aislada de discos de phytagel inmersos en la bahía de Santa Marta (Mar Caribe Colombiano).	+	Bacilos	Bacteria redonda, elevada, cremosa, de color crema y brillante.	-	+	+	+	A/K	-	K/K	-	-	m+ s- i-	+
29-C	Bacterias aisladas del *biofilm* que recubría la concha de un bivalvo *Donax* sp, recolectada en la bahía de Santa Marta (Mar Caribe Colombiano).	+	Cocos	Bacteria redonda, brillante, elevada, de coloración amarilla	+	-	+	+	A/K	-	K/K	+	-	m- s- i-	+
31-C		+	Bacilos	Bacteria redonda, de color blanco y opaco.	-	+	+	-	A/K	-	K/K	-	-	m- s- i-	-
11-C1		+	Cocos	Bacteria redonda, de color blanco y opaco.	+	-	+	+	K/A/K	-	K/A	-	-	m- s- i-	-

A los aislamientos de bacterias marinas sujeto de estudio, se les realizaron pruebas de tinción de Gram, y pruebas bioquímicas en medios sólidos y en caldos, tales como: fermentación de diferentes carbohidratos (Maltosa (MAL), Lactosa (LAC), Glucosa (GLU), Sacarosa (SAC) y Triple azúcar Hierro (Agar TSI)) en caldos usando rojo de fenol como indicador de pH del medio, citratos (CIT- Agar Simmons), Lisina descarboxilasa (Agar LIA), Prueba acidez (Rojo metilo (MR)), Prueba acetoína (Voges Proskaver (VP)), Sulfuros/Indol/Movilidad (Medio SIM), Reducción de nitrato y Producción de H_2S. Para lo anterior se usaron los procedimientos descritos por Valencia, H.A., 2004 y los resultados obtenidos se analizaron haciendo uso del manual de determinación bacteriológica de Bergey[41]. Pruebas positivas (+), pruebas negativas (-), sin producción de gas (gas-), ácido (A), base (K), motilidad negativa (m-), motilidad positiva (m+), sulfuros negativo (s-), sulfuros positivo (s+), indol negativo (i-), indol positivo (i+).

[41] Holt, J.G; Krieg, N.R. Sneath, P.; Staley, J.T. **1994.** Bergey's manual of determinative bacteriology. 9th ed. Williams and Wilkins. Baltimore. Maryland. p 1388.

2.2. CURVAS DE CRECIMIENTO DE LOS AISLAMIENTOS BACTERIANOS

Para la realización de las curvas, se adaptó el procedimiento descrito por Valencia, H.A., 2004.[42] De los aislamientos de bacterias marinas se realizaron preinóculos, para ello se cultivaron aeróbicamente en 50mL de caldo marino (CM) con agitación constante 200rpm, las condiciones de temperatura probadas fueron 37ºC y temperatura ambiente. Los tiempos de incubación ensayados se encuentran entre 12-35 horas. Ambas condiciones se ajustaron para cada uno de los aislamientos, hasta obtener las mejores condiciones de crecimiento (temperatura y tiempo).

De éste preinóculo se tomó 1 mL que se llevó a 100 mL de CM, homogenizando el cultivo mediante agitación y midiendo la densidad óptica a 600 nm. El cultivo restante se incubó a las condiciones encontradas para el preinóculo y se tomaron alícuotas de 1mL cada hora para medir su densidad óptica hasta tener una medida de absorbancia constante. Por último se graficó la absorbancia en función del tiempo (horas). (Anexo 1) Adicionalmente, a cuatro tiempos representativos dentro de las zonas de la curva de crecimiento, se realizó el cálculo de u.f.c. con el fin de conocer la población aproximada en dicho momento. Para ello, se tomaron 100 µL que se cultivaron en una caja con MM y también se realizaron diluciones del preinóculo en CM de las que se tomaron 100 µL que se cultivaron en cajas con MM y se incubaron a las condiciones encontradas. Para el cálculo de u.f.c. se seleccionaron las placas que presentaban entre 30 y 300 colonias.

2.3. DETECCIÓN DEL *QUORUM SENSING* POR MEDIO DE BIOSENSORES

En cajas de Petri con medio marino modificado,[43] se realizó un estriado en cruz del biosensor *E. coli* (pSB401), un control positivo que produce HSLs (*Pseudomonas putida*

[42] Valencia, H.A. **2004.** Manual de prácticas de microbiología básica. Notas de Clase, Facultad de Ciencias. Universidad Nacional de Colombia. 53-62.
[43] Castro, V. Detección de comunicación intercelular, mediada por acil-homoserinlactonas, en bacterias marinas del mar Caribe Colombiano. Trabajo de Grado en Química, Universidad Nacional de Colombia, 2008.

IsoF), un control negativo (*P. putida* 117 mutante incapaz de sintetizar HSLs) y cada uno de los aislamientos bacterianos objeto de estudio, como se observa en la figura 7a. Con el fin de tomar fotografías de la bioluminiscencia producida en el ensayo, se realizó el estriado únicamente del biosensor *E. coli* (pSB401) con cada uno de los aislamientos bacterianos objeto de estudio, el control positivo y el control negativo, como se observa en la figura 7b, esto con el fin de evitar que la bioluminiscencia inducida por el control positivo enmascarara la producida por el asilamiento bacteriano. En ambos casos se incubaron las cajas aeróbicamente a 32°C durante 24 horas, la luminiscencia que presentó el biosensor se registró con una fotografía tomada con un digitalizador de geles. (Anexo 2)

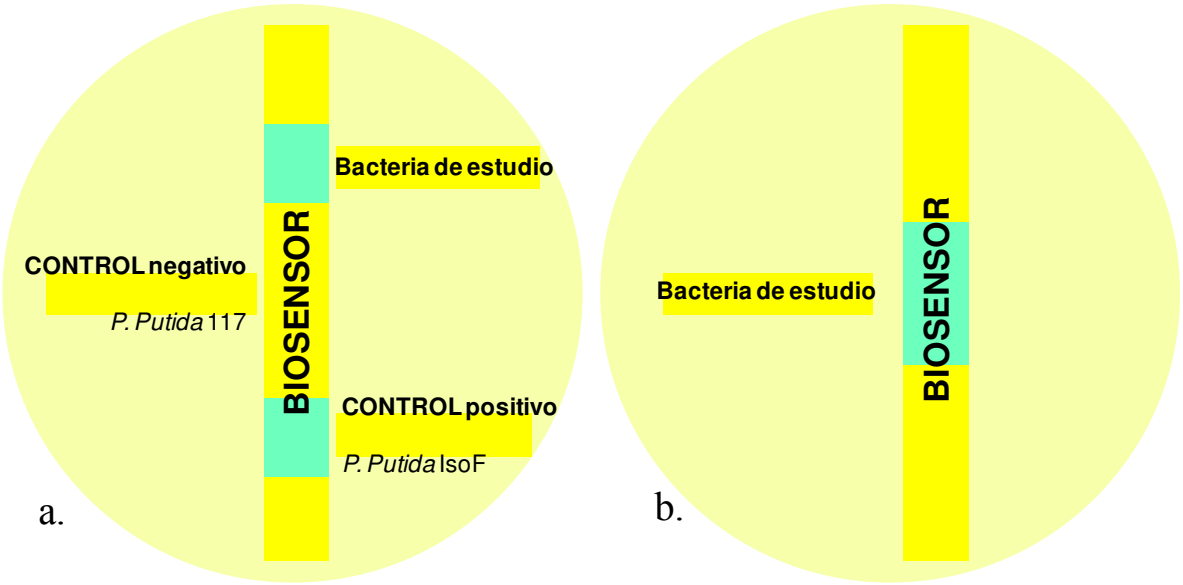


Figura 7. Ensayo de estriado en cruz usando como biosensor *E. coli* (pSB401). a. Ensayo con el biosensor en donde se tiene el control positivo (*P. putida* IsoF), control negativo (*P. putida* 117) y la bacteria de estudio. b. Ensayo con el biosensor y la bacteria de estudio. En verde se representa la luminiscencia inducida por las AHLs en el biosensor.

2.4. EXTRACCIÓN DE LAS *N*-ACILHOMOSERINLACTONAS

Los aislamientos de bacterias marinas se cultivaron aeróbicamente en 500 mL de CM, con agitación constante 200 rpm, las condiciones de temperatura y tiempo de incubación, fueron las que se encontraron para cada una de ellas al realizar las curvas de crecimiento.

Cuando cada uno de los cultivos alcanzó su fase exponencial se centrifugaron a 5000 rpm durante 15 minutos y a una temperatura de 15°C. El líquido sobrenadante se filtró al vacío con membranas Millipore de celulosa de 0,8, 0,6 y 0,45 μm, consecutivamente. El filtrado se sometió a partición líquido-líquido con tres volúmenes de 200 mL de AcOEt acidificado con ácido fórmico al 0,5%.[32,33] El extracto orgánico se sometió a partición líquido-líquido con 300mL de agua destilada, luego la fase orgánica se secó sobre sulfato de sodio anhidro, se filtró y el solvente se eliminó por destilación a presión reducida, las cantidades obtenidas de extracto para cada aislamiento bacteriano se muestran en la tabla 1.

Adicionalmente, se seleccionó el aislamiento bacteriano etiquetado como 21-6PIN para hacer un cultivo en gran volumen, y buscar un aislamiento de las HSLs producidas. Para lo anterior la bacteria se cultivó aeróbicamente en 2 L de CM, con agitación constante 200 rpm, a 37°C durante 24 horas, con el fin de realizar la extracción de las HSLs haciendo uso de la anterior metodología (centrifugación, filtración y extracción) y manteniendo las proporciones descritas (partición con 800 mL de AcOEt acidificado con ácido fórmico al 0,5%). Éste procedimiento se realizó por triplicado obteniendo 225,8 mg de extracto. Una porción del extracto (205,3 mg) fue separada mediante CC-FR, para esto se empleó un gradiente discontinuo de polaridad desde H_2O/MeOH proporción 92:8 hasta MeOH y luego ACN. Se obtuvieron 66 fracciones de 5 mL, las cuales se agruparon en 8 fracciones, teniendo en cuenta el perfil obtenido por CCD-FR revelando con lámpara UV (longitud de onda 254 nm y 366 nm). Cada fracción se analizó por CCD-FR revelada con el biosensor y por CLAE-EM (*vide infra*).

Las bacterias utilizadas como control positivo (*P. putida* IsoF) y control negativo (*P. putida* 117) se extrajeron del mismo modo que las bacterias marinas objeto de estudio. Se cultivaron aeróbicamente en 500 mL de cLB, con agitación constante 200 rpm, a 37ºC durante 24 h. Luego se centrifugó y el líquido sobrenadante se filtró al vacío. El filtrado se sometió a partición líquido-líquido con tres volúmenes de 200 mL de AcOEt acidificado con ácido fórmico al 0,5%, el procedimiento restante se llevo a cabo de la misma manera que se describió anteriormente.

2.5. DETECCIÓN DE HSLs POR TÉCNICAS CROMATOGRÁFICAS Y ESPECTROSCÓPICAS

De los extractos de las bacterias marinas, del control positivo (*P. putida* IsoF), y del control negativo (*P. putida* 117) se tomaron 100 µL de concentración 0,01% en acetona y se pusieron en una placa de CCD-FR, así mismo del patrón C6-HSL se tomaron 5 µL de concentración 0,01%. La placa se eluyó con MeOH/H_20 en proporción 7:3 y se reveló con el biosensor de la siguiente manera: El biosensor *E. coli* (pSB401) se dejó crecer en cLB durante 12 horas a 37°C, a continuación se mezcló con la misma proporción de mLBr ajustando la concentración al 0,8% de agar. Luego se sirvieron alrededor de 5 mL de este medio sobre las placas cromatográficas que se encuentran dentro de una caja de petri. En seguida las cajas se incubaron durante 12 horas a 37°C, hasta observar luminiscencia la cual se registró con una fotografía tomada con un digitalizador de geles.

De otro lado los extractos se analizaron por medio de espectroscopía de infrarrojo, haciendo seguimiento de las bandas características de las HSLs (1777, 1545, 1173, 1014, 947 y 726 cm^{-1}).

2.6. CARACTERIZACIÓN DE LAS *N*-ACILHOMOSERINLACTONAS

La identificación de las HSLs se llevó a cabo utilizando cromatografía de gases acoplada a espectrometría de masas (CG-IE-EM), y cromatografía líquida de alta eficiencia acoplada a espectrometría de masas (CLAE-ESI-EM). Lo anterior se hace para obtener los espectros de masas de las HSLs tanto por ionización electrónica como por ionización suave (ESI) y de esta manera contar con más elementos de juicio para llevar a cabo la elucidación estructural.

3. RESULTADOS Y DISCUSIÓN DE RESULTADOS

Las bacterias objeto de estudio fueron aisladas a partir de *biofilm*s naturales que recubrían superficies tanto animadas como inanimadas sumergidas en el mar Caribe. Las bacterias provenientes de la esponja *Aplysina insularis*, codificadas como #-AINS, y de discos de phytagel, codificadas como PHY-#, corresponden a bacterias provenientes de superficies limpias de *macrofouling*, mientras las bacterias aisladas de la concha *Donax* sp, codificadas como #-C, y de la esponja *Aplysina lacunosa*, codificadas como (#-DEP y #-PIN) corresponden a bacterias provenientes de superficies recubiertas de macroorganismos. Por tal motivo, las bacterias objeto de estudio han venido siendo utilizadas en nuestro ensayo *antifouling* y es de nuestra necesidad hacer su caracterización determinando su identidad, las condiciones apropiadas de crecimiento y la presencia de circuitos de QS, porque como se mencionó anteriormente este es fundamental para la consolidación de los *biofilms* primer paso en la formación del *biofouling*. Para la identificación preliminar de las bacterias marinas a nivel de género, se realizó una observación macroscópica de la apariencia de los cultivos de cada uno de los aislamientos bacterianos, encontrando que en su mayoría corresponden a bacterias que forman colonias redondeadas y son de aspecto cremoso. Al realizar la tinción de Gram se infirió que 5 de los aislamientos bacterianos son Gram-positivos y 8 son Gram-negativos. Dentro de las bacterias Gram-positivas se encuentran bacilos y cocos, mientras que en las bacterias Gram-negativas se encuentran básicamente bacilos.

Uno de los sistemas más importantes y utilizados para la caracterización de microorganismos corresponde a la observación de su crecimiento en diversos medios de cultivo, esta técnica se conoce como pruebas bioquímicas. Teniendo en cuenta lo anterior,

se realizaron las pruebas bioquímicas más representativas, cambio en el pH, producción de gas, producción de H_2S y producción de algunos metabolitos. Con los resultados conjuntos de las pruebas bioquímicas, descripción macro, tinción de Gram y morfología, se realizó el reconocimiento parcial de los aislamientos bacterianos, comparando con las descripciones presentes en el manual de Bergey[41] (Tabla 3). Encontrando que dentro de las bacterias Gram-negativas 4 de ellas corresponden al género *Vibrio*, 2 corresponden al género *Ochrobactrum* y las otras 2 corresponden al género *Pseudomonacea* y *Alteromonas*. Adicionalmente, dentro de las bacterias Gram-positivas se encontraron los géneros *Bacillus* y *Staphylococcus*. Estos géneros se han aislado comúnmente a partir de superficies inmersas en el mar.[11] Teniendo en cuenta la superficie de la cual fueron aisladas, se tiene que las bacterias aisladas del pinacodermo de la esponja *Aplysina lacunosa* corresponden solamente a bacterias Gram-negativas de los géneros *Vibrio* y *Ochrobactrum*, en cambio las bacterias aisladas de la depresión inhalante de la misma esponja corresponden tanto a bacterias Gram-negativas como bacterias Gram-positivas de los géneros *Vibrio*, *Ochrobactrum*, *Bacillus* y *Staphylococcus*. Por otro lado las bacterias aisladas tanto de la esponja *Aplysina insularis*, la concha *Donax* sp y los discos de phytagel, muestran variada morfología y una gran diversidad de géneros bacterianos los cuales corresponden a *Pseudomonacea*, *Vibrio*, *Alteromonas*, *Bacillus* y *Staphylococcus*.

Los resultados obtenidos mediante pruebas bioquímicas, morfología y tinción de Gram, fueron comparados con los obtenidos en nuestro grupo de investigación[44] haciendo uso de la caracterización molecular (comparación de la similitud de las secuencias del gen 16S rRNA con las bases de datos EMBL y GenBank). Encontrando total correspondencia en el género de 7 de las 8 cepas Gram-negativas, en la determinación de la identidad de la otra cepa Gram-negativa no se pudo llegar hasta la determinación de género por medio de las pruebas bioquímicas solo se llegó hasta la determinación de la familia (*Pseudomonacea*), a la cual pertenece la especie determinada por 16S (*Schewanella* sp). De acá se puede concluir que la aproximación de la identidad a la que se llegó por medio de técnicas

[44] Mora, J.A.; Arévalo-Ferro, C.; Ramos, F.A.; Tello, E.; Duque, C. Antifouling activities of extracts of marine invertebrates collected at the Colombian Caribbean Sea against marine surface colonizer bacteria. Sometido a Aquatic Toxicology.

sencillas como lo son la tinción de Gram, las pruebas bioquímicas y la observación morfológica de las bacterias es bastante cercana a la obtenida por técnicas moleculares, las limitantes para conocer en algunos casos el género y la especie, vienen dados por la bibliografía con la cual se compara, ya que no existe una clasificación para todas las especies por medio de pruebas bioquímicas, para ello se deben realizar pruebas de identificación específicas para género o especie haciendo uso de medios selectivos.

TABLA 3. Reconocimiento parcial de las 13 cepas de bacterias marinas, haciendo uso de pruebas bioquímicas, morfología y tinción de Gram.

CÓDIGO CEPA	GRAM	APROXIMACIÓN A LA IDENTIDAD POR PRUEBAS BIOQUÍMICAS	CARACTERIZADAS MEDIANTE EL ANÁLISIS DE LAS SECUENCIAS DEL GEN 16S rRNA[44]
21-6 PIN	-	*Ochrobactrum*	*Ochrobactrum* sp
23-6 PIN	-	*Vibrio*	*Vibrio* sp (23-6PIN)
6-8PIN	-	*Vibrio*	*Vibrio campbellii*
11-6 DEP	-	*Vibrio*	*Vibrio* sp (11-6DEP)
4-4 DEP	-	*Ochrobactrum*	*Ochrobactrum pseudogringnonense*
12-AINS-6B	-	*Pseudomonacea*	*Schewanella* sp
PHY-11	-	*Vibrio*	*Vibrio harveyi*
29-C	-	*Alteromonas*	*Alteromona* sp
16-AINS-5	+	*Bacillus*	*Bacillus megaterium*
10-6 DEP	+	*Bacillus*	
10-4 DEP	+	*Staphylococcus*	*Kokuria* sp
31-C	+	*Bacillus*	*Oceanobacillus iheyensis*
11-C1	+	*Staphylococcus*	

Curvas de crecimiento de los aislamientos bacterianos

Una vez identificadas las bacterias Gram-negativas, se continuó el estudio solo con dichas bacterias, ya que son las que poseen circuitos de QS regulados por HSLs que son los compuestos objeto de nuestro estudio.

Con el fin de conocer las condiciones apropiadas de crecimiento de nuestras bacterias marinas, se realizaron las curvas de crecimiento de las cepas Gram-negativas, ya que para nosotros es importante saber a que tiempo la bacteria se encuentra en su fase exponencial debido a que, si nuestras bacterias poseen circuitos de QS mediados con HSLs, en éste lapso de tiempo la producción de estas moléculas se incrementa, ya que

aqui las HSLs producidas y acumuladas se unen de manera estable al factor de transcripción que regula la expresión de la sintasa, de aquí en adelante se da el proceso de transcripción y por consiguiente la expresión genética regulada por QS. Por este motivo las HSLs son también conocidas como autoinductores.

Para realizar las curvas de crecimiento se usó caldo marino, este medio se encuentra enriquecido en sales, con el fin de simular el ambiente natural del cual fueron aisladas las bacterias. Éste caldo fue apropiado ya que permitió el crecimiento de todas las bacterias marinas aunque los tiempos de crecimiento fueron variados, ya que hay bacterias que crecieron rápidamente como la cepa *Ochrobactrum* sp, que alcanzó su fase estacionaria alrededor de 10 horas, mientras que hay bacterias de crecimiento lento, como la cepa *Ochrobactrum pseudogringnonense* que a 24 horas aún no ha alcanzado su fase estacionaria (Fig. 8). Esto se debe a la diversidad en el metabolismo de las cepas bacterianas, ya que en su fase de adaptación ellas van a depender de la actividad metabólica remanente, crecen en tamaño, almacenan nutrientes y sintetizan las enzimas necesarias para su reproducción, que se da en su fase exponencial de crecimiento y en ella se da la expresión genética. En el caso de la cepa *Ochrobactrum pseudogringnonense* que es la cepa de crecimiento más lento, se debe buscar otro sustrato (ensayar otros tipos de caldos) con el que la maquinaria genética no requiera una adaptación tan larga, de ese modo disminuir el tiempo de cultivo y así mismo facilitar las condiciones de crecimiento para la posterior extracción de las HSLs. En la siguiente gráfica se muestran las curvas de crecimiento de las bacterias mencionadas anteriormente, todas las curvas y las condiciones de crecimiento que se encontraron apropiadas estan en el anexo 1.

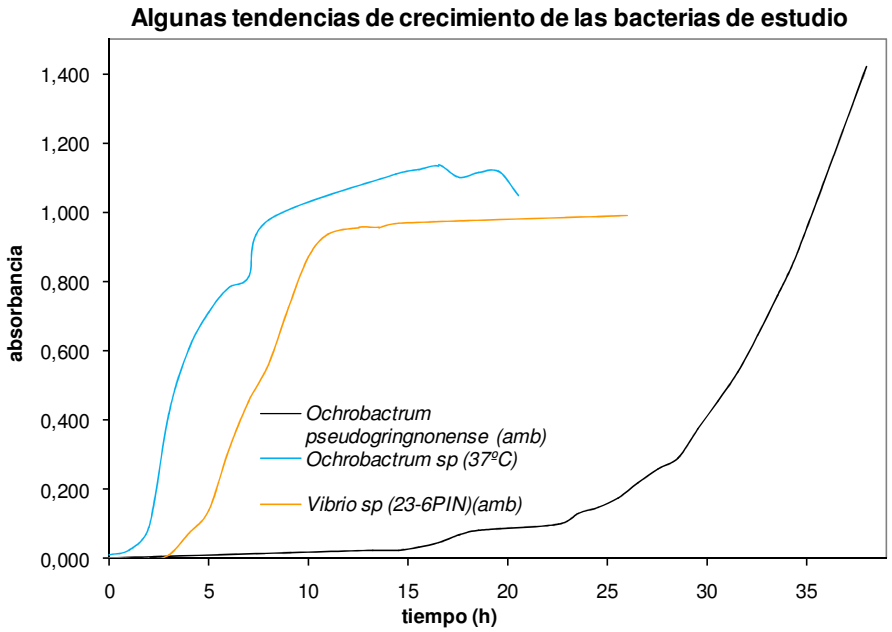


Figura 8. Curvas de crecimiento de algunas de los aislamientos bacterianos de estudio.

Para establecer unas condiciones adecuadas de crecimiento, se debieron ajustar otros parámetros a parte del medio de cultivo, tales como la agitación y la temperatura de incubación. Se ensayaron diferentes velocidades de agitación desde 0 hasta 200 rpm (que es la velocidad estándar para el cultivo de bacterias), encontrando ésta última la más conveniente para agitar las bacterias, debido a que se distribuye la población bacteriana a lo largo del caldo de cultivo facilitando la adquisición de los nutrientes y de oxígeno, ya que las bacterias son aeróbias y requieren de una concentración de oxígeno mínima para su crecimiento. Se ensayaron dos temperaturas (temperatura ambiente y 37ºC), encontrando que no hay una temperatura que sea óptima para todas las bacterias; es decir que unas bacterias crecen mejor a temperatura ambiente y otras crecen mejor a 37ºC. Teniendo en cuenta el rango de temperatura al cual crecen todas las bacterias se dice que las bacterias objeto de estudio son mesófilas. En la tabla 4 se resumen las condiciones de crecimiento encontradas para cada una de las bacterias objeto de estudio, cabe resaltar que las bacterias crecen mejor a temperatura ambiente y en general se tardan el mismo tiempo en crecer (alrededor de 12 h).

TABLA 4. Determinación de las condiciones de crecimiento de las bacterias marinas.

CÓDIGO CEPA	GRAM	TIEMPO DE INCUBACIÓN PREINÓCULO (h)	TEMPERATURA DE INCUBACIÓN (^{0}C)
Ochrobactrum sp	-	10	37
Vibrio sp (23-6PIN)	-	12	ambiente
Vibrio campbellii	-	12	ambiente
Vibrio sp (11-6DEP)	-	12	ambiente
Ochrobactrum pseudogringnonense	-	> 24	ambiente
Schewanella sp	-	12	ambiente
Vibrio harveyi	-	12	ambiente
Alteromona sp	-	12	ambiente

Detección del *Quorum sensing*

Los datos de tiempo de incubación y temperatura, obtenidos a partir de las curvas de crecimiento, fueron los usados en el ensayo de estriado en cruz para la detección del QS haciendo uso del biosensor *E. coli* (pSB401). Los resultados obtenidos y la discusión se describen a continuación.

Con el fin de detectar si las bacterias objeto de estudio producen HSLs, se usó en primera medida el ensayo de estriado en cruz (Fig. 7a), en donde se hizo uso de un control positivo (*P. putida* IsoF), un control negativo (*P. putida* 117), la bacteria de estudio y el biosensor. Los resultados obtenidos en este ensayo no eran operativos, especialmente cuando se trataban de registrar las fotografías, ya que en la mayoría de los casos la luminiscencia producida frente al estriado del control positivo se difundía tanto que hacía parecer que el estriado frente a la bacteria de estudio presentara luminiscencia, esto se debe a problemas de espacio en la caja, ya que las bacterias se difunden y los resultados de luminiscencia no permiten conclusiones certeras. Por tal motivo, y para solucionar el problema se optó por realizar un ensayo adicional, en el cual solamente se usa el biosensor y la bacteria de estudio (Fig. 7b), de este modo se eliminó la posible interferencia que generaba la bioluminiscencia producida por el biosensor frente al control positivo. En la figura 9 se

tienen las fotografías tomadas al ensayo con el control positivo y a la cepa *Ochrobactrum* sp, las fotografías de todos los ensayos se encuentran en el anexo 2.

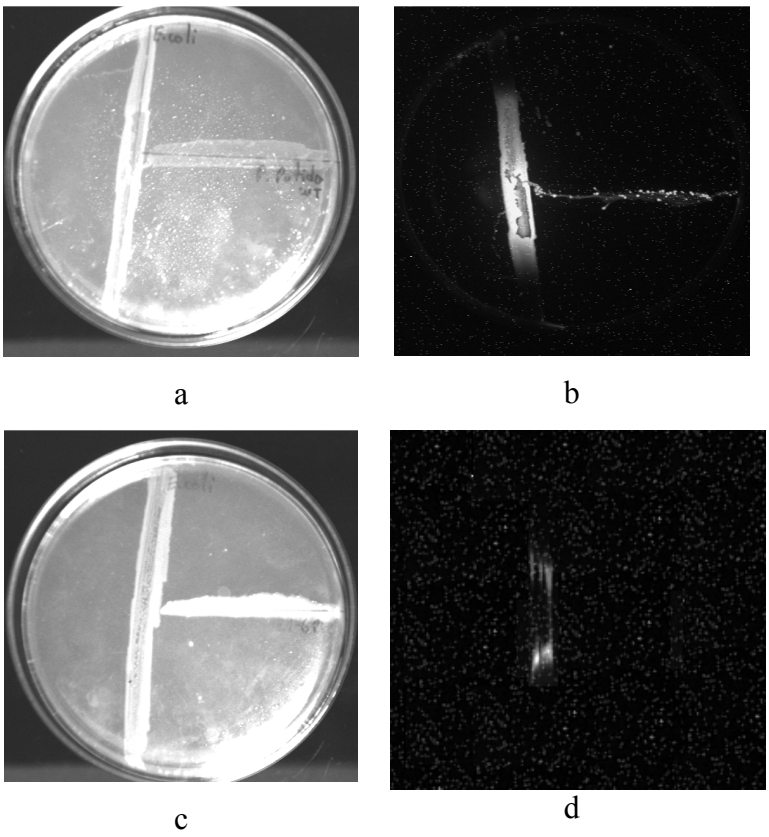


Figura 9. Fotografías tomadas al ensayo de estriado en cruz. a. Fotografía en blanco y negro del estriado del biosensor con el control positivo (*P. putida* IsoF). b. Fotografía de la bioluminiscencia producida por el biosensor en respuesta al control positivo. c. Fotografía en blanco y negro del estriado del biosensor con la cepa *Ochrobactrum* sp. d. Fotografía de la bioluminiscencia producida por el biosensor en respuesta a la cepa *Ochrobactrum* sp.

Se encontró que 7 de las 8 cepas Gram-negativas poseen sistemas de QS que son detectados con *E. coli* (pSB401), cabe aclarar que el biosensor usado tiene excelente respuesta hacia las HSLs: C_6-3-oxo-HSL, C_6-HSL, C_8-3-oxo-HSL y C_8-HSL, y es poco sensible frente a las otras HSLs. De lo anterior se concluye que las cepas que dieron actividad deben tener al menos una de las HSLs anteriores. La cepa *Vibrio* sp (23-6PIN) no presentó actividad en este bioensayo, lo cual resulta inusual tratándose de este género, esto puede interpretarse como que no tiene las HSLs mencionadas o las produce en baja concentración, o produce

otras que no detecta el biosensor, o en esta cepa ocurrieron problemas de liberación (segregación) de las HSLs por lo cual los compuestos no pudieron ser detectados por el biosensor (tabla 5).

TABLA 5. Detección de circuitos de QS por medio del bioensayo de estriado en cruz con el biosensor *E. coli* (pSB401).

CÓDIGO CEPA	BIOENSAYO DE ESTRIADO EN CRUZ
P. putida IsoF	+
P. putida 116	-
Ochrobactrum sp	+
Vibrio sp (23-6PIN)	-
Vibrio campbellii	+
Vibrio sp (11-6DEP)	+
Ochrobactrum pseudogringnonense	+
Schewanella sp	+
Vibrio harveyi	+
Alteromona sp	+

Una vez se determinó que las bacterias objeto de estudio poseían circuitos de QS mediados por HSLs, se realizaron cultivos de éstas, teniendo en cuenta su curva de crecimiento, para posteriormente realizar la extracción del sobrenadante del cultivo en la fase exponencial de crecimiento, y de esta manera obtener la máxima cantidad de HSLs, puesto que en esta fase es en donde mayor cantidad de ellas hay. Los extractos obtenidos con AcOEt presentaron cantidades inferiores a los 25 mg en el caso de las bacterias marinas y alrededor de 40 mg en el caso de las bacterias control. En la tabla 6 se encuentran las cantidades de extracto obtenidas a partir de cada una de las cepas de estudio, así como las condiciones de cultivo.

TABLA 6. Cantidad de extracto obtenido a partir de las bacterias utilizadas en el estudio.

CÓDIGO CEPA	GRAM	TIEMPO DE INCUBACIÓN PREINÓCULO (h)	TEMPERATURA DE INCUBACIÓN (^{0}C)	CALDO DE CULTIVO	Extracto (mg)
Ochrobactrum sp	-	12	37	CM	13,7
Vibrio sp (23-6PIN)	-	12	ambiente	CM	23,7
Vibrio campbellii	-	12	ambiente	CM	11,5
Vibrio sp (11-6DEP)	-	12	ambiente	CM	16,5
Ochrobactrum pseudogringnonense	-	> 24	ambiente	CM	6,7

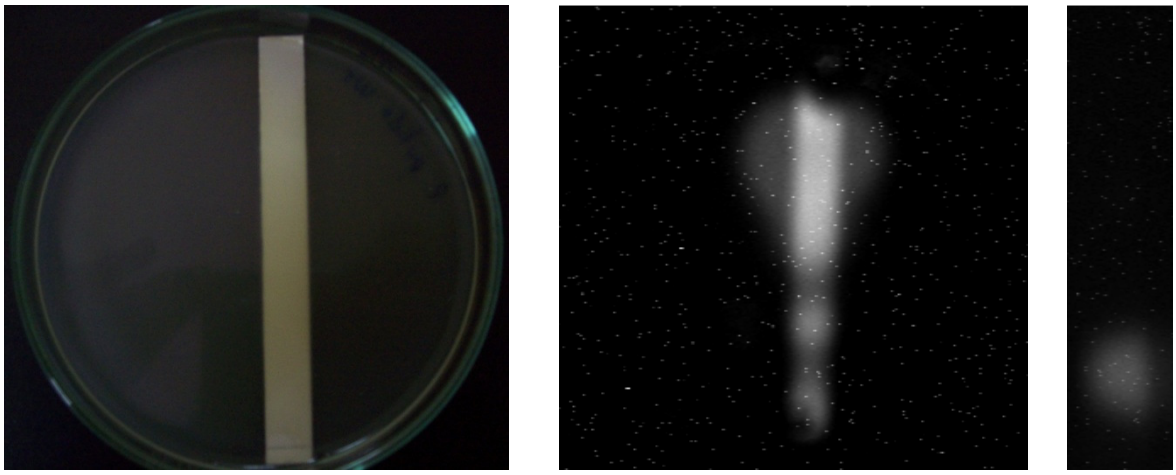
Schewanella sp	-	12	ambiente	CM	15,8
Vibrio harveyi	-	12	ambiente	CM	10,9
Alteromona sp	-	12	ambiente	CM	5,6
CM					4,4
P. putida WT	-	24	37	cLB	40,9
P. putida 117	-	24	37	cLB	48,4

Una primera aproximación espectroscópica para la caracterización de las HSLs, fue el registro de los espectros infrarrojo de los extractos realizados a las bacterias Gram-negativas. Se pensó en ésta técnica por su practicidad, y facilidad de recuperación de la muestra. La frecuencia de estiramiento del carbonilo de estos compuestos suele ser 1777 cm^{-1} que es una frecuencia alta en la que se esperaba que no hubiese interferentes. Los espectros obtenidos para los extractos presentan bandas demasiado anchas y en algunos casos se solapan con otras bandas, debido a la presencia de otros compuestos en los extractos. Por lo que ésta técnica no permitió obtener resultados claros al parecer por las concentraciones bajas a las que se encuentran las HSLs en el extracto (Ver anexo 3). Por tal motivo se requieren de técnicas más sensibles como el ensayo de CCD-FR revelada con el biosensor *E. coli* (pSB401), el cual no se necesita grandes cantidades de compuesto, ya que el límite de detección del biosensor se encuentra en la escala de las femtomoles.

Análisis cromatográfico de HSLs

En este punto se pretendían implementar las condiciones cromatográficas de análisis para detectar las HSLs, de ahí que se hayan usado extractos de bacterias ya caracterizados y patrones. Una revisión bibliográfica de las condiciones de CCD para el análisis de HSLs mostró que éste se suele llevar a cabo sobre una fase estacionaria reversa como es la RP-18, empleando como fase móvil MeOH/H_20 en proporciones 6:4 y 7:3.[32] Así, al realizar la CCD-FR con el extracto de *P. putida* IsoF se observaron cuatro manchas luminiscentes al revelar la placa con el biosensor *E. coli* (pSB401) (Fig. 10). Estas manchas corresponden en orden creciente de Rf a C6-HSL, C8-oxo-HSL, C6-oxo-HSL y C4-HSL. Lo anterior se

afirma porque estas son las HSLs reportadas para *P. putida* IsoF,[45] y a que la primera mancha tiene el mismo Rf del patrón sintético de C6-HSL. Adicionalmente, se ensayaron diferentes reveladores químicos tales como: yodo, ácido sulfúrico/MeOH, sulfato cérico amónico, vainillina, y ácido fosfomolíbdico. Sin que ninguno de ellos diera resultados positivos, por lo que todos los controles cromatográficos se hicieron con el biosensor que tiene una alta sensibilidad, aunque es una técnica dispendiosa de revelado. En la figura 10 se observan las fotografías tomadas a las CCD-FR del extracto de *P. putida* IsoF y el patrón de C6-HSL antes y después de ser reveladas con el biosensor.



a b c

Figura 10. Fotografías tomadas a las CCD-FR del extracto del control positivo *P. putida* IsoF y el patrón de HSL. a. Visible. b. Luminiscencia producida por el biosensor debido a las HSLs presentes en la placa cromatográfica. c. Luminiscencia producida por el biosensor debido a la presencia de C6-HSL en la placa cromatográfica.

Una vez estandarizada la CCD-FR se procedió a analizar cada uno de los extractos de las bacterias Gram-negativas obtenidos por extracción con AcOEt (0,5% ácido fórmico) de su cultivo en caldo marino en la fase de crecimiento, como ya se explicó. Este análisis mostró que casi todas las bacterias producían HSLs, como se puede observar en la figura 11 por las manchas de luminiscencia y confirma los resultados obtenidos por el ensayo de estriado en

[45] Steidle, A.; Allesen-Holm, M.; Riedel, K.; Berg, G.; Givskov, M.; Molin, S.; Eberl, L. **2002.** Identification and Characterization of an *N*-Acylhomoserine Lactone-Dependent Quorum-Sensing System in *Pseudomonas putida* Strain IsoF. *Appl. Environment. Microbiol.* 68, 6371–6382.

cruz. La bacteria *Vibrio harveyi* no presentó luminosidad en CCD-FR, aunque en el ensayo de estriado en cruz si lo había hecho, esto podría ser explicado porque la HSL de 4 átomos de carbono es muy soluble en agua y por tanto la extracción con solvente orgánico no garantiza una completa recuperación de esta HLS. También es importante resaltar que la bacteria *Vibrio* sp (23-6PIN) que no presentó bioluminiscencia en el ensayo de estriado en cruz, si presentó luminiscencia al revelar la CCD-FR con el biosensor, aunque esta es muy débil y parece deberse a una HSL de alto peso molecular por su baja movilidad en la placa.

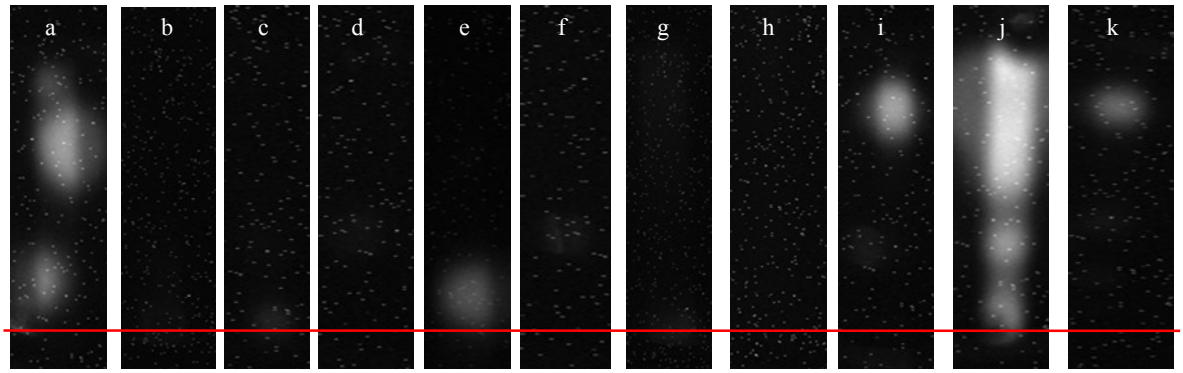


Figura 11. Fotografías tomadas a la CCD-FR de todos los extractos de las bacterias de estudio, los controles positivo y negativo, y el patrón de C6-HSL. a. *Ochrobactrum* sp. b. *Vibrio* sp (23-6PIN). c. *Vibrio campbellii.* d. *Vibrio* sp (11-6DEP). e. C6-HSL. f. *Ochrobactrum pseudogringnonense*. g. *Schewanella* sp. h. *Vibrio harveyi*. i. *Alteromona* sp. j. *P. putida* WT (control positivo). k. *P. putida* 117 (control negativo).

Es importante resaltar que a pesar de haberse aplicado la misma cantidad de todos los extractos en la placa, en algunas de ellas la luminiscencia observada es muy débil, en cambio para otras se observan manchas definidas y de gran luminosidad. Esto nos da un indicio de la concentración a la que se encuentran las HSLs dentro del extracto, ya que a cantidades más grandes de compuesto, el biosensor responde emitiendo mayor luminiscencia y durante un tiempo más prolongado. Las bacterias que presentan una mayor concentración de HSLs son: *Ochrobactrum* sp, y *Alteromona* sp. Adicionalmente, la mayoría de los extractos que indujeron luminiscencia en el biosensor solamente presentaron una mancha, es decir que con este biosensor solo se pudo detectar una HSL. Es importante aclarar que podría haber otras no detectables con este biosensor, para afrontar este problema se pueden usar técnicas químicas que se mencionaran posteriormente o un

biosensor de amplio espectro como el *Agrobacterium tumefeciens* A136. El único extracto bacteriano que en CCD-FR presenta más de una mancha es el correspondiente a la cepa *Ochrobactrum* sp. Una información adicional que se puede obtener de la CCD-FR es una aproximación a la identidad de la HSL, para esto se comparó el Rf de los extractos bacterianos con el del carril e en la figura 11 que corresponde al patrón de C6-HSL. Se encontró que las bacterias *Ochrobactrum* sp, *Vibrio* sp (23-6PIN), *Vibrio campbellii*, y *Schewanella* sp producen la C6-HSL. Sin embargo, en el caso de la cepa *Alteromona* sp se observa una mancha con un Rf diferente al del patrón que coincide con una de las manchas del extracto de *P. putida* IsoF, éste compuesto por dicho Rf parece corresponder a la C4-HSL. Con este mismo criterio se identificó de manera preliminar como C4-HSL la mancha de mayor Rf de la cepa *Ochrobactrum* sp. Los resultados anteriores se resumen en la tabla 7. En la figura 11 se destaca encontrar que el extracto del control negativo *P. putida* 117 presenta una mancha bioluminiscente que indica la presencia de HSLs (identificada tentativamente como C4-HSL), esto no se esperaba ya que esta bacteria se encuentra mutada en el gen *ppuI* que codifica para la síntesis de las HSLs por tanto debería ser incapaz de producir HSLs. Lo anterior se puede interpretar como una impureza en el cultivo bacteriano, o como la pérdida de la mutación existente, ya que este cultivo produce una HSLs característica de la *P. putida* IsoF de la cual proviene la cepa mutada.

TABLA 7. Detección de circuitos de QS por medio de CCD-FR revelado con el biosensor *E. coli* (pSB401).

CÓDIGO CEPA	**CCD-FR revelado con *E. coli* (pSB401)**
P. putida IsoF	+
P. putida 116	+
Ochrobactrum sp	+ (C4-HSL, C6-HSL)
Vibrio sp (23-6PIN)	+ (C6-HSL)
Vibrio campbellii	+ (C6-HSL)
Vibrio sp (11-6DEP)	+
Ochrobactrum pseudogringnonense	+
Schewanella sp	+ (C6-HSL)
Vibrio harveyi	-
Alteromona sp	+ (C4-HSL)

Identificación de HSLs

Los análisis llevados a cabo usando la CCD-FR revelada con el biosensor, nos permitieron una aproximación a la identificación de las HSLs. Esta es una metodología conveniente para una valoración preliminar de los extractos, pero que no resulta satisfactoria para confirmar la identidad de las HSLs, puesto que por un lado la resolución cromatográfica es muy pobre, y por otro lado la técnica de detección (biosensor) si bien es muy sensible, no es universal para todas las HSLs y según el biosensor usado se puede estar perdiendo información sobre la presencia de HSLs de cadenas largas o con sustituyentes hidroxilo o carbonilo. Así, para complementar los estudios por CCD-FR se analizaron los extractos por CG-EM. La ventaja de utilizar CG-EM, radica en la posibilidad de obtener una mejor separación de los compuestos presentes en los extractos de las bacterias, y por sobre todo conocer su identidad por el análisis de sus espectros de masas y la comparación de los tiempos de retención con los patrones establecidos. Por tal motivo se realizaron inyecciones en modo de detección SCAN del patrón de *N*-hexanoil-DL-homoserinlactona (Fluka), el extracto de *Pseudomonas putida* IsoF y de todos los extractos objeto de estudio, con el fin de determinar la composición de cada uno de ellos. Las condiciones cromatográficas de análisis se ajustaron haciendo uso del patrón de C6-HSL y teniendo en cuenta que éste debía presentar un tiempo de retención mayor a 10 minutos, con el fin de asegurar que las HSLs oxigenadas de 6 átomos de carbono y las de 4 átomos de carbono pudieran ser detectadas, porque en estas condiciones ellas presentan un tiempo de retención menor al del patrón C6-HSL.[28] Las condiciones de análisis utilizadas para satisfacer las condiciones anteriores fueron: una columna RPX-5 (30 m, 0,32 mm, 0,25 μm) y un programa de temperatura de 50°C durante 3 minutos y posterior incremento a 8,3°C min^{-1} hasta alcanzar una temperatura de 300°C. Estas condiciones permitieron asegurar que se podían observar las HSLs de diferente tamaño y obtener una buena separación en poco tiempo. En la figura 12 se ilustra el cromatograma obtenido por CG-EM en modo SCAN para el patrón C6-HSL.

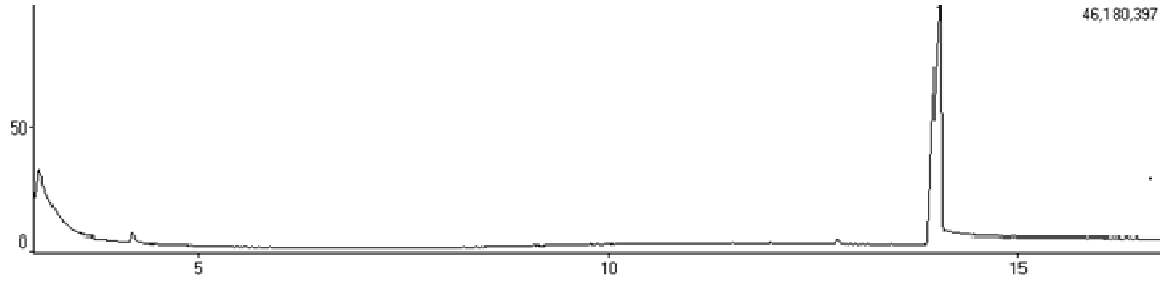


Figura 12. Cromatograma obtenido por CG-EM en modo SCAN para el patrón C6-HSL.

También es importante conocer la fragmentación típica de las HSLs, por tal motivo se analizó el espectro de masas del patrón C6-HSL obtenido mediante la inyección en CG-EM. El espectro de masas en modo de impacto electrónico obtenido para el patrón concuerda con lo esperado, como se observa en el Anexo 4. Así, mostró un ión molecular en *m/z* 199 $[M]^+$, el cual es consistente con una fórmula $C_{10}H_{17}NO_3$ indicando un IDH de 3 (índice de deficiencia de hidrógenos), adicionalmente se observaron fragmentos correspondientes a la pérdida del agua *m/z* 181 $[M\text{-}H_2O]^+$, grupo etilo *m/z* 170 $[M\text{-}CH_3CH_2]^+$, y consecutivas pérdidas de la cadena acílica (*m/z* 170, 156 y 102), el rearreglo de McLafferty sobre el grupo carbonilo de la amida correspondiente al ión *m/z* 143$[M\text{-}CHCH_2C_3H_7]^+$ y la pérdida completa de la cadena acílica con protonación doble o sencilla de la aminolactona (iones *m/z* 102 y 101, respectivamente). Los últimos fragmentos mencionados son comunes en todas las HSLs (Fig. 13).[28] Las razones expuestas anteriormente permiten el análisis de estos compuestos por CG-EM en modo SIM, con el fin de caracterizar estas moléculas inductoras aún en bajas concentraciones. Así, en el caso de las HSLs de cadena acílica hidrocarbonada suele seguirse el ion *m/z* 143, mientras que en las HSLs que tienen sustituciones con grupo OH o carbonilo suele seguirse el ión *m/z* 102, antes descrito.

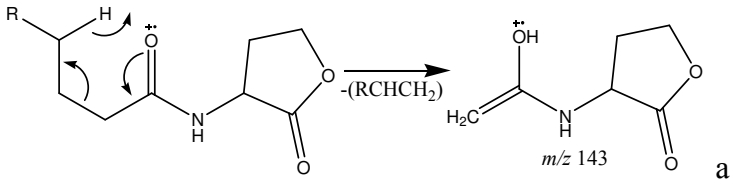


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Figura 13. Principales fragmentos observados en los espectros de masas en modo de ionización electrónica de las HSLs. a. Rearreglo de McLafferty, correspondiente al ión *m/z* 143. b. Pérdida de toda la cadena. acílica con doble protonación, correspondiente al ión *m/z* 102. c. Pérdida de toda la cadena acílica con protonación sencilla, correspondiente al ión *m/z* 101.

Por CG-EM en modo SCAN se observó que los cromatogramas obtenidos para los extractos bacterianos eran bastante complejos, ya que presentaba gran cantidad de picos cromatográficos. Dentro de la composición de los extractos se encontraron ácidos grasos tales como el ácido hexadecanóico, y el ácido octadecanóico, además se encontraron compuestos nitrogenados no caracterizados. Se observó la presencia de HSLs en bajas concentraciones, con el fin de limitar la cantidad de picos graficados en el cromatograma, para hacer más sencilla y rápida la identificación de las HSLs, se graficaron únicamente los fragmentos *m/z* 143 y 102. De esta manera se determinó la presencia de *N*-butanoilhomoserinlactona (C4-HSL) en *Ochrobactrum* sp, *Vibrio* sp (11-6DEP), *Schewanella* sp y *Vibrio harveyi*, y de *N*-hexanoilhomoserinlactona (C6-HSL) en *Vibrio* sp (23-6PIN), *Ochrobactrum pseudogringnonense*, *Ochrobactrum* sp y *Vibrio harveyi*, los resultados se resumen en la tabla 8 y los cromatogramas con los respectivos espectros de masas se pueden observar en el anexo 4.

TABLA 8. Detección e identificación de HSLs por medio de CG-EM en modo SCAN y modo SIM.

CÓDIGO CEPA	CG-EM (SCAN)	CG-EM (SIM)
Ochrobactrum sp	+ (C4-HSL y C6-HSL)	+
Vibrio sp (23-6PIN)	+ (C6-HSL)	+
Vibrio campbellii	-	+
Vibrio sp (11-6DEP)	+ (C4-HSL)	+
Ochrobactrum pseudogringnonense	+ (C6-HSL)	+
Schewanella sp	+ (C4-HSL y C6-HSL)	+
Vibrio harveyi	+ (C4-HSL y C6-HSL)	+

Alteromona sp	-	+
P. putida IsoF	+	+
Patrón C6-HSL	+	+

Como se ha venido mencionando a lo largo del texto, las HSLs se encuentran en bajas concentraciones, por tal motivo se decidió hacer CG-EM en modo SIM haciendo seguimiento a los iones *m/z* 143 y 102 que son iones característicos de las HSLs y que están presentes en altas intensidades en su espectro, con el fin de aumentar la sensibilidad de detección en el análisis. En la mayoría de los extractos se observaron en el modo SIM varios picos a diferentes tiempos de retención (Anexo 5), sin embargo estos no necesariamente corresponden a HSLs, ya que mediante esta técnica podemos observar solo los fragmentos monitoreados, y no todos los presentes en el pico cromatográfico, por tal motivo procedimos a analizar el espectro de masas obtenido en el modo SCAN para el pico obtenido en el mismo tiempo de retención en el modo SIM. Adicionalmente, este análisis nos permitiría ver el ion molecular en el espectro de masas en modo SCAN, lo cual no se puede hacer en modo SIM, y que es un ion diagnóstico para la identificación de las HSLs. Con este análisis se esperaba ver un mayor número de HSLs, sin embargo solo se pudo determinar la presencia de C6-HSL, y C4-HSL, previamente identificadas por CCD-FR y CG-EM en modo SCAN. En la figura 14 se presenta un ejemplo típico de lo discutido anteriormente, pudiéndose observar que el extracto contiene una gran cantidad de picos (Fig. 14a), en la figura 14b se observa el cromatograma obtenido en modo SIM siguiendo los fragmentos *m/z* 143 y 102, el cual es claramente menos complejo. Al observar el espectro de masas del pico 8 que contiene los fragmentos *m/z* 143 y 102, éste no presenta la fragmentación característica de una HSL (Fig. 14c), mientras que el pico 9 que también contiene los fragmentos *m/z* 143 y 102 si tiene el patrón de fragmentación de una HSL, siendo la C6-HSL (Fig. 14d).

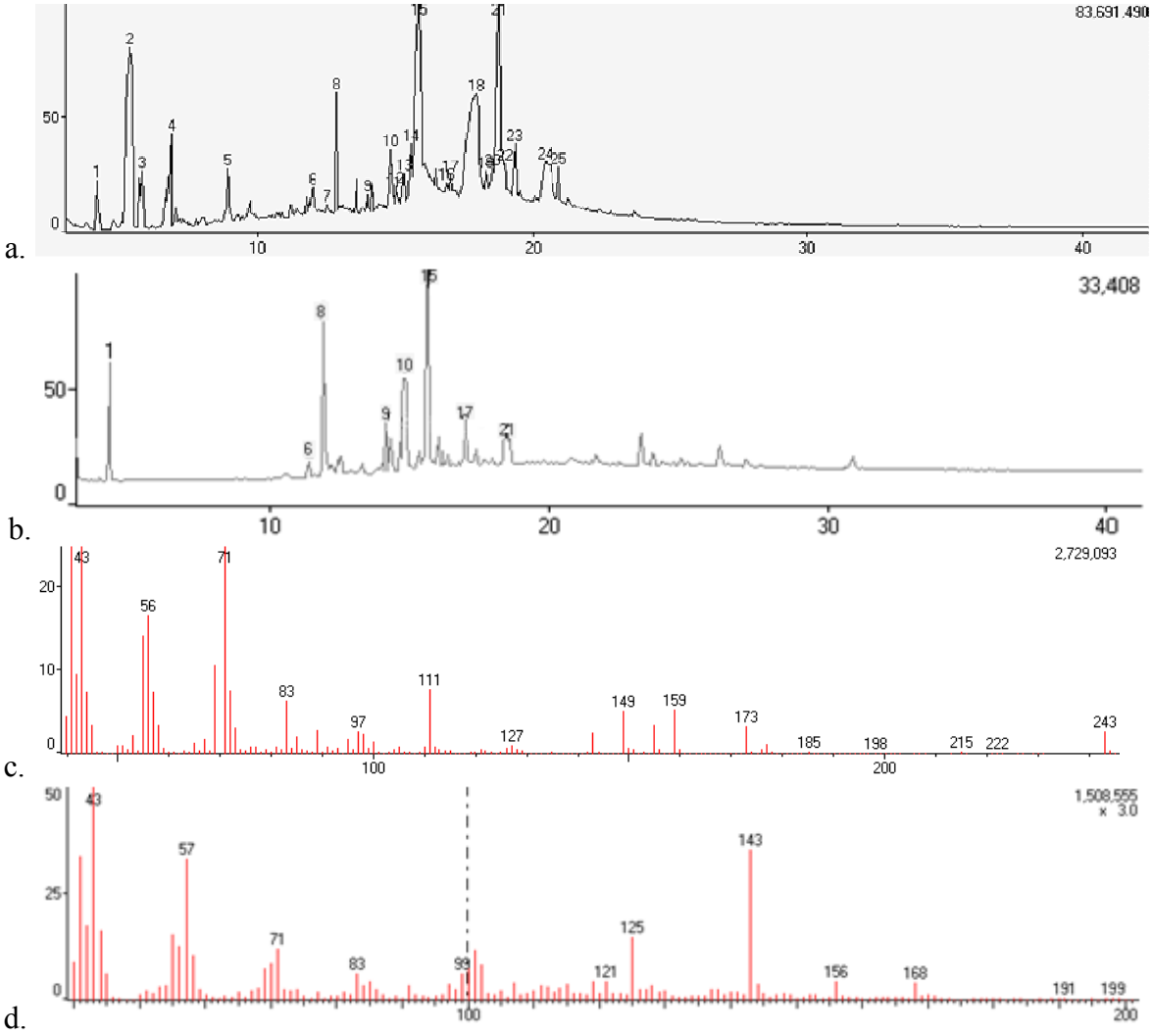

Figura 14. a. Cromatograma obtenido por CG-EM en modo SCAN para el extracto de la bacteria *Ochrobactrum* sp. b. Cromatograma obtenido por CG-EM en modo SIM para el extracto de la bacteria *Ochrobactrum* sp. c. Espectro de masas obtenido por CG-EM en modo SCAN para el pico 8 del extracto de la bacteria *Ochrobactrum* sp. Tiempo de retención 12.9 minutos y EM-IE (70 eV), *m/z*: 243 $[M]^+$. d. Espectro de masas obtenido por CG-EM en modo SCAN para el pico 9 del extracto de la bacteria *Ochrobactrum* sp. Tiempo de retención 14.2 minutos y EM-IE (70 eV), *m/z*: 199 $[M]^+$.

El análisis de los extractos bacterianos por CG-EM en modo SCAN y modo SIM, permitió corroborar los resultados obtenidos en el ensayo de estriado en cruz y de CCD-FR, ya que aquellas cepas en las que se había detectado preliminarmente la presencia de HSLs, y en las que se presumía su identidad por la correspondencia de Rf con los patrones, presentaron por CG-EM las mismas HSLs (C4-HSL y C6-HSL) confirmando la identificación inicial.

Cabe resaltar que la cepa *Vibrio* sp (23-6PIN) que en el ensayo de estriado en cruz no presentó luminiscencia, pero que había exhibido la presencia de C6-HSL por CCD-FR, en el análisis realizado por CG-EM mostró la misma HSL. Así mismo, el extracto de la cepa *Vibrio harveyi* que no presentó luminiscencia en CCD-FR, en el análisis por CG-EM se determinó la presencia de C6-HSL y C4-HSL, aunque en concentraciones muy bajas.

Análisis de HSLs por CLAE-EM

Como se ha expuesto hasta ahora la CG-EM en modo de impacto electrónico es una técnica muy útil para la identificación de las HSLs. Sin embargo, existen otras técnicas analíticas para su análisis como la CLAE-EM que deseamos explorar en este trabajo. Esta técnica puede ofrecer la ventaja de una correcta identificación del ion molecular, que con la anterior técnica no se logra, y además hace posible el análisis de compuestos tanto no volátiles como volátiles, aunque éste factor no parece ser una limitante en el estudio de las HSLs porque todas ellas se pueden volatilizar.

El primer paso para realizar el estudio por CLAE-EM, consistió en ajustar las condiciones de operación del equipo. Por tal motivo, luego de múltiples ensayos se estableció que las condiciones apropiadas para el funcionamiento del espectrómetro de masas, en las que se obtuvieran espectros en donde se apreciara claramente el ion $[M+H]^+$ fueron las siguientes: el equipo se operó en modo de ionización por electrospray (ESI) a 1,5 kV utilizando como gas nebulizador nitrógeno a un flujo de 1,5 L min^{-1}. A pesar de haber ensayado diferentes voltajes (1 a 3 kV) no se logró fragmentación. Con el fin de encontrar un tiempo de retención apropiado, que permitiera identificar un amplio rango de HSLs, se variaron las proporciones de la fase móvil y el flujo, hasta obtener un tiempo de retención para C6-HSL de 7,9 minutos, que es un tiempo que nos permite el análisis de HSLs de cadena más corta o con las sustituciones mencionadas a lo largo del texto, que eluyen a un menor tiempo de retención. Las condiciones de operación del cromatógrafo con las que se suplió esta necesidad fueron las siguientes: una columna RP-18 phenomenex Luna 5µ 100A, como fase móvil se utilizó MeOH/(H_2O/ácido fórmico 0,1%) en gradiente lineal, el cual inició

con MeOH al 15% hasta llegar al 100% en 15 minutos, el flujo se fijó en 0,3 mL min^{-1}. En la figura 15 se observa el cromatograma y el espectro de masas obtenido para el patrón por CLAE-EM empleando las condiciones antes descritas. En él se observa el ión molecular *m/z*: 200,05 $[M+H]^+$, el cual es consistente con una fórmula $C_{10}H_{17}NO_3$ indicando un IDH de 3, y que es lo que se esperaba para el patrón.

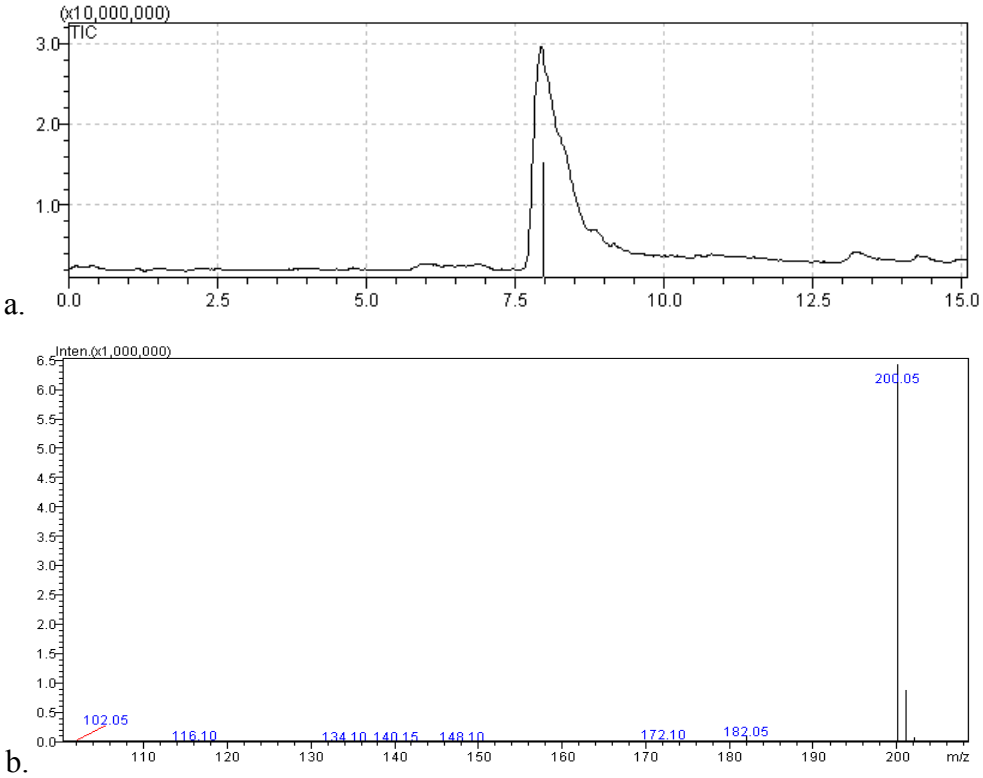

Figura 15. a. Cromatograma obtenido por CLAE-EM para el patrón C6-HSL. b. Espectro de masas obtenido por CLAE-EM para el patrón C6-HSL. Tiempo de retención 7,9 minutos, *m/z*: 200,05 $[M+H]^+$.

Si bien la técnica CLAE-EM es adecuada para determinar la masa de las HSLs, no es la más apropiada para realizar análisis de diferentes HSLs al mismo tiempo, ya que no existe un patrón de fragmentación común a ellas (ion característico) que permita el monitoreo de las HSLs en una matriz compleja como lo es un extracto bacteriano. Este mismo fenómeno

se ha observado en ensayos reportados anteriormente.[46,47] Una forma de solucionar el problema de la fragmentación es o bien el uso de nano-ESI[32] o el uso de masas de ultra-alta-resolución, que permite el seguimiento de los iones moleculares de las HSLs probables (las HSLs corresponden a series homólogas)[23] o bien técnicas de masas-masas en donde se selecciona el ion molecular y el ion *m/z* 102,[48,49] estás metodologías no estaban a nuestra disposición pero se recomienda su uso para futuros estudios. Adicionalmente, éste permitiría detecciones a muy baja concentración, tal y como se espera para este tipo de compuestos recuperados de aislamientos bacterianos ambientales.

Los cromatogramas obtenidos para los extractos bacterianos por CLAE-EM son bastante complejos (Anexo 6), y no se observaron picos definidos, lo que hubiese permitido comparar los perfiles cromatográficos entre ellos y éstos con los obtenidos para el patrón C6-HSL y el perfil del extracto de *P. putida* IsoF. No obstante se pudo identificar la C6-HSL en tres de los extractos de bacterianos (*Ochrobactrum* sp, *Vibrio campbellii*, y *Schewanella* sp), siendo la única HSL que fue posible identificar, esto se hizo tanto por su Rf como por su espectro de masas. Al contrastar estos resultados con los obtenidos por CCD-FR y CG-EM se encontró que en las bacterias *Vibrio* sp (23-6PIN), *Ochrobactrum pseudogringnonense*, y *Vibrio harveyi*, sí se había detectado previamente la C6-HSL, mientras por la técnica CLAE-EM no fue posible, lo que junto con la baja resolución de los cromatogramas, pone en evidencia que esta técnica es menos útil para la identificación de las HSLs de las bacterias estudiadas en éste trabajo. También es importante resaltar que no fue posible detectar la C4-HSL en ninguno de los extractos bacterianos y que sí había sido

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[46] Flodgaard, L.R.; Dalgaard, P.; Andersen, J.B.; Nielsen, K.F.; Givskov, M.; Gram, L. **2005**. Nonbioluminescent Strains of *Photobacterium phosphoreum* Produce the Cell-to-Cell Communication Signal N-(3-Hydroxyoctanoyl)homoserine Lactone. *Appl. Environ. Microbiol. 71*, 2113–2120.

[47] Buchholtz, Ch.; Nielsenb, K.F.; Miltonc, D.L.; Larsend, J.L.; Gram L. **2006**. Profiling of acylated homoserine lactones of *Vibrio anguillarum* in vitro and in vivo: Influence of growth conditions and serotype. *System. Appl. Microbiol. 29*, 433–445.

[48] Frommberger M.; Schmitt-Kopplin, P.; Ping, G.; Frisch, H.; Schmid, M.; Zhang, Y.; Hartmann, A.; Kettrup, A. **2004.** A simple and robust set-up for on-column sample preconcentration – nano-liquid chromatography – electrospray ionization mass spectrometry for the analysis of N-acylhomoserine lactones *Anal. Bioanal. Chem. 378*, 1014–1020.

[49] Khan, S.R.; Herman, J.; Krank, J.; Serkova, N.J.; Churchill, M.E.A.; Suga, H.; Farrand, S.K. **2007**. *N*-(3-Hydroxyhexanoyl)-L-Homoserine Lactone Is the Biologically Relevant Quormone That Regulates the phz Operon of *Pseudomonas chlororaphis* Strain30-84. *Appl.environ.microbiol. 73*, 7443–7455.

identificada por otras técnicas en las bacterias *Ochrobactrum* sp, *Vibrio* sp (11-6DEP), *Schewanella* sp, *Vibrio harveyi*, y *Alteromona* sp (Tabla 9). Ésto se puede interpretar de dos maneras o como se ha venido discutiendo el sistema de ionización y detección (ESI-TOF) no es el más adecuado o a que el sistema cromatográfico no haya sido el apropiado, puesto que la C4-HSL es muy polar y pudo haber eluído con el frente del solvente. En cualquier caso queda claro en esta discusión que la técnica a elegir es la CG-EM siendo más potente utilizar un analizador que nos permita hacer masas a la n (tándem) y sea de alta resolución, aunque no se cuenta con este equipo en el país.

TABLA 9. Resumen de todos los resultados obtenidos para la detección de HSLs por las distintas técnicas.

CÓDIGO CEPA	ESTRIADO EN CRUZ	CCD-FR	IR	CG-EM (SCAN)	CG-EM (SIM)	CLAE-EM
Ochrobactrum sp	+	+ (C_4, C_6)	+	+ (C_4, C_6)	+	+ (C_6)
Vibrio sp (23-6PIN)	-	+ (C_6)	+	+ (C_6)	+	-
Vibrio campbellii	+	+ (C_6)	+	-	+	+(C_6)
Vibrio sp (11-6DEP)	+	+	+	+ (C_4)	+	-
Ochrobactrum pseudogringnonense	+	+	+	+ (C_6)	+	-
Schewanella sp	+	+ (C_6)	+	+ (C_4, C_6)	+	+ (C_6)
Vibrio harveyi	+	-	+	+ (C_4, C_6)	+	-
Alteromona sp	+	+ (C_4)	+	-	+	-
P. putida IsoF	+	+	+	+	+	+
Patrón C6-HSL		+	+	+	+	+

En resumen, se encontró que las 8 cepas Gram-negativas poseen circuitos de QS mediados por HSLs (Tabla 9), identificando la *N*-butanoilhomoserinlactona (C4-HSL) y la *N*-hexanoilhomoserinlactona (C6-HSL). La mayor parte de los resultados fue concordante entre todas las técnicas utilizadas. Como se observa a lo largo del trabajo las técnicas utilizadas son complementarias, ya que al realizar el estriado en cruz tenemos una primera aproximación de las cepas que poseen circuitos de QS, sin conocer cuáles son las moléculas señalizadoras, pues los biosensores tienen una alta sensibilidad y especificidad frente a este tipo de compuestos. Al realizar los extractos se puede hacer la identificación de las moléculas señalizadoras que han sido detectadas, es así que en primera instancia se hace uso de una técnica cromatográfica (CCD) acoplada a un ensayo biológico (revelado con biosensor), lo cual nos permite avanzar en la identificación de las HSLs a partir de los Rfs

de las manchas que se revelan específicamente con el biosensor; pero si no se cuenta con una amplia gama de patrones no se pueden identificar los compuestos presentes. Por este motivo se utilizan otro tipo de técnicas que unen tanto la cromatografía como la espectroscopía. Estas técnicas son CG-EM y CLAE-EM en donde podemos separar los compuestos presentes en los extractos, de los cuales podemos obtener el espectro de masas ya sea por ionización fuerte o ionización débil para cada uno de los compuestos presentes en el extracto. La técnica más apropiada para la correcta identificación de las HSLs presentes en los extractos bacterianos es la CG-EM. Los mejores resultados se obtendrían si el detector fuese o bien de alta resolución o se utilizaran técnicas tándem.

HSLs de la bacteria *Ochrobactrum* sp

Como una segunda fase en nuestro estudio químico de las bacterias involucradas en *biofilms* naturales provenientes del mar Caribe Colombiano, quisimos realizar una búsqueda más profunda de las HSLs que la que permite el análisis de los extractos en pequeña cantidad. Por tal motivo, se realizó la extracción de un cultivo en gran volumen (6 L de CM) de la bacteria *Ochrobactrum* sp. Se escogió esta bacteria porque su extracto mostró ser el más rico en HSLs según su comportamiento en CCD-FR revelada con el biosensor. Así, el cultivo en su fase de crecimiento exponencial fue extraído con AcOEt, obteniendo 225,8 mg de extracto. Este fue separado por CC-FR, utilizando como eluente mezclas H_2O/MeOH (H_2O/MeOH proporción 92:8 hasta MeOH), obteniendo 8 fracciones, de las cuales la única fracción que presentó luminiscencia con el biosensor fue la correspondiente a la fracción F7 (18,0 mg, eluída con H_2O/MeOH 20:80). También se analizaron todas las fracciones por CLAE-EM pudiendo identificar las HSLs caracterizadas en el extracto inicial. No obstante este esfuerzo por obtener un extracto en grande no nos fue posible detectar e identificar otras HSLs diferentes a las identificadas en el extracto hecho en pequeño.

HSLs de aislamientos marinos

Con los resultados anteriores determinamos por primera vez la presencia de circuitos de QS para los aislamientos bacterianos con los que en nuestro grupo de investigación realizamos el ensayo *antifouling*, esto es muy relevante porque como ya se ha mencionado el QS determina la formación del *biofilm* y este a su vez es fundamental en el proceso del *biofouling* marino, a continuación contrastaremos nuestros estudios con los realizados por otros autores para los mismos géneros de bacterias, así como la implicación ecológica que tienen estas HSLs desde el punto de vista del *fouling*.

La búsqueda por SciFinder (Anexo 7)[50] para los géneros bacterianos objeto de estudio con relación al QS mostró que:

El género *Shewanella* tiene 11 referencias. Dentro de estas se ha evidenciado la presencia de circuitos de QS mediados por HSLs y el autoinductor AI-2, y también la capacidad de degradar HSLs. Las HSLs caracterizadas para este género en la bibliografía son del tipo 3-oxo (3-oxo-C4-HSL, 3-oxo-C10-HSL y 3-oxo-C12-HSL), que no fueron identificadas por nosotros en el extracto de la bacteria *Schewanella* sp (12-AINS-6B) a pesar de que se hizo una búsqueda intensiva de ellas mediante CG-EM siguiendo los iones *m/z* 143 y 102 que son característicos. De esta manera este es el primer reporte de circuitos de QS para bacterias del género *Schewanella* mediados por C4-HSL y C6-HSL.

Para el género *Ochrobactrum* hay solo 2 referencias, y ambas muestran que dicho género tiene la habilidad de degradar HSLs y no se han reportado sistemas de QS. Por lo tanto, las *N*-acilhomoserinlactonas C4-HSL y C6-HSL identificadas en los extractos de las bacterias *Ochrobactrum* sp (21-6PIN) y *Ochrobactrum pseudogringnonense* (4-4DEP), corresponden al primer reporte de HSLs existente para este género.

[50] SciFinder/Medline database search, www.cas.org/SCIFINDER/SCHOLAR/index.html, Septiembre 2009.

El género *Alteromona* tiene 5 referencias en las cuales se destaca la presencia de circuitos de comunicación mediados por 2-alquil-4-quinolonas (AHQs). Por tal razón la C4-HSL identificada en el extracto de la bacteria *Alteromona* sp (29-C), indica que nuestro estudio corresponde al primer reporte de circuitos de comunicación mediados por HSLs para el género *Alteromona*.

Por último el género *Vibrio* tiene 306 referencias, ya que, como se mencionó anteriormente, es el género bacteriano que tiene más estudios de QS. En este género se ha evidenciado la presencia de circuitos de QS mediados por HSLs y el autoinductor AI-2. Las HSLs caracterizadas para este género tienen una gran variedad estructural, ya que además de las HSLs de cadena normal, se encuentran algunas que tienen sustituciones de grupos hidroxilo y carbonilo (C4-HSL, 3-OH-C4-HSL, C6-HSL, 3-oxo-C6-HSL, 3-OH-C6-HSL, C8-HSL, 3-oxo-C8-HSL y 3-oxo-C10-HSL). En este trabajo se determinó la presencia de la C4-HSL y la C6-HSL en los extractos de las bacterias *Vibrio* sp (23-6PIN), *Vibrio campbellii* (6-8PIN), *Vibrio* sp (11-6DEP) y *Vibrio harveyi* (PHY-11), pero no se encontraron las HSLs con sustituciones.

Como último punto queremos discutir la relevancia que tienen las HSLs dentro de los *biofilms* naturales, y la inducción que generan éstas en el asentamiento de otros organismos marinos. En estudios previos se ha reportado que la C6-HSL, aislada del *biofilm* natural de la bacteria marina *Vibrio anguillarum*, induce el asentamiento de las zooesporas del alga verde *Enteromorpha*,[20] ya que las zooesporas se unen o buscan *biofilms* bacterianos para empezar a crecer. Las moléculas señalizadoras que el alga reconoce son: la 3-oxo-C10-HSL, 3-hidroxi-C6-HSL, y C6-HSL. Además, se determinó que cuando falta alguna de estas moléculas las zooesporas no se adhieren.[20] Así mismo, se ha observado que la C6-HSL, aislada del *biofilm* natural de *Vibrio* sp, induce el asentamiento de las larvas del poliqueto *Hydroides elegans*.[51] Por otro lado, se ha encontrado que la C4-HSL induce el asentamiento de las esporas del alga *Acrochaetium* sp.[11] Por tal motivo, teniendo en cuenta

[51] Huang, Y-L.; Dobretsov, S.; Ki, J-S.; Yang L-H.; Qian, P-Y. **2007**. Presence of Acyl-Homoserine Lactone in Subtidal Biofilm and the Implication in Larval Behavioral Response in the Polychaete *Hydroides elegans*. *Microbial Ecology*. *54*, 384–392.

estos reportes se puede decir que las *N*-acilhomoserinlactonas C4-HSL y C6-HSL, inducen el asentamiento de organismos marinos y se encuentran relacionadas en la comunicación inter-reinos. Es importante resaltar que 7 de las 8 bacterias objeto de estudio en las que se demostró la presencia de C4 y C6-HSL provienen de superficies altamente colonizadas (cepas denominadas como #-PIN, #-DEP, #-C, y PHY-#, Tabla 3) y solamente una proviene de una superficie no colonizada (*Schewanella* sp), lo que puede estar relacionado con la presencia de HSLs. Con todo lo anterior, la inducción del asentamiento de macroorganismos y la procedencia de las bacterias, hace que se soporte de mejor manera el ensayo *antifouling* que estamos desarrollando con el uso de nuestras bacterias. Adicionalmente, el hecho de encontrar las mismas HSLs en los distintos géneros bacterianos aislados a partir de la misma superficie, indica que se puede estar dando una comunicación interespecies entre las bacterias, lo que puede generar un *biofilm* estratificado y estable, que es el primer paso para la formación del *biofouling*.

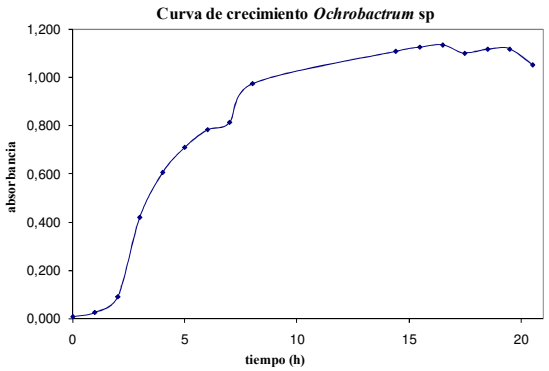
CONCLUSIONES Y RECOMENDACIONES

En este trabajo se detecta por primera vez la presencia de circuitos de QS en los géneros *Ochrobactrum* y *Alteromona*, además se encontraron HSLs diferentes a las que habían sido reportadas para el género *Shewanella* y las mismas HSLs para el género *Vibrio* ya que es el género que ha sido más ampliamente estudiado. El hecho de haber encontrado HSLs en las bacterias objeto de estudio nos indica que se está dando comunicación entre ellas es decir hay comunicación dentro de la misma especie, y que además hay comunicación entre las bacterias de distinto género, puesto que se identificaron las mismas HSLs en bacterias diferentes provenientes de una misma superficie (comunicación interespecies). Se recomienda hacer ensayos con otros biosensores con el fin de detectar la existencia de otras HSLs, ya que el biosensor que se utilizó es específico para HSLs de cadena corta; así mismo, se recomienda realizar los análisis por CG-EM en tándem, debido a la complejidad de los extractos y la poca cantidad presente en ellos de las moléculas señalizadoras.

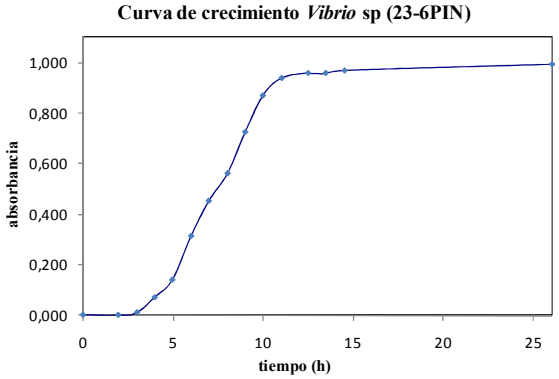
ANEXOS

ANEXO 1. Curvas de crecimiento de las bacterias marinas Gram-negativas

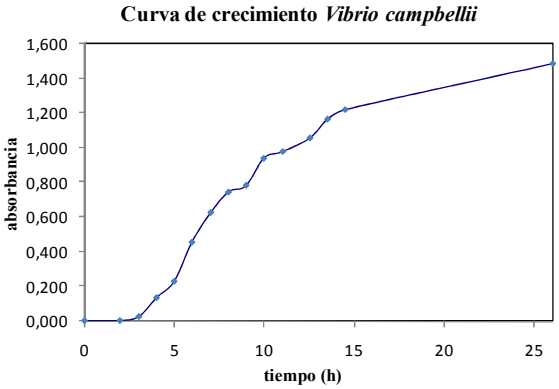
***Ochrobactrum* sp**
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento:600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 10h
Temperatura de incubación: 37º



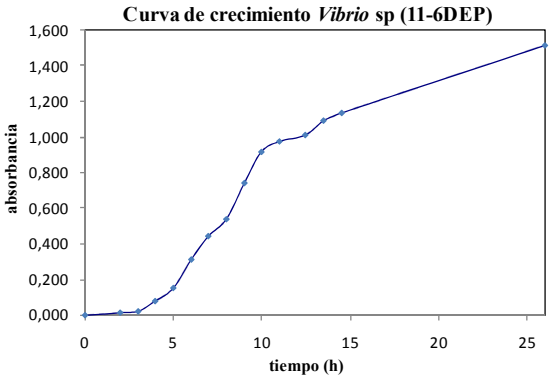
***Vibrio* sp (23-6 PIN)**
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento:600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 12h
Temperatura de incubación: ambiente



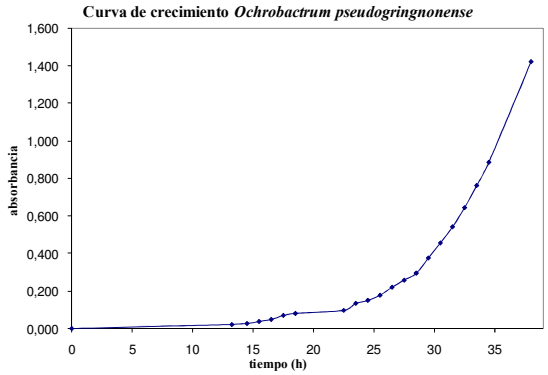
Vibrio campbellii
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento: 600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 12h
Temperatura de incubación: ambiente



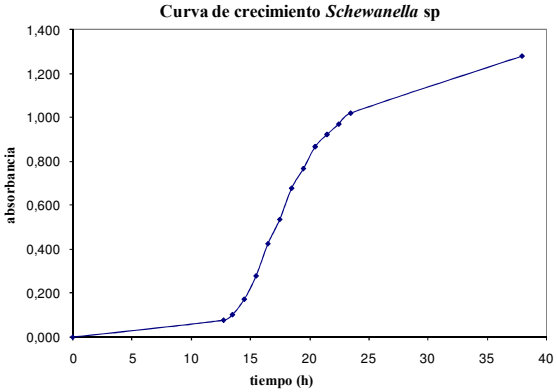
***Vibrio* sp (11-6 DEP)**
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento: 600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 12h
Temperatura de incubación: ambiente



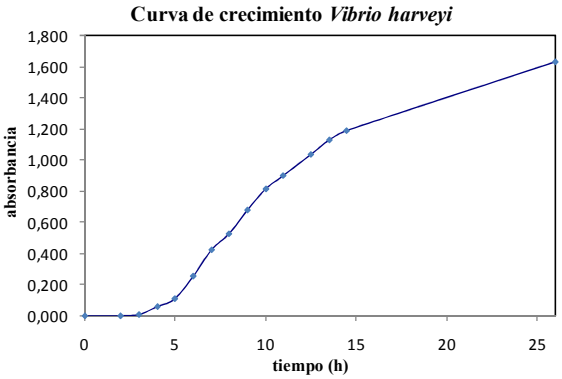
Ochrobactrum pseudogringnonense
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento: 600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 24h
Temperatura de incubación: ambiente



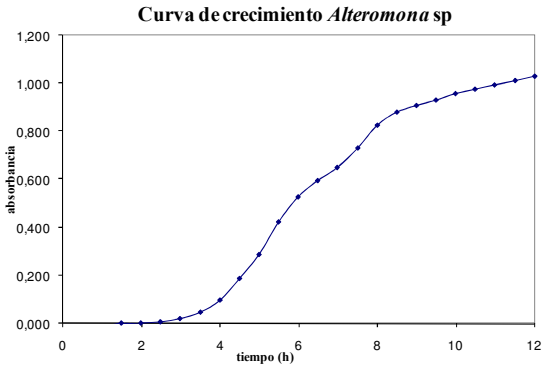
***Schewanella* sp**
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento: 600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 12h
Temperatura de incubación: ambiente



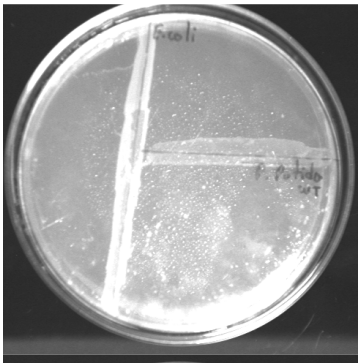
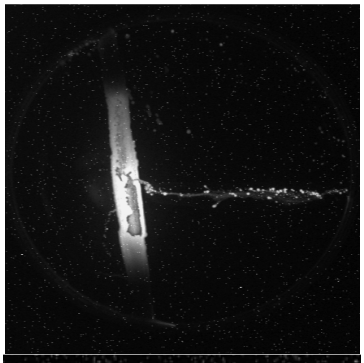
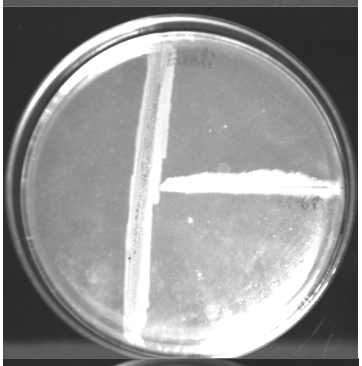
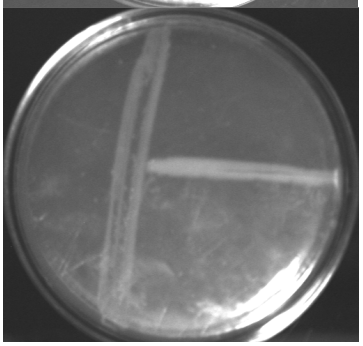
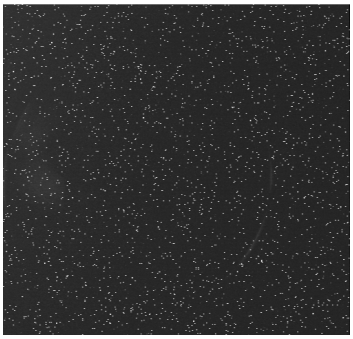
Vibrio harveyi
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento: 600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 12h
Temperatura de incubación: ambiente



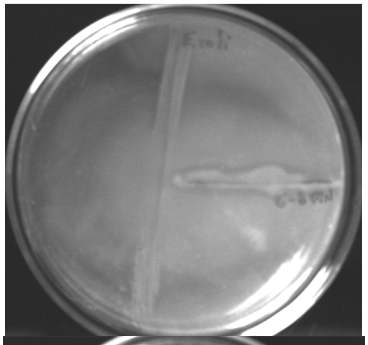
***Alteromona* sp**
Tinción de Gram: Gram-negativa
λ para leer la curva de crecimiento: 600nm
Agitación: 200 rpm
Medio de cultivo: Caldo marino
Tiempo de incubación preinóculo: 12h
Temperatura de incubación: ambiente

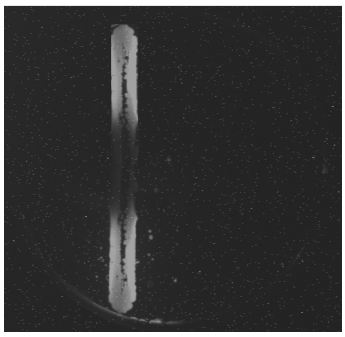


ANEXO 2: DETECCIÓN DEL *QUORUM SENSING* POR MEDIO DE BIOSENSORES

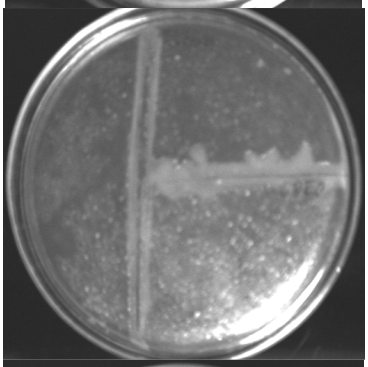
CEPA	FOTO ESTRIADO	FOTO LUMINISCENCIA
***P. putida* IsoF**		
***Ochrobactrum* sp**		
***Vibrio* sp (23-6PIN)**		

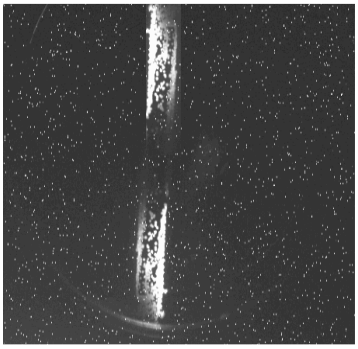
Vibrio campbellii



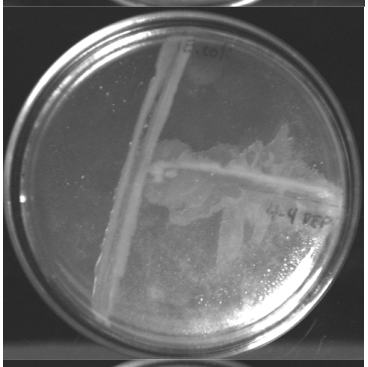


***Vibrio* sp (11-6DEP)**

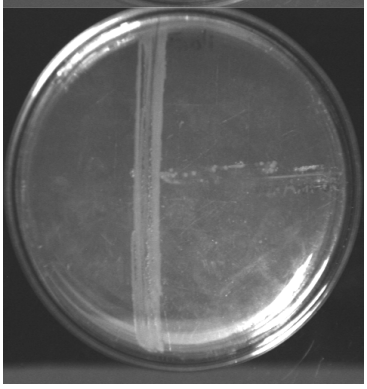




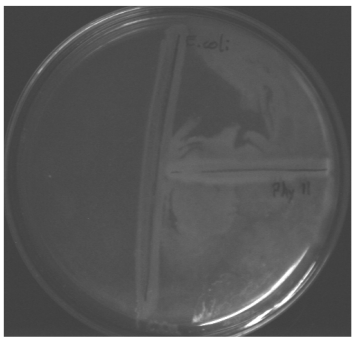
Ochrobactrum pseudogringnonense

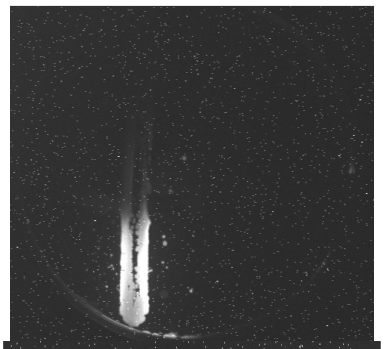


***Schewanella* sp**

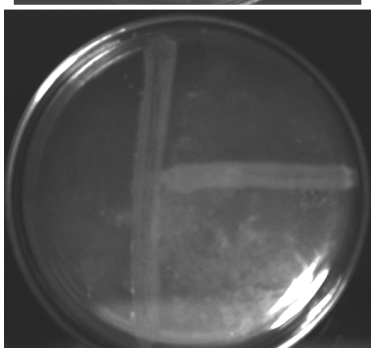


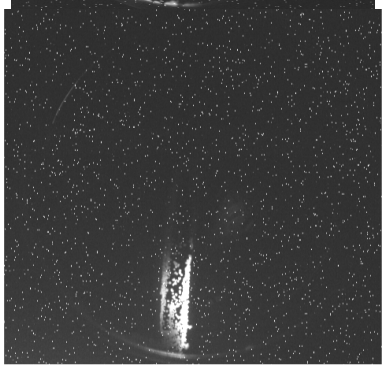
Vibrio harveyi





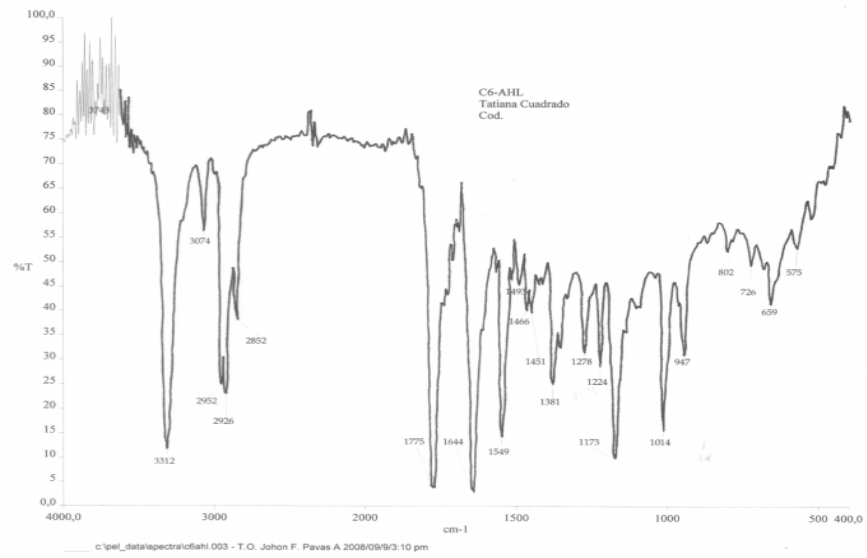
Alteromona **sp**



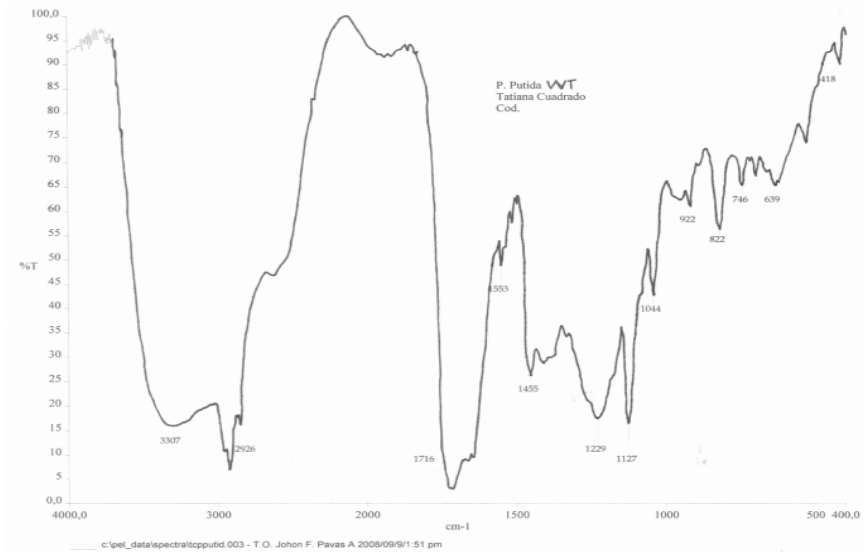


ANEXO 3: DETECCIÓN DEL *QUORUM SENSING* POR MEDIO DE ESPECTROMETRÍA INFRARROJA

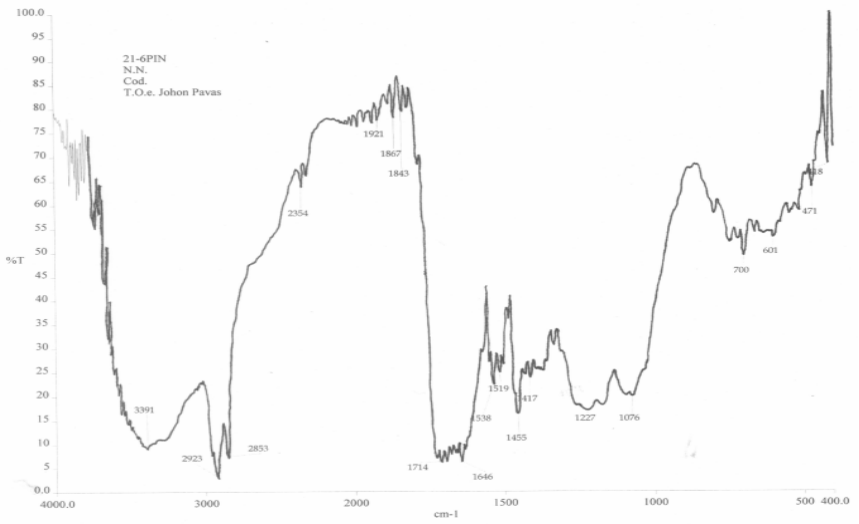
C6-HSL



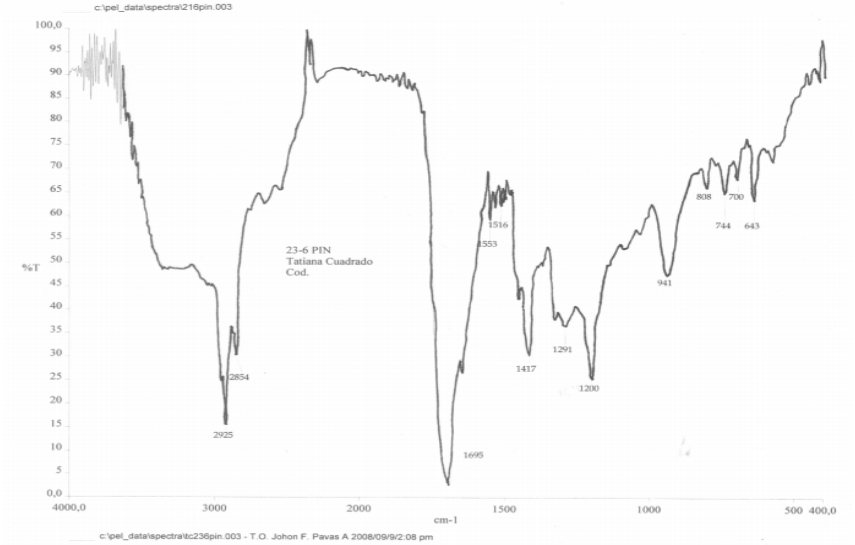
***P. putida* IsoF**



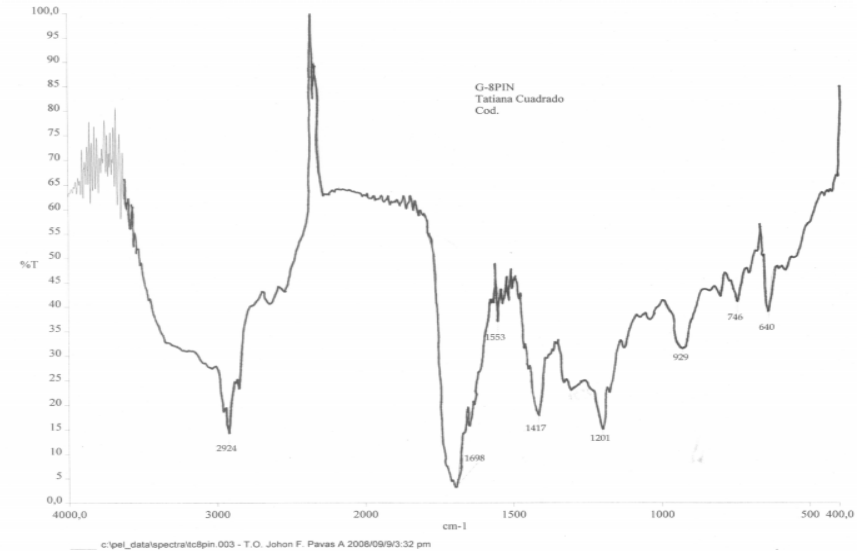
***Ochrobactrum* sp**



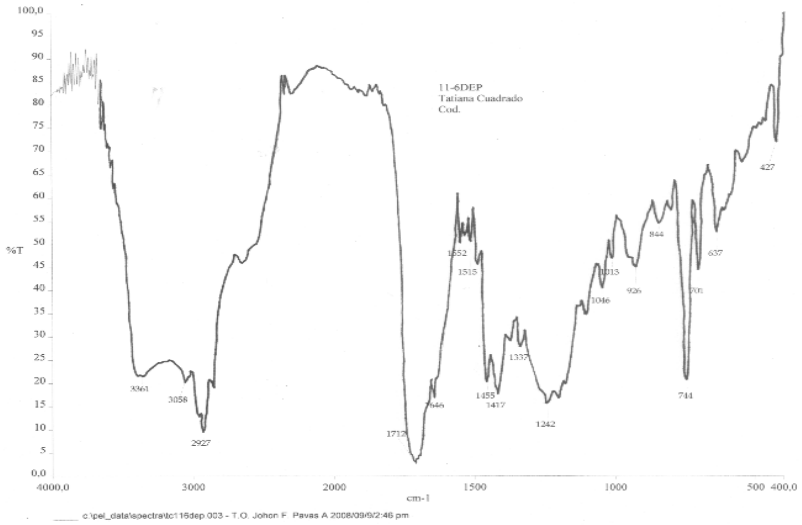
***Vibrio* sp (23-6PIN)**



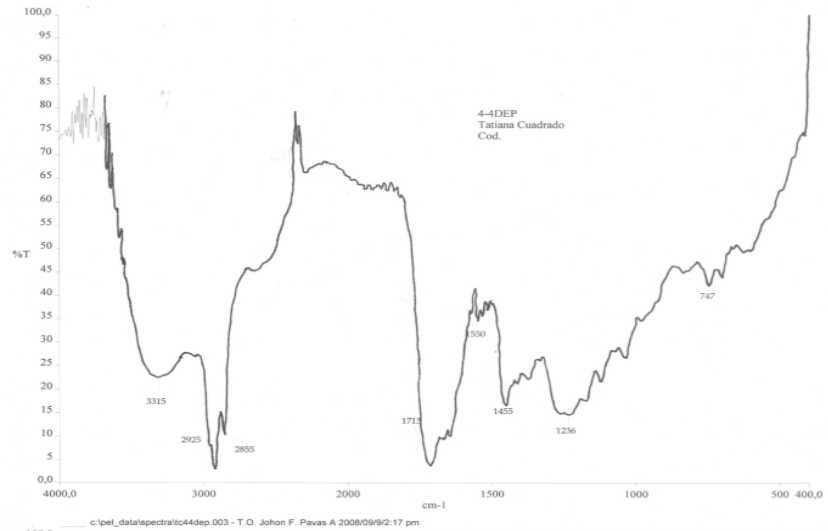
Vibrio campbellii



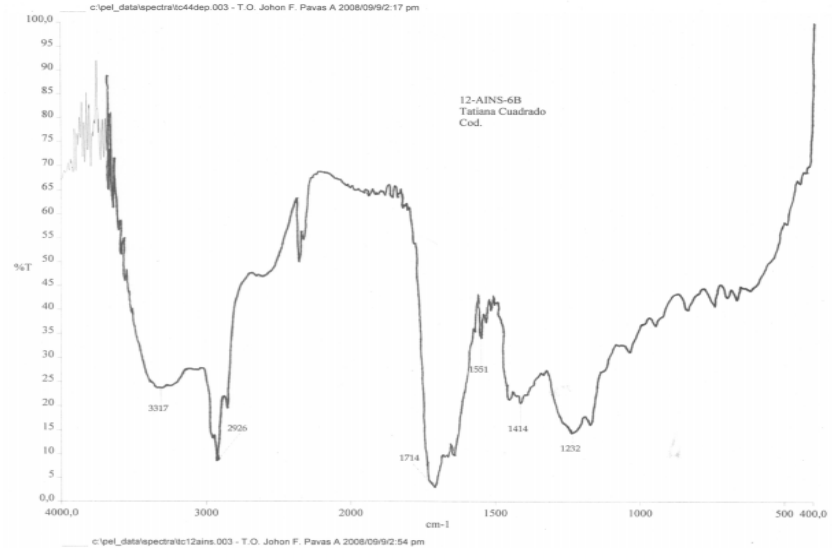
***Vibrio* sp (11-6DEP)**



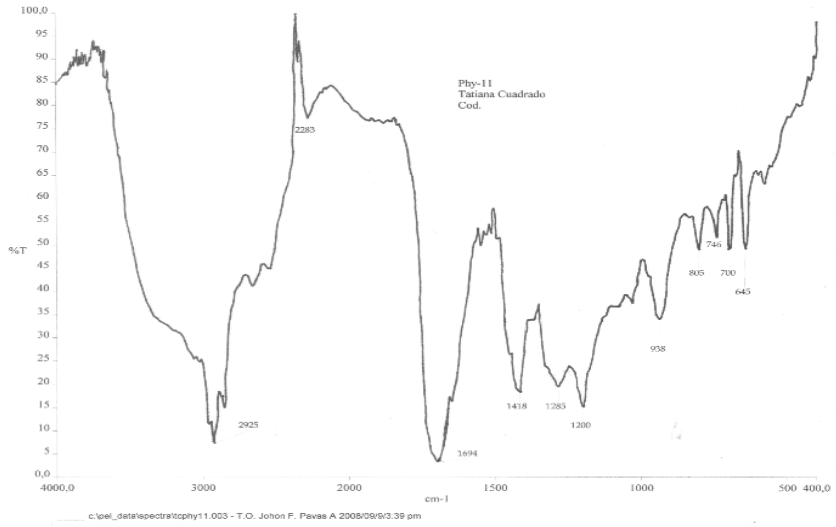
Ochrobactrum pseudogringnonense



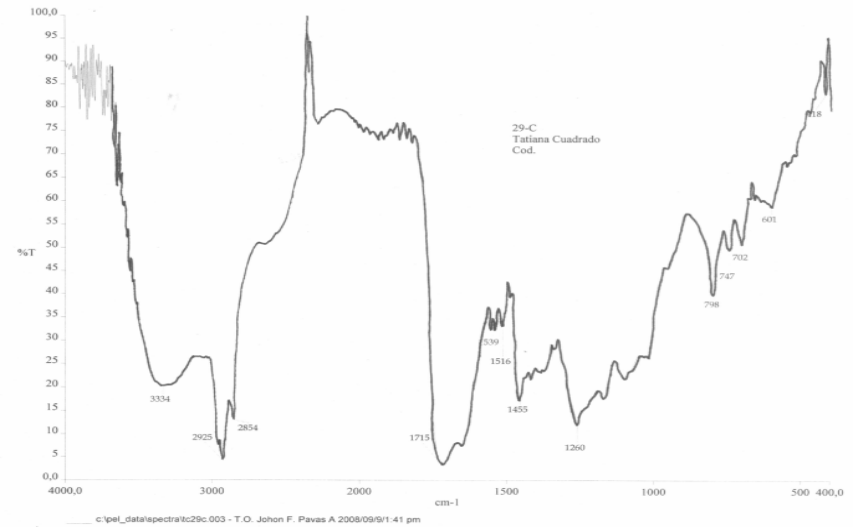
***Schewanella* sp**



Vibrio harveyi



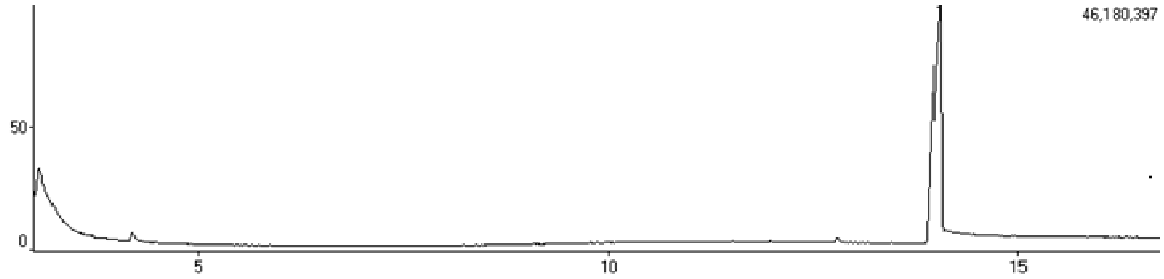
***Alteromona* sp**



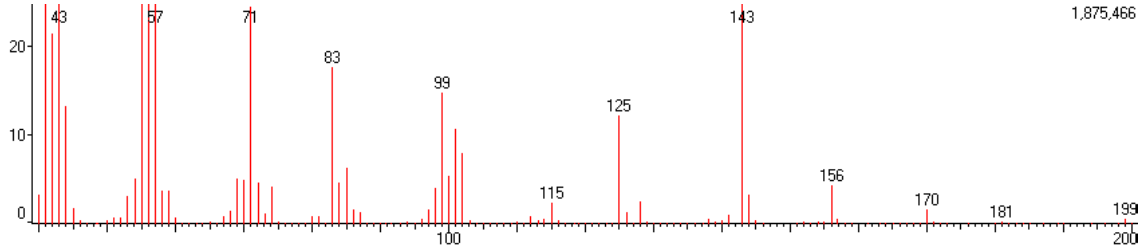
ANEXO 4: DETECCIÓN Y CARACTERIZACIÓN DE LAS HSL PRESENTES EN LOS EXTRACTOS, HACIENDO USO DE CG-EM EN MODO SCAN

Los análisis por CGAR-EM se realizaron en un cromatógrafo de gases GC-17A Shimadzu acoplado a espectrómetro de masas QP-5050A Shimadzu. La inyección de la muestra se realizó en modo *split* (10:1) dentro de una columna RPX-5 (30 m, 0,32 mm, 0,25 µm). Se inyectó en todos los casos 1 µg de muestra disuelta en 1 µL de acetona. Como gas de arrastre se empleó Helio (grado UAP) a una velocidad de 1,9 mL min^{-1}. Durante los análisis se mantuvo la temperatura del inyector a 200ºC y el detector a 300°C. La temperatura programada para el horno se optimizó manteniéndolo durante 3 minutos a 50°C e incrementando la temperatura 8,3°C min^{-1} hasta alcanzar una temperatura de 300°C. El espectrómetro de masas operó en modo de ionización por impacto electrónico (IE) a 70 eV, el monitoreo de los iones se llevó a cabo en modo SCAN detectando los iones positivos con masas entre 40 y 800 u.

C6-HSL

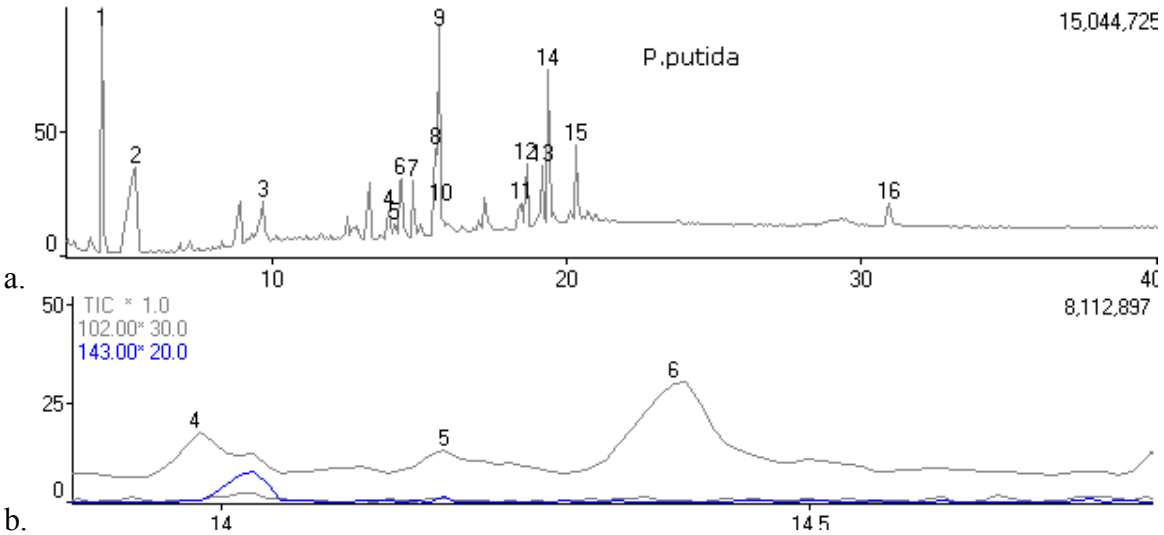


Cromatograma obtenido por CG-EM en modo SCAN.



Espectro de masas obtenido por CG-EM en modo SCAN. Tiempo de retención 14.2 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

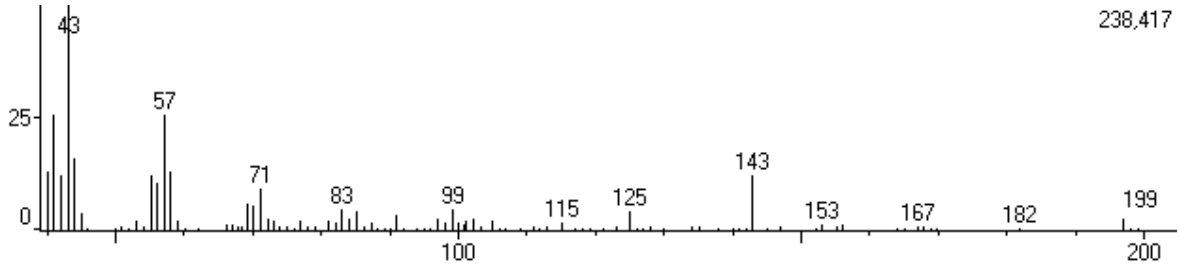
P. putida IsoF



a.

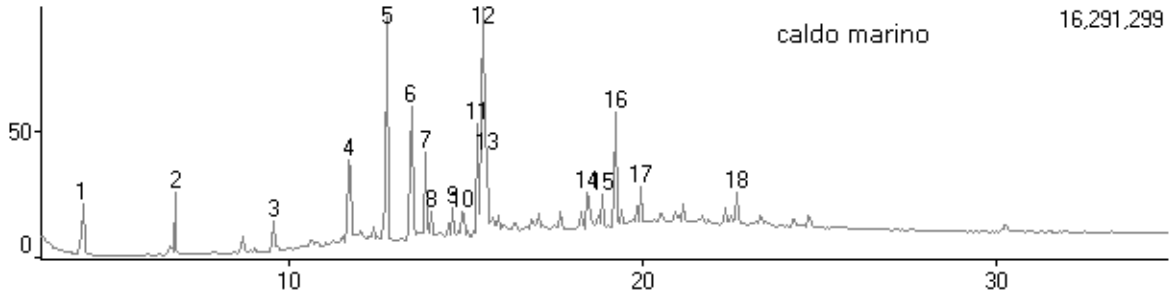
b.

a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.



Espectro de masas obtenido por CG-EM en modo SCAN para el pico 4. Tiempo de retención 14.0 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

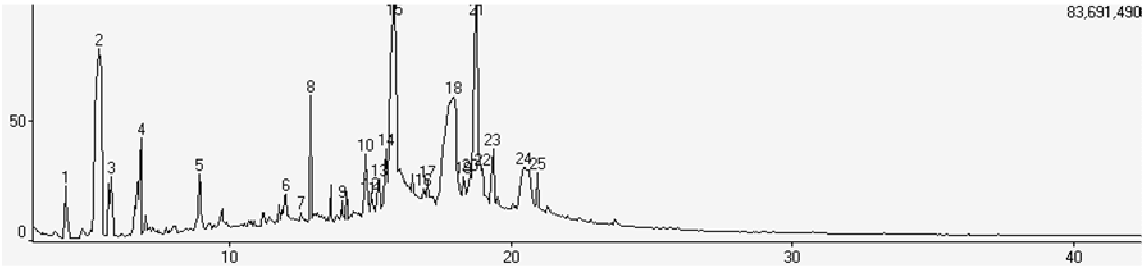
Extracto caldo marino



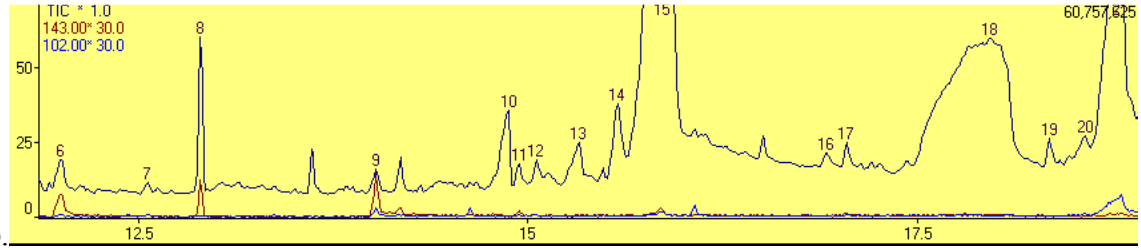
Cromatograma obtenido por CG-EM en modo SCAN.

Ochrobactrum sp

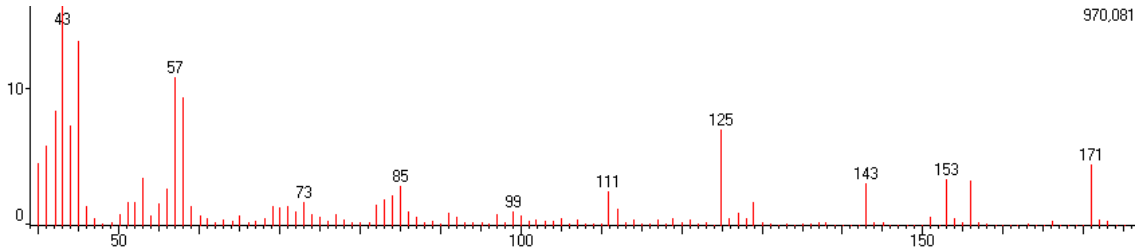
a.



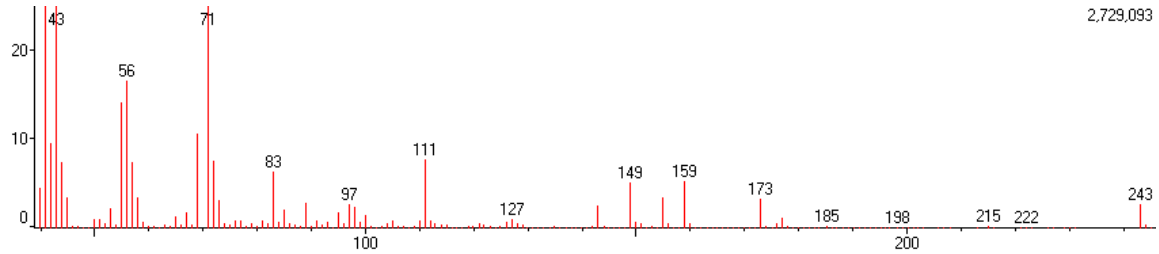
b.



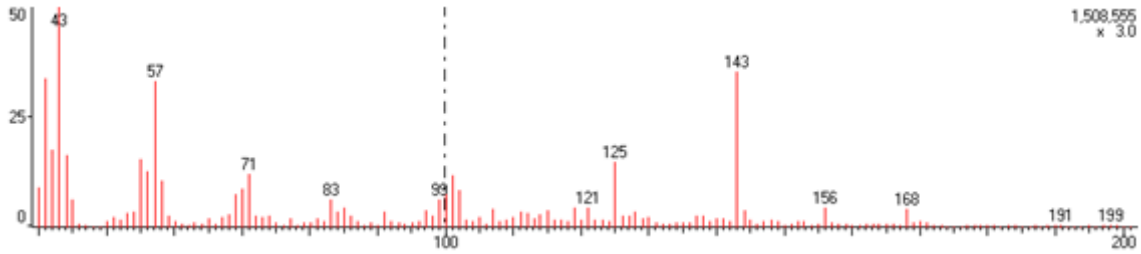
a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/*z 143 y 102.



Espectro de masas obtenido por CG-EM en modo SCAN para el pico 6. Tiempo de retención 11,9 minutos y EM-IE (70eV), *m/z*: 171 $[M]^+$.

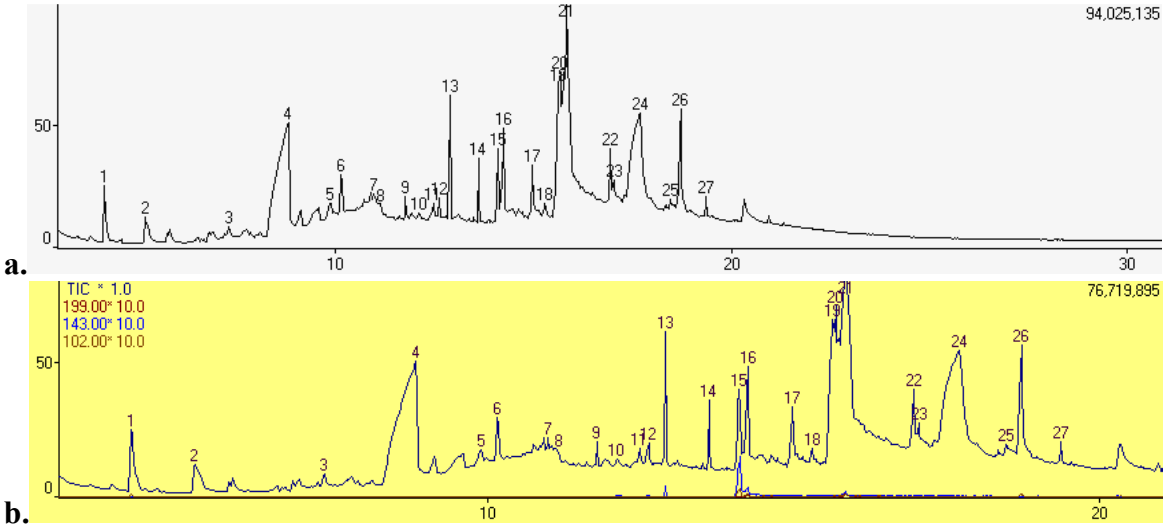


Espectro de masas obtenido por CG-EM en modo SCAN para el pico 8. Tiempo de retención 12.9 minutos y EM-IE (70eV), *m/z*: 243 $[M]^+$.



Espectro de masas obtenido por CG-EM en modo SCAN para el pico 9. Tiempo de retención 14.2 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

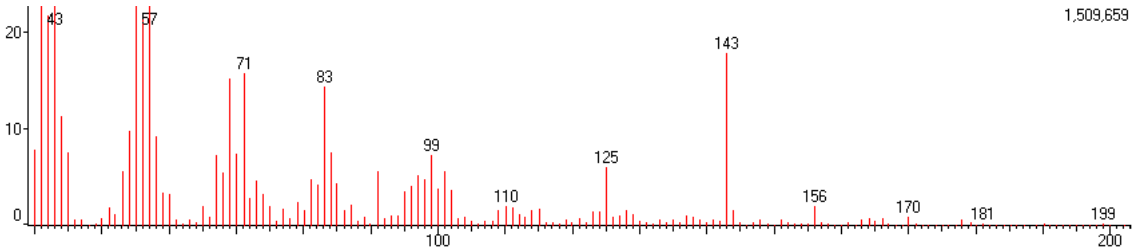
Vibrio sp (23-6PIN)



a.

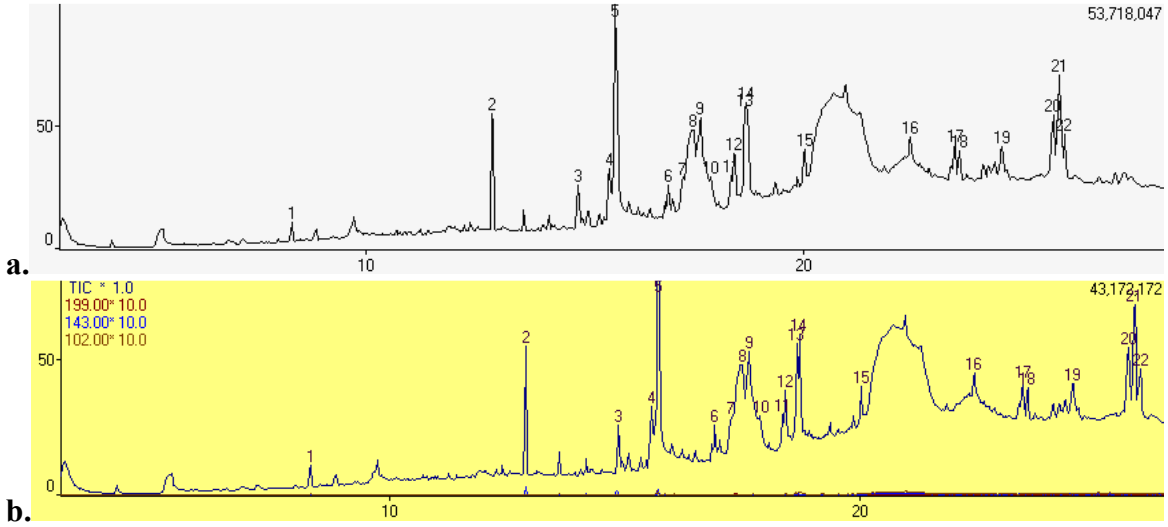
b.

a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.



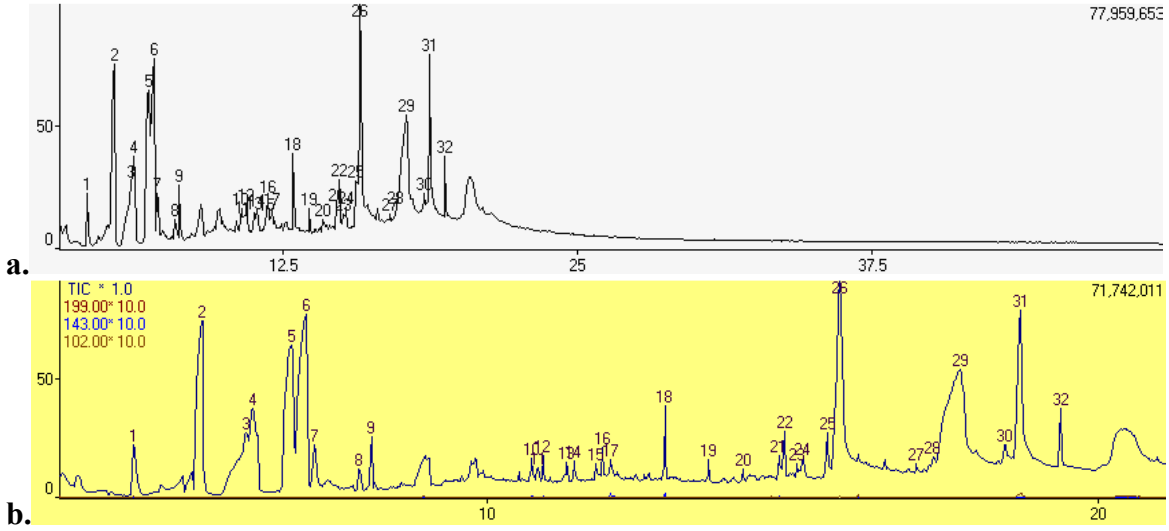
Espectro de masas obtenido por CG-EM en modo SCAN para el pico 15. Tiempo de retención 14.1 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

Vibrio campbellii

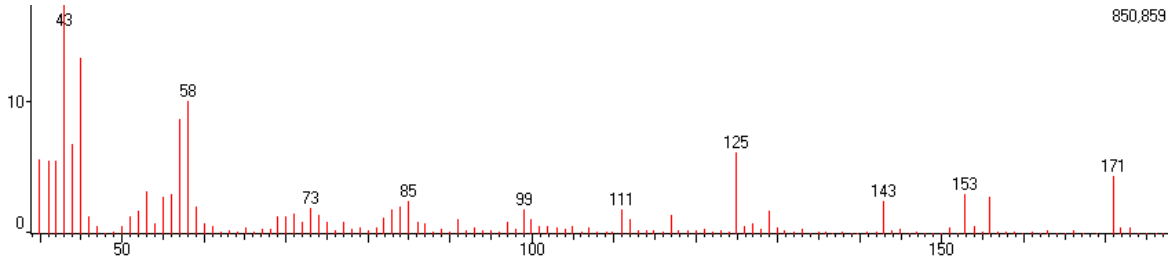


a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.

Vibrio sp (11-6DEP)

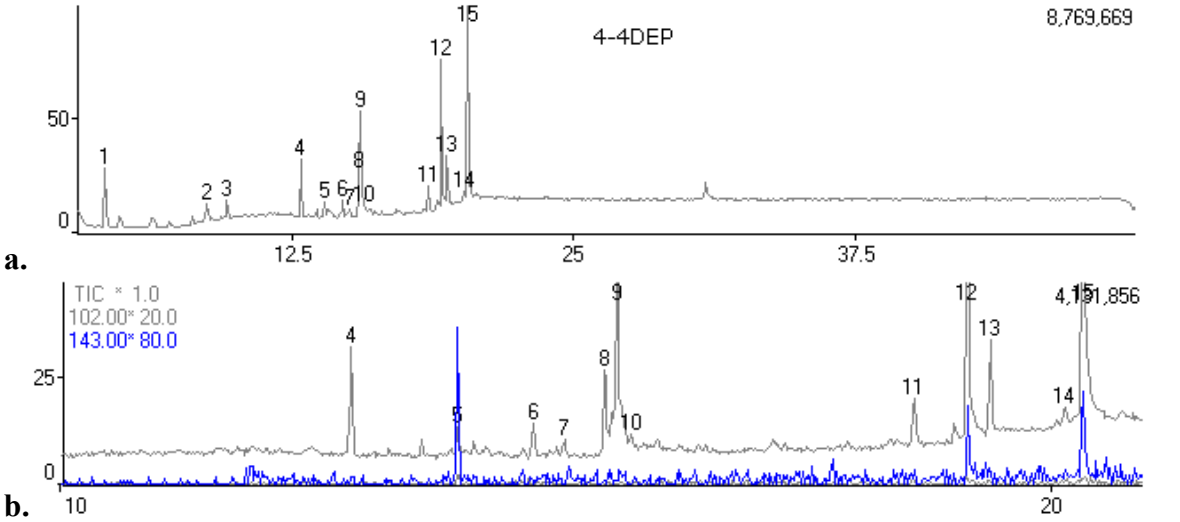


a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.



Espectro de masas obtenido por CG-EM en modo SCAN para el pico 17. Tiempo de retención 12.0 minutos y EM-IE (70eV), *m/z*: 171 $[M]^+$.

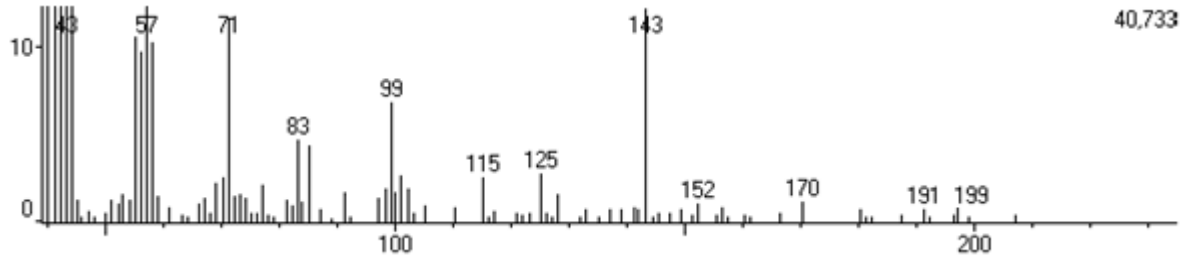
Ochrobactrum pseudogringnonense



a.

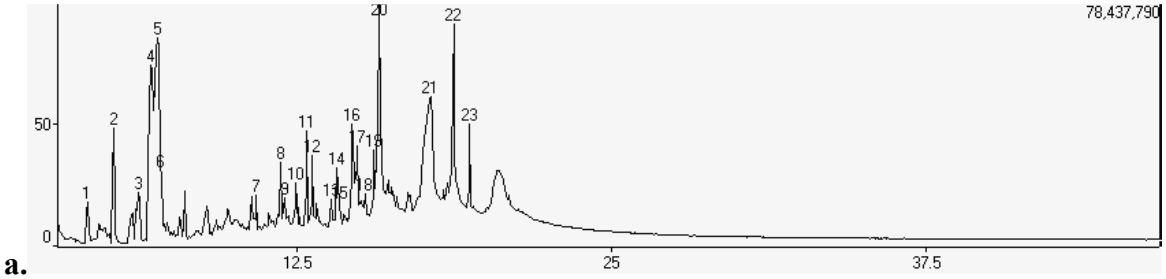
b.

a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.

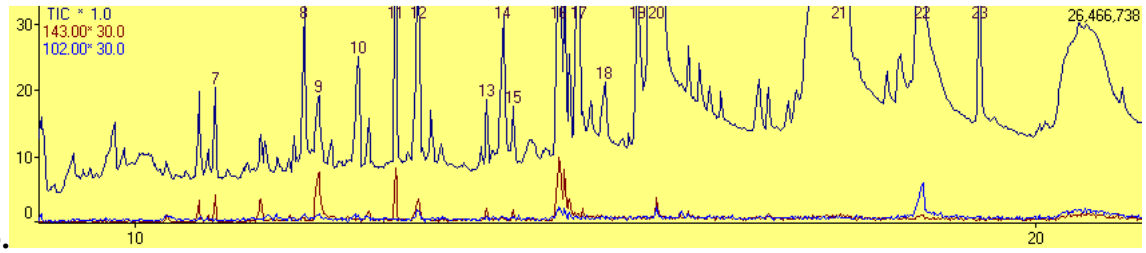


Espectro de masas obtenido por CG-EM en modo SCAN para el pico 5. Tiempo de retención 14.0 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

Schewanella sp

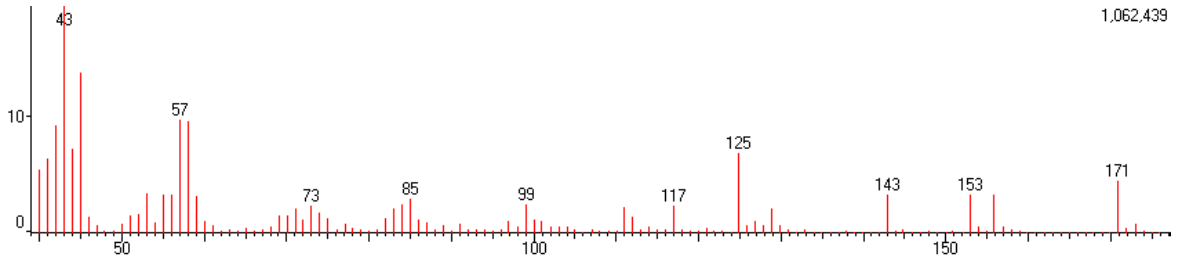


a.

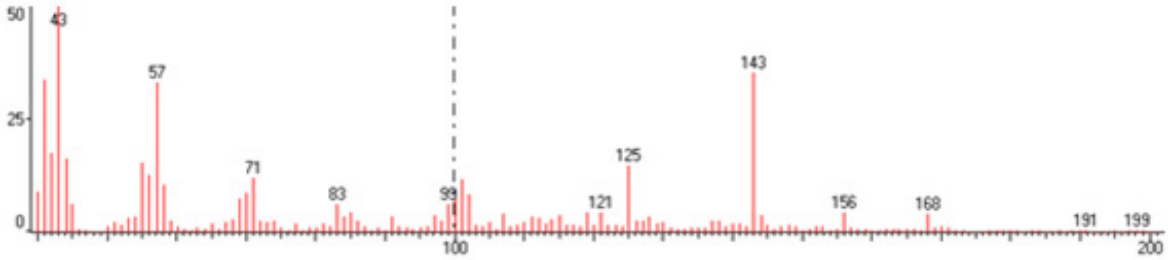


b.

a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.

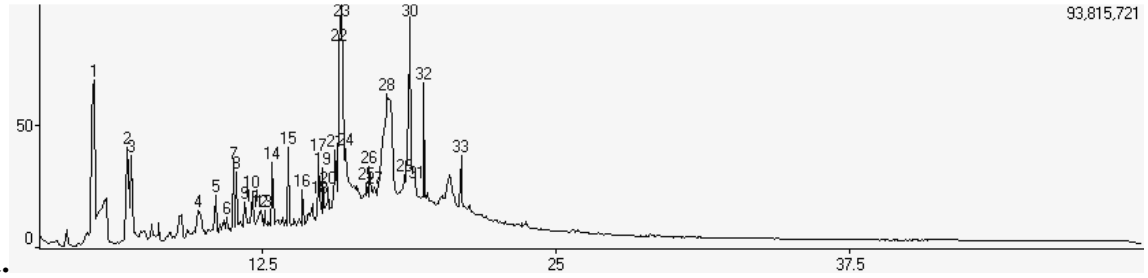


Espectro de masas obtenido por CG-EM en modo SCAN para el pico 9. Tiempo de retención 12.0 minutos y EM-IE (70eV), *m/z*: 171 $[M]^+$.

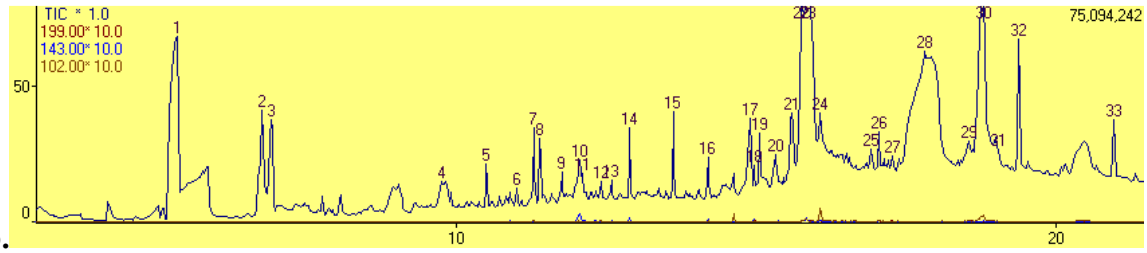


Espectro de masas obtenido por CG-EM en modo SCAN para el pico 15. Tiempo de retención 14.2 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

Vibrio harveyi

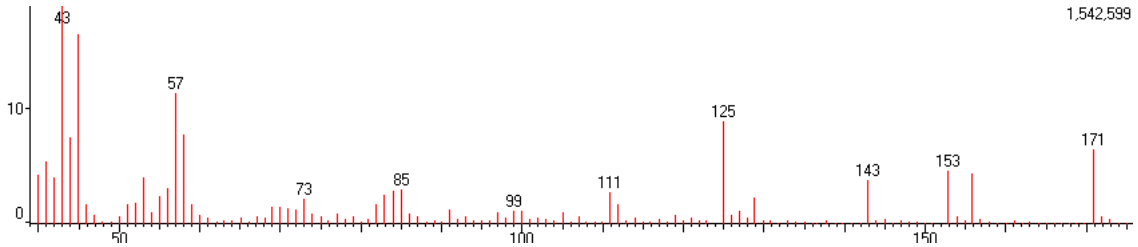


a.

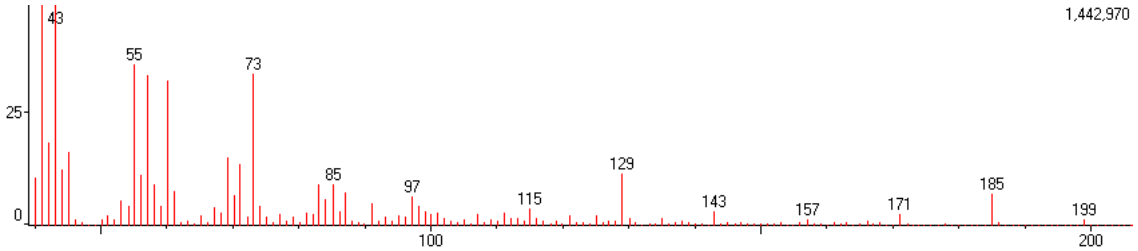


b.

a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.

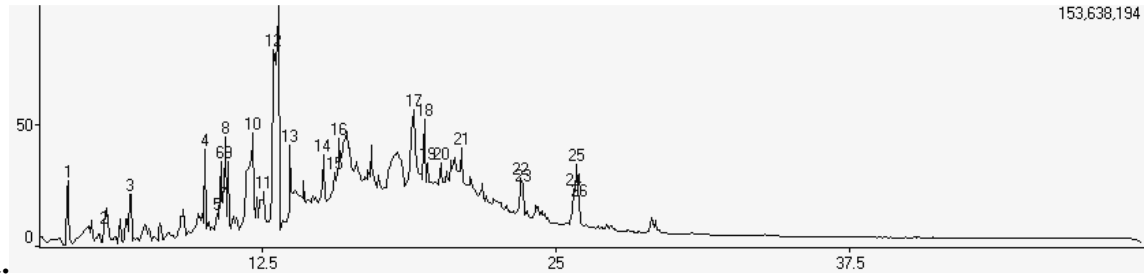


Espectro de masas obtenido por CG-EM en modo SCAN para el pico 10. Tiempo de retención 12.0 minutos y EM-IE (70eV), *m/z*: 171 $[M]^+$.



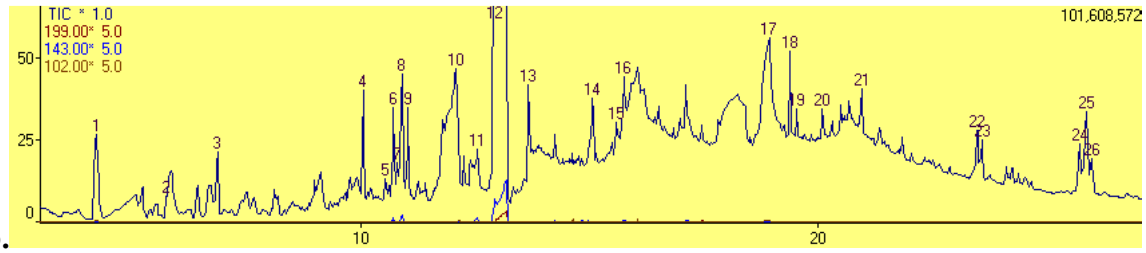
Espectro de masas obtenido por CG-EM en modo SCAN para el pico 16. Tiempo de retención 14.2 minutos y EM-IE (70eV), *m/z*: 199 $[M]^+$.

Alteromona sp



a.

b.

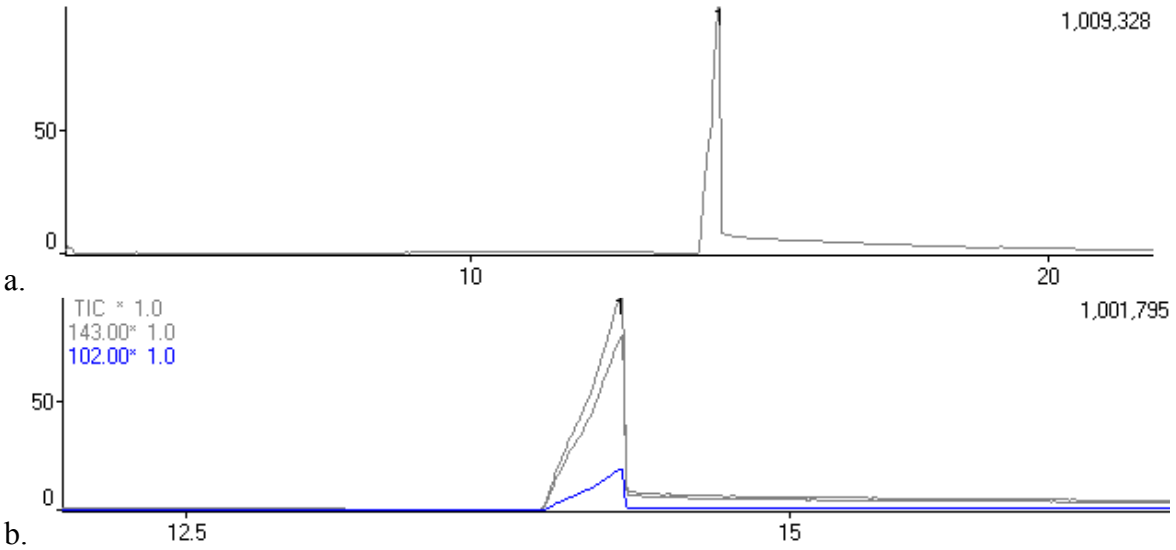


a. Cromatograma obtenido por CG-EM en modo SCAN b. Cromatograma ampliado, buscando los picos que contienen los iones *m/z* 143 y 102.

ANEXO 5: DETECCIÓN Y CARACTERIZACIÓN DE LAS HSL PRESENTES EN LOS EXTRACTOS, HACIENDO USO DE CG-EM EN MODO SIM

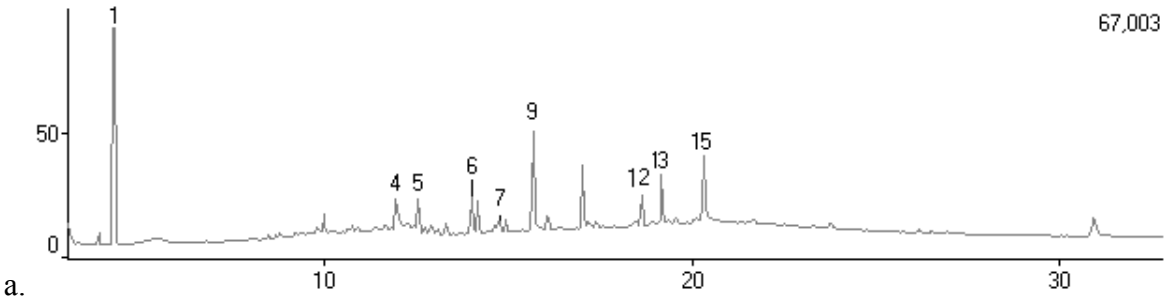
Se llevó a cabo el análisis en modo SIM monitoreando los iones *m/z* 102 y 143.

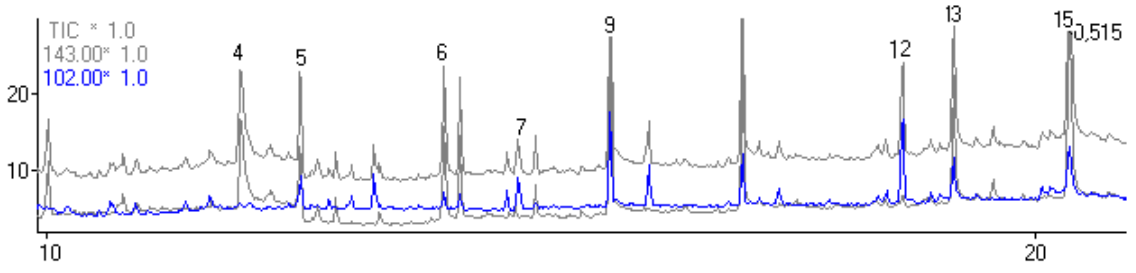
C6-HSL



a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/z* 143 y 102.

P. putida Iso F

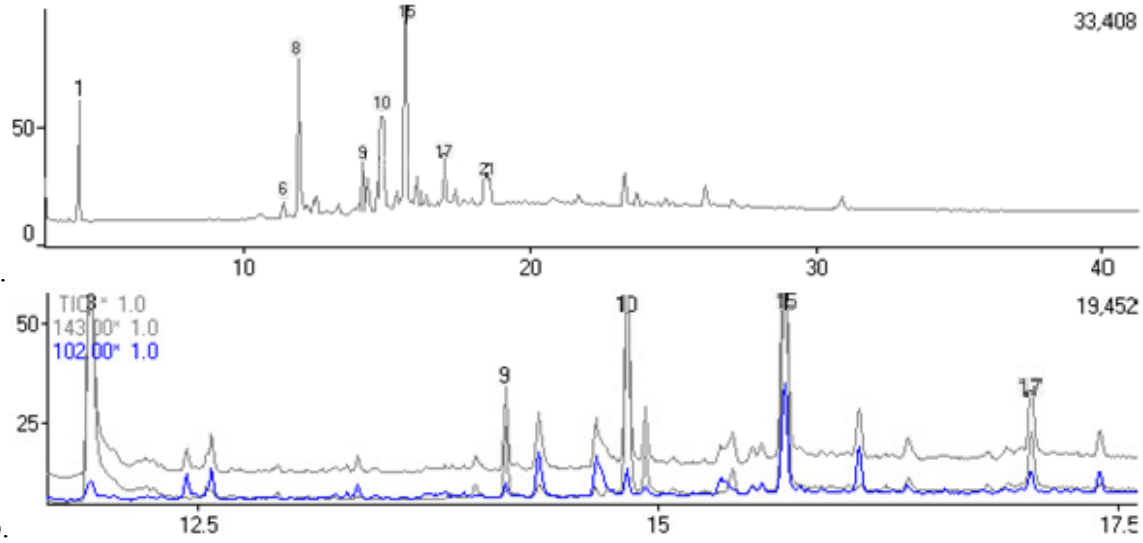




b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Ochrobactrum sp

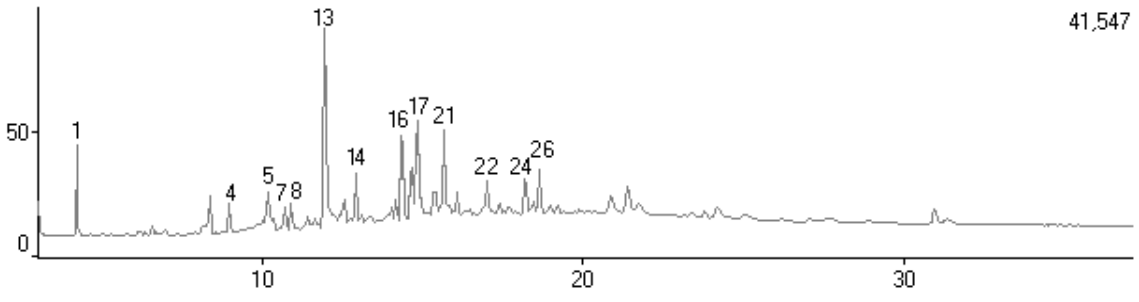


a.

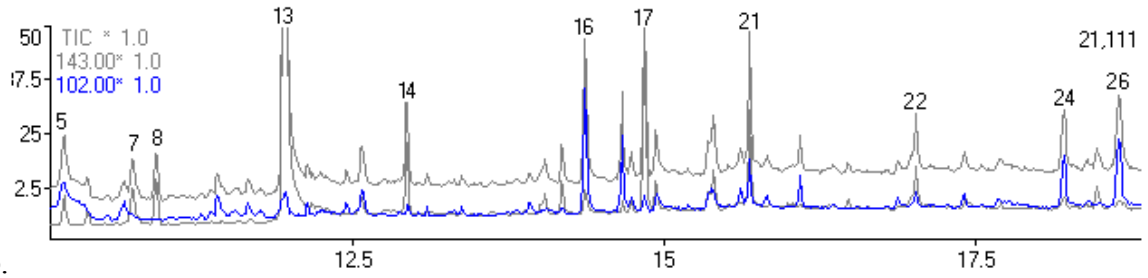
b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Vibrio sp (23-6PIN)



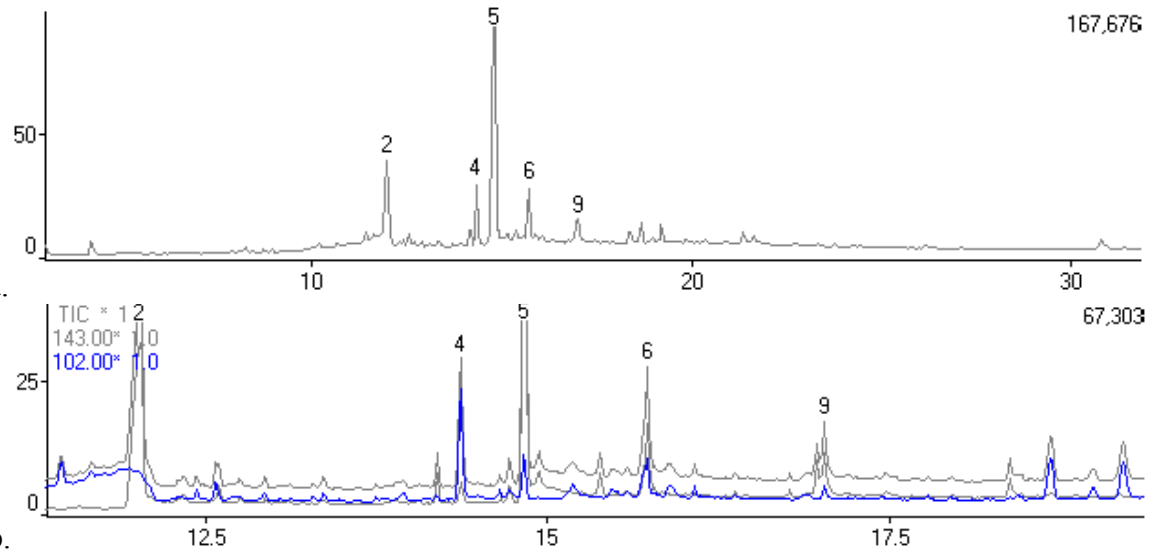
a.



b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Vibrio campbellii

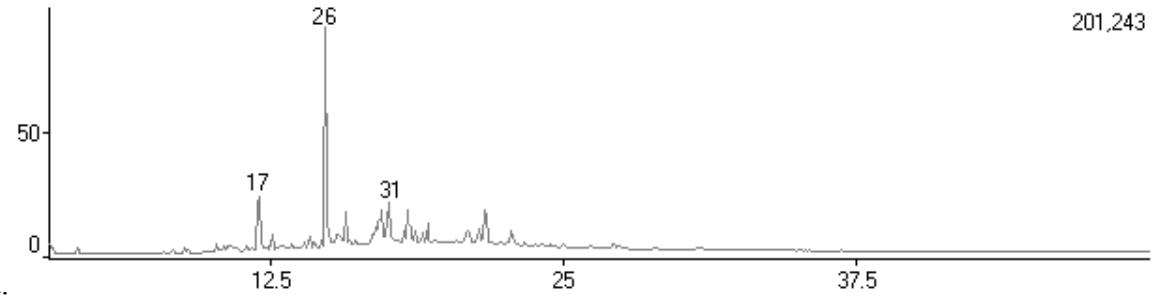


a.

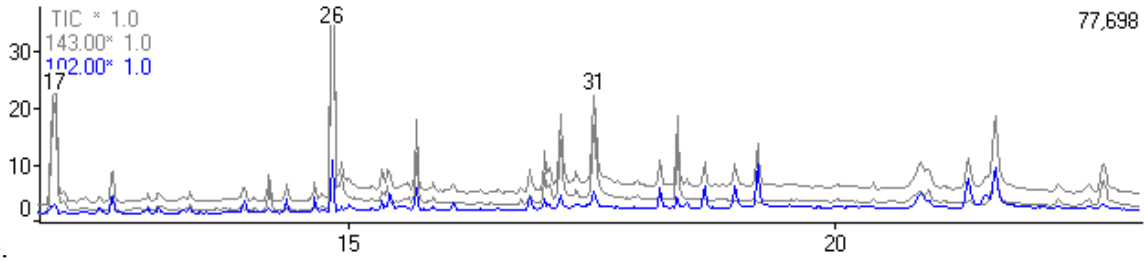
b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Vibrio sp (11-6DEP)



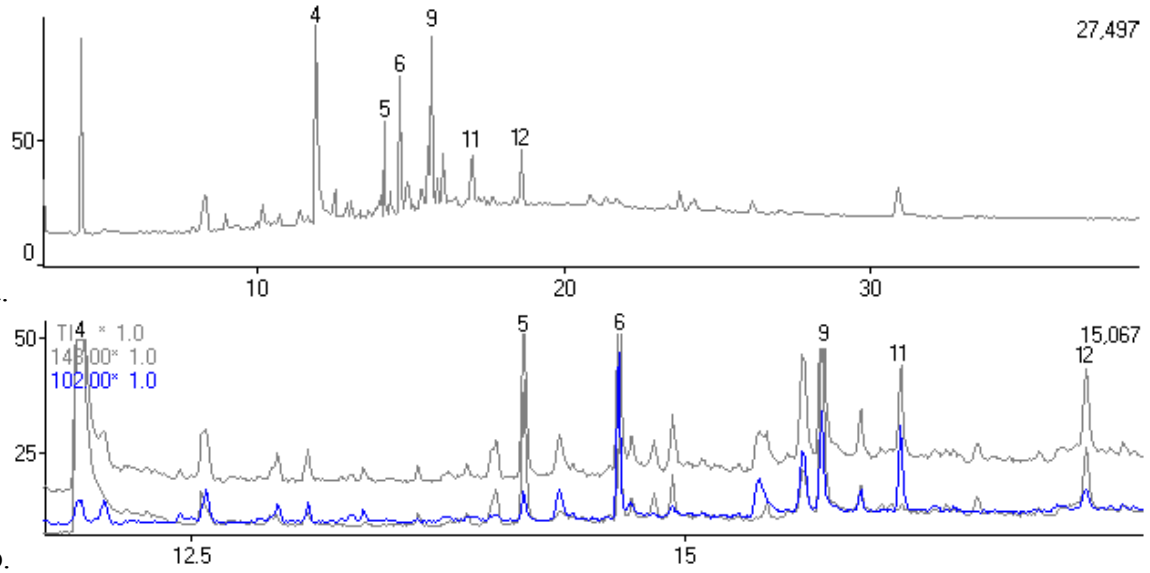
a.



b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Ochrobactrum pseudogringnonense

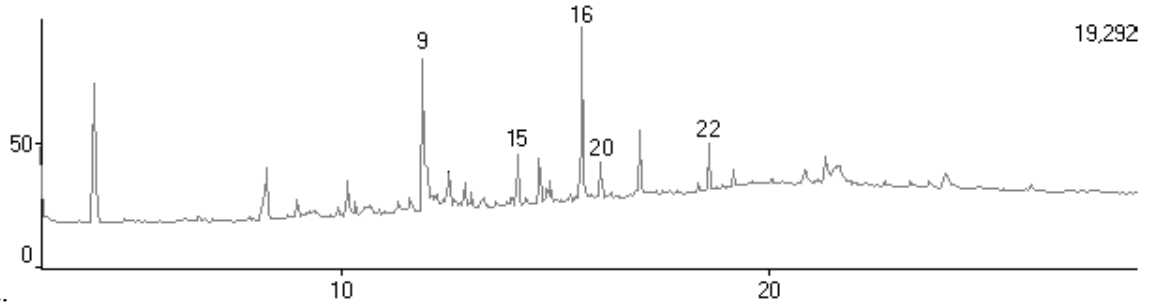


a.

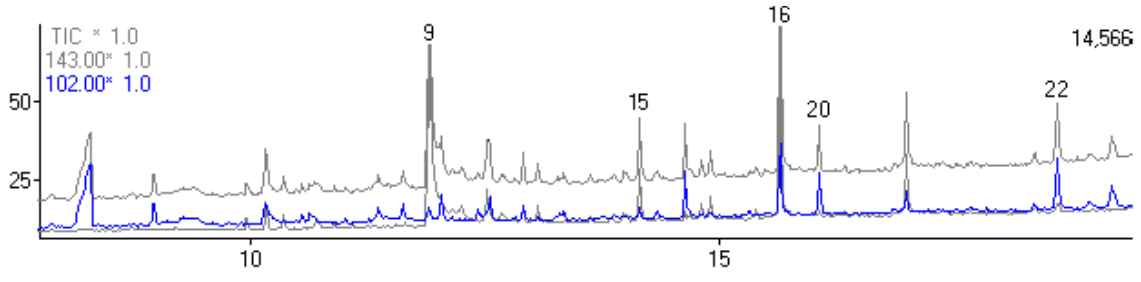
b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Schewanella sp



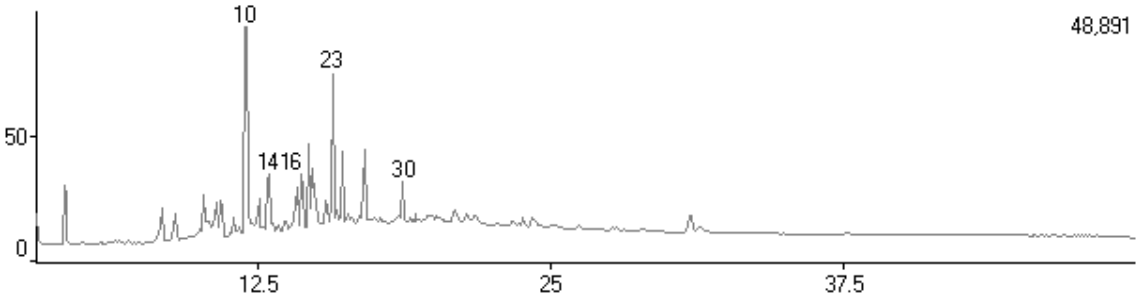
a.



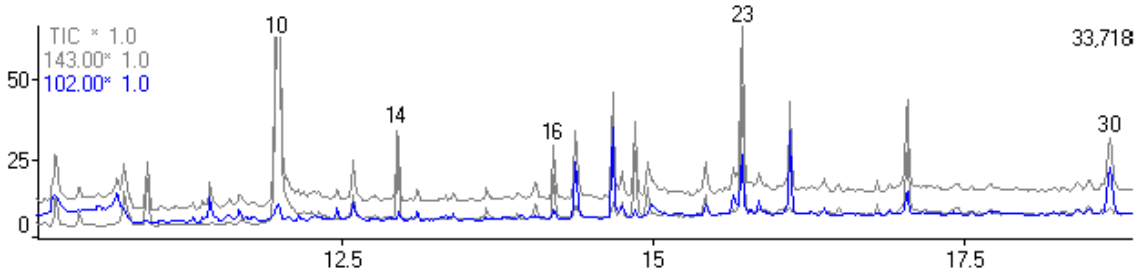
b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Vibrio harveyi



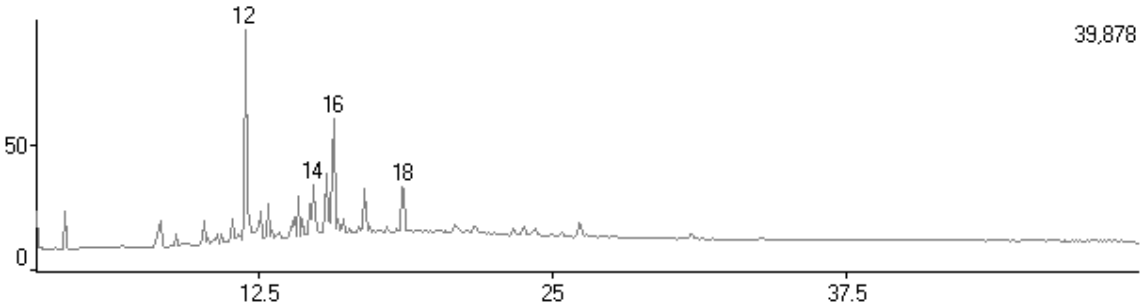
a.



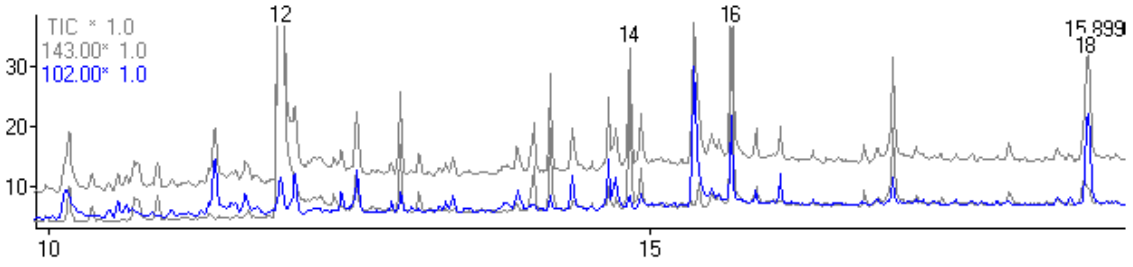
b.

a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

Alteromona **sp**



a.



b.

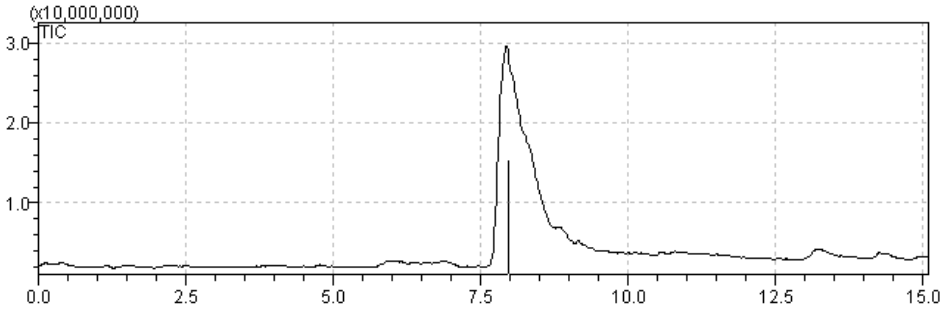
a. Cromatograma obtenido por CG-EM en modo SIM. b. Cromatograma ampliado, siguiendo los iones *m/*z 143 y 102.

ANEXO 6: DETECCIÓN Y CARACTERIZACIÓN DE LAS HSL PRESENTES EN LOS EXTRACTOS, HACIENDO USO DE CLAE-EM

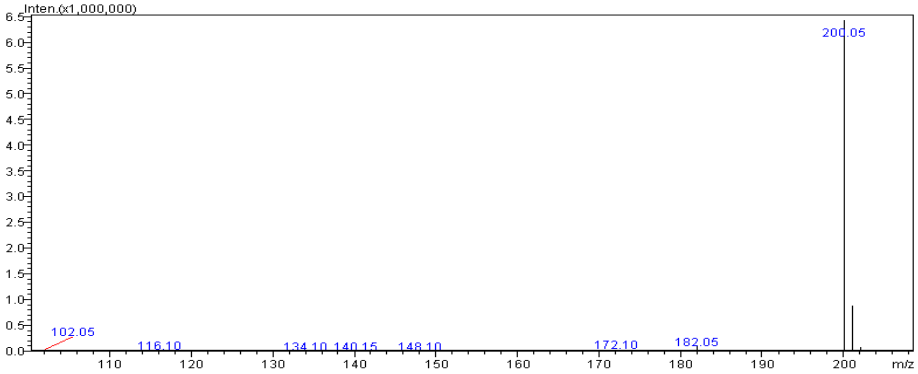
Los análisis por CLAE-EM se realizaron en un cromatógrafo líquido LC-10AD Shimadzu acoplado a espectrómetro de masas LCMS-2010 EV Shimadzu. La inyección de la muestra se realizó dentro de una columna RP-18 phenomenex Luna 5 µ 100A de dimensiones150 x 2,0 nm. Se inyectaron 2 µL del patrón C6-HSL, de una disolución de 1 mg en 60 µL de MeOH/Agua (1:1). En el caso de los extractos y fracciones se inyectaron 5 µL de una disolución de 5 mg en 100 µL de MeOH/Agua (1:1). Como fase móvil se utilizaron MeOH grado CLAE-EM y agua/ác. fórmico 0,1%. Las muestras fueron eluídas con un gradiente lineal, el cual inició con metanol al 15% hasta llegar al 100% en 15 minutos, el flujo se fijó en 0,3 mL min^{-1}. El espectrómetro de masas operó en modo de ionización por electrospray (ESI) a 1,5 kV, utilizando como gas nebulizador nitrógeno a un flujo de 1,5 L min^{-1}, se detectaron los iones positivos con masas entre 100 y 300 u.

C6-HSL

a.



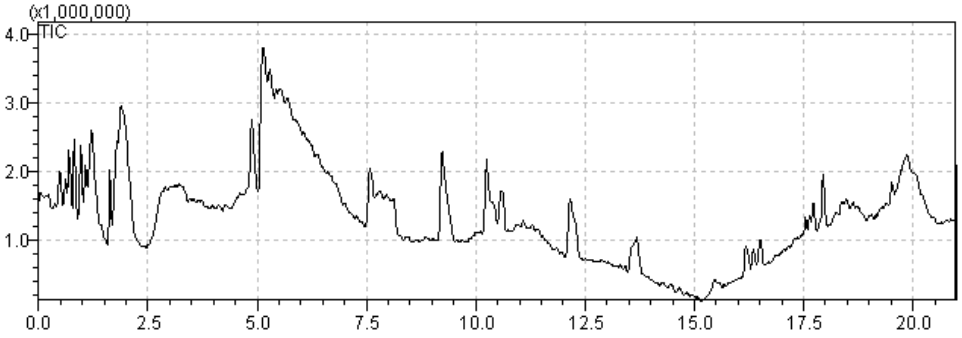
b.



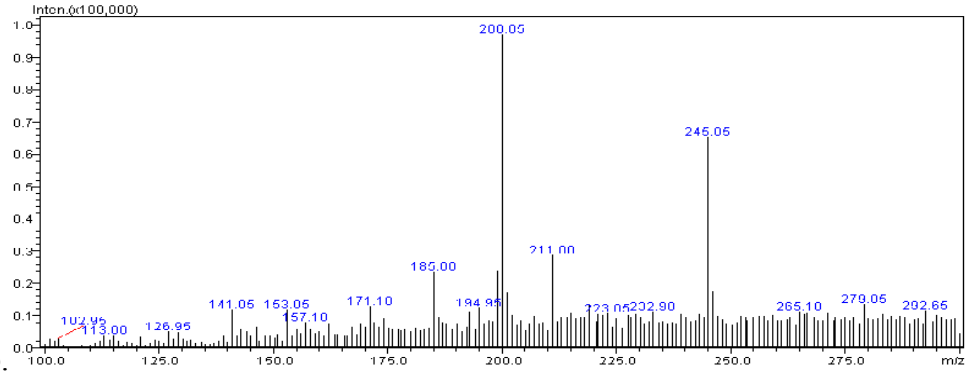
a. Cromatograma obtenido por CLAE-EM. b. Espectro de masas obtenido por CLAE-EM. Tiempo de retención 7,9 minutos, *m/z*: 200,05 $[M+H]^+$.

P. putida IsoF

a.

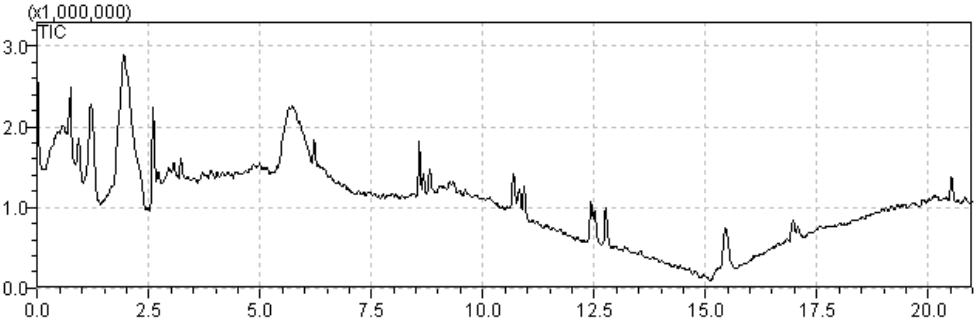


b.

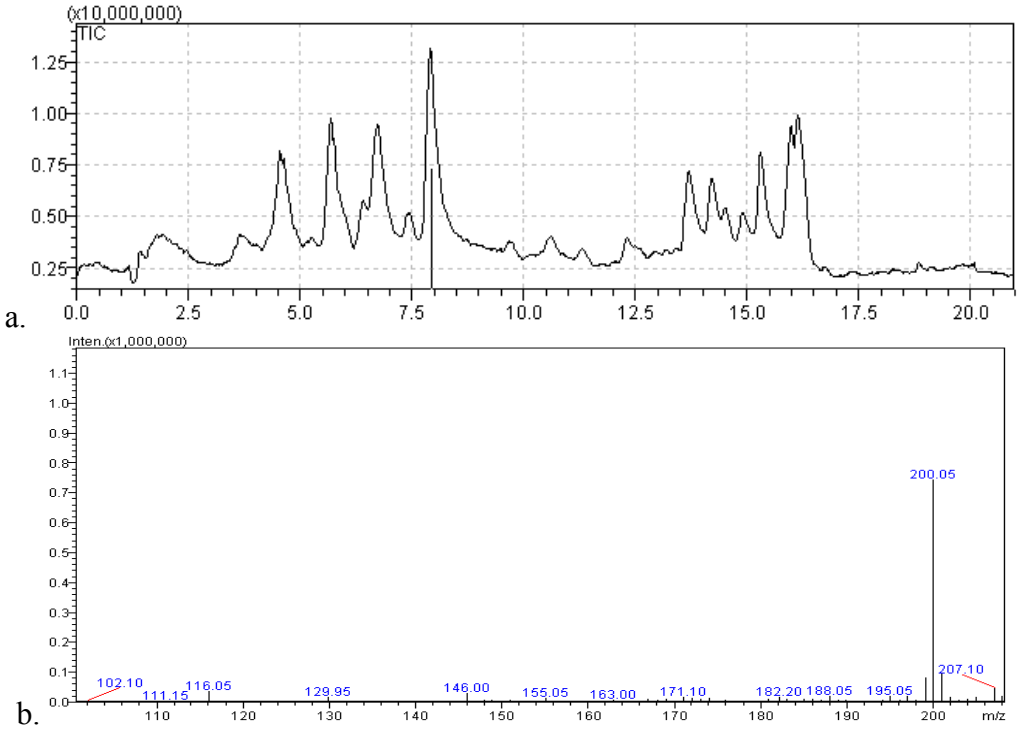


a. Cromatograma obtenido por CLAE-EM. b. Espectro de masas obtenido por CLAE-EM. Tiempo de retención 7,9 minutos, *m/z*: 200,05 $[M+H]^+$.

Extracto caldo marino



Ochrobactrum sp

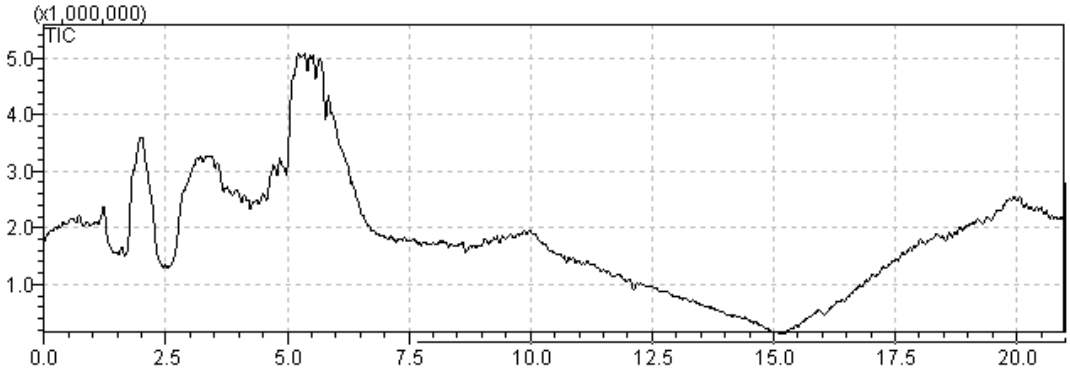


a.

b.

a. Cromatograma obtenido por CLAE-EM. b. Espectro de masas obtenido por CLAE-EM. Tiempo de retención 7,9 minutos, *m/z*: 200,05 $[M+H]^+$.

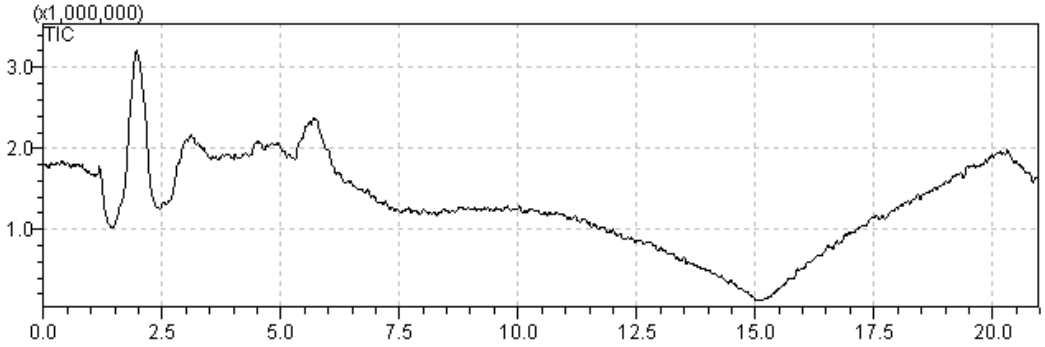
Vibrio sp (23-6PIN)



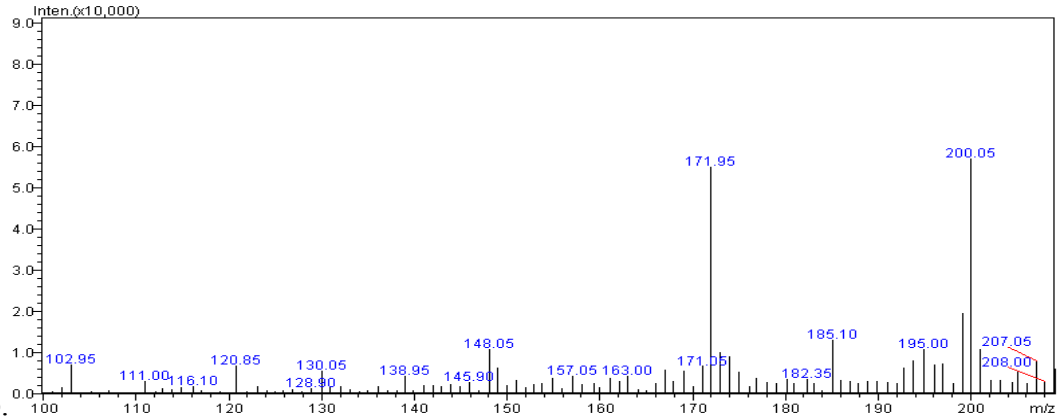
Cromatograma obtenido por CLAE-EM.

Vibrio campbellii

a.

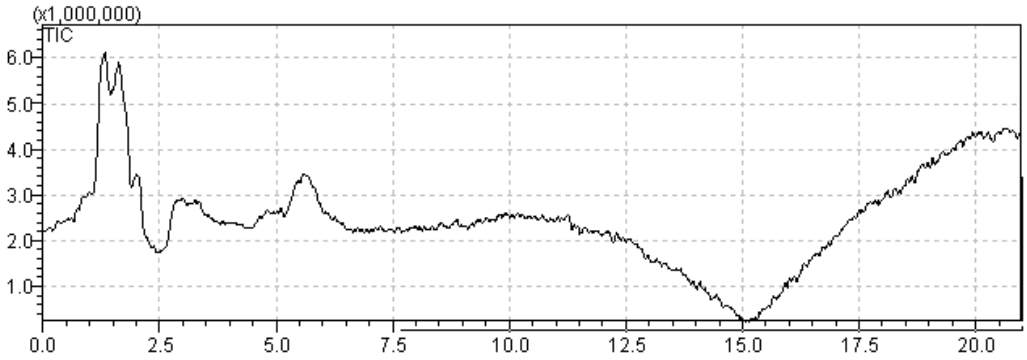


b.



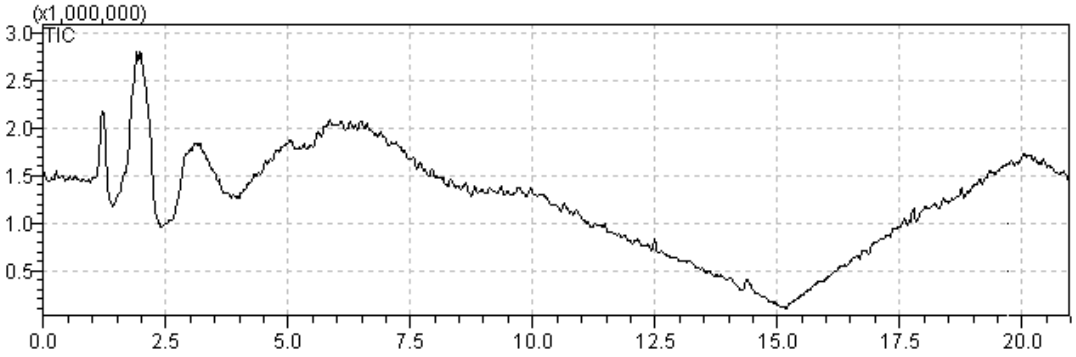
a. Cromatograma obtenido por CLAE-EM. b. Espectro de masas obtenido por CLAE-EM. Tiempo de retención 7,9 minutos, *m/z*: 200,05 $[M+H]^+$.

Vibrio sp (11-6DEP)



Cromatograma obtenido por CLAE-EM.

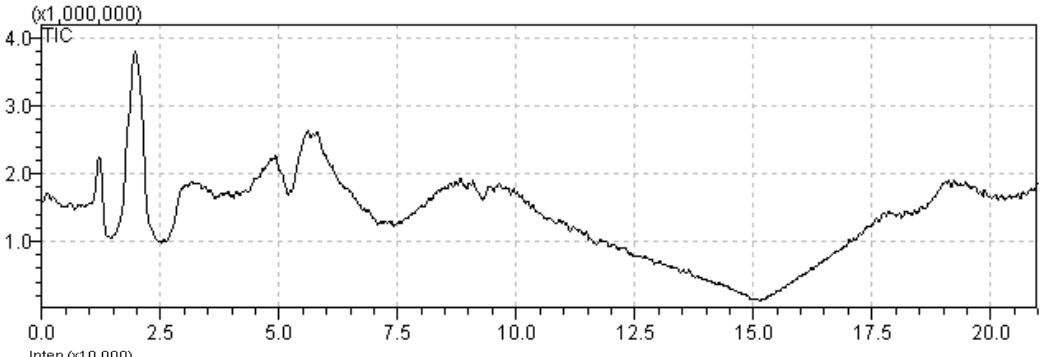
Ochrobactrum pseudogringnonense



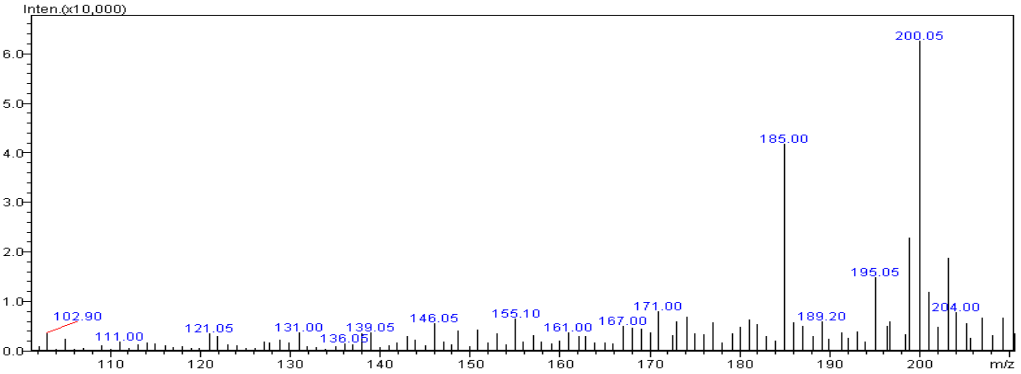
Cromatograma obtenido por CLAE-EM.

Schewanella sp

a.

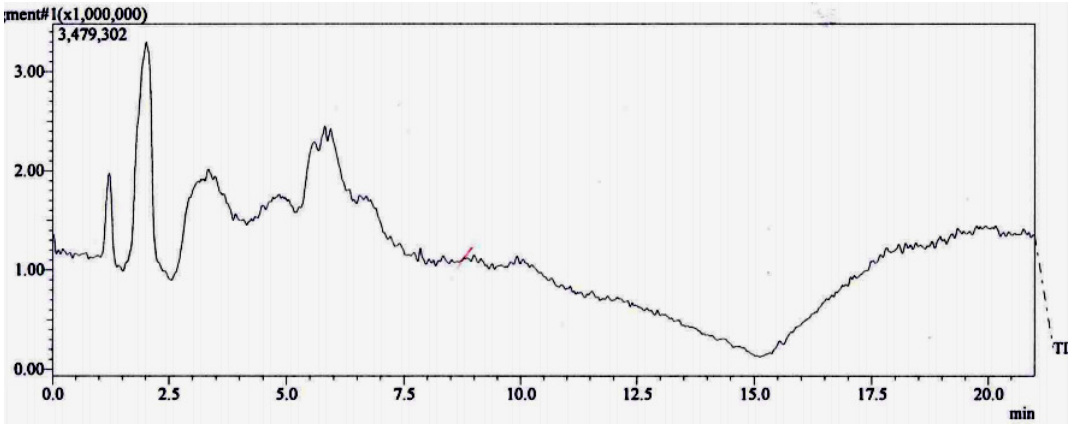


b.



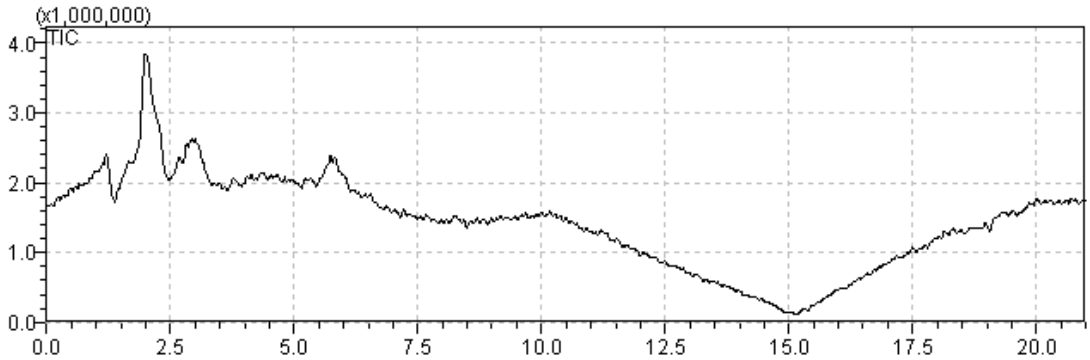
a. Cromatograma obtenido por CLAE-EM. b. Espectro de masas obtenido por CLAE-EM. Tiempo de retención 7,9 minutos, *m/z*: 200,05 $[M+H]^+$.

Vibrio harveyi



Cromatograma obtenido por CLAE-EM.

Alteromona **sp**



Cromatograma obtenido por CLAE-EM.

ANEXO 7: BÚSQUEDA POR SciFinder DE LOS GÉNEROS BACTERIANOS DE ESTUDIO

Género *Shewanella*

Research Topic task started on Wed Sep 23, 2009 at 2:54 PM
3 Research Topic candidates were identified in CAPLUS and MEDLINE.
using the phrase "Shewanella quorum sensing"

Selected 3 of 3 candidate topics.
1 reference was found containing **"Shewanella quorum sensing"** as entered.
6 references were found containing the concept **"Shewanella quorum sensing"**.
14 references were found containing all of the concepts **"Shewanella"**, **"quorum"** and **"sensing"**.

Remove Duplicates
11 references were found (3 duplicates removed)

Bibliographic Information

Turnover of quorum sensing signal molecules modulates cross-kingdom signalling. Tait, Karen; Williamson, Holly; Atkinson, Steve; Williams, Paul; Camara, Miguel; Joint, Ian. Plymouth Marine Laboratory, Plymouth, UK. Environmental Microbiology (2009), 11(7), 1792-1802. Publisher: Wiley-Blackwell, CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. AN 2009:938296 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
N-acylhomoserine lactone (AHL) quorum-sensing mols. modulate the swimming behavior of zoospores of the macroalga Ulva to facilitate the location of bacterial biofilms. Here we show that the intertidal surfaces colonized by Ulva are dominated by Alphaproteobacteria, particularly the Rhodobacteraceae family, and the Bacteroidetes family Flavobacteriaceae, and that this diverse assemblage both produces and degrades AHLs. N-acylhomoserine lactones could also be extd. from the surfaces of pebbles recovered from intertidal rock-pools. Bacteria representative of this assemblage were isolated and tested for the prodn. and degrdn. of AHLs, and for their ability to modulate zoospore settlement at different biofilm densities. Of particular interest was a Shewanella sp. This strain produced three major AHLs (OC4, OC10 and OC12) in the late exponential phase, but the longer-chain AHLs were rapidly degraded in the stationary phase. Degrdn. occurred via both lactonase and amidase activity. A close relationship was found between AHL synthesis and Ulva zoospore settlement. The Shewanella isolate also interfered with AHL prodn. by a Sulfitobacter isolate and its ability to enhance zoospore settlement in a polymicrobial biofilm. This influence on the attachment of Ulva zoospores suggests that AHL-degrading strains can affect bacterial community behavior by interfering with quorum sensing between neighboring bacteria. More importantly, these interactions may exert wider ecol. effects across different kingdoms.

Bibliographic Information

Involvement of Shewanella oneidensis MR-1 LuxS in biofilm development and sulfur metabolism. Learman, Deric R.; Yi, Haakrho; Brown, Steven D.; Martin, Stanton L.; Geesey, Gill G.; Stevens, Ann M.; Hochella, Michael F., Jr. Department of Geosciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. Applied and Environmental Microbiology (2009), 75(5), 1301-1307. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 150:369841 AN 2009:308910 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The role of LuxS in Shewanella oneidensis MR-1 has been examd. by transcriptomic profiling, biochem., and physiol. expts. The results indicate that a mutation in luxS alters biofilm development, not by altering quorum-sensing abilities but by disrupting the activated Me cycle (AMC). The S. oneidensis wild type can produce a luminescence response in the AI-2 reporter strain Vibrio harveyi MM32. This luminescence response is abolished upon the deletion of luxS. The deletion of luxS also alters biofilm formations in static and flowthrough conditions. Genetic complementation restores the mutant biofilm defect, but the addn. of synthetic AI-2 has no effect. These results suggest that AI-2 is not used as a quorum-sensing signal to regulate biofilm development in S. oneidensis. Growth on various sulfur sources was examd. because of the involvement of LuxS in the AMC. A mutation in luxS produced a reduced ability to grow with

methionine as the sole sulfur source. Methionine is a key metabolite used in the AMC to produce a Me source in the cell and to recycle homocysteine. These data suggest that LuxS is important to metabolizing methionine and the AMC in S. oneidensis.

Bibliographic Information

Quorum Sensing by the Bacterial Signal Auto-inducer-2: Phylogenetic Distribution of the Synthesis Gene LuxS, and its Role in Shewanella oneidensis. Bodor, Agnes Maria. Germany. Avail. Metadata on Internet Documents, Order No. 386484. (2008), No pp. From: Metadata Internet Doc. [Ger. Diss.] 2008, (D1105-4), No pp. given. http://www.meind.de/search.py?recid=386484 Dissertation written in English. CAN 151:237379 AN 2008:1326210 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

Identification and characterization of N-acylhomoserine lactone-acylase from the fish intestinal Shewanella sp. strain MIB015. Morohoshi, Tomohiro; Nakazawa, Shigehisa; Ebata, Atsushi; Kato, Norihiro; Ikeda, Tsukasa. Department of Applied Chemistry, Faculty of Engineering, Utsunomiya University, 7-1-2 Yoto, Utsunomiya, Japan. Bioscience, Biotechnology, and Biochemistry (2008), 72(7), 1887-1893. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry, CODEN: BBBIEJ ISSN: 0916-8451. Journal written in English. CAN 149:241078 AN 2008:957078 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

N-Acylhomoserine lactones (AHLs) are used as quorum-sensing signal mols. by many gram-neg. bacteria. We have reported that Shewanella sp. strain MIB015 degrades AHLs. In the present study, we cloned the aac gene from MIB015 by PCR with specific primers based on the aac gene in Shewanella oneidensis strain MR-1, which showed high homol. with the known AHL-acylases. Escherichia coli expressing Aac showed high degrading activity of AHLs with long acyl chains. HPLC anal. revealed that Aac worked as AHL-acylase, which hydrolyzed the amide bond of AHL. In addn., expression of Aac in fish pathogen Vibrio anguillarum markedly reduced AHL prodn. and biofilm formation. In conclusion, this study indicates that Aac might be effective in quenching quorum sensing of fish pathogens.

Bibliographic Information

Potential for luxS related signalling in marine bacteria and production of autoinducer-2 in the genus Shewanella. Bodor, Agnes; Elxnat, Bettina; Thiel, Verena; Schulz, Stefan; Wagner-Doebler, Irene. Division of Cell Biology, Helmholtz-Center for Infection Research, Group Microbial Communication, Braunschweig, Germany. BMC Microbiology (2008), 8 No pp. given. Publisher: BioMed Central Ltd., CODEN: BMMIBC ISSN: 1471-2180. http://www.biomedcentral.com/content/pdf/1471-2180-8-13.pdf Journal; Online Computer File written in English. CAN 150:186453 AN 2008:611949 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The autoinducer-2 (AI-2) group of signalling mols. are produced by both Gram pos. and Gram neg. bacteria as the byproduct of a metabolic transformation carried out by the LuxS enzyme. They are

the only non species-specific quorum sensing compds. presently known in bacteria. The luxS gene coding for the AI-2 synthase enzyme was found in many important pathogens. Here, we surveyed its occurrence in a collection of 165 marine isolates belonging to abundant marine phyla using conserved degenerated PCR primers and sequencing of selected pos. bands to det. if the presence of the luxS gene is phylogenetically conserved or dependent on the habitat. The luxS gene was not present in any of the Alphaproteobacteria (n = 71) and Bacteroidetes strains (n = 29) tested; by contrast, these bacteria harboured the sahH gene, coding for an alternative enzyme for the detoxification of S-adenosylhomocysteine (SAH) in the activated Me cycle. Within the Gammaproteobacteria (n = 76), luxS was found in all Shewanella, Vibrio and Alteromonas isolates and some Pseudoalteromonas and Halomonas species, while sahH was detected in Psychrobacter strains. A no. of Gammaproteobacteria (n = 27) appeared to have neither the luxS nor the sahH gene. We then studied the prodn. of AI-2 in the genus Shewanella using the Vibrio harveyi bioassay. All ten species of Shewanella tested produced a pronounced peak of AI-2 towards the end of the exponential growth phase in several media investigated. The max. of AI-2 activity was different in each Shewanella species, ranging from 4% to 46% of the pos. control. The data are consistent with those of fully sequenced bacterial genomes and show that the potential for luxS related signalling is dependent on phylogenetic affiliation rather than ecol. niche and is largest in certain groups of Gammaproteobacteria in the marine environment. This is the first report on AI-2 prodn. in Shewanella species; its signalling role in these organisms remains to be elucidated.

Bibliographic Information

Production of antibacterial compounds and biofilm formation by Roseobacter species are influenced by culture conditions. Bruhn, Jesper Bartholin; Gram, Lone; Belas, Robert. Department of Seafood Research, Soeltofts Plads, Danish Institute for Fisheries Research, Kgs. Lyngby, Den. Applied and Environmental Microbiology (2007), 73(2), 442-450. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 146:270104 AN 2007:114898 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Bacterial communities assocd. with marine algae are often dominated by members of the Roseobacter clade, and in the present study, we describe Roseobacter phenotypes that may provide this group of bacteria with selective advantages when colonizing this niche. Nine of 14 members of the Roseobacter clade, of which half were isolated from cultures of the dinoflagellate Pfiesteria piscicida, produced antibacterial compds. Many non-Roseobacter marine bacteria were inhibited by sterile filtered supernatants of Silicibacter sp. TM1040 and Phaeobacter (formerly Roseobacter) strain 27-4, which had the highest prodn. of antibacterial compd. In contrast, Roseobacter strains were susceptible only when exposed to concd. compd. The prodn. of antibacterial compd. was influenced by the growth conditions, as prodn. was most pronounced when bacteria were grown in liq. medium under static conditions. Under these conditions, Silicibacter sp. TM1040 cells attached to one another, forming rosettes, as has previously been reported for Phaeobacter 27-4. A spontaneous Phaeobacter 27-4 mutant unable to form rosettes was also defective in biofilm formation and the prodn. of antibacterial compd., indicating a possible link between these phenotypes. Rosette formation was obsd. in 8 of 14 Roseobacter clade strains examd. and was very pronounced under static growth in 5 of these strains. Attachment to surfaces and biofilm formation at the air-liq. interface by these five strains was greatly facilitated by growth conditions that favored rosette formation, and rosette-forming strains were 13 to 30 times more efficient in attaching to glass compared to strains under conditions where rosette formation was not pronounced. We

hypothesize that the ability to produce antibacterial compds. that principally inhibit non-Roseobacter species, combined with an enhancement in biofilm formation, may give members of the Roseobacter clade a selective advantage and help to explain the dominance of members of this clade in assocn. with marine algal microbiota.

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Quorum sensing signaling in enteric flora of fish. Morohoshi, Tomohiro; Kato, Norihiro; Ikeda, Osamu. Dep. of Engineering, Utsunomiya Univ., Japan. Seibutsu Kogaku Kaishi (2006), 84(11), 436-439. Publisher: Nippon Seibutsu Kogakkai, CODEN: SEKAEA ISSN: 0919-3758. Journal; General Review written in Japanese. CAN 146:478356 AN 2007:55244 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review discussed the mechanism underlying quorum sensing signaling system of the intestinal flora in fish. The mechanism using AHL (acylated homoserine lactone) as the signaling compds. and the regulation of crit. factors involved in the AHL biosynthesis (AHL synthase, lactonase and acylase and the genes encoding these enzymes) were discussed. Screening of the AHL producing microorganisms such as Aeromonas by using reporter organism Chromobacterium violaceum strain CV026 that can produce purple pigment violacein from AHL was described. Screening of the AHL degradating microorganisms such as Shewanella was also discussed. Clonings of the genes for the enzymes involved in AHL metab. from microorganisms of Ayu fish enteric flora were also discussed.

Bibliographic Information

A model for anoxic iron corrosion by Sh. oneidensis MR-1 based on cell density related H2 consumption and Fe(II) precipitation. De Windt, W.; Dick, J.; Boon, N.; Verstraete, W.; Siciliano, S.; Gao, H.; Zhou, J. Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belg. Editor(s): Verstraete, W. European Symposium on Environmental Biotechnology, ESEB 2004, Proceedings, Oostende, Belgium, Apr. 25-28, 2004 (2004), 169-173. Publisher: A. A. Balkema, Lisse, Neth CODEN: 69FKFT Conference written in English. CAN 141:202933 AN 2004:401594 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
In the absence of oxygen, a protective H2 film is formed around an Fe(0) surface, inhibiting the electron flow from this surface. A study of anoxic corrosion of Fe(0) beads revealed that, in the presence of Shewanella oneidensis MR-1, H2 removal and pptn. of Fe mineral particles on the cell surface are detg. processes for corrosion. These two biol. mediated processes were governed by cell d. Addn. of supernatant of a corrosion assay with high cell concn. induced metabolic activity in a corrosion assay with low cell concn., resulting in increased H2 consumption and Fe release from Fe(0) beads. Homoserine lactone-like mols. were detected in the supernatant by a bio-assay, suggesting the involvement of a quorum-sensing regulatory mechanism. The interaction of Sh. oneidensis MR-1 with mineral particles was further investigated by means of the mutant Sh. oneidensis COAG. The COAG mutant exhibited increased biomineralization (up to 50% increase) and the putative interactions between COAG cells and mineral ppts. were validated by means of transcriptional anal. and Proteomics.

Bibliographic Information

Cell density related H2 consumption in relation to anoxic Fe(0) corrosion and precipitation of corrosion products by Shewanella oneidensis MR-1. De Windt, Wim; Boon, Nico; Siciliano, Steven D.; Verstraete, Willy. Laboratory of Microbial Ecology and Technology, Ghent University, Ghent, Belg. Environmental Microbiology (2003), 5(11), 1192-1202. Publisher: Blackwell Publishing Ltd., CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 140:426652 AN 2003:975256 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
In the absence of oxygen, a protective H2 film is formed around an Fe(0) surface, inhibiting the electron flow from this surface. Our study of anoxic corrosion of Fe(0) beads revealed that, in the presence of Shewanella oneidensis MR-1, H2 removal and pptn. of Fe mineral particles on the cell surface are detg. processes for corrosion. These two biol. mediated processes were governed by cell d. H2 removal by Shewanella oneidensis was detected at cell concns. of 1.0 □ 106 live cells ml-1 and higher and H2 was electron donor for denitrification of NO3-. The removal of the protective H2 layer from Fe(0) beads by Shewanella oneidensis, resulted in an increase of Fe release out of the Fe(0) beads from 153 □ 25 mg l-1 to 196 □ 7 mg l-1 after 20 h. When the cell concn. exceeded 1.0 □ 106 live cells ml-1, pptn. of iron minerals on the cell surface was characteristic for the greatest percentage of MR-1 cells, whereas micrometer-scale iron ppts. not assocd. with culturable cell biomass significantly decreased in no. Addn. of supernatant of a corrosion assay with high cell concn. induced metabolic activity in a corrosion assay with low cell concn., resulting in increased H2 consumption and Fe release from Fe(0) beads. Homoserine lactone-like mols. were detected in the supernatant by a bio-assay, suggesting the involvement of a quorum-sensing regulatory mechanism.

Bibliographic Information

Bacterial response to siderophore and quorum-sensing chemical signals in the seawater microbial community. Guan, Le Luo; Kamino, Kei. Marine Biotechnology Institute, Shimizu Laboratories, Shimizu City, Shizuoka, Japan. BMC Microbiology [online computer file] (2001), 1 No pp. given. Publisher: BioMed Central Ltd., CODEN: BMMIBC ISSN: 1471-2180. http://www.biomedcentral.com/1471-2180/1/27 Journal; Online Computer File written in English. CAN 136:196691 AN 2001:919872 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Background: Oceans are iron-deficient and nutrient-poor environments. These conditions impart limitations on our understanding of and our ability to identify microorganisms from the marine environment. However, less of knowledge on the influence of siderophores and N-acyl homoserine lactone as interspecies communication signals on the bacterial diversity of seawater has been understood. Results: In the presence of 0.1 nM of the com. siderophore desferrioxamine and the known quorum-sensing chem. signals, synthetic N-(3-oxo)-hexanoylhomoserine lactone (0.1 nM) or N-octanoyl homoserine lactone (0.1 nM), the total nos. of bacteria in S9905 seawater increased nearly three-fold, and nearly eight-fold in S0011 seawater as detd. by DAPI staining and counting, and increased three-fold by counting colony forming units in S9905 seawater after 7 days of incubation. Similar bacterial changes in bacterial abundance were obsd. when high concn. of desferroixamine (1 □M) and each of homoserine lactone compds. (1 □M) were presented in seawater samples. The no. of cultivable bacterial species obsd. was also found to increase from 3 (without addn.) to 8 (with addns.) including three unknown species which were identified by

phylogenetic anal. of 16S rDNA sequences. The growth of unknown species was related to their siderophore prodn. with response to the addn. of desferroixamine and N-acyl homoserine lactones under iron-limited conditions. Conclusion: Artificial addn. of siderophores and HSLs may be a possible method to aid in the identification and isolation of marine bacterial species which are thought to be unknown.

Bibliographic Information

Potential for luxS related signalling in marine bacteria and production of autoinducer-2 in the genus Shewanella. Bodor Agnes; Elxnat Bettina; Thiel Verena; Schulz Stefan; Wagner-Dobler Irene Helmholtz-Center for Infection Research, Group Microbial Communication, Division of Cell Biology, Inhoffenstr, 7, 38124 Braunschweig, Germany. agb@gbf.de BMC microbiology (2008), 8 13. Journal code: 100966981. E-ISSN:1471-2180. Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 18215278 AN 2008097236 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
BACKGROUND: The autoinducer-2 (AI-2) group of signalling molecules are produced by both Gram positive and Gram negative bacteria as the by-product of a metabolic transformation carried out by the LuxS enzyme. They are the only non species-specific quorum sensing compounds presently known in bacteria. The luxS gene coding for the AI-2 synthase enzyme was found in many important pathogens. Here, we surveyed its occurrence in a collection of 165 marine isolates belonging to abundant marine phyla using conserved degenerated PCR primers and sequencing of selected positive bands to determine if the presence of the luxS gene is phylogenetically conserved or dependent on the habitat. RESULTS: The luxS gene was not present in any of the Alphaproteobacteria (n = 71) and Bacteroidetes strains (n = 29) tested; by contrast, these bacteria harboured the sahH gene, coding for an alternative enzyme for the detoxification of S-adenosylhomocysteine (SAH) in the activated methyl cycle. Within the Gammaproteobacteria (n = 76), luxS was found in all Shewanella, Vibrio and Alteromonas isolates and some Pseudoalteromonas and Halomonas species, while sahH was detected in Psychrobacter strains. A number of Gammaproteobacteria (n = 27) appeared to have neither the luxS nor the sahH gene. We then studied the production of AI-2 in the genus Shewanella using the Vibrio harveyi bioassay. All ten species of Shewanella tested produced a pronounced peak of AI-2 towards the end of the exponential growth phase in several media investigated. The maximum of AI-2 activity was different in each Shewanella species, ranging from 4% to 46% of the positive control. CONCLUSION: The data are consistent with those of fully sequenced bacterial genomes and show that the potential for luxS related signalling is dependent on phylogenetic affiliation rather than ecological niche and is largest in certain groups of Gammaproteobacteria in the marine environment. This is the first report on AI-2 production in Shewanella species; its signalling role in these organisms remains to be elucidated.

Género *Ochrobactrum*

Research Topic task started on Wed Sep 23, 2009 at 2:53 PM
2 Research Topic candidates were identified in CAPLUS and MEDLINE.
using the phrase "Ochrobactrum quorum sensing"

Selected 2 of 2 candidate topics.
1 reference was found containing the concept **"Ochrobactrum quorum sensing"**.
3 references were found containing all of the concepts **"Ochrobactrum"**, **"quorum"** and **"sensing"**.

Remove Duplicates
2 references were found (1 duplicate removed)

Bibliographic Information

Tetrahydrofuran derivatives promoting the growth of N-acylhomoserine lactone-degrading bacteria, useful as soil additives for control of bacterial plant pathogens. Faure, Denis; Cirou, Amelie; Dessaux, Yves. (Centre National de la Recherche Scientifique, Fr.; Comite Economique Agricole Production des Plants du Nord (Comite Nord Pomme de Terre)). PCT Int. Appl. (2008), 33pp. CODEN: PIXXD2 WO 2008090479 A2 20080731 Designated States W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO,

RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, MT, NL, NO, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2008-IB1156 20080118. Priority: US 2007-885727 20070119. CAN 149:169435 AN 2008:916668 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2008090479	A2	20080731	WO2008-IB1156	20080118
WO2008090479	A3	20090430		

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

Priority Application		
US 2007-885727P	P	20070119

Abstract

The invention concerns certain THF derivs. (Markush given), such as □-caprolactone, which control quorum-sensing-dependent plant pathogens. This is carried out by growth promotion of N-acylhomoserine lactone-degrading bacteria, such as Delftia acidovorans and Rhodococcus erythropolis. The THF derivs. are used as soil additives. Other uses include control of colonizing bacteria in plumbing materials, pipes, silos fermenters and colander.

Bibliographic Information

Detection and characterization of bacteria from the potato rhizosphere degrading N-acyl-homoserine lactone. Jafra, S.; Przysowa, J.; Czajkowski, R.; Michta, A.; Garbeva, P.; Van der Wolf, J. M. Plant Research International, Wageningen, Neth. Canadian Journal of Microbiology (2006), 52(10), 1006-1015. Publisher: National Research Council of Canada, CODEN: CJMIAZ ISSN: 0008-4166. Journal written in English. CAN 146:247213 AN 2006:1330383 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing plays a role in the regulation of soft rot diseases caused by the plant pathogenic bacterium Pectobacterium carotovorum subsp. carotovorum. The signal mols. involved in quorum sensing in P. carotovorum subsp. carotovorum belong to the group of N-acyl homoserine lactones (AHLs). In our study, we screened bacteria isolated from the potato rhizosphere for the ability to degrade AHLs produced by P. carotovorum subsp. carotovorum. Six isolates able to degrade AHLs were selected for further studies. According to 16S rDNA sequence anal. and fatty acid Me ester profiling, the isolates belonged to the genera Ochrobactrum, Rhodococcus, Pseudomonas, Bacillus,

and Delftia. For the genera Ochrobactrum and Delftia, for the first time AHL-degrading isolates were found. Data presented in this study revealed for the first time that Ochrobactrum sp. strain A44 showed the capacity to inactivate various synthetic AHL mols.; the substituted AHLs were inactivated with a lower efficiency than the unsubstituted AHLs. Compared with the other isolates, A44 was very effective in the degrdn. of AHLs produced by P. carotovorum subsp. carotovorum. It was verified by polymerase chain reaction, DNA-DNA hybridization, and a lactone ring reconstruction assay that Ochrobactrum sp. strain A44 did not possess AHL lactonase activity. AHL degrdn. in Ochrobactrum sp. strain A44 occurred intracellularly; it was not found in the culture supernatant. AHL-degrading activity of A44 was thermosensitive. Expts. in planta revealed that Ochrobactrum sp. strain A44 significantly inhibited the maceration of potato tuber tissue. Since A44 did not produce antibiotics, the attenuation of the decay might be due to the quenching of quorum- sensing-regulated prodn. of pectinolytic enzymes. The strain can potentially serve to control P. carotovorum subsp. carotovorum in potato.

Género *Alteromona*

Research Topic task started on Wed Sep 23, 2009 at 2:55 PM
2 Research Topic candidates were identified in CAPLUS and MEDLINE.
using the phrase "Alteromonas quorum sensing"

Selected 2 of 2 candidate topics.
2 references were found containing the concept **"Alteromonas quorum sensing"**.
6 references were found containing all of the concepts **"Alteromonas"**, **"quorum"** and **"sensing"**.

Remove Duplicates
5 references were found (1 duplicate removed)

Bibliographic Information

Potential for luxS related signalling in marine bacteria and production of autoinducer-2 in the genus Shewanella. Bodor, Agnes; Elxnat, Bettina; Thiel, Verena; Schulz, Stefan; Wagner-Doebler, Irene. Division of Cell Biology, Helmholtz-Center for Infection Research, Group Microbial Communication, Braunschweig, Germany. BMC Microbiology (2008), 8 No pp. given. Publisher: BioMed Central Ltd., CODEN: BMMIBC ISSN: 1471-2180. http://www.biomedcentral.com/content/pdf/1471-2180-8-13.pdf Journal; Online Computer File written in English. CAN 150:186453 AN 2008:611949 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The autoinducer-2 (AI-2) group of signalling mols. are produced by both Gram pos. and Gram neg. bacteria as the byproduct of a metabolic transformation carried out by the LuxS enzyme. They are the only non species-specific quorum sensing compds. presently known in bacteria. The luxS gene coding for the AI-2 synthase enzyme was found in many important pathogens. Here, we surveyed its occurrence in a collection of 165 marine isolates belonging to abundant marine phyla using conserved degenerated PCR primers and sequencing of selected pos. bands to det. if the presence of the luxS gene is phylogenetically conserved or dependent on the habitat. The luxS gene was not present in any of the Alphaproteobacteria (n = 71) and Bacteroidetes strains (n = 29) tested; by contrast, these bacteria harboured the sahH gene, coding for an alternative enzyme for the detoxification of S-adenosylhomocysteine (SAH) in the activated Me cycle. Within the Gammaproteobacteria (n = 76), luxS was found in all Shewanella, Vibrio and Alteromonas isolates and some Pseudoalteromonas and Halomonas species, while sahH was detected in Psychrobacter strains. A no. of Gammaproteobacteria (n = 27) appeared to have neither the luxS nor the sahH gene. We then studied the prodn. of AI-2 in the genus Shewanella using the Vibrio harveyi bioassay. All ten species of Shewanella tested produced a pronounced peak of AI-2 towards the end of the exponential growth phase in several media investigated. The max. of AI-2 activity was different in each Shewanella species, ranging from 4% to 46% of the pos. control. The data are consistent with those of fully sequenced bacterial genomes and show that the potential for luxS related signalling is dependent on phylogenetic affiliation rather than ecol. niche and is largest in certain groups of Gammaproteobacteria in the marine environment. This is the first report on AI-2 prodn. in Shewanella species; its signalling role in these organisms remains to be elucidated.

Bibliographic Information

A dual biosensor for 2-alkyl-4-quinolone quorum-sensing signal molecules. Fletcher, Matthew P.; Diggle, Stephen P.; Crusz, Shanika A.; Chhabra, Siri Ram; Camara, Miguel; Williams, Paul. Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK. Environmental Microbiology (2007), 9(11), 2683-2693. Publisher: Blackwell Publishing Ltd., CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 148:186078 AN 2007:1372566 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Pseudomonas, Burkholderia and Alteromonas species produce diverse 2-alkyl-4-quinolones (AHQs) which inhibit the growth of bacteria, algae and phytoplankton, chelate iron, modulate mammalian host immune defences and act as quorum-sensing (QS) signal mols. To facilitate the detection, identification and quantification of the major Pseudomonas aeruginosa AHQs 2-heptyl-3-hydroxy-4-quinolone (PQS) and 2-heptyl-4-quinolone (HHQ) we developed two different AHQ biosensors. These were constructed by introducing either a lecA::luxCDABE or a pqsA::luxCDABE reporter gene fusion into a P. aeruginosa pqsA mutant which cannot synthesize AHQs. While both biosensors responded similarly to PQS (EC50 18 □ 4 □M), the pqsA::luxCDABE biosensor was most sensitively activated by HHQ (EC50 0.44 □ 0.1 □M). This biosensor was also activated albeit less sensitively by (i) PQS analogs with alkyl chains varying from C1 to C11, (ii) HHQ analogs with C9 and C11 alkyl chains and (iii) 2-heptyl-4-hydroxyquinoline-N-oxide (HHQNO). The AHQ biosensor also responded differentially to the AHQs present in cell free culture supernatants prepd. from PAO1 and isogenic strains carrying mutations in genes (pqsA, pqsH, lasR, lasI, rhlR, rhlI) known to influence AHQ prodn. The AHQ profiles of P. aeruginosa strains was also evaluated by overlaying thin layer chromatogram (TLC) plates with the pqsA::luxCDABE biosensor. In PAO1, three major bioluminescent spots were obsd.

which correspond to PQS, HHQ and a mixt. of 2 nonyl-4-quinolone and HHQNO. We also noted that on TLC plates the biosensor not only produced bioluminescence in response to AHQs but also the green pigment, pyocyanin which offers an alternative visual indicator for AHQ prodn.

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Possible quorum sensing in marine snow bacteria: Production of acylated homoserine lactones by Roseobacter strains isolated from marine snow. Gram, Lone; Grossart, Hans-Peter; Schlingloff, Andrea; Kiorboe, Thomas. Department of Seafood Research, Danish Institute for Fisheries Research, Kgs. Lyngby, Den. Applied and Environmental Microbiology (2002), 68(8), 4111-4116. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 137:307283 AN 2002:603024 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

We report here, for the first time, that bacteria assocd. with marine snow produce communication signals involved in quorum sensing in gram-neg. bacteria. Four of 43 marine microorganisms isolated from marine snow were found to produce acylated homoserine lactones (AHLs) in well diffusion and thin-layer chromatog. assays based on the Agrobacterium tumefaciens reporter system. Three of the AHL-producing strains were identified by 16S ribosomal DNA gene sequence anal. as Roseobacter spp., and this is the first report of AHL prodn. by these □-Proteobacteria. It is likely that AHLs in Roseobacter species and other marine snow bacteria govern phenotypic traits (biofilm formation, exoenzyme prodn., and antibiotic prodn.) which are required mainly when the population reaches high densities, e.g., in the marine snow community.

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Bioactive food complex, method for making bioactive food complex product and method for controlling disease. Villamar, Daniel F.; Moriarty, David J. W. (Acuabiotec Llc, USA). PCT Int. Appl. (2002), 38 pp. CODEN: PIXXD2 WO 2002000035 A1 20020103 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2001-US16489 20010622. Priority: US 2000-213538 20000623. CAN 136:69139 AN 2002:10213 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2002000035	A1	20020103	WO2001-US16489	20010622

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CN 100456949	C	20090204	CN 2001-812813	20010622
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US 20040009160	A1	20040115	US 2003-312039	20030715

Priority Application

US 2000-213538P	P	20000623
WO 2001-US16489	W	20010622

Abstract

A bioactive food complex product, method for prepg. a bioactive food complex product and method for controlling disease uses probiotics and quorum sensing inhibitors such as inhibitory furanones and other bioactive compds. included in both the continuous and dispersed phases of a bioactive food complex product. The product is comprised of a solids-in-oil or an oil-in-solids emulsion forming a first emulsion that is itself emulsified in polymer forming oil-in-polymer or solids-in-polymer emulsion complex. The bioactive complex is formed of two emulsions with the first emulsion comprising the dispersed phase and a hydrocolloid polymer serving as the continuous phase. The second emulsion complex is then crosslinked to form a phys. stable matrix. The bioactive food complex or the first emulsion of the bioactive food complex then serve to deliver different bioactive components including probiotic bacteria and quorum sensing inhibitor mols. to the digestive tract and environment of animals such as shrimp or fish or other livestock raised com. to effectively control bacterial disease by a novel combination of mechanisms including: competitive exclusion, direct inhibition, digestion of cell-to-cell signaling mols. and direct inhibition of homoserine lactone and (acyl) homoserine lactone-regulated processes of pathogenic bacteria. Thus, effective disease prevention and control is accomplished through the novel combined delivery and use of probiotic bacteria and quorum sensing inhibitory furanones.

Bibliographic Information

Potential for luxS related signalling in marine bacteria and production of autoinducer-2 in the genus Shewanella. Bodor Agnes; Elxnat Bettina; Thiel Verena; Schulz Stefan; Wagner-Dobler Irene Helmholtz-Center for Infection Research, Group Microbial Communication, Division of Cell Biology, Inhoffenstr, 7, 38124 Braunschweig, Germany. agb@gbf.de BMC microbiology (2008), 8 13. Journal code: 100966981. E-ISSN:1471-2180. Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 18215278 AN 2008097236 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

BACKGROUND: The autoinducer-2 (AI-2) group of signalling molecules are produced by both Gram positive and Gram negative bacteria as the by-product of a metabolic transformation carried out by the LuxS enzyme. They are the only non species-specific quorum sensing compounds presently known in bacteria. The luxS gene coding for the AI-2 synthase enzyme was found in many important pathogens. Here, we surveyed its occurrence in a collection of 165 marine isolates belonging to abundant marine phyla using conserved degenerated PCR primers and sequencing of selected positive bands to determine if the presence of the luxS gene is phylogenetically conserved or dependent on the habitat. RESULTS: The luxS gene was not present in any of the Alphaproteobacteria (n = 71) and Bacteroidetes strains (n = 29) tested; by contrast, these bacteria

harboured the sahH gene, coding for an alternative enzyme for the detoxification of S-adenosylhomocysteine (SAH) in the activated methyl cycle. Within the Gammaproteobacteria (n = 76), luxS was found in all Shewanella, Vibrio and Alteromonas isolates and some Pseudoalteromonas and Halomonas species, while sahH was detected in Psychrobacter strains. A number of Gammaproteobacteria (n = 27) appeared to have neither the luxS nor the sahH gene. We then studied the production of AI-2 in the genus Shewanella using the Vibrio harveyi bioassay. All ten species of Shewanella tested produced a pronounced peak of AI-2 towards the end of the exponential growth phase in several media investigated. The maximum of AI-2 activity was different in each Shewanella species, ranging from 4% to 46% of the positive control. CONCLUSION: The data are consistent with those of fully sequenced bacterial genomes and show that the potential for luxS related signalling is dependent on phylogenetic affiliation rather than ecological niche and is largest in certain groups of Gammaproteobacteria in the marine environment. This is the first report on AI-2 production in Shewanella species; its signalling role in these organisms remains to be elucidated.

Género *Vibrio*

Research Topic task started on Wed Sep 23, 2009 at 2:57 PM
2 Research Topic candidates were identified in CAPLUS and MEDLINE.
using the phrase "Vibrio quorum sensing"
Selected 2 of 2 candidate topics.

44 references were found containing **"Vibrio quorum sensing"** as entered.
457 references were found containing the concept **"Vibrio quorum sensing"**.
Remove Duplicates
306 references were found (151 duplicates removed)

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Bacterial Attraction and Quorum Sensing Inhibition in Caenorhabditis elegans Exudates. Kaplan, Fatma; Badri, Dayakar V.; Zachariah, Cherian; Ajredini, Ramadan; Sandoval, Francisco J.; Roje, Sanja; Levine, Lanfang H.; Zhang, Fengli; Robinette, Steven L.; Alborn, Hans T.; Zhao, Wei; Stadler, Michael; Nimalendran, Rathika; Dossey, Aaron T.; Brueschweiler, Rafael; Vivanco, Jorge M.; Edison, Arthur S. Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL, USA. Journal of Chemical Ecology (2009), 35(8), 878-892. Publisher: Springer, CODEN: JCECD8 ISSN: 0098-0331. Journal written in English. AN 2009:1141436 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Caenorhabditis elegans, a bacterivorous nematode, lives in complex rotting fruit, soil, and compost environments, and chem. interactions are required for mating, monitoring population d., recognition of food, avoidance of pathogenic microbes, and other essential ecol. functions. Despite being one of the best-studied model organisms in biol., relatively little is known about the signals that C. elegans uses to interact chem. with its environment or as defense. C. elegans exudates were analyzed by using several anal. methods and found to contain 36 common metabolites that include org. acids, amino acids, and sugars, all in relatively high abundance. Furthermore, the concns. of amino acids in the exudates were dependent on developmental stage. The C. elegans exudates were tested for bacterial chemotaxis using Pseudomonas putida (KT2440), a plant growth promoting rhizobacterium, Pseudomonas aeruginosa (PAO1), a soil bacterium pathogenic to C. elegans, and Escherichia coli (OP50), a non-motile bacterium tested as a control. The C. elegans exudates attracted the two Pseudomonas species, but had no detectable antibacterial activity against P. aeruginosa. To our surprise, the exudates of young adult and adult life stages of C. elegans exudates inhibited quorum sensing in the reporter system based on the LuxR bacterial quorum sensing (QS) system, which regulates bacterial virulence and other factors in Vibrio fischeri. We were able to fractionate the QS inhibition and bacterial chemotaxis activities, thus demonstrating that these activities are chem. distinct. Our results demonstrate that C. elegans can attract its bacterial food and has the potential of partially regulating the virulence of bacterial pathogens by inhibiting specific QS systems.

Bibliographic Information

Insights into the Biosynthesis of the Vibrio cholerae Major Autoinducer CAI-1 from the Crystal Structure of the PLP-Dependent Enzyme CqsA. Jahan, Nasrin; Potter, Jane A.; Sheikh, Md. Arif; Botting, Catherine H.; Shirran, Sally L.; Westwood, Nicholas J.; Taylor, Garry L. University of St. Andrews, Centre for Biomolecular Sciences, St. Andrews, Fife, Journal of Molecular Biology (2009), 392(3), 763-773. Publisher: Elsevier Ltd., CODEN: JMOBAK ISSN: 0022-2836. Journal written in English. AN 2009:1097947 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
CqsA is an enzyme involved in the biosynthesis of cholerae autoinducer-1 (CAI-1), the major Vibrio cholerae autoinducer engaged in quorum sensing. The amino acid sequence of CqsA suggests that it belongs to the family of □-oxoamine synthases that catalyze the condensation of an amino acid to an acyl-CoA substrate. Here we present the apo- and PLP-bound crystal structures of CqsA and confirm that it shares structural homol. with the dimeric □-oxoamine synthases, including a conserved PLP-binding site. The chem. structure of CAI-1 suggests that decanoyl-CoA may be one substrate of CqsA and that another substrate may be -threonine or -2-aminobutyric acid. A crystal structure of CqsA at 1.9-.ANG. resoln. obtained in the presence of PLP and -threonine reveals an external aldimine that has lost the -threonine side chain. Similarly, a 1.9-.ANG.-resoln. crystal structure of CqsA in the presence of PLP, -threonine, and decanoyl-CoA shows a trapped external aldimine intermediate, suggesting that the condensation and decarboxylation steps have occurred, again with loss of the -threonine side chain. It is suggested that this side-chain loss, an observation supported by mass spectrometry, is due to a retro-aldol reaction. Although no structural data have been obtained on CqsA using -2-aminobutyric acid and decanoyl-CoA as substrates, mass spectrometry confirms the expected product of the enzyme reaction. It is proposed that a region of structure that is disordered in the apo structure is involved in the release of product. While not confirming if CqsA alone is able to synthesize CAI-1, these results suggest possible synthetic routes.

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Defining the roles of multiple small RNAs and feedback regulation in the Vibrio harveyi quorum sensing network. Tu, Chia-En Kimberly. Princeton Univ., Princeton, NJ, USA. Avail. UMI, Order No. DA3341310. (2009), 208 pp. From: Diss. Abstr. Int., B 2009, 70(1), 113. Dissertation written in English. AN 2009:1070789 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

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Synthesis and Evaluation of New Antagonists of Bacterial Quorum Sensing in Vibrio harveyi. Peng, Hanjing; Cheng, Yunfeng; Ni, Nanting; Li, Minyong; Choudhary, Gaurav; Chou, Han Ting; Lu, Chung-Dar; Tai, Phang C.; Wang, Binghe. Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA, USA. ChemMedChem (2009), 4(9), 1457-1468. Publisher: Wiley-VCH Verlag GmbH & Co. KGaA, CODEN: CHEMGX ISSN: 1860-7179. Journal written in English. AN 2009:1063702 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacterial quorum sensing has received much attention in recent years because of its relevance to pathol. events such as biofilm formation. Based on the structures of two lead inhibitors (IC50: 35-55 □M) against autoinducer-2-mediated quorum sensing identified through virtual screening, we synthesized 39 analogs and examd. their inhibitory activities. Twelve of these new analogs showed equal or better inhibitory activities than the lead inhibitors. The best compd. showed an IC50 value of .apprx.6 □M in a whole-cell assay using Vibrio harveyi as the model organism. The structure-activity relationship is discussed herein.

Bibliographic Information

Quorum sensing regulation of the two hcp alleles in Vibrio cholerae O1 strains. Ishikawa, Takahiko; Rompikuntal, Pramod Kumar; Lindmark, Barbro; Milton, Debra L.; Wai, Sun Nyunt. Department of Molecular Biology, Umeaa University, Umeaa, Swed. PLoS One (2009), 4(8), No pp. given. Publisher: Public Library of Science, CODEN: POLNCL ISSN: 1932-6203. http://www.plosone.org/article/fetchObjectAttachment.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0006734&representation=PDF Journal; Online Computer File written in English. AN 2009:1053811 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Background: The type VI secretion system (T6SS) has emerged as a protein secretion system important to several Gram-neg. bacterial species. One of the common components of the system is Hcp, initially described as a hemolysin co-regulated protein in a serotype O17 strain of Vibrio cholerae. Homologs to V. cholerae hcp genes have been found in all characterized type VI secretion systems and they are present also in the serotype O1 strains of V. cholerae that are the cause of cholera diseases but seemed to have non-functional T6SS. Methodol./Principal Findings: The serotype O1 V. cholerae strain A1552 was shown to express detectable levels of Hcp as detd. by immunoblot analyses using polyclonal anti-Hcp antiserum. We found that the expression of Hcp was growth phase dependent. The levels of Hcp in quorum sensing deficient mutants of V. cholerae were compared with the levels in wild type V. cholerae O1 strain A1552. The expression of Hcp

was pos. and neg. regulated by the quorum sensing regulators HapR and LuxO, resp. In addn., we obsd. that expression of Hcp was dependent on the cAMP-CRP global transcriptional regulatory complex and required the RpoN sigma factor. Conclusion/Significance: Our results show that serotype O1 strains of V. cholerae do express Hcp which is regarded as one of the important T6SS components and is one of the secreted substrates in non-O1 non-O139 V. cholerae isolates. We found that expression of Hcp was strictly regulated by the quorum sensing system in the V. cholerae O1 strain. In addn., the expression of Hcp required the alternative sigma factor RpoN and the cAMP-CRP global regulatory complex. Interestingly, the environmental isolates of V. cholerae O1 strains that showed higher levels of the HapR quorum sensing regulator in comparison with our lab. std. serotype O1 strain A1552 where also expressing higher levels of Hcp.

Bibliographic Information

Synthesis and evaluation of new antagonists of bacterial quorum sensing in Vibrio harveyi. Peng, Hanjing; Cheng, Yunfeng; Ni, Nanting; Li, Minyong; Choudhary, Gaurav; Chou, HanTing; Lu, Chung-dar; Tai, Phang C.; Wang, Binghe. Department of Chemistry, Georgia State University, Atlanta, GA, USA. Abstracts of Papers, 238th ACS National Meeting, Washington, DC, United States, August 16-20, 2009 (2009), MEDI-272. Publisher: American Chemical Society, Washington, D. C CODEN: 69LVCL Conference; Meeting Abstract; Computer Optical Disk AN 2009:984456 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacterial quorum sensing has received much attention in recent years because of its relevance to pathol. events such as biofilm formation. Based on the structures of two lead inhibitors (IC50: 35-55 micromolar) against AI-2 mediated quorum sensing identified through virtual screening, we have synthesized 39 analogs and examd. their inhibitory activities. Twelve of the new analogs showed equal or better inhibitory activities compared with the lead inhibitors. The best compd. showed an IC50 of about 6 micromolar in a whole cell assay using Vibrio harveyi as the model organism. The structure-activity relationship is also described.

Bibliographic Information

Heterogeneity in quorum sensing-regulated bioluminescence of Vibrio harveyi. Anetzberger, Claudia; Pirch, Torsten; Jung, Kirsten. Munich Center for integrated Protein Science (CiPSM) at the Department of Biology I, Microbiology, Ludwig-Maximilians-Universitaet Munich, Planegg-Martinsried, Germany. Molecular Microbiology (2009), 73(2), 267-277. Publisher: Wiley-Blackwell, CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 151:283383 AN 2009:949660 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing (QS) refers to the ability of bacterial populations to read out the local environment for cell d. and to collectively activate gene expression. Vibrio harveyi, one of the best characterized model organisms in QS, was used to address the question how single cells behave within a QS-activated community in a homogeneous environment. Anal. of the QS-regulated bioluminescence of a wild type strain revealed that even at high cell densities only 69% of the cells of the population produced bioluminescence, 25% remained dark and 6% were dead. Moreover, light intensities greatly varied from cell to cell at high population d. Addn. of autoinducer to a bright liq. culture of V. harveyi increased the percentage of luminescent cells up to 98%, suggesting that V. harveyi produces and/or keeps the autoinducers at non-satg. concns. In contrast, all living cells of a

constitutive QS-active mutant (□luxO) produced light. QS also affects biofilm formation in V. harveyi. The data provide first evidence that a heterogeneous population produces more biofilm than a homogeneous one. It is suggested that even a QS-committed population of V. harveyi takes advantage of heterogeneity, which extends the current view of QS-regulated uniformity.

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Redundant small RNAs and feedback control in Vibrio cholerae and Vibrio harveyi quorum sensing. Lo Svenningsen, Sine. Princeton Univ., Princeton, NJ, USA. Avail. UMI, Order No. DA3333867. (2008), 224 pp. From: Diss. Abstr. Int., B 2009, 69(10), 5931. Dissertation written in English. AN 2009:945281 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

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Sugar synthesis in a protocellular model leads to a cell signalling response in bacteria. Gardner, Paul M.; Winzer, Klaus; Davis, Benjamin G. Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Oxford, UK. Nature Chemistry (2009), 1(5), 377-383, S377/1-S377/50. Publisher: Nature Publishing Group, CODEN: NCAHBB ISSN: 1755-4330. Journal written in English. AN 2009:900008 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The design of systems with life-like properties from simple chem. components may offer insights into biol. processes, with the ultimate goal of creating an artificial chem. cell that would be considered to be alive. Most efforts to create artificial cells have concd. on systems based on complex natural mols. such as DNA and RNA. Here we have constructed a lipid-bound protometabolism that synthesizes complex carbohydrates from simple feedstocks, which are capable of engaging the natural quorum sensing mechanism of the marine bacterium Vibrio harveyi and stimulating a proportional bioluminescent response. This encapsulated system may represent the first step towards the realization of a cellular mimic' and a starting point for bottom-up' designs of other chem. cells, which could perhaps display complex behaviors such as communication with natural cells.

Bibliographic Information

Distinct sensory pathways in Vibrio cholerae El Tor and classical biotypes modulate cyclic dimeric GMP levels to control biofilm formation. Hammer, Brian K.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Journal of Bacteriology (2009), 191(1), 169-177. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. AN 2009:874201 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing (QS), or cell-cell communication in bacteria, is achieved through the prodn. and subsequent response to the accumulation of extracellular signal mols. called autoinducers (AIs). To identify AI-regulated target genes in Vibrio cholerae El Tor (V. choleraeEl), the strain responsible for the current cholera pandemic, luciferase expression was assayed in an AI- strain carrying a random lux transcriptional reporter library in the presence and absence of exogenously added AIs. Twenty-three genes were identified and shown to require the QS transcription factor, HapR, for

their regulation. Several of the QS-dependent target genes, annotated as encoding hypothetical proteins, in fact encode HD-GYP proteins, phosphodiesterases that degrade the intracellular second messenger cyclic dimeric GMP (c-di-GMP), which is important for controlling biofilm formation. Indeed, overexpression of a representative QS-activated HD-GYP protein in V. choleraeEl reduced the intracellular concn. of c-di-GMP, which in turn decreased exopolysaccharide prodn. and biofilm formation. The V. cholerae classical biotype (V. choleraeCl), which caused previous cholera pandemics and is HapR-, controls c-di-GMP levels and biofilm formation by the VieA signaling pathway. We show that the VieA pathway is dispensable for biofilm formation in V. choleraeEl but that restoring HapR in V. choleraeCl reestablishes QS-dependent repression of exopolysaccharide prodn. Thus, different pandemic strains of V. cholerae modulate c-di-GMP levels and control biofilm formation in response to distinct sensory pathways.

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Identification of bacterial autoinducer and use in treating bacterial pathogenicity. Bassler, Bonnie; Semmelhack, Martin; Higgins, Douglas A.; Pomianek, Megan A.; Kraml, Kristina M.; Ng, Wai-Leung. (Princeton University, USA). PCT Int. Appl. (2009), 54pp. CODEN: PIXXD2 WO 2009088402 A2 20090716 Designated States W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, MT, NL, NO, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2008-US11336 20081001. Priority: US 2007-976587 20071001; US 2008-189844 20080822. CAN 151:181535 AN 2009:859686 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2009088402	A2	20090716	WO2008-US11336	20081001

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

Priority Application		
US 2007-976587P	P	20071001
US 2008-189844P	P	20080822

Abstract

A bacterial autoinducer, CAI-1, was purified and its structure identified. Methods for synthesis of the autoinducer and its analogs were elucidated. Methods of using the autoinducer or its analogs for treating bacterial pathogenicity and biofilm formation are described. Methods for prevention and treatment of cholera are described. Synthetic (S)-3-hydroxytridecan-4-one functions as well as natural CAI-1 in repressing prodn. of the virulence factor toxin co-regulated pilus (TCP). Strategies are described to manipulate bacterial quorum sensing in the clin. arena.

Bibliographic Information

Inhibition of Pseudomonas aeruginosa quorum sensing by AI-2 analogs. Ganin, Hadas; Tang, Xu; Meijler, Michael M. Department of Chemistry, Ben-Gurion University of the Negev, Be'er-Sheva, Israel. Bioorganic & Medicinal Chemistry Letters (2009), 19(14), 3941-3944. Publisher: Elsevier B.V., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. AN 2009:845452 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Autoinducer-2 (AI-2) has been suggested to serve as a universal interspecies quorum sensing signaling mol. We have synthesized a set of AI-2 analogs with small incremental changes in alkyl substitution on C-2 and evaluated them for their agonistic and antagonistic potential as quorum sensing (QS) attenuators in two different bacterial species: Pseudomonas aeruginosa and Vibrio harveyi. Unexpectedly, several of the analogs were found to function as synergistic QS agonists in V. harveyi, while two of these analogs inhibit QS in P. aeruginosa.

Bibliographic Information

Direct Quantitation of the Quorum Sensing Signal, Autoinducer-2, in Clinically Relevant Samples by Liquid Chromatography-Tandem Mass Spectrometry. Campagna, Shawn R.; Gooding, Jessica R.; May, Amanda L. Department of Chemistry, The University of Tennessee, Knoxville, TN, USA. Analytical Chemistry (Washington, DC, United States) (2009), 81(15), 6374-6381. Publisher: American Chemical Society, CODEN: ANCHAM ISSN: 0003-2700. Journal written in English. CAN 151:166872 AN 2009:841566 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum Sensing is a type of bacterial cell-to-cell signaling that allows for cell d. dependent regulation of gene expression. Many of the behaviors mediated by quorum sensing are crit. for bacterial colonization or infection, and autoinducer-2 has been proposed as a universal interspecies signaling mol. that allows multispecies colonies of bacteria, e.g., biofilms or dental plaque, to behave as pseudomulticellular organisms. However, the direct detection of autoinducer-2 has been difficult, leaving the in vivo relevance of this signal in question. Herein the authors report a liq. chromatog.-tandem mass spectrometric technique that enables reproducible, quant., and sensitive measurement of the concn. of autoinducer-2 from a variety of sources. This technique was applied to the detection of autoinducer-2 from Escherichia coli and Vibrio harveyi in proof-of-concept studies and was then used to directly measure the concn. of the signal produced by oral bacteria in human saliva.

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Inhibition of quorum sensing in Vibrio harveyi by boronic acids. Ni, Nanting; Choudhary, Gaurav; Peng, Hanjing; Li, Minyong; Chou, Han-Ting; Lu, Chung-Dar; Gilbert, Eric S.; Wang, Binghe. Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA, USA. Chemical Biology & Drug Design (2009), 74(1), 51-56. Publisher: Wiley-Blackwell, CODEN: CBDDAL ISSN: 1747-0277. Journal written in English. CAN 151:192787 AN 2009:816055 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Bacterial quorum sensing refers to the ability of bacteria to control gene expression through the detection of a threshold concn. of certain chems. called autoinducer(s), which are secreted by self and/or other bacteria. Quorum sensing is implicated in the regulation of pathol. relevant events such as biofilm formation, virulence, conjugation, sporulation, and swarming mobility. Inhibitors of bacterial quorum sensing are valuable research tools and potential antimicrobial agents. Here, the authors describe the discovery of several boronic acid inhibitors of bacterial quorum sensing in Vibrio harveyi with IC50 values in the low to sub-micromolar range in whole cell assays.

Bibliographic Information

Vibrio vulnificus produces quorum sensing signals of the AHL-class. Valiente, Esmeralda; Bruhn, Jesper Bartholin; Nielsen, Kristian Fog; Larsen, Jens Laurits; Roig, Francisco J.; Gram, Lone; Amaro, Carmen. Department of Microbiology and Ecology, University of Valencia, Burjasot, Spain. FEMS Microbiology Ecology (2009), 69(1), 16-26. Publisher: Wiley-Blackwell, CODEN: FMECEZ ISSN: 0168-6496. Journal written in English. CAN 151:262848 AN 2009:775156 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio vulnificus is an aquatic pathogenic bacterium that can cuase vibriosis in humans and fish. The species is subdivided into three biotypes with the fishvirulent strains belonging to biotype 2. The quorum sensing (QS) phenomenon mediated by furanosyl borate diester or autoinducer 2 (AI-2) has been described in human strains of biotype 1, and here we show that the luxS gene which encodes AI-2 is present in all strains of V. vulnificus regardless of origin, biotype or serovar. In this study, we also demonstrate that V. vulnificus produces QS signals of the acylated homoserine lactone (AHL) class (AI-1). AHLs were detected in strains of biotype 1 and 2 from water, fish and human wound infections but not in strains isolated from human septicemic cases. The AHL compd. was identified as N-butanoyl-homoserine-lactone (C4-HL) by both reporter strains and by HPLC-high-resoln. MS. C4-HL was detected when AHL-pos. strains were grown in low-nutrient medium [modified sea water yeast ext. (MSWYE)] but not in rich media (tryptic soy broth or brain-heart infusion) and its prodn. was enhanced when blood factors were added to MSWYE. C4-HL was detected in vivo, in eels infected with AHL-pos. biotype 2 strains. No known AHL-related gene was detected by PCR or Southern blot suggesting that AHL-related genes in V. vulnificus are different from those found in other Gram-neg. bacteria.

Bibliographic Information

Molecular diversification in the quorum-sensing system of Vibrio cholerae: role of natural selection in the emergence of pandemic strains. Talyzina, Nina M.; Ingvarsson, Paer K.; Zhu, Jun; Wai, Sun N.; Andersson, Agneta. Department of Ecology and Environmental Science, Umeaa University, Umeaa, Swed. Applied and Environmental Microbiology (2009), 75(11), 3808-3812. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240.

Journal written in English. CAN 151:98459 AN 2009:730360 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Two haplotypes of the Vibrio cholerae quorum-sensing system regulator hapR are described: hapR1, common among nonpandemic, non-O1, non-O139 strains, and hapR2, assocd. with pandemic O1 and O139 and epidemic O37 V. cholerae strains. The hapR2 has evolved under strong natural selection, implying that its fixation was influenced by conditions that led to cholera pandemics.

Bibliographic Information

Illuminating cell signaling: using Vibrio harveyi in an introductory biology laboratory. Hrizo, Stacy L.; Kaufmann, Nancy. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA. Biochemistry and Molecular Biology Education (2009), 37(3), 164-169. Publisher: John Wiley & Sons, Inc., CODEN: BMBECE ISSN: 1470-8175. Journal written in English. AN 2009:704323 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Cell signaling is an essential cellular process that is performed by all living organisms. Bacteria communicate with each other using a chem. language in a signaling pathway that allows bacteria to evaluate the size of their population, det. when they have reached a crit. mass (quorum sensing), and then change their behavior in unison to carry out processes that require many cells acting together to be effective. Here, we describe a lab. exercise in which the students observe the induction of bioluminescence or light prodn. as an output of the quorum sensing pathway in Vibrio harveyi. Using both wildtype and mutant bacterial strains they explore the induction of community behavior via cell-cell communication by detg. whether there is a correlation between the d. of the bacterial population and the prodn. of light by the bacterial culture. Using data from a cross-feeding assay the students make predictions about the identity of their strains and directly test these predictions using conditioned media from various liq. cultures. This two part exercise is designed for an introductory biol. course to begin familiarizing students with collecting data, making predictions based upon the data and directly testing their hypotheses using a model organism with a cell signaling pathway that has a simple visual output: light prodn.

Bibliographic Information

Information processing and signal integration in bacterial quorum sensing. Mehta, Pankaj; Wingreen, Ned S.; Bassler, Bonnie L.; Goyal, Sidhartha; Long, Tao. Dept. of Molecular Biology, Princeton University, Princeton, NJ, USA. arXiv.org, e-Print Archive, Quantitative Biology (2009), 1-38, arXiv:0905.4092v1 [q-bio.MN]. Publisher: Cornell University Library, CODEN: AQBRAY http://aps.arxiv.org/PS_cache/arxiv/pdf/0905/0905.4092v1.pdf Preprint written in English. AN 2009:664522 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Bacteria communicate using secreted chem. signaling mols. called autoinducers in a process known as quorum sensing. The quorum-sensing network of the marine bacterium Vibrio harveyi employs three autoinducers, each known to encode distinct ecol. information. Yet how cells integrate and interpret the information contained within the three autoinducer signals remains a mystery. Here, we develop a new framework for analyzing signal integration based on Information Theory and use

it to analyze quorum sensing in V. harveyi. We quantify how much the cells can learn about individual autoinducers and explain the exptl. obsd. input-output relation of the V. harveyi quorum-sensing circuit. Our results suggest that the need to limit interference between input signals places strong constraints on the architecture of bacterial signal-integration networks, and that bacteria likely have evolved active strategies for minimizing this interference. Here we analyze two such strategies: manipulation of autoinducer prodn. and feedback on receptor no. ratios.

Bibliographic Information

Evaluation and monitoring of quorum sensing soluble mediators implicated in the regulation of bacterial growth in Vibrio strains. Israil, Anca-Michaela; Chifiriuc, Mariana-Carmen; Delcaru, Cristina; Iordache, Carmen; Pelinescu, Diana; Sasarman, Elena. National Institute for Research and Development in Microbiology and Immunology, Bucharest, Rom. Romanian Biotechnological Letters (2009), 14(2), 4211-4224. Publisher: Ars Docendi, CODEN: RBLEFU ISSN: 1224-5984. Journal written in English. AN 2009:612995 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing (QS) is an ubiquitary regulation mechanism in the bacterial world implicated in intra and inter-bacterial communication and dependent upon the cellular d. In the present study, the authors have tried to elucidate the influence of sol. mediators accumulated in stationary phase cultures on the multiplication rate and growth curve of the homologous strains belonging to Vibrionaceae family. In this purpose, in the first step of the expt., the growth curves of two bacterial strains, one non halophilic and one halophilic were comparatively established for the simple, control culture, culture treated by the homologous filtrate (contg. sol. mediators) and culture treated by autoclaved filtrate (at 115□ C and 130□ C, resp.), incubated in small/big vols. at 4□ C, 28□ C and 37□ C. Taking into account that the sol. mediators remain active in filtrate as well as in cultures treated at 115□ C, for further expts., there were used only simple, control cultures compared with cultures treated by simple filtrate, all of them being cultivated in small vols. and incubated at 37□ C. The present study demonstrated that in stationary phase, bacterial cultures are accumulating sol. factors influencing the duration and aspect of the bacterial growth curve. In the most cases of the tested strains this influence consisted in the redn. of the multiplication rate and subsequently, of the culture d., the shortening of the lag phase and of the total duration of the growth curve. The synthesis of autoinducers proved to be dependent upon the bacterial strain, source of isolation (clin. case or aquatic environment), incubation temp., vol. of the culture medium, influencing the oxygenation surface.

Bibliographic Information

Characterization of a new plasmid-like prophage in a pandemic Vibrio parahaemolyticus O3:K6 strain. Lan, Shih-Feng; Huang, Chung-Ho; Chang, Chuan-Hsiung; Liao, Wei-Chao; Lin, I.-Hsuan; Jian, Wan-Neng; Wu, Yueh-Gin; Chen, Shau-Yan; Wong, Hin-chung. Department of Microbiology, Soochow University, Taipei, Taiwan. Applied and Environmental Microbiology (2009), 75(9), 2659-2667. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 151:94597 AN 2009:591183 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio parahaemolyticus is a common food-borne pathogen that is normally assocd. with seafood. In 1996, a pandemic O3:K6 strain abruptly appeared and caused the first pandemic of this pathogen to spread throughout many Asian countries, America, Europe, and Africa. The role of temperate bacteriophages in the evolution of this pathogen is of great interest. In this work, a new temp. phage, VP882, from a pandemic O3:K6 strain of V. parahaemolyticus was purified and characterized after mitomycin C induction. VP882 was a Myoviridae bacteriophage with a polyhedral head and a long rigid tail with a sheath-like structure. It infected and lysed high proportions of V. parahaemolyticus, Vibrio vulnificus, and Vibrio cholerae strains. The genome of phage VP882 was sequenced and was 38,197 bp long, and 71 putative open reading frames were identified, of which 27 were putative functional phage or bacterial genes. VP882 had a linear plasmid-like genome with a putative protelomerase gene and cohesive ends. The genome does not integrate into the host chromosome but was maintained as a plasmid in the lysogen. Anal. of the reaction sites of the protelomerases in different plasmid-like phages revealed that VP882 and □HAP-1 were highly similar, while N15, □KO2, and PY54 made up another closely related group. The presence of DNA adenine methylase and quorum-sensing transcriptional regulators in VP882 may play a specific role in this phage or regulate physiol. or virulence-assocd. traits of the hosts. These genes may also be remnants from the bacterial chromosome following transduction.

Bibliographic Information

Phenotypic and genetic differences between opaque and translucent colonies of Vibrio alginolyticus. Chang, Chen; Jin, Xie; Hu, Chaoqun. LMB and LAMB of the South China Sea Institute of Oceanology, The Chinese Academy of Sciences, Guangzhou, Peop. Rep. China. Biofouling (2009), 25(6), 525-531. Publisher: Taylor & Francis Ltd., CODEN: BFOUEC ISSN: 0892-7014. Journal written in English. AN 2009:575162 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Many pathogens undergo phase variation between rugose and smooth colony morphol. or between opaque and translucent colony morphol., which is mainly due to the variation in the surface polysaccharides. In this study, Vibrio alginolyticus ZJ-51 displayed phase variation between opaque, rugose colonies (Op) and translucent, smooth colonies (Tr). Unlike the vibrios reported previously, Tr cells of ZJ-51 enhanced biofilm formation and motility, but they did not differ from Op cells in the quantity of surface polysaccharides produced. Real time PCR was used to analyze the expression of the genes involved in polysaccharide biosynthesis, flagellar synthesis, and the AI-2 quorum-sensing system. The results revealed that the K-antigen capsule gene cluster (which consists of homologs to the cpsA-K in Vibrio parahaemolyticus) and O-antigen polysaccharide gene cluster (which contains homologs to the wza-wzb-wzc) were significantly more transcribed in Tr cells. The AI-2 quorum-sensing genes showed enhanced expression in the Tr variant which also exhibited greater expression of genes assocd. with polar flagellar biosynthesis. These results suggest that colony phase variation might affect the virulence and survival ability in the stressful environment inhabited by V. alginolyticus.

Bibliographic Information

Regulatory targets of quorum sensing in Vibrio cholerae: evidence for two distinct HapR-binding motifs. Tsou, Amy M.; Cai, Tao; Liu, Zhi; Zhu, Jun; Kulkarni, Rahul V. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. Nucleic Acids Research (2009), 37(8), 2747-2756. Publisher: Oxford University Press, CODEN:

NARHAD ISSN: 0305-1048. Journal written in English. CAN 151:117283 AN 2009:573882 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The quorum-sensing pathway in Vibrio cholerae controls the expression of the master regulator HapR, which in turn regulates several important processes such as virulence factor prodn. and biofilm formation. While HapR is known to control several important phenotypes, there are only a few target genes known to be transcriptionally regulated by HapR. In this work, we combine bioinformatic anal. with exptl. validation to discover a set of novel direct targets of HapR. Our results provide evidence for two distinct binding motifs for HapR-regulated genes in V. cholerae. The first binding motif is similar to the motifs recently discovered for orthologs of HapR in V. harveyi and V. vulnificus. However, our results demonstrate that this binding motif can be of variable length in V. cholerae. The second binding motif shares common elements with the first motif, but is of fixed length and lacks dyad symmetry at the ends. The contributions of different bases to HapR binding for this second motif were demonstrated using systematic mutagenesis expts. The current anal. presents an approach for systematically expanding our knowledge of the quorum-sensing regulon in V. cholerae and other related bacteria.

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Vibrio vulnificus: disease and pathogenesis. Jones, Melissa K.; Oliver, James D. Department of Biology, University of North Carolina at Charlotte, Charlotte, NC, USA. Infection and Immunity (2009), 77(5), 1723-1733. Publisher: American Society for Microbiology, CODEN: INFIBR ISSN: 0019-9567. Journal; General Review written in English. CAN 150:467196 AN 2009:550752 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review on mechanism od virulence and disease of Vibrio vulnificus. Virulence factors like CPS, hemolysin, and extracellular polysaccharides and quorum sensing of virulence and cytotoxicity are described.

Bibliographic Information

A new phenothiazine structural scaffold as inhibitors of bacterial quorum sensing in Vibrio harveyi. Ni, Nanting; Choudhary, Gaurav; Li, Minyong; Wang, Binghe. Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA, USA. Biochemical and Biophysical Research Communications (2009), 382(1), 153-156. Publisher: Elsevier B.V., CODEN: BBRCA9 ISSN: 0006-291X. Journal written in English. CAN 151:3776 AN 2009:419450 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing has attracted much attention due to its involvement in pathol. relevant events such as biofilm formation, virulence factor prodn., and sporulation. Inhibitors of quorum sensing are important research tools and potential therapeutic agents. Here, the authors describe a phenothiazine structural scaffold as a new type of quorum sensing inhibitors with IC50 values in the single digit micro molar range in Vibrio harveyi.

Bibliographic Information

Quantifying the integration of quorum-sensing signals with single-cell resolution. Long, Tao; Tu, Kimberly C.; Wang, Yufang; Mehta, Pankaj; Ong, N. P.; Bassler, Bonnie L.; Wingreen, Ned S. Department of Physics, Princeton University, Princeton, NJ, USA. PLoS Biology (2009), 7(3), No pp. given. Publisher: Public Library of Science, CODEN: PBLIBG ISSN: 1545-7885. http://biology.plosjournals.org/perlserv/?request=get-pdf&file=10.1371_journal.pbio.1000068-S.pdf Journal; Online Computer File written in English. CAN 150:417421 AN 2009:406802 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Cell-to-cell communication in bacteria is a process known as quorum sensing that relies on the prodn., detection, and response to the extracellular accumulation of signaling mols. called autoinducers. Often, bacteria use multiple autoinducers to obtain information about the vicinal cell d. However, how cells integrate and interpret the information contained within multiple autoinducers remains a mystery. Using single-cell fluorescence microscopy, we quantified the signaling responses to and analyzed the integration of multiple autoinducers by the model quorum-sensing bacterium Vibrio harveyi. Our results revealed that signals from two distinct autoinducers, AI-1 and AI-2, are combined strictly additively in a shared phosphorelay pathway, with each autoinducer contributing nearly equally to the total response. We found a coherent response across the population with little cell-to-cell variation, indicating that the entire population of cells can reliably distinguish several distinct conditions of external autoinducer concn. We speculate that the use of multiple autoinducers allows a growing population of cells to synchronize gene expression during a series of distinct developmental stages.

Bibliographic Information

Systems, methods and kits for diagnosis and monitoring of bacteria-related conditions and detg. quorum sensing mol. in biol. sample. Daunert, Sylvia; Deo, Sapna K.; Pasini, Patrizia; Kumari, Anjali; Shashidhar, Harohalli; Auer Flomenhof, Deborah R.; Raut, Nilesh. (University of Kentucky Research Foundation, USA). PCT Int. Appl. (2009), 61pp. CODEN: PIXXD2 WO 2009036081 A1 20090319 Designated States W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, MT, NL, NO, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2008-US75882 20080910. Priority: US 2007-971228 20070910. CAN 150:347885 AN 2009:331996 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2009036081	A1	20090319	WO2008-US75882	20080910

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO,

RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

Priority Application
US 2007-971228P P 20070910

Abstract
The presently-disclosed subject matter provides systems, methods, and kits for diagnosing and/or monitoring a bacteria-related condition of interest in a subject by providing a cell sensing system, each system contg. a reporter mol. capable of detecting binding of a quorum sensing mol. and capable of generating a detectable signal.

Bibliographic Information

Transition state analogs of 5'-methylthioadenosine nucleosidase disrupt quorum sensing. Gutierrez, Jemy A.; Crowder, Tamara; Rinaldo-Matthis, Agnes; Ho, Meng-Chiao; Almo, Steven C.; Schramm, Vern L. Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY, USA. Nature Chemical Biology (2009), 5(4), 251-257. Publisher: Nature Publishing Group, CODEN: NCBABT ISSN: 1552-4450. Journal written in English. CAN 150:509993 AN 2009:286574 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
5'-Methylthioadenosine/S-adenosylhomocysteine nucleosidase (MTAN) is a bacterial enzyme involved in S-adenosylmethionine-related quorum sensing pathways that induce bacterial pathogenesis factors. Transition state analogs MT-DADMe-Immucillin-A, EtT-DADMe-Immucillin-A and BuT-DADMe-Immucillin-A are slow-onset, tight-binding inhibitors of Vibrio cholerae MTAN (VcMTAN), with equil. dissocn. consts. of 73, 70 and 208 pM, resp. Structural anal. of VcMTAN with BuT-DADMe-Immucillin-A revealed interactions contributing to the high affinity. In V. cholerae cells, these compds. are potent MTAN inhibitors with IC50 values of 27, 31 and 6 nM for MT-, EtT- and BuT-DADMe-Immucillin-A, resp.; the compds. disrupt autoinducer prodn. in a dose-dependent manner without affecting growth. MT- and BuT-DADMe-Immucillin-A also inhibited autoinducer-2 prodn. in enterohemorrhagic Escherichia coli O157:H7 with IC50 values of 600 and 125 nM, resp. BuT-DADMe-Immucillin-A inhibition of autoinducer-2 prodn. in both strains persisted for several generations and caused redn. in biofilm formation. These results support MTAN's role in quorum sensing and its potential as a target for bacterial anti-infective drug design.

Bibliographic Information

Vibrio biofilms: so much the same yet so different. Yildiz, Fitnat H.; Visick, Karen L. Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, CA, USA. Trends in Microbiology (2009), 17(3), 109-118. Publisher: Elsevier B.V., CODEN: TRMIEA ISSN: 0966-842X. Journal; General Review written in English. CAN 150:490044 AN 2009:286049 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Vibrios are natural inhabitants of aquatic environments and form symbiotic or pathogenic relationships with eukaryotic hosts. Recent studies reveal that the ability of vibrios to form biofilms (i.e. matrix-enclosed, surface-assocd. communities) depends upon specific structural genes (flagella, pili and exopolysaccharide biosynthesis) and regulatory processes (two-component regulators, quorum sensing and c-di-GMP signaling). Here, we compare and contrast mechanisms and regulation of biofilm formation by Vibrio species, with a focus on Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus and Vibrio fischeri. Although many aspects are the same, others differ dramatically. Crucial questions that remain to be answered regarding the mol. underpinnings of Vibrio biofilm formation are also discussed.

Bibliographic Information

Compositions for regulating or modulating quorum sensing in bacteria, methods of using the compounds, and methods of regulating or modulating quorum sensing in bacteria. Wang, Binghe; Ni, Nanting; Wang, Junfeng; Lu, Chung-Dar; Chou, Han-Ting; Li, Minyong; Zheng, Shilong; Cheng, Yunfeng; Peng, Hanjing. (Georgia State University Research Foundation, Inc., USA). PCT Int. Appl. (2009), 75pp. CODEN: PIXXD2 WO 2009029317 A2 20090305 Designated States W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, MT, NL, NO, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2008-US66028 20080606. Priority: US 2007-933735 20070608. CAN 150:302176 AN 2009:264669 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2009029317	A2	20090305	WO2008-US66028	20080606
WO2009029317	A3	20090820		

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

Priority Application		
US 2007-933735P	P	20070608

Abstract

The present disclosure encompasses compds. and compns. that are useful as specific AI-2 antagonists for the control of bacterial quorum sensing. Although the AI-2 antagonists according to the present disclosure may not have bactericidal effect, their ability to attenuate virulence, drug resistance, and/or biofilm formation have therapeutic benefits. In addn., the AI-2 antagonists of the present disclosure can also be used as tools to probe bacterial AI-2 functions. The present disclosure also encompasses methods for inhibiting or attenuating microbial virulence, biofilm formation, and drug resistance. The methods are suitable for preventing bacteria from accruing and forming extensive biofilms that may be a health or hygiene hazard or a phys. issue, such as in the blockage of water or fuel lines.

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Identification, quantification, and determination of the absolute configuration of the bacterial quorum-sensing signal auto-inducer-2 by gas chromatography-mass spectrometry. Thiel, Verena; Vilchez, Ramiro; Sztajer, Helena; Wagner-Doebler, Irene; Schulz, Stefan. Institut fuer Organische Chemie, Technische Universitaet Braunschweig, Braunschweig, Germany. ChemBioChem (2009), 10(3), 479-485. Publisher: Wiley-VCH Verlag GmbH & Co. KGaA, CODEN: CBCHFX ISSN: 1439-4227. Journal written in English. CAN 151:239169 AN 2009:262522 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Autoinducer-2 (AI-2) is an important, small extracellular signaling mol. that is used by many bacteria. It is part of the AI-2 pool, a group of equil.-connected compds. derived from (S)-4,5-dihydroxy-2,3-pentanedione [(S)-DPD, 1]. Currently, these compds. are analyzed by indirect methods relying on the luminescence of sensor strains, the fluorescence of receptor proteins modified with fluorophores, or by isolation procedures not practical for quant. anal. Herein, we report a direct anal. procedure that allows for the unambiguous identification and quantification of mol. species by mass spectrometry. Phenylenediamine reacts readily and quant. with 1 to form the quinoxalinediol 12 under aq. conditions. The extn. and silylation of this compd. results in the formation of a silyl ether (13), which is amenable for anal. by gas chromatog.-mass spectrometry. The use of an isotopically labeled variant (16) of 12 as an internal std. opens the possibility for the accurate quantification of samples contg. AI-2 or its equil. products. The anal. of cell-free culture supernatants of Vibrio harveyi and Streptococcus mutans allowed for the accurate quantification of the AI-2 concn. above the limit of detection (0.7 ng mL-1). No compds. were detected in mutants lacking the capability to produce AI-2. In addn., the abs. configuration of 1 can be analyzed using the deriv. 13 by chiral gas chromatog.

Bibliographic Information

Inhibition of Lux quorum-sensing system by synthetic N-acyl-L-homoserine lactone analogous. Wang, Wenzhao; Morohoshi, Tomohiro; Ikeda, Tsukasa; Chen, Liang. Department of Material and Environmental Chemistry, Utsunomiya University, Utsunomiya, Tochigi, Japan. Acta Biochimica et Biophysica Sinica (2008), 40(12), 1023-1028. Publisher: Wiley-Blackwell, CODEN: ABBSC2 ISSN: 1672-9145. Journal written in English. AN 2009:201471 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In the present study, we investigated the inhibition of the Lux quorum-sensing system by N-acyl cyclopentylamine (Cn-CPA). The Lux quorum-sensing system regulates luminescence gene

expression in Vibrio fischeri. We have already reported on the synthesis of Cn-CPA and their abilities as inhibitors of the quorum-sensing systems in Pseudomonas aeruginosa and Serratia marcescens. In the case of Pseudomonas aeruginosa (Las and Rhl quorum-sensing system) and Serratia marcescens (Spn quorum-sensing system), specific Cn-CPA with a particular acyl chain length showed the strongest inhibitory effect. In the case of the Lux quorum-sensing system, it was found that several kinds of Cn-CPA with a range from C5 to C10 showed similar strong inhibitory effects. Moreover, the inhibitory effect of Cn-CPA on the Lux quorum-sensing system was stronger than that of halogenated furanone, a natural quorum-sensing inhibitor.

Bibliographic Information

Gene dosage compensation calibrates four regulatory RNAs to control Vibrio cholerae quorum sensing. Svenningsen, Sine L.; Tu, Kimberly C.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. EMBO Journal (2009), 28(4), 429-439. Publisher: Nature Publishing Group, CODEN: EMJODG ISSN: 0261-4189. Journal written in English. CAN 150:232322 AN 2009:84169 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing is a mechanism of cell-to-cell communication that allows bacteria to coordinately regulate gene expression in response to changes in cell-population d. At the core of the Vibrio cholerae quorum-sensing signal transduction pathway reside four homologous small RNAs (sRNAs), named the quorum regulatory RNAs 1-4 (Qrr1-4). The four Qrr sRNAs are functionally redundant. That is, expression of any one of them is sufficient for wild-type quorum-sensing behavior. Here, we show that the combined action of two feedback loops, one involving the sRNA-activator LuxO and one involving the sRNA-target HapR, promotes gene dosage compensation between the four qrr genes. Gene dosage compensation adjusts the total Qrr1-4 sRNA pool and provides the mol. mechanism underlying sRNA redundancy. The dosage compensation mechanism is exquisitely sensitive to small perturbations in Qrr levels. Precisely maintained Qrr levels are required to direct the proper timing and correct patterns of expression of quorum-sensing-regulated target genes.

Bibliographic Information

A small-RNA-mediated negative feedback loop controls quorum-sensing dynamics in Vibrio harveyi. Tu, Kimberly C.; Waters, Christopher M.; Svenningsen, Sine L.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2008), 70(4), 896-907. Publisher: Wiley-Blackwell, CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 150:93417 AN 2008:1428840 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The bioluminescent marine bacterium Vibrio harveyi uses a cell-to-cell communication process called quorum sensing (QS) to coordinate behaviors in response to changes in population d. QS is accomplished through the secretion and detection of extracellular signalling mols. called autoinducers. At the center of the V. harveyi QS circuit are five small regulatory RNAs called Qrr1-5 which destabilize the mRNA of luxR, encoding LuxR, the master transcriptional regulator of QS target genes. Here we show that LuxR directly activates transcription of qrr2, qrr3 and qrr4, leading to the rapid downregulation of luxR. The LuxR-binding sites in the promoters of qrr2, qrr3

and qrr4 were identified and mutated to det. the consequences of this regulatory loop on QS dynamics. Disruption of the loop delays the transition from high to low cell d., and more significantly, decreases the cell d. at which the population reaches a quorum. Our results suggest that feedback is essential for optimizing the dynamics of the transitions between individual and group behaviors.

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Evaluation of a focused library of N-aryl -homoserine lactones reveals a new set of potent quorum sensing modulators. Geske, Grant D.; Mattmann, Margrith E.; Blackwell, Helen E. Department of Chemistry, University of Wisconsin-Madison, Madison, WI, USA. Bioorganic & Medicinal Chemistry Letters (2008), 18(22), 5978-5981. Publisher: Elsevier Ltd., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. CAN 150:93456 AN 2008:1372566 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A focused library of N-aryl -homoserine lactones was designed around known lactone leads and evaluated for antagonistic and agonistic activity against quorum-sensing receptors in Agrobacterium tumefaciens, Pseudomonas aeruginosa, and Vibrio fischeri. Several compds. were identified with significantly heightened activities relative to the lead compds., and new structure-activity relationships (SARs) were delineated. Notably, 4-substituted N-phenoxyacetyl and 3-substituted N-phenylpropionyl -homoserine lactones were identified as potent antagonists of TraR and LuxR, resp.

Bibliographic Information

Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for luxS in most bacteria. Rezzonico, Fabio; Duffy, Brion. Division of Plant Protection, Agroscope Changins-Wadenswil ACW, Wadenswil, Switz. BMC Microbiology (2008), 8 No pp. given. Publisher: BioMed Central Ltd., CODEN: BMMIBC ISSN: 1471-2180. http://www.biomedcentral.com/content/pdf/1471-2180-8-154.pdf Journal; Online Computer File written in English. CAN 150:276137 AN 2008:1330251 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Great excitement accompanied discoveries over the last decade in several Gram-neg. and Gram-pos. bacteria of the LuxS protein, which catalyzes prodn. of the AI-2 autoinducer mol. for a second quorum sensing system (QS-2). Since the luxS gene was found to be widespread among the most diverse bacterial taxa, it was hypothesized that AI-2 may constitute the basis of a universal microbial language, a kind of bacterial Esperanto. Many of the studies published in this field have drawn a direct correlation between the occurrence of the luxS gene in a given organism and the presence and functionality of a QS-2 therein. However, rarely hath existence of potential AI-2 receptors been examd. This is important, since it is now well recognized that LuxS also holds a central role as a metabolic enzyme in the activated Me cycle which is responsible for the generation of S-adenosyl-L-methionine, the major Me donor in the cell. In order to assess whether the role of LuxS in these bacteria is indeed related to AI-2 mediated quorum sensing we analyzed genomic databases searching for established AI-2 receptors (i.e., LuxPQ-receptor of Vibrio harveyi and Lsr ABC-transporter of Salmonella typhimurium) and other presumed QS-related proteins and compared the outcome with published results about the role of QS-2 in these organisms. An

unequivocal AI-2 related behavior was restricted primarily to organisms bearing known AI-2 receptor genes, while phenotypes of luxS mutant bacteria lacking these genes could often be explained simply by assuming deficiencies in sulfur metab. Genomic anal. shows that while LuxPQ is restricted to Vibrionales, the Lsr-receptor complex is mainly present in pathogenic bacteria assocd. with endotherms. This suggests that QS-2 may play an important role in interactions with animal hosts. In most other species, however, the role of LuxS appears to be limited to metab., although in a few cases the presence of yet unknown receptors or the adaptation of pre-existent effectors to QS-2 must be postulated.

Bibliographic Information

Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in Vibrio spp. by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR. Brackman, Gilles; Defoirdt, Tom; Miyamoto, Carol; Bossier, Peter; Van Calenbergh, Serge; Nelis, Hans; Coenye, Tom. Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belg. BMC Microbiology (2008), 8 No pp. given. Publisher: BioMed Central Ltd., CODEN: BMMIBC ISSN: 1471-2180. http://www.biomedcentral.com/content/pdf/1471-2180-8-149.pdf Journal; Online Computer File written in English. CAN 149:524674 AN 2008:1330246 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Background: To date, only few compds. targeting the AI-2 based quorum sensing (QS) system are known. In the present study, we screened cinnamaldehyde and substituted cinnamaldehydes for their ability to interfere with AI-2 based QS. The mechanism of QS inhibition was elucidated by measuring the effect on bioluminescence in several Vibrio harveyi mutants. We also studied in vitro the ability of these compds. to interfere with biofilm formation, stress response and virulence of Vibrio spp. The compds. were also evaluated in an in vivo assay measuring the redn. of Vibrio harveyi virulence towards Artemia shrimp. Results: Our results indicate that cinnamaldehyde and several substituted derivs. interfere with AI-2 based QS without inhibiting bacterial growth. The active compds. neither interfered with the bioluminescence system as such, nor with the prodn. of AI-2. Study of the effect in various mutants suggested that the target protein is LuxR. Mobility shift assays revealed a decreased DNA-binding ability of LuxR. The compds. were further shown to (i) inhibit biofilm formation in several Vibrio spp., (ii) result in a reduced ability to survive starvation and antibiotic treatment, (iii) reduce pigment and protease prodn. in Vibrio anguillarum and (iv) protect gnotobiotic Artemia shrimp against virulent Vibrio harveyi BB120. Conclusion: Cinnamaldehyde and cinnamaldehyde derivs. interfere with AI-2 based QS in various Vibrio spp. by decreasing the DNA-binding ability of LuxR. The use of these compds. resulted in several marked phenotypic changes, including reduced virulence and increased susceptibility to stress. Since inhibitors of AI-2 based quorum sensing are rare, and considering the role of AI-2 in several processes these compds. may be useful leads towards antipathogenic drugs.

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Detection of quorum-sensing-related molecules in Vibrio scophthalmi. Garcia-Aljaro, Cristina; Eberl, Leo; Riedel, Kathrin; Blanch, Anicet R. Department of Microbiology, University of Barcelona, Barcelona, Spain. BMC Microbiology (2008), 8 No pp. given. Publisher: BioMed Central Ltd., CODEN: BMMIBC ISSN: 1471-2180. http://www.biomedcentral.com/content/pdf/1471-2180-8-138.pdf Journal; Online Computer File

written in English. CAN 150:278316 AN 2008:1330235 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Background: Cell-to-cell communication (also referred to as quorum sensing) based on N-acyl-homoserine lactones (AHLs) is a widespread response to environmental change in Gram-neg. bacteria. AHLs seem to be highly variable, both in terms of the acyl chain length and in the chem. structure of the radicals. Another quorum sensing pathway, the autoinducer-2-based system, is present both in Gram-pos. and Gram-neg. bacteria. In this study the presence of signal mols. belonging to both quorum sensing signaling pathways was analyzed in the marine symbiotic species Vibrio scophthalmi. Results: Three AHL-like signal mols. were detected in V. scophthalmi supernatants with the Agrobacterium tumefaciens sensor assay. This observation was further supported by the decrease in the presence of these signal mols. after cloning and expression of lactonase AiiA from Bacillus cereus in the V. scophthalmi strains. One of the signal mols. was identified as N-(3-hydroxy dodecanoyl)-L-homoserine lactone. V. scophthalmi was also shown to carry a functional LuxS synthase. The coding sequence for a luxS-like gene was obtained showing a max. similarity of 78% with Vibrio vulnificus. Anal. of the translated sequence revealed that the sequenced luxS gene carried the conserved domain, which is common to luxS sequences found in other species, and which is essential for LuxS enzymic activity. Conclusion: The data are consistent with the presence of quorum-sensing signal mols. from both AHL- and autoinducer 2-based quorum sensing systems in V. scophthalmi, which are homologous to others previously described in various Vibrio species. How this bacterium interacts with other bacteria and eukaryotic cells to compete ecol. with other intestinal bacteria present in the fish Scophthalmus maximus warrants further investigation.

Bibliographic Information

Cyclic AMP post-transcriptionally regulates the biosynthesis of a major bacterial autoinducer to modulate the cell density required to activate quorum sensing. Liang, Weili; Sultan, Syed Zafar; Silva, Anisia J.; Benitez, Jorge A. Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA, USA. FEBS Letters (2008), 582(27), 3744-3750. Publisher: Elsevier B.V., CODEN: FEBLAL ISSN: 0014-5793. Journal written in English. CAN 149:572920 AN 2008:1329460 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
In Vibrio cholerae, expression of the quorum sensing regulator HapR is induced by the accumulation of a major autoinducer synthesized by the activity of CqsA. Here we show that the cAMP-cAMP receptor protein complex regulates cqsA expression at the post-transcriptional level. This conclusion is supported by the anal. of cqsA-lacZ fusions, the ectopic expression of cqsA in □crp mutants and by Northern blot anal. showing that cqsA mRNA is unstable in □crp and □cya (adenylate cyclase) mutants. Addn. of cAMP to the culture of a □cya mutant restored cqsA mRNA stability and cholera autoinducer 1 prodn. Lowering intracellular cAMP levels by addn. of D-glucose increased the cell d. required to activate HapR. These results indicate that cAMP acts as a quorum modulator.

Bibliographic Information

Analysis of gene expression in mouse alveolar macrophages stimulated with quorum-sensing mutants of Vibrio vulnificus. Shin, Na-Ri; Lee, Deog-Yong; Yoo, Han Sang. Department of Infectious Diseases, College of Veterinary Medicine, KRF Zoonotic Priority Research Institute and Brain Korea, Seoul National University, Seoul, S. Korea. Japanese Journal of Infectious Diseases (2008), 61(5), 402-406. Publisher: National Institute of Infectious Diseases, CODEN: JJIDFE ISSN: 1344-6304. Journal written in English. CAN 150:370891 AN 2008:1327417 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Bacterial pathogens manipulate host cells to promote pathogen survival and dissemination. In this study, microarray technol. was used to identify the genes that are affected by the Vibrio vulnificus quorum-sensing genes, luxS and smcR. By comparing the expression profiles of mouse macrophage cell lines stimulated with either the parent strains or a luxS smcR mutant, differentially expressed genes were identified. The genes included those that affect host cell death, stress, signaling transduction, inflammation, and immune response. Macrophages stimulated with the luxS smcR mutant differentially expressed genes assocd. with removal of toxins, the complement pathway, regulation of cytokine expression, and antigen presentation, indicating that macrophages stimulated with the luxS smcR mutant induced an appropriate inflammation reaction and immune response for removal of bacteria. In summary, quorum-sensing in V. vulnificus could contribute to bacterial survival and increased pathogenesis by inducing a changed expression profile in macrophages.

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The Vibrio harveyi master quorum-sensing regulator, LuxR, a TetR-type protein is both an activator and a repressor: DNA recognition and binding specificity at target promoters. Pompeani, Audra J.; Irgon, Joseph J.; Berger, Michael F.; Bulyk, Martha L.; Wingreen, Ned S.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2008), 70(1), 76-88. Publisher: Wiley-Blackwell, CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 149:571210 AN 2008:1225202 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing is the process of cell-to-cell communication by which bacteria communicate via secreted signal mols. called autoinducers. As cell population d. increases, the accumulation of autoinducers leads to coordinated changes in gene expression across the bacterial community. The marine bacterium, Vibrio harveyi, uses three autoinducers to achieve intra-species, intra-genera and inter-species cell-cell communication. The detection of these autoinducers ultimately leads to the prodn. of LuxR, the quorum-sensing master regulator that controls expression of the genes in the quorum-sensing regulon. LuxR is a member of the TetR protein superfamily; however, unlike other TetR repressors that typically repress their own gene expression and that of an adjacent operon, LuxR is capable of activating and repressing a large no. of genes. Here, we used protein binding microarrays and a two-layered bioinformatics approach to show that LuxR binds a 21 bp consensus operator with dyad symmetry. In vitro and in vivo analyses of two promoters directly regulated by LuxR allowed us to identify those bases that are crit. for LuxR binding. Together, the in silico and biochem. results enabled us to scan the genome and identify novel targets of LuxR in V. harveyi and thus expand the understanding of the quorum-sensing regulon.

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Metabolic role of LuxS, putative autoinducer-2 synthase, in Erwinia amylovora. Rezzonico, F.; Duffy, B. Agroscope Changins-Wadenswil, Swiss National Competence Centre for Fire Blight, Wadenswil, Switz. Acta Horticulturae (2008), 793(Proceedings of the XIth International Workshop on Fire Blight, 2007), 61-65. Publisher: International Society for Horticultural Science, CODEN: AHORA2 ISSN: 0567-7572. Journal written in English. CAN 151:27560 AN 2008:1199480 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The putative autoinducer-2 (AI-2) synthase gene LuxS was sequenced from Erwinia amylovora. Phylogenetic anal. indicated conservation of LuxS among genotypically diverse E. amylovora strains. Quorum sensing mediated by LuxS, has been implicated in coordinated gene expression, growth and virulence in other enterobacteria, but our evidence suggests that this is not the function in E. amylovora. Mutational anal. pointed to a role in colonization of apple blossoms, the primary infection court for fire blight but little or no role in virulence on apple shoots and pear fruit. Expression of key virulence genes, hrpL and dspA/E, was reduced in mutants of two E. amylovora strains, but stronger up-regulatory effects were obsd. for expression of metabolic genes involved in the activated Me cycle (AMC) in those two mutants. Supplementation with key sulfur amino-acids in the AMC pathway complemented likely disruptions in the mutant. Despite a pos. reaction with the AI-2 biosensor Vibrio harveyi BB170, quorum sensing was not confirmed in co-culture expts. with wild-type and mutant strains either in vitro or in apple blossoms. Known receptors essential for AI-2 quorum sensing, the LuxPQ sensor kinase or the Lsr ABC transporter, are absent in E. amylovora (and in many LuxS carrying bacteria) further indicating a primarily metabolic role for LuxS in this and other bacteria.

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Quorum-sensing control of gene expression in Vibrio fischeri. Antunes, Luis Caetano Martha. Univ. of Iowa, Iowa City, IA, USA. Avail. UMI, Order No. DA3301684. (2007), 74 pp. From: Diss. Abstr. Int., B 2008, 69(2), 816. Dissertation written in English. CAN 149:466032 AN 2008:1197216 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

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Modulation of bacterial quorum sensing with synthetic ligands. Blackwell, Helen E.; Geske, Grant D.; O'Neill, Jennifer C. (Wisconsin Alumni Research Foundation, USA). PCT Int. Appl. (2008), 198pp. CODEN: PIXXD2 WO 2008116029 A1 20080925 Designated States W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, MT, NL, NO, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2008-US57562 20080319. Priority: US 2007-895598 20070319; US 2007-912345 20070417; US 2007-974026 20070920. CAN 149:394606 AN 2008:1154815 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2008116029	A1	20080925	WO2008-US57562	20080319

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

US 20080312319	A1	20081218	US 2008-51826	20080319

Priority Application

US 2007-895598P	P	20070319
US 2007-912345P	P	20070417
US 2007-974026P	P	20070920

Abstract

The present invention provides compds. and methods for modulation of the quorum sensing of bacteria. In an embodiment, the compds. of the present invention are able to act as replacements for naturally occurring bacterial quorum sensing ligands in a ligand-protein binding system; i.e., they imitate the effect of natural ligands and produce an agonistic effect. In another embodiment, the compds. of the present invention are able to act in a manner which disturbs or inhibits the naturally occurring ligand-protein binding system in quorum sensing bacteria; i.e., they produce an antagonistic effect. The compds. of the present invention comprise N-acylated-homoserine lactones (AHLs) comprised of a wide range of acyl groups.

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Transition state analogues in quorum sensing and SAM recycling. Schramm, Vern L.; Gutierrez, Jemy A.; Cordovano, Grace; Basu, Indranil; Guha, Chandan; Belbin, Thomas J.; Evans, Gary B.; Tyler, Peter C.; Furneaux, Richard H. Albert Einstein College of Medicine, Bronx, NY, USA. Nucleic Acids Symposium Series (2008), 52(1), 75-76. Publisher: Oxford University Press, CODEN: NASSCJ ISSN: 1746-8272. http://nass.oxfordjournals.org/content/vol52/issue1/index.dtl Journal; Online Computer File written in English. CAN 150:298047 AN 2008:1153684 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Transition state structures can be derived from kinetic isotope effects and computational chem. Mol. electrostatic potential maps of transition states serve as blueprints to guide synthesis of transition state analog inhibitors of target enzymes. 5'-Methylthioadenosine phosphorylase (MTAP) functions in the polyamine pathway by recycling methylthioadenosine (MTA) and maintaining cellular S-adenosylmethionine (SAM). Its transition state structure was used to guide synthesis of MT-DADMe-ImmA, a picomolar inhibitor that shows anticancer effects against solid tumors. Biochem. and genomic anal. suggests that MTAP inhibition acts by altered DNA methylation and

gene expression patterns. A related bacterial enzyme, 5'-methylthioadenosine nucleosidase (MTAN), functions in pathways of quorum sensing involving AI-1 and AI-2 mols. Transition states have been solved for several bacterial MTANs and used to guide synthesis of powerful inhibitors with dissocn. consts. in the femtomolar to picomolar range. BuT-DADMe-ImmA blocks quorum sensing in Vibrio cholerae without changing bacterial growth rates. Transition state analog inhibitors show promise as anticancer and antibacterial agents.

Bibliographic Information

Coordinated regulation of gene expression in Vibrio cholerae. Bhadra, Rupak K.; Das, Bhabatosh. Infectious Diseases & Immunology Division, Indian Institute of Chemical Biology, Kolkata, India. Editor(s): Faruque, Shah M.; Nair, G. Balakrish. Vibrio cholerae (2008), 153-167. Publisher: Caister Academic Press, Norwich, UK CODEN: 69LAJX Conference; General Review written in English. CAN 150:464846 AN 2008:1129269 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Vibrio cholerae, the causative agent of the severe diarrhoeal disease cholera, has evolved with intricate signal transduction and gene regulatory systems to survive and grow under various environmental conditions. The virulence regulon of V. cholerae, which involves multiple genes working in a coordinated manner, represents a regulatory paradigm for extracellular bacterial pathogens. Availability of the whole genome sequence has allowed microarray based transcriptome analyses of V. cholerae cells isolated directly from cholera patients. Such studies indicate that quite a large no. of genes are involved in the disease process and their expression pattern changes as the infection progresses. Further understanding of the process came with the recent discoveries of small non-coding RNAs and intracellular signal mol. c-di-GMP as modulators of gene expression in V. cholerae. Transcriptome anal. has also shed light on synchronized gene expression related to chitin utilization and development of natural competence when the organism exists in the natural aquatic environment. Thus, the survival, evolution and pathogenesis of V. cholerae appear to be controlled by several intricate overlapping regulatory circuits.

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Genetics of Vibrio cholerae colonization and motility. Jude, Brooke A.; Taylor, Ronald K. Colby College, Waterville, ME, USA. Editor(s): Faruque, Shah M.; Nair, G. Balakrish. Vibrio cholerae (2008), 67-99. Publisher: Caister Academic Press, Norwich, UK CODEN: 69LAJX Conference; General Review written in English. CAN 150:464843 AN 2008:1129265 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Survival of Vibrio cholerae, either in the aquatic environment or in the human host, is mediated by appropriate expression of factors that control motility, colonization, prodn. of virulence factors, as well as sensing the cell d. (quorum sensing). Successful transition of the organism between the aquatic and the host intestinal environments thus depends on the coordinated activity of a no. of genes and regulatory circuits. Recent developments in our understanding of the complex gene regulation supporting the cyclic transitions and the dual lifestyle of V. cholerae has been presented and discussed.

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Signal Discrimination by Differential Regulation of Protein Stability in Quorum Sensing. Smith, Cameron; Song, Hao; You, Lingchong. Department of Biomedical Engineering, Duke University, Durham, NC, USA. Journal of Molecular Biology (2008), 382(5), 1290-1297. Publisher: Elsevier Ltd., CODEN: JMOBAK ISSN: 0022-2836. Journal written in English. CAN 149:551399 AN 2008:1116581 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing (QS) is a communication mechanism exploited by a large variety of bacteria to coordinate gene expression at the population level. In Gram-neg. bacteria, QS occurs via synthesis and detection of small chem. signals, most of which belong to the acyl-homoserine lactone class. In such a system, binding of an acyl-homoserine lactone signal to its cognate transcriptional regulator (R-protein) often induces stabilization and subsequent dimerization of the R-protein, which results in the regulation of downstream gene expression. Existence of diverse QS systems within and among species of bacteria indicates that each bacterium needs to distinguish among a myriad of structurally similar chem. signals. We show, using a math. model, that fast degrdn. of an R-protein monomer can facilitate discrimination of signals that differentially stabilize it. Furthermore, our results suggest an inverse correlation between the stability of an R-protein and the achievable limits of fidelity in signal discrimination. In particular, an unstable R-protein tends to be more specific to its cognate signal, whereas a stable R-protein tends to be more promiscuous. These predictions are consistent with exptl. data on well-studied natural and engineered R-proteins and thus have implications for understanding the functional design of QS systems.

Bibliographic Information

Functional independence of a variant LuxOPL91 from a non-O1 non-O139 Vibrio cholerae over the activity of CsrA and Fis. Dongre, Mitesh; Tripathi, Ranjana; Jain, Vibhu; Raychaudhuri, Saumya. Institute of Microbial Technology, Chandigarh, India. Journal of Medical Microbiology (2008), 57(8), 1041-1045. Publisher: Society for General Microbiology, CODEN: JMMIAV ISSN: 0022-2615. Journal written in English. CAN 150:441782 AN 2008:1114551 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A variant LuxO mol. from a non-O1, non-O139 Vibrio cholerae PL91 (designated LuxOPL91) was previously identified whose function remains unaffected in the absence of LuxU, thus conferring the protease-neg. phenotype exhibited by this strain. The present study was conducted to evaluate whether LuxO functioning at high cell d. in PL91 is due to a gain of function mutation or whether there exists a continuous input from CsrA and Fis to quorum sensing system in PL91, which in turn maintain the activity of LuxOPL91 at high cell d. and thus render this strain protease neg. For this purpose, csrA and fis single mutants of V. cholerae strain PL91 were generated. Results confirmed that LuxOPL91 is gain of function variant, and the absence of CsrA and Fis does not perturb its activity. It can also exert its constitutive activity in strains other than PL91. It appears that the quorum sensing system of V. cholerae requires the involvement of two global regulators, CsrA and Fis.

Bibliographic Information

Structure-based discovery and experimental verification of novel Al-2 quorum sensing inhibitors against Vibrio harveyi. Li, Minyong; Ni, Nanting; Chou, Han-Ting; Lu, Chung-Dar;

Tai, Phang C.; Wang, Binghe. Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA, USA. ChemMedChem (2008), 3(8), 1242-1249. Publisher: Wiley-VCH Verlag GmbH & Co. KGaA, CODEN: CHEMGX ISSN: 1860-7179. Journal written in English. CAN 149:462080 AN 2008:1072193 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing has been implicated in the control of pathol. relevant bacterial behavior such as secretion of virulence factors, biofilm formation, sporulation, and swarming motility. The Al-2 quorum sensing pathway is found in both Gram-pos. and Gram-neg. bacteria. Therefore, antagonizing Al-2 quorum sensing is a possible approach to modifying bacterial behavior. However, efforts in developing inhibitors of Al-2-mediated quorum sensing are esp. lacking. High-throughput virtual screening using the V. harveyi LuxP crystal structure identified two compds. that were found to antagonize Al-2-mediated quorum sensing in V. harveyi without cytotoxicity. The sulfone functionality of these inhibitors was identified as crit. to their ability to mimic the natural ligand in their interactions with Arg215 and Arg310 of the active site.

Bibliographic Information

AinS quorum sensing regulates the Vibrio fischeri acetate switch. Studer, Sarah V.; Mandel, Mark J.; Ruby, Edward G. Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. Journal of Bacteriology (2008), 190(17), 5915-5923. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 149:302679 AN 2008:1046670 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The marine bacterium Vibrio fischeri uses two acyl-homoserine lactone (acyl-HSL) quorum-sensing systems. The earlier signal, octanoyl-HSL, produced by AinS, is required for normal colonization of the squid Euprymna scolopes and, in culture, is necessary for a normal growth yield. In examg. the latter requirement, we found that during growth in a glycerol/tryptone-based medium, wild-type V. fischeri cells initially excrete acetate but, in a metabolic shift termed the acetate switch, they subsequently utilize the acetate, removing it from the medium. In contrast, an ainS mutant strain grown in this medium does not remove the excreted acetate, which accumulates to lethal levels. The acetate switch is characterized by the induction of acs, the gene encoding acetyl CoA (acetyl-CoA) synthetase, leading to uptake of the excreted acetate. Wild-type cells induce an acs transcriptional reporter 25-fold, coincident with the disappearance of the extracellular acetate; in contrast, the ainS mutant did not display significant induction of the acs reporter. Supplementation of the medium of an ainS mutant with octanoyl-HSL restored normal levels of acs induction and acetate uptake. Addnl. mutant analyses indicated that acs regulation was accomplished through the regulator LitR but was independent of the LuxIR quorum-signaling pathway. Importantly, the acs mutant of V. fischeri has a competitive defect when colonizing the squid, indicating the importance of proper control of acetate metab. in the light of organ symbiosis. This is the first report of quorum-sensing control of the acetate switch, and it indicates a metabolic connection between acetate utilization and cell d.

Bibliographic Information

Quorum sensing "flips" the acetate switch. Wolfe, Alan J. Department of Microbiology and Immunology, Loyola University Medical School, Maywood, IL, USA. Journal of Bacteriology (2008), 190(17), 5735-5737. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal; General Review written in English. CAN 149:262266 AN 2008:1046651 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. In this issue of the Journal of Bacteriol., S. V. Studer et al. (ibid. 5915-5923) connect the bacterial phenomena of acetate switching and quorum sensing. Specifically, they demonstrate that the luminescent marine bacterium Vibrio fischeri uses quorum sensing to control its transition from acetate prodn. (dissimilation) to acetate utilization (assimilation). This finding raises the distinct possibility that acetate assimilation is a group behavior that contributes to the symbiosis of V. fischeri with its eukaryotic host, the Hawaiian squid Euprymna scolopes.

Bibliographic Information

Quorum sensing and cell-to-cell communication in the dental biofilm. Demuth, Donald R.; Lamont, Richard J. School of Dentistry, University of Louisville, KY, USA. Advances in Molecular and Cellular Microbiology (2006), 11(Bacterial Cell-to-Cell Communication), 175-197. Publisher: Cambridge University Press, CODEN: AMCMDX Journal; General Review written in English. CAN 150:325424 AN 2008:1044263 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review on the recent work that elucidates two mechanisms of interspecies communication among the oral bacteria, cell-contact-dependent communication and intra- and interspecies signaling that is mediated by a sol. quorum sensing signal related to autoinducer 2, the cyclic borate diester produced by Vibrio harveyi.

Bibliographic Information

LuxS in cellular metabolism and cell-to-cell signaling. Duan, Kangmin; Surette, Michael G. Molecular Microbiology Laboratory, Northwest University, Xian, Peop. Rep. China. Advances in Molecular and Cellular Microbiology (2006), 11(Bacterial Cell-to-Cell Communication), 117-149. Publisher: Cambridge University Press, CODEN: AMCMDX Journal; General Review written in English. CAN 150:369679 AN 2008:1044261 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. This was a review on the biol. of LuxS and autoinducer-2 (AI-2) prodn. and the recent evidence for and against a role in cell-to-cell signaling. Topics covered include identification of LuxS, homoserine lactone quorum sensing systems, oligopeptide quorum sensing pathways, identification of LuxS, metabolic pathway of AI-2 prodn., the structures of AI-2, the controversy of AI-2 and cell-to-cell signaling, AI-2 as a classical quorum sensing signal, AI-2 as a quorum sensing signal in bacteria other than Vibrio spp., bacteria with ambiguous roles for AI-2, AI-2 as an interspecies quorum sensing signal, and AI-2 as a reporter of metabolic status.

Bibliographic Information

Quorum sensing mediated regulation of biofilm growth and virulence of Vibrio cholerae. Zhu, Jun; Mekalanos, John J. University of Pennsylvania School of Medicine, USA. Advances in Molecular and Cellular Microbiology (2006), 11(Bacterial Cell-to-Cell Communication), 101-116. Publisher: Cambridge University Press, CODEN: AMCMDX Journal; General Review written in English. CAN 150:325423 AN 2008:1044260 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review on the recent studies on Vibrio cholerae quorum sensing from various labs. and the significance of quorum-sensing-mediated regulation of virulence gene expression and biofilm formation.

Bibliographic Information

Roles of LuxR in regulating extracellular alkaline serine protease A, extracellular polysaccharide and mobility of Vibrio alginolyticus. Rui, Haopeng; Liu, Qin; Ma, Yue; Wang, Qiyao; Zhang, Yuanxing. State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, Peop. Rep. China. FEMS Microbiology Letters (2008), 285(2), 155-162. Publisher: Wiley-Blackwell, CODEN: FMLED7 ISSN: 0378-1097. Journal written in English. CAN 149:528385 AN 2008:1038192 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In marine Vibrio species, the Vibrio harveyi-type LuxR protein, a key player in a quorum-sensing system, controls the expression of various genes. In this study, the luxR homolog in Vibrio alginolyticus was identified and named luxRval, whose expression was greatly induced by the increase of cell no. The luxRval in-frame deletion mutant showed a significant downregulation of total extracellular protease activity, and esp. caused a 70% decrease in the transcript levels of extracellular alk. serine protease A (proA), which was an important virulent factor of V. alginolyticus. Complementation in trans with luxRval could restore the expression of proA to the level of the wild-type strain. Deletion of the luxRval gene also resulted in changes of colony morphol., extracellular polysaccharide prodn. and mobility. Therefore, another member of the V. harveyi-type LuxR regulator family has been characterized in V. alginolyticus.

Bibliographic Information

A consensus sequence for binding of SmcR, a Vibrio vulnificus LuxR homologue, and genome-wide identification of the SmcR regulon. Lee, Dong Hwan; Jeong, Hye Sook; Jeong, Hee Gon; Kim, Kyung Mo; Kim, Heebal; Choi, Sang Ho. National Research Laboratory of Molecular Microbiology and Toxicology, Center for Agricultural Biomaterials, Seoul National University, Seoul, S. Korea. Journal of Biological Chemistry (2008), 283(35), 23610-23618. Publisher: American Society for Biochemistry and Molecular Biology, CODEN: JBCHA3 ISSN: 0021-9258. Journal written in English. CAN 149:464038 AN 2008:1017404 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing has been implicated as an important global regulatory system controlling the expression of numerous virulence factors in bacterial pathogens. In the present study, DNA targets of SmcR, a Vibrio vulnificus LuxR homolog, were selected from a random pool of DNA fragments

by using a cycle selection procedure consisting of in vitro DNA-SmcR interaction, purifn. of SmcR-DNA complexes, and PCR amplification of SmcR-bound DNA. The amplified DNA fragments were cloned and analyzed sep. by electrophoretic mobility shift assay to verify the specific binding of SmcR to the DNA. The DNA sequences bound by SmcR were detd. by DNase I footprinting, and alignment of the resulting 29 sequences revealed a 22-bp consensus SmcR-binding sequence, 5'-TTATTGATWWRWTWNTNAATAA-3' (where W represents A or T, R is G or A, and N is any nucleotide), with an 8-bp (TTATTGAT) inverted repeat. The consensus sequence revealed greater efficiency for the binding of SmcR than the SmcR-binding sequence previously identified within PvvpE. Mutational anal. demonstrated that the 9th and 10th bases from the center are the most essential for SmcR binding. A genome-wide search using the consensus sequence predicted that at least 121 genes are under the control of SmcR, and 10 of these newly identified SmcR regulon members were verified as being regulated by SmcR in V. vulnificus as well as in vitro. The consensus sequence and newly identified genes should be of use for elucidating the regulatory mechanism of SmcR and provide further insight into the role of the quorum sensing in V. vulnificus pathogenesis.

Bibliographic Information

Deducing receptor signaling parameters from in vivo analysis: LuxN/Al-1 quorum sensing in Vibrio harveyi. Swem, Lee R.; Swem, Danielle L.; Wingreen, Ned S.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Cell (Cambridge, MA, United States) (2008), 134(3), 461-473. Publisher: Cell Press, CODEN: CELLB5 ISSN: 0092-8674. Journal written in English. CAN 149:371498 AN 2008:1001113 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing, a process of bacterial cell-cell communication, relies on prodn., detection, and response to autoinducer signaling mols. LuxN, a nine-transmembrane domain protein from Vibrio harveyi, is the founding example of membrane-bound receptors for acyl-homoserine lactone (AHL) autoinducers. We used mutagenesis and suppressor analyses to identify the AHL-binding domain of LuxN and discovered LuxN mutants that confer both decreased and increased AHL sensitivity. Our anal. of dose-response curves of multiple LuxN mutants pins these inverse phenotypes on quantifiable opposing shifts in the free-energy bias of LuxN for occupying its kinase and phosphatase states. To understand receptor activation and to characterize the pathway signaling parameters, we exploited a strong LuxN antagonist, one of fifteen small-mol. antagonists we identified. We find that quorum-sensing-mediated communication can be manipulated pos. and neg. to control bacterial behavior and, more broadly, that signaling parameters can be deduced from in vivo data.

Bibliographic Information

Dissecting the quorum-sensing receptor LuxN. Bonneau, Richard. New York University Center for Comparative Functional Genomics, New York, NY, USA. Cell (Cambridge, MA, United States) (2008), 134(3), 390-391. Publisher: Cell Press, CODEN: CELLB5 ISSN: 0092-8674. Journal; General Review written in English. CAN 149:326871 AN 2008:1001100 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Quorum sensing plays a key role in the behavior of many bacteria and is carried out by a wide diversity of secreted mols. and their receptors. In this issue, Swem et al. (2008) provide a detailed site-specific anal. of the functioning of the quorum-sensing receptor LuxN from Vibrio harveyi.

Bibliographic Information

Natural Products Chemistry and Taxonomy of the Marine Cyanobacterium Blennothrix cantharidosmum. Clark, Benjamin R.; Engene, Niclas; Teasdale, Margaret E.; Rowley, David C.; Matainaho, Teatulohi; Valeriote, Frederick A.; Gerwick, William H. Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA. Journal of Natural Products (2008), 71(9), 1530-1537. Publisher: American Chemical Society-American Society of Pharmacognosy, CODEN: JNPRDF ISSN: 0163-3864. Journal written in English. CAN 149:302608 AN 2008:973358 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A Papua New Guinea field collection of the marine cyanobacterium Blennothrix cantharidosmum was investigated for its cytotoxic constituents. Bioassay-guided isolation defined the cytotoxic components as the known compds. lyngbyastatins 1 and 3. However, six new acyl proline derivs., tumonoic acids D-I, plus the known tumonoic acid A were also isolated. Their planar structures were defined from NMR and MS data, while their stereostructures followed from a series of chiral chromatogs., degrdn. sequences, and synthetic approaches. The new compds. were tested in an array of assays, but showed only modest antimalarial and inhibition of quorum sensing activities. Nevertheless, these are the first natural products to be reported from this genus, and this inspired a detailed morphol. and 16S rDNA-based phylogenetic anal. of the producing organism.

Bibliographic Information

Chemical synthesis and biological characterization of structural analogs of the Vibrio cholerae autoinducer CAI-1. Semmelhack, Martin F.; Pomianek, Megan E.; Kraml, Christina M.; Higgins, Douglas A.; Bassler, Bonnie L. Department of Chemistry, Princeton University, Princeton, NJ, USA. Abstracts of Papers, 236th ACS National Meeting, Philadelphia, PA, United States, August 17-21, 2008 (2008), AGRO-031. Publisher: American Chemical Society, Washington, D. C CODEN: 69KXQ2 Conference; Meeting Abstract; Computer Optical Disk written in English. AN 2008:949031 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The bacterium Vibrio cholerae, the causative agent of the disease cholera, uses the cell-to-cell communication system of quorum sensing to regulate its life cycle and to control the expression of virulence factors. Quorum sensing is a process by which groups of bacteria control collective behaviors according to population d. which is perceived through the secretion and detection of small org. mols. called autoinducers. The principal autoinducer of V. cholerae is CAI-1 (cholerae autoinducer-1) which is synthesized by CqsA (cholerae quorum sensing synthase) and detected by the transmembrane sensor-kinase CqsS (cholerae quorum sensing sensor). We have recently identified CAI-1 as 3-(S)-hydroxy-4-tridecanone. Chem.-synthesized CAI-1 initiates quorum sensing in V. cholerae and has a pronounced effect on the formation of the toxin-coregulated pilus crucial to virulence. To det. the effect of small structural changes on the ability of the autoinducer to communicate population d. information and influence virulence factor prodn., we now implement straightforward synthetic strategies to access a variety of structural analogs of CAI-1. We evaluate

the ability of CAI-1-like mols. that differ in characteristics such as stereochem., functional groups, acyl tail length, and steric bulk to activate the quorum sensing circuit of V. cholerae.

Bibliographic Information

Chemical synthesis and biological characterization of structural analogs of the Vibrio cholerae autoinducer CAI-1. Semmelhack, Martin F.; Pomianek, Megan E.; Kraml, Christina M.; Higgins, Douglas A.; Bassler, Bonnie L. Department of Chemistry, Princeton University, Princeton, NJ, USA. Abstracts of Papers, 236th ACS National Meeting, Philadelphia, PA, United States, August 17-21, 2008 (2008), AEI-055. Publisher: American Chemical Society, Washington, D. C CODEN: 69KXQ2 Conference; Meeting Abstract; Computer Optical Disk written in English. AN 2008:948691 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The bacterium Vibrio cholerae, the causative agent of the disease cholera, uses the cell-to-cell communication system of quorum sensing to regulate its life cycle and to control the expression of virulence factors. Quorum sensing is a process by which groups of bacteria control collective behaviors according to population d., which is perceived through the secretion and detection of small org. mols. called autoinducers. The principal autoinducer of V. cholerae, CAI-1, is synthesized by the CqsA protein and detected by the transmembrane sensor-kinase CqsS. We have recently identified CAI-1 as 3-(S)-hydroxy-4-tridecanone. Chem.-synthesized CAI-1 initiates quorum sensing in V. cholerae and has a pronounced effect on the formation of the toxin-coregulated pilus crucial to virulence. To det. the effect of small structural changes on the ability of the autoinducer to communicate population d. information and influence virulence factor prodn., we now implement straightforward synthetic strategies to access a variety of structural analogs of CAI-1. We evaluate the ability of CAI-1-like mols. that differ in characteristics such as stereochem., functional groups, acyl tail length, and steric bulk to activate the quorum sensing circuit of V. cholerae.

Bibliographic Information

Detection of possible AI-2-mediated quorum sensing system in commensal intestinal bacteria. Lukas, F.; Gorenc, G.; Kopecny, J. Institute of Animal Physiology and Genetics, v.v.i., Academy of Science of the Czech Republic, Prague, Czech Rep. Folia Microbiologica (Prague, Czech Republic) (2008), 53(3), 221-224. Publisher: Institute of Microbiology, Academy of Sciences of the Czech Republic, CODEN: FOMIAZ ISSN: 0015-5632. Journal written in English. CAN 149:326911 AN 2008:899206 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The Vibrio harveyi strain BB170-autoinducer bioassay was used to detect possible quorum sensing autoinducer-2 mol. (AI-2) in culture fluids of commensal intestinal bacteria. Culture fluids of Bacteroides vulgatus, Clostridium proteoclasticum, Escherichia coli, Eubacterium rectale, Lachnospira multipara, Pseudobutyrivibrio ruminis, Roseburia intestinalis, Ruminococcus albus and Ruminococcus flavefaciens contained AI-2-like mols. The PCR bands from some of the tested strains could be also amplified using primers designed for the luxS gene. These findings suggest that AI-2 is present in the gastrointestinal tract; however, it has not yet been proved whether it is used for bacterial cell-to-cell communication.

Bibliographic Information

Mucosal penetration primes Vibrio cholerae for host colonization by repressing quorum sensing. Liu, Zhi; Miyashiro, Tim; Tsou, Amy; Hsiao, Ansel; Goulian, Mark; Zhu, Jun. Departments of Microbiology, University of Pennsylvania, Philadelphia, PA, USA. Proceedings of the National Academy of Sciences of the United States of America (2008), 105(28), 9769-9774. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 149:302776 AN 2008:886616 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
To successfully infect a host and cause the diarrheal disease cholera, Vibrio cholerae must penetrate the intestinal mucosal layer and express virulence genes. Previous studies have demonstrated that the transcriptional regulator HapR, which is part of the quorum sensing network in V. cholerae, represses the expression of virulence genes. Here, we show that hapR expression is also modulated by the regulatory network that governs flagellar assembly. Specifically, FliA, which is the alternative □-factor (□28) that activates late-class flagellin genes in V. cholerae, represses hapR expression. In addn., we show that mucin penetration by V. cholerae is sufficient to break flagella and so cause the secretion of FlgM, the anti-□ factor that inhibits FliA activity. During initial colonization of host intestinal tissue, hapR expression is repressed because of low cell d. However, full repression of hapR expression does not occur in fliA mutants, which results in attenuated colonization. Our results suggest that V. cholerae uses flagellar machinery to sense particular intestinal signals before colonization and enhance the expression of virulence genes by modulating the output of quorum sensing signaling.

Bibliographic Information

A mutational analysis defines Vibrio fischeri LuxR binding sites. Antunes, Luis Caetano M.; Ferreira, Rosana B. R.; Lostroh, C. Phoebe; Greenberg, E. Peter. Department of Microbiology, The University of Iowa, Iowa City, IA, USA. Journal of Bacteriology (2008), 190(13), 4392-4397. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 149:218951 AN 2008:819219 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio fischeri quorum sensing involves the LuxI and LuxR proteins. The LuxI protein generates the quorum-sensing signal N-3-oxohexanoyl-L-homoserine lactone (3OC6-HSL), and LuxR is a signal-responsive transcriptional regulator which activates the luminescence (lux) genes and 17 other V. fischeri genes. For activation of the lux genes, LuxR binds to a 20-base-pair inverted repeat, the lux box, which is centered 42.5 base pairs upstream of the transcriptional start of the lux operon. Similar lux box-like elements have been identified in only a few of the LuxR-activated V. fischeri promoters. To better understand the DNA sequence elements required for LuxR binding and to identify binding sites in LuxR-regulated promoters other than the lux operon promoter, we have systematically mutagenized the lux box and evaluated the activity of many mutants. By doing so, we have identified nucleotides that are crit. for promoter activity. Interestingly, certain lux box mutations allow a 3OC6-HSL-independent LuxR activation of the lux operon promoter. We have used the results of the mutational anal. to create a consensus lux box, and we have used this consensus sequence to identify LuxR binding sites in 3OC6-HSL-activated genes for which lux boxes could not be identified previously.

Bibliographic Information

Mucosal penetration primes Vibrio cholerae for host colonization by repressing quorum sensing. Liu, Zhi; Miyashiro, Tim; Tsou, Amy; Hsiao, Ansel; Goulian, Mark; Zhu, Jun. Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA. Proceedings of the National Academy of Sciences of the United States of America, Early Edition (2008), (July 7 2008), 1-6, 6 pp. Publisher: National Academy of Sciences, CODEN: PNASC8 http://www.pnas.org/cgi/reprint/0802241105v1 Journal; Online Computer File written in English. AN 2008:816865 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
To successfully infect a host and cause the diarrheal disease cholera, Vibrio cholerae must penetrate the intestinal mucosal layer and express virulence genes. Previous studies have demonstrated that the transcriptional regulator HapR, which is part of the quorum sensing network in V. cholerae, represses the expression of virulence genes. Here, we show that hapR expression is also modulated by the regulatory network that governs flagellar assembly. Specifically, FliA, which is the alternative □-factor (□28) that activates late-class flagellin genes in V. cholerae, represses hapR expression. In addn., we show that mucin penetration by V. cholerae is sufficient to break flagella and so cause the secretion of FlgM, the anti-□ factor that inhibits FliA activity. During initial colonization of host intestinal tissue, hapR expression is repressed because of low cell d. However, full repression of hapR expression does not occur in fliA mutants, which results in attenuated colonization. Our results suggest that V. cholerae uses flagellar machinery to sense particular intestinal signals before colonization and enhance the expression of virulence genes by modulating the output of quorum sensing signaling.

Bibliographic Information

An Unexpected Switch in the Modulation of AI-2-Based Quorum Sensing Discovered through Synthetic 4,5-Dihydroxy-2,3-pentanedione Analogues. Lowery, Colin A.; Park, Junguk; Kaufmann, Gunnar F.; Janda, Kim D. The Skaggs Institute for Chemical Biology and Departments of Chemistry and Immunology and the Worm Institute for Research and Medicine (WIRM), The Scripps Research Institute, La Jolla, CA, USA. Journal of the American Chemical Society (2008), 130(29), 9200-9201. Publisher: American Chemical Society, CODEN: JACSAT ISSN: 0002-7863. Journal written in English. CAN 149:218944 AN 2008:779777 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing (QS) has traditionally referred to a mechanism of communication within a species of bacteria. However, emerging research implicates QS in interspecies communication and competition, and such systems have been proposed in a wide variety of bacteria. The AI-2-based QS system represents the most studied of these proposed interspecies systems, and has been proposed to regulate diverse functions such as bioluminescence, expression of virulence factors, and biofilm formation. As such, the development of modulatory compds., both agonists and antagonists, is of great interest for the treatment of bacterial infections and the study of unknown AI-2-based QS systems. Toward this end, the authors have designed and synthesized a panel of 4,5-dihydroxy-2,3-pentanedione/AI-2 analogs and evaluated their effects on the AI-2 QS of various bacteria. The panel of compds. exhibited differential effects in the bacterial cell lines examd., providing a platform for the development of broad-spectrum modulators of AI-2-based QS.

Bibliographic Information

The Legionella autoinducer synthase LqsA produces an □ -hydroxyketone signaling molecule. Spirig, Thomas; Tiaden, Andre; Kiefer, Patrick; Buchrieser, Carmen; Vorholt, Julia A.; Hilbi, Hubert. Institute of Microbiology, ETH Zurich, Zurich, Switz. Journal of Biological Chemistry (2008), 283(26), 18113-18123. Publisher: American Society for Biochemistry and Molecular Biology, CODEN: JBCHA3 ISSN: 0021-9258. Journal written in English. CAN 149:262291 AN 2008:744389 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The opportunistic pathogen Legionella pneumophila replicates in human lung macrophages and in free-living amebas. To accommodate the transfer between host cells, L. pneumophila switches from a replicative to a transmissive phase. L. pneumophila harbors a gene cluster homologous to the Vibrio cholerae cqsAS quorum sensing system, encoding a putative autoinducer synthase (lqsA) and a sensor kinase (lqsS), which flank a response regulator (lqsR). LqsR is an element of the L. pneumophila virulence regulatory network, which promotes pathogen-host cell interactions and inhibits entry into the replicative growth phase. Here, we show that lqsA functionally complements a V. cholerae cqsA autoinducer synthase deletion mutant and, upon expression in L. pneumophila or Escherichia coli, produces the diffusible signaling mol. LAI-1 (Legionella autoinducer-1). LAI-1 is distinct from CAI-1 (Cholerae autoinducer-1) and was identified as 3-hydroxypentadecan-4-one using liq. chromatog. coupled to high resoln. tandem mass spectrometry. The activity of both LqsA and CqsA was abolished upon mutation of a conserved lysine, and covalent binding of the cofactor pyridoxal 5'-phosphate to this lysine was confirmed by mass spectrometry. Thus, LqsA and CqsA belong to a family of pyridoxal 5'-phosphate-dependent autoinducer synthases, which produce the □-hydroxyketone signaling mols. LAI-1 and CAI-1.

Bibliographic Information

Quorum sensing antagonist, method of preventing a biofilm formation using the quorum sensing antagonist and method of reducing a bacterial contamination using the quorum sensing antagonist. Yoon, Je-Yong; Kim, Cheol-Jin; Kim, Jae-Eun; Park, Hyung-Yeon. (Seoul National University Industry Foundation, S. Korea). PCT Int. Appl. (2008), 38pp. CODEN: PIXXD2 WO 2008069374 A1 20080612 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, MT, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2007-KR2169 20070503. Priority: KR 1216-50 20061204. CAN 149:53857 AN 2008:700061 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2008069374	A1	20080612	WO2007-KR2169	20070503

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL,

IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

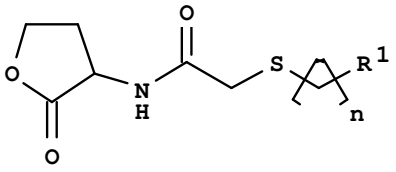
KR 2008050844	A	20080610	KR 2006-121650	20061204
KR 841294	B1	20080625		

Priority Application

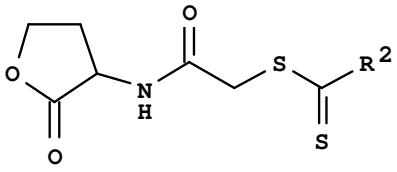
KR 2006-121650	A	20061204

Abstract

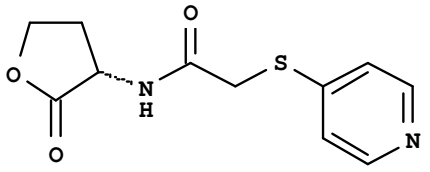
In the quorum sensing antagonist blocking the communication in bacteria, the method for preventing biofilm formation using this quorum sensing antagonist of formula I and II and the method for reducing the bacterial contamination, the quorum sensing antagonist contains the homoserine lactone moiety and sulfanylethanoyl group, and has a similar chem. structure to that of the autoinducer which is produced by bacteria as a signal, whereby the quorum sensing antagonist can inhibit the formation of biofilm and reduce the bacterial contamination as well. Compds. of formula I and II wherein n is 0 to 10; R1 is H, carboxy, nitrogen-contg. heteroaryl, and C1-10 carboxyalkylthio; R2 is aryl and C1-10 carboxyalkylthio; are claimed. Example compd. III was prepd. by a general procedure (procedure given). ALl the invention compds. were evaluated for their quorum sensing antagonistic activity (data given).



I



II



III

Bibliographic Information

Small Talk: Molecules That Control Quorum Sensing in Vibrio Cholerae. Semmelhack, Martin F.; Pomianek, Megan E.; Brow, William E.; Campagna, Shawn R.; Higgins, Douglas A.; Bassler, Bonnie L. Chemistry, Princeton University, Princeton, NJ, USA. Abstracts, 40th Middle Atlantic Regional Meeting of the American Chemical Society, Queens, NY, United States, May 17-21 (2008), MRM-182. Publisher: American Chemical Society, Washington, D. C CODEN: 69KSUP Conference; Meeting Abstract written in English. AN 2008:587900 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio cholerae, the causative agent of the human disease cholera, uses cell-to-cell communication to control pathogenicity and biofilm formation. This process, known as quorum sensing, relies on the secretion and detection of signalling mols. called autoinducers. At low cell d. V. cholerae activates the expression of virulence factors and forms biofilms. At high cell d. the accumulation of two quorum-sensing autoinducers represses these traits. These two autoinducers, cholerae autoinducer-1 (CAI-1) and autoinducer-2 (AI-2), function synergistically to control gene regulation, although CAI-1 is the stronger of the two signals. V. cholerae AI-2 is a furanosyl borate diester, which was identified and fully characterized in Vibrio harveyi. CAI-1 is (S)-3- hydroxytridecan-4-one, a new type of bacterial signal. The structure elucidation, synthesis, mechanism of action, and structure-activity relations for both AI-2 and CAI-1 will be discussed. CAI-1 represses prodn. of the canonical virulence factor TCP (toxin co-regulated pilus) which suggests that CAI-1 could be used as a therapy to prevent cholera infection and, furthermore, that strategies to manipulate bacterial quorum sensing hold promise in the clin. arena.

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Modeling the quorum sensing signaling regulatory network in Vibrio fischeri. Yan, Ling. Univ. of Tennessee, Knoxville, TN, USA. Avail. UMI, Order No. DA3286738. (2007), 120 pp. From: Diss. Abstr. Int., B 2008, 68(10), 6885. Dissertation written in English. CAN 149:37434 AN 2008:535669 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

Signal integration in the Vibrio harveyi and Vibrio cholerae quorum-sensing circuits. Hammer, Brian; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Editor(s): Winans, Stephen Carlyle; Bassler, Bonnie L. Chemical Communication among Bacteria (2008), 323-332. Publisher: American Society for Microbiology, Washington, D. C CODEN: 69KOK3 Conference; General Review written in English. CAN 149:528285 AN 2008:476399 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review. This was a review on the Vibrio harveyi and Vibrio cholerae quorum-sensing circuits, effect of chem. and microbiota on the Vibrio QS responses, and differences in the regulation of the Qrr sRNAs in V. harveyi and V. cholerae.

Bibliographic Information

Quorum signaling and symbiosis in the marine luminous bacterium Vibrio fischeri. Stabb, E. V.; Schaefer, A.; Bose, J. L.; Ruby, E. G. Department of Microbiology, University of Georgia, Athens, GA, USA. Editor(s): Winans, Stephen Carlyle; Bassler, Bonnie L. Chemical Communication among Bacteria (2008), 233-250. Publisher: American Society for Microbiology, Washington, D. C CODEN: 69KOK3 Conference; General Review written in English. CAN 149:528281 AN 2008:476394 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review on the role of quorum sensing in Vibrio fischeri, focusing on recent developments in the understanding of the genetics and physiol. of cell-cell signaling by populations of these bacteria, both in culture and in their light-organ symbioses. Particular emphasis is placed on outlining the regulatory factors and pathways by which quorum sensing coordinates the biol. activities of this bioluminescent microbe.

Bibliographic Information

Quorum sensing in Vibrio cholerae pathogenesis. Stirling, Fiona R.; Liu, Zhi; Zhu, Jun. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. Editor(s): Winans, Stephen Carlyle; Bassler, Bonnie L. Chemical Communication among Bacteria (2008), 145-160. Publisher: American Society for Microbiology, Washington, D. C CODEN: 69KOK3 Conference; General Review written in English. CAN 149:528276 AN 2008:476389 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. This was a review on the role of quorum sensing during the various phases of the life cycle of the human pathogen Vibrio cholerae. The mechanisms of quorum sensing in V. cholerae and quorum-sensing-controlled phenotypes in this pathogen are discussed.

Bibliographic Information

Synthetic homoserine lactone-derived sulfonylureas as inhibitors of Vibrio fischeri quorum sensing regulator. Frezza, Marine; Soulere, Laurent; Reverchon, Sylvie; Guiliani, Nicolas; Jerez, Carlos; Queneau, Yves; Doutheau, Alain. Institut de Chimie et Biochimie Moleculaires et Supramoleculaires, Laboratoire de Chimie Organique, INSA Lyon, Villeurbanne, Fr. Bioorganic & Medicinal Chemistry (2008), 16(7), 3550-3556. Publisher: Elsevier Ltd., CODEN: BMECEP ISSN: 0968-0896. Journal written in English. CAN 149:48531 AN 2008:465897 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A series of 9 homoserine lactone-derived sulfonylureas substituted by an alkyl chain, some of them bearing a Ph group at the extremity, have been prepd. All compds. were found to inhibit the action of 3-oxo-hexanoyl-L-homoserine lactone, the natural inducer of bioluminescence in the bacterium Vibrio fischeri, the aliph. compds. being more active than their phenyl-substituted counterparts. Mol. modeling studies performed on the most active compd. in each series suggest that the antagonist activity could be related to the perturbation of the hydrogen-bond network in the ligand-protein complexes.

Bibliographic Information

Quorum sensing influences Vibrio harveyi growth rates in a manner not fully accounted for by the marker effect of bioluminescence. Nackerdien, Zeena E.; Keynan, Alexander; Bassler, Bonnie L.; Lederberg, Joshua; Thaler, David S. Raymond and Beverly Sackler Laboratory of Molecular Genetics and Informatics, Rockefeller University, New York, NY, USA. PLoS One (2008), 3(2), No pp. given. Publisher: Public Library of Science, CODEN: POLNCL ISSN: 1932-6203. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0001671 Journal; Online Computer File written in English. CAN 148:397897 AN 2008:453345 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The light-emitting vibrios provide excellent material for studying the interaction of cellular communication with growth rate because bioluminescence is a convenient marker for quorum sensing. However, the use of bioluminescence as a marker is complicated because bioluminescence itself may affected growth rate, e.g. by diverting energy. The marker effect was explored via growth rate studies in isogenic Vibrio harveyi (Vh) strains altered in quorum sensing on the one hand, and bioluminescence on the other. By hypothesis, growth rate is energy limited: mutants deficient in quorum sensing grow faster because wild type quorum sensing unleashes bioluminescence and bioluminescence diverts energy. Findings reported here confirm a role for bioluminescence in limiting Vh growth rate, at least under the conditions tested. However, the results argue that the bioluminescence is insufficient to explain the relationship of growth rate and quorum sensing in Vh. A Vh mutant null for all genes encoding the bioluminescence pathway grew faster than wild type but not as fast as null mutants in quorum sensing. Vh quorum sensing mutants showed altered growth rates that do not always rank with their relative increase or decrease in bioluminescence. In addn., the cell-free culture fluids of a rapidly growing Vibrio parahaemolyticus (Vp) strain increased the growth rate of wild type Vh without significantly altering Vh's bioluminescence. The same cell-free culture fluid increased the bioluminescence of Vh quorum mutants. Conclusions/Significance: The effect of quorum sensing on Vh growth rate can be either pos. or neg. and includes both bioluminescence-dependent and independent components. Bioluminescence tends to slow growth rate but not enough to account for the effects of quorum sensing on growth rate.

Bibliographic Information

Quorum sensing controls biofilm formation in Vibrio cholerae through modulation of cyclic Di-GMP levels and repression of vpsT. Waters, Christopher M.; Lu, Wenyun; Rabinowitz, Joshua D.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Journal of Bacteriology (2008), 190(7), 2527-2536. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 148:421399 AN 2008:408601 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Two chem. signaling systems, quorum sensing (QS) and 3',5'-cyclic diguanylic acid (c-di-GMP), reciprocally control biofilm formation in Vibrio cholerae. QS is the process by which bacteria communicate via the secretion and detection of autoinducers, and in V. cholerae, QS represses biofilm formation. C-di-GMP is an intracellular second messenger that contains information regarding local environmental conditions, and in V. cholerae, c-di-GMP activates biofilm formation. Here we show that HapR, a major regulator of QS, represses biofilm formation in V. cholerae through two distinct mechanisms. HapR controls the transcription of 14 genes encoding a group of proteins that synthesize and degrade c-di-GMP. The net effect of this transcriptional program is a redn. in cellular c-di-GMP levels at high cell d. and, consequently, a decrease in biofilm formation. Increasing the c-di-GMP concn. at high cell d. to the level present in the low-cell-d. QS state restores biofilm formation, showing that c-di-GMP is epistatic to QS in the control of biofilm formation in V. cholerae. In addn., HapR binds to and directly represses the expression of the biofilm transcriptional activator, vpsT. Together, our results suggest that V. cholerae integrates information about the vicinal bacterial community contained in extracellular QS autoinducers with the intracellular environmental information encoded in c-di-GMP to control biofilm formation.

Bibliographic Information

Implications of rewiring bacterial quorum sensing. Haseltine, Eric L.; Arnold, Frances H. Division of Chemistry and Chemical Engineering 210-41, California Institute of Technology, Pasadena, CA, USA. Applied and Environmental Microbiology (2008), 74(2), 437-445. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. AN 2008:408498 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria employ quorum sensing, a form of cell-cell communication, to sense changes in population d. and regulate gene expression accordingly. This work investigated the rewiring of one quorum-sensing module, the lux circuit from the marine bacterium Vibrio fischeri. Steady-state expts. demonstrate that rewiring the network architecture of this module can yield graded, threshold, and bistable gene expression as predicted by a math. model. The expts. also show that the native lux operon is most consistent with a threshold, as opposed to a bistable, response. Each of the rewired networks yielded functional population sensors at biol. relevant conditions, suggesting that this operon is particularly robust. These findings (i) permit prediction of the behaviors of quorum-sensing operons in bacterial pathogens and (ii) facilitate forward engineering of synthetic gene circuits.

Bibliographic Information

Regulation of Vibrio alginolyticus virulence by the LuxS quorum-sensing system. Ye, J.; Ma, Y.; Liu, Q.; Zhao, D. L.; Wang, Q. Y.; Zhang, Y. X. State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, Peop. Rep. China. Journal of Fish Diseases (2008), 31(3), 161-169. Publisher: Blackwell Publishing Ltd., CODEN: JFIDDI ISSN: 0140-7775. Journal written in English. CAN 149:218992 AN 2008:400335 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing (QS) is a bacterial intercommunication system that controls the expression of multiple genes in response to population d. The LuxS QS system regulates the expression of several virulence factors in a wide variety of pathogenic bacteria. LuxS has been characterized to be responsible for producing a type of autoinducer, AI-2, which stimulates the expression of the luciferase operon in Vibrio harveyi. Vibrio alginolyticus is established as an opportunistic pathogen of several marine animals, and its LuxS QS system remains undefined. To investigate the pathogenic role of luxS in V. alginolyticus, the luxS mutants of both the std. strain ATCC 33787 and a fish-clin. isolate MVP01, named MYJS and MYJM, resp., were constructed. The mutation resulted in reduced lethality to Pagrus major. I.p. LD50 of MYJS and MYJM increased by 15- and 93-fold, resp. The two luxS mutants exhibited a lower growth rate and defective flagellar biosynthesis. They also showed a significant decrease in protease prodn. and an increase in both extracellular polysaccharide prodn. and biofilm development. The results suggest that the LuxS QS system plays an important role in regulating the expression of virulence factors in V. alginolyticus.

Bibliographic Information

RpoS induces expression of the Vibrio anguillarum quorum-sensing regulator VanT. Weber, Barbara; Croxatto, Antony; Chen, Chang; Milton, Debra L. Department of Molecular Biology, Umeaa University, Umeaa, Swed. Microbiology (Reading, United Kingdom) (2008), 154(3), 767-780. Publisher: Society for General Microbiology, CODEN: MROBEO ISSN: 1350-0872. Journal written in English. CAN 149:25796 AN 2008:393816 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In vibrios, regulation of the Vibrio harveyi-like LuxR transcriptional activators occurs post-transcriptionally via small regulatory RNAs (sRNAs) that destabilize the luxR mRNA at a low cell population, eliminating expression of LuxR. Expression of the sRNAs is modulated by the vibrio quorum-sensing phosphorelay systems. However, vanT mRNA, which encodes a LuxR homolog in Vibrio anguillarum, is abundant at low and high cell d., indicating that VanT expression may be regulated via addnl. mechanisms. In this study, Western analyses showed that VanT was expressed throughout growth with a peak of expression during late exponential growth. VanO induced partial destabilization of vanT mRNA via activation of at least one Qrr sRNA. Interestingly, the sigma factor RpoS significantly stabilized vanT mRNA and induced VanT expression during late exponential growth. This induction was in part due to RpoS repressing expression of Hfq, an RNA chaperone. RpoS is not part of the quorum-sensing regulatory cascade since RpoS did not regulate expression or activity of VanO, and RpoS was not regulated by VanO or VanT. VanT and RpoS were needed for survival following UV irradn. and for pigment and metalloprotease prodn., suggesting that RpoS works with the quorum-sensing systems to modulate expression of VanT, which regulates survival and stress responses.

Bibliographic Information

Pyrogallol and its analogs can antagonize bacterial quorum sensing in Vibrio harveyi. Ni, Nanting; Choudhary, Gaurav; Li, Minyong; Wang, Binghe. Department of Chemistry, Georgia State University, Atlanta, GA, USA. Abstracts of Papers, 235th ACS National Meeting, New Orleans, LA, United States, April 6-10, 2008 (2008), MEDI-034. Publisher: American Chemical Society, Washington, D. C CODEN: 69KNN3 Conference; Meeting Abstract; Computer Optical Disk written in English. AN 2008:389588 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria can coordinate community-wide behaviors through quorum sensing, i.e., the secretion and sensing of autoinducer (AI) mols. Bacterial quorum sensing is implicated in the regulation of pathol. relevant events such as biofilm formation, bacterial virulence and drug resistance. Inhibitors of bacterial quorum sensing could therefore be useful therapeutics. We are interested in finding quorum sensing antagonists by using Vibrio harveyi a model organism. We have found several catechol compds. capable of antagonizing AI-2-mediated pathway in V. harvey with pyrogallol having the lowest IC50 at 2 □M. We postulate that the inhibitory effect of catechols was due to their ability to chelate boric acid the same way as DPD, the natural AI-2 mol. It is interesting to note that these compds. also inhibit AI-1-mediated bacterial quorum sensing. Further studies are needed to fully understand their mechanisms of action.

Bibliographic Information

Synthesis and biological activity of antagonists of AI-2-mediated bacterial quorum sensing in Vibrio harveyi. Cheng, Yunfeng; Ni, Nanting; Li, Minyong; Chou, Han-Ting; Lu, Chung-dar; Tai, Phang C.; Wang, Binghe. Department of Chemistry, Georgia State University, Atlanta, GA, USA. Abstracts of Papers, 235th ACS National Meeting, New Orleans, LA, United States, April 6-10, 2008 (2008), MEDI-033. Publisher: American Chemical Society, Washington, D. C CODEN: 69KNN3 Conference; Meeting Abstract; Computer Optical Disk written in English. AN 2008:389587 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacterial quorum sensing is a process of community-wide regulation of behaviors through the secretion and sensing of chem. autoinducers (AI). Because quorum sensing is implicated in pathol. relevant bacterial traits such as biofilm formation, conjugation, virulence factor prodn., and drug resistance, we are interested in developing quorum sensing inhibitors as potential therapeutic agents. In this effort, we conducted virtual screening against the audoinducer-2 (AI-2) receptor protein in Vibrio harveyi, LuxP. Among the 26 candidates selected for evaluation of their ability to inhibit AI-2 mediated quorum sensing, several showed good inhibitory activities in a bioluminescence assay (IC50: 40-50 micromolar). From these promising hits, 12 analogs were designed, synthesized, and evaluated. Several synthetic analogs showed improved activities. This presentation will discuss the design, synthesis, and structure-activity relationship studies of these AI-2 inhibitors.

Bibliographic Information

Detection of quorum-sensing pathway and construction of luxS gene allelic exchange plasmid of Streptococcus mutans. Yu, Danni; Han, Yuzhi; Han, Fusheng; Chen, Jie. Department of Stomatology, Second Hospital of Tianjin Medical University, Tianjin, Peop. Rep. China. Zhonghua Kouqiang Yixue Zazhi (2008), 43(1), 37-40. Publisher: Zhonghua Yixuehui Zazhishe, CODEN: ZKYZE2 ISSN: 1002-0098. Journal written in Chinese. CAN 149:216704 AN 2008:383044 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

This paper aims to detect the AI-2 quorum-sensing pathway and construct the luxS gene allelic exchange plasmid of Streptococcus mutans. To detect AI-2 pathway in Streptococcus mutans, the Vibrio harveyi BB170 was used as reporter strain. The PCR fragments of the upstream and downstream regions of luxS and the erythromycin resistance gene were amplified with the primers resp., and these fragments were ligated into pUC19 vector with double endonuclease reaction sequentially, and the ligated DNAs were transformed into Escherichia coli DH5□, then the reconstructed plasmids wee isolated and identified by restricted endonuclease digestions. Streptococcus mutans Ingbritt C could induce luminescence of BB170, suggesting the presence of AI-2 quorum sensing pathway in Streptococcus mutans, and such stimulatory activity was maximal at the mid-log growth phase. The recombinant plasmid pUCluxKO was digested by PstI-BamHI, and the digested product were 1000bp and 5000bp. When the pUCluxKO was digested by BamHI-KpnI, the digested product were 1500bp and 4500bp. While it was digested by KpnI-EcoRI, the digested product were 1000bp and 5000bp. All PCR product was in a single belt resp. The recombinant plasmid was cloned effectively and can be used in the construction of S. mutans luxS mutant.

Bibliographic Information

Identification of boronic acids as antagonists of bacterial quorum sensing in Vibrio harveyi. Ni, Nanting; Chou, Han-Ting; Wang, Junfeng; Li, Minyong; Lu, Chung-Dar; Tai, Phang C.; Wang, Binghe. Department of Chemistry, Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA, USA. Biochemical and Biophysical Research Communications (2008), 369(2), 590-594. Publisher: Elsevier, CODEN: BBRCA9 ISSN: 0006-291X. Journal written in English. CAN 148:421357 AN 2008:369390 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacterial quorum sensing plays a very important role in the regulation of biofilm formation, virulence, conjugation, sporulation, and swarming mobility. Inhibitors of bacterial quorum sensing are important research tools and potential therapeutic agents. In this paper, we describe for the 1st time the discovery of several boronic acids as single digit micromolar inhibitors of bacterial quorum sensing in V. harveyi.

Bibliographic Information

Pyrogallol and its analogs can antagonize bacterial quorum sensing in Vibrio harveyi. Ni, Nanting; Choudhary, Gaurav; Li, Minyong; Wang, Binghe. Department of Chemistry, Georgia State University, Atlanta, GA, USA. Bioorganic & Medicinal Chemistry Letters (2008), 18(5), 1567-1572. Publisher: Elsevier Ltd., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. CAN 148:532992 AN 2008:324730 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria can coordinate community-wide behaviors through quorum sensing, i.e., the secretion and sensing of autoinducer (AI) mols. Bacterial quorum sensing is implicated in the regulation of pathol. relevant events such as biofilm formation, bacterial virulence, and drug resistance. Inhibitors of bacterial quorum sensing could therefore be useful therapeutics. Here, the authors report for the first time the discovery of several pyrogallol compds. as single digit micromolar inhibitors of bacterial quorum sensing in Vibrio harveyi.

Bibliographic Information

Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges. Mohamed, Naglaa M.; Cicirelli, Elisha M.; Kan, Jinjun; Chen, Feng; Fuqua, Clay; Hill, Russell T. Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA. Environmental Microbiology (2008), 10(1), 75-86. Publisher: Blackwell Publishing Ltd., CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 149:99299 AN 2008:254382 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Marine sponges are hosts to diverse and dense bacterial communities and thus provide a potential environment for quorum sensing. Quorum sensing, a key factor in cell-cell communication and bacterial colonization of higher animals, might be involved in the symbiotic interactions between bacteria and their sponge hosts. Given that marine Proteobacteria are known to produce N-acyl homoserine lactone (AHL) signal mols., we tested the prodn. of AHLs by Alpha- and Gammaproteobacteria isolated from marine sponges Mycale laxissima and Ircinia strobilina and the surrounding water column. We used three different AHL biodetection systems in diffusion assays: Chromobacterium violaceum, Agrobacterium tumefaciens and Sinorhizobium meliloti with optimal sensitivity to short-chain (C4-C6), moderate-chain (C8-C12) and long-chain (C14) AHLs resp. Thirteen of 23 isolates from M. laxissima and five of 25 isolates from I. strobilina were found to produce AHLs. Signals were detected from two of eight proteobacterial strains from the water column. Thin-layer chromatog. assays based on the A. tumefaciens reporter system were utilized to det. the AHL profiles of the pos. isolates. The types and amts. of AHLs synthesized varied considerably among the strains. Small ribosomal rRNA gene sequencing revealed that the AHL-producing alphaproteobacterial isolates were mainly from the Silicibacter-Ruegeria subgroup of the Roseobacter clade. Two-dimensional gel electrophoresis (2DGE)-based proteomic analyses were congruent with phylogenetic relationships but provided higher resoln. to differentiate these closely related AHL-producing strains.

Bibliographic Information

Platinum colloid for antibacterial regulation of quorum-sensing. Sato, Takuya; Nagao, Kiyoka. (G-C Corp., Japan). Jpn. Kokai Tokkyo Koho (2008), 4pp. CODEN: JKXXAF JP 2008043209 A 20080228 Patent written in Japanese. Application: JP 2006-219118 20060811. Priority: . CAN 148:233855 AN 2008:244807 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
JP 2008043209	A	20080228	JP 2006-219118	20060811

Priority Application	
JP 2006-219118	20060811

Abstract

The nano platinum colloid is useful for control of bacteria by down-regulation of quorum-sensing. The method prevents formation of resistant bacteria. It is useful for control of bacteria, esp. oral bacteria such as Streptococcus mutans by down-regulation of the signaling. Solvent for prepn. of the nano platinum colloid is selected from polyvinyl pyrrolidone, polyacrylic acid.

Bibliographic Information

Localized Quorum Sensing in Vibrio fischeri. Parent, Mary E.; Snyder, Charles E.; Kopp, Nathaniel D.; Velegol, Darrell. Department of Chemical Engineering, The Pennsylvania State University, University Park, PA, USA. Colloids and Surfaces, B: Biointerfaces (2008), 62(2),

180-187. Publisher: Elsevier B.V., CODEN: CSBBEQ ISSN: 0927-7765. Journal written in English. AN 2008:241042 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing is almost always regarded as a population d. effect in three-dimensional bulk samples of bacteria. Here we create two-dimensional samples of Vibrio fischeri cells adhered onto glass surfaces to examine the effect of local population densities on quorum sensing. This is done by measuring the luminescent response. The 2-D bacterial populations enable us to simultaneously account for time and distance effects on quorum sensing, which were previously very challenging to access in typical three-dimensional bulk samples. Thus, we are able to consider quorum sensing in terms of signal diffusion. A diffusion model of quorum sensing signals guides the expts. and shows that for a given cell spacing (d.) and diffusion time there exists a "true quorum"- a no. of cells necessary for quorum sensing. We find that quorum sensing can occur locally in 2-D surface samples and is a function of cell population d. as well as signal diffusion time.

Bibliographic Information

Quorum sensing and quorum quenching in Vibrio harveyi: lessons learned from in vivo work. Defoirdt, Tom; Boon, Nico; Sorgeloos, Patrick; Verstraete, Willy; Bossier, Peter. Laboratory of Microbial Ecology and Technology, Ghent University, Ghent, Belg. ISME Journal (2008), 2(1), 19-26. Publisher: Nature Publishing Group, CODEN: IJSOCF ISSN: 1751-7362. Journal; General Review written in English. CAN 148:512888 AN 2008:209221 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Luminescent vibrios, bacteria belonging to the species Vibrio harveyi and closely related species, are important pathogens in aquaculture that can affect almost all types of cultured animals. Due to large-scale use of antibiotics, many luminescent vibrios have acquired (multiple) resistance, which render antibiotic treatments ineffective. One of the alternative strategies that has recently been developed to control infections caused by antibiotic-resistant bacteria is the disruption of quorum sensing, bacterial cell-to-cell communication. The quorum sensing system of V. harveyi has been studied quite intensively in vitro. Recent studies have been directed towards understanding the impact of quorum sensing and quorum sensing disruption on the virulence of luminescent vibrios towards different host organisms in vivo. This mini-review aims at discussing the current knowledge of quorum sensing in luminescent vibrios in vivo. Subsequently, quorum quenching by halogenated furanones is discussed and finally, some directions for further research are presented.

Bibliographic Information

Oxazaborolidine derivatives inducing autoinducer-2 signal transduction in Vibrio harveyi. Aharoni, R.; Bronstheyn, M.; Jabbour, A.; Zaks, B.; Srebnik, M.; Steinberg, D. Institute of Dental Sciences, Faculty of Dental Medicine, Hebrew University-Hadassah, Jerusalem, Israel. Bioorganic & Medicinal Chemistry (2008), 16(4), 1596-1604. Publisher: Elsevier Ltd., CODEN: BMECEP ISSN: 0968-0896. Journal written in English. CAN 148:373873 AN 2008:207060 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The bioluminescence of the marine bacterium Vibrio harveyi is controlled by quorum sensing. This effect is mediated by prodn., accumulation, and auto-detection of the species-specific autoinducer 1 (AI-1), autoinducer 2 (AI-2), and the V. cholerae autoinducer 1 (CAI-1). The V. harveyi AI-2 was recently identified as furanosyl borate diester. The authors synthesized several oxazaborolidine derivs. that chem. resemble the structure of AI-2. Five oxazaborolidine derivs. (BNO-1 to BNO-5) were tested, however only BNO-1 (3,4-dimethyl-2,5-diphenyl-1,3,2-oxazaborolidine), and BNO-5 (2-butyl-3,4-dimethyl-5-phenyl-1,3,2-oxazaborolidine) strongly induced V. harveyi bioluminescence in V. harveyi mutant (BB170) lacking sensor 1. A dose-dependent relationship between those oxazaborolidine derivs. and bioluminescence induction was obsd. with this V. harveyi strain (BB170). BNO-1 and BNO-5 did not affect V. harveyi BB886 lacking sensor 2. Using a mutant strain which produces neither AI-1 nor AI-2 (V. harveyi MM77) the authors showed that the presence of spent medium contg. AI-2 is essential for BNO-1 and BNO-5 activity. This effect was similar when introducing the spent medium and the BNOs together or at a 3-h interval. A comparable induction of bioluminescence was obsd. when using synthetic DPD (pre-AI-2) in the presence of BNO-1 or BNO-5. The mode of action of BNO-1 and BNO-5 on bioluminescence of V. harveyi is of a co-agonist category. BNO-1 and BNO-5 enhanced AI-2 signal transduction only in the presence of AI-2 and only via sensor 2 cascade. BNO-1 and BNO-5 are the first oxazaborolidines reported to affect AI-2 activity. Those derivs. represent a new class of borates which may become prototypes of novel agonists of quorum sensing mediated by AI-2 in V. harveyi.

Bibliographic Information

The evolution of quorum sensing in bacterial biofilms. Nadell, Carey D.; Xavier, Joao B.; Levin, Simon A.; Foster, Kevin R. Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA. PLoS Biology (2008), 6(1), 171-179. Publisher: Public Library of Science, CODEN: PBLIBG ISSN: 1545-7885. http://biology.plosjournals.org/archive/1545-7885/6/1/pdf/10.1371_1545-7885_6_1_complete.pdf Journal; Online Computer File written in English. CAN 148:303322 AN 2008:180612 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria have fascinating and diverse social lives. They display coordinated group behaviors regulated by quorum sensing systems that detect the d. of other bacteria around them. A key example of such group behavior is biofilm formation, in which communities of cells attach to a surface and envelope themselves in secreted polymers. Curiously, after reaching high cell d., some bacterial species activate polymer secretion, whereas others terminate polymer secretion. Here, the authors investigate this striking variation in the first evolutionary model of quorum sensing in biofilms. They use detailed individual-based simulations to investigate evolutionary competitions between strains that differ in their polymer prodn. and quorum-sensing phenotypes. The benefit of activating polymer secretion at high cell d. is relatively straightforward: secretion starts upon biofilm formation, allowing strains to push their lineages into nutrient-rich areas and suffocate neighboring cells. But why use quorum sensing to terminate polymer secretion at high cell d. The authors find that deactivating polymer prodn. in biofilms can yield an advantage by redirecting resources into growth, but that this advantage occurs only in a limited time window. They predict, therefore, that down-regulation of polymer secretion at high cell d. will evolve when it can coincide with dispersal events, but it will be disfavored in long-lived (chronic) biofilms with sustained competition among strains. Our model suggests that the obsd. variation in quorum-sensing

behavior can be linked to the differing requirements of bacteria in chronic vs. acute biofilm infections. This is well illustrated by the case of Vibrio cholerae, which competes within biofilms by polymer secretion, terminates polymer secretion at high cell d., and induces an acute disease course that ends with mass dispersal from the host. More generally, this work shows that the balance of competition within and among biofilms can be pivotal in the evolution of quorum sensing.

Bibliographic Information

A negative feedback loop involving small RNAs accelerates Vibrio cholerae's transition out of quorum-sensing mode. Svenningsen, Sine L.; Waters, Christopher M.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Genes & Development (2008), 22(2), 226-238. Publisher: Cold Spring Harbor Laboratory Press, CODEN: GEDEEP ISSN: 0890-9369. Journal written in English. CAN 148:303312 AN 2008:117811 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing is a cell-to-cell communication process that allows bacteria to measure their population nos. and to synchronously alter gene expression in response to changes in cell population d. At the core of the Vibrio cholerae quorum-sensing signal transduction pathway lie four redundant small RNAs (sRNAs), named the Quorum Regulatory RNAs (Qrr1-4). Expression of qrr1-4 is cell population d.-dependent due to a requirement for the quorum-sensing controlled phosphorylated response regulator LuxO-P, which is abundant only at low cell population d. When expressed, Qrr1-4 repress translation of HapR, the "master" quorum-sensing transcription factor. Here we show a neg. feedback loop in which HapR activates transcription of the qrr genes, which indirectly leads to hapR repression. Efficient feedback activation of the qrr genes requires the simultaneous presence of LuxO-P (present only at low cell population d.) and HapR (present only at high cell population d.). For this reason, the feedback loop does not influence quorum sensing at steady-state low or high cell population d. However, LuxO-P and HapR are simultaneously present immediately following the switch from high to low cell d. conditions. In this state, the HapR feedback loop dramatically accelerates V. cholerae's transition from the high to the low cell d. mode.

Bibliographic Information

The biological characteristics, epidemiology and detection techniques of Vibrio harveyi. Zhang, Xiao-Hua; Zhong, Ying-Bin; Chen, Ji-Xiang. College of Marine Life Sciences, Ocean University of China, Qingdao, Peop. Rep. China. Zhongguo Haiyang Daxue Xuebao, Ziran Kexueban (2007), 37(5), 740-748. Publisher: Zhongguo Haiyang Daxue Xuebao Bianjibu, CODEN: ZHDXB3 ISSN: 1672-5174. Journal; General Review written in Chinese. CAN 149:262240 AN 2008:84442 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review. Vibrio harveyi is a gram-neg., luminous, marine bacterium, and it is ubiquitous in marine habitats. It was only recognized as the major causal agent of aquaculture animals in recent decades. In this paper, the biol. characteristics, epidemiol., pathogenicity mechanisms, quorum sensing system, detection techniques, disease control and applications of Vibrio harveyi was discussed.

Bibliographic Information

The Cyclic AMP receptor protein modulates colonial morphology in Vibrio cholerae. Liang, Weili; Silva, Anisia J.; Benitez, Jorge A. Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA, USA. Applied and Environmental Microbiology (2007), 73(22), 7482-7487. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 148:233805 AN 2008:75842 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Inactivation of the quorum-sensing regulator HapR causes Vibrio cholerae El Tor biotype strain C7258 to adopt a rugose colonial morphol. that correlates with enhanced biofilm formation. V. cholerae mutants lacking the cAMP receptor protein (CRP) produce very little HapR, which results in elevated expression of Vibrio exopolysaccharide (vps) genes and biofilm compared to the wild type. However, □crp mutants still exhibited smooth colonial morphol. and expressed reduced levels of vps genes compared to isogenic hapR mutants. In this study we demonstrate that deletion of crp and cya (adenylate cyclase) converts a rugose □hapR mutant to a smooth one. The smooth □hapR □crp and □hapR □cya double mutants could be converted back to rugose by complementation with crp and cya, resp. CRP was found to enhance the expression of VpsR, a strong activator of vps expression, but to diminish transcription of VpsT. Ectopic expression of VpsR in smooth □hapR □crp and □hapR □cya double mutants restored rugose colonial morphol. Lowering intracellular cAMP levels in a □hapR mutant by the addn. of glucose diminished VpsR expression and colonial rugosity. On the basis of our results, we propose a model for the regulatory input of CRP on exopolysaccharide biosynthesis.

Bibliographic Information

Disruption of quorum sensing in Vibrio harveyi by the AiiA protein of Bacillus thuringiensis. Bai, Fangfang; Han, Yin; Chen, Jixiang; Zhang, Xiao-Hua. Department of Marine Biology, Ocean University of China, Qingdao, Peop. Rep. China. Aquaculture (2008), 274(1), 36-40. Publisher: Elsevier B.V., CODEN: AQCLAL ISSN: 0044-8486. Journal written in English. CAN 149:99009 AN 2008:15258 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing is a mechanism in which bacteria coordinate the expression of certain genes in response to their population d. by producing, releasing and detecting signal mols. called autoinducers. Quorum sensing is responsible for controlling a plethora of virulence genes in several bacterial pathogens. Disruption of the quorum sensing system of Vibrio harveyi has been proposed as a new anti-infective strategy. AiiA is a protein which can block the bacterial quorum sensing by hydrolyzing AHL-lactone, and could greatly attenuate the disease caused by many bacterial pathogens in which quorum sensing regulate the expression of virulence genes. In this study, primers were designed from the conserved sequences of aiiA gene in the genomes of several Bacillus strains, and the gene was amplified from Bacillus thuringiensis BF1 by PCR. AiiA gene was cloned to a cloning vector pUC and sequenced. The open reading frame of the aiiA gene was 753 bp, and the similarity of aiiA gene from B. thuringiensis BF1 to other sequences in the GenBank was as high as 99%. This gene was subsequently cloned into an expression vector pET-24d(+), and the recombinant AiiA protein was overexpressed in Escherichia coli overexpression strain BL21(DE3). The mol. wt. of the expressed AiiA protein was estd. to be 28 kDa by SDS-PAGE. The optimized expression condition for the recombinant AiiA protein was at 25 □C for 6 h

with 1 mM IPTG induction. The lysate of the recombinant E. coli could not only repress the pigment synthesize of the quorum sensing system reporter strain Chromobacterium violaceum ATCC 12472, but also attenuate the intensity of bioluminescence of V. harveyi VIB391 by 85%. This study was very important for further research on the disruption of infections caused by V. harveyi in aquaculture.

Bibliographic Information

The Legionella pneumophila response regulator LqsR promotes host cell interactions as an element of the virulence regulatory network controlled by RpoS and LetA. Tiaden, Andre; Spirig, Thomas; Weber, Stefan S.; Bruggemann, Holger; Bosshard, Rachel; Buchrieser, Carmen; Hilbi, Hubert. Institute of Microbiology, ETH Zurich, Zurich, Switz. Cellular Microbiology (2007), 9(12), 2903-2920. Publisher: Blackwell Publishing Ltd., CODEN: CEMIF5 ISSN: 1462-5814. Journal written in English. CAN 148:279867 AN 2007:1431390 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Legionella pneumophila is an opportunistic human pathogen that replicates within environmental amoebae including Acanthamoeba castellanii and Dictyostelium discoideum. The Icm/Dot type IV secretion system promotes phagocytosis and intracellular replication of L. pneumophila in an endoplasmic reticulum-derived 'Legionella-contg. vacuole' (LCV). L. pneumophila adopts a biphasic life cycle consisting of a replicative growth phase and a transmissive (stationary) phase, the latter of which is characterized by the preferential expression of genes required for motility and virulence. A bioinformatic anal. of the L. pneumophila genome revealed a gene cluster homologous to the Vibrio cholerae cqsAS genes, encoding a putative quorum sensing autoinducer synthase (IqsA) and a sensor kinase (IqsS), which flank a novel response regulator (IqsR). We report here that an L. pneumophila IqsR deletion mutant grew in broth with the same rate as wild-type bacteria, but entered the replicative growth phase earlier. Overexpression of IqsR led to an elongated morphol. of the bacteria. The IqsR mutant strain was found to be more salt-resistant and impaired for intracellular growth in A. castellanii, D. discoideum and macrophages, formation of the ER-derived LCV and toxicity. Moreover, L. pneumophila lacking LqsR, as well as strains lacking the stationary sigma factor RpoS or the two-component response regulator LetA, were phagocytosed less efficiently by A. castellanii, D. discoideum or macrophages. The expression of IqsR was dependent on RpoS and, to a lesser extent, also on LetA. DNA microarray expts. revealed that IqsR regulates the expression of genes involved in virulence, motility and cell division, consistent with a role for LqsR in the transition from the replicative to the transmissive (virulent) phase. Our findings indicate that LqsR is a novel pleiotropic regulator involved in RpoS- and LetA-controlled interactions of L. pneumophila with phagocytes.

Bibliographic Information

Identification of poultry meat-derived fatty acids functioning as quorum sensing signal inhibitors to autoinducer-2 (AI-2). Widmer, K. W.; Soni, K. A.; Hume, M. E.; Beier, R. C.; Jesudhasan, P.; Pillai, S. D. Food Safety and Environmental Microbiology Program, Dept. of Poultry Science and Nutrition & Food Science, Texas A and M Univ., College Station, TX, USA. Journal of Food Science (2007), 72(9), M363-M368. Publisher: Blackwell Publishing, Inc., CODEN: JFDSAZ ISSN: 0022-1147. Journal written in English. CAN 148:283461 AN 2007:1426761 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Autoinducer-2 (AI-2) is a compd. that plays a key role in bacterial cell-to-cell communication (quorum sensing). Previous research has shown certain food matrixes inhibit this signaling compd. Using the reporter strain, Vibrio harveyi BB170, quorum-sensing inhibitors contained in poultry meat wash (PMW) samples were characterized by mol. wt. and hydrophobic properties using liq. chromatog. systems. Most fractions that demonstrated AI-2 inhibition were 13.7 kDa or less, and had hydrophobic properties. Hexane was used to ext. inhibitory compds. from a PMW prepn. and the ext. was further sepd. by gas chromatog. (GC). Several fatty acids were identified and quantified. Linoleic acid, oleic acid, palmitic acid, and stearic acid were each tested for inhibition at 0.1, 1, and 10 mM concns. All samples expressed AI-2 inhibition (ranging from .apprx.25% to 99%). Fatty acids, combined in concns. equiv. to those detd. by GC anal., expressed inhibition at 59.5%, but higher combined concns. (10- and 100-fold) had inhibition at 84.4% and 69.5%, resp. The combined fatty acids (100-fold) did not demonstrate a substantial decrease in colony plate counts, despite presenting high AI-2 inhibition. These fatty acids, through modulating quorum sensing by inhibition, may offer a unique means to control food-borne pathogens and reduce microbial spoilage.

Bibliographic Information

LuxO controls extracellular protease, haemolytic activities and siderophore production in fish pathogen Vibrio alginolyticus. Wang, Q.; Liu, Q.; Ma, Y.; Rui, H.; Zhang, Y. State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, Peop. Rep. China. Journal of Applied Microbiology (2007), 103(5), 1525-1534. Publisher: Blackwell Publishing Ltd., CODEN: JAMIFK ISSN: 1364-5072. Journal written in English. CAN 148:418850 AN 2007:1406265 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Aims: To characterize the luxO gene in fish pathogen Vibrio alginolyticus MVP01 and investigate its roles in regulation of extracellular products (ECP) and siderophore prodn. Methods and Results: The luxO gene was cloned from V. alginolyticus MVP01. Genetic anal. revealed that it encoded a protein with high similarity to other LuxO homologues. The luxO in-frame deletion mutant and rpoN null mutant were constructed with suicide plasmids. We demonstrated that sole deletion in LuxO increased the secretion of extracellular protease and hemolytic products, but decreased siderophore prodn. for V. alginolyticus MVP01. Mutants with null rpoN displayed significantly enhanced protease level and siderophore prodn. while notable redn. in hemolytic activities of ECP. Conclusions: Vibrio alginolyticus harbors functional luxO gene that regulates the secretion of extracellular protease and hemolytic materials as well as siderophore prodn. in either □54 dependent or independent manners. Significance and Impact of the Study: The current study demonstrated that V. alginolyticus MVP01 produces extracellular protease and hemolytic activity material as well as siderophore, which may be characteristics of the virulence of the strain. Revelations that secretion of these products is under the regulation of LuxO and □54 as well as the potential quorum sensing systems in V. alginolyticus MVP01 will expedite the understanding of vibriosis pathogenesis.

Bibliographic Information

The major Vibrio cholerae autoinducer and its role in virulence factor production. Higgins, Douglas A.; Pomianek, Megan E.; Kraml, Christina M.; Taylor, Ronald K.; Semmelhack,

Martin F.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Nature (London, United Kingdom) (2007), 450(7171), 883-886. Publisher: Nature Publishing Group, CODEN: NATUAS ISSN: 0028-0836. Journal written in English. CAN 148:186685 AN 2007:1393373 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio cholerae, the causative agent of the human disease cholera, uses cell-to-cell communication to control pathogenicity and biofilm formation. This process, known as quorum sensing, relies on the secretion and detection of signaling mols. called autoinducers. At low cell d. V. cholerae activates the expression of virulence factors and forms biofilms. At high cell d. the accumulation of two quorum-sensing autoinducers represses these traits. These two autoinducers, cholerae autoinducer-1 (CAI-1) and autoinducer-2 (AI-2), function synergistically to control gene regulation, although CAI-1 is the stronger of the two signals. V. cholerae AI-2 is the furanosyl borate diester (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate. Here the authors describe the purifn. of CAI-1 and identify the mol. as (S)-3-hydroxytridecan-4-one, a new type of bacterial autoinducer. The authors provide a synthetic route to both the R and S isomers of CAI-1 as well as simple homologs, and the authors evaluate their relative activities. Synthetic (S)-3-hydroxytridecan-4-one functions as effectively as natural CAI-1 in repressing prodn. of the canonical virulence factor TCP (toxin co-regulated pilus). These findings suggest that CAI-1 could be used as a therapy to prevent cholera infection and, furthermore, that strategies to manipulate bacterial quorum sensing hold promise in the clin. arena.

Bibliographic Information

Transcriptome analysis of the Vibrio fischeri LuxR-LuxI regulon. Antunes, Luis Caetano M.; Schaefer, Amy L.; Ferreira, Rosana B. R.; Qin, Nan; Stevens, Ann M.; Ruby, Edward G.; Greenberg, E. Peter. Department of Microbiology, University of Iowa, Iowa City, IA, USA. Journal of Bacteriology (2007), 189(22), 8387-8391. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 148:136978 AN 2007:1382573 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The Vibrio fischeri quorum-sensing signal N-3-oxohexanoyl-L-homoserine lactone (3OC6-HSL) activates expression of the seven-gene luminescence operon. We used microarrays to unveil 18 addnl. 3OC6-HSL-controlled genes, 3 of which had been identified by other means previously. We show most of these genes are regulated by the 3OC6-HSL-responsive transcriptional regulator LuxR directly. This demonstrates that V. fischeri quorum sensing regulates a substantial no. of genes other than those involved in light prodn.

Bibliographic Information

The natural furanone (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone disrupts quorum sensing-regulated gene expression in Vibrio harveyi by decreasing the DNA-binding activity of the transcriptional regulator protein luxR. Defoirdt, Tom; Miyamoto, Carol M.; Wood, Thomas K.; Meighen, Edward A.; Sorgeloos, Patrick; Verstraete, Willy; Bossier, Peter. Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belg. Environmental Microbiology (2007), 9(10), 2486-2495. Publisher: Blackwell Publishing Ltd.,

CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 148:350726 AN 2007:1259213 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
This study aimed at getting a deeper insight in the mol. mechanism by which the natural furanone (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone disrupts quorum sensing in Vibrio harveyi. Bioluminescence expts. with signal mol. receptor double mutants revealed that the furanone blocks all three channels of the V. harveyi quorum sensing system. In further expts. using mutants with mutations in the quorum sensing signal transduction pathway, the compd. was found to block quorum sensing-regulated bioluminescence by interacting with a component located downstream of the Hfq protein. Furthermore, reverse transcriptase real-time polymerase chain reaction with specific primers showed that there was no effect of the furanone on luxRVh mRNA levels in wild-type V. harveyi cells. In contrast, mobility shift assays showed that in the presence of the furanone, significantly lower levels of the LuxRVh response regulator protein were able to bind to its target promoter sequences in wild-type V. harveyi. Finally, tests with purified LuxRVh protein also showed less shifts with furanone-treated LuxRVh, whereas the LuxRVh concn. was found not to be altered by the furanone (as detd. by SDS-PAGE). Therefore, our data indicate that the furanone blocks quorum sensing in V. harveyi by rendering the quorum sensing master regulator protein LuxRVh unable to bind to the promoter sequences of quorum sensing-regulated genes.

Bibliographic Information

Methods in cell-to-cell signaling in Salmonella. Ahmer, Brian M. M.; Smith, Jenee N.; Dyszel, Jessica L.; Lindsay, Amber. Department of Microbiology, Ohio State University, Columbus, OH, USA. Methods in Molecular Biology (Totowa, NJ, United States) (2007), 394(Salmonella), 307-322. Publisher: Humana Press Inc., CODEN: MMBIED ISSN: 1064-3745. Journal written in English. CAN 148:95649 AN 2007:1214088 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Many bacteria can sense their population d. This has been termed "quorum sensing.". The bacteria use this information to coordinate their behavior, essentially behaving as multicellular organisms. The paradigm of Gram-neg. quorum sensing is the LuxI/LuxR-type system employed by Vibrio fischeri to regulate luminescence. The LuxR transcription factor detects the presence of N-acylhomoserine lactones (AHLs) produced by LuxI. The AHL diffuses freely across the cell wall, and its accumulation signals a high population d. within a confined space. Upon binding AHL, the LuxR transcription factor activates the luminescence genes. Homologous systems are used by numerous Gram-neg. pathogens to regulate host interaction genes. The AHLs produced by different LuxI homologs can vary in the length and modification of their acyl side chain. In the first section of this chapter, we describe the use of bacterial biosensors to det. whether a particular bacterial species synthesizes AHLs. The second section describes how to identify AHL-responsive genes in Salmonella typhimurium, an organism that detects but does not synthesize AHLs. The approach described can be modified for use with any organism that responds to AHLs but does not synthesize them. The third section describes the use of recombination-based in vivo expression technol. (RIVET) to study AHL detection in vitro and in vivo, in this case the mouse gut.

Bibliographic Information

Interacting microhabitat array for microorganisms and uses thereof. Keymer, Juan; Galajda, Peter; Austin, Robert H. (USA). U.S. Pat. Appl. Publ. (2007), 19pp. CODEN: USXXCO US 2007243572 A1 20071018 Patent written in English. Application: US 2007-623595 20070116. Priority: US 2006-759607 20060117; US 2006-849076 20061003. CAN 147:443113 AN 2007:1175717 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
US 20070243572	A1	20071018	US 2007-623595	20070116
Priority Application				
US 2006-759607P	P	20060117		
US 2006-849076P	P	20061003		

Abstract
The invention is directed to an interacting microhabitat array (IMA) for microorganisms having more than one microhabitat in a substrate in which at least two microhabitats are connected in series by at least one corridor. The corridor is of sufficient size to allow the microorganism to move between microhabitats in a restricted manner. The invention is also directed to uses of the device for screening for method of modulating biofilms and identifying drug candidates. An IMA having 0.1 □m deep nanofeed channels was used to study Synechocystis PCC 6803, engineered to express green fluorescent protein. The IMA was inoculated at a very low d. of 10 bacteria per microhabitat. The population dynamics were complex in that bacteria moved between chambers in both wave-like and chaotic manner.

Bibliographic Information

The cyclic AMP receptor protein modulates quorum sensing, motility and multiple genes that affect intestinal colonization in Vibrio cholerae. Liang, Weili; Pascual-Montano, Alberto; Silva, Anisia J.; Benitez, Jorge A. Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA, USA. Microbiology (Reading, United Kingdom) (2007), 153(9), 2964-2975. Publisher: Society for General Microbiology, CODEN: MROBEO ISSN: 1350-0872. Journal written in English. CAN 148:49321 AN 2007:1168600 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio cholerae is the causative agent of cholera, which continues to be a major public health concern in Asia, Africa and Latin America. The bacterium can persist outside the human host and alternates between planktonic and biofilm community lifestyles. Transition between the different lifestyles is mediated by multiple signal transduction pathways including quorum sensing. Expression of the Zn-metalloprotease hemagglutinin (HA)/protease is subject to a dual regulation which involves the quorum sensing regulator HapR and the cAMP receptor protein. In a previous study, the authors obsd. that a mutant defective in the cAMP-receptor protein (CRP) expressed lower levels of HapR. To further investigate the role of CRP in modulating HapR and other signal transduction pathways, they performed global gene expression profiling of a □crp mutant of El Tor biotype V. cholerae. Here, they show that CRP is required for the biosynthesis of cholera autoinducer 1 (CAI-1) and affects the expression of multiple HapR-regulated genes. As expected, the □crp mutant produced more cholera toxin and enhanced biofilm. Expression of flagellar genes,

reported to be affected in □hapR mutants, was diminished in the □crp mutant. However, an epistasis anal. indicated that cAMP-CRP affects motility by a mechanism independent of HapR. Inactivation of crp inhibited the expression of multiple genes reported to be strongly induced in vivo and to affect the ability of V. cholerae to colonize the small intestine and cause disease. These genes included ompU, ompT and ompW encoding outer membrane proteins, the alternative sigma factor □E required for intestinal colonization, and genes involved in anaerobic energy metab. The results indicate that CRP plays a crucial role in the V. cholerae life cycle by affecting quorum sensing and multiple genes required for survival of V. cholerae in the human host and the environment.

Bibliographic Information

N-acyl homoserine lactone-degrading microbial enrichment cultures isolated from Penaeus vannamei shrimp gut and their probiotic properties in Brachionus plicatilis cultures. Tinh, Nguyen Thi Ngoc; Gunasekara, R. A. Y. S. Asanka; Boon, Nico; Dierckens, Kristof; Sorgeloos, Patrick; Bossier, Peter. Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belg. FEMS Microbiology Ecology (2007), 62(1), 45-53. Publisher: Blackwell Publishing Ltd., CODEN: FMECEZ ISSN: 0168-6496. Journal written in English. CAN 148:73746 AN 2007:1137432 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Three bacterial enrichment cultures (ECs) were isolated from the digestive tract of Pacific white shrimp Penaeus vannamei, by growing the shrimp microbial communities in a mixt. of N-acyl homoserine lactone (AHL) mols. The ECs, characterized by denaturing gradient gel electrophoresis anal. and subsequent rRNA sequencing, degraded AHL mols. in the degrdn. assays. Apparently, the resting cells of the ECs also degraded one of the three types of quorum-sensing signal mols. produced by Vibrio harveyi in vitro [i.e. harveyi autoinducer 1 (HAI-1)]. The most efficient AHL-degrading ECs, EC5, was tested in Brachionus expts. EC5 degraded the V. harveyi HAI-1 autoinducer in vivo, neutralizing the neg. effect of V. harveyi autoinducer 2 (AI-2) mutant, in which only the HAI-1- and CAI-1-mediated components of the quorum-sensing system are functional on the growth of Brachionus. This suggests that EC5 interferes with HAI-1-regulated metab. in V. harveyi. These AHL-degrading ECs need to be tested in other aquatic systems for their probiotic properties, preferably in combination with specific AI-2-degrading bacteria.

Bibliographic Information

Directed evolution of Vibrio fischeri LuxR for improved response to butanoyl-homoserine lactone. Hawkins, Andrew C.; Arnold, Frances H.; Stuermer, Rainer; Hauer, Bernhard; Leadbetter, Jared R. Environmental Science & Engineering, California Institute of Technology, Pasadena, CA, USA. Applied and Environmental Microbiology (2007), 73(18), 5775-5781. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 147:498551 AN 2007:1093652 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
LuxR is the 3-oxohexanoyl-homoserine lactone (3OC6HSL)-dependent transcriptional activator of the prototypical acyl-homoserine lactone (AHL) quorum-sensing system of Vibrio fischeri. Wild-type LuxR exhibits no response to butanoyl-HSL (C4HSL) in quant. bioassays at concns. of up to 1 □M; a previously described LuxR variant (LuxR-G2E) exhibits a broadened response to diverse

AHLs, including pentanoyl-HSL (C5HSL), but not to C4HSL. Here, two rounds of directed evolution of LuxR-G2E generated variants of LuxR that responded to C4HSL at concns. as low as 10 nM. One variant, LuxR-G4E, had only one change, I45F, relative to the parent LuxR-G2E, which itself differs from the wild type at three residues. Dissection of the four mutations within LuxR-G4E demonstrated that at least three of these changes were simultaneously required to achieve any measurable C4HSL response. The four changes improved both sensitivity and specificity towards C4HSL relative to any of the other 14 possible combinations of those residues. These data confirm that LuxR is evolutionarily pliable and suggest that LuxR is not intrinsically asym. in its response to quorum-sensing signals with different acyl-side-chain lengths.

Bibliographic Information

Cell-to-cell communication in bacteria: a chemical discourse. Bassler, Bonnie L. Howard Hughes Medical Institute and Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Harvey Lectures (2006), 100 123-142. Publisher: Wiley-Liss, Inc., CODEN: HALEAA ISSN: 0073-0874. Journal; General Review written in English. CAN 148:116358 AN 2007:1073489 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. A review on cell-cell communication, or quorum sensing, in bacteria. The canonical quorum-sensing systems are discussed, along with the multichannel quorum-sensing circuits of Vibrio harveyi and V. cholerae, role of small RNAs in triggering quorum-sensing response, and intra- and interspecies bacterial communication.

Bibliographic Information

Interference with the quorum sensing systems in a Vibrio harveyi strain alters the growth rate of gnotobiotically cultured rotifer Brachionus plicatilis. Tinh, N. T. N.; Linh, N. D.; Wood, T. K.; Dierckens, K.; Sorgeloos, P.; Bossier, P. Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belg. Journal of Applied Microbiology (2007), 103(1), 194-203. Publisher: Blackwell Publishing Ltd., CODEN: JAMIFK ISSN: 1364-5072. Journal written in English. CAN 148:326316 AN 2007:887947 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Aims: To evaluate the effect of Vibrio harveyi strains on the growth rate of the gnotobiotically cultured rotifer Brachionus plicatilis, and to establish whether quorum sensing is involved in the obsd. phenomena. Methods and Results: Gnotobiotic B. plicatilis sensu strictu, obtained by hatching glutaraldehyde-treated amictic eggs, were used as test organisms. Challenge tests were performed with 11 V. harveyi strains and different quorum sensing mutants derived from the V. harveyi BB120 strain. Brominated furanone [(5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone] as a quorum sensing inhibitor was tested in Brachionus challenge tests. Some V. harveyi strains, such as strain BB120, had a significantly neg. effect on the Brachionus growth rate. In the challenge test with MM77, an isogenic strain of BB120 in which the two autoinducers (HAI-1 and AI-2) are both inactivated, no neg. effect was obsd. The effect of single mutants was the same as that obsd. in the BB120 strain. This indicates that both systems are responsible for the growth-retarding (GR) effect of the BB120 strain towards Brachionus. Moreover, the addn. of an exogenous source of HAI-1 or AI-2 could restore the GR effect in the HAI-1 and AI-2 nonproducing mutant MM77. The addn. of brominated furanone at a concn. of 2□5 mg l-1 could

neutralize the GR effect of some strains such as BB120 and VH-014. Conclusions: Two quorum sensing systems in V. harveyi strain BB120 (namely HAI-1 and AI-2-mediated) are necessary for its GR effect on B. plicatilis. With some other V. harveyi strains, however, growth inhibition towards Brachionus does not seem to be related to quorum sensing. Significance and Impact of the Study: Interference with the quorum sensing system might help to counteract the GR effect of some V. harveyi strains on Brachionus. However, further studies are needed to demonstrate the pos. effect of halogenated furanone in nongnotobiotic Brachionus cultures and eventually, in other segments of the aquaculture industry.

Bibliographic Information

3-Hydroxy-4-tridecanone is the quorum sensing small molecule CAI-1 that controls virulence in Vibrio cholerae. Higgins, Douglas A.; Pomianek, Megan E.; Kraml, Christina M.; Taylor, Ronald K.; Semmelhack, Martin F.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Abstracts of Papers, 234th ACS National Meeting, Boston, MA, United States, August 19-23, 2007 (2007), MEDI-014. Publisher: American Chemical Society, Washington, D. C CODEN: 69JNR2 Conference; Meeting Abstract; Computer Optical Disk written in English. AN 2007:883380 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing is a process by which groups of bacteria control collective behaviors according to population d. Many of these collective behaviors, including virulence factor prodn. and biofilm formation, play significant roles in the development of infection in human hosts. Such is the case with the bacterium Vibrio cholerae, the causative agent of the disease cholera. To date, the chem. structure of V. cholerae autoinducer-1 (CAI-1) that mediates System 1 quorum sensing in this species has not been identified. In this work, we det. the structure of CAI-1, as isolated from cell supernatants, to be 3-hydroxy-4-tridecanone. We implement a straightforward synthetic strategy to easily access CAI-1 and a variety of structural analogs. Using chem.-synthesized CAI-1, we show that this small mol. initiates quorum sensing behavior in V. cholerae and has a pronounced effect on the formation of the toxin-coregulated pilus crucial to V. cholerae virulence. Our results demonstrate that CAI-1, a small mol. of a novel structural type for bacterial autoinducers, exerts direct control of virulence in V. cholerae through the System 1 quorum sensing circuit.

Bibliographic Information

Crystal structure of the Vibrio cholerae quorum-sensing regulatory protein HapR. De Silva, Rukman S.; Kovacikova, Gabriela; Lin, Wei; Taylor, Ronald K.; Skorupski, Karen; Kull, F. Jon. Department of Chemistry, Dartmouth College, Hanover, NH, USA. Journal of Bacteriology (2007), 189(15), 5683-5691. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 147:337954 AN 2007:864890 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing in Vibrio cholerae involves signaling between two-component sensor protein kinases and the response regulator LuxO to control the expression of the master regulator HapR. HapR, in turn, plays a central role in regulating a no. of important processes, such as virulence gene expression and biofilm formation. We have detd. the crystal structure of HapR to 2.2-.ANG. resoln. Its structure reveals a dimeric, two-domain mol. with an all-helical structure that is strongly

conserved with members of the TetR family of transcriptional regulators. The N-terminal DNA-binding domain contains a helix-turn-helix DNA-binding motif and alteration of certain residues in this domain completely abolishes the ability of HapR to bind to DNA, alleviating repression of both virulence gene expression and biofilm formation. The C-terminal dimerization domain contains a unique solvent accessible tunnel connected to an amphipathic cavity, which by analogy with other TetR regulators, may serve as a binding pocket for an as-yet-unidentified ligand.

Bibliographic Information

Regulatory small RNAs circumvent the conventional quorum sensing pathway in pandemic Vibrio cholerae. Hammer, Brian K.; Bassler, Bonnie L. Department of Molecular Biology, and Howard Hughes Medical Institute, Princeton University, Princeton, NJ, USA. Proceedings of the National Academy of Sciences of the United States of America (2007), 104(27), 11145-11149. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 147:295670 AN 2007:795360 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Using a process called quorum sensing (QS), bacteria communicate with extracellular signal mols. called auto-inducers (AIs). Response to Als allows bacteria to coordinate gene expression on a population-wide scale and thereby carry out particular behaviors in unison, much like multicellular organisms. In Vibrio cholerae El Tor, the etiol. agent of the current cholera pandemic, AI information is transduced internally through a phospho-relay circuit that impinges on the transcription of multiple small regulatory RNAs (sRNAs). These RNAs base-pair with, and repress the translation of, the mRNA encoding the master transcriptional regulator HapR. In V. cholerae, HapR controls virulence factor expression and biofilm formation. Here we identify a sRNA-dependent, HapR-independent QS pathway in which the sRNAs base-pair with a new target mRNA and activate translation by preventing formation of a translation-inhibiting stem-loop structure. We show that the classical V. cholerae strain, which caused previous pandemics and is reportedly incapable of QS because of a nonfunctional HapR, nonetheless exhibits QS-controlled gene expression through this new HapR-independent pathway.

Bibliographic Information

N-Phenylacetanoyl-L-homoserine lactones can strongly antagonize or superagonize quorum sensing in Vibrio fischeri. [Erratum to document cited in CA147:090792]. Geske, Grant D.; O'Neill, Jennifer C.; Blackwell, Helen E. Dep. Chemistry, Univ. Wisconsin-Madison, Madison, WI, USA. ACS Chemical Biology (2007), 2(6), 426. Publisher: American Chemical Society, CODEN: ACBCCT ISSN: 1554-8929. Journal written in English. CAN 151:27827 AN 2007:651117 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In the version published online on May 4, 2007, the DOI was incorrectly given. The online version has been cor. as of May 22, 2007. This error does not affect the scientific integrity of the article.

Bibliographic Information

Quorum sensing enhances the stress response in Vibrio cholerae. Joelsson, Adam; Kan, Biao; Zhu, Jun. Department of Microbiology, University of Pennsylvania School of Medicine,

Philadelphia, PA, USA. Applied and Environmental Microbiology (2007), 73(11), 3742-3746. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 147:184444 AN 2007:636785 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio cholerae lives in aquatic environments and causes cholera. Quorum sensing enhances V. cholerae viability under certain stress conditions by upregulating the expression of RpoS, and this regulation acts through HapR, suggesting that a quorum-sensing-enhanced stress response plays a role in V. cholerae environmental survival.

Bibliographic Information

The quorum sensing regulator HapR downregulates the expression of the virulence gene transcription factor AphA in Vibrio cholerae by antagonizing Lrp- and VpsR-mediated activation. Lin, Wei; Kovacikova, Gabriela; Skorupski, Karen. Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA. Molecular Microbiology (2007), 64(4), 953-967. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 147:184487 AN 2007:636615 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
HapR is a quorum sensing-regulated transcription factor that represses the virulence cascade in Vibrio cholerae by binding to a specific site centered at -71 in the aphA promoter, ultimately preventing activation of the tcpPH promoter on the Vibrio pathogenicity island. In an effort to elucidate the mechanism by which HapR represses aphA expression, the authors identified two transcriptional regulators, Lrp and VpsR, both of which activate the aphA promoter. Lrp, the leucine-responsive regulatory protein, binds to a region between -136 and -123 in the promoter to initiate aphA expression. VpsR, the response regulator that controls biofilm formation, binds to a region between -123 and -73 to activate aphA expression. HapR represses aphA expression by antagonizing the functions of both of these activators. The HapR binding site at -71 lies downstream of the Lrp binding site and overlaps the VpsR binding site. HapR binding thus directly blocks access of VpsR to the promoter. A naturally occurring point mutation in the aphA promoter (G-77T), which has previously been shown to prevent HapR binding, also prevents VpsR binding. In the absence of HapR, either Lrp or VpsR is capable of achieving nearly full expression of the aphA promoter, but when present together their effects are to some degree additive. The aphA promoter is also neg. autoregulated and an AphA binding site is centered at -20. The results here provide a model for the dual activation of the aphA promoter by Lrp and VpsR as well as its dual repression by HapR and AphA.

Bibliographic Information

Analysis of LuxR regulon gene expression during quorum sensing in Vibrio fischeri. Qin, Nan; Callahan, Sean M.; Dunlap, Paul V.; Stevens, Ann M. Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA. Journal of Bacteriology (2007), 189(11), 4127-4134. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 147:205185 AN 2007:623974 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The regulation of the lux operon (luxICDABEG) of Vibrio fischeri has been intensively studied as a model for quorum sensing in proteobacteria. Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis anal. previously identified several non-Lux proteins in V. fischeri MJ-100 whose expression was dependent on LuxR and 3-oxo-hexanoyl-L-homoserine lactone (3-oxo-C6-HSL). To det. if the LuxR-dependent regulation of the genes encoding these proteins was due to direct transcriptional control by LuxR and 3-oxo-C6-HSL or instead was due to indirect control via an unidentified regulatory element, promoters of interest were cloned into a lacZ reporter and tested for their LuxR and 3-oxo-C6-HSL dependence in recombinant Escherichia coli. The promoters for qsrP, acfA, and ribB were found to be directly activated via LuxR-3-oxo-C6-HSL. The sites of transcriptional initiation were established via primer extension anal. Based on this information and the position of the lux box-binding site near position -40, all three promoters appear to have a class II-type promoter structure. In order to more fully characterize the LuxR regulon in V. fischeri MJ-100, real-time reverse transcription-PCR was used to study the temporal expression of qsrP, acfA, and ribB during the exponential and stationary phases of growth, and electrophoretic mobility shift assays were used to compare the binding affinities of LuxR to the promoters under investigation. Taken together, the results demonstrate that regulation of the prodn. of QsrP, RibB, and AcfA is controlled directly by LuxR at the level of transcription, thereby establishing that there is a LuxR regulon in V. fischeri MJ-100 whose genes are coordinately expressed during mid-exponential growth.

Bibliographic Information

Identification of quorum sensing-related regulons in Vibrio vulnificus by two-dimensional gel electrophoresis and differentially displayed reverse transcriptase PCR. Shin, Na-Ri; Lee, Deog Yong; Yoo, Han Sang. Department of Infectious Diseases, BK21 for Veterinary Science and KRF Zoonotic Priority Research Institute, College of Veterinary Medicine, Seoul National University, S. Korea. FEMS Immunology and Medical Microbiology (2007), 50(1), 94-103. Publisher: Blackwell Publishing Ltd., CODEN: FIMIEV ISSN: 0928-8244. Journal written in English. CAN 147:270093 AN 2007:607240 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio vulnificus is thought to employ a quorum-sensing system to control the expression of a global gene. In this study, proteomes and transcriptomes of a lacZ null mutant, VvSR□Z, and a luxS-smcR double mutant, VvSR□ZSR, were compared with the parent strain, VvAR, by means of two-dimensional gel electrophoresis (2D-PAGE) and differentially displayed reverse transcriptase PCR (DDRT-PCR). 2D-PAGE anal. showed that 36 protein spots were differentially expressed, 14 of which have been identified by peptide-mass fingerprinting. The expression of eight cellular proteins was repressed by luxS and smcR mutation: Zn-dependent protease, 6-phosophofructokinase, periplasmic ABC-type Fe3+ transport system, deoxyribose-phosphate aldolase, phosphomannomutase, orotidine-5'-phosphate decarboxylase, uridylate kinase, and an unidentified protein. These proteins are involved in virulence, adaptation to environmental stress, biosynthesis of LPS, and cell multiplication. Phage shock protein A, a chemotaxis signal transduction protein, and an uncharacterized low-complexity protein were activated in the cellular components of the luxS-smcR mutant. However, only three proteins, of unknown function, were identified in the extracellular components of the mutants. Anal. of transcriptomes with DDRT-PCR showed that two genes, phosphoribosylformylglycinamidine synthase and ATP-dependent protease HslVU protease were regulated at the transcriptional level by luxS and smcR gene mutation. The

results from this study show conclusively that luxS/smcR quorum sensing endows a global change in gene expression to V. vulnificus.

Bibliographic Information

Expression of foreign proteins in a Vibrio cholerae vaccine strain using the stationary phase hemagglutinin/protease promoter. Hazra, Anupam; Silva, Anisia J.; Benitez, Jorge A. Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA, USA. Biotechnology Letters (2007), 29(7), 1093-1097. Publisher: Springer, CODEN: BILED3 ISSN: 0141-5492. Journal written in English. CAN 147:251359 AN 2007:560208 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The use of the hemagglutinin(HA)/protease promoter and secretion signals to drive expression and secretion of a foreign antigen in a live genetically attenuated cholera vaccine candidate is demonstrated. A Vibrio cholerae vaccine strain, contg. a HA/protease-tetanus toxin C fragment (TCF) fusion, produced sol.-and cell-assocd. TCF. The fraction of TCF secreted to the culture medium was degraded unless expressed in a HA/protease-defective vaccine strain. Comparison of the hapA promoter with the strong Tac promoter using quant. real time PCR revealed that at least five times more TCF mRNA was produced when expressed from the hapA promoter.

Bibliographic Information

Making bacteria behave: new agonists and antagonists of quorum sensing. Pomianek, Megan E.; Semmelhack, M. F. Dep. Chem., Princeton University, Princeton, NJ, USA. ACS Chemical Biology (2007), 2(5), 293-295. Publisher: American Chemical Society, CODEN: ACBCCT ISSN: 1554-8929. Journal; General Review written in English. CAN 146:496499 AN 2007:550629 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review. Small-mol. agonists and antagonists of bacterial quorum sensing can enhance our understanding of this form of cell-cell communication. A recent effort has discovered effective modulators of the autoinducer-1 circuit for bacterial quorum sensing by the synthesis and evaluation of a small library of aryl-substituted acyl-homoserine lactone analogs. This series highlights the sensitivity to structure of the contrasting responses of agonism and antagonism of the natural signal and identifies an analog that provokes the same response as the natural signal but at 10-fold lower concn., a "superagonist".

Bibliographic Information

luxS and smcR quorum sensing system of Vibrio vulnificus as an important factor for in vivo survival. Shin, Na-Ri; Baek, Chang-Ho; Lee, Deog-Yong; Cho, Young-Wook; Park, Dae-Kyun; Lee, Ko-Eun; Kim, Kun-Soo; Yoo, Han-Sang. Department of Infectious Diseases, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Seoul, S. Korea. Journal of Microbiology and Biotechnology (2005), 15(6), 1197-1206. Publisher: Korean Society for Microbiology and Biotechnology, CODEN: JOMBES ISSN: 1017-7825. Journal written in English. CAN 147:401354 AN 2007:548312 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio vulnificus is an opportunistic pathogen that causes a septicemia and expresses numerous virulence factors, in which luxS and smcR are genes encoding for components responsible for quorum-sensing regulation. In the present study, null mutants were constructed with lesions in each or both of these two genes from the V. vulnificus Vv□Z strain, which is a lacZ and chloramphenicol/streptomycin-resistant deriv. of the wild-type ATCC29307 strain, and their phenotypes related to virulence were compared with those of the parental cells. LD50 and histopathol. findings of luxS-, smcR-, or luxS- smcR- deficient mutant were not different from those of the parent strain, a lacZ-deficient streptomycin-resistant strain in mice. However, time of death in mice was delayed, and nos. of bacteria survived in bloodstream after i.p. injection in mice were decreased by mutation, esp. luxS and smcR double mutant (VvSR□ZSR). These phenomena were supported by increased serum sensitivity and delayed bacterial proliferation in both murine blood and iron-restricted medium. These results suggest that the luxS and luxR homologous genes in V. vulnificus could play a role in bacterial survival in host by enhancing proliferation and adjusting to changed environment.

Bibliographic Information

N-Phenylacetanoyl-L-homoserine lactones can strongly antagonize or superagonize quorum sensing in Vibrio fischeri. Geske, Grant D.; O'Neill, Jennifer C.; Blackwell, Helen E. Dep. Chemistry, Univ. Wisconsin-Madison, Madison, WI, USA. ACS Chemical Biology (2007), 2(5), 315-319. Publisher: American Chemical Society, CODEN: ACBCCT ISSN: 1554-8929. Journal written in English. CAN 147:90792 AN 2007:486454 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria monitor their population densities using low-mol.-wt. ligands in a process known as quorum sensing. At sufficient cell densities, bacteria can change their mode of growth and behave as multicellular communities that play crit. roles in both beneficial symbioses and the pathogenesis of infectious disease. The development of non-native ligands that can block quorum-sensing signals has emerged as a promising new strategy to attenuate these divergent outcomes. Here, we report that N-phenylacetanoyl-L-homoserine lactones are capable of either inhibiting or, in some cases, strongly inducing quorum sensing in the bacterial symbiont V. fischeri. Moreover, simple structural modifications to these ligands have marked effects on activity. These studies have revealed one of the 1st synthetic superagonists of quorum sensing, N-(3-nitro-phenylacetanoyl)-L-homoserine lactone. Together, these ligands represent a powerful new class of chem. probes with the potential to significantly expand the current understanding of quorum sensing and its role in host/bacteria interactions.

Bibliographic Information

The quorum-sensing hybrid histidine kinase LuxN of Vibrio harveyi contains a periplasmically located N terminus. Jung, Kirsten; Odenbach, Tina; Timmen, Melanie. Department Biologie I, Bereich Mikrobiologie, Ludwig-Maximilians-Universitaet Muenchen, Munich, Germany. Journal of Bacteriology (2007), 189(7), 2945-2948. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 146:495770 AN 2007:387104 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Hydropathy profile analyses of the amino acid sequence of the quorum-sensing hybrid histidine kinase LuxN of Vibrio harveyi predict a periplasmic location of the N terminus. To test this, two-hybrid proteins consisting of LuxN and an N-terminally fused maltose-binding protein with or without a leader sequence were analyzed with regard to the enzymic activities of LuxN, protease accessibility, and complementation of an Escherichia coli malE mutant. The results strongly support a periplasmic location of the N terminus, implying that LuxN is anchored with nine transmembrane domains in the cytoplasmic membrane.

Bibliographic Information

Identification and analysis of the Vibrio cholerae quorum sensing circuit. Lenz, Derrick Harold Manfred. Princeton Univ., Princeton, NJ, USA. Avail. UMI, Order No. DA3223817. (2006), 190 pp. From: Diss. Abstr. Int., B 2006, 67(6), 2952. Dissertation written in English. CAN 146:354405 AN 2007:374604 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

A novel lux operon in the cryptically bioluminescent fish pathogen Vibrio salmonicida is associated with virulence. Nelson, Eric J.; Tunsjo, Hege S.; Fidopiastis, Pat M.; Sorum, Henning; Ruby, Edward G. Molecular and Microbiology Department, Tufts University School of Medicine, Boston, MA, USA. Applied and Environmental Microbiology (2007), 73(6), 1825-1833. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 147:2972 AN 2007:362260 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The cold-water-fish pathogen Vibrio salmonicida expresses a functional bacterial luciferase but produces insufficient levels of its aliph.-aldehyde substrate to be detectably luminous in culture. Our goals were to (i) better explain this cryptic bioluminescence phenotype through mol. characterization of the lux operon and (ii) test whether the bioluminescence gene cluster is assocd. with virulence. Cloning and sequencing of the V. salmonicida lux operon revealed that homologs of all of the genes required for luminescence are present: luxAB (luciferase) and luxCDE (aliph.-aldehyde synthesis). The arrangement and sequence of these structural lux genes are conserved compared to those in related species of luminous bacteria. However, V. salmonicida strains have a novel arrangement and no. of homologs of the luxR and luxI quorum-sensing regulatory genes. Reverse transcriptase PCR anal. suggests that this novel arrangement of quorum-sensing genes generates antisense transcripts that may be responsible for the reduced prodn. of bioluminescence. In addn., infection with a strain in which the luxA gene was mutated resulted in a marked delay in mortality among Atlantic salmon relative to infection with the wild-type parent in single-strain challenge expts. In mixed-strain competition between the luxA mutant and the wild type, the mutant was attenuated up to 50-fold. It remains unclear whether the attenuation results from a direct loss of luciferase or a polar disturbance elsewhere in the lux operon. Nevertheless, these findings document for the first time an assocn. between a mutation in a structural lux gene and virulence, as well as provide a new mol. system to study Vibrio pathogenesis in a natural host.

Bibliographic Information

A LuxP-FRET-Based Reporter for the Detection and Quantification of AI-2 Bacterial Quorum-Sensing Signal Compounds. Rajamani, Sathish; Zhu, Jinge; Pei, Dehua; Sayre,

Richard. Biophysics Program, Department of Chemistry, and Department of Plant Cellular and Molecular Biology, Ohio State University, Columbus, OH, USA. Biochemistry (2007), 46(13), 3990-3997. Publisher: American Chemical Society, CODEN: BICHAW ISSN: 0006-2960. Journal written in English. CAN 146:397585 AN 2007:269738 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Various bacterial species produce and monitor low-mol. wt. signaling mols. that regulate specific sets of genes in a population d.-dependent manner. This process is known as quorum sensing (QS). To date, the detection of QS signaling mols. from Gram-neg. bacteria has relied primarily on bacterial reporter strains. These bioassays are subject to substantial interference by compds. that affect the growth and metab. of the reporter strains. In addn., the sensitivity of reporter strains to QS signaling mols. is population d.-dependent. Here, the authors describe the development of an in vitro assay system for the rapid detection and quantification of the furanosyl borate diester (BAI-2) subclass of autoinducer 2 (AI-2), QS mols. The sensor is based on ligand binding-induced changes in fluorescence resonance energy transfer (FRET) between a cyan and yellow variant of GFP fused to the termini of the BAI-2 receptor, LuxP. Unexpectedly, the addn. of synthetic BAI-2 to the purified biosensor induces a decrease in the level of FRET between the terminal fluorophores. Several lines of evidence, including mutation of the ligand binding sites, indicate that the obsd. FRET changes are BAI-2-dependent. The FRET-based BAI-2 biosensor responded to the addn. of culture filtrates from wild-type Vibrio harveyi but exhibited no response to culture filtrates from V. harveyi mutants defective in BAI-2 synthesis. The sensitivity of the biosensor to BAI-2 (apparent Kd = 270 nM) was similar to that of BAI-2 bioassay systems. The limitations of microbial bioassay systems and the advantages and potential applications for the FRET-based BAI-2 biosensor are discussed.

Bibliographic Information

The small nucleoid protein Fis is involved in Vibrio cholerae quorum sensing. Lenz, Derrick H.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2007), 63(3), 859-871. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 146:398036 AN 2007:254672 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing is a process of cell-cell communication that bacteria use to relay information to one another about the cell d. and species compn. of the bacterial community. Quorum sensing involves the prodn., secretion and population-wide detection of small signalling mols. called autoinducers. This process allows bacteria to synchronize group behaviors and act as multicellular units. The human pathogen, Vibrio cholerae, uses quorum sensing to coordinate such complex behaviors as pathogenicity and biofilm formation. The quorum-sensing circuit of V. cholerae consists of two autoinducer/sensor systems, CAI-1/CqsS and AI-2/LuxPQ, and the VarS/A-CsrA/BCD growth-phase regulatory system. Genetic anal. suggests that an addnl. regulatory arm involved in quorum sensing exists in V. cholerae. All of these systems channel information into the histidine phosphotransfer protein, LuxU, and/or the response regulator, LuxO. LuxO, when phosphorylated, activates the expression of four genes encoding the Qrr (quorum regulatory RNAs) small RNAs (sRNAs). The Qrr sRNAs destabilize the hapR transcript encoding the master regulator of quorum sensing, HapR. Here we identify the nucleoid protein Fis as playing a major role in the V. cholerae quorum-sensing circuit. Fis fulfils the predictions required to be the putative

addnl. component that inputs information into the cascade: its expression is regulated in a growth phase-dependent manner; it requires LuxO but acts independently of LuxU, and it regulates all four qrr genes and, in turn, HapR by directly binding to the qrr gene promoters and modulating their expression.

Bibliographic Information

Ac2-DPD, the bis-(O)-acetylated derivative of 4,5-dihydroxy-2,3-pentanedione (DPD) is a convenient stable precursor of bacterial quorum sensing autoinducer AI-2. Frezza, Marine; Soulere, Laurent; Balestrino, Damien; Gohar, Michel; Deshayes, Christian; Queneau, Yves; Forestier, Christiane; Doutheau, Alain. Laboratoire de Chimie Organique, UMR 5181 CNRS, INSA, Institut National des Sciences Appliquees, Universite Lyon 1, Villeurbanne, Fr. Bioorganic & Medicinal Chemistry Letters (2007), 17(5), 1428-1431. Publisher: Elsevier Ltd., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. CAN 146:437946 AN 2007:188062 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Ac2-DPD, the bis-(O)-acetylated deriv. of 4,5-dihydroxy-2,3-pentanedione (DPD), was prepd. both as a racemic mixt. and in the optically active form found in naturally occurring DPD. It was shown to exhibit the same ability as DPD to induce bioluminescence in Vibrio Harveyi and □-galactosidase activity in Salmonella enterica Typhimurium, both Gram-neg. bacteria. Likewise, it was also shown to inhibit biofilm formation in Gram-pos. Bacillus cereus. The most likely hypothesis is that Ac2-DPD activity is due to the release of DPD by in situ hydrolysis of the ester groups. Importantly, by contrast with DPD, Ac2-DPD proved to be a stable compd. which can be purified and stored.

Bibliographic Information

Multiple small RNAs act additively to integrate sensory information and control quorum sensing in Vibrio harveyi. Tu, Kimberly C.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Genes & Development (2007), 21(2), 221-233. Publisher: Cold Spring Harbor Laboratory Press, CODEN: GEDEEP ISSN: 0890-9369. Journal written in English. CAN 146:310379 AN 2007:115104 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing is a cell-cell communication mechanism that bacteria use to collectively regulate gene expression and, at a higher level, to coordinate group behavior. In the bioluminescent marine bacterium Vibrio harveyi, sensory information from three independent quorum-sensing systems converges on the shared response regulator LuxO. When LuxO is phosphorylated, it activates the expression of a putative repressor that destabilizes the mRNA encoding the master quorum-sensing transcriptional regulator LuxR. In the closely related species Vibrio cholerae, this repressor was revealed to be the RNA chaperone Hfq together with four small regulatory RNAs (sRNAs) called Qrr1-4 (quorum regulatory RNA). Here, we identify five Qrr sRNAs that control quorum sensing in V. harveyi. Mutational anal. reveals that only four of the five Qrrs are required for destabilization of the luxR mRNA. Surprisingly, unlike in V. cholerae where the sRNAs act redundantly, in V. harveyi, the Qrr sRNAs function additively to control quorum sensing. This latter mechanism produces a gradient of LuxR that, in turn, enables differential regulation of quorum-sensing target genes. Other regulators appear to be involved in control of V. harveyi qrr

expression, allowing the integration of addnl. sensory information into the regulation of quorum-sensing gene expression.

Bibliographic Information

Production of antibacterial compounds and biofilm formation by Roseobacter species are influenced by culture conditions. Bruhn, Jesper Bartholin; Gram, Lone; Belas, Robert. Department of Seafood Research, Soeltofts Plads, Danish Institute for Fisheries Research, Kgs. Lyngby, Den. Applied and Environmental Microbiology (2007), 73(2), 442-450. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 146:270104 AN 2007:114898 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Bacterial communities assocd. with marine algae are often dominated by members of the Roseobacter clade, and in the present study, we describe Roseobacter phenotypes that may provide this group of bacteria with selective advantages when colonizing this niche. Nine of 14 members of the Roseobacter clade, of which half were isolated from cultures of the dinoflagellate Pfiesteria piscicida, produced antibacterial compds. Many non-Roseobacter marine bacteria were inhibited by sterile filtered supernatants of Silicibacter sp. TM1040 and Phaeobacter (formerly Roseobacter) strain 27-4, which had the highest prodn. of antibacterial compd. In contrast, Roseobacter strains were susceptible only when exposed to concd. compd. The prodn. of antibacterial compd. was influenced by the growth conditions, as prodn. was most pronounced when bacteria were grown in liq. medium under static conditions. Under these conditions, Silicibacter sp. TM1040 cells attached to one another, forming rosettes, as has previously been reported for Phaeobacter 27-4. A spontaneous Phaeobacter 27-4 mutant unable to form rosettes was also defective in biofilm formation and the prodn. of antibacterial compd., indicating a possible link between these phenotypes. Rosette formation was obsd. in 8 of 14 Roseobacter clade strains examd. and was very pronounced under static growth in 5 of these strains. Attachment to surfaces and biofilm formation at the air-liq. interface by these five strains was greatly facilitated by growth conditions that favored rosette formation, and rosette-forming strains were 13 to 30 times more efficient in attaching to glass compared to strains under conditions where rosette formation was not pronounced. We hypothesize that the ability to produce antibacterial compds. that principally inhibit non-Roseobacter species, combined with an enhancement in biofilm formation, may give members of the Roseobacter clade a selective advantage and help to explain the dominance of members of this clade in assocn. with marine algal microbiota.

Bibliographic Information

Direct quantification of N-(3-oxo-hexanoyl)-L-homoserine lactone in culture supernatant using a whole-cell bioreporter. Yan, Ling; Allen, Michael S.; Simpson, Michael L.; Sayler, Gary S.; Cox, Chris D. Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN, USA. Journal of Microbiological Methods (2007), 68(1), 40-45. Publisher: Elsevier B.V., CODEN: JMIMDQ ISSN: 0167-7012. Journal written in English. CAN 147:66903 AN 2007:49925 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The autoinducer N-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxo-C6-HSL) plays a significant role in the quorum-sensing system of the marine bacterium Vibrio fischeri. Upon forming a

transcriptional activation complex with LuxR, 3-oxo-C6-HSL induces transcription of the luxICDABEG operon, leading to the increased prodn. of both the 3-oxo-C6-HSL synthase (LuxI) and the bioluminescent proteins. In order to quant. analyze this regulatory mechanism, a novel approach was developed to measure 3-oxo-C6-HSL concns. in V. fischeri cell culture supernatant. A bioluminescent strain of Escherichia coli that responds to 3-oxo-C6-HSL was used as a bioreporter. Although a linear response of the bioreporter to exogenously added synthetic 3-oxo-C6-HSL was found over several orders of magnitude, we show that bioreporter performance was dramatically impacted by variations in the supernatants using samples from a V. fischeri LuxI- strain. However, when maintained in the same supernatant background, the normalized peak bioluminescence maintained a linear response to 3-oxo-C6-HSL concns. Therefore, a std. addns. technique was developed in which a known concn. of 3-oxo-C6-HSL was added to supernatant samples from wild-type V. fischeri cultures, and the incremental increase of the normalized peak bioluminescence relative to the untreated sample was detd. The concn. of 3-oxo-C6-HSL in the supernatant of the unknown sample was then quantified from the slope of the response between the normalized bioluminescent peaks with and without the addn. of 3-oxo-C6-HSL. Advantages of this method are that it is rapid, does not require concn. or extn., uses a small sample vol. (ca. 2 mL), and accounts for effects caused by the compn. of the supernatant. Furthermore, the findings can be broadly applicable to other bioreporter systems involving variable background conditions.

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Temporal quorum-sensing induction regulates Vibrio cholerae biofilm architecture. Liu, Zhi; Stirling, Fiona R.; Zhu, Jun. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. Infection and Immunity (2007), 75(1), 122-126. Publisher: American Society for Microbiology, CODEN: INFIBR ISSN: 0019-9567. Journal written in English. CAN 146:202003 AN 2007:34876 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio cholerae, the pathogen that causes cholera, also survives in aq. reservoirs, probably in the form of biofilms. Quorum sensing neg. regulates V. cholerae biofilm formation through HapR, whose expression is induced at a high cell d. In this study, we show that the concn. of the quorum-sensing signal mol. CAI-1 is higher in biofilms than in planktonic cultures. By measuring hapR expression and activity, we found that the induction of quorum sensing in biofilm-assocd. cells occurs earlier. We further demonstrate that the timing of hapR expression is crucial for biofilm thickness, biofilm detachment rates, and intestinal colonization efficiency. These results suggest that V. cholerae is able to regulate its biofilm architecture by temporal induction of quorum-sensing systems.

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Compounds which interfere with microbial quorum sensing mechanism for use as anti-infective and immunomodulatory agents. Rahme, Laurence; Lepine, Francois; Deziel, Eric. (The General Hospital Corporation, USA; Inrs - Institut Armand-Frappier). PCT Int. Appl. (2006), 111pp. CODEN: PIXXD2 WO 2006130832 A2 20061207 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ,

TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2006-US21454 20060602. Priority: US 2005-686816 20050602. CAN 146:20249 AN 2006:1285902 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2006130832	A2	20061207	WO2006-US21454	20060602
WO2006130832	A3	20090507		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

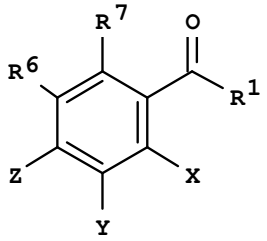
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

US 20090215819	A1	20090827	US 2008-916379	20081028

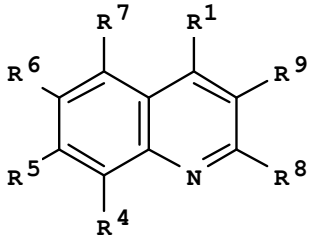
Priority Application		
US 2005-686816P	P	20050602
WO2006-US21454	W	20060602

Abstract

The present invention provides compds. I (R1 = (substituted)C1-12-alkyl, C1-4-alkaryl, etc. ; R2,R3 = H, (substituted))C1-6-alkyl, C1-4-alkaryl, etc., or R2, R3 and the N to which they are bound form NO2; R4 = H, halo, OH, C1-6-alkoxy; R5-7 = R4, C1-6-alkyl; X = R4, NR2R3; Y = R4, R5, NR2R3; Z = R5, NR2R3) and II (R1,R4-6 same as in I; R8 = R1; R9 = H, OH, (substituted)C1-6-alkoxy, etc.), pharmaceutical compns. contg. I and II, and methods that include the use of I and II as anti-infective compds. that potentiate the host immune response or limit or prevent the expression or activity of individual virulence factors. In addn., the compns. have immunomodulatory activity, and therefore can be used to prime host defenses to prevent or limit bacterial, fungal, and viral viability. In the compns. and methods of the inventions, the quorum sensing mechanism is targeted to prevent pathogenesis (e.g., infection). Such an approach should prevent pathogenic organisms from acquiring resistance to the protective anti-infective compds. Thus, compds. such as 6-fluoro-2-aminobenzoic acid and 4-chloro-2-aminobenzoic acid were potent inhibitors of Pseudomonas aeruginosa 4-hydroxy-2-alkylquinoline prodn. The compd. 2-aminoacetophenone (2AA), when injected into the burn eschar of burned and P. aeruginosa-infected mice, reduced mortality. 2AA inhibited expression of the pqs operon by competing with transcription factor MvfR activators 4-hydroxy-2-heptylquinoline and 3,4-dihydroxy-2-heptylquinoline. P. aeruginosa genes downregulated by 2AA and mouse genes induced by 2AA were identified.



I



II

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Capsular polysaccharide phase variation in Vibrio vulnificus. Hilton, Tamara; Rosche, Tom; Froelich, Brett; Smith, Benjamin; Oliver, James. University of North Carolina at Charlotte, Charlotte, NC, USA. Applied and Environmental Microbiology (2006), 72(11), 6986-6993. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 146:117823 AN 2006:1256997 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Commonly found in raw oysters, Vibrio vulnificus poses a serious health threat to immunocompromised individuals and those with serum iron overload, with a fatality rate of approx. 50%. An essential virulence factor is its capsular polysaccharide (CPS), which is responsible for a significant increase in virulence compared to nonencapsulated strains. However, this bacterium is known to vary the amt. of CPS expressed on the cell surface, converting from an opaque (Op) colony phenotype to a translucent (Tr) colony phenotype. In this study, the consistency of CPS conversion was detd. for four strains of V. vulnificus. Environmental conditions including variations in aeration, temp., incubation time, oxidative stress, and media (heart infusion or modified maintenance medium agar) were investigated to det. their influence on CPS conversion. All conditions, with the exception of variations in media and oxidative stress, significantly affected the conversion of the population, with high ranges of CPS expression found even within cells from a single colony. The global quorum-sensing regulators RpoS and AI-2 were also examd. While RpoS was found to significantly mediate phenotypic conversion, quorum sensing was not. Finally, 12 strains that comprise the recently found clin. (C) and environmental (E) genotypes of V. vulnificus were examd. to det. their rates of population conversion. C-genotype strains, which are most often assocd. with infection, had a significantly lower rate of population conversion from Op to Tr phenotypes than did E-genotype strains (.apprx.38% vs. .apprx.14%, resp.). Biofilm capabilities of these strains, however, were not correlated with increased population conversion.

Bibliographic Information

Subinhibitory concentrations of cinnamaldehyde interfere with quorum sensing. Niu, C.; Afre, S.; Gilbert, E. S. Department of Biology, Georgia State University, Atlanta, GA, USA. Letters in Applied Microbiology (2006), 43(5), 489-494. Publisher: Blackwell Publishing Ltd., CODEN: LAMIE7 ISSN: 0266-8254. Journal written in English. CAN 146:456303 AN 2006:1254791 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

To investigate the effect of cinnamaldehyde (CA) on transcription from selected quorum sensing (QS) promoters. The action of CA on QS was assayed using three Escherichia coli green fluorescent protein (GFP) based bioreporters (two inducible and the other constitutive) and two Vibrio harveyi bioluminescent reporter strains. LuxR-mediated transcription from the PluxI promoter, which is induced by 3-oxo-C6-homoserine lactone (HSL), was reduced by 70% following exposure to 200 □mol/L-1 CA (26 ppm). The bioluminescence of Vibrio harveyi BB886, which is mediated by 3-hydroxy-C4-HSL, was reduced by 55% after exposure to 60 □mol/L-1 CA (8 ppm), and 100 □mol/L-1 CA (13 ppm) inhibited the bioluminescence of the autoinducer-2 (AI-2) responsive reporter strain V. harveyi BB170 by nearly 60%. CA did not inhibit the growth of the bioreporter strains at these concns. CA had a minimal effect on LasR promoter activity, induced by 3-oxo-C12-HSL. Low concns. of CA were effective at inhibiting two types of acyl homoserine lactone mediated QS, and also autoinducer-2 mediated QS. Because CA is widely used in the food and flavor industries, its potential to affect bacterial QS regulated processes should be recognized.

Bibliographic Information

Profiling of acylated homoserine lactones of Vibrio anguillarum in vitro and in vivo: influence of growth conditions and serotype. Buchholtz, Christiane; Nielsen, Kristian Fog; Milton, Debra L.; Larsen, Jens Laurits; Gram, Lone. Department of Seafood Research, Danish Institute for Fisheries Research, Kgs. Lyngby, Den. Systematic and Applied Microbiology (2006), 29(6), 433-445. Publisher: Elsevier GmbH, CODEN: SAMIDF ISSN: 0723-2020. Journal written in English. CAN 146:517772 AN 2006:1160461 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio anguillarum produces several interlinked acylate homoserine lactone (AHL) signal mols. which may influence expression of its virulence factors such as exoprotease prodn. and biofilm formation. Using both thin layer chromatog. and HPLC-high resoln. mass spectrometry (HPLC-HRMS), we demonstrate in this study that the same types of AHLs are produced by many serotypes of V. anguillarum and that altering in vitro growth conditions (salinity, temp. and iron concn.) has little influence on the AHL-profile. Most strains produced N-(3-oxodecanoyl)-L-homoserine lactone (3-oxo-C10-HSL) and N-(3-hydroxy-hexanoyl)-L-homoserine lactone (3-hydroxy-C6-HSL) as the dominant mols. Also, two spots with AHL activity appeared on TLC plates, which could not be identified as AHL structures. Trace amts. of N-(3-hydroxy-octanoyl)-L-homoserine lactone, N-(3-hydroxy-decanoyl)-L-homoserine lactone and N-(3-hydroxy-dodecanoyl)-L-homoserine lactone (3-hydroxy-C8-HSL, 3-hydroxy-C10-HSL and 3-oxo-C12-HSL, resp.) were also detected by HPLC-HRMS anal. from in vitro cultures. Most studies of quorum sensing (QS) systems have been conducted in vitro, the purpose of our study was to det. if the same acylated homoserine lactones were produced in vivo during infection. Exts. from infected fish were purified using several solid phase extn. strategies to allow chromatog. detection and sepn. by both TLC and HPLC-HRMS. 3-Oxo-C10-HSL and 3-hydroxy-C6-HSL were detected in organs from fish dying from vibriosis, however, compared to in vitro culturing where 3-oxo-C10-HSL is the dominant mol., 3-hydroxy-C6-HSL was prominent in the infected fish tissues. Hence, the balance between the QS systems may be different during infection compared to in vitro cultures. For future studies of QS systems and the possible specific interference with expression of virulence factors, in vitro cultures should be optimized to reflect the in vivo situation.

Bibliographic Information

The role of quorum sensing and the effect of environmental conditions on biofilm formation by strains of Vibrio vulnificus. McDougald, D.; Lin, W. H.; Rice, S. A.; Kjelleberg, S. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia. Biofouling (2006), 22(3), 133-144. Publisher: Taylor & Francis Ltd., CODEN: BFOUEC ISSN: 0892-7014. Journal written in English. CAN 146:496589 AN 2006:1136559 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
It has been suggested that Vibrio vulnificus attaches to plankton and algae and is found in large nos. in the environment. Factors affecting attachment, biofilm formation and morphol. of V. vulnificus have not been thoroughly investigated. This study evaluated the role of quorum sensing (QS) and environmental conditions on biofilm development of V. vulnificus. It was found that biofilm development by V. vulnificus was affected by nutrient and glucose concn., but not by NaCl concn. or temp. under the conditions used here. Moreover, biofilm development of a QS mutant strain proceeded rapidly and sloughing occurred earlier than for the isogenic parent strain. There was a significant loss of viability for the QS mutant biofilm early in development. Hence, it is hypothesised that factors regulated by the QS system play a role in proper biofilm development and maintenance of V. vulnificus. Furthermore, it is shown that biofilm development varied among isolates.

Bibliographic Information

The Vibrio harveyi quorum-sensing system uses shared regulatory components to discriminate between multiple autoinducers. Waters, Christopher M.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Genes & Development (2006), 20(19), 2754-2767. Publisher: Cold Spring Harbor Laboratory Press, CODEN: GEDEEP ISSN: 0890-9369. Journal written in English. CAN 146:21147 AN 2006:1092264 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The quorum-sensing bacterium Vibrio harveyi produces and responds to three autoinducers (AIs), and this sensory information converges to control the expression of bioluminescence, biofilm formation, type III secretion (TTS), and protease prodn. The AIs are detected by cognate sensor histidine kinases that all relay phosphate to the shared response regulator LuxO. LuxO indirectly represses the master regulator of quorum sensing, LuxR, through the activation of multiple genes encoding small regulatory RNAs (called qrr genes for Quorum Regulatory RNA). Here we use differential fluorescence induction to identify 50 quorum-sensing-controlled promoters. Some promoters only showed significant responses in the simultaneous presence of all three AIs, while others displayed substantial responses to the individual AIs. A differential response to each AI input state was also obsd. for qrr and luxR expression and LuxR protein prodn. Individual cell analyses revealed that, in each case, all the bacteria in the population respond in unison to the various AI inputs. We propose that the V. harveyi quorum-sensing transition is not switch-like but rather operates in a graded manner, and that this signaling arrangement, which uses shared regulatory proteins, nonetheless provides V. harveyi a mechanism to respond uniquely to different AI input states.

Bibliographic Information

Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing. Neiditch, Matthew B.; Federle, Michael J.; Pompeani, Audra J.; Kelly, Robert C.; Swem, Danielle L.; Jeffrey, Philip D.; Bassler, Bonnie L.; Hughson, Frederick M. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Cell (Cambridge, MA, United States) (2006), 126(6), 1095-1108. Publisher: Cell Press, CODEN: CELLB5 ISSN: 0092-8674. Journal written in English. CAN 146:2577 AN 2006:1044046 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria sense their environment using receptors of the histidine sensor kinase family, but how kinase activity is regulated by ligand binding is not well understood. Autoinducer-2 (AI-2), a secreted signaling mol. originally identified in studies of the marine bacterium Vibrio harveyi, regulates quorum-sensing responses and allows communication between different bacterial species. AI-2 signal transduction in V. harveyi requires the integral membrane receptor LuxPQ, comprised of periplasmic binding protein (LuxP) and histidine sensor kinase (LuxQ) subunits. Combined X-ray crystallog. and functional studies show that AI-2 binding causes a major conformational change within LuxP, which in turn stabilizes a quaternary arrangement in which two LuxPQ monomers are asym. assocd. We propose that formation of this asym. quaternary structure is responsible for repressing the kinase activity of both LuxQ subunits and triggering the transition of V. harveyi into quorum-sensing mode.

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Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp Artemia franciscana from pathogenic Vibrio harveyi, Vibrio campbellii, and Vibrio parahaemolyticus isolates. Defoirdt, Tom; Crab, Roselien; Wood, Thomas K.; Sorgeloos, Patrick; Verstraete, Willy; Bossier, Peter. Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belg. Applied and Environmental Microbiology (2006), 72(9), 6419-6423. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 145:502169 AN 2006:986096 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Autoinducer 2 (AI-2) quorum sensing was shown before to regulate the virulence of V. harveyi towards the brine shrimp A. franciscana. In this study, several different pathogenic V. harveyi, V. campbellii, and V. parahaemolyticus isolates were shown to produce AI-2. Furthermore, disruption of AI-2 quorum sensing by a natural and a synthetic brominated furanone protected gnotobiotic Artemia from the pathogenic isolates in in vivo challenge tests.

Bibliographic Information

Involvement of LuxR, a quorum sensing regulator in Vibrio harveyi, in the promotion of metabolic genes: argA, purM, lysE and rluA. Miyamoto, Carol M.; Meighen, Edward A. Department of Biochemistry, McGill University, Montreal, QC, Can. Biochimica et Biophysica Acta, Gene Structure and Expression (2006), 1759(6), 296-307. Publisher: Elsevier Ltd., CODEN: BBGSD5 ISSN: 0167-4781. Journal written in English. CAN 145:432918 AN 2006:905472 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing, involving signal transduction via the two-component response regulator LuxO to its downstream target LuxR, controls luminescence in the marine bacterium Vibrio harveyi. LuxR is a DNA binding protein that acts as both activator of the lux operon and repressor of its own gene. In order to det. if any other genes are affected by quorum sensing in V. harveyi, an assay for luxR-dependent promotion was devised using a genomic library maintained in a novel luxAB (luciferase) reporter. Screening in Escherichia coli DH-21 (lacI sq) entailed the addn. of a second plasmid contg. luxR under plac control. Four out of 5000 colonies showed luminescence stimulation upon IPTG induction of luxR. The four luxR-dependent promoters were upstream of argA, purM, lysE, and rluA, genes involved in arginine and purine biosyntheses, amino acid efflux, and pseudouridine synthesis, resp. Based on anal. of luxR-dependent promoters, particularly that of argA, we describe a LuxR binding site, and implicate the coordination of LuxR with ArgR.

Bibliographic Information

Localized bacterial biofilm formation as a diffusion-mediated process. Parent, Mary E.; Velegol, Darrell. Chemical Engineering, Penn State University, University Park, PA, USA. Abstracts of Papers, 232nd ACS National Meeting, San Francisco, CA, United States, Sept. 10-14, 2006 (2006), ENVR-251. Publisher: American Chemical Society, Washington, D. C CODEN: 69IHRD Conference; Meeting Abstract; Computer Optical Disk written in English. AN 2006:859627 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing is a necessary mechanism for biofilm formation in many species, for example, Burkholderia cepacia and Vibrio fischeri. In quorum sensing, cells produce and sense signaling mols. that trigger the activation of target genes at a crit. signal (and, thus, cell) concn. Quorum sensing signals are commonly acylated homoserine lactones (AHLs). We hypothesize that due to the phys. phenomenon of the diffusion of AHLs from adhered cells, biofilms form on a local scale in space and time. Through B. cepacia cell adhesion measurements and V. fischeri luminescence monitoring of cells in varying surface concns. with time, we aim to show that biofilms form at discrete localized points. Exptl. results are compared to a model of the AHL signal diffusion. It is expected that larger surface cell concns. and longer times will result in more biofilm development (more and stronger adhesion and luminescence). These results will test our hypothesis that biofilms form locally due to the phys. diffusion of quorum sensing signals.

Bibliographic Information

AI-1 Influences the Kinase Activity but Not the Phosphatase Activity of LuxN of Vibrio harveyi. Timmen, Melanie; Bassler, Bonnie L.; Jung, Kirsten. Department Biologie I, Bereich Mikrobiologie, Ludwig-Maximilians-Universitaet Muenchen, Munich, Germany. Journal of Biological Chemistry (2006), 281(34), 24398-24404. Publisher: American Society for Biochemistry and Molecular Biology, CODEN: JBCHA3 ISSN: 0021-9258. Journal written in English. CAN 145:412955 AN 2006:823930 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The Gram-neg. bacterium Vibrio harveyi produces and responds to three autoinducers, AI-1, AI-2, and CAI-1 to regulate cell d. dependent gene expression by a process referred to as quorum sensing. The concn. of the autoinducers is sensed by three cognate hybrid sensor kinases, and information is channeled via the HPt protein LuxU to the response regulator LuxO. Here, a detailed biochem.

study on the enzymic activities of the membrane-integrated hybrid sensor kinase LuxN, the sensor for N-(D-3-hydroxybutanoyl)homoserine lactone (AI-1), is provided. LuxN was heterologously overproduced as the full-length protein in Escherichia coli. LuxN activities were characterized in vitro and are an autophosphorylation activity with an unusually high ATP turnover rate, stable LuxU phosphorylation, and a slow phosphatase activity with LuxU.apprx.P as substrate. The presence of AI-1 affected the kinase but not the phosphatase activity of LuxN. The influence of AI-1 on the LuxN LuxU signaling step was monitored, and in the presence of AI-1, the kinase activity of LuxN, and hence the amt. of LuxU.apprx.P produced, were significantly reduced. Half-maximal inhibition of kinase activity by AI-1 occurred at 20 □M. Together, these results indicate that AI-1 directly interacts with LuxN to down-regulate its autokinase activity and suggest that the key regulatory step of the AI-1 quorum sensing system of Vibrio harveyi is AI-1-mediated repression of the LuxN kinase activity.

Bibliographic Information

In vitro reconstruction of quorums sensing signal transduction cascade for characterization of the hybrid sensor kinase LuxN from Vibrio harveyi. Timmen, Melanie. Germany. Avail. Metadata on Internet Documents, Order No. 362199. (2006), No pp. given. From: Metadata Internet Doc. [Ger. Diss.] 2006, (D0723-2), No pp. given. http://www.meind.de/search.py?recid=362199 Dissertation written in German. CAN 145:140024 AN 2006:718901 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

Enzymic degradation of acyl-L-homoserine lactones and its possible use in biological control and suppression of infection development. Czajkowski, Robert; Jafra, Sylwia. Zakl. Ochrony i Biotechnol. Roslin, Miedzyuczelniany Wydz. Biotechnol., Uniw. Gdanski i Akad. Med., Gdansk, Pol. Biotechnologia (2006), (2), 49-64. Publisher: Instytut Chemii Bioorganicznej PAN, CODEN: BIECEV ISSN: 0860-7796. Journal; General Review written in Polish. CAN 146:248288 AN 2006:696843 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Various bacteria control diverse metabolic processes through prodn. and distribution of specific signal mols.; their concns. in the environment depend on bacterial cell d. and the concns. increase when the bacterial populations enlarge. This microbial strategy is known as quorum sensing (QS) and was first described in the Gram-neg. marine bacterium Vibrio fischeri. QS is a mechanism of gene expression regulation dependent on bacterial cell d. and is widely found in Gram-neg. bacteria; it controls different physiol. processes, such as prodn. of virulence factors, conjugal plasmid transfer, antibiotic prodn., replication, swarming, or luminescence. QS functions via signal mols.; in Gram-neg. bacteria the signal mols. belong to the acyl-L-homoserine lactones (AHL). Many bacteria can interfere in QS via quorum quenching (QQ) by enzymic degrdn. of AHL. So far 2 classes of enzymes degrading AHL have been described: AHL-lactonases and AHL-acylases. AHL-lactonases hydrolyze the ester bond in the lactone ring of AHL. AHL-acylases hydrolyze the amide bond between the acyl side-chain and the lactone ring in AHL. Both reactions lead to the inhibition of signal transfer in QS as the degrdn. products do not act as signal mols. QS has a major role in infection pathogenesis and is a potential target for modern antimicrobial therapy in human and veterinary medicine and in biol. control of plant diseases.

Bibliographic Information

Presence of LuxS/AI-2 based quorum-sensing system in Vibrio mimicus: LuxO controls protease activity. Sultan, Zafar; Miyoshi, Shin-ichi; Shinoda, Sumio. Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan. Microbiology and Immunology (2006), 50(5), 407-417. Publisher: Center for Academic Publications Japan, CODEN: MIIMDV ISSN: 0385-5600. Journal written in English. CAN 145:227238 AN 2006:664942 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Presence of the quorum-sensing regulation system in Vibrio mimicus was investigated. The culture supernatants of V. mimicus strains were found to possess AI-2 autoinducer like activity, and the strains were found to harbor the genes which are homologous to luxS, luxO, and luxR of V. harveyi. These genes of V. harveyi have been shown to be important components of V. harveyi-like quorum-sensing system. The luxO gene homolog known to encode LuxO, the central component of the regulation system, was disrupted, and effects on protease and hemolysin activity were studied. Disruption of luxO gene resulted in the increased protease activity, but the hemolysin activity did not vary considerably.

Bibliographic Information

Adaptive response of Vibrios. McDougald, Diane; Kjelleberg, Staffan. School of Biotechnology and Biomolecular Sciences, Centre for Marine Biofouling and Bio-Innovation, University of New South Wales, Sydney, Australia. Editor(s): Thompson, Fabiano L.; Austin, Brian; Swings, Jean. Biology of Vibrios (2006), 133-155. Publisher: American Society for Microbiology, Washington, D. C CODEN: 69IFUA Conference; General Review written in English. CAN 146:354278 AN 2006:636438 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review discusses the several aspects of adaptation that were suggested to play a role in the survival of vibrios, in particular, starvation adaptation, the viable but nonculturable response, and biofilm formation. In addn., quorum sensing, which has been shown to control many phenotypes assocd. with survival under different conditions, is described.

Bibliographic Information

Swarming differentiation of Vibrio vulnificus downregulates the expression of the vvhBA hemolysin gene via the LuxS quorum-sensing system. Kim, Moon-Young; Park, Ra-Young; Choi, Mi-Hwa; Sun, Hui-Yu; Kim, Choon-Mee; Kim, Soo-Young; Rhee, Joon-Haeng; Shin, Sung-Heui. Research Center for Resistant Cells, Chosun University Medical School, Gwangju, S. Korea. Journal of Microbiology (Seoul, Republic of Korea) (2006), 44(2), 226-232. Publisher: Microbiological Society of Korea, CODEN: JOMIFG ISSN: 1225-8873. Journal written in English. CAN 146:77702 AN 2006:536395 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Swarming has proven to be a good in vitro model for bacterial surface adherence and colonization, and the swarming differentiation of a bacterium has been shown to be coupled with changes in the expression of virulence factors assocd. with its invasiveness, particularly in the early stages of

infection. In this study, we attempted to det. whether the expression of vvhA, which encodes for hemolysin/cytolysin (VvhA), is either upregulated or downregulated during the swarming differentiation of V. vulnificus. The insertional inactivation of vvhA itself exerted no detectable effect on the expression of V. vulnificus swarming motility. However, in our lacZ-fused vvhA transcriptional reporter assay, vvhA expression decreased in swarming V. vulnificus as compared to non-swarming or planktonic V. vulnificus. The reduced expression of vvhA in swarming V. vulnificus increased as a result of the deletional inactivation of luxS, a gene assocd. with quorum sensing. These results show that vvhA expression in swarming V. vulnificus is downregulated via the activity of the LuxS quorum-sensing system, suggesting that VvhA performs no essential role in the invasiveness of V. vulnificus via the adherence to and colonization on the body surfaces required in the early stages of the infection. However, VvhA may play a significant role in the pathophysiol. deterioration occurring after swarming V. vulnificus is differentiated into planktonic V. vulnificus.

Bibliographic Information

Synthesis and biological evaluation of homoserine lactone derived ureas as antagonists of bacterial quorum sensing. Frezza, Marine; Castang, Sandra; Estephane, Jane; Soulere, Laurent; Deshayes, Christian; Chantegrel, Bernard; Nasser, William; Queneau, Yves; Reverchon, Sylvie; Doutheau, Alain. Laboratoire de Chimie Organique, UMR CNRS-UCBL-INSA 5181, Institut National des Sciences Appliquees, Villeurbanne, Fr. Bioorganic & Medicinal Chemistry (2006), 14(14), 4781-4791. Publisher: Elsevier B.V., CODEN: BMECEP ISSN: 0968-0896. Journal written in English. CAN 145:141026 AN 2006:517570 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A series of 15 racemic alkyl- and aryl-N-substituted ureas, derived from homoserine lactone, were synthesized and tested for their ability to competitively inhibit the action of 3-oxohexanoyl-L-homoserine lactone, the natural inducer of bioluminescence in the bacterium Vibrio fischeri. N-alkyl ureas with an alkyl chain of at least 4 carbon atoms, as well as certain ureas bearing a Ph group at the extremity of the alkyl chain, were found to be significant antagonists. In the case of N-Bu urea, it has been shown that the antagonist activity was related to the inhibition of the dimerization of the N-terminal domain of ExpR, a protein of the receptor LuxR family. Mol. modeling suggested that this would result from the formation of an addnl. hydrogen bond in the protein acylhomoserine lactone binding cavity.

Bibliographic Information

Quorum sensing in vibrios: complexity for diversification. Milton, Debra L. Department of Molecular Biology, Umeaa University, Umeaa, Swed. International Journal of Medical Microbiology (2006), 296(2-3), 61-71. Publisher: Elsevier GmbH, CODEN: IMEMFV ISSN: 1438-4221. Journal; General Review written in English. CAN 146:23107 AN 2006:450883 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. N-acylhomoserine lactone-dependent quorum sensing was first discovered in two luminescent marine bacteria, Vibrio fischeri and Vibrio harveyi. The LuxI/R system of V. fischeri is the paradigm of Gram-neg. quorum-sensing systems; however, it is not found in all vibrios. A more complex quorum-sensing regulation is found in V. harveyi. Three parallel systems transmit

signals via phosphorylase that converge onto one regulatory protein LuxO. Components of the three systems are found only in vibrios. Of the five Vibrio strains analyzed, the no. and types of signal circuits found in each strain are diverse. The signalling systems have different regulatory responses depending on the type of assocn. the Vibrio strains have with an animal host, which may reflect the diverse roles the vibrios have in structuring and maintaining microniches within the aquatic milieu. Further studies are likely to show that the diversity and complexity of the Vibrio quorum-sensing systems coordinate intraspecies behavior, niche occupation, and possibly evolution.

Bibliographic Information

Research progress and application of bacterial quorum-sensing system. Li, Chengguang; Jia, Zhenhua; Qiu, Jian; Zhang, Xia; Ma, Hong; Ji, Yinggguang; Song, Shuishan. Department of Biotechnology Engineering, Hebei University of Technology, Tianjin, Peop. Rep. China. Shengwu Jishu Tongbao (2006), (1), 5-8. Publisher: Shengwu Jishu Tongbao Bianjibu, CODEN: SJTHA5 ISSN: 1002-5464. Journal; General Review written in Chinese. CAN 145:120125 AN 2006:427036 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Quorum-sensing refers to a regulating system, in which bacteria secret small signal mols., sense their concn. change, and regulate bacterial population behavior. Bacterial quorum-sensing is involved in the regulation of biol. processes including the virulence of human and plant pathogens. This article outlines the latest research of quorum-sensing, and describes the prosperity of application in biotechnol.

Bibliographic Information

Assessment of the roles of LuxS, S-ribosyl homocysteine, and autoinducer 2 in cell attachment during biofilm formation by Listeria monocytogenes EGD-e. Belval, Sylvain Challan; Gal, Laurent; Margiewes, Sylvain; Garmyn, Dominique; Piveteau, Pascal; Guzzo, Jean. Laboratoire de Microbiologie, ENSBANA, UMR INRA UB 1232, Dijon, Fr. Applied and Environmental Microbiology (2006), 72(4), 2644-2650. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 145:79481 AN 2006:412394 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

LuxS is responsible for the prodn. of autoinducer 2 (AI-2), which is involved in the quorum-sensing response of Vibrio harveyi. AI-2 is found in several other Gram-neg. and Gram-pos. bacteria and is therefore considered a good candidate for an interspecies communication signal mol. In order to det. if this system is functional in the gastrointestinal pathogen Listeria monocytogenes EGD-e, an AI-2 bioassay was performed with culture supernatants. The results indicated that this bacterium produces AI-2 like mols. A potential ortholog of V. harveyi luxS, lmo1288, was found by performing sequence similarity searches and complementation expts. with Escherichia coli DH5□, a luxS null strain. Lmo1288 was found to be a functional luxS ortholog involved in AI-2 synthesis. Indeed, interruption of lmo1288 resulted in loss of the AI-2 signal. Although no significant differences were obsd. between Lux1 and EGD-e with regard to planktonic growth (at 10□C, 15□C, 25□C, and 42□C), swimming motility, and phospholipase and hemolytic activity, biofilm culture expts. showed that under batch conditions between 25% and 58% more Lux1 cells than EGD-e cells were attached to the surface depending on the incubation time. During biofilm growth in continuous conditions after 48 h of culture, Lux1 biofilms were 17 times denser than EGD-e

biofilms. Finally, the results showed that Lux1 accumulates more S-adenosyl homocysteine (SAH) and S-ribosyl homocysteine (SRH) in culture supernatant than the parental strain accumulates and that SRH, but not SAH or AI-2, is able to modify the no. of attached cells.

Bibliographic Information

The transcriptional regulator VqmA increases expression of the quorum-sensing activator HapR in Vibrio cholerae. Liu, Zhi; Hsiao, Ansel; Joelsson, Adam; Zhu, Jun. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. Journal of Bacteriology (2006), 188(7), 2446-2453. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 144:446136 AN 2006:302479 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio cholerae is the causative agent of the severe diarrheal disease cholera. A no. of environmental stimuli regulate virulence gene expression in V. cholerae, including quorum-sensing signals. At high cell densities, quorum sensing in V. cholerae invokes a series of signal transduction pathways in order to activate the expression of the master regulator HapR, which then represses the virulence regulon and biofilm-related genes and activates protease prodn. In this study, we identified a transcriptional regulator, VqmA (VCA1078), that activates hapR expression at low cell densities. Under in vitro inducing conditions, constitutive expression of VqmA represses the virulence regulon in a HapR-dependent manner. VqmA increases hapR transcription as measured by the activity of the hapR-lacZ reporter, and it increases HapR prodn. as measured by Western blotting. Using a heterogenous luxCDABE cosmid, we found that VqmA stimulates quorum-sensing regulation at lower cell densities and that this stimulation bypasses the known LuxO-small-RNA regulatory circuits. Furthermore, we showed that VqmA regulates hapR transcription directly by binding to its promoter region and that expression of vqmA is cell d. dependent and autoregulated. The physiol. role of VqmA is also discussed.

Bibliographic Information

Linking bacteriophage infection to quorum sensing signalling and bioluminescent bioreceptor monitoring for direct detection of bacterial agents. Ripp, S.; Jegier, P.; Birmele, M.; Johnson, C. M.; Daumer, K. A.; Garland, J. L.; Sayler, G. S. Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN, USA. Journal of Applied Microbiology (2006), 100(3), 488-499. Publisher: Blackwell Publishing Ltd., CODEN: JAMIFK ISSN: 1364-5072. Journal written in English. CAN 145:370455 AN 2006:275420 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

To incorporate into the lambda phage genome, a luxI-based acyl-homoserine lactone (AHL) synthase genetic construct and exploit the autoamplified power of quorum sensing to translate a phage infection event into a chem. signature detectable by a lux-based bioluminescent bioreporter, with focus towards facile detection of microbial pathogens. The luxI gene from Vibrio fischeri was inserted into the lambda phage genome to construct a model phage-based biosensor system for the general detection of Escherichia coli. The AHL signalling mols. synthesized upon phage infection are detected by an AHL-specific bioluminescent bioreporter based on the luxCDABE gene cassette of V. fischeri. The assay generates target-specific visible light signals with no requisite addn. of extraneous substrate. This binary reporter system was able to autonomously respond to lambda

phage infection events at target E. coli concns. ranging from 1 □ 108 to 1 CFU ml-1 within 1.5-10.3 h, resp., in pure culture. When assayed against artificially contaminated lettuce leaf washings, detection within an E. coli inoculum range from 1 □ 108 to 130 CFU ml-1 was achieved within 2.6-22.4 h, resp. The initial feasibility of binary phage-based reporter assays indicates that quorum sensing can be used to translate a phage infection event into an autoamplified chem. signature. With further modification, binary phage-based reporter assays may be capable of rapidly and cost effectively detecting pathogenic agents at very low population densities.

Bibliographic Information

Evidence for quorum sensing in Clostridium botulinum 56A. Zhao, L.; Montville, T. J.; Schaffner, D. W. Department of Food Science, Cook College, The New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA. Letters in Applied Microbiology (2006), 42(1), 54-58. Publisher: Blackwell Publishing Ltd., CODEN: LAMIE7 ISSN: 0266-8254. Journal written in English. CAN 145:331452 AN 2006:262841 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Expts. were designed to detect quorum-sensing signals produced by Clostridium botulinum. Clostridium botulinum 56A cell-free supernatants obtained at the end of lag phase, the mid-exponential phase and early stationary phase of growth were assayed for bioluminescence in the Vibrio harveyi quorum-sensing assay system. Twelve and 16-h culture supernatants induced bioluminescence in the auto-inducer 2 (AI-2) but not the auto-inducer 1 (AI-1) assay. Intra-species quorum sensing was also assayed as the ability of the supernatants to promote spore germination and outgrowth in a microtitre plate system. Spore populations exposed to C. botulinum supernatant from the end of lag phase became pos. for growth sooner than controls. The influence of cell-free supernatant on ungerminated spores and detection of bioluminescence in the AI-2 assay are evidence for a signalling mol.(s) and provide a first step in characterizing C. botulinum quorum sensing. Significance and Impact of the Study: This study suggests that spores do not behave independently of each other and may explain the inocula size effects obsd. in challenge studies. Whether AI-2 prodn. in C. botulinum serves as an inter-species signal or as a detoxification mechanism remains to be detd.

Bibliographic Information

Increased chatter: cyclic dipeptides as molecules of chemical communication in Vibrio spp. Klose, Karl E. South Texas Center for Emerging Infectious Diseases and Department of Biology, University of Texas at San Antonio, San Antonio, TX, USA. Journal of Bacteriology (2006), 188(6), 2025-2026. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal; General Review written in English. CAN 144:366036 AN 2006:256510 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Bacteria use a wide variety of mechanisms and overlapping systems communicate with each other and coordinate population-dependent gene expression. This type of cell-to-cell communication, called quorum sensing (QS), is involved in modulating bioluminescence, biofilm formation, and pathogenesis (for a review see ref. 18). Several different QS signaling pathways have been characterized in Vibrio spp. In the current issue, Park et al. (14a) describe a signaling

mol., cyclo-L-Phe-L-Pro (cFP) that fulfills some of the definitions of a QS mol. and may represent yet another means by which Vibrio spp. communicate.

Bibliographic Information

Identification of a constitutively active variant of LuxO that affects production of HA/protease and biofilm development in a non-O1, non-O139 Vibrio cholerae O110. Raychaudhuri, Saumya; Jain, Vibhu; Dongre, Mitesh. Molecular Biology Division, Institute of Microbial Technology, Chandigarh, India. Gene (2006), 369 126-133. Publisher: Elsevier B.V., CODEN: GENED6 ISSN: 0378-1119. Journal written in English. CAN 144:406641 AN 2006:199474 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Pathogenesis of Vibrio cholerae depends on the concerted action of numerous virulence factors that includes a secreted hemagglutinin (HA) protease. Recent studies have evidenced that the expression of these virulence factors as well as the genes responsible for biofilm development is subject to control by quorum sensing in this organism. At low cell d., LuxO, the pivotal regulator of quorum-sensing circuit, has been shown to be phosphorylated at aspartate-47. Working in concert with sigma-54, LuxO-P activates the downstream repressor, which turned out to be four sRNAs [Lenz, D.H., Mok, K.C., Lilley, B.N., Kulkarni, R.V., Wingreen, N.S., Bassler, B.L., 2004. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in Vibrio harveyi and Vibrio cholerae. Cell 118, 69-82]. Subsequently, these sRNAs form complex with sRNA chaperone, Hfq. The Hfq-sRNA complex causes the destabilization of hapR mRNA transcript. HapR is a pos. regulator of hapA that encodes HA/protease. At high cell d., dephosphorylation of LuxO impairs its function to activate the expression of sRNA, which in turn promotes HapR expression and causes protease prodn. It has been demonstrated that conversion of aspartate to glutamate (D47E) renders the LuxO mol. active without being phosphorylated. This variant of LuxO is referred as constitutively active LuxO or con-LuxO [Freeman, J.A., Bassler, B.L., 1999. A genetic anal. of the function of LuxO, a two-component response regulator involved in quorum sensing in Vibrio harveyi. Mol Microbiol 31, 665-677]. Other than D47E, mutation at L104Q also develops con-LuxO [Vance, R.E., Zhu, J., Mekalanos, J.J., 2003. A constitutively active variant of the quorum-sensing regulator LuxO affects protease prodn. and biofilm formation in Vibrio cholerae. Infect. Immun. 71, 2571-2576]. The purpose of this study was to investigate the cause of protease neg. phenotype of a non-O1, non-O139 strain of V. cholerae O110.

In the process of exploring the nature of the phenotype, a constitutively active variant of LuxO mol. was characterized which represses protease prodn. and enhances biofilm formation by this strain. Unlike luxU, disruption of luxO restored the protease prodn., which showed the constitutively active nature of LuxO protein in this strain.

Bibliographic Information

Genetic and phenotypic diversity of quorum-sensing systems in clinical and environmental isolates of Vibrio cholerae. Joelsson, Adam; Liu, Zhi; Zhu, Jun. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. Infection and Immunity (2006), 74(2), 1141-1147. Publisher: American Society for Microbiology, CODEN: INFIBR ISSN: 0019-9567. Journal written in English. CAN 144:344493 AN 2006:132253 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio cholerae is the causative agent of cholera, a severe and devastating diarrheal disease. V. cholerae lives naturally in various aquatic habitats during interepidemic periods. Recent studies reveal that quorum-sensing systems, which exist in many bacteria and help them monitor their population densities and regulate various cellular functions, control V. cholerae pathogenesis, biofilm formation, and protease prodn. In this study we surveyed quorum-sensing systems in 16 geog. diverse V. cholerae strains from epidemic-causing O1 and O139 strains as well as non-O1/non-O139 and environmental isolates and discovered an unexpectedly high rate of dysfunctional components. We also found that a functional quorum-sensing system conferred a survival advantage on bacteria in biofilms when the bacteria were exposed to seawater, though quorum sensing was less important to survival in a planktonic state under the same conditions. These findings suggest that variations in quorum-sensing systems are due to environmental selective pressures and might be beneficial to V. cholerae's fitness under certain conditions found in its natural reservoirs.

Bibliographic Information

Environmentally controlled invasion of cancer cells by engineered bacteria. Anderson, J. Christopher; Clarke, Elizabeth J.; Arkin, Adam P.; Voigt, Christopher A. Howard Hughes Medical Institute, California Institute of Quantitative Biology, Department of Bioengineering, University of California, Berkeley, CA, USA. Journal of Molecular Biology (2006), 355(4), 619-627. Publisher: Elsevier B.V., CODEN: JMOBAK ISSN: 0022-2836. Journal written in English. CAN 144:208272 AN 2005:1348516 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria can sense their environment, distinguish between cell types, and deliver proteins to eukaryotic cells. Here, we engineer the interaction between bacteria and cancer cells to depend on heterologous environmental signals. We have characterized invasin from Yersinia pseudotuberculosis as an output module that enables Escherichia coli to invade cancer-derived cells, including HeLa, HepG2, and U2OS lines. To environmentally restrict invasion, we placed this module under the control of heterologous sensors. With the Vibrio fischeri lux quorum sensing circuit, the hypoxia-responsive fdhF promoter, or the arabinose-inducible araBAD promoter, the bacteria invade cells at densities greater than 108 bacteria/mL, after growth in an anaerobic growth chamber or in the presence of 0.02% arabinose, resp. In the process, we developed a technique to tune the linkage between a sensor and output gene using ribosome binding site libraries and genetic selection. This approach could be used to engineer bacteria to sense the microenvironment of a tumor and respond by invading cancerous cells and releasing a cytotoxic agent.

Bibliographic Information

Structure of the Escherichia coli Quorum Sensing Protein SdiA: Activation of the Folding Switch by Acyl Homoserine Lactones. Yao, Yong; Martinez-Yamout, Maria A.; Dickerson, Tobin J.; Brogan, Andrew P.; Wright, Peter E.; Dyson, H. Jane. Department of Molecular Biology, the Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, USA. Journal of Molecular Biology (2006), 355(2), 262-273. Publisher: Elsevier B.V., CODEN: JMOBAK ISSN: 0022-2836. Journal written in English. CAN 144:186687 AN 2005:1300583 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The three-dimensional structure of a complex between the N-terminal domain of the quorum sensing protein SdiA of Escherichia coli and a candidate autoinducer N-octanoyl-L-homoserine lactone (C8-HSL) has been calcd. in soln. from NMR data. The SdiA-HSL system shows the "folding switch" behavior that has been seen for quorum-sensing factors produced by other bacterial species. In the presence of C8-HSL, a significant proportion of the SdiA protein is produced in a folded, sol. form in an E. coli expression system, whereas in the absence of acyl homoserine lactones, the protein is expressed into insol. inclusion bodies. In the three-dimensional structure, the autoinducer mol. is sequestered in a deep pocket in the hydrophobic core, forming an integral part of the core packing of the folded SdiA. The NMR spectra of the complex show that the bound C8-HSL is conformationally heterogeneous, either due to motion within the pocket or to heterogeneity of the bound structure. The C8-HSL conformation is defined by NOEs to the protein only at the terminal Me group of the octanoyl chain. Unlike other well-studied bacterial quorum sensing systems such as LuxR of Vibrio fischeri and TraR of Agrobacterium tumefaciens, there is no endogenous autoinducer for SdiA in E. coli: the E. coli genome does not contain a gene analogous to the LuxI and TraI autoinducer synthetases. We show that two other homoserine lactone derivs. are also capable of acting as a folding-switch autoinducers for SdiA. The obsd. structural heterogeneity of the bound C8-HSL in the complex, together with the variety of autoinducer-type mols. that can apparently act as folding switches in this system, are consistent with the postulated biol. function of the SdiA protein as a detector of the presence of other species of bacteria.

Bibliographic Information

CsrA and three redundant small RNAs regulate quorum sensing in Vibrio cholerae. Lenz, Derrick H.; Miller, Melissa B.; Zhu, Jun; Kulkarni, Rahul V.; Bassler, Bonnie L. Department of Molecular Biology, Howard Hughes Medical Institute, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2005), 58(4), 1186-1202. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 144:146251 AN 2005:1295084 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria communicate using a process called quorum sensing which involves prodn., secretion and detection of signaling mols. called autoinducers. Quorum sensing allows populations of bacteria to simultaneously regulate gene expression in response to changes in cell d. The human pathogen, Vibrio cholerae, uses a quorum-sensing circuit composed of parallel systems that transduce information through four redundant regulatory small RNAs (sRNAs) called quorum regulatory RNAs (Qrr) to control the expression of numerous genes, most notably those required for virulence. We show that the VarS/VarA two-component sensory system comprises an addnl. regulatory input controlling quorum-sensing-dependent gene expression in V. cholerae. VarS/VarA controls transcription of three previously unidentified small regulatory RNAs (sRNAs) that are similar to the sRNAs CsrB and CsrC of Escherichia coli. The three V. cholerae sRNAs, which we name CsrB, CsrC and CsrD, act redundantly to control the activity of the global regulatory protein, CsrA. The VarS/VarA-CsrA/BCD system converges with the V. cholerae quorum-sensing systems to regulate the expression of the Qrr sRNAs, and thus, the entire quorum-sensing regulon.

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Solution structure and dynamics of Vibrio harveyi LuxU, a phosphotransferase protein involved in bacterial quorum sensing. Ulrich, Dagny Lorraine. Yale Univ., New Haven,

CT, USA. Avail. UMI, Order No. DA3169006. (2005), 130 pp. From: Diss. Abstr. Int., B 2005, 66(3), 1314. Dissertation written in English. CAN 144:407784 AN 2005:1266004 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

Vibrio qrr genes and small RNAs which bind to Hfq and regulate quorum sensing and Vibrio mutants for indentifying quorum-sensing regulators. Lenz, Derrick H.; Mok, Kenny C.; Wingreen, Ned S.; Bassler, Bonnie L. (The Trustees of Princeton University, USA). PCT Int. Appl. (2005), 58 pp. CODEN: PIXXD2 WO 2005100544 A2 20051027 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2005-US12462 20050412. Priority: US 2004-561660 20040412. CAN 143:417277 AN 2005:1154669 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2005100544	A2	20051027	WO2005-US12462	20050412
WO2005100544	A3	20060427		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 20060057607	A1	20060316	US 2005-104705	20050412
US 7405050	B2	20080729		

Priority Application		
US 2004-561660P	P	20040412

Abstract

Quorum-sensing bacteria communicate with extracellular signal mols. called autoinducers to allow community-wide synchronization of gene expression. The present invention relates to the identification of Vibrio harveyi and Vibrio cholerae protein Hfq as mediating interactions between small, regulatory RNAs (sRNAs) and specific mRNA targets. Accordingly, the present invention provides nucleic acids encoding the Vibrio sRNAs, strains having various deletions and mutations of one or more qrr genes encoding these sRNA, as well as methods of identifying quorum-sensing regulators. Addnl., the invention relates to an isolated V. harveyi Hfq protein and conservative

amino acid substitutions thereof as well as nucleic acids encoding those proteins, recombinant methods of producing those proteins and antibodies against those proteins.

Bibliographic Information

Molecular Communication through Stochastic Synchronization Induced by Extracellular Fluctuations. Zhou, Tianshou; Chen, Luonan; Aihara, Kazuyuki. School of Mathematics and Computational Sciences, Zhongshan University, Guangzhou, Peop. Rep. China. Physical Review Letters (2005), 95(17), 178103/1-178103/4. Publisher: American Physical Society, CODEN: PRLTAO ISSN: 0031-9007. Journal written in English. CAN 144:48075 AN 2005:1145433 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The authors model a synthetic gene regulatory network in a microbial cell, and investigate the effect of noises on cell-cell communication in a well-mixed multicellular system. A biol. plausible model is developed for cellular communication in an indirectly coupled multicellular system. Without extracellular noises, all cells, in spite of interaction among them, behave irregularly due to independent intracellular noises. Extracellular noises that are common to all cells can induce collective dynamics and stochastically synchronize the multicellular system by actively enhancing the integrated interchange of signaling mols.

Bibliographic Information

Interkingdom signaling: Deciphering the language of acyl homoserine lactones. Shiner, Erin K.; Rumbaugh, Kendra P.; Williams, Simon C. Departments of Microbiology & Immunology, Texas Tech University Health Sciences Center, Lubbock, TX, USA. FEMS Microbiology Reviews (2005), 29(5), 935-947. Publisher: Elsevier B.V., CODEN: FMREE4 ISSN: 0168-6445. Journal; General Review written in English. CAN 144:103574 AN 2005:1101274 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Bacteria use small secreted chems. or peptides as autoinducers to coordinately regulate gene expression within a population in a process called quorum sensing. Quorum sensing controls several important functions in different bacterial species, including the prodn. of virulence factors and biofilm formation in Pseudomonas aeruginosa and bioluminescence in Vibrio fischeri. Many gram-neg. bacterial species use acyl homoserine lactones as autoinducers that function as ligands for transcriptional regulatory proteins. Several recent reports indicate that bacterial acyl homoserine lactones can also affect gene expression in host cells. Direct signaling also appears to function in the opposite direction as some eukaryotic cell types produce mimics that interact with quorum sensing systems in bacteria. Here, the authors describe the evidence to support the existence of bi-directional interkingdom signaling via acyl homoserine lactones and eukaryotic mimics and discuss the potential mol. mechanisms that mediate these responses. The functional consequences of interkingdom signaling will be discussed in relation to both pathogenic and non-pathogenic bacterial-host interactions.

Bibliographic Information

A furanosyl-carbonate autoinducer in cell-to-cell communication of V. harveyi. McKenzie, Kathleen M.; Meijler, Michael M.; Lowery, Colin A.; Boldt, Grant E.; Janda, Kim D. Skaggs

Institute for Chemical Biology and Departments of Chemistry and Immunology, The Scripps Research Institute, La Jolla, CA, USA. Chemical Communications (Cambridge, United Kingdom) (2005), (38), 4863-4865. Publisher: Royal Society of Chemistry, CODEN: CHCOFS ISSN: 1359-7345. Journal written in English. CAN 144:2993 AN 2005:1039154 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
An autoinducer arising from reaction of cyclized S-DPD and carbonate is shown to induce light in V. harveyi and thus may play a previously unknown role in quorum sensing.

Bibliographic Information

Quorum sensing signal molecules (acylated homoserine lactones) in gram-negative fish pathogenic bacteria. Bruhn, Jesper B.; Dalsgaard, Inger; Nielsen, Kristian F.; Buchholtz, Christiane; Larsen, Jens L.; Gram, Lone. Department of Seafood Research, Danish Institute for Fisheries Research, Kgs. Lyngby, Den. Diseases of Aquatic Organisms (2005), 65(1), 43-52. Publisher: Inter-Research, CODEN: DAOREO ISSN: 0177-5103. Journal written in English. CAN 145:23956 AN 2005:1037900 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The aim of the present study was to investigate the prodn. of quorum sensing signals (specifically acylated homoserine lactones, AHLs) among a selection of strains of Gram-neg. fish bacterial pathogens. These signals are involved in the regulation of virulence factors in some human and plant-pathogenic bacteria. A total of 59 strains, representing 9 different fish pathogenic species, were tested against 2 AHL monitor bacteria (Agrobacterium tumefaciens NT1 [pZLR4] and Chromobacterium violaceum CV026) in a well diffusion assay and by thin-layer chromatog. (TLC). Representative samples were further characterized by high performance liq. chromatog.-high resoln. mass spectrometry (HPLC-HR-MS). AHLs were produced by all strains of Aeromonas salmonicida, Aeromonas hydrophila, Yersinia ruckeri, Vibrio salmonicida, and Vibrio vulnificus. Some strains of atypical Aeromonas salmonicida and Vibrio splendidus were also pos. Aeromonas species produced N-butanoyl homoserine lactone (BHL) and N-hexanoyl homoserine lactone (HHL) and 1 addnl. product, whereas N-3-oxo-hexanoyl homoserine lactone (OHHL) and HHL were detected in Vibrio salmonicida. N-3-oxo-octanoyl homoserine lactone (OOHL) and N-3-octanoyl homoserine lactone (OHL) were detected in Y. ruckeri. AHLs were not detected from strains of Photobacterium damselae, Flavobacterium psychrophilum or Moritella viscosa. AHLs were extd. from fish infected with Y. ruckeri but not from fish infected with A. salmonicida. In conclusion, the prodn. of quorum sensing signals, AHLs, is common among the strains that we examd. If the AHL mols. regulate the expression of the virulence phenotype in these bacteria, as shown to occur in some bacterial pathogens, novel disease control measures may be developed by blocking AHL-mediated communication and suppressing virulence.

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Construction and analysis of a Mannheimia haemolytica A1 luxS mutant. van der Vinne, Amanda N.; Lo, Reggie Y. C.; Shewen, Patricia E. Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Can. Veterinary Microbiology (2005), 110(1-2), 53-66. Publisher: Elsevier B.V., CODEN: VMICDQ ISSN: 0378-1135. Journal written in English. CAN 144:344427 AN 2005:989541 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Mannheimia haemolytica A1 is the causative agent of bovine pneumonic pasteurellosis, a major cause of sickness, death, and economic loss to the feedlot cattle industry. M. haemolytica A1 produces autoinducer-2 (AI-2) like mols. that are capable of inducing quorum sensing system 2 of Vibrio harveyi. This interspecies quorum sensing system has been shown to regulate the expression of virulence genes in several pathogenic bacteria. The protein central to the prodn. of AI-2 is LuxS. To det. if quorum sensing is involved in the regulation of virulence genes in M. haemolytica A1, a luxS mutant was constructed by replacing luxS with a cat cassette. This mutant was verified by PCR anal., Southern hybridization, as well as its inability to induce bioluminescence in the V. harveyi reporter strain. RT-PCR anal. showed there was no difference in leukotoxin (lktC) mRNA levels, however there were increased mRNA levels of putative virulence assocd. genes, transferrin binding protein B (tbpB), adhesin (ahs) and capsule biosynthesis (nmaA). Electron microscopy showed that the level of encapsulation in the mutant is higher than the parent. Addnl., the mutant was slightly more adherent to bovine tracheal cells than the parent. In vitro competition assays showed the mutant out-competed the parent under iron-restricted conditions. However, in a calf challenge, the parent was the dominant isolate recovered.

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LuxS/autoinducer-2 quorum sensing molecule regulates transcriptional virulence gene expression in Clostridium difficile. Lee, Alex Sek Yew; Song, Keang Peng. Microbial Pathogenesis Laboratory, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. Biochemical and Biophysical Research Communications (2005), 335(3), 659-666. Publisher: Elsevier, CODEN: BBRCA9 ISSN: 0006-291X. Journal written in English. CAN 143:402358 AN 2005:944371 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Toxigenic Clostridium difficile CCUG19126 produces the autoinducer-2 (AI-2) quorum sensing mol. that induces bioluminescence in Vibrio harveyi BB170 reporter strain. AI-2-contg. cell-free supernatants from mid-log phase C. difficile and Escherichia coli DH5□ expressing recombinant luxScd upregulated the transcript levels of tcdA (7-10-fold), tcdB (4-6-fold), and tcdE (2-3-fold) in early-log C. difficile. In contrast, no induction occurred when cells were exposed to sterile medium or to cell-free supernatant from E. coli DH5□. AI-2 did not significantly increase the level of toxin A in early-log C. difficile. These results suggest that LuxScd-dependent signaling regulates virulence gene expression at the transcriptional level in C. difficile.

Bibliographic Information

The impact of mutations in the quorum sensing systems of Aeromonas hydrophila, Vibrio anguillarum and Vibrio harveyi on their virulence towards gnotobiotically cultured Artemia franciscana. Defoirdt, Tom; Bossier, Peter; Sorgeloos, Patrick; Verstraete, Willy. Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belg. Environmental Microbiology (2005), 7(8), 1239-1247. Publisher: Blackwell Publishing Ltd., CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 144:146330 AN 2005:924118 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Disruption of quorum sensing, bacterial cell-to-cell communication by means of small signal mols., has been suggested as a new anti-infective strategy for aquaculture. However, data about the impact of quorum sensing on the virulence of aquatic pathogens are scarce. In this study, a model system using gnotobiotically cultured Artemia franciscana was developed in order to det. the impact of mutations in the quorum sensing systems of Aeromonas hydrophila, Vibrio anguillarum and V. harveyi on their virulence. Mutations in the autoinducer 2 (AI-2) synthase gene luxS, the AI-2 receptor gene luxP or the response regulator gene luxO of the dual channel quorum sensing system of V. harveyi abolished virulence of the strain towards Artemia. Moreover, the addn. of an exogenous source of AI-2 could restore the virulence of an AI-2 non-producing mutant. In contrast, none of the mutations in either the acylated homoserine lactone (AHL)-mediated component of the V. harveyi system or the quorum sensing systems of Ae. hydrophila and V. anguillarum had an impact on virulence of these bacteria towards Artemia. The results indicate that disruption of quorum sensing could be a good alternative strategy to combat infections caused by V. harveyi.

Bibliographic Information

A novel Vibrio harveyi mutant with altered quorum sensing regulation. Czyz, Agata; Zielke, Ryszard; Wegrzyn, Grzegorz. Laboratory of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Gdansk, Pol. Oceanological and Hydrobiological Studies (2005), 34(2), 55-61. Publisher: University of Gdansk, Institute of Oceanography, CODEN: OHSCBU ISSN: 1730-413X. Journal written in English. CAN 144:146328 AN 2005:889491 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The expression of genes involved in the luminescence (lux genes) of many light-emitting bacteria, including the marine bacterium Vibrio harveyi, is regulated by a phenomenon called quorum sensing. The expression of the lux genes, and thus the efficiency of light emission, depends on the concn. of cells in the environment. Bacterial luminescence is effective when cells occur at high d., whereas light emission is negligible in dild. cultures. Quorum sensing regulation is a complex process which requires the functioning of many genes. The current paper describes the recently isolated novel V. harveyi mutant, which now appears to be impaired in quorum sensing. The mutant produces autoinducers normally, but it is partially defective in responding to these mols., thus its quorum sensing reaction is delayed relative to wild-type bacteria.

Bibliographic Information

Bacteroides species produce Vibrio harveyi autoinducer 2-related molecules. Antunes, Luis Caetano Martha; Ferreira, Livia Queiroz; Ferreira, Eliane Oliveira; Miranda, Karla Rodrigues; Avelar, Katia Eliane Santos; Domingues, Regina Maria Cavalcanti Pilotto; Ferreira, Maria Candida de Souza. Departmento de Microbiologia Medica, Instituto de Microbiologia Prof. Paulo de Goes, Universidade Federal do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. Anaerobe (2005), 11(5), 295-301. Publisher: Elsevier B.V., CODEN: ANAEF8 ISSN: 1075-9964. Journal written in English. CAN 144:428584 AN 2005:778649 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing is a d.-dependent gene regulation mechanism that has been described in many bacterial species in the last decades. Bacteria that use quorum sensing as part of their gene regulation circuits produce mols. called autoinducers that accumulate in the environment and

activate target genes in a quorum-dependent way. Some specific clues led the authors to hypothesize that Bacteroides species can produce autoinducers and possess a quorum sensing system. First, Bacteroides are anaerobic bacteria that are frequently involved in polymicrobial infections. These infections often involve Pseudomonas aeruginosa and Staphylococcus aureus, two of the best understood examples of bacteria that employ quorum sensing systems as part of their pathogenesis. Also, studies have detected the presence of a quorum sensing gene involved in the prodn. of autoinducers in Porphyromonas gingivalis, a species closely related to the Bacteroides genus. These and other evidences prompted us to investigate if Bacteroides strains could produce autoinducer mols. that could be detected by a Vibrio harveyi reporter system. In this paper, we show that supernatants of B. fragilis, B. vulgatus and B. distasonis strains are able to stimulate the V. harveyi quorum sensing system 2. Also, we were able to demonstrate that the stimulation detected is due to the prodn. of autoinducer mols. and not the growth of reporter strains after addn. of supernatant. Moreover, the phenomenon obsd. does not seem to represent the degrdn. of repressors possibly present in the culture medium used. The authors could also amplify bands from some of the strains tested using primers designed to the luxS gene of Escherichia coli. The results show that B. fragilis, B. vulgatus and B. distasonis (but possibly some other species) can produce V. harveyi autoinducer 2-related mols. However, the role of such mols. in the biol. of these organisms remains unknown.

Bibliographic Information

Dual regulation of genes involved in acetoin biosynthesis and motility/biofilm formation by the virulence activator AphA and the acetate-responsive LysR-type regulator AlsR in Vibrio cholerae. Kovacikova, Gabriela; Lin, Wei; Skorupski, Karen. Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA. Molecular Microbiology (2005), 57(2), 420-433. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 143:282376 AN 2005:646078 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

AphA is a quorum sensing-regulated activator that initiates the virulence cascade in Vibrio cholerae by cooperating with the LysR-type regulator AphB at the tcpPH promoter on the Vibrio pathogenicity island (VPI). To identify the ancestral chromosomal genes in V. cholerae regulated by AphA, we carried out a microarray anal. and show here that AphA influences the expression of 15 genes not assocd. with the VPI. One set of genes strongly repressed by AphA is involved in the biosynthesis of acetoin, a product synthesized by a variety of bacteria that plays a role in preventing intracellular acidification and which is essential for the viability of V. cholerae in the presence of glucose. Also present in this operon are two putative signal transduction proteins with EAL and GGDEF domains that oppositely influence motility and biofilm formation. Gel mobility shift assays show that AphA binds to a site upstream of the first gene in the acetoin operon. Transcriptional lacZ fusions indicate that at low cell d. AphA represses the expression of the acetoin genes up to 15-fold. Voges Proskauer tests confirm that deletion of AphA increases the prodn. of acetoin under non-inducing conditions and also that the LysR-type regulator AlsR divergently transcribed from the operon is required for its prodn. This is the first report of a specific repressor protein involved in the transcriptional control of acetoin prodn. as well as the co-regulation of these genes with those that influence motility and biofilm formation. The results here provide a model for the dual regulation of these processes by acetate and quorum sensing through AlsR and AphA.

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Defining the Vibrio harveyi quorum sensing network. Mok, Kenny Chee-Kin. Princeton Univ., Princeton, NJ, USA. Avail. UMI, Order No. DA3142349. (2004), 140 pp. From: Diss. Abstr. Int., B 2005, 65(7), 3312. Dissertation written in English. CAN 143:148611 AN 2005:629195 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

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Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts. Rasch, Maria; Andersen, Jens Bo; Nielsen, Kristian Fog; Flodgaard, Lars Ravn; Christensen, Henrik; Givskov, Michael; Gram, Lone. Department of Seafood Research, Soltofts Plads, Danish Institute for Fisheries Research, Kgs. Lyngby, Den. Applied and Environmental Microbiology (2005), 71(6), 3321-3330. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 143:208812 AN 2005:527802 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacterial communication signals, acylated homoserine lactones (AHLs), were extd. from samples of com. bean sprouts undergoing soft-rot spoilage. Bean sprouts produced in the lab. did not undergo soft-rot spoilage and did not contain AHLs or AHL-producing bacteria, although the bacterial population reached levels similar to those in the com. sprouts, 108 to 109 CFU/g. AHL-producing bacteria (Enterobacteriaceae and pseudomonads) were isolated from com. sprouts, and strains that were both proteolytic and pectinolytic were capable of causing soft-rot spoilage in bean sprouts. Thin-layer chromatog. and liq. chromatog.-high-resoln. mass spectrometry revealed the presence of N-3-oxohexanoyl-L-homoserine lactone in spoiled bean sprouts and in exts. from pure cultures of bacteria. During normal spoilage, the pH of the sprouts increased due to proteolytic activity, and the higher pH probably facilitated the activity of pectate lyase. The AHL synthetase gene (I gene) from a spoilage Pectobacterium was cloned, sequenced, and inactivated in the parent strain. The predicted amino acid sequence showed 97% homol. to HslI and CarI in Erwinia carotovora. Spoilage of lab. bean sprouts inoculated with the AHL-neg. mutant was delayed compared to sprouts inoculated with the wild type, and the AHL-neg. mutant did not cause the pH to rise. Compared to the wild-type strain, the AHL-neg. mutant had significantly reduced protease and pectinase activities and was neg. in an iron chelation (siderophore) assay. This is the first study demonstrating AHL regulation of iron chelation in Enterobacteriaceae. The present study clearly demonstrates that the bacterial spoilage of some food products is influenced by quorum-sensing-regulated phenotypes, and understanding these processes may be useful in the development of novel food preservation additives that specifically block the quorum-sensing systems.

Bibliographic Information

Vibrio fischeri uses two quorum-sensing systems for the regulation of early and late colonization factors. Lupp, Claudia; Ruby, Edward G. Pacific Biomedical Research Center, University of Hawaii at Manoa, Honolulu, HI, USA. Journal of Bacteriology (2005), 187(11), 3620-3629. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 143:129714 AN 2005:469450 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio fischeri possesses two quorum-sensing systems, ain and lux, using acyl homoserine lactones as signaling mols. We have demonstrated previously that the ain system activates luminescence gene expression at lower cell densities than those required for lux system activation and that both systems are essential for persistent colonization of the squid host, Euprymna scolopes. Here, we asked whether the relative contributions of the two systems are also important at different colonization stages. Inactivation of ain, but not lux, quorum-sensing genes delayed initiation of the symbiotic relationship. In addn., our data suggest that lux quorum sensing is not fully active in the early stages of colonization, implying that this system is not required until later in the symbiosis. The V. fischeri luxI mutant does not express detectable light levels in symbiosis yet initiates colonization as well as the wild type, suggesting that ain quorum sensing regulates colonization factors other than luminescence. We used a recently developed V. fischeri microarray to identify genes that are controlled by ain quorum sensing and could be responsible for the initiation defect. We found 30 differentially regulated genes, including the repression of a no. of motility genes. Consistent with these data, ain quorum-sensing mutants displayed an altered motility behavior in vitro. Taken together, these data suggest that the sequential activation of these two quorum-sensing systems with increasing cell d. allows the specific regulation of early colonization factors (e.g., motility) by ain quorum sensing, whereas late colonization factors (e.g., luminescence) are preferentially regulated by lux quorum sensing.

Bibliographic Information

Layers of signaling in a bacterium-host association. Visick, Karen L. Department of Microbiology and Immunology, Maywood, IL, USA. Journal of Bacteriology (2005), 187(11), 3603-3606. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal; General Review written in English. CAN 143:129600 AN 2005:469448 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review with commentary on aspects of Vibrio fischeri quorum sensing in its symbiotic interaction with Euprymna scolopes.

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Modulation of quorum sensing in bacteria with peptide hydrolase and peptide hydrolase inhibitors. Robinson, Gary Kevin; Rising, Hannah. (University of Kent, UK). PCT Int. Appl. (2005), 21 pp. CODEN: PIXXD2 WO 2005047514 A1 20050526 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2004-GB4739 20041110. Priority: GB 2003-26194 20031110. CAN 143:4084 AN 2005:451552 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date

WO2005047514 A1 20050526 WO2004-GB4739 20041110
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
EP 1685250 A1 20060802 EP 2004-798462 20041110
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK, IS
US 20070264715 A1 20071115 US 2007-577119 20070413

Priority Application
GB 2003-26194 A 20031110
WO2004-GB4739 W 20041110

Abstract
The method relates to a method of modulating quorum sensing in bacteria with peptide hydrolase and peptide hydrolase inhibitors. Quorum sensing is inhibited using peptide hydrolases while it is upregulated with peptide hydrolase inhibitor. This inhibition is used to prevent biofilm formation or to break down established biofilms and may also be used to downregulate the prodn. of virulence determinants by pathogenic bacteria. The invention also relates to the use of peptide hydrolase inhibitors for the upregulation of quorum sensing in bacteria, resulting in the overprodn. of proteins and the use of this system as an expression system. The invention further provides a method of inhibiting biofilms. Proteins such as LuxR and homologs involved in quorum sensing are provided.

Bibliographic Information

Proteins involved in quorum sensing. Robinson, Gary Kevin; Rising, Hannah. (University of Kent, UK). PCT Int. Appl. (2005), 47 pp. CODEN: PIXXD2 WO 2005046713 A1 20050526 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IS, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2004-GB4775 20041110. Priority: GB 2003-26192 20031110. CAN 142:480791 AN 2005:451219 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2005046713	A1	20050526	WO2004-GB4775	20041110

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,

KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

EP 1682163	A1	20060726	EP 2004-798498	20041110

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK, IS

US 20070298032	A1	20071227	US 2007-577124	20070501

Priority Application

GB 2003-26192	A	20031110
WO2004-GB4775	W	20041110

Abstract
The invention relates to a method of regulating quorum sensing in bacteria by modulating the activation of LuxR or a homolog thereof. Such modulation generally occurs when the bacteria are in the pre-quorate or quorate stage and may be achieved by targeting antibodies to LuxR or a homolog thereof.

Bibliographic Information

Quorum sensing in Clostridium difficile: analysis of a luxS-type signalling system. Carter, Glen P.; Purdy, Des; Williams, Paul; Minton, Nigel P. Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK. Journal of Medical Microbiology (2005), 54(2), 119-127. Publisher: Society for General Microbiology, CODEN: JMMIAV ISSN: 0022-2615. Journal written in English. CAN 143:435673 AN 2005:433818 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The increasing incidence of Clostridium difficile-assocd. disease, and the problems assocd. with its control, highlight the need for addnl. countermeasures. The attenuation of virulence through the blockade of bacterial cell-to-cell communication (quorum sensing) is one potential therapeutic target. Preliminary studies have shown that C. difficile produces at least one potential signalling mol. Through the mol.'s ability to induce bioluminescence in a Vibrio harveyi luxS reporter strain, it has been shown to correspond to autoinducer 2 (Al-2). In keeping with this observation, a homolog of luxS has been identified in the genome of C. difficile. Adjacent to luxSCd a potential transcriptional regulator and sensor kinase, rolA and rolB, have been located. RT-PCR has been used to confirm the genetic organization of the luxSCd locus. While Al-2 prodn. has not been blocked so far using antisense technol., Al-2 levels could be modulated by controlling expression of the putative transcriptional regulator rolA. RolA, therefore, acts as a neg. regulator of Al-2 prodn. Finally, it has been shown that the exogenous addn. of Al-2 or 4-hydroxy-5-methyl-3(2H) furanone has no discernible effect on the prodn. of toxins by C. difficile.

Bibliographic Information

Requirements for Vibrio cholerae HapR binding and transcriptional repression at the hapR promoter are distinct from those at the aphA promoter. Lin, Wei; Kovacikova, Gabriela; Skorupski, Karen. Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA. Journal of Bacteriology (2005), 187(9), 3013-3019. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 143:91865 AN 2005:385954 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Virulence gene expression in certain strains of Vibrio cholerae is regulated in response to cell d. by a quorum-sensing cascade that influences the levels of the LuxR homolog HapR through small regulatory RNAs that control the stability of its message. At high cell d., HapR represses the expression of the gene encoding the virulence gene activator AphA by binding to a site between -85 and -58 in the aphA promoter. The authors show here that a second binding site for HapR lies within the hapR promoter from which it functions to repress its own transcription. This site, as detd. by gel mobility shift assay and DNaseI footprinting, is located between +8 and +36 from the transcriptional start and is not strongly conserved with the site at the aphA promoter. At low cell d., when the expression of a transcriptional hapR-lacZ fusion was low, no autorepression was obsd. However, at high cell d., when the expression of the hapR-lacZ fusion was approx. 15-fold higher, the presence of HapR reduced its expression. Introduction of a single base pair change within the binding site at +18 prevented HapR binding in gel mobility shift assays. In the absence of HapR, this mutation did not significantly influence the expression of the hapR promoter, but in its presence, the expression of the promoter was increased at high cell d. These results indicate that HapR autorepresses from a single binding site in the hapR promoter and suggest a model for the temporal regulation of its expression as its intracellular levels increase.

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Quorum sensing in Vibrio harveyi: Probing the specificity of the LuxP binding site. Lowery, Colin A.; McKenzie, Kathleen M.; Qi, Longwu; Meijler, Michael M.; Janda, Kim D. Department of Chemistry, Institute for Chemical Biology, The Scripps Research Institute and The Skaggs, La Jolla, CA, USA. Bioorganic & Medicinal Chemistry Letters (2005), 15(9), 2395-2398. Publisher: Elsevier B.V., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. CAN 143:3908 AN 2005:331947 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing activity was investigated in the bacterium Vibrio harveyi using a series of both natural and nonnatural analogs of DPD, the penultimate precursor to autoinducer AI-2. The progression of mols. that were both synthesized and investigated includes enantiomeric variants, carbon-chain extension, and hydroxyl-functional group addn./deletions of DPD. The compilation of these studies reveals a binding cleft that can accommodate a no. of different structural variants of DPD, albeit with invariably lower activities.

Bibliographic Information

Solution Structure and Dynamics of LuxU from Vibrio harveyi, a Phosphotransferase Protein Involved in Bacterial Quorum Sensing. Ulrich, Dagny L.; Kojetin, Douglas; Bassler, Bonnie L.; Cavanagh, John; Loria, J. Patrick. Department of Chemistry, Yale University, New Haven, CT, USA. Journal of Molecular Biology (2005), 347(2), 297-307. Publisher: Elsevier

B.V., CODEN: JMOBAK ISSN: 0022-2836. Journal written in English. CAN 142:425543 AN 2005:185162 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The marine bacterium Vibrio harveyi controls its bioluminescence by a process known as quorum sensing. In this process, autoinducer mols. are detected by membrane-bound sensor kinase/response regulator proteins (LuxN and LuxQ) that relay a signal via a series of protein phosphorylation reactions to another response regulator protein, LuxO. Phosphorylated LuxO indirectly represses the expression of the proteins responsible for bioluminescence. Integral to this quorum sensing process is the function of the phosphotransferase protein, LuxU. LuxU acts to shuttle the phosphate from the membrane-bound proteins, LuxN and LuxQ, to LuxO. LuxU is a 114 amino acid residue monomeric protein. Soln. NMR was used to det. the three-dimensional structure of LuxU. LuxU contains a four-helix bundle topol. with the active-site histidine residue (His58) located on □-helix C and exposed to soln. The active site represents a cluster of pos. charged residues located on an otherwise hydrophobic protein face. NMR spin-relaxation expts. identify a collection of flexible residues localized on the same region of LuxU as His58. The studies described here represent the first structural characterization of an isolated, monomeric bacterial phosphotransferase protein.

Bibliographic Information

Disruption of quorum sensing in seawater abolishes attraction of zoospores of the green alga Ulva to bacterial biofilms. Tait, Karen; Joint, Ian; Daykin, Mavis; Milton, Debra L.; Williams, Paul; Camara, Miguel. Plymouth Marine Laboratory, Plymouth, UK. Environmental Microbiology (2005), 7(2), 229-240. Publisher: Blackwell Publishing Ltd., CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 143:263363 AN 2005:170657 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Zoospores of the eukaryotic green seaweed Ulva respond to bacterial N-acylhomoserine lactone (AHL) quorum sensing signal mols. for the selection of surface sites for permanent attachment. In this study we have investigated the prodn. and destruction of AHLs in biofilms of the AHL-producing marine bacterium, Vibrio anguillarum and their stability in seawater. While wild type V. anguillarum NB10 was a strong attractor of zoospores, inactivation of AHL prodn. in this strain by either expressing the recombinant Bacillus lactonase coding gene aiiA, or by mutating the AHL biosynthetic genes, resulted in the abolition of zoospore attraction. In seawater, with a pH of 8.2, the degrdn. of AHL mols. was temp.-dependent, indicating that the AHLs produced by marine bacterial biofilms have short half-lives. The Ulva zoospores sensed a range of different AHL mols. and in particular more zoospores settled on surfaces releasing AHLs with longer (>six carbons) N-linked acyl chains. However, this finding is likely to be influenced by the differential diffusion rates of AHLs from the exptl. surface matrix. Mols. with longer N-acyl chains, such as N-(3-oxodecanoyl)-L-homoserine lactone, diffused more slowly than those with shorter N-acyl chains such as N-(3-hydroxy-hexanoyl)-L-homoserine lactone. Image anal. using GFP-tagged V. anguillarum biofilms revealed that spores settle directly on bacterial cells and in particular on microcolonies which we show are sites of concd. AHL prodn.

Bibliographic Information

Optimization of Vibrio harveyi luminometry assay for detecting quorum sensing inhibitors. Kim, Yeon-Hee; Kim, Young-Hee; Park, Sunghoon; Kim, Jung Sun. Department of Chemical Engineering and Institute for Environmental Technology and Industry, Pusan National University, Pusan, S. Korea. Key Engineering Materials (2005), 277-279(Pt. 1, On the Convergence of Bio-, Information-, Environmental-, Energy-, Space- and Nano-Technologies), 19-22. Publisher: Trans Tech Publications Ltd., CODEN: KEMAEY ISSN: 1013-9826. Journal written in English. CAN 143:149223 AN 2005:65778 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The luminometry assay using the wild-type Vibrio harveyi BB120 was evaluated as a possible detection method for quorum sensing inhibitors. The effects of the concn. of the quorum sensing signal mol. (AHL) as well as the cell d. of the reporter strain and the different AHL analogs on luminescence expressed as relative light units (RLU) were examd. Inhibition of V harveyi luminescence was obsd. in a dose dependent manner for all five AHL analogs. The RLU values exhibited linearity within the range of 2.9□102 .apprx. 3.2□105. Detection up to 102nM was possible for dodecanoyl-DL-homoserine lactone and AHLs with alkyl chain lengths of C-8.apprx.C-14 were more active than the shorter chain-lengthen hexanoyl-homoserine lactones. Lipophilicity of the AHL seems to affect the sensitivity of the assay.

Bibliographic Information

The Aeromonas hydrophila LuxR homologue AhyR regulates the N-acyl homoserine lactone synthase, AhyI positively and negatively in a growth phase-dependent manner. Kirke, David F.; Swift, Simon; Lynch, Martin J.; Williams, Paul. Centre for Biomolecular Sciences, Institute of Infection, Immunity and Inflammation, University of Nottingham, Nottingham, UK. FEMS Microbiology Letters (2004), 241(1), 109-117. Publisher: Elsevier B.V., CODEN: FMLED7 ISSN: 0378-1097. Journal written in English. CAN 142:276602 AN 2004:1008641 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A. hydrophila is a pathogen of fish, amphibians and humans which produces N-acylhomoserine lactone quorum sensing signal mols. and possesses homologs of the Vibrio fischeri luxI and luxR quorum sensing genes termed ahyI and ahyR, resp. The ahyI and ahyR genes of A. hydrophila comprise a divergon with a 62-bp intergenic region and control biofilm maturation and extracellular protease prodn. Stationary phase culture supernatants from an ahyR but not an ahyI mutant contain N-butanoylhomoserine lactone (C4-HSL), which is shown to be required for max. ahyI expression. To det. whether AhyR regulates ahyI, the expression of AhyI was followed throughout growth by Western blot anal. This revealed that AhyI can be detected in the exponential phase but appears to be degraded in stationary phase in the parent A. hydrophila strain. In an ahyR mutant however, the AhyI protein is produced only in stationary phase, but prodn. is sustained, suggesting that AhyR controls the timing of AhyI prodn. and turnover. By using RT-PCR, we mapped the transcriptional start site of ahyI, which revealed that the 12-bp sym. lux-box like sequence present in the 62 bp ahyRI intergenic region overlaps with the -10 region of the ahyI promoter. To det. whether AhyR could bind to the ahyRI intergenic region, the ahyR gene was expressed and purified as a maltose binding protein (MalE) fusion. Electrophoretic mobility shift assays demonstrated that MalE-AhyR specifically bound to this sequence in both the presence and absence of N-butanoylhomoserine lactone (C4-HSL). Taken together, these data suggest that AhyR acts as both a neg. and a pos. regulator of ahyI and hence C4-HSL prodn. in a growth phase dependent manner.

Bibliographic Information

Vibrio cholerae proteins expressed during infection. Calderwood, Stephen B.; Hang, Long; John, Manohar; Ryan, Edward T.; Progulske-Fox, Ann; Handfield, Martin; Hillman, Jeffrey D.; Asaduzzaman, Muhammad. (The General Hospital Corporation, USA; University of Florida Research Foundation, Inc.; Ivigene Corporation). PCT Int. Appl. (2004), 95 pp. CODEN: PIXXD2 WO 2004094644 A2 20041104 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2004-US11817 20040416. Priority: US 2003-463819 20030417. CAN 141:389908 AN 2004:934506 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2004094644	A2	20041104	WO2004-US11817	20040416
WO2004094644	A3	20050506		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

Priority Application		
US 2003-463819P	P	20030417

Abstract

Disclosed are compns. comprising in vivo expressed polynucleotides of V. cholerae. Also disclosed are methods of using such polynucleotides and the corresponding expression products to treat V. cholerae infection.

Bibliographic Information

Is autoinducer-2 a universal signal for interspecies communication: a comparative genomic and phylogenetic analysis of the synthesis and signal transduction pathways. Sun, Jibin; Daniel, Rolf; Wagner-Dobler, Irene; Zeng, An-Ping. Department of Genome Analysis, GBF - German Research Center for Biotechnology, Braunschweig, Germany. BMC Evolutionary Biology (2004), 4 No pp. given. Publisher: BioMed Central Ltd., CODEN: BEBMCG ISSN: 1471-2148. http://www.biomedcentral.com/content/pdf/1471-2148-4-36.pdf Journal; Online

Computer File written in English. CAN 141:376935 AN 2004:922048 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Background: Quorum sensing is a process of bacterial cell-to-cell communication involving the prodn. and detection of extracellular signaling mols. called autoinducers. Recently, it has been proposed that autoinducer-2 (AI-2), a furanosyl borate diester derived from the recycling of S-adenosyl-homocysteine (SAH) to homocysteine, serves as a universal signal for interspecies communication. Results: In this study, 138 completed genomes were examd. for the genes involved in the synthesis and detection of AI-2. Except for some symbionts and parasites, all organisms have a pathway to recycle SAH, either using a two-step enzymic conversion by the Pfs and LuxS enzymes or a one-step conversion using SAH-hydrolase (SahH). 51 Organisms including most Gamma-, Beta-, and Epsilonproteobacteria, and Firmicutes possess the Pfs-LuxS pathway, while Archaea, Eukarya, Alphaproteobacteria, Actinobacteria and Cyanobacteria prefer the SahH pathway. In all 138 organisms, only the three Vibrio strains had strong, bidirectional matches to the periplasmic AI-2 binding protein LuxP and the central signal relay protein LuxU. The initial two-component sensor kinase protein LuxQ, and the terminal response regulator luxO are found in most Proteobacteria, as well as in some Firmicutes, often in several copies. Conclusions: The genomic anal. indicates that the LuxS enzyme required for AI-2 synthesis is widespread in bacteria, while the periplasmic binding protein LuxP is only present in Vibrio strains. Thus, other organisms may either use components different from the AI-2 signal transduction system of Vibrio strains to sense the signal of AI-2, or they do not have such a quorum sensing system at all.

Bibliographic Information

Three parallel quorum-sensing systems regulate gene expression in Vibrio harveyi. Henke, Jennifer M.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Journal of Bacteriology (2004), 186(20), 6902-6914. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 142:34121 AN 2004:906843 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
In a process called quorum sensing, bacteria communicate using extracellular signal mols. termed autoinducers. Two parallel quorum-sensing systems have been identified in the marine bacterium Vibrio harveyi. System 1 consists of the LuxM-dependent autoinducer HAI-1 and the HAI-1 sensor, LuxN. System 2 consists of the LuxS-dependent autoinducer AI-2 and the AI-2 detector, LuxPQ. The related bacterium, Vibrio cholerae, a human pathogen, possesses System 2 (LuxS, AI-2, and LuxPQ) but does not have obvious homologues of V. harveyi System 1. Rather, System 1 of V. cholerae is made up of the CqsA-dependent autoinducer CAI-1 and a sensor called CqsS. Using a V. cholerae CAI-1 reporter strain we show that many other marine bacteria, including V. harveyi, produce CAI-1 activity. Genetic anal. of V. harveyi reveals cqsA and cqsS, and phenotypic anal. of V. harveyi cqsA and cqsS mutants shows that these functions comprise a third V. harveyi quorum-sensing system that acts in parallel to Systems 1 and 2. Together these communication systems act as a three-way coincidence detector in the regulation of a variety of genes, including those responsible for bioluminescence, type III secretion, and metalloprotease prodn.

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Regulation system for protease production in Vibrio vulnificus. Kawase, Tomoka; Miyoshi, Shin-ichi; Sultan, Zafar; Shinoda, Sumio. Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-Naka, Okayama, Japan. FEMS Microbiology Letters (2004), 240(1), 55-59. Publisher: Elsevier B.V., CODEN: FMLED7 ISSN: 0378-1097. Journal written in English. CAN 142:110197 AN 2004:889115 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio vulnificus is a causative agent of serious food-borne diseases in humans related to consumption of raw seafoods. This human pathogen secretes a metalloprotease (VVP) that evokes enhancement of the vascular permeability and disruption of the capillaries. Prodn. of microbial proteases is generally induced at early stationary phase of its growth. This cell d. dependent regulation of VVP prodn. in V. vulnificus known to be the quorum-sensing. When V. vulnificus was cultivated in Luria-Bertani (LB) medium, accumulation of the autoinducer, the signal mol. operating the quorum-sensing system, was detected. Moreover, expression of the vvp gene encoding VVP was found to be closely related with expression of the luxS gene that encode the synthase of the autoinducer precursor (luxS). These findings may indicate VVP prodn. is controlled by the quorum-sensing system in LB medium. Furthermore, this system functioned more effectively at 26 □C than at 37 □C. When incubated at 37 □C in human serum supplemented with ferric chloride, prodn. of VVP and expression of vvp increased in proportion to the concn. of ferric ion; whereas, expression of luxS was not increased. This suggests that VVP prodn. in human serum contg. ferric ion may be regulated mainly by the system other than the quorum-sensing system.

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Quorum sensing in the Vibrio fischeri-Euprymna scolopes symbiosis. Lupp, Claudia. Univ. of Hawaii, Honolulu, HI, USA. Avail. UMI, Order No. DA3110022. (2003), 186 pp. From: Diss. Abstr. Int., B 2004, 64(10), 4756. Dissertation written in English. CAN 142:388993 AN 2004:813539 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

N-Sulfonyl homoserine lactones as antagonists of bacterial quorum sensing. Castang, Sandra; Chantegrel, Bernard; Deshayes, Christian; Dolmazon, Rene; Gouet, Patrice; Haser, Richard; Reverchon, Sylvie; Nasser, William; Hugouvieux-Cotte-Pattat, Nicole; Doutheau, Alain. Unite de Microbiologie et Genetique, UMR CNRS-INSA-UCB 5122, Villeurbanne, Fr. Bioorganic & Medicinal Chemistry Letters (2004), 14(20), 5145-5149. Publisher: Elsevier B.V., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. CAN 142:6774 AN 2004:767293 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A series of 11 new analogs of N-acylhomoserine lactones in which the carboxamide bond was replaced by a sulfonamide one, has been synthesized. These compds. were evaluated for their ability to competitively inhibit the action of 3-oxohexanoyl-L-homoserine lactone, the natural ligand of the quorum sensing transcriptional regulator LuxR, which in turn activates expression of bioluminescence in the model bacterium Vibrio fischeri. Several compds. were found to display antagonist activity. Mol. modeling suggests that the latter prevent a cascade of structural rearrangements necessary for the formation of the active LuxR dimer.

Bibliographic Information

An inhibitor of bacterial quorum sensing reduces moralities caused by vibriosis in rainbow trout (Oncorhynchus mykiss, Walbaum). Rasch, Maria; Buch, Christiane; Austin, Brian; Slierendrecht, Wilhelmina J.; Ekmann, Kim S.; Larsen, Jens Laurits; Johansen, Charlotte; Riedel, Kathrin; Eberl, Leo; Givskov, Michael; Gram, Lone. Danish Institute for Fisheries Research, Department of Seafood Research, Technical University of Denmark, Lyngby, Den. Systematic and Applied Microbiology (2004), 27(3), 350-359. Publisher: Elsevier GmbH, CODEN: SAMIDF ISSN: 0723-2020. Journal written in English. CAN 142:32490 AN 2004:646863 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The fish pathogen Vibrio anguillarum produces quorum sensing signal mols., N-acyl homoserine lactones (AHLs), which in several Gram-neg. human and plant pathogenic bacteria regulate virulence factors. Expression of these factors can be blocked using specific quorum-sensing inhibitors (QSIs). The purpose of this study was to investigate the effect of a QSI, furanone C-30, on mortality of rainbow trout during challenge with V. anguillarum. Addn. of 0.01 or 0.1 □M furanone C-30 to rainbow trout infected by cohabitation caused a significant redn. in accumulated mortality from 80-100% in challenge controls to 4-40% in treated groups. Furanone C-30 had no effect in an immersion challenge system, probably due to a very high water exchange and a rapid diln. of furanone C-30. Growth and survival of V anguillarum were not affected by the concns. of furanone C-30 used in the challenge expts., thus avoiding selection for resistance. To elucidate the mechanism of disease control by furanone C-30, we detd. its effect on the bacterial proteome, motility, and respiration. No effects were seen of furanone C-30 in any of these expts. Although no cytotoxic effect on HeLa cells were obsd., exposure to 1 □M (or higher) concns. of furanone C-30 had detrimental effects on the rainbow trout. Our results indicate that QSIs can be used in non-antibiotic based control of fish diseases. However, they also underline the need for development of novel, less toxic QSI compds. and the need for understanding the exact mechanism(s) of action.

Bibliographic Information

Regulation of Vibrio anguillarum empA metalloprotease expression and its role in virulence. Denkin, Steven M.; Nelson, David R. Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI, USA. Applied and Environmental Microbiology (2004), 70(7), 4193-4204. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 141:292037 AN 2004:611475 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Atlantic salmon (Salmo salar) were challenged with Vibrio anguillarum strains M93Sm and NB10 and empA null mutants M99 and NB12. Both wild types were virulent when administered by i.p. injection or anal intubation. NB12 was avirulent via either route of infection. M99 virulence was attenuated when delivered by intubation, but fully virulent by i.p. injection. Northern blot anal. revealed empA expression in M93Sm and NB10 cells incubated in mucus, while incubation in Luria-Bertani broth plus 2% NaCl (LB20) induced empA expression only in NB10. Nucleotide differences between M93Sm and NB10 empA sequences were found in regions located 207 and 229 bp upstream of the empA translational start. Reverse transcription-PCR and 5' rapid amplification of cDNA ends revealed the empA transcriptional start site 85 bp upstream of the translational start for both strains. A putative □S-dependent promoter was identified upstream of the transcriptional start in both strains. Site-directed mutagenesis was used to create rpoS mutants of M93Sm and

NB10. Neither rpoS mutant exhibited protease activity. Since empA is expressed during stationary phase, the effects of conditioned medium on protease activity were examd. M99 conditioned LB20 supernatants stimulated protease activity in NB10 while allowing M93Sm to produce protease in LB20. Neither acyl homoserine lactones nor AI-2 induced protease activity. Conditioned LB20 supernatant from a V. anguillarum luxS mutant caused a more rapid induction of protease activity in wild-type cells. Our data show that expression of empA is differentially regulated in V. anguillarum strains NB10 and M93Sm and requires □S, quorum-sensing mols., and gastrointestinal mucus.

Bibliographic Information

Vibrio cholerae AphA uses a novel mechanism for virulence gene activation that involves interaction with the LysR-type regulator AphB at the tcpPH promoter. Kovacikova, Gabriela; Lin, Wei; Skorupski, Karen. Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA. Molecular Microbiology (2004), 53(1), 129-142. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 141:255363 AN 2004:593443 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

AphA is required for expression of the Vibrio cholerae virulence cascade and for its regulation by quorum sensing. In order to activate transcription, AphA functions together with a second protein, the LysR-type regulator AphB, at the tcpPH promoter. As AphA is a member of a new and largely uncharacterized regulator family, random mutagenesis was used to gain insights into how this protein activates transcription. As shown here, 17 amino acid substitutions were identified in AphA that reduced expression of the tcpPH promoter and prevented the protein from binding DNA. The amino acids involved in DNA recognition inferred from a dominant-neg. anal. were located throughout the N-terminal domain from amino acids 18 to 67. This region of AphA has a conserved domain architecture similar to that of MarR, a multiple antibiotic resistance repressor. The analogous positions of the dominant-neg. mutations in AphA and MarR confirm that the DNA-binding domains of these proteins are similar and indicate that AphA is a new member of the winged helix family of transcription factors. The authors also show that AphB is capable of rescuing two of the DNA binding-defective AphA mutants, suggesting that the proteins interact directly on the DNA. Disruption of this interaction by insertion of half a helical turn between the two binding sites prevented AphB from rescuing the mutants and prevented the expression of the virulence cascade in a wild-type background. These results provide a novel mechanism for the initiation of virulence gene expression at tcpPH.

Bibliographic Information

The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in Vibrio harveyi and Vibrio cholerae. Lenz, Derrick H.; Mok, Kenny C.; Lilley, Brendan N.; Kulkarni, Rahul V.; Wingreen, Ned S.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Cell (Cambridge, MA, United States) (2004), 118(1), 69-82. Publisher: Cell Press, CODEN: CELLB5 ISSN: 0092-8674. Journal written in English. CAN 141:257130 AN 2004:593075 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum-sensing bacteria communicate with extracellular signal mols. called autoinducers. This process allows community-wide synchronization of gene expression. A screen for addnl. components of the Vibrio harveyi and Vibrio cholerae quorum-sensing circuits revealed the protein Hfq. Hfq mediates interactions between small, regulatory RNAs (sRNAs) and specific mRNA (mRNA) targets. These interactions typically alter the stability of the target transcripts. Hfq mediates the destabilization of the mRNA encoding the quorum-sensing master regulators LuxR (V. harveyi) and HapR (V. cholerae), implicating an sRNA in the circuit. A bioinformatics approach to identifying putative sRNAs identified four candidate sRNAs in V. cholerae. The simultaneous deletion of all four sRNAs is required to stabilize hapR mRNA. The authors propose that Hfq, together with these sRNAs, creates an ultrasensitive regulatory switch that controls the crit. transition into the high cell d., quorum-sensing mode.

Bibliographic Information

Small RNAs shed some light. Gottesman, Susan. Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD, USA. Cell (Cambridge, MA, United States) (2004), 118(1), 1-2. Publisher: Cell Press, CODEN: CELLB5 ISSN: 0092-8674. Journal; General Review written in English. CAN 141:237178 AN 2004:593067 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review. Small regulatory RNAs can act by pairing with their target messages, targeting themselves and the mRNA for degrdn.; Lenz et al. (this issue of Cell) now report that multiple small RNAs are essential regulators of the quorum-sensing systems of Vibrio species, including the regulation of virulence in V. cholerae.

Bibliographic Information

A distinctive dual-channel quorum-sensing system operates in Vibrio anguillarum. Croxatto, Antony; Pride, John; Hardman, Andrea; Williams, Paul; Camara, Miguel; Milton, Debra L. Department of Molecular Biology, Umea University, Umea, Swed. Molecular Microbiology (2004), 52(6), 1677-1689. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 141:219812 AN 2004:547403 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Many bacterial cells communicate using diffusible signal mols. to monitor cell population d. via a process termed quorum sensing. In marine Vibrio species, the Vibrio harveyi-type LuxR protein is a key player in a quorum-sensing phosphorelay cascade, which controls the expression of virulence, symbiotic and survival genes. Previously, we characterized Vibrio anguillarum homologues of LuxR (VanT) and LuxMN (VanMN) and, in this study, we have identified homologues of LuxPQ (VanPQ) and LuxOU (VanOU). In contrast to other Vibrio species, vanT was expressed at low cell d. and showed no significant induction as the cell no. increased. In addn., although the loss of VanO increased vanT expression, the loss of VanU, unexpectedly, decreased it. Both VanN and VanQ were required for repression of vanT even in a vanU mutant, suggesting an alternative route for VanNQ signal transduction other than via VanU. VanT neg. regulated its own expression by binding and repressing the vanT promoter and by binding and activating the vanOU promoter. The signal relay results in a cellular response as expression of the metalloprotease, empA, was altered similar to that of vanT in all the mutants. Consequently, the V. anguillarum quorum-sensing

phosphorelay systems work differently from those of V. harveyi and may be used to limit rather than induce vanT expression.

Bibliographic Information

Boron binding with the quorum sensing signal AI-2 and analogues. Semmelhack, Martin F.; Campagna, Shawn R.; Hwa, Charlotte; Federle, Michael J.; Bassler, Bonnie L. Departments of Chemistry and Molecular Biology, Princeton University, Princeton, NJ, USA. Organic Letters (2004), 6(15), 2635-2637. Publisher: American Chemical Society, CODEN: ORLEF7 ISSN: 1523-7060. Journal written in English. CAN 141:221592 AN 2004:534557 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The unstable bacterial metabolic product, DPD, and the related natural product, laurencione, are shown to have a high affinity for borate complexation, through the hydrated analog. The boron complex of DPD is Vibrio harveyi AI-2, an interspecies quorum sensing signal in bacteria, and an affinity column with a borate resin is effective in providing the 1st method for concg. and purifying V. harveyi AI-2 from the biosynthetic product.

Bibliographic Information

Vibrio fischeri LuxS and AinS: Comparative study of two signal synthases. Lupp, Claudia; Ruby, Edward G. Pacific Biomedical Research Center, University of Hawaii at Manoa, Honolulu, HI, USA. Journal of Bacteriology (2004), 186(12), 3873-3881. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 141:221435 AN 2004:497816 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio fischeri possesses two acyl-homoserine lactone quorum-sensing systems, ain and lux, both of which are involved in the regulation of luminescence gene expression and are required for persistent colonization of the squid host, Euprymna scolopes. We have previously demonstrated that the ain system induces luminescence at cell densities that precede lux system activation. Our data suggested that the ain system both relieves repression and initially induces the lux system, thereby achieving sequential induction of gene expression by these two systems. Anal. of the V. fischeri genome revealed the presence of a putative third system based on the enzyme LuxS, which catalyzes the synthesis of the Vibrio harveyi autoinducer 2 (AI-2). In this study, we investigated the impact of V. fischeri LuxS on luminescence and colonization competence in comparison to that of the ain system. Similar to the ain system, inactivation of the AI-2 system decreased light prodn. in culture, but not in the squid host. However, while an ainS mutant produces no detectable light in culture, a luxS mutant expressed approx. 70% of wild-type luminescence levels. A mutation in luxS alone did not compromise symbiotic competence of V. fischeri; however, levels of colonization of an ainS luxS double mutant were reduced to 50% of the already diminished level of ainS mutant colonization, suggesting that these two systems regulate colonization gene expression synergistically through a common pathway. Introduction of a luxO mutation into the luxS and ainS luxS background could relieve both luminescence and colonization defects, consistent with a model in which LuxS, like AinS, regulates gene expression through LuxO. Furthermore, while luxS transcription appeared to be constitutive and the AI-2 signal concn. did not change dramatically, our

data suggest that ainS transcription is autoregulated, resulting in an over 2,000-fold increase in signal concn. as culture d. increased. Taken together, these data indicate that V.
fischeri LuxS affects both luminescence regulation and colonization competence; however, its quant. contribution is small when compared to that of the AinS signal.

Bibliographic Information

Quorum sensing regulates type III secretion in Vibrio harveyi and Vibrio parahaemolyticus. Henke, Jennifer M.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Journal of Bacteriology (2004), 186(12), 3794-3805. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 141:187542 AN 2004:497808 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In a process known as quorum sensing, bacteria communicate with one another by producing, releasing, detecting, and responding to signal mols. called autoinducers. Vibrio harveyi, a marine pathogen, uses two parallel quorum-sensing circuits, each consisting of an autoinducer-sensor pair, to control the expression of genes required for bioluminescence and a no. of other target genes. Genetic screens designed to discover autoinducer-regulated targets in V. harveyi have revealed genes encoding components of a putative type III secretion (TTS) system. Using transcriptional reporter fusions and TTS protein localization studies, we show that the TTS system is indeed functional in V. harveyi and that expression of the genes encoding the secretion machinery requires an intact quorum-sensing signal transduction cascade. The newly completed genome of the closely related marine bacterium Vibrio parahaemolyticus, which is a human pathogen, shows that it possesses the genes encoding both of the V. harveyi-like quorum-sensing signaling circuits and that it also has a TTS system similar to that of V. harveyi. We show that quorum sensing regulates TTS in V. parahaemolyticus. Previous reports connecting quorum sensing to TTS in enterohemorrhagic and enteropathogenic Escherichia coli show that quorum sensing activates TTS at high cell d. Surprisingly, we find that at high cell d. (in the presence of autoinducers), quorum sensing represses TTS in V. harveyi and V. parahaemolyticus.

Bibliographic Information

Reciprocal regulation of bioluminescence and type III protein secretion in Vibrio harveyi and Vibrio parahaemolyticus in response to diffusible chemical signals. Winans, Stephen C. Department of Microbiology, Cornell University, Ithaca, NY, USA. Journal of Bacteriology (2004), 186(12), 3674-3676. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal; General Review written in English. CAN 141:187368 AN 2004:497794 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A review with commentary on bioluminescence, type III protein secretion and signaling in Vibrio harveyi and Vibrio parahaemolyticus.

Bibliographic Information

Programmable cells: Interfacing natural and engineered gene networks. Kobayashi, Hideki; Kaern, Mads; Araki, Michihiro; Chung, Kristy; Gardner, Timothy S.; Cantor, Charles R.;

Collins, James J. Department of Biomedical Engineering, Center for BioDynamics, and Center for Advanced Biotechnology, Boston University, Boston, MA, USA. Proceedings of the National Academy of Sciences of the United States of America (2004), 101(22), 8414-8419. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 141:168848 AN 2004:497537 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Novel cellular behaviors and characteristics can be obtained by coupling engineered gene networks to the cell's natural regulatory circuitry through appropriately designed input and output interfaces. Here, we demonstrate how an engineered genetic circuit can be used to construct cells that respond to biol. signals in a predetd. and programmable fashion. We employ a modular design strategy to create Escherichia coli strains where a genetic toggle switch is interfaced with: (i) the SOS signaling pathway responding to DNA damage, and (ii) a transgenic quorum sensing signaling pathway from Vibrio fischeri. The genetic toggle switch endows these strains with binary response dynamics and an epigenetic inheritance that supports a persistent phenotypic alteration in response to transient signals. These features are exploited to engineer cells that form biofilms in response to DNA-damaging agents and cells that activate protein synthesis when the cell population reaches a crit. d. Our work represents a step toward the development of "plug-and-play" genetic circuitry that can be used to create cells with programmable behaviors.

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Cell-to-cell signalling in Escherichia coli and Salmonella enterica. Ahmer, Brian M. M. Department of Microbiology, The Ohio State University, Columbus, OH, USA. Molecular Microbiology (2004), 52(4), 933-945. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal; General Review written in English. CAN 141:119854 AN 2004:436741 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review. Cell-to-cell signalling in prokaryotes that leads to coordinated behavior has been termed quorum sensing. This type of signalling can have profound impacts on microbial community structure and host-microbe interactions. The Gram-neg. quorum-sensing systems were first discovered and extensively characterized in the marine Vibrios. Some components of the Vibrio systems are present in the classical genetic model organisms Escherichia coli and Salmonella enterica. Both organisms encode a signal receptor of the LuxR family, SdiA, but not a corresponding signal-generating enzyme. Instead, SdiA of Salmonella detects and responds to signals generated only by other microbial species. Conversely, E. coli and Salmonella encode the signal-generating component of a second system (a LuxS homolog that generates AI-2), but the sensory app. for AI-2 differs substantially from the Vibrio system. The only genes currently known to be regulated by AI-2 in Salmonella encode an active uptake and modification system for AI-2. Therefore, it is not yet clear whether Salmonella uses AI-2 as a signal mol. or whether AI-2 has some other function. In E. coli, the functions of both SdiA and AI-2 are unclear due to pleiotropy. Genetic strategies to identify novel signalling systems have been performed with E. coli and Providencia stuartii. Several putative signalling systems have been identified, one that uses indole as a signal and another that releases what appears to be a peptide. The latter system has homologues in E. coli and Salmonella, as well as other bacteria, plants and animals. In fact, the protease components from Providencia and Drosophila are functionally interchangeable.

Bibliographic Information

A model system for pathogen detection using a two-component bacteriophage/bioluminescent signal amplification assay. Bright, Nathan G.; Carroll, Richard J.; Applegate, Bruce M. Department of Food Science, Purdue Univ., West Lafayette, IN, USA. Proceedings of SPIE-The International Society for Optical Engineering (2004), 5271(Monitoring Food Safety, Agriculture, and Plant Health), 13-19. Publisher: SPIE-The International Society for Optical Engineering, CODEN: PSISDG ISSN: 0277-786X. Journal written in English. CAN 142:92384 AN 2004:431422 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
This research investigates a detection approach utilizing bacteriophage pathogen specificity coupled with a bacterial bioluminescent bioreporter utilizing the quorum sensing mol. from Vibrio fischeri, N-(3-oxohexanoyl)-homoserine lactone (3-oxo-C6-HSL). The 3-oxo-C6-HSL mols. diffuse out of the target cell after infection and induce bioluminescence from a population of 3-oxo-C6-HSL bioreporters (ROLux). E. coli phage M13, a well-characterized bacteriophage, offers a model system testing the use of bacteriophage for pathogen detection through cell-to-cell communication via a LuxR/3-oxo-C6-HSL system. Simulated temperate phage assays tested functionality of the ROLux reporter and prodn. of 3-oxo-C6-HSL by various test strains. These assays showed detection limits of 102cfu after 24 h in a variety of detection formats. Assays incorporating the bacteriophage M13-luxI with the ROLux reporter and a known population of target cells were subsequently developed and have shown consistent detection limits of 105cfu target organisms. Measurable light response from high concns. of target cells was almost immediate, suggesting an enrichment step to further improve detection limits and reduce assay time.

Bibliographic Information

Stationary-phase quorum-sensing signals affect autoinducer-2 and gene expression in Escherichia coli. Ren, Dacheng; Bedzyk, Laura A.; Ye, Rick W.; Thomas, Stuart M.; Wood, Thomas K. Departments of Chemical Engineering and Molecular & Cell Biology, University of Connecticut, Storrs, CT, USA. Applied and Environmental Microbiology (2004), 70(4), 2038-2043. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 140:386935 AN 2004:337169 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing via autoinducer-2 (AI-2) has been identified in different strains, including those from Escherichia, Vibrio, Streptococcus, and Bacillus species, and previous studies have suggested the existence of addnl. quorum-sensing signals working in the stationary phase of Escherichia coli cultures. To investigate the presence and global effect of these possible quorum-sensing signals other than AI-2, DNA microarrays were used to study the effect of stationary-phase signals on the gene expression of early exponential-phase cells of the AI-2-deficient strain E. coli DH5□. For statistically significant differential gene expression ($P < 0.05$), 14 genes were induced by supernatants from a stationary culture and 6 genes were repressed, suggesting the involvement of indole (induction of tnaA and tnaL) and phosphate (repression of phoA, phoB, and phoU). To study the stability of the signals, the stationary-phase supernatant was autoclaved and was used to study its effect on E. coli gene expression. Three genes were induced by autoclaved stationary-phase supernatant, and 34 genes were repressed. In total, three genes (ompC, ptsA, and btuB) were induced and five genes (nupC, phoB, phoU, argT, and ompF) were repressed by both fresh and autoclaved stationary-phase supernatants. Furthermore, supernatant from E. coli DH5□ stationary

culture was found to repress E. coli K-12 AI-2 concns. by 4.8-fold 0.4-fold, suggesting that an addnl. quorum-sensing system in E. coli exists and that gene expression is controlled as a network with different signals working at different growth stages.

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Quorum sensing controls biofilm formation in Vibrio cholerae. [Erratum to document cited in CA140:038599]. Hammer, Brian K.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2004), 51(5), 1521. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 141:310454 AN 2004:251663 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In Figure 1 on page 101, AphA was incorrectly depicted as a repressor of tcpPH expression; the cor. figure is given. AphA activates the expression of tcpPH (Skorupski and Taylor, 1999 Mol. Microbiol 31: 763-771).

Bibliographic Information

Lead precipitation by Vibrio harveyi: Evidence for novel quorum-sensing interactions. Mire, Chad E.; Tourjee, Jeanette A.; O'Brien, William F.; Ramanujachary, Kandalam V.; Hecht, Gregory B. Department of Biological Sciences, Rowan University, Glassboro, NJ, USA. Applied and Environmental Microbiology (2004), 70(2), 855-864. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 141:119868 AN 2004:164291 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Three pleiotropic, quorum sensing-defective V. harveyi mutants were obsd. to ppt. sol. Pb2+ as an insol. compd. The compd. was purified and subjected to X-ray diffraction and elemental analyses. These assays identified the pptd. compd. as Pb9(PO4)6, an unusual and complex lead phosphate salt that is produced synthetically at temps. of .apprx.200 . Regulation of the pptn. phenotype was also examd. Introduction of a luxO::kan allele into one of the mutants abolished lead pptn., indicating that the well-characterized autoinducer 1 (AI1)-AI2 quorum-sensing system can block lead pptn. in dense cell populations. Interestingly, the V. harveyi D1 mutant, a strain defective for secretion of both AI1 and AI2, was shown to be an effective trans inhibitor of lead pptn. This suggests that a previously undescribed V. harveyi autoinducer, referred to as AI3, can also neg. regulate lead pptn. Expts. with heterologous bacterial populations demonstrated that many different spp. are capable of trans regulating the V. harveyi lead pptn. phenotype. Moreover, one of the V. harveyi mutants in this study exhibited little or no response to intercellular signals from other V. harveyi inocula but was quite responsive to some of the heterologous bacteria. Based on these observations, we propose that V. harveyi carries 1 quorum sensor that is specifically dedicated to receiving cross-species communication.

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Compns. and methods for identifying compounds which modulate bacterial response to autoinducers and pathogenesis. Bassler, Bonnie L.; Surette, Michael G. (Princeton University, USA; University Technologies International, Inc.). U.S. Pat. Appl. Publ. (2004), 60 pp., Cont.-

in-part of U.S. Ser. No. 961,507. CODEN: USXXCO US 2004033548 A1 20040219 Patent written in English. Application: US 2003-387345 20030310. Priority: US 2001-961507 20010921; US 99-453976 19991202; US 98-110570 19981202. CAN 140:194388 AN 2004:142694 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
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WO2004101826	A2	20041125	WO2004-US7181	20040308
WO2004101826	A3	20050120		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

Priority Application		
US 1998-110570P	P	19981202
US 1999-453976	A3	19991202
US 2001-961507	A2	20010921
US 2003-387345	A	20030310

Abstract

The prodn. of a purified extracellular bacterial signal called autoinducer-2 is regulated by changes in environmental conditions assocd. with a shift from a free-living existence to a colonizing or pathogenic existence in a host organism. Autoinducer-2 stimulates LuxQ luminescence genes, and is believed also to stimulate a variety of pathogenesis-related genes in the bacterial species that produce it. The examples explore quorum sensing system 2 in Escherichia coli, Salmonella typhimurium and Vibrio harveyi. A new family of LuxS genes, responsible for AI-2 prodn., is identified. The encoded product of the LuxS genes catalyzes an essential step in the biosynthesis of autoinducer-2.

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Chlamydomonas reinhardtii secretes compounds that mimic bacterial signals and interfere with quorum sensing regulation in bacteria. Teplitski, Max; Chen, Hancai; Rajamani, Sathish; Gao, Mengsheng; Merighi, Massimo; Sayre, Richard T.; Robinson, Jayne B.; Rolfe, Barry G.; Bauer, Wolfgang D. Ohio State University, Columbus, OH, USA. Plant Physiology (2004), 134(1), 137-146. Publisher: American Society of Plant Biologists, CODEN: PLPHAY ISSN: 0032-0889. Journal written in English. CAN 140:300163 AN 2004:102382 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The unicellular soil-freshwater alga Chlamydomonas reinhardtii was found to secrete substances that mimic the activity of the N-acyl-L-homoserine lactone (AHL) signal mols. used by many bacteria for quorum sensing regulation of gene expression. More than a dozen chem. separable but unidentified substances capable of specifically stimulating the LasR or CepR but not the LuxR, AhyR, or CviR AHL bacterial quorum sensing reporter strains were detected in Et acetate exts. of C. reinhardtii culture filtrates. Colonies of C. reinhardtii and Chlorella spp. stimulated quorum sensing-dependent luminescence in Vibrio harveyi, indicating that these algae may produce compds. that affect the AI-2 furanosyl borate diester-mediated quorum sensing system of Vibrio spp. Treatment of the soil bacterium Sinorhizobium meliloti with a partially purified LasR mimic from C. reinhardtii affected the accumulation of 16 of the 25 proteins that were altered in response to the bacterium's own AHL signals, providing evidence that the algal mimic affected quorum sensing-regulated functions in this wild-type bacterium. Peptide mass fingerprinting identified 32 proteins affected by the bacterium's AHLs or the purified algal mimic, including GroEL chaperonins, the nitrogen regulatory protein PII, and a GTP-binding protein. The algal mimic was able to cancel the stimulatory effects of bacterial AHLs on the accumulation of seven of these proteins, providing evidence that the secretion of AHL mimics by the alga could be effective in disruption of quorum sensing in naturally encountered bacteria.

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Reversible acyl-homoserine lactone binding to purified Vibrio fischeri LuxR protein. Urbanowski, M. L.; Lostroh, C. P.; Greenberg, E. P. Department of Microbiology and W. M. Keck Microbial Communities and Cell Signaling Program, Carver College of Medicine, University of Iowa, Iowa City, IA, USA. Journal of Bacteriology (2004), 186(3), 631-637. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 140:265425 AN 2004:82218 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The Vibrio fischeri LuxR protein is the founding member of a family of acyl-homoserine lactone-responsive quorum-sensing transcription factors. Previous genetic evidence indicates that in the presence of its quorum-sensing signal, N-(3-oxohexanoyl) homoserine lactone (3OC6-HSL), LuxR binds to lux box DNA within the promoter region of the luxI gene and activates transcription of the luxICDABEG luminescence operon. We have purified LuxR from recombinant Escherichia coli. Purified LuxR binds specifically and with high affinity to DNA contg. a lux box. This binding requires addn. of 3OC6-HSL to the assay reactions, presumably forming a LuxR-3OC6-HSL complex. When bound to the lux box at the luxI promoter in vitro, LuxR-3OC6-HSL enables E. coli RNA polymerase to initiate transcription from the luxI promoter. Unlike the well-characterized LuxR homolog TraR in complex with its signal (3-oxo-octanoyl-HSL), the LuxR-30C6-HSL complex can be reversibly inactivated by diln., suggesting that 3OC6-HSL in the complex is not

tightly bound and is in equil. with the bulk solvent. Thus, although LuxR and TraR both bind 3-oxoacyl-HSLs, the binding is qual. different. The differences have implications for the ways in which these proteins respond to decreases in signal concns. or rapid drops in population d.

Bibliographic Information

GacA regulates symbiotic colonization traits of Vibrio fischeri and facilitates a beneficial association with an animal host. Whistler, Cheryl A.; Ruby, Edward G. Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI, USA. Journal of Bacteriology (2003), 185(24), 7202-7212. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 140:142349 AN 2003:982937 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The GacS/GacA two-component system regulates the expression of bacterial traits during host assocn. Although the importance of GacS/GacA as a regulator of virulence is well established, its role in benign assocns. is not clear, as mutations in either the gacS or gacA gene have little impact on the success of colonization in nonpathogenic assocns. studied thus far. Using as a model the symbiotic assocn. of the bioluminescent marine bacterium Vibrio fischeri with its animal host, the Hawaiian bobtail squid, Euprymna scolopes, we investigated the role of GacA in this beneficial animal-microbe interaction. When grown in culture, gacA mutants were defective in several traits important for symbiosis, including luminescence, growth in defined media, growth yield, siderophore activity, and motility. However, gacA mutants were not deficient in prodn. of acylated homoserine lactone signals or catalase activity. The ability of the gacA mutants to initiate squid colonization was impaired but not abolished, and they reached lower-than-wild-type population densities within the host light organ. In contrast to their dark phenotype in culture, gacA mutants that reached population densities above the luminescence detection limit had normal levels of luminescence per bacterial cell in squid light organs, indicating that GacA is not required for light prodn. within the host. The gacA mutants were impaired at competitive colonization and could only successfully cocolonize squid light organs when present in the seawater at higher inoculum densities than wild-type bacteria. Although severely impaired during colonization initiation, gacA mutants were not displaced by the wild-type strain in light organs that were colonized with both strains. This study establishes the role of GacA as a regulator of a beneficial animal-microbe assocn. and indicates that GacA regulates utilization of growth substrates as well as other colonization traits.

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Involvement of region 4 of the □ 70 subunit of RNA polymerase in transcriptional activation of the lux operon during quorum sensing. Johnson, Deborah C.; Ishihama, Akira; Stevens, Ann M. Department of Biology, Virginia Tech., Blacksburg, VA, USA. FEMS Microbiology Letters (2003), 228(2), 193-201. Publisher: Elsevier Science B.V., CODEN: FMLED7 ISSN: 0378-1097. Journal written in English. CAN 140:158375 AN 2003:923759 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Quorum sensing-dependent activation of the luminescence (lux) genes of Vibrio fischeri relies on the formation of a complex between the autoinducer mol., N-(3-oxohexanoyl)-L-homoserine lactone, and the autoinducer-dependent transcriptional activator LuxR. In its active conformation,

LuxR binds to a site known as the lux box centered at position -42.5 relative to the luxI transcriptional start site and is thought to function as an ambidextrous activator capable of making multiple contacts with RNA polymerase (RNAP). The specific role of region 4 of the Escherichia coli □70 subunit of RNAP in LuxR-dependent activation of the luxI promoter has been investigated. Single-round transcription assays were performed in the presence of purified LuxR□N, the autoinducer-independent C-terminal domain of LuxR, and a variant RNAP which contained a C-terminally truncated □70 subunit devoid of region 4. Results indicated that region 4 is essential for LuxR□N-dependent luxI transcription, therefore 16 single and two triple alanine substitutions in region 4.2 of □70 between amino acid residues 590 and 613 were examd. for their effects on LuxR- and LuxR□N-dependent transcription at the luxI promoter. Taken together, the analyses performed on these variants of RpoD suggest that some individual residues in region 4.2 are important to the mechanism of activator-dependent transcription initiation under investigation.

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Quorum sensing-dependent biofilms enhance colonization in Vibrio cholerae. Zhu, Jun; Mekalanos, John J. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA, USA. Developmental Cell (2003), 5(4), 647-656. Publisher: Cell Press, CODEN: DCEEBE ISSN: 1534-5807. Journal written in English. CAN 140:54356 AN 2003:831003 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio cholerae is the causative agent of the diarrheal disease cholera. By an incompletely understood developmental process, V. cholerae forms complex surface-assocd. communities called biofilms. Here we show that quorum sensing-deficient mutants of V. cholerae produce thicker biofilms than those formed by wild-type bacteria. Microarray anal. of biofilm-assocd. bacteria shows that expression of the Vibrio polysaccharide synthesis (vps) operons is enhanced in hapR mutants. CqsA, one of two known autoinducer synthases in V. cholerae, acts through HapR to repress vps gene expression. Vibrio biofilms are more acid resistant than planktonic cells. However, quorum sensing-deficient biofilms have lower colonization capacities than those of wild-type biofilms, suggesting that quorum sensing may promote cellular exit from the biofilm once the organisms have traversed the gastric acid barrier of the stomach. These results shed light on the relationships among biofilm development, quorum sensing, infectivity, and pathogenesis in V. cholerae.

Bibliographic Information

The Vibrio fischeri quorum-sensing systems ain and lux sequentially induce luminescence gene expression and are important for persistence in the squid host. Lupp, Claudia; Urbanowski, Mark; Greenberg, E. Peter; Ruby, Edward G. Pacific Biomedical Research Center, University of Hawaii, Manoa, Honolulu, HI, USA. Molecular Microbiology (2003), 50(1), 319-331. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 140:25326 AN 2003:817294 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacterial quorum sensing using acyl-homoserine lactones (acyl-HSLs) as cell-d. dependent signaling mols. is important for the transcriptional regulation of many genes essential in the establishment and the maintenance of bacteria-host assocns. Vibrio fischeri, the symbiotic partner

of the Hawaiian bobtail squid Euprymna scolopes, possesses two distinct acyl-HSL synthase proteins, LuxI and AinS. Whereas the cell d.-dependent regulation of luminescence by the LuxI-produced signal is a well-described phenomenon, and its role in light organ symbiosis has been defined, little is known about the ain system. We have investigated the impact of the V. fischeri acyl-HSL synthase AinS on both luminescence and symbiotic colonization. Through phenotypic studies of V. fischeri mutants we have found that the AinS-signal is the predominant inducer of luminescence expression in culture, whereas the impact of the LuxI-signal is apparent only at the high cell densities occurring in symbiosis. Furthermore, our studies revealed that ainS regulates activities essential for successful colonization of E. scolopes, i.e. the V. fischeri ainS mutant failed to persist in the squid light organ. Mutational inactivation of the transcriptional regulator protein LuxO in the ainS mutant partially or completely reversed all the obsd. phenotypes, demonstrating that the AinS-signal regulates expression of downstream genes through the inactivation of LuxO. Taken together, our results suggest that the two quorum-sensing systems in V. fischeri, ain and lux, sequentially induce the expression of luminescence genes and possibly other colonization factors.

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Quorum sensing controls biofilm formation in Vibrio cholerae. Hammer, Brian K.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2003), 50(1), 101-114. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 140:38599 AN 2003:817278 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Multiple quorum-sensing circuits function in parallel to control virulence and biofilm formation in Vibrio cholerae. In contrast to other bacterial pathogens that induce virulence factor prodn. and/or biofilm formation at high cell d. in the presence of quorum-sensing autoinducers, V. cholerae represses these behaviors at high cell d. Consistent with this, we show here that V. cholerae strains "locked" in the regulatory state mimicking low cell d. are enhanced for biofilm prodn. whereas mutants "locked" in the regulatory state mimicking high cell d. are incapable of producing biofilms. The quorum-sensing cascade we have identified in V. cholerae regulates the transcription of genes involved in exopolysaccharide prodn. (EPS), and variants that produce EPS and form biofilms arise at high frequency from non-EPS, non-biofilm producing strains. Our data show that spontaneous mutation of the transcriptional regulator hapR is responsible for this effect. Several toxigenic strains of V. cholerae possess a naturally occurring frameshift mutation in hapR. Thus, the distinct environments occupied by this aquatic pathogen presumably include niches where cell-cell communication is crucial, as well as ones where loss of quorum sensing via hapR mutation confers a selective advantage. Bacterial biofilms could represent a complex habitat where such differentiation occurs.

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Analysis of noise in quorum sensing. Cox, Chris D.; Peterson, Gregory D.; Allen, Michael S.; Lancaster, Joseph M.; McCollum, James M.; Austin, Derek; Yan, Ling; Sayler, Gary S.; Simpson, Michael L. Tennessee and Center for Environmental Biotechnology, Department of Civil and Environmental Engineering, University of Tennessee, Knoxville, TN, USA. OMICS (2003), 7(3), 317-334. Publisher: Mary Ann Liebert, Inc., CODEN: OMICAE ISSN: 1536-2310. Journal written in English. CAN 140:36578 AN 2003:804609 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Noise may play a pivotal role in gene circuit functionality, as demonstrated for the genetic switch in the bacterial phage □. Like the □ switch, bacterial quorum sensing (QS) systems operate within a population and contain a bistable switching element, making it likely that noise plays a functional role in QS circuit operation. Therefore, a detailed anal. of the noise behavior of QS systems is needed. We have developed a set of tools generally applicable to the anal. of gene circuits, with an emphasis on investigations in the frequency domain (FD), that we apply here to the QS system in the marine bacterium Vibrio fischeri. We demonstrate that a tight coupling between exact stochastic simulation and FD anal. provides insights into the structure/function relationships in the QS circuit. Furthermore, we argue that a noise anal. is incomplete without consideration of the power spectral densities (PSDs) of the important mol. output signals. As an example we consider reversible reactions in the QS circuit, and show through anal. and exact stochastic simulation that these circuits make significant and dynamic modifications to the noise spectra. In particular, we demonstrate a "whitening" effect, which occurs as the noise is processed through these reversible reactions.

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Bacterial cell-to-cell channeling system. Tateda, Kazuhiro. Department of Microbiology, Toho University School of Medicine, Japan. Saishin Igaku (2003), 58(8), 1945-1951. Publisher: Saishin Igakusha, CODEN: SAIGAK ISSN: 0370-8241. Journal; General Review written in Japanese. CAN 140:177898 AN 2003:725048 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review on the bacterial response to environmental fluctuation such as pathogenesis and expression of virulence, synchronized prodn. of fluorescent substance in Vibrio [Photobacterium] fischeri and quorum sensing, quorum sensing mechanism for Pseudomonas aeruginosa, bioreaction modification with quorum sensing mol.(s), drug design based on quorum sensing inhibition.

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Production of substances by Medicago truncatula that affect bacterial quorum sensing. Gao, Mengsheng; Teplitski, Max; Robinson, Jayne B.; Bauer, Wolfgang D. Department of Horticulture and Crop Science, Ohio State University, Columbus, OH, USA. Molecular Plant-Microbe Interactions (2003), 16(9), 827-834. Publisher: APS Press, CODEN: MPMIEL ISSN: 0894-0282. Journal written in English. CAN 139:378124 AN 2003:683857 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Earlier work showed that higher plants produce unidentified compds. that specifically stimulate or inhibit quorum sensing (QS) regulated responses in bacteria. The ability of plants to produce substances that affect QS regulation may provide plants with important tools to manipulate gene expression and behavior in the bacteria they encounter. In order to examine the kinds of QS active substances produced by the model legume M. truncatula, young seedlings and seedling exudates were systematically extd. with various org. solvents, and the exts. were fractionated by reverse phase C18 high-performance liq. chromatog. M. truncatula appears to produce at least 15 to 20 separable substances capable of specifically stimulating or inhibiting responses in QS reporter

bacteria, primarily substances that affect QS regulation dependent on N-acyl homoserine lactone (AHL) signals. The secretion of AHL QS mimic activities by germinating seeds and seedlings was found to change substantially with developmental age. The secretion of some mimic activities may be dependent upon prior exposure of the plants to bacteria.

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Detection of pathogenic bacteria by exploiting bacteriophage specificity and the Vibrio fischeri quorum sensing system. Applegate, Bruce; Bright, Nathan G. Department of Food Science, Purdue University, West Lafayette, IN, USA. Abstracts of Papers, 226th ACS National Meeting, New York, NY, United States, September 7-11, 2003 (2003), AGFD-100. Publisher: American Chemical Society, Washington, D. C CODEN: 69EKY9 Conference; Meeting Abstract written in English. AN 2003:629482 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

This research investigates pathogenic bacteria detection utilizing bacteriophage specificity coupled with a bacterial bioluminescent bioreporter which responds to the quorum sensing mol. from Vibrio fischeri, N-(3-oxohexanoyl)-homoserine lactone (3-oxo-C6-HSL). This two-component system incorporates luxI into the bacteriophage genome. Phage host specificity is exploited to infect a pathogenic target cell and introduce/express luxI and subsequently produce 3-oxo-C6-HSL. The 3-oxo-C6-HSL diffuses out of the target cell and induces bioluminescence from a population of 3-oxo-C6-HSL inducible bioreporters (ROlux). The model system incorporating the bacteriophage M13-luxI with the ROlux reporter has shown consistent detection limits of 105cfu target organisms. However, measurable light response from high concns. of target cells at the beginning of these expts. was rapid, suggesting an enrichment step to further improve detection limits. Bacteriophage contg. luxI have been constructed for E. coli O157 and Salmonella spp. and are being evaluated. Addnl. bacteriophages are currently being constructed for Listeria monocytogenes, and Bacillus anthracis.

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The virulence activator AphA links quorum sensing to pathogenesis and physiology in Vibrio cholerae by repressing the expression of a penicillin amidase gene on the small chromosome. Kovacikova, Gabriela; Lin, Wei; Skorupski, Karen. Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA. Journal of Bacteriology (2003), 185(16), 4825-4836. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 139:271894 AN 2003:625034 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Activation of the tcpPH promoter on the Vibrio pathogenicity island by AphA and AphB initiates the Vibrio cholerae virulence cascade and is regulated by quorum sensing through the repressive action of HapR on aphA expression. To further understand how the chromosomally encoded AphA protein activates tcpPH expression, site-directed mutagenesis was used to identify the base pairs crit. for AphA binding and transcriptional activation. This anal. revealed a region of partial dyad symmetry, TATGCA-N6-TNCNNA, that is important for both of these activities. Searching the V. cholerae genome for this binding site permitted the identification of a second one upstream of a penicillin V amidase (PVA) gene on the small chromosome. AphA binds to and footprints this site,

which overlaps the pva transcriptional start, consistent with its role as a repressor at this promoter. Since aphA expression is under quorum-sensing control, the response regulators LuxO and HapR also influence pva expression. Thus, pva is repressed at low cell d. when AphA levels are high, and it is derepressed at high cell d. when AphA levels are reduced. Penicillin amidases are thought to function as scavengers for phenylacetylated compds. in the nonparasitic environment. That AphA oppositely regulates the expression of pva from that of virulence, together with the observation that PVA does not play a role in virulence, suggests that these activities are coordinated to serve V. cholerae in different biol. niches.

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Lsr operon and its genes and their use in identifying inhibitors of autoinducer transporters in bacterial quorum sensing systems. Taga, Michiko E.; Bassler, Bonnie L.; McKenzie, Douglas T. (Quorex Pharmaceuticals, Inc., USA; Princeton University). PCT Int. Appl. (2003), 172 pp. CODEN: PIXXD2 WO 2003064592 A2 20030807 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2002-US34656 20021028. Priority: US 2001-336324 20011029. CAN 139:160834 AN 2003:610580 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2003064592	A2	20030807	WO2002-US34656	20021028
WO2003064592	A3	20041014		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

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US 7183099	B2	20070227		
US 20060269951	A1	20061130	US 2006-439812	20060524

Priority Application		
US 2001-336324P	P	20011029
US 2002-284084	A3	20021028
WO2002-US34656	A	20021028

Abstract

The present invention relates to the discovery of the lsr operon, the genes therein, and the polypeptides encoded by these genes involved in autoinducer-2 biosynthesis and transport in the

quorum sensing systems of Salmonella typhimurium, Vibrio harveyi, and other bacteria. A gene responsible for autoinducer-2 prodn., designated luxS, is highly homologous in V. harveyi, S. typhimurium, and Escherichia coli, and has been identified in many species of bacteria by genome sequencing projects, but until now no function has been ascribed to this gene in any organism. The present invention also includes strains with altered expression levels of the polypeptides encoded by the genes and the lsr operon relative to wild-type cells. In some embodiments, the strains express a transporter that transports an autoinducer into the cell at a level higher than that of wild-type cells. The present invention also includes methods for identifying compds. that modulate the transport of the autoinducer into cells.

Bibliographic Information

Assessing the quorum sensing potential of microbial communities by screening for homologs of the luxI and luxR genes of Vibrio fischeri. Fuhrmann, Jeffry J.; Romesser, James A. (Fraunhofer Usa Inc., USA). PCT Int. Appl. (2003), 86 pp. CODEN: PIXXD2 WO 2003057902 A2 20030717 Designated States W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2003-US479 20030108. Priority: US 2002-346531 20020108; US 2003-338110 20030107. CAN 139:112739 AN 2003:551672 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Patent Family Information

Patent No.	Kind	Date	Application No.	Date
WO2003057902	A2	20030717	WO2003-US479	20030108
WO2003057902	A3	20040325		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 20040023254	A1	20040205	US 2003-338110	20030107
AU 2003216044	A1	20030724	AU 2003-216044	20030108

Priority Application		
US 2002-346531P	P	20020108
US 2003-338110	A	20030107
WO2003-US479	W	20030108

Abstract

A method of assessing the ability of a microbial community to operate by quorum sensing by searching for homologs of genes known to be involved in quorum sensing is described. The method involves searching for homologs of the luxI and luxR genes of Vibrio fischeri by PCR using degenerate primers derived from consensus sequences of the luxI and luxR genes. The primers are at least 15 bases long. Total DNA from a community is extd. and amplified using these primers. Amplification products are size fractionated to look for products in the size range expected for homologs of these genes. Methods of amplifying fragments of lux genes and homologs thereof are also provided as well as isolated nucleic acid fragments of lux and kits for performing PCR reactions.

Bibliographic Information

Regulation of Vibrio vulnificus virulence by the LuxS quorum-sensing system. Kim, Soo Young; Lee, Shee Eun; Kim, Young Ran; Kim, Choon Mee; Ryu, Phil Youl; Choy, Hyon E.; Chung, Sun Sik; Rhee, Joon Haeng. National Research Laboratory of Molecular Microbial Pathogenesis, Chonnam National University Medical School, Kwangju, S. Korea. Molecular Microbiology (2003), 48(6), 1647-1664. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 139:194163 AN 2003:494576 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio vulnificus is a halophilic estuarine bacterium that causes fatal septicemia and necrotizing wound infections. We tested whether V. vulnificus produces signalling mols. (autoinducer 1 and/or 2) stimulating Vibrio harveyi quorum-sensing system 1 and/or 2. Although there was no evidence for signalling system 1, we found that V. vulnificus produced a signalling activity in the culture supernatant that induced luminescence expression in V. harveyi through signalling system 2. Maximal autoinducer 2 (Al-2) activity was obsd. during mid-exponential to early stationary phase and disappeared in the late stationary phase when V. vulnificus was grown in heart infusion broth contg. 2.5% NaCl. V. vulnificus showed increased signalling activity when it was cultured in the presence of glucose (0.5%) and at low pH (pH 6.0). From a cosmid library of V. vulnificus type strain ATCC 29307, we have identified the Al-2 synthase gene (luxSVv) showing 80% identity with that of V. harveyi (luxSVh) at the amino acid level. To investigate the pathogenic role of luxSVv, a deletion mutant of the clin. isolate V. vulnificus MO6-24/O was constructed. The luxSVv mutant showed a significant delay in protease prodn. and an increase in haemolysin prodn. The decreased protease and increased haemolysin activities were restored to the isogenic wild-type level by complementation with the wild-type luxSVv allele. The change in phenotypes was also complemented by logarithmic phase spent media produced by the wild-type bacteria. Transcriptional activities of the haemolysin gene (vvhA) and protease gene (vvpE) were also obsd. in the mutant using chromosomal PvvhA::lacZ and PvvpE::lacZ transcriptional reporter constructs: transcription of vvhA was increased and of vvpE decreased by the mutation. The mutation resulted in an attenuation of lethality to mice. I.p. LD50 of the luxSVv mutant increased by 10- and 750-fold in ferric ammonium citrate-non-overloaded and ferric ammonium citrate-overloaded mice resp. The time required for the death of mice was also significantly delayed in the luxSVv mutant. Cytotoxic activity of the organism against HeLa cells, measured by lactate dehydrogenase (LDH) release assay, was also decreased significantly by the mutation. Taken together, the V. vulnificus LuxS quorum-sensing system seems to play an important role in co-ordinating the expression of virulence factors.

Bibliographic Information

On-line high-performance liquid chromatography-mass spectrometric detection and quantification of N-acylhomoserine lactones, quorum sensing signal molecules, in the presence of biological matrices. Morin, Daniele; Grasland, Beatrice; Vallee-Rehel, Karine; Dufau, Chrystele; Haras, Dominique. Laboratoire de Biologie et Chimie Moleculaires, Universite de Bretagne-Sud, Lorient, Fr. Journal of Chromatography, A (2003), 1002(1-2), 79-92. Publisher: Elsevier Science B.V., CODEN: JCRAEY ISSN: 0021-9673. Journal written in English. CAN 140:195772 AN 2003:459321 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

A protocol using reversed-phase liq. chromatog. coupled with pos.-ion electrospray ionization and ion trap mass spectrometry is described for the identification and quantification of N-acylhomoserine lactones (HSLs) in crude cell-free supernatants of bacterial cultures. The HSLs are produced by Gram-neg. bacteria and act as intercellular signals inducing d.-dependent gene expression. Compared with the multi-step procedures previously reported, which included chem. extn., purifn. and the use of Escherichia coli HSL biosensors, this online LC-MS-MS method is fast and detects 11 HSLs. Its speed and robustness allow the anal. of a large no. of samples without loss of performance (no signal variation for a control sample after 90 chromatog. injections). The selectivity is based on the MS-MS fragment ions of the mol. [M+H]+ ions and on their relative intensities. For quantification, the m/z 102 ion, specific for the lactone ring and detected with a good signal-to-noise ratio, allows low detection limits even in complex matrix samples (0.28 up to 9.3 pmol). Moreover, this method allows the quantification of 11 HSLs whatever their chem. structure, substituted or not. The protocol was applied to Vibrio vulnificus, a marine bacterium. Six HSLs were detected and quantified with relative std. deviations for repeatability of <10%.

Bibliographic Information

Identification and analysis of multiple quorum sensing systems in Vibrio cholerae. Miller, Melissa Blair. Princeton Univ., Princeton, NJ, USA. Avail. UMI, Order No. DA3062506. (2002), 190 pp. From: Diss. Abstr. Int., B 2003, 63(8), 3591. Dissertation written in English. CAN 139:176432 AN 2003:351241 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

LuxO controls luxR expression in Vibrio harveyi: Evidence for a common regulatory mechanism in Vibrio. Miyamoto, Carol M.; Dunlap, Paul V.; Ruby, Edward G.; Meighen, Edward A. Department of Biochemistry, McGill University, Montreal, QC, Can. Molecular Microbiology (2003), 48(2), 537-548. Publisher: Blackwell Publishing Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 139:114334 AN 2003:342076 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum-sensing control of luminescence in Vibrio harveyi, which involves an indirect autoinducer-mediated phosphorelay signal transduction system, contrasts with the prototypical quorum-sensing system of Vibrio fischeri, in which the autoinducer and the transcriptional activator LuxR directly activate lux operon expression. In V. harveyi, a regulator not homologous to V. fischeri LuxR and also designated LuxR (LuxRvh), binds specifically to the lux operon promoter region and activates the expression of luminescence. A direct connection has not been identified previously between V. harveyi LuxRvh and the autoinducer-mediated phosphorelay system. Here, we demonstrate by

mobility shift assays and measurement of luxRvh mRNA levels with luxO+ and luxO- cells that the central response regulator of the V. harveyi phosphorelay system (LuxO) represses the level of LuxRvh. Expression of a luxRvh-bearing plasmid strongly stimulated luminescence of a luxO- mutant but had no effect on luminescence of wild-type luxO+ cells, indicating tight regulation of luxRvh by LuxO. Furthermore, luxO null mutants of V. fischeri MJ-1 and 2 autoinducer mutants, MJ-211 (luxI-) and MJ-215 (luxI- ainS-), emitted more light and exhibited more elevated levels of litR, a newly identified V. harveyi luxRvh homolog, than their luxO+ counterparts. These results suggest that activity of the autoinducer-mediated phosphorelay system is coupled to LuxRvh/LitR control of luminescence through LuxO in V. harveyi and V. fischeri. The presence of homologs of V. harveyi LuxRvh, LuxO, and other phosphorelay system proteins in various Vibrio spp. and the control of LuxRvh and its homologs by LuxO identified here in V. harveyi and V. fischeri and recently in Vibrio cholerae suggest that the luxO-luxRvh couple is a central feature of this quorum-sensing system in members of the genus Vibrio.

Bibliographic Information

A constitutively active variant of the quorum-sensing regulator LuxO affects protease production and biofilm formation in Vibrio cholerae. Vance, Russell E.; Zhu, Jun; Mekalanos, John J. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA, USA. Infection and Immunity (2003), 71(5), 2571-2576. Publisher: American Society for Microbiology, CODEN: INFIBR ISSN: 0019-9567. Journal written in English. CAN 139:144797 AN 2003:339177 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Vibrio cholerae normally inhabits aquatic habitats but can cause a severe diarrheal illness in humans. Its arsenal of virulence factors includes a secreted hemagglutinin (HA) protease. An HA protease-deficient mutant of V. cholerae was isolated and designated E7946 mpc. E7946 mpc was found to contain a point mutation in the luxO quorum-sensing regulator. In accordance with this finding, E7946 mpc exhibits a defect in quorum sensing. The mutant luxO allele [luxO(Con)] produces a protein with a leucine-to-glutamine substitution at amino acid 104. Transfer of the luxO(Con) allele to an otherwise wild-type background was sufficient to eliminate HA protease expression; conversely, deletion of luxO(Con) from E7946 mpc restored protease activity. We demonstrate that LuxO(Con) constitutively represses the transcription of hapR, an essential pos. regulator of HA protease. Interestingly, strains harboring luxO(Con) form enhanced biofilms, and enhanced biofilm formation does not appear to be dependent on reduced HA protease expression. Taken together, the results confirm the role of LuxO as a central switch that coordinately regulates virulence-related phenotypes such as protease prodn. and biofilm formation.

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Vibrio harveyi quorum sensing: a coincidence detector for two autoinducers controls gene expression. Mok, Kenny C.; Wingreen, Ned S.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. EMBO Journal (2003), 22(4), 870-881. Publisher: Oxford University Press, CODEN: EMJODG ISSN: 0261-4189. Journal written in English. CAN 139:3347 AN 2003:228306 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In a process called quorum sensing, bacteria communicate with one another by exchanging chem. signals called autoinducers. In the bioluminescent marine bacterium Vibrio harveyi, two different auto inducers (AI-1 and AI-2) regulate light emission. Detection of and response to the V.harveyi autoinducers are accomplished through two two-component sensory relay systems: AI-1 is detected by the sensor LuxN and AI-2 by LuxPQ. Here we further define the V.harveyi quorum-sensing regulon by identifying 10 new quorum-sensing-controlled target genes. Our examn. of signal processing and integration in the V.harveyi quorum-sensing circuit suggests that AI-1 and AI-2 act synergistically, and that the V.harveyi quorum-sensing circuit may function exclusively as a coincidence detector' that discriminates between conditions in which both autoinducers are present and all other conditions.

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Crystal structure of Vibrio harveyi quorum sensing regulator LuxP complex with autoinducer-2 and its use of rational drug design. Bassler, Bonnie L.; Schauder, Stephan; Chen, Xin; Hughson, Frederick M.; Cooper, Stephen R. (Quorex Pharmaceuticals, Inc., USA; Princeton University). PCT Int. Appl. (2003), 74 pp. CODEN: PIXXD2 WO 2003018046 A1 20030306 Designated States W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG. Designated States RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, ML, MR, NE, SN, TD, TG. Patent written in English. Application: WO 2002-US26579 20020822. Priority: US 2001-314705 20010824. CAN 138:217167 AN 2003:173456 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

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Patent No.	Kind	Date	Application No.	Date
WO 2003018046	A1	20030306	WO 2002-US26579	20020822
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RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
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WO 2002-US26579	W	20020822
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Abstract
A crystal comprising Vibrio harveyi protein LuxP is obtained, and a binding site for autoinducer-2 (AI-2) identified. X-ray crystallog. data for LuxP and a LuxP-AI-2 complex is detd. and refined to 1.5 .ANG. resoln. and used in a drug discovery method. Pharmaceutical compns. comprising ligands identified by such drug discovery methods are used to treat bacterial infections.

Bibliographic Information

Possible quorum sensing in the rumen microbial community: detection of quorum-sensing signal molecules from rumen bacteria. Mitsumori, Makoto; Xu, Liming; Kajikawa, Hiroshi; Kurihara, Mitsunori; Tajima, Kiyoshi; Hai, Jin; Takenaka, Akio. National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan. FEMS Microbiology Letters (2003), 219(1), 47-52. Publisher: Elsevier Science B.V., CODEN: FMLED7 ISSN: 0378-1097. Journal written in English. CAN 138:381833 AN 2003:126563 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The bioluminescence assay using Vibrio harveyi BB170 was used to examine quorum-sensing autoinducer 2 (AI-2) activity from cell-free culture fluids of rumen bacteria. The assay showed that the culture fluids of four species of rumen bacteria, Butyrivibrio fibrisolvens, Eubacterium ruminantium, Ruminococcus flavefaciens, and Succinimonas amylolytica, contained AI-2-like mols. Furthermore, homologues for luxS genes were detected in rumen fluids collected from three cows and in bacterial cells of P. ruminicola subsp. ruminicola and R. flavefaciens. These findings suggest that the quorum-sensing system mediated by AI-2 is present in the rumen.

Bibliographic Information

Common features of the quorum sensing systems in Vibrio species. Miyamoto, C.; Skouris, N.; Hosseinkhani, S.; Lin, L. Y.; Meighen, E. A. Dept of Biochemistry, McGill University, Montreal, QC, Can. Editor(s): Stanley, Philip E.; Kricka, Larry J. Bioluminescence & Chemiluminescence: Progress & Current Applications, [Proceedings of the Symposium on Bioluminescence and Chemiluminescence], 12th, Cambridge, United Kingdom, Apr. 5-9, 2002 (2002), 97-100. Publisher: World Scientific Publishing Co. Pte. Ltd., Singapore, Singapore CODEN: 69DPGZ Conference written in English. CAN 139:319920 AN 2003:108779 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The LuxO system is a large family of quorum sensing proteins involved in □54-dependent transcriptional control. LuxO is only present in vibrios and that even within the genus, all the components are not completely conserved. V. harveyi luxO is organized as in V. harveyi:uvrB-luxO-luxU. Other quorum sensing proteins include LuxU, LuxOU, LuxMN, LuxPQ, LuxQ, and LuxN.

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RP4-based plasmids for conjugation between Escherichia coli and members of the Vibrionaceae. Stabb, Eric V.; Ruby, Edward G. Department of Microbiology, University of Georgia, Athens, GA, USA. Methods in Enzymology (2002), 358(Bacterial Pathogenesis, Part C), 413-426. Publisher: Elsevier Science, CODEN: MENZAU ISSN: 0076-6879. Journal;

General Review written in English. CAN 138:281697 AN 2003:101226 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review describing the construction and improved utility of RP4-based plasmids for conjugation with Vibrionaceae. Improvement was achieved by modifying conjugal tools to meet 3 criteria: (1) an ideal conjugal helper should not introduce insertion elements into recipient cells, except where that function is intended; (2) for many purposes, a conjugal helper that replicates in the donor, but not in the recipient, is desirable; (3) optimally, mobilizable vectors should contain only a defined, minimal oriT. The Vibrionaceae family includes both important pathogens and useful model organisms in the study of processes ranging from the regulation of light prodn. by quorum sensing to the establishment of mutualistic animal-bacteria assocns. (c) 2002 Academic Press.

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luxS and arcB control aerobic growth of Actinobacillus actinomycetemcomitans under iron limitation. Fong, Karen P.; Gao, Ling; Demuth, Donald R. Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA. Infection and Immunity (2003), 71(1), 298-308. Publisher: American Society for Microbiology, CODEN: INFIBR ISSN: 0019-9567. Journal written in English. CAN 138:251260 AN 2003:18488 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
LuxS is responsible for the prodn. of autoinducer 2 (AI-2), which functions in Vibrio harveyi as a quorum-sensing signal that controls the cell d.-dependent expression of the lux operon. In nonluminescent organisms, the physiol. role of AI-2 is not clear. We report that inactivation of luxS in Actinobacillus actinomycetemcomitans JP2 results in reduced growth of the mutant, but not the wild-type organism, under aerobic, iron-limited conditions. Stunted cultures of the luxS mutant A. actinomycetemcomitans JP2-12 grew to high cell d. when subcultured under iron-replete conditions. In addn., the mutant strain grew to high cell d. under iron limitation after transformation with a plasmid contg. a functional copy of luxS. Results of real-time PCR showed that A. actinomycetemcomitans JP2-12 exhibited significantly reduced expression of afuA (eightfold), fecBCDE (10-fold), and ftnAB (>50-fold), which encode a periplasmic ferric transport protein, a putative ferric citrate transporter, and ferritin, resp. The expressions of putative receptors for transferrin, Hb, and hemophore binding protein were also reduced at more modest levels (two- to threefold). In contrast, expressions of sidD and frpB (encoding putative siderophore receptors) were increased 10- and 3-fold, resp., in the luxS mutant. To better understand the mechanism of the AI-2 response, the A. actinomycetemcomitans genome was searched for homologs of the V. harveyi signal transduction proteins, LuxP, LuxQ, LuxU, and LuxO. Interestingly, ArcB was found to be most similar to LuxQ sensor/kinase. To det. whether arcB plays a role in the response of A. actinomycetemcomitans to AI-2, an arcB-deficient mutant was constructed. The isogenic arcB mutant grew poorly under anaerobic conditions but grew normally under aerobic iron-replete conditions. However, the arcB mutant failed to grow aerobically under iron limitation, and reverse transcriptase PCR showed that inactivation of arcB resulted in decreased expression of afuA and ftnAB.
Thus, isogenic luxS and arcB mutants of A. actinomycetemcomitans exhibit similar phenotypes when cultured aerobically under iron limitation, and both mutants exhibit reduced expression of a common set of genes involved in the transport and storage of iron. These results suggest that LuxS

and ArcB may act in concert to control the adaptation of A. actinomycetemcomitans to iron-limiting conditions and its growth under such conditions.

Bibliographic Information

Regulation of virulence gene expression in Vibrio cholerae by quorum sensing: HapR functions at the aphA promoter. Kovacikova, Gabriela; Skorupski, Karen. Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH, USA. Molecular Microbiology (2002), 46(4), 1135-1147. Publisher: Blackwell Science Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 138:118928 AN 2002:900029 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Quorum sensing neg. influences virulence gene expression in certain toxigenic Vibrio cholerae strains. At high cell densities, the response regulator LuxO fails to reduce the expression of HapR, which in turn represses the expression of the virulence cascade. A crit. regulatory step in the cascade is activation of tcpPH expression by AphA and AphB. We show here that HapR influences the virulence cascade by directly repressing aphA expression. In strain C6706, aphA expression was increased in a □hapR mutant and decreased in a □luxO mutant, indicating a neg. and pos. influence, resp., of these gene products on the promoter. Overexpression of HapR also reduced aphA expression in both C6706 and Escherichia coli. DNase I footprinting showed that purified HapR binds to the aphA promoter between -85 and -58. Although it appears that quorum sensing does not influence virulence gene expression in strain O395 solely because of a frameshift in hapR, overproduced HapR did not repress expression from the O395 aphA promoter in either Vibrio or E. coli, nor did the protein bind to the promoter. Two basepair differences from C6706 are present in the O395 HapR binding site at -85 and -77. Introducing the -77 change into C6706 prevented HapR binding and repression of aphA expression. This mutation also eliminated the repression of toxin-co-regulated pilus (TCP) and cholera toxin (CT) that occurs in a □luxO mutant, indicating that HapR function at aphA is crit. for d.-dependent regulation of virulence genes.

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Quorum sensing in Vibrio cholerae. Camara, Miguel; Hardman, Andrea; Williams, Paul; Milton, Debra. School of Pharmaceutical Sciences, University of Nottingham, Nottingham, UK. Nature Genetics (2002), 32(2), 217-218. Publisher: Nature Publishing Group, CODEN: NGENEC ISSN: 1061-4036. Journal written in English. AN 2002:832112 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Bacteria can communicate with members of their own species and others to coordinate their behavior in response to cell d. This phenomenon, known as quorum sensing, relies on the prodn. and sensing of one or more secreted signal mols. A recent study identifies a complex quorum sensing network in the human pathogen Vibrio cholerae.

Bibliographic Information

MetR and CRP bind to the Vibrio harveyi lux promoters and regulate luminescence. Chatterjee, Jaidip; Miyamoto, Carol M.; Zouzoulas, Athina; Lang, B. Franz; Skouris, Nicolas; Meighen, Edward A. Department of Biochemistry, McGill University, Montreal, QC, Can.

Molecular Microbiology (2002), 46(1), 101-111. Publisher: Blackwell Science Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 138:101890 AN 2002:810205 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The induction of luminescence in Vibrio harveyi at the later stages of growth is controlled by a quorum-sensing mechanism in addn. to nutritional signals. However, the mechanism of transmission of these signals directly to the lux promoters is unknown and only one regulatory protein, LuxR, has been shown to bind directly to lux promoter DNA. In this report, we have cloned and sequenced two genes, crp and metR, coding for the nutritional regulators, CRP (cAMP receptor protein) and MetR (a LysR homolog), involved in catabolite repression and methionine biosynthesis resp. The metR gene was cloned based on a general strategy to detect lux DNA-binding proteins expressed from a genomic library, whereas the crp gene was cloned based on its complementation of an Escherichia coli crp mutant. Both CRP and MetR were shown to bind to lux promoter DNA, with CRP being dependent on the presence of cAMP. Expression studies indicated that the two regulators had opposite effects on luminescence: CRP was an activator and MetR a repressor. Disruption of crp decreased luminescence by about 1000-fold showing that CRP is a major activator of luminescence the same as LuxR, whereas disruption of MetR resulted in activation of luminescence over 10-fold, confirming its function as a repressor. Comparison of the levels of the autoinducers involved in quorum sensing excreted by V. harveyi, and the crp and metR mutants, showed that autoinducer prodn. was not significantly different, thus indicating that the nutritional signals do not affect luminescence by changing the levels of the signals required for quorum sensing. Indeed, the large effects of these nutritional sensors show that luminescence is controlled by multiple signals related to the environment and the cell d. which must be integrated at the mol. level to control expression at the lux promoters.

Bibliographic Information

A LuxR homolog controls production of symbiotically active extracellular polysaccharide II by Sinorhizobium meliloti. Pellock, Brett J.; Teplitski, Max; Boinay, Ryan P.; Bauer, W. Dietz; Walker, Graham C. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA. Journal of Bacteriology (2002), 184(18), 5067-5076. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 137:380816 AN 2002:678775 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Prodn. of complex extracellular polysaccharides (EPSs) by the nitrogen-fixing soil bacterium Sinorhizobium meliloti is required for efficient invasion of root nodules on the host plant alfalfa. Any one of three S. meliloti polysaccharides, succinoglycan, EPS II, or K antigen, can mediate infection thread initiation and extension (root nodule invasion) on alfalfa. Of these three polysaccharides, the only symbiotically active polysaccharide produced by S. meliloti wild-type strain Rm1021 is succinoglycan. The expR101 mutation is required to turn on prodn. of symbiotically active forms of EPS II in strain Rm1021. In this study, we have detd. the nature of the expR101 mutation in S. meliloti. The expR101 mutation, a spontaneous dominant mutation, results from precise, reading frame-restoring excision of an insertion sequence from the coding region of expR, a gene whose predicted protein product is highly homologous to the Rhizobium leguminosarum bv. viciae RhiR protein and a no. of other homologs of Vibrio fischeri LuxR that function as receptors for N-acylhomoserine lactones (AHLs) in quorum-sensing regulation of gene expression. S. meliloti ExpR activates transcription of genes involved in EPS II prodn. in a d.-

dependent fashion, and it does so at much lower cell densities than many quorum-sensing systems. High-pressure liq. chromatog. fractionation of S. meliloti culture filtrate exts. revealed at least three peaks with AHL activity, one of which activated ExpR-dependent expression of the expE operon.

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Parallel quorum sensing systems converge to regulate virulence in Vibrio cholerae. Miller, Melissa B.; Skorupski, Karen; Lenz, Derrick H.; Taylor, Ronald K.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Cell (Cambridge, MA, United States) (2002), 110(3), 303-314. Publisher: Cell Press, CODEN: CELLB5 ISSN: 0092-8674. Journal written in English. CAN 137:335084 AN 2002:639806 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The marine bacterium Vibrio harveyi possesses 2 quorum sensing systems (System 1 and System 2) that regulate bioluminescence. Although the V. cholerae genome sequence reveals that a V. harveyi-like System 2 exists, it does not predict the existence of a V. harveyi-like System 1 or any obvious quorum sensing-controlled target genes. In this report we identify and characterize the genes encoding an addnl. V. cholerae autoinducer synthase and its cognate sensor. Anal. of double mutants indicates that a 3rd as yet unidentified sensory circuit exists in V. cholerae. This quorum sensing app. is unusually complex, as it is composed of □3 parallel signaling channels. We show that in V. cholerae these communication systems converge to control virulence.

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Role of the C-terminal domain of the alpha subunit of RNA polymerase in LuxR-dependent transcriptional activation of the lux operon during quorum sensing. Finney, Angela H.; Blick, Robert J.; Murakami, Katsuhiko; Ishihama, Akira; Stevens, Ann M. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. Journal of Bacteriology (2002), 184(16), 4520-4528. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 137:380776 AN 2002:589315 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

During quorum sensing in Vibrio fischeri, the luminescence, or lux, operon is regulated in a cell d.-dependent manner by the activator LuxR in the presence of an acylated homoserine lactone autoinducer mol. [N-(3-oxohexanoyl) homoserine lactone]. LuxR, which binds to the lux operon promoter at a position centered at -42.5 relative to the transcription initiation site, is thought to function as an ambidextrous activator making multiple contacts with RNA polymerase (RNAP). The specific role of the □-subunit C-terminal domain (□CTD) of RNAP in LuxR-dependent transcriptional activation of the lux operon promoter has been investigated. The effects of 70 alanine substitution variants of the □ subunit were detd. in vivo by measuring the rate of transcription of the lux operon via luciferase assays in recombinant Escherichia coli. The mutant RNAPs from strains exhibiting at least twofold-increased or -decreased activity in comparison to the wild type were further examd. by in vitro assays. Since full-length LuxR has not been purified, an autoinducer-independent N-terminally truncated form of LuxR, LuxR□N, was used for in vitro studies. Single-round transcription assays were performed using reconstituted mutant RNAPs in the presence of LuxR□N, and 14 alanine substitutions in the □CTD were identified as having neg. effects on the rate of transcription from the lux operon promoter. Five of these 14 □ variants were

also involved in the mechanisms of both LuxR- and LuxR□N-dependent activation in vivo. The positions of these residues lie roughly within the 265 and 287 determinants in □ that have been identified through studies of the cAMP receptor protein and its interactions with RNAP. This suggests a model where residues 262, 265, and 296 in □ play roles in DNA recognition and residues 290 and 314 play roles in □-LuxR interactions at the lux operon promoter during quorum sensing.

Bibliographic Information

Quorum sensing in Campylobacter jejuni: detection of a luxS encoded signalling molecule. Elvers, Karen T.; Park, Simon F. School of Biomedical and Life Sciences, University of Surrey, Surrey, UK. Microbiology (Reading, United Kingdom) (2002), 148(5), 1475-1481. Publisher: Society for General Microbiology, CODEN: MROBEO ISSN: 1350-0872. Journal written in English. CAN 137:122059 AN 2002:410245 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The expression of a wide variety of physiol. functions in many bacterial species is modulated by quorum sensing, a population-dependent signaling mechanism that involves the prodn. and detection of extracellular signaling mols. The genome sequence of Campylobacter jejuni NCTC 11168 contains a gene encoding an orthologue of LuxS, which is required for autoinducer-2 (Al-2) prodn. in other bacterial species, but does not contain genes predicted to encode any known acyl-homoserine lactone synthetase. This study demonstrates that C. jejuni produces functional Al-2 activity through the ability of cell-free exts. to specifically induce bioluminescence in Vibrio harveyi BB170, a reporter strain for quorum-sensing system 2. Prodn. of this signaling compd. was shown to be dependent upon the product of the C. jejuni luxS gene (Cj1198). While the luxS mutant showed comparable growth rate, resistance to oxidative stress and ability to invade Caco-2 cell monolayers to the parental strain, it exhibited decreased motility haloes in semisolid media, suggesting a role for quorum sensing in the regulation of motility.

Bibliographic Information

Quorum sensing in vibrio cholerae. Zhou, Xin. Trends in Microbiology (2002), 10(5), 213. Publisher: Elsevier Science Ltd., CODEN: TRMIEA ISSN: 0966-842X. Journal written in English. AN 2002:355450 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

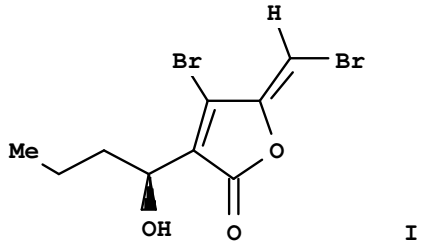
Bibliographic Information

Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Manefield, Michael; Rasmussen, Thomas Bovbjerg; Henzter, Morten; Andersen, Jens Bo; Steinberg, Peter; Kjelleberg, Staffan; Givskov, Michael. School of Microbiology and Immunology, University of New South Wales, Sydney, Australia. Microbiology (Reading, United Kingdom) (2002), 148(4), 1119-1127. Publisher: Society for General Microbiology, CODEN: MROBEO ISSN: 1350-0872. Journal written in English. CAN 137:60034 AN 2002:323519 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

N-acyl-L-homoserine lactones (AHLs) are co-regulatory ligands required for control of the expression of genes encoding virulence traits in many Gram-neg. bacterial species. Recent studies

have indicated that AHLs modulate the cellular concns. of LuxR-type regulatory proteins by binding and fortifying these proteins against proteolytic degrdn. (Zhu & Winans, 2001). Halogenated furanones produced by the macroalga Delisea pulchra, e.g. I, inhibit AHL-dependent gene expression. This study assayed for an in vivo interaction between a tritiated halogenated furanone and the LuxR protein of Vibrio fischeri overproduced in Escherichia coli. While a stable interaction between the algal metabolite and the bacterial protein was not found, it was noted by Western anal. that the half-life of the protein is reduced up to 100-fold in the presence of halogenated furanones (including I, II, III, and IV). This suggests that halogenated furanones modulate LuxR activity but act to destabilize, rather than protect, the AHL-dependent transcriptional activator. The furanone-dependent redn. in the cellular concn. of the LuxR protein was assocd. with a redn. in expression of a plasmid-encoded Pluxl-gfp(ASV) fusion, suggesting that the redn. in LuxR concn. is the mechanism by which furanones control expression of AHL-dependent phenotypes. The mode of action by which halogenated furanones reduce cellular concns. of the LuxR protein remains to be characterized.



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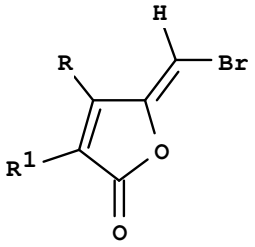

II R=Br, R ^{1}=H
III R=Br, R ^{1}=butyl
IV R=R ^{1}=H

Bibliographic Information

Quorum-sensing regulators control virulence gene expression in Vibrio cholerae. Zhu, Jun; Miller, Melissa B.; Vance, Russell E.; Dziejman, Michelle; Bassler, Bonnie L.; Mekalanos, John J. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA, USA. Proceedings of the National Academy of Sciences of the United States of America (2002), 99(5), 3129-3134. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 137:150988 AN 2002:224914 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The prodn. of virulence factors including cholera toxin and the toxin-coregulated pilus in the human pathogen Vibrio cholerae is strongly influenced by environmental conditions. The well-characterized ToxR signal transduction cascade is responsible for sensing and integrating the environmental information and controlling the virulence regulon. We show here that, in addn. to the known components of the ToxR signaling circuit, quorum-sensing regulators are involved in regulation of V. cholerae virulence. We focused on the regulators LuxO and HapR because

homologs of these two proteins control quorum sensing in the closely related luminous marine bacterium Vibrio harveyi. Using an infant mouse model, we found that a luxO mutant is severely defective in colonization of the small intestine. Gene arrays were used to profile transcription in the V. cholerae wild type and the luxO mutant. These studies revealed that the ToxR regulon is repressed in the luxO mutant, and that this effect is mediated by another neg. regulator, HapR. We show that LuxO represses hapR expression early in log-phase growth, and constitutive expression of hapR blocks ToxR-regulon expression. Addnl., LuxO and HapR regulate a variety of other cellular processes including motility, protease prodn., and biofilm formation. Together these data suggest a role for quorum sensing in modulating expression of blocks of virulence genes in a reciprocal fashion in vivo.

Bibliographic Information

VanT, a homologue of Vibrio harveyi LuxR, regulates serine, metalloprotease, pigment, and biofilm production in Vibrio anguillarum. Croxatto, Antony; Chalker, Victoria J.; Lauritz, Johan; Jass, Jana; Hardman, Andrea; Williams, Paul; Camara, Miguel; Milton, Debra L. Department of Molecular Biology, Umea University, Umea, Swed. Journal of Bacteriology (2002), 184(6), 1617-1629. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 137:135802 AN 2002:210241 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio anguillarum possesses at least two N-acylhomoserine lactone (AHL) quorum-sensing circuits, one of which is related to the luxMN system of Vibrio harveyi. In this study, we have cloned an addnl. gene of this circuit, vanT, encoding a V. harveyi LuxR-like transcriptional regulator. A V. anguillarum □vanT null mutation resulted in a significant decrease in total protease activity due to loss of expression of the metalloprotease EmpA, but no changes in either AHL prodn. or virulence. Addnl. genes pos. regulated by VanT were identified from a plasmid-based gene library fused to a promoterless lacZ. Three lacZ fusions (serA::lacZ, hpdA-hgdA::lacZ, and sat-vps73::lacZ) were identified which exhibited decreased expression in the □vanT strain. SerA is similar to 3-phosphoglycerate dehydrogenases and catalyzes the first step in the serine-glycine biosynthesis pathway. HgdA has identity with homogentisate dioxygenases, and HpdA is homologous to 4-hydroxyphenylpyruvate dioxygenases (HPPDs) involved in pigment prodn. V. anguillarum strains require an active VanT to produce high levels of an L-tyrosine-induced brown color via HPPD, suggesting that VanT controls pigment prodn. Vps73 and Sat are related to Vibrio cholerae proteins encoded within a DNA locus required for biofilm formation. A V. anguillarum □vanT mutant and a mutant carrying a polar mutation in the sat-vps73 DNA locus were shown to produce defective biofilms. Hence, a new member of the V. harveyi LuxR transcriptional activator family has been characterized in V. anguillarum that pos. regulates serine, metalloprotease, pigment, and biofilm prodn.

Bibliographic Information

Structural identification of a bacterial quorum-sensing signal containing boron. Chen, Xin; Schauder, Stephan; Potier, Noelle; Van Dorsselaer, Alain; Pelczer, Istvan; Bassler, Bonnie L.; Hughson, Frederick M. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Nature (London, United Kingdom) (2002), 415(6871), 545-549. Publisher: Nature Publishing Group, CODEN: NATUAS ISSN: 0028-0836. Journal written in English. CAN 136:275055 AN 2002:125272 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Cell-cell communication in bacteria is accomplished through the exchange of extracellular signaling mols. called autoinducers. This process, termed quorum sensing, allows bacterial populations to coordinate gene expression. Community cooperation probably enhances the effectiveness of processes such as bioluminescence, virulence factor expression, antibiotic prodn. and biofilm development. Unlike other autoinducers, which are specific to a particular species of bacteria, a recently discovered autoinducer (AI-2) is produced by a large no. of bacterial species. AI-2 has been proposed to serve as a 'universal' signal for inter-species communication. However, the chem. identity of AI-2 has proved elusive. Here we present the crystal structure of an AI-2 sensor protein, LuxP, in a complex with autoinducer. The bound ligand is a furanosyl borate diester that bears no resemblance to previously characterized autoinducers. Our findings suggest that addn. of naturally occurring borate to an AI-2 precursor generates active AI-2. Furthermore, they indicate a potential biol. role for boron, an element required by a no. of organisms but for unknown reasons.

Bibliographic Information

Synthesis of new 3- and 4-substituted analogues of acyl homoserine lactone quorum sensing autoinducers. Olsen, J. A.; Severinsen, R.; Rasmussen, T. B.; Hentzer, M.; Givskov, M.; Nielsen, J. Department of Chemistry, Technical University of Denmark, 2800 Kgs., Lyngby, Den. Bioorganic & Medicinal Chemistry Letters (2002), 12(3), 325-328. Publisher: Elsevier Science Ltd., CODEN: BMCLE8 ISSN: 0960-894X. Journal written in English. CAN 136:386358 AN 2002:97664 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The quorum sensing mechanism in Gram-neg. bacteria uses small intercellular signal mols., N-acyl-homoserine lactones (AHLs), to control transcription of specific genes in relation to population d. In this communication, we describe the parallel synthesis of new AHL analogs, in which substituents have been introduced into the 3- and 4-positions of the lactone ring. These analogs have been screened for their ability to activate and inhibit a Vibrio fischeri LuxI/LuxR-derived quorum sensing reporter system.

Bibliographic Information

Inhibition of biofilm formation and swarming of Escherichia coli by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. Ren, Dacheng; Sims, James J.; Wood, Thomas K. Departments of Chemical Engineering and Molecular and Cellular Biology, University of Connecticut, Storrs, CT, USA. Environmental Microbiology (2001), 3(11), 731-736. Publisher: Blackwell Science Ltd., CODEN: ENMIFM ISSN: 1462-2912. Journal written in English. CAN 136:259882 AN 2002:58655 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The quorum-sensing disrupter (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone (furanone) of the alga Delisea pulchra was found to inhibit the swarming motility of Escherichia coli completely at 13 □g cm-2 (also at 20 □g ml-1) but did not inhibit its growth rate at 13-52 □g cm-2 or from 20 to 100 □g ml-1. Swimming was not inhibited by the furanone at 20-40 □g ml-1. In addn., confocal scanning laser microscopy revealed that this furanone at 60 □g ml-1 inhibited the biofilm formation of E. coli, as it decreased its thickness by 55%, reduced the no. of water channels

and decreased the percentage of live cells by 87%. This suggests that natural furanone may be used as a new method to control bacterial biofilms that does not involve toxicity. Furanone at 10 □g ml-1 also inhibited by 3300-fold the quorum sensing of Vibrio harveyi via autoinducer 1 (AI-1) and inhibited by 5500-fold that of V. harveyi via autoinducer 2 (AI-2) as well as inhibited by 26,600-fold the quorum sensing of E. coli via AI-2; hence, this furanone is a non-specific intercellular signal antagonist.

Bibliographic Information

Studies on the production of quorum-sensing signal molecules in Mannheimia haemolytica A1 and other Pasteurellaceae species. Malott, Rebecca J.; Lo, Reggie Y. C. Department of Microbiology, University of Guelph, Guelph, ON, Can. FEMS Microbiology Letters (2002), 206(1), 25-30. Publisher: Elsevier Science B.V., CODEN: FMLED7 ISSN: 0378-1097. Journal written in English. CAN 136:259677 AN 2002:21214 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The bioluminescence assay system using Vibrio harveyi reporter strains were used to examine quorum-sensing autoinducer (AI) activity from Mannheimia haemolytica A1 cell-free culture supernatant. We showed that M. haemolytica A1 cell-free culture supernatant contains mols. that can stimulate the quorum-sensing system that regulates the expression of the luciferase operon in V. harveyi. Specifically, M. haemolytica A1 can stimulate only the quorum system 2 but not system 1, suggesting that the culture supernatant only contains mols. similar to AI-2 of V. harveyi. The bioluminescence assay was also used to show that culture supernatants from related Pasteurellaceae organisms, Pasteurella multocida, Pasteurella trehalosi, Actinobacillus suis and Actinobacillus pleuropneumoniae, also contain AI-2-like mols. This is consistent with the presence of a luxS homolog in the genomes of P. multocida and A. pleuropneumoniae. A luxS homolog was cloned by PCR from M. haemolytica A1 using sequencing data from the ongoing genome sequencing project. The cloned luxSM.h. was able to complement AI-2 prodn. in the Escherichia coli DH5□ luxS mutant. This is the first report of a quorum-sensing activity in M. haemolytica A1 and suggests that this bacterium utilizes this mechanism to regulate expression of genes under specific conditions.

Bibliographic Information

The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in Salmonella typhimurium. Taga, Michiko E.; Semmelhack, Julia L.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2001), 42(3), 777-793. Publisher: Blackwell Science Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 136:337638 AN 2001:866359 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In a process called quorum sensing, bacteria communicate with one another using secreted chem. signaling mols. termed autoinducers. A novel autoinducer called AI-2, originally discovered in the quorum-sensing bacterium Vibrio harveyi, is made by many species of Gram-neg. and Gram-pos. bacteria. In every case, prodn. of AI-2 is dependent on the LuxS autoinducer synthase. The genes regulated by AI-2 in most of these luxS-contg. species of bacteria are not known. Here, we describe the identification and characterization of AI-2-regulated genes in Salmonella typhimurium. We find

that LuxS and AI-2 regulate the expression of a previously unidentified operon encoding an ATP binding cassette (ABC)-type transporter. We have named this operon the lsr (luxS regulated) operon. The Lsr transporter has homol. to the ribose transporter of Escherichia coli and S. typhimurium. A gene encoding a DNA-binding protein that is located adjacent to the Lsr transporter structural operon is required to link AI-2 detection to operon expression. This gene, which we have named lsrR, encodes a protein that represses lsr operon expression in the absence of AI-2. Mutations in the lsr operon render S. typhimurium unable to eliminate AI-2 from the extracellular environment, suggesting that the role of the Lsr app. is to transport AI-2 into the cells. It is intriguing that an operon regulated by AI-2 encodes functions resembling the ribose transporter, given recent findings that AI-2 is derived from the ribosyl moiety of S-ribosylhomocysteine.

Bibliographic Information

The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Schauder, Stephan; Shokat, Kevan; Surette, Michael G.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (2001), 41(2), 463-476. Publisher: Blackwell Science Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 135:300836 AN 2001:584686 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Many bacteria control gene expression in response to cell population d., and this phenomenon is called quorum sensing. In Gram-neg. bacteria, quorum sensing typically involves the prodn., release and detection of acylated homoserine lactone signaling mols. called autoinducers. Vibrio harveyi, a Gram-neg. bioluminescent marine bacterium, regulates light prodn. in response to two distinct autoinducers (AI-1 and AI-2). AI-1 is a homoserine lactone. The structure of AI-2 is not known. We have suggested previously that V. harveyi uses AI-1 for intraspecies communication and AI-2 for interspecies communication. Consistent with this idea, we have shown that many species of Gram-neg. and Gram-pos. bacteria produce AI-2 and, in every case, prodn. of AI-2 is dependent on the function encoded by the luxS gene. We show here that LuxS is the AI-2 synthase and that AI-2 is produced from S-adenosylmethionine in three enzymic steps. The substrate for LuxS is S-ribosylhomocysteine, which is cleaved to form two products, one of which is homocysteine, and the other is AI-2. In this report, we also provide evidence that the biosynthetic pathway and biochem. intermediates in AI-2 biosynthesis are identical in Escherichia coli, Salmonella typhimurium, V. harveyi, Vibrio cholerae and Enterococcus faecalis. This result suggests that, unlike quorum sensing via the family of related homoserine lactone autoinducers, AI-2 is a unique, "universal" signal that could be used by a variety of bacteria for communication among and between species.

Bibliographic Information

Signaling system in Porphyromonas gingivalis based on a LuxS protein. Chung, Whasun O.; Park, Yoonsuk; Lamont, Richard J.; McNab, Rod; Barbieri, Bruno; Demuth, Donald R. Department of Oral Biology, University of Washington, Seattle, WA, USA. Journal of Bacteriology (2001), 183(13), 3903-3909. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 136:50939 AN 2001:464792 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The luxS gene of quorum-sensing Vibrio harveyi is required for type 2 autoinducer prodn. We identified a Porphyromonas gingivalis open reading frame encoding a predicted peptide of 161 aa that shares 29% identity with the amino acid sequence of the LuxS protein of V. harveyi. Conditioned medium from a late-log-phase P. gingivalis culture induced the luciferase operon of V. harveyi, but that from a luxS insertional mutant did not. In P. gingivalis, the expression of luxS mRNA was environmentally controlled and varied according to the cell d. and the osmolarity of the culture medium. In addn., differential display PCR showed that the inactivation of P. gingivalis luxS resulted in up-regulation of a hemin acquisition protein and an arginine-specific protease and reduced expression of a hemin-regulated protein, a TonB homolog, and an excinuclease. The data suggest that the luxS gene in P. gingivalis may function to control the expression of genes involved in the acquisition of hemin.

Bibliographic Information

The luxM homologue vanM from Vibrio anguillarum directs the synthesis of N-(3-hydroxyhexanoyl)homoserine lactone and N-hexanoylhomoserine lactone. Milton, Debra L.; Chalker, Victoria J.; Kirke, David; Hardman, Andrea; Camara, Miguel; Williams, Paul. Department of Cell and Molecular Biology, Umea University, Umea, Swed. Journal of Bacteriology (2001), 183(12), 3537-3547. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 135:177810 AN 2001:430358 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Vibrio anguillarum, which causes terminal hemorrhagic septicemia in fish, was previously shown to possess a LuxRI-type quorum-sensing system (vanRI) and to produce N-(3-oxodecanoyl)homoserine lactone (3-oxo-C10-HSL). However, a vanI null mutant still activated N-acylhomoserine lactone (AHL) biosensors, indicating the presence of an addnl. quorum-sensing circuit in V. anguillarum. In this study, we have characterized this second system. Using high-pressure liq. chromatog. in conjunction with mass spectrometry and chem. anal., we identified two addnl. AHLs as N-hexanoylhomoserine lactone (C6-HSL) and N-(3-hydroxyhexanoyl)homoserine lactone (3-hydroxy-C6-HSL). Quantification of each AHL present in stationary-phase V. anguillarum spent culture supernatants indicated that 3-oxo-C10-HSL, 3-hydroxy-C6-HSL, and C6-HSL are present at approx. 8.5, 9.5, and 0.3 nM, resp. Furthermore, vanM, the gene responsible for the synthesis of these AHLs, was characterized and shown to be homologous to the luxL and luxM genes, which are required for the prodn. of N-(3-hydroxybutanoyl)homoserine lactone in Vibrio harveyi. However, resequencing of the V. harveyi luxL/luxM junction revealed a sequencing error present in the published sequence, which when cor. resulted in a single open reading frame (termed luxM). Downstream of vanM, we identified a homolog of luxN (vanN) that encodes a hybrid sensor kinase which forms part of a phosphorelay cascade involved in the regulation of bioluminescence in V. harveyi. A mutation in vanM abolished the prodn. of C6-HSL and 3-hydroxy-C6-HSL. In addn., prodn. of 3-oxo-C10-HSL was abolished in the vanM mutant, suggesting that 3-hydroxy-C6-HSL and C6-HSL regulate the prodn. of 3-oxo-C10-HSL via vanRI. However, a vanN mutant displayed a wild-type AHL profile. Neither mutation affected either the prodn. of proteases or virulence in a fish infection model. These data indicate that V.
anguillarum possesses a hierarchical quorum sensing system consisting of regulatory elements homologous to those found in both V. fischeri (the LuxRI homologues VanRI) and V. harveyi (the LuxMN homologues, VanMN).

Bibliographic Information

Quorum sensing, or how bacteria "talk" to each other. Zavilgelsky, G. B.; Manukhov, I. V. State Research Center GosNIIGenetika, Moscow, Russia. Molecular Biology (Moscow, Russian Federation, English Language)(Translation of Molekulyarnaya Biologiya) (2001), 35(2), 224-232. Publisher: MAIK Nauka/Interperiodica Publishing, CODEN: MOLBBJ ISSN: 0026-8933. Journal; General Review written in English. CAN 135:104747 AN 2001:368053 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review with 88 refs. on recent advances in studying the quorum sensing systems that regulate bacterial gene expression depending on population d. Low-mol.-wt. acyl derivs. of L-homoserine lactone (N-AHL) freely diffuse through cell membranes and det. cell-to-cell communication in bacteria. The quorum sensing systems were first found to regulate bioluminescence in the marine bacteria Photobacterium (Vibrio) fischeri and Vibrio harveyi. Such systems are widespread and control expression of genes for virulence factors, proteases, antibiotics, etc., in various Gram-neg. bacteria, including plant, animal, and human pathogens. Quorum sensing is a prominent example of social behavior in bacteria, as signal exchange among individual cells allows the entire population to choose an optimal way of interaction with the environment and with higher organisms.

Bibliographic Information

Mechanism of quorum sensing: transcriptional activation of the vibrio fischeri luminescence genes by luxr. Egland, Kristi Ann. Univ. of Iowa, Iowa City, IA, USA. Avail. UMI, Order No. DA9975809. (2000), 88 pp. From: Diss. Abstr. Int., B 2000, 61(6), 2896. Dissertation written in English. CAN 135:41722 AN 2001:325211 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Bibliographic Information

gfp-based N-acyl homoserine-lactone sensor systems for detection of bacterial communication. Andersen, Jens Bo; Heydorn, Arne; Hentzer, Morten; Eberl, Leo; Geisenberger, Otto; Christensen, Bjarke Bak; Molin, Soren; Givskov, Michael. Department of Microbiology, The Technical University of Denmark, Lyngby, Den. Applied and Environmental Microbiology (2001), 67(2), 575-585. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 134:249019 AN 2001:110572 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
In order to perform single-cell anal. and online studies of N-acyl homoserine lactone (AHL)-mediated communication among bacteria, components of the Vibrio fischeri quorum sensor encoded by luxR-PluxI have been fused to modified versions of gfpmut3* genes encoding unstable green fluorescent proteins. Bacterial strains harboring this green fluorescent sensor detected a broad spectrum of AHL mols. and were capable of sensing the presence of 5 nM N-3-oxohexanoyl-L-homoserine lactone in the surroundings. In combination with epifluorescent microscopy, the sensitivity of the sensor enabled AHL detection at the single-cell level and allowed for real-time measurements of fluctuations in AHL concns. This green fluorescent AHL sensor provides a state-of-the-art tool for studies of communication between the individuals present in mixed bacterial communities.

Bibliographic Information

Amino acid residues in LuxR critical for its mechanism of transcriptional activation during quorum sensing in Vibrio fischeri. Trott, Amy E.; Stevens, Ann M. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. Journal of Bacteriology (2001), 183(1), 387-392. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 135:87891 AN 2001:11569 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
PCR-based site-directed mutagenesis has been used to generate 38 alanine-substitution mutations in the C-terminal 41 amino acid residues of LuxR. This region plays a crit. role in the mechanism of LuxR-dependent transcriptional activation of the Vibrio fischeri lux operon during quorum sensing. The ability of the variant forms of LuxR to activate transcription of the lux operon was examd. by using in vivo assays in recombinant Escherichia coli. Eight recombinant strains produced luciferase at levels less than 50% of that of a strain expressing wild-type LuxR. Western immunoblotting anal. verified that the altered forms of LuxR were expressed at levels equiv. to those of the wild type. An in vivo DNA binding-repression assay in recombinant E. coli was subsequently used to measure the ability of the variant forms of LuxR to bind to the lux box, the binding site of LuxR at the lux operon promoter. All eight LuxR variants found to affect cellular luciferase levels were unable to bind to the lux box. An addnl. 11 constructs that had no effect on cellular luciferase levels were also found to exhibit a defect in DNA binding. None of the alanine substitutions in LuxR affected activation of transcription of the lux operon without also affecting DNA binding. These results support the conclusion that the C-terminal 41 amino acids of LuxR are important for DNA recognition and binding of the lux box rather than pos. control of the process of transcription initiation.

Bibliographic Information

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Abstract
LuxR is the transcriptional activator for quorum-sensing control of luminescence in Vibrio fischeri. A series of alanine-scanning mutants spanning a predicted helix-turn-helix region in the DNA binding domain of LuxR was constructed, and the activity of each of the LuxR mutant proteins in recombinant Escherichia coli was investigated. The region covered by the mutagenesis spanned residues 190 to 224. About half of the alanine-scanning mutants showed activities similar to that of the wild-type LuxR: at least two were pos.-control mutants, four appeared to be defective in DNA binding, and several others were characterized as DNA binding affinity mutants. This anal., taken together with information about other bacterial transcription factors, provides insights into amino acid residues in LuxR that are involved in DNA binding and transcriptional activation.

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Cloning and functional studies of a luxO regulator LuxT from Vibrio harveyi. Lin, Y. H.; Miyamoto, C.; Meighen, E. A. Department of Biochemistry, Room 813, McIntyre Medical Sciences Building, McGill University, Montreal, QC, Can. Biochimica et Biophysica Acta, Gene Structure and Expression (2000), 1494(3), 226-235. Publisher: Elsevier B.V., CODEN: BBGSD5 ISSN: 0167-4781. Journal written in English. CAN 135:29749 AN 2000:828633 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

LuxO is the central regulator integrating the quorum sensing signals controlling autoinduction of luminescence in Vibrio harveyi. We have previously purified to homogeneity a new lux regulator, LuxT, that binds to the luxO promoter. Based on the sequence of the tryptic peptides of LuxT, degenerate oligonucleotides were designed for PCR of the genomic DNA. A 273 bp PCR DNA fragment contg. sequences encoding the tryptic peptides was extended by inverse PCR to obtain the complete gene (luxT) encoding a protein of 153 amino acids which shares homol. with the AcrR/TetR family of transcriptional regulators. The recombinant and native LuxT gave the same footprint binding between 117 and 149 bp upstream from the luxO initiation codon. Gene disruption of luxT in V. harveyi increased luxO expression and affected the cell d. dependent induction of luminescence showing that LuxT was a repressor of luxO. As LuxT also affected the survival of the V. harveyi cells at high salt concn. and homologous proteins are present in other bacterial species, including the pathogen, Vibrio cholerae, the LuxT regulatory protein appears to be a general rather than a lux-specific regulator.

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Abstract

A review with 45 refs. Recent advances in studies of bacterial gene expression and light microscopy show that cell-to-cell communication and community behavior are the rule rather than the exception. One type of cell-cell communication, quorum sensing in Gram-neg. bacteria, involves acyl-homoserine lactone signals. This type of quorum sensing represents a dedicated communication system that enables a given species to sense when it has reached a crit. population d., and to respond by activating expression of specific genes. The LuxR and LuxI proteins of Vibrio fischeri are the founding members of the acylhomoserine lactone quorum sensing signal receptor and signal generator families of proteins. Acylhomoserine lactone signaling in Pseudomonas aeruginosa is one model for the relationship between quorum sensing, community behavior, and virulence. In the P. aeruginosa model, quorum sensing is required for normal biofilm maturation and virulence. There are multiple quorum-sensing circuits that control the expression of dozens of specific genes in P. aeruginosa.

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The quorum-sensing regulon of Vibrio fischeri: Novel components of the autoinducer/LuxR regulatory circuit. Callahan, Sean M. Massachusetts Institute of Technology, USA. Avail.

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Assay of autoinducer activity with luminescent Escherichia coli sensor strains harboring a modified Vibrio fischeri lux regulon. Devine, Jerry H.; Shadel, Gerald S. Department of Cell Biology and Biochemistry, Texas Tech University Health Science Center, Lubbock, TX, USA. Methods in Enzymology (2000), 305(Bioluminescence and Chemiluminescence, Pt. C), 279-287. Publisher: Academic Press, CODEN: MENZAU ISSN: 0076-6879. Journal written in English. CAN 134:159783 AN 2000:510872 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The anal. of various quorum sensing bacteria has revealed the existence of a relatively diverse family of autoinducers that differ in the type of acyl group attached to the homoserine lactone ring. Also, it is now clear that more than one autoinducer is produced by Vibrio harveyi, Vibrio fischeri, Pseudomonas aeruginosa, and probably others, making simple models for quorum sensing based on one LuxR/Lx-like protein pair largely inadequate. Another complicating factor is that genes that exhibit no homol. to luxl may also participate in the synthesis of compds. with autoinducer activity. Finally, the detection of autoinducers in biofilms suggests that such mols. may also have signaling roles in the external environment. Given the general use of the N-(acyl) homoserine lactones as signaling mols. in nature and the complexity added to the study of quorum sensing by the presence of multiple autoinducers in each system, the development of simple assays for autoinducer activity has become increasingly important. A general strategy for assaying autoinducer activity using recombinant plasmids harboring an engineered V. fischeri lux regulon as a sensor in Escherichia coli, is described. (c) 2000 Academic Press.

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Evidence for a signaling system in Helicobacter pylori: detection of a luxS-encoded autoinducer. Joyce, Elizabeth A.; Bassler, Bonnie L.; Wright, Andrew. Department of Microbiology and Molecular Biology, Tufts University School of Medicine, Boston, MA, USA. Journal of Bacteriology (2000), 182(13), 3638-3643. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 133:190249 AN 2000:446269 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Helicobacter pylori possesses a homolog of the luxS gene, initially identified by its role in autoinducer prodn. for the quorum-sensing system 2 in Vibrio harveyi. The genomes of several other species of bacteria, notably Escherichia coli, Salmonella enterica serovar Typhimurium, and Vibrio cholerae, also include luxS homologs. All of these bacteria have been shown to produce

active autoinducers capable of stimulating the expression of the luciferase operon in V. harveyi. In this report, we demonstrate that H. pylori also synthesizes a functional autoinducer (AI-2) that can specifically activate signaling system 2 in V. harveyi. Maximal activity is produced during early log phase, and the activity is diminished when cells enter stationary phase. We show that AI-2 is not involved in modulating any of the known or putative virulence factors in H. pylori and that a luxS null mutant has a two-dimensional protein profile identical to that of its isogenic parent strain. We discuss the implications of having an AI-2-like quorum-sensing system in H. pylori and suggest possible roles that it may play in H. pylori infection.

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Abstract

The bioluminescent marine bacterium V. harveyi controls light prodn. (lux) by an elaborate quorum-sensing circuit. V. harveyi produces and responds to 2 different autoinducer signals (Al-1 and Al-2) to modulate the luciferase structural operon (luxCDABEGH) in response to changes in cell-population d. Unlike all other Gram-neg. quorum-sensing organisms, V. harveyi regulates quorum sensing using a 2-component phosphorylation-dephosphorylation cascade. Each autoinducer is recognized by a cognate hybrid sensor kinase (called LuxN and LuxQ). Both sensors transduce information to a shared phosphorelay protein called LuxU, which in turn conveys the signal to the response regulator protein LuxO. Phospho-LuxO is responsible for repression of luxCDABEGH expression at low cell d. The present study demonstrates that LuxO functions as an activator protein via interaction with the alternative sigma factor, □54 (encoded by rpoN). These results suggest that LuxO, together with □54, activates the expression of a neg. regulator of luminescence. It is also shown that phenotypes other than lux are regulated by LuxO and □54, demonstrating that in V. harveyi, quorum sensing controls multiple processes.

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Abstract

Bioluminescence in the marine bacterium Vibrio fischeri is controlled by the excretion of a N-acyl homoserine lactone (HSL) autoinducer which interacts with a regulator, LuxR, and activates transcription of the lux operon at high-cell d. This system has become the prototype for quorum sensing in many bacteria. Although light emission in Vibrio harveyi is also regulated by a N-acyl-HSL inducer, in sharp contrast, a completely different and more complex system is involved in quorum sensing which is mediated via LuxO, the response regulator of a phosphorelay signal transduction system. In the present work, luxO and the overlapping luxU gene, also involved in the phosphorelay system in V. harveyi, have been discovered in V. fischeri. By gene replacement

technol., a V. fischeri luxO- mutant was generated whose phenotype was similar to that of V. harveyi luxO- showing that LuxO is involved in control of luminescence in V. fischeri. This mutant could be complemented with luxO from either V. fischeri or V. harveyi resulting in the restoration of the dependence of luminescence intensity on cell d. In contrast to V. harveyi luxO-, light emission of V. fischeri luxO- was stimulated by the N-acyl-HSL autoinducer indicating that luxO is part of a second signal transduction system controlling luminescence in this species. The presence of a luxO-based phosphorelay regulatory system as well as the luxR-based system in V. fischeri suggests that the former system, originally discovered in V. harveyi, may be a general regulatory mechanism in luminescent bacteria.

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Inhibition of luminescence and virulence in the black tiger prawn (Penaeus monodon) pathogen Vibrio harveyi by intercellular signal antagonists. Manefield, Michael; Harris, Lachlan; Rice, Scott A.; De Nys, Rocky; Kjelleberg, Staffan. School of Microbiology and Immunology, University of New South Wales, Sydney, Australia. Applied and Environmental Microbiology (2000), 66(5), 2079-2084. Publisher: American Society for Microbiology, CODEN: AEMIDF ISSN: 0099-2240. Journal written in English. CAN 133:71343 AN 2000:309688 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Expression of luminescence in the Penaeus monodon pathogen Vibrio harveyi is regulated by an intercellular quorum sensing mechanism involving the synthesis and detection of two signaling mols., one of which is N-hydroxy butanoyl-L-homoserine lactone and the other of which is uncharacterized. Indirect evidence has suggested that virulence, assocd. with a toxic extracellular protein, and luminescence in V. harveyi are coregulated. In this study, the effects of an acylated homoserine lactone antagonist produced by the marine alga Delisea pulchra on luminescence and toxin prodn. in a virulent strain of V. harveyi were analyzed. Luminescence and toxin prodn. were both inhibited by the signal antagonist at concns. that had no impact on growth. Toxin prodn. was found to be prematurely induced in V. harveyi cultures incubated in a 10% conditioned medium. Addnl., a significant redn. in the toxicity of concd. supernatant exts. from V. harveyi cultures incubated in the presence of the signal antagonist, as measured by in vivo toxicity assays in mice and prawns, was obsd. These results suggest that intercellular signaling antagonists have potential utility in the control of V. harveyi prawn infections.

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LuxR- and acyl-homoserine-lactone-controlled non-lux genes define a quorum-sensing regulon in Vibrio fischeri. Callahan, Sean M.; Dunlap, Paul V. Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA. Journal of Bacteriology (2000), 182(10), 2811-2822. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 134:26036 AN 2000:296922 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The luminescence (lux) operon (luxICDABEG) of the symbiotic bacterium Vibrio fischeri is regulated by the transcriptional activator LuxR and two acyl-homoserine lactone (acyl-HSL) autoinducers (the luxI-dependent 3-oxo-hexanoyl-HSL [3-oxo-C6-HSL] and the aniS-dependent octanoyl-HSL [C8-HSL]) in a population d.-responsive manner called quorum sensing. To identify

quorum-sensing-regulated (QSR) proteins different from those encoded by lux genes, we examd. the protein patterns of V. fischeri quorum-sensing mutants defective in luxI, ainS, and luxR by two-dimensional polyacrylamide gel electrophoresis. Five non-lux QSR proteins, QsrP, RibB, AcfA, QsrV, and QSR 7, were identified; their prodn. occurred preferentially at high population d., required both LuxR and 3-oxo-C6-HSL, and was inhibited by C8-HSL at low population d. The genes encoding two of the QSR proteins were characterized: qsrP directs cells to synthesize an apparently novel periplasmic protein, and ribB is a homolog of the Escherichia coli gene for 3,4-dihydroxy-2-butanone 4-phosphate synthase, a key enzyme for riboflavin synthesis. The qsrP and ribB promoter regions each contained a sequence similar to the lux operon lux box, a 20-bp region of dyad symmetry necessary for LuxR/3-oxo-C6-HSL-dependent activation of lux operon transcription. V. fischeri qsrP and ribB mutants exhibited no distinct phenotype in culture. However, a qsrP mutant, in competition with its parent strain, was less successful in colonizing Euprymna scolopes, the symbiotic host of V. fischeri. The newly identified QSR genes, together with the lux operon, define a LuxR/acyl-HSL-responsive quorum-sensing regulon in V. fischeri.

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Abstract

The bioluminescent marine bacterium Vibrio harveyi controls light prodn. using two parallel quorum-sensing systems. V. harveyi produces two autoinducers (Al-1 and Al-2), which are recognized by cognate membrane-bound two-component hybrid sensor kinases called LuxN and LuxQ resp. Under conditions of low cell d., in the absence of autoinducer, the hybrid sensors are kinases, and under conditions of high cell d., in the presence of autoinducer, the sensors are phosphatases. These activities allow LuxN and LuxQ to modulate the level of phosphorylation of the response regulator protein LuxO. LuxO, in turn, controls the transcription of the genes encoding luciferase. The phosphorelay protein LuxU is required for signalling to LuxO. In this report, we present a genetic anal. of the activities of the Al-1 sensor LuxN. Point mutations and in frame deletions were constructed in luxN and recombined onto the chromosome of V. harveyi for in vivo phenotypic anal. We show that the conserved histidine (H471) in the sensor kinase domain of LuxN is required for kinase activity but not for phosphatase activity. In contrast, the conserved aspartate (D771) in the response regulator domain of LuxN is required for both activities. Furthermore, the LuxN phosphatase activity is localized to the response regulator domain. Our results indicate that the LuxN kinase activity is regulated by the presence of Al-1, whereas the LuxN phosphatase activity is constitutive. We also show that signalling from the two V. harveyi

quorum-sensing systems is not equiv. Al-1 and LuxN have a much greater effect on the level of LuxO phosphate and therefore Lux expression than do Al-2 and LuxQ.

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Conversion of the Vibrio fischeri transcriptional activator, LuxR, to a repressor. Egland, Kristi A.; Greenberg, E. P. Department of Microbiology and Graduate Program in Molecular Biology, University of Iowa, Iowa City, IA, USA. Journal of Bacteriology (2000), 182(3), 805-811. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 132:232662 AN 2000:51507 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

The Vibrio fischeri luminescence (lux) operon is regulated by a quorum-sensing system that involves the transcriptional activator (LuxR) and an acyl-homoserine lactone signal. Transcriptional activation requires the presence of a 20-base inverted repeat termed the lux box at a position centered 42.5 bases upstream of the transcriptional start of the lux operon. LuxR has proven difficult to study in vitro. A truncated form of LuxR has been purified, and together with □70 RNA polymerase it can activate transcription of the lux operon. Both the truncated LuxR and RNA polymerase are required for binding to lux regulatory DNA in vitro. We have constructed an artificial lacZ promoter with the lux box positioned between and partially overlapping the consensus -35 and -10 hexamers of an RNA polymerase binding site. LuxR functioned as an acyl-homoserine lactone-dependent repressor at this promoter in recombinant Escherichia coli. Furthermore, multiple lux boxes on an independent replicon reduced the repressor activity of LuxR. Thus, it appears that LuxR can bind to lux boxes independently of RNA polymerase binding to the promoter region. A variety of LuxR mutant proteins were studied, and with one exception there was a correlation between function as a repressor of the artificial promoter and activation of a native lux operon. The exception was the truncated protein that had been purified and studied in vitro. This protein functioned as an activator but not as a repressor in E. coli. The data indicate that the mutual dependence of purified, truncated LuxR and RNA polymerase on each other for binding to the lux promoter is a feature specific to the truncated LuxR and that full-length LuxR by itself can bind to lux box-contg. DNA.

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Abstract

Enterohemorrhagic Escherichia coli O157:H7 and enteropathogenic E. coli cause a characteristic histopathol. in intestinal cells known as attaching and effacing. The attaching and effacing lesion is encoded by the Locus of Enterocyte Effacement (LEE) pathogenicity island, which encodes a type III secretion system, the intimin intestinal colonization factor, and the translocated intimin receptor

protein that is translocated from the bacterium to the host epithelial cells. Using lacZ reporter gene fusions, the authors show that expression of the LEE operons encoding the type III secretion system, translocated intimin receptor, and intimin is regulated by quorum sensing in both enterohemorrhagic E. coli and enteropathogenic E. coli. The luxS gene recently shown to be responsible for prodn. of autoinducer in the Vibrio harveyi and E. coli quorum-sensing systems is responsible for regulation of the LEE operons, as shown by the mutation and complementation of the luxS gene. Regulation of intestinal colonization factors by quorum sensing could play an important role in the pathogenesis of disease caused by these organisms. These results suggest that intestinal colonization by E. coli O157:H7, which has an unusually low infectious dose, could be induced by quorum sensing of signals produced by nonpathogenic E. coli of the normal intestinal flora.

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Abstract
A review with 37 refs.

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Quorum regulation of luminescence in Vibrio fischeri. Dunlap, Paul V. Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA. Journal of Molecular Microbiology and Biotechnology (1999), 1(1), 5-12. Publisher: Horizon Scientific Press, CODEN: JMMBFF ISSN: 1464-1801. Journal; General Review written in English. CAN 131:295946 AN 1999:569890 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
A review with 102 refs. Luminescence in Vibrio fischeri is controlled by a population d.-responsive regulatory mechanism called quorum sensing. Elements of the mechanism include: LuxI, an acyl-homoserine lactone (acyl-HSL) synthase that directs synthesis of the diffusible signal mol., 3-oxo-hexanoyl-HSL (V. fischeri auto-inducer-1, VAI-1); LuxR, a transcriptional activator protein necessary for response to VAI-1; GroEL, which is necessary for prodn. of active LuxR; and AinS, an acyl-HSL synthase that catalyzes the synthesis of octanoyl-HSL (VAI-2). The population d.-dependent accumulation of VAI-1 triggers induction of lux operon (luxICDABEG; genes for luminescence enzymes and for LuxI) transcription and luminescence by binding to LuxR, forming a complex that facilitates the assocn. of RNA polymerase with the lux operon promoter. VAI-2, which apparently interferes with VAI-1 binding to LuxR, operates to limit premature lux operon induction. Hierarchical control is imposed on the system by 3':5'-cAMP and cAMP receptor protein (CRP), which are necessary for activated expression of luxR. Several non-lux genes in V. fischeri are controlled by LuxR and VAI-1. Quorum regulation in V. fischeri serves as a model for LuxI/LuxR-type quorum sensing systems in other Gram-neg. bacteria.

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Abstract
A review with 102 refs. Luminescence in Vibrio fischeri is controlled by a population d.-responsive regulatory mechanism called quorum sensing. Elements of the mechanism include: LuxI, an acyl-homoserine lactone (acyl-HSL) synthase that directs synthesis of the diffusible signal mol., 3-oxo-hexanoyl-HSL (V. fischeri auto-inducer-1, VAI-1); LuxR, a transcriptional activator protein necessary for response to VAI-1; GroEL, which is necessary for prodn. of active LuxR; and AinS, an acyl-HSL synthase that catalyzes the synthesis of octanoyl-HSL (VAI-2). The population d.-dependent accumulation of VAI-1 triggers induction of lux operon (luxICDABEG; genes for luminescence enzymes and for LuxI) transcription and luminescence by binding to LuxR, forming a complex that facilitates the assocn. of RNA polymerase with the lux operon promoter. VAI-2, which apparently interferes with VAI-1 binding to LuxR, operates to limit premature lux operon induction. Hierarchical control is imposed on the system by 3':5'-cAMP and cAMP receptor protein (CRP), which are necessary for activated expression of luxR. Several non-lux genes in V. fischeri are controlled by LuxR and VAI-1. Quorum regulation in V. fischeri serves as a model for LuxI/LuxR-type quorum sensing systems in other Gram-neg. bacteria.

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Abstract
Although cell d.-dependent regulation of the luminescence genes in Vibrio fischeri (Moritella marina) is a model for quorum sensing in Gram-neg. bacteria, relatively little is known about the promoter of the luminescence operon. The luminescence operon is activated by the LuxR protein, which requires a diffusible acyl homoserine lactone signal. The lux box, a 20 bp inverted repeat, is located in the luxI promoter region and is required for LuxR-dependent induction of the luminescence genes. Using primer extension, we mapped the LuxR-dependent transcriptional start site of the lux operon to 19 bp upstream of the luxI start codon. This indicates that the lux box is centered at -42.5 bp from the start of transcription. To gain evidence about the location of the -10

sequence, we placed a consensus -35 hexamer at different locations relative to the luxI transcriptional start site and measured constitutive levels of luminescence in recombinant Escherichia coli. The strongest constitutive promoter contained a TATAGT hexamer 17 bp from the -35 consensus sequence and 6 bp from the transcriptional start site. We propose that this is the -10 hexamer. Also in recombinant E. coli, both half-sites of the lux box were required for LuxR-dependent gene activation and for activation by an autoinducer-independent, monomeric LuxR deletion protein. LuxR-dependent activation of luminescence was eliminated when the lux box was centered at -47.5, -52.5 and -62.5 with respect to the luxI transcriptional start site. Our evidence, taken together with other information, points to a model in which a LuxR dimer overlaps the -35 region of the luxI promoter and functions as an ambidextrous activator with each LuxR subunit interacting with a different region of RNA polymerase.

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Quorum sensing in Escherichia coli, Salmonella typhimurium, and Vibrio harveyi: a new family of genes responsible for autoinducer production. Surette, Michael G.; Miller, Melissa B.; Bassler, Bonnie L. Department of microbiology and Infectious Diseases, University of Calgary, Calgary, AB, Can. Proceedings of the National Academy of Sciences of the United States of America (1999), 96(4), 1639-1644. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 130:333524 AN 1999:150922 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

In bacteria, the regulation of gene expression in response to changes in cell d. is called quorum sensing. Quorum-sensing bacteria produce, release, and respond to hormone-like mols. (autoinducers) that accumulate in the external environment as the cell population grows. In the marine bacterium Vibrio harveyi two parallel quorum-sensing systems exist, and each is composed of a sensor-autoinducer pair. V. harveyi reporter strains capable of detecting only autoinducer 1 (AI-1) or autoinducer 2 (AI-2) have been constructed and used to show that many species of bacteria, including Escherichia coli MG1655, E. coli O157:H7, Salmonella typhimurium 14028, and S. typhimurium LT2 produce autoinducers similar or identical to the V. harveyi system 2 autoinducer AI-2. However, the domesticated lab. strain E. coli DH5□ does not produce this signal mol. Here we report the identification and anal. of the gene responsible for AI-2 prodn. in V. harveyi, S. typhimurium, and E. coli. The genes, which we have named luxSV.h., luxSS.t., and luxSE.c. resp., are highly homologous to one another but not to any other identified gene. E. coli DH5□ can be complemented to AI-2 prodn. by the introduction of the luxS gene from V. harveyi or E. coli O157:H7. Anal. of the E. coli DH5□ luxSE.c. gene shows that it contains a frameshift mutation resulting in premature truncation of the LuxSE.c. protein. Our results indicate that the luxS genes define a new family of autoinducer-prodn. genes.

Bibliographic Information

A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in Vibrio harveyi. Freeman, Jeremy A.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Molecular Microbiology (1999), 31(2), 665-677. Publisher: Blackwell Science Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 130:279098 AN 1999:118307 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Two independent quorum-sensing systems control the expression of bioluminescence (lux) in the marine bacterium Vibrio harveyi. Each system is composed of an autoinducer (Al-1 or Al-2) and its cognate sensor (LuxN or LuxQ). The sensors are two-component hybrid kinases, contg. both sensor kinase domains and response regulator domains. Sensory information from the two systems is relayed by a phosphotransfer mechanism to a shared integrator protein called LuxO. LuxO is a member of the response regulator class of the two-component family of signal transduction proteins, and LuxO acts neg. to control luminescence. In this report, missense and in frame deletion mutations were constructed in luxO that encoded proteins mimicking either the phosphorylated or the unphosphorylated form, and these mutations were introduced into the V. harveyi chromosome at the luxO locus. Phenotypical analyses of the resulting mutant V. harveyi strains indicate that the phosphorylated form of LuxO is the repressor, and that the unphosphorylated form of the protein is inactive. Anal. of the lux phenotypes of V. harveyi strains contg. single and double luxN and luxQ mutations indicate that LuxN and LuxQ have two activities on LuxO. They act as LuxO protein kinases at low cell d. in the absence of autoinducers, and they switch to LuxO protein phosphatases at high cell d. in the presence of autoinducers. Furthermore, the timing and potency of inputs from the two systems into regulation of quorum sensing are different.

Bibliographic Information

Regulation of autoinducer production in Salmonella typhimurium. Surette, Michael G.; Bassler, Bonnie L. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, AB, Can. Molecular Microbiology (1999), 31(2), 585-595. Publisher: Blackwell Science Ltd., CODEN: MOMIEE ISSN: 0950-382X. Journal written in English. CAN 130:264614 AN 1999:118300 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Salmonella typhimurium strain LT2 secretes an org. signalling mol. that can be assayed by its ability to activate one of two specific quorum-sensing systems in Vibrio harveyi. Maximal activity is produced during mid- to late exponential phase when S. typhimurium is grown in the presence of glucose or other preferred carbohydrates. The signal is degraded by the onset of stationary phase or when the carbohydrate is depleted from the medium. Presumably, quorum sensing in S. typhimurium is operational during periods of rapid, nutrient-rich growth. Protein synthesis is required for degrdn. of the activity, suggesting that a complex regulatory circuitry controls signal prodn. and detection in S. typhimurium. Increased signalling activity is obsd. if, after growth in the presence of glucose, S. typhimurium is transferred to a high-osmolarity (0.4 M NaCl) or to a low-pH (pH 5.0) environment. Degrdn. of the signal is induced by conditions of low osmolarity (0.1 M NaCl). High osmolarity and low pH are two conditions encountered by S. typhimurium cells when they undergo the transition to a pathogenic existence inside a host organism, suggesting that quorum sensing may have a role in the regulation of virulence in S. typhimurium.

Bibliographic Information

Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in Vibrio harveyi. Freeman, Jeremy A.; Bassler, Bonnie L. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Journal of Bacteriology (1999), 181(3), 899-906. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 130:264535 AN 1999:105098 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
V. harveyi regulates the expression of bioluminescence (lux) in response to cell d., a phenomenon known as quorum sensing. In V. harveyi, 2 independent quorum-sensing systems exist, and each produces, detects, and responds to a specific cell d.-dependent autoinducer signal. The autoinducers are recognized by 2-component hybrid sensor kinases called LuxN and LuxQ, and sensory information from both systems is transduced by a phosphorelay mechanism to the response regulator protein LuxO. Genetic evidence suggests that LuxO-phosphate neg. regulates the expression of luminescence at low cell d. in the absence of autoinducers. At high cell d., interaction of the sensors with their cognate autoinducers results in dephosphorylation and inactivation of the LuxO repressor. The present report shows that LuxN and LuxQ channel sensory information to LuxO via a newly identified phosphorelay protein, named LuxU. LuxU shows sequence similarity to other described phosphorelay proteins, including BvgS, ArcB, and Ypd1. A crit. His residue (His 58) of LuxU is required for phosphorelay function.

Bibliographic Information

A negative regulator mediates quorum-sensing control of exopolysaccharide production in Pantoea stewartii subsp. stewartii. Von Bodman, Susanne Beck; Majerczak, Doris R.; Coplin, David L. Department of Biology, University of Puerto Rico, San Juan, USA. Proceedings of the National Academy of Sciences of the United States of America (1998), 95(13), 7687-7692. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 129:146714 AN 1998:414310 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Classical quorum-sensing (autoinduction) regulation, as exemplified by the lux system of Vibrio fischeri, requires N-acyl homoserine lactone (AHL) signals to stimulate cognate transcriptional activators for the cell d.-dependent expression of specific target gene systems. For Pantoea stewartii subsp. stewartii, a bacterial pathogen of sweet corn and maize, the extracellular polysaccharide (EPS) stewartan is a major virulence factor, and its prodn. is controlled by quorum sensing in a population d.-dependent manner. Two genes, esaI and esaR, encode essential regulatory proteins for quorum sensing. EsaI is the AHL signal synthase, and EsaR is the cognate gene regulator. EsaI, □esaR, and □esaI-esaR mutations were constructed to establish the regulatory role of EsaR. Strains contg. an esaR mutation produce high levels of EPS independently of cell d. and in the absence of the AHL signal. Data indicate that quorum-sensing regulation in P. s. subsp. stewartii, in contrast to most other described systems, uses EsaR to repress EPS synthesis at low cell d., and that derepression requires micromolar amts. of AHL. In addn., derepressed esaR strains, which synthesize EPS constitutively at low cell densities, were significantly less virulent than the wild-type parent. This finding suggests that quorum sensing in P. s. subsp. stewartii may be a mechanism to delay the expression of EPS during the early stages of infection so that it does not interfere with other mechanisms of pathogenesis.

Bibliographic Information

Cross-species induction of luminescence in the Quorum-sensing bacterium Vibrio harveyi. Bassler, Bonnie L.; Greenberg, E. Peter; Stevens, Ann M. Department of Molecular Biology, Princeton University, Princeton, NJ, USA. Journal of Bacteriology (1997), 179(12), 4043-4045. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193.

Journal written in English. AN 1997:406377 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Different species of bacteria were tested for prodn. of extracellular autoinducer-like activities that could stimulate the expression of the luminescence genes in Vibrio harveyi. Several species of bacteria, including the pathogens Vibrio cholerae and Vibrio parahaemolyticus, were found to produce such activities. Possible physiol. roles for the two V. harveyi detection-response systems and their joint regulation are discussed.

Bibliographic Information

Quorum sensing in Vibrio anguillarum: characterization of the vanI/vanR locus and identification of the autoinducer N-(3-oxodecanoyl)-L-homoserine lactone. Milton, Debra L.; Hardman, Andrea; Camara, Miguel; Chhabra, Siri Ram; Bycroft, Barrie W.; Stewart, Gordon S. A. B.; Williams, Paul. Dep. Cell and Mol.Biology, Umea Univ., Umea, Swed. Journal of Bacteriology (1997), 179(9), 3004-3012. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 127:31300 AN 1997:304582 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Certain gram-neg. pathogens are known to control virulence gene expression through cell-cell communication via small mols. termed autoinducers. This intercellular signal transduction mechanism termed quorum sensing depends on the interaction of an N-acylhomoserine lactone (AHL) auto-inducer mol. with a receptor protein belonging to the LuxR family of pos. transcriptional activators. Vibrio anguillarum is a gram-neg. pathogen capable of causing a terminal hemorrhagic septicemia known as vibriosis in fish such as rainbow trout. In this study, we sought to det. whether V. anguillarum employs AHLs to regulate virulence gene expression. Spent V. anguillarum culture supernatants stimulated bioluminescence in recombinant lux-based Escherichia coli AHO biosensor strain, whereas they both stimulated and inhibited AHL-mediated violacein pigment prodn. in Chromobacterium violaceum. This finding suggested that V. anguillarum may produce multiple AHL signal mols. Using high-performance liq. chromatog. and high-resoln. tandem mass spectrometry, we identified the major V. anguillarum AHL as N-(3-oxodecanoyl)-L-homoserine lactone (ODHL), a structure which was unequivocally confirmed by chem. synthesis. The gene (vanI) responsible for ODHL synthesis was cloned and sequenced and shown to belong to the LuxI family of putative AHL synthases. Further sequencing downstream of vanI revealed a second gene (vanR) related to the LuxR family of transcriptional activators. Although deletion of vanI abolished ODHL synthesis, no redn. of either metalloprotease prodn. or virulence in a fish infection model was obsd. However, the vanI mutant remained capable of weakly activating both bioluminescence and violacein in the E. coli and C. violaceum biosensors, resp., indicating the existence of addnl. layers of AHL-mediated regulatory complexity.

Bibliographic Information

Quorum sensing in Vibrio fischeri: essential elements for activation of the luminescence genes. Stevens, Ann M.; Greenberg, E. P. Dep. Microbiol., Univ. Iowa, Iowa City, IA, USA. Journal of Bacteriology (1997), 179(2), 557-562. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 126:100209 AN 1997:53257 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
LuxR is required for cell d.-dependent activation of the V. fischeri luminescence (lux) genes. It has not been possible to study full-length LuxR in vitro, but a polypeptide contg. the C-terminal transcriptional-activator domain of LuxR (LuxR□N) has been purified, and its binding to lux regulatory DNA has been investigated. By itself, LuxR□N interacts with a region of lux regulatory DNA that is upstream of the lux box, which is a 20-bp element that is required for LuxR activation of the luminescence operon. Individually, neither the purified LuxR□N nor RNA polymerase binds to the lux box region, but together the 2 proteins bind in synergy to the lux box-luxI promoter region. It is shown here that binding of LuxR□N to the upstream region is not a prerequisite for its synergistic binding with RNA polymerase to the lux box and the luxI promoter region. Also, LuxR□N and RNA polymerase are both required and sufficient for transcriptional activation of the lux operon. This argues against the hypothesis that LuxR functions to alleviate repression of the lux operon by another cellular factor. These data support the view that LuxR functions as an accessory factor that enables RNA polymerase to bind to and initiate transcription from the promoter of the lux operon.

Bibliographic Information

Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified Vibrio fischeri LuxI protein. Schaefer, Amy L.; Val, Dale L.; Hanzelka, Brian L.; Cronan, John E., Jr.; Greenberg, E. P. Dep. Microbiol., Univ. Iowa, Iowa City, IA, USA. Proceedings of the National Academy of Sciences of the United States of America (1996), 93(18), 9505-9509. Publisher: National Academy of Sciences, CODEN: PNASA6 ISSN: 0027-8424. Journal written in English. CAN 125:215351 AN 1996:553145 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Many bacteria use acyl homoserine lactone signals to monitor cell d. in a type of gene regulation termed quorum sensing and response. Synthesis of these signals is directed by homologs of the luxI gene of Vibrio fischeri. This communication resolves two crit. issues concerning the synthesis of the V. fischeri signal. The luxI product is directly involved in signal synthesis-the protein is an acyl homoserine lactone synthase; and the substrates for acyl homoserine lactone synthesis are not amino acids from biosynthetic pathways or fatty acid degrdn. products, but rather they are S-adenosylmethionine (SAM) and an acylated acyl carrier protein (ACP) from the fatty acid biosynthesis pathway. The authors purified a maltose binding protein-LuxI fusion polypeptide and showed that, when provided with the appropriate substrates, it catalyzes the synthesis of an acyl homoserine lactone. In V. fischeri, luxI directs the synthesis of N-(3-oxohexanoyl)homoserine lactone and hexanoyl homoserine lactone. The purified maltose binding protein-LuxI fusion protein catalyzes the synthesis of hexanoyl homoserine lactone from hexanoyl-ACP and SAM. There is a high level of specificity for hexanoyl-ACP over ACPs with differing acyl group lengths, and hexanoyl homoserine lactone was not synthesized when SAM was replaced with other amino acids, such as methionine, S-adenosylhomocysteine, homoserine, or homoserine lactone, or when hexanoyl-SAM was provided as the substrate. This provides direct evidence that the LuxI protein is an autoinducer synthase that catalyzes the formation of an amide bond between SAM and a fatty acyl-ACP and then catalyzes the formation of the acyl homoserine lactone from the acyl-SAM intermediate.

Bibliographic Information

Quorum sensing in Vibrio fischeri: evidence that S-adenosylmethionine is the amino acid substrate for autoinducer synthesis. Hanzelka, Brian L.; Greenberg, E. P. Department Microbiology, University Iowa, Iowa City, IA, USA. Journal of Bacteriology (1996), 178(17), 5291-5294. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 125:190288 AN 1996:532851 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Synthesis of the autoinducer signal involved in the cell d.-dependent activation of Vibrio fischeri luminescence is directed by luxI. The autoinducer is N-(3-oxohexanoyl)homoserine lactone, and little is known about its synthesis. The authors have measured autoinducer synthesis by amino acid auxotrophs of Escherichia coli that contained luxI on a high-copy-no. plasmid. Expts. with cell suspensions starved for methionine or homoserine show that either methionine or S-adenozylmethionine, but not homoserine or homoserine lactone, is required for autoinducer synthesis. The S-adenosylmethionine synthesis inhibitor cycloleucine blocks methionine-dependent autoinducer synthesis. Thus, it appears that S-adenosylmethionine rather than methionine is the mol. required for autoinducer synthesis. The amt. of 15N-labeled methionine incorporated into the autoinducer by growing cultures of a homoserine and a methionine auxotroph was measured by mass spectrometry. The labeling studies show that even in the presence of homoserine, almost all of the autoinducer produced contains the 15N label from methionine. Thus, it appears that S-adenosylmethionine serves as the amino acid substrate in the luxI-dependent synthesis of the V. fischeri autoinducer.

Bibliographic Information

Quorum sensing in Vibrio fischeri: probing autoinducer-LuxR interactions with autoinducer analogs. Schaefer, Amy L.; Hanzelka, Brian L.; Eberhard, Anatol; Greenberg, E. P. Dep. Microbiology, Univ. Iowa, Iowa City, IA, USA. Journal of Bacteriology (1996), 178(10), 2897-2901. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 125:5356 AN 1996:295790 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
The Vibrio fischeri luminescence genes are activated by the transcription factor LuxR in combination with a diffusible signal compd., N-(3-oxohexanoyl)homoserine lactone, termed the autoinducer. We have synthesized a set of autoinducer analogs. Many analogs with alterations in the acyl side chain showed evidence of binding to LuxR. Some appeared to bind with an affinity similar to that of the autoinducer, but none showed a higher affinity, and many did not bind as tightly as the autoinducer. For the most part, compds. with substitutions in the homoserine lactone ring did not show evidence of binding to LuxR. The exceptions were compds. with a homocysteine thiolactone ring in place of the homoserine lactone ring. Many but not all of the analogs showing evidence of LuxR binding had some ability to activate the luminescence genes. None were as active as the autoinducer. While most showed little ability to induce luminescence, a few analogs with rather conservative substitutions had appreciable activity. Under the conditions employed, some of the analogs showing little or no ability to induce luminescence were inhibitors of the autoinducer.

Bibliographic Information

Bacteriocin small of Rhizobium leguminosarum belongs to the class of N-acyl-L-homoserine lactone molecules, known as autoinducers and as quorum sensing co-transcription factors. Schripsema, Jan; de Rudder, Karel E. E.; van Vliet, Theo B.; Lankhorst, Peter P.; de Vroom, Erik; Kijne, Jan W.; van Brussel, Anton A. N. Div. Pharmacognosy, Gorlaeus Lab., Delft, Neth. Journal of Bacteriology (1996), 178(2), 366-71. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 124:81661 AN 1996:36134 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Small bacteriocin was isolated from the culture broth of the Gram-neg. bacterium Rhizobium leguminosarum, which forms symbiotic nitrogen-fixing root nodules on a no. of leguminous plants. The structure of the mol. was elucidated by spectroscopic methods and identified as N-(3R-hydroxy-7-cis-tetradecanoyl)-L-homoserine lactone. The abs. configuration of both asym. carbon atoms in the mol. was detd. by the use of the chiral solvating agents S-(+)- and R-(-)-2,2,2-trifluoro-1-(9-anthryl)-ethanol. Small bacteriocin is structurally related to the quorum sensing co-transcription factors for genes from other bacteria, such as Vibrio fischeri, Pseudomonas aeruginosa, Erwinia carotovora, and Agrobacterium tumefaciens, which are involved in animal-microbe or plant-microbe interactions. The mechanism of regulation of such interactions by this kind of co-transcription factors is still unknown in R. leguminosarum.

Bibliographic Information

Synthesis of multiple exoproducts in Pseudomonas aeruginosa is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-response LuxR-LuxI family. Brint, J. Mark; Ohman, Dennis E. Dep. Med., Univ. Tennessee and Vet. Affairs Med. Cent., Memphis, TN, USA. Journal of Bacteriology (1995), 177(24), 7155-63. Publisher: American Society for Microbiology, CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 124:137480 AN 1995:987873 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract
Mutants of Pseudomonas aeruginosa PAO1 that were deficient in the ability to produce proteases that degrade casein were detected among the survivors of chem. mutagenesis. One such mutant (PDO31) showed reduced prodn. of elastolytic activity, □-hemolytic activity, and pyocyanin. A 4.3-kb EcoRI fragment from a gene bank of PAO1 that complemented defects in PDO31 was found. Transposon mutagenesis and deletion derivs. of the clone were used in conjunction with complementation tests to det. the phys. location of the gene of interest. Nucleotide sequence anal. revealed an open reading frame (rhlR) encoding a putative 27.6-kDa protein (RhlR) with homol. to autoinducer-responsive regulators of quorum sensing systems such as LuxR of Vibrio fischeri and LasR of P. aeruginosa. Further sequence anal. downstream of rhlR revealed an independently transcribed gene (rhlI) that encodes a putative 22.2-kDa protein with homol. to members of the family of autoinducer synthetases, such as LuxI of V. fischeri and LasI of P. aeruginosa. The rhlRI sequences were also recently reported by others (U. A. Ochsner and J. Reiser, Proc. Natl. Acad. Sci. USA 92:6424-6428, 1995) as an autoinducer-mediated regulation mechanism for rhamnolipid biosurfactant synthesis in P. aeruginosa PG201. Mutants with defects in rhlR or rhlI were constructed in PAO1 by gene replacement, using clones modified by Tn501 insertion. Compared with the wild type, the rhlR and rhlI mutants both showed defects in the prodn. of elastase, LasA protease, rhamnolipid, and pyocyanin. Transcription from the gene for elastase, as measured with a

lasB-cat fusion, demonstrated that prodn. of elastase was subject to cell d.-dependent gene activation in PAO1. However, transcription of lasB-cat in the rhlI mutant, which had lost the presumptive autoinducer synthetase (predicted to activate RhlR), showed low basal activity and had lost all cell d.-dependent transcription of lasB. Thus, RhlR-RhlI represent the second autoinducer-responsive regulatory mechanism found in p.
aeruginosa that controls expression of multiple virulence factor exoproducts, including elastase.

Bibliographic Information

Interchangeability and specificity of component from the quorum-sensing regulatory systems of Vibrio fischeri and Pseudomonas aeruginosa. Gray, Kendall M.; Paasador, Luciano; Iglewski, Barbara H.; Greenberg, E. P. Dep. Microbiol., Univ. Iowa, Iowa City, IA, USA. Journal of Bacteriology (1994), 176(10), 3076-80. CODEN: JOBAAY ISSN: 0021-9193. Journal written in English. CAN 121:75099 AN 1994:475099 CAPLUS (Copyright (C) 2009 ACS on SciFinder (R))

Abstract

Autoinduction is a conserved mechanism of cell d.-dependent gene regulation that occurs in a variety of gram-neg. bacteria. Autoinducible luminescence in Vibrio fischeri requires a transcriptional activator, LuxR, while a LuxR homolog, LasR, activates elastase expression in Pseudomonas aeruginosa. Both LuxR and LasR require specific signal mols., called autoinducers, for activity. The authors show here the activation in Escherichia coli of the V. fischeri luminescence (lux) operon by LasR and of the P. aeruginosa elastase gene (lasB) by LuxR when each is in the presence of its cognate autoinducer. Neither LuxR nor LasR showed appreciable activity with the heterologous V. fischeri or P. aeruginosa autoinducer. This supports the view that there is a direct interaction of each transcriptional activator with its proper autoinducer and suggests that there are conserved, autoinduction-related elements within the promoter regions of these genes.

Bibliographic Information

Coordinated regulation of virulence by quorum sensing and motility pathways during the initial stages of Vibrio cholerae infection. Tsou Amy M; Frey Erin M; Hsiao Ansel; Liu Zhi; Zhu Jun Department of Microbiology; University of Pennsylvania School of Medicine; Philadelphia, Pennsylvania USA Communicative & integrative biology (2008), 1(1), 42-4. Journal code: 101478473. E-ISSN:1942-0889. Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 19704787 AN 2009579267 In-process for MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

Pathogenic bacteria, such as Vibrio cholerae, must be capable of adapting to diverse living conditions, especially when transitioning from life in environmental reservoirs to life in a host. The abilities to sense arrival at a site suitable for colonization or infection and to respond with appropriate alterations in gene expression are crucial for a pathogen's success. Recently, we have shown that V. cholerae is able to recognize that it has reached its colonization site in the small intestine by sensing breakage of its flagellum as it penetrates the mucosal layer overlaying the intestinal epithelium. Flagellar loss results in the release of the anti-sigma factor FlgM and subsequent activation of the alternative sigma-factor FliA. FliA represses the quorum sensing-controlled transcriptional regulator, HapR, allowing increased expression of virulence factors such as Cholera Toxin (CT) and the Toxin Coregulated Pilus (TCP). In this way, the de-repression of

virulence factor expression coincides with the arrival of bacteria at the site of infection at the intestinal mucosa. Our work reveals an interesting interplay between motility and quorum sensing signaling pathways to precisely time virulence gene expression during colonization.

Bibliographic Information

Quorum sensing regulation of the two hcp alleles in Vibrio cholerae O1 strains. Ishikawa Takahiko; Rompikuntal Pramod Kumar; Lindmark Barbro; Milton Debra L; Wai Sun Nyunt Department of Molecular Biology, Umea University, Umea, Sweden PloS one (2009), 4(8), e6734. Journal code: 101285081. E-ISSN:1932-6203. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 19701456 AN 2009573930 In-process for MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

BACKGROUND: The type VI secretion system (T6SS) has emerged as a protein secretion system important to several gram-negative bacterial species. One of the common components of the system is Hcp, initially described as a hemolysin co-regulated protein in a serotype O17 strain of Vibrio cholerae. Homologs to V. cholerae hcp genes have been found in all characterized type VI secretion systems and they are present also in the serotype O1 strains of V. cholerae that are the cause of cholera diseases but seemed to have non-functional T6SS. METHODOLOGY/PRINCIPAL FINDINGS: The serotype O1 V. cholerae strain A1552 was shown to express detectable levels of Hcp as determined by immunoblot analyses using polyclonal anti-Hcp antiserum. We found that the expression of Hcp was growth phase dependent. The levels of Hcp in quorum sensing deficient mutants of V. cholerae were compared with the levels in wild type V. cholerae O1 strain A1552. The expression of Hcp was positively and negatively regulated by the quorum sensing regulators HapR and LuxO, respectively. In addition, we observed that expression of Hcp was dependent on the cAMP-CRP global transcriptional regulatory complex and required the RpoN sigma factor. CONCLUSION/SIGNIFICANCE: Our results show that serotype O1 strains of V. cholerae do express Hcp which is regarded as one of the important T6SS components and is one of the secreted substrates in non-O1 non-O139 V. cholerae isolates. We found that expression of Hcp was strictly regulated by the quorum sensing system in the V. cholerae O1 strain. In addition, the expression of Hcp required the alternative sigma factor RpoN and the cAMP-CRP global regulatory complex. Interestingly, the environmental isolates of V. cholerae O1 strains that showed higher levels of the HapR quorum sensing regulator in comparison with our laboratory standard serotype O1 strain A1552 where also expressing higher levels of Hcp.

Bibliographic Information

Quantifying the integration of quorum-sensing signals with single-cell resolution. Long Tao; Tu Kimberly C; Wang Yufang; Mehta Pankaj; Ong N P; Bassler Bonnie L; Wingreen Ned S Department of Physics, Princeton University, Princeton, NJ, USA PLoS biology (2009), 7(3), e68. Journal code: 101183755. E-ISSN:1545-7885. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, N.I.H., EXTRAMURAL); (RESEARCH SUPPORT, NON-U.S. GOV'T); (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.) written in English. PubMed ID 19320539 AN 2009251670 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

Cell-to-cell communication in bacteria is a process known as quorum sensing that relies on the production, detection, and response to the extracellular accumulation of signaling molecules called autoinducers. Often, bacteria use multiple autoinducers to obtain information about the vicinal cell density. However, how cells integrate and interpret the information contained within multiple autoinducers remains a mystery. Using single-cell fluorescence microscopy, we quantified the signaling responses to and analyzed the integration of multiple autoinducers by the model quorum-sensing bacterium Vibrio harveyi. Our results revealed that signals from two distinct autoinducers, AI-1 and AI-2, are combined strictly additively in a shared phosphorelay pathway, with each autoinducer contributing nearly equally to the total response. We found a coherent response across the population with little cell-to-cell variation, indicating that the entire population of cells can reliably distinguish several distinct conditions of external autoinducer concentration. We speculate that the use of multiple autoinducers allows a growing population of cells to synchronize gene expression during a series of distinct developmental stages.

Bibliographic Information

Virulence of luminescent and non-luminescent isogenic vibrios towards gnotobiotic Artemia franciscana larvae and specific pathogen-free Litopenaeus vannamei shrimp. Phuoc L H; Defoirdt T; Sorgeloos P; Bossier P Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Belgium Journal of applied microbiology (2009), 106(4), 1388-96. Journal code: 9706280. E-ISSN:1365-2672. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 19187135 AN 2009210811 In-process for MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

AIMS: This study was conducted to test the virulence of luminescent (L) and non-luminescent (NL) isogenic strains of Vibrio campbellii LMG21363, Vibrio harveyi BB120 (wild type) and quorum-sensing mutant strains derived from the wild type such as Vibrio harveyi BB152, BB170, MM30 and BB886. METHODS AND RESULTS: The NL strains could be obtained by culturing rifampicin-resistant luminescent strains in the dark under static condition. The virulence of the L and NL strains was tested in gnotobiotic Artemia franciscana larvae challenged with 10(4) CFU ml(-1) of bacteria. All luminescent isogenic tested strains showed higher virulence compared to the NL strains. The virulence of L and NL V. campbellii and V. harveyi BB120 was also tested in specific pathogen-free juvenile shrimp upon intramuscular injection with 10(6) CFU of bacteria. In contrast with Artemia, there was no significant difference in mortality between the groups challenged with L and NL strains ($P > 0.05$). The non-luminescent strains were not able to revert back to the luminescent state and quorum sensing did not influence this phenotypic shift. CONCLUSIONS: Luminescent Vibrio strains can switch to a non-luminescent state by culturing them in static conditions. The NL strains become less virulent as verified in Artemia. SIGNIFICANCE AND IMPACT OF THE STUDY: The luminescent state of Vibrio cells in a culture needs to be verified in order to assure maintenance of virulence.

Bibliographic Information

Robust and sensitive control of a quorum-sensing circuit by two interlocked feedback loops. Williams Joshua W; Cui Xiaohui; Levchenko Andre; Stevens Ann M Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA Molecular systems biology (2008), 4 234. Journal code: 101235389. E-ISSN:1744-4292. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, N.I.H., EXTRAMURAL); (RESEARCH SUPPORT, NON-U.S.

GOV'T); (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.) written in English. PubMed ID 19096361 AN 2009041138 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
The quorum-sensing (QS) response of Vibrio fischeri involves a rapid switch between low and high induction states of the lux operon over a narrow concentration range of the autoinducer (AI) 3-oxo-hexanoyl-L-homoserine lactone. In this system, LuxR is an AI-dependent positive regulator of the lux operon, which encodes the AI synthase. This creates a positive feedback loop common in many bacterial species that exhibit QS-controlled gene expression. Applying a combination of modeling and experimental analyses, we provide evidence for a LuxR autoregulatory feedback loop that allows LuxR to increase its concentration in the cell during the switch to full lux activation. Using synthetic lux gene fragments, with or without the AI synthase gene, we show that the buildup of LuxR provides more sensitivity to increasing AI, and promotes the induction process. Elevated LuxR levels buffer against spurious variations in AI levels ensuring a robust response that endows the system with enhanced hysteresis. LuxR autoregulation also allows for two distinct responses within the same cell population.

Bibliographic Information

Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for luxS in most bacteria. Rezzonico Fabio; Duffy Brion Agroscope Changins-Wadenswil ACW, Division of Plant Protection, CH-8820 Wadenswil, Switzerland. fabio.rezzonico@acw.admin.ch BMC microbiology (2008), 8 154. Journal code: 100966981. E-ISSN:1471-2180. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 18803868 AN 2008642882 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
BACKGROUND: Great excitement accompanied discoveries over the last decade in several Gram-negative and Gram-positive bacteria of the LuxS protein, which catalyzes production of the AI-2 autoinducer molecule for a second quorum sensing system (QS-2). Since the luxS gene was found to be widespread among the most diverse bacterial taxa, it was hypothesized that AI-2 may constitute the basis of a universal microbial language, a kind of bacterial Esperanto. Many of the studies published in this field have drawn a direct correlation between the occurrence of the luxS gene in a given organism and the presence and functionality of a QS-2 therein. However, rarely hathe existence of potential AI-2 receptors been examined. This is important, since it is now well recognized that LuxS also holds a central role as a metabolic enzyme in the activated methyl cycle which is responsible for the generation of S-adenosyl-L-methionine, the major methyl donor in the cell. RESULTS: In order to assess whether the role of LuxS in these bacteria is indeed related to AI-2 mediated quorum sensing we analyzed genomic databases searching for established AI-2 receptors (i.e., LuxPQ-receptor of Vibrio harveyi and Lsr ABC-transporter of Salmonella typhimurium) and other presumed QS-related proteins and compared the outcome with published results about the role of QS-2 in these organisms. An unequivocal AI-2 related behavior was restricted primarily to organisms bearing known AI-2 receptor genes, while phenotypes of luxS mutant bacteria lacking these genes could often be explained simply by assuming deficiencies in sulfur metabolism. CONCLUSION: Genomic analysis shows that while LuxPQ is restricted to Vibrionales, the Lsr-receptor complex is mainly present in pathogenic bacteria associated with endotherms. This suggests that QS-2 may play an important role in interactions with animal hosts.

In most other species, however, the role of LuxS appears to be limited to metabolism, although in a few cases the presence of yet unknown receptors or the adaptation of pre-existent effectors to QS-2 must be postulated.

Bibliographic Information

Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in Vibrio spp. by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR. Brackman Gilles; Defoirdt Tom; Miyamoto Carol; Bossier Peter; Van Calenbergh Serge; Nelis Hans; Coenye Tom Laboratory of Pharmaceutical Microbiology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium. gilles.brackman@ugent.be BMC microbiology (2008), 8 149. Journal code: 100966981. E-ISSN:1471-2180. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 18793453 AN 2008622297 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

BACKGROUND: To date, only few compounds targeting the AI-2 based quorum sensing (QS) system are known. In the present study, we screened cinnamaldehyde and substituted cinnamaldehydes for their ability to interfere with AI-2 based QS. The mechanism of QS inhibition was elucidated by measuring the effect on bioluminescence in several Vibrio harveyi mutants. We also studied in vitro the ability of these compounds to interfere with biofilm formation, stress response and virulence of Vibrio spp. The compounds were also evaluated in an in vivo assay measuring the reduction of Vibrio harveyi virulence towards Artemia shrimp. RESULTS: Our results indicate that cinnamaldehyde and several substituted derivatives interfere with AI-2 based QS without inhibiting bacterial growth. The active compounds neither interfered with the bioluminescence system as such, nor with the production of AI-2. Study of the effect in various mutants suggested that the target protein is LuxR. Mobility shift assays revealed a decreased DNA-binding ability of LuxR. The compounds were further shown to (i) inhibit biofilm formation in several Vibrio spp., (ii) result in a reduced ability to survive starvation and antibiotic treatment, (iii) reduce pigment and protease production in Vibrio anguillarum and (iv) protect gnotobiotic Artemia shrimp against virulent Vibrio harveyi BB120. CONCLUSION: Cinnamaldehyde and cinnamaldehyde derivatives interfere with AI-2 based QS in various Vibrio spp. by decreasing the DNA-binding ability of LuxR. The use of these compounds resulted in several marked phenotypic changes, including reduced virulence and increased susceptibility to stress. Since inhibitors of AI-2 based quorum sensing are rare, and considering the role of AI-2 in several processes these compounds may be useful leads towards antipathogenic drugs.

Bibliographic Information

Transition state analogues in quorum sensing and SAM recycling. Schramm Vern L; Gutierrez Jemy A; Cordovano Grace; Basu Indranil; Guha Chandan; Belbin Thomas J; Evans Gary B; Tyler Peter C; Furneaux Richard H Albert Einstein College of Medicine, Bronx, New York, NY 10805, USA. vern@aecom.yu.edu Nucleic acids symposium series (2004) (2008), (52), 75-6. Journal code: 101259965. E-ISSN:1746-8272. Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 18776260 AN 2008588361 In-process for MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

Transition state structures can be derived from kinetic isotope effects and computational chemistry. Molecular electrostatic potential maps of transition states serve as blueprints to guide synthesis of transition state analogue inhibitors of target enzymes. 5'- Methylthioadenosine phosphorylase (MTAP) functions in the polyamine pathway by recycling methylthioadenosine (MTA) and maintaining cellular S-adenosylmethionine (SAM). Its transition state structure was used to guide synthesis of MT-DADMe-ImmA, a picomolar inhibitor that shows anticancer effects against solid tumors. Biochemical and genomic analysis suggests that MTAP inhibition acts by altered DNA methylation and gene expression patterns. A related bacterial enzyme, 5'-methylthioadenosine nucleosidase (MTAN), functions in pathways of quorum sensing involving AI-1 and AI-2 molecules. Transition states have been solved for several bacterial MTANs and used to guide synthesis of powerful inhibitors with dissociation constants in the femtomolar to picomolar range. BuT-DADMe-ImmA blocks quorum sensing in Vibrio cholerae without changing bacterial growth rates. Transition state analogue inhibitors show promise as anticancer and antibacterial agents.

Bibliographic Information

Detection of quorum-sensing-related molecules in Vibrio scophthalmi. Garcia-Aljaro Cristina; Eberl Leo; Riedel Kathrin; Blanch Anicet R Department of Microbiology, University of Barcelona, Barcelona, Spain. cristina.garcia@cnm.es BMC microbiology (2008), 8 138. Journal code: 100966981. E-ISSN:1471-2180. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 18700048 AN 2008550026 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
BACKGROUND: Cell-to-cell communication (also referred to as quorum sensing) based on N-acyl-homoserine lactones (AHLs) is a widespread response to environmental change in Gram-negative bacteria. AHLs seem to be highly variable, both in terms of the acyl chain length and in the chemical structure of the radicals. Another quorum sensing pathway, the autoinducer-2-based system, is present both in Gram-positive and Gram-negative bacteria. In this study the presence of signal molecules belonging to both quorum sensing signalling pathways was analysed in the marine symbiotic species Vibrio scophthalmi. RESULTS: Three AHL-like signal molecules were detected in V. scophthalmi supernatants with the Agrobacterium tumefaciens sensor assay. This observation was further supported by the decrease in the presence of these signal molecules after cloning and expression of lactonase AiiA from Bacillus cereus in the V. scophthalmi strains. One of the signal molecules was identified as N-(3-hydroxy dodecanoyl)-L-homoserine lactone. V. scophthalmi was also shown to carry a functional LuxS synthase. The coding sequence for a luxS-like gene was obtained showing a maximum similarity of 78% with Vibrio vulnificus. Analysis of the translated sequence revealed that the sequenced luxS gene carried the conserved domain, which is common to luxS sequences found in other species, and which is essential for LuxS enzymatic activity. CONCLUSION: The data are consistent with the presence of quorum-sensing signal molecules from both AHL- and autoinducer 2-based quorum sensing systems in V. scophthalmi, which are homologous to others previously described in various Vibrio species. How this bacterium interacts with other bacteria and eukaryotic cells to compete ecologically with other intestinal bacteria present in the fish Scophthalmus maximus warrants further investigation.

Bibliographic Information

Dissecting the quorum-sensing receptor LuxN. Comment on: Cell. 2008 Aug 8;134(3):461-73. PubMed ID: 18692469 Bonneau Richard New York University Center for Comparative Functional Genomics, 100 Washington Sq. E., New York, NY, 10003, USA. bonneau@cs.nyu.edu Cell (2008), 134(3), 390-1. Journal code: 0413066. E-ISSN:1097-4172. Commentary; Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 18692463 AN 2008509700 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
Quorum sensing plays a key role in the behavior of many bacteria and is carried out by a wide diversity of secreted molecules and their receptors. In this issue, Swem et al. (2008) provide a detailed site-specific analysis of the functioning of the quorum-sensing receptor LuxN from Vibrio harveyi.

Bibliographic Information

Luminescence, virulence and quorum sensing signal production by pathogenic Vibrio campbellii and Vibrio harveyi isolates. Defoirdt T; Verstraete W; Bossier P Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belgium. Tom.Defoirdt@UGent.be Journal of applied microbiology (2008), 104(5), 1480-7. Journal code: 9706280. E-ISSN:1365-2672. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 18070032 AN 2008251004 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
AIMS: To study the relationship between luminescence, autoinducer production and virulence of pathogenic vibrios. METHODS AND RESULTS: Luminescence, quorum sensing signal production and virulence towards brine shrimp nauplii of 13 Vibrio campbellii and Vibrio harveyi strains were studied. Although only two of the tested strains were brightly luminescent, all of them were shown to produce the three different types of quorum sensing signals known to be produced by Vibrio harveyi. Cell-free culture fluids of all strains significantly induced bioluminescence in the cholerae autoinducer 1, autoinducer 2 and harveyi autoinducer 1 reporter strains JAF375, JMH597 and JMH612, respectively. There was no relation between luminescence and signal production and virulence towards brine shrimp. CONCLUSIONS: There is a large difference between different strains of Vibrio campbellii and Vibrio harveyi with respect to bioluminescence. However, this is not reflected in signal production and virulence towards gnotobiotic brine shrimp. Moreover, there seems to be no relation between quorum sensing signal production and virulence towards brine shrimp. SIGNIFICANCE AND IMPACT OF THE STUDY: The results presented here indicate that strains that are most brightly luminescent are not necessarily the most virulent ones and that the lower virulence of some of the strains is not due to a lack of autoinducer production.

Bibliographic Information

Interplay of two quorum sensing regulation systems of Vibrio fischeri. Kuttler Christina; Hense Burkhard A GSF-National Research Center for Environment and Health, Institute of Biomathematics and Biometry, Ingolstadter Landstr. 1, 85764 Oberschleissheim, Germany. christina.kuttler@gsf.de Journal of theoretical biology (2008), 251(1), 167-80. Journal code: 0376342. E-ISSN:1095-8541. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT,

NON-U.S. GOV'T) written in English. PubMed ID 18164038 AN 2008147011 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
Many bacteria developed a possibility to recognise aspects of their environment or to communicate with each other by chemical signals. An important strategy is the so-called quorum sensing (QS), a regulatory mechanism for the gene expression, where the bacteria measure their own cell density by means of this signalling pathway. One of the best-studied species using QS is the marine luminescent bacterium Vibrio fischeri which is considered here as a model organism. The two main regulatory pathways (lux and ain) are combined to a regulation system, the dynamics is modelled by an ODE system. This system is analysed thoroughly, considering stationary states, dynamical behaviour and the possible biological meaning of it. The influence of different parameter values on the behaviour is examined, the same basic system is able to reflect the peculiarities of different bacteria strains (respectively, their mutants).

Bibliographic Information

Quorum sensing influences Vibrio harveyi growth rates in a manner not fully accounted for by the marker effect of bioluminescence. Nackerdien Zeena E; Keynan Alexander; Bassler Bonnie L; Lederberg Joshua; Thaler David S Raymond and Beverly Sackler Laboratory of Molecular Genetics and Informatics, Rockefeller University, New York, New York, USA PloS one (2008), 3(2), e1671. Journal code: 101285081. E-ISSN:1932-6203. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, N.I.H., EXTRAMURAL); (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.) written in English. PubMed ID 18301749 AN 2008143165 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
BACKGROUND: The light-emitting Vibrios provide excellent material for studying the interaction of cellular communication with growth rate because bioluminescence is a convenient marker for quorum sensing. However, the use of bioluminescence as a marker is complicated because bioluminescence itself may affect growth rate, e.g. by diverting energy. METHODOLOGY/PRINCIPAL FINDINGS: The marker effect was explored via growth rate studies in isogenic Vibrio harveyi (Vh) strains altered in quorum sensing on the one hand, and bioluminescence on the other. By hypothesis, growth rate is energy limited: mutants deficient in quorum sensing grow faster because wild type quorum sensing unleashes bioluminescence and bioluminescence diverts energy. Findings reported here confirm a role for bioluminescence in limiting Vh growth rate, at least under the conditions tested. However, the results argue that the bioluminescence is insufficient to explain the relationship of growth rate and quorum sensing in Vh. A Vh mutant null for all genes encoding the bioluminescence pathway grew faster than wild type but not as fast as null mutants in quorum sensing. Vh quorum sensing mutants showed altered growth rates that do not always rank with their relative increase or decrease in bioluminescence. In addition, the cell-free culture fluids of a rapidly growing Vibrio parahaemolyticus (Vp) strain increased the growth rate of wild type Vh without significantly altering Vh's bioluminescence. The same cell-free culture fluid increased the bioluminescence of Vh quorum mutants. CONCLUSIONS/SIGNIFICANCE: The effect of quorum sensing on Vh growth rate can be either positive or negative and includes both bioluminescence-dependent and independent components. Bioluminescence tends to slow growth rate but not enough to account for the effects of quorum sensing on growth rate.

Bibliographic Information

A model of the quorum sensing system in Vibrio fischeri using P systems. Romero-Campero Francisco J; Perez-Jimenez Mario J Research Group on Natural Computing, Department of Computer Science and Artificial Intelligence, University of Seville, Avenida Reina Mercedes s/n, 41012 Sevilla, Spain. fran@us.es Artificial life (2008), 14(1), 95-109. Journal code: 9433814. ISSN:1064-5462. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 18171133 AN 2008005755 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

Quorum sensing is a cell-density-dependent gene regulation system that allows an entire population of bacterial cells to communicate in order to regulate the expression of certain or specific genes in a coordinated way depending on the size of the population. We present a model of the quorum sensing system in Vibrio fischeri using a variant of membrane systems called P systems. In this framework each bacterium and the environment are represented by membranes, and the rules are applied according to an extension of Gillespie's algorithm called the multicompartmental Gillespie's algorithm. This algorithm runs on more than one compartment and takes into account the disturbance produced when chemical substances diffuse from one compartment or region to another one. Our approach allows us to examine the individual behavior of each bacterium as an agent as well as the emergent behavior of the colony as a whole and the processes of swarming and recruitment. Our simulations show that at low cell densities bacteria remain dark, while at high cell densities some bacteria start to produce light and a recruitment process takes place that makes the whole colony of bacteria do so. Our computational modeling of quorum sensing could provide insights leading to new applications where multiple agents need to robustly and efficiently coordinate their collective behavior based only on very limited information about the local environment.

Bibliographic Information

N-phenylacetanoyl-L-homoserine lactones can strongly antagonize or superagonize quorum sensing in Vibrio fischeri. Erratum in: ACS Chem Biol. 2007 Jul 15;2(6):426 Geske Grant D; O'Neill Jennifer C; Blackwell Helen E ACS chemical biology (2007), 2(5), 315-9. Journal code: 101282906. E-ISSN:1554-8937. Letter written in English. PubMed ID 17480049 AN 2007309882 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

Bacteria monitor their population densities using low-molecular-weight ligands in a process known as quorum sensing. At sufficient cell densities, bacteria can change their mode of growth and behave as multicellular communities that play critical roles in both beneficial symbioses and the pathogenesis of infectious disease. The development of non-native ligands that can block quorum-sensing signals has emerged as a promising new strategy to attenuate these divergent outcomes. Here, we report that N-phenylacetanoyl-L-homoserine lactones are capable of either inhibiting or, in some cases, strongly inducing quorum sensing in the bacterial symbiont Vibrio fischeri. Moreover, simple structural modifications to these ligands have remarkable effects on activity. These studies have revealed one of the first synthetic superagonists of quorum sensing, N-(3-nitro-phenylacetanoyl)-L-homoserine lactone. Together, these ligands represent a powerful new class of

chemical probes with the potential to significantly expand the current understanding of quorum sensing and its role in host/bacteria interactions.

Bibliographic Information

Is autoinducer-2 a universal signal for interspecies communication: a comparative genomic and phylogenetic analysis of the synthesis and signal transduction pathways. Sun Jibin; Daniel Rolf; Wagner-Dobler Irene; Zeng An-Ping Department of Genome Analysis, GBF - German Research Center for Biotechnology, Braunschweig, Germany. jsu@gbf.de <jsu@gbf.de> BMC evolutionary biology (2004), 4 36. Journal code: 100966975. E-ISSN:1471-2148. (COMPARATIVE STUDY); Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 15456522 AN 2004527393 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
BACKGROUND: Quorum sensing is a process of bacterial cell-to-cell communication involving the production and detection of extracellular signaling molecules called autoinducers. Recently, it has been proposed that autoinducer-2 (AI-2), a furanosyl borate diester derived from the recycling of S-adenosyl-homocysteine (SAH) to homocysteine, serves as a universal signal for interspecies communication. RESULTS: In this study, 138 completed genomes were examined for the genes involved in the synthesis and detection of AI-2. Except for some symbionts and parasites, all organisms have a pathway to recycle SAH, either using a two-step enzymatic conversion by the Pfs and LuxS enzymes or a one-step conversion using SAH-hydrolase (SahH). 51 organisms including most Gamma-, Beta-, and Epsilonproteobacteria, and Firmicutes possess the Pfs-LuxS pathway, while Archaea, Eukarya, Alphaproteobacteria, Actinobacteria and Cyanobacteria prefer the SahH pathway. In all 138 organisms, only the three Vibrio strains had strong, bidirectional matches to the periplasmic AI-2 binding protein LuxP and the central signal relay protein LuxU. The initial two-component sensor kinase protein LuxQ, and the terminal response regulator luxO are found in most Proteobacteria, as well as in some Firmicutes, often in several copies. CONCLUSIONS: The genomic analysis indicates that the LuxS enzyme required for AI-2 synthesis is widespread in bacteria, while the periplasmic binding protein LuxP is only present in Vibrio strains. Thus, other organisms may either use components different from the AI-2 signal transduction system of Vibrio strains to sense the signal of AI-2, or they do not have such a quorum sensing system at all.

Bibliographic Information

Small RNAs shed some light. Comment on: Cell. 2004 Jul 9;118(1):69-82. PubMed ID: 15242645 Gottesman Susan Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892, USA Cell (2004), 118(1), 1-2. Journal code: 0413066. ISSN:0092-8674. Commentary; Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 15242637 AN 2004340195 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract
Small regulatory RNAs can act by pairing with their target messages, targeting themselves and the mRNA for degradation; Lenz et al. (this issue of Cell) now report that multiple small RNAs are essential regulators of the quorum-sensing systems of Vibrio species, including the regulation of virulence in V. cholerae.

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Quorum sensing in Vibrio cholerae. Camara Miguel; Hardman Andrea; Williams Paul; Milton Debra Nature genetics (2002), 32(2), 217-8. Journal code: 9216904. ISSN:1061-4036. News Announcement written in English. PubMed ID 12355076 AN 2002493754 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Bibliographic Information

"Quorum sensing", or how bacteria "talk" to each other. Zavil'gel'skii G B; Manukhov I V Department of Biochemistry, New York University Medical Center, New York, NY 10016, USA. zavilgel@genetika.ru Molekuliarnaia biologiia (2001), 35(2), 268-77. Journal code: 0105454. ISSN:0026-8984. (ENGLISH ABSTRACT); Journal; Article; (JOURNAL ARTICLE); General Review; (REVIEW) written in Russian. PubMed ID 11357409 AN 2001266065 MEDLINE (Copyright (C) 2009 U.S. National Library of Medicine on SciFinder (R))

Abstract

Recent advances in studying the quorum-sensing systems, which regulate gene expression depending on population density, are reviewed. Low-molecular-weight acyl derivatives of L-homoserine lactone (N-AHL) freely diffuse through cell membranes and determine cell-to-cell communications in bacteria. The quorum-sensing systems have first been found to regulate bioluminescence in marine bacteria Photobacterium (Vibrio) fischeri and Vibrio harveyi. Such systems are widespread and control expression of genes for virulence factors, proteases, antibiotics, etc., in various Gram-negative bacteria, including plant, animal, and human pathogens. Quorum sensing is a prominent example of social behavior in bacteria, as signal exchange among individual cells allows the entire population to choose an optimal way of interaction with the environment and with higher organisms.