Short Communication

The Response of *Pseudomonas aeruginosa* PAO1 Efflux Pump-Defective Mutants to *N*-Octanoyl-L-Homoserine Lactone

YUTAKA YAWATA^{1,†}, HIDEAKI MASEDA^{1,2,‡*}, SATOSHI OKABE³, AKINOBU ITO³, ISAO SAWADA^{1†}, HIROAKI KURASHIMA¹, HIROO UCHIYAMA¹, and NOBUHIKO NOMURA^{1*}

¹Institute of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan; ²Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, Minamijosanjima-cho, Tokushima 770–8506, Japan and ³Department of Urban and Environmental Engineering, Graduate School of Engineering, Hokkaido University, Sapporo, Hokkaido 060–8628, Japan

(Received May 25, 2009—Accepted August 5, 2009—Published online September 16, 2009)

N-Octanoyl-L-homoserine lactone (C8-HSL) is an acyl-homoserine-lactone signal utilized in the quorum-sensing (QS) systems of *Burkholderia cenocepacia* and other bacterial species. Although also produced by *Pseudomonas aeruginosa*, its role in this species has not been elucidated. Here, we report that C8-HSL modulated antibiotic resistance and pyocyanin production in a *P. aeruginosa* efflux pump-deficient mutant. The *rhl/las* quorum-sensing system and *qscR* gene were both shown to be nonessential in the C8-HSL-induced changes in ofloxacin resistance, suggesting that *P. aeruginosa* possesses a distinct pathway to respond to C8-HSL.

Key words: cell-cell communication, *Pseudomonas aeruginosa*, acyl-homoserine lactone

Pseudomonas aeruginosa is a Gram-negative ubiquitous bacterium that causes infections in immunocompromised hosts and colonizes the lungs of individuals with cystic fibrosis (CF) (7). *P. aeruginosa* and other bacteria control gene expression globally via cell-cell signalling systems that are termed quorum sensing (QS) systems (28). Gram-negative bacteria mainly utilize acyl-homoserine lactone (AHL) as a cell-cell signal in their QS systems (28). *P. aeruginosa* possesses at least two AHL-mediated QS systems, the LasR-LasI (*las*) and RhIR-RhII (*rhl*) systems (21). LasI directs the synthesis of the AHL signal *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) (25), while RhII directs the synthesis of another AHL signal, *N*-butyryl-L-homoserine lactone (C4-HSL) (18). In *P. aeruginosa*, the homoserine lactone ring of C4-HSL or 3-oxo-C12 HSL originates from *S*-adenosyl-methionine and the acyl side chains are derived from fatty acids loaded onto acyl carrier proteins (8). RhII or LasI catalyzes the reaction. The transcriptional regulatory proteins LasR (5) and RhIR (17) are specifically activated by 3-oxo-C12-HSL and C4-HSL, respectively, and induce expression of their own target genes, including *lasI* and *rhlI*. Studies have shown that the production of virulence factors is regulated by QS in *P. aeruginosa* (9), suggesting that QS plays a significant role in the pathogenicity of *P. aeruginosa* (6, 29).

N-Octanoyl-L-homoserine lactone (C8-HSL) is an AHL signal utilized in the QS systems of *Burkholderia cenocepacia* and other species (23, 28). In CF lungs, *P. aeruginosa* is reported to frequently coexist with *B. cenocepacia* (7, 26). Accordingly, it can be presumed that *P. aeruginosa* cells are exposed to exogenous C8-HSL in these multispecies complexes. This idea is supported by the detection of various AHLs from CF sputum (3). In addition, *P. aeruginosa* also produces C8-HSL (3, 23). Nevertheless, unlike C4-HSL or 3-oxo-C12-HSL, C8-HSL is unable to trigger the *P. aeruginosa* QS circuit (23). Therefore, the role and effect of C8-HSL in *P. aeruginosa* have not yet been elucidated. Here, we report that exogenous C8-HSL modulated ofloxacin resistance and pyocyanin production in *P. aeruginosa* MexAB-OprM efflux pump-deficient mutants.

The strains and plasmids used in this study are listed in Table 1. The oligonucleotides used in the construction of plasmids and *P. aeruginosa* mutants are listed in Table S1. For routine culturing, bacterial strains were grown in LB media or on LB agar plates. *Escherichia coli* DH5 α (Takara Bio, Otsu, Japan) was the host used for DNA manipulation. When necessary, gentamicin was added at a concentration of 15 $\mu\text{g mL}^{-1}$ for *Escherichia coli* DH5 α , S17-1 (15) and 100 $\mu\text{g mL}^{-1}$ for *P. aeruginosa*.

DNA manipulations followed standard procedures (24). A *mexAB-oprM* mutant of PAO1 (TNP1001) was constructed by conjugating a pG19II derivative carrying a defective *mexAB-oprM* into the strain PAO1, followed by homologous recombination, as described (15). The pG19II derivative was constructed by inserting a defective *mexAB-oprM* derived from the chromosome of *P. aeruginosa* TNP077 into a multicloning site of pG19II. Deletion of *mexAB-oprM* in *P. aeruginosa* PAO1 was confirmed by PCR and Western blot analyses (data not shown). The *rhlI/lasI*, *lasR/rhlR*

* Corresponding authors. Hideaki Maseda; E-mail: maseda@bio.tokushima-u.ac.jp; Tel: +81–88–656–7524; Fax: +81–88–656–7524. and Nobuhiko Nomura; E-mail: nomunobu@sakura.cc.tsukuba.ac.jp; Tel: +81–29–853–6627; Fax: +81–29–853–6627.

† Y.Y. and H.M. contributed equally to this study.

† Present address: Department of Chemical Science and Engineering, Kobe University, 1–1 Rokkodaicho, Nada-ku, Kobe 657–8501, Japan

Table 1. Strains and plasmids used in this study

Strain or plasmid	Relevant properties	Reference
Strains		
<i>P. aeruginosa</i>		
PAO1	Wild-type	(14)
PS1090	$\Delta rhII\Delta lasI$; PAO1 derivative	This study
PS1091	$\Delta rhIRI\Delta lasRI$; PAO1 derivative	This study
TNP1001	$\Delta mexAB-oprM$; PAO1 derivative	This study
TNP077	$\Delta mexAB-oprM$; derivative of PA4290	(13)
PS1104	$\Delta mexAB-oprM \Delta qscR$; TNP1001 derivative	This study
PS1107	$\Delta mexAB-oprM \Delta rhII\Delta lasI$; PS1101 derivative	This study
PS1108	$\Delta mexAB-oprM \Delta rhIRI\Delta lasRI$; PS1101 derivative	This study
<i>E. coli</i>		
DH5 α	<i>E. coli</i> strain for transformation	Takara bio
S17-1	Mobilizer strain	(13)
Plasmids		
pG19II	pK19mobsac derivative carrying a Gm ^r gene as a marker	(15)
pG19-dellas	pG19II derivative carrying a deficient <i>lasI</i>	(15)
pG19-delrhl	pG19II derivative carrying a deficient <i>rhlI</i>	(15)

or *qscR* mutants of strain PAO1 or TNP1001 (PS1090, PS1091, PS1104, PS1107 and PS1108) were constructed by transferring pG19II derivatives carrying a defective *rhlI*, *lasI*, *lasR*, *rhlR* or *qscR* using the same procedure as for TNP1001. The pG19-dellas or pG19-delrhl plasmid (15) was used to construct *rhlI/lasI* mutants. The pG19II derivatives carrying a defective *lasR*, *rhlR* or *qscR* were constructed by inverted PCR as described previously (15). The deletions of *rhlI/lasI*, *lasR*, *rhlR* or *qscR* in *P. aeruginosa* TNP1001 were confirmed by PCR analyses and the presence of the known mutant phenotypes (data not shown).

The minimal inhibition concentration (MIC) of the strains was determined by the agar-dilution method using Muller-Hinton agar (Becton Dickinson Microbiology Systems, Cokeseville, MD, USA). Ofloxacin was added at a final concentration of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, or 25.6 $\mu\text{g mL}^{-1}$. Gentamicin or aztreonam were added at a final concentration of 0.006, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 or 6.4 $\mu\text{g mL}^{-1}$. C4-HSL, C8-HSL or 3-oxo-C12-HSL was added in the agar medium at a final concentration of 50 μM unless noted otherwise. DMSO was used as a con-

trol. Additionally, a modified cup method was developed to evaluate the effect of AHL on antibiotic resistance. Liquid LB agar (1.5%) medium at 45°C was mixed with precultured cells and ofloxacin, aztreonam or gentamicin. The medium was then poured into a Petri dish and left static until the agar solidified. Before examining the effect of AHLs, the minimal antibiotic concentration required to inhibit cell growth in the mixed agar was determined. For examination of the effects of AHLs, bottomless, stainless steel cylinders (6.0 mm internal diameter, 8.0 mm outer diameter, 10.0 mm height) were carefully placed on the agar plate. Cylinders were filled with 100 μL of AHL solution, and the plates were incubated at 37°C. After 24 h, the effect of the AHL solution was evaluated by the presence of a “growth circle” around the cylinder on the plate containing antibiotics at minimal concentrations to inhibit cell growth of the strains.

Phenazine compounds were quantified by extracting pyocyanin with chloroform and measuring the A_{520} of the aqueous phase as described (2). The elastolytic activity of *P. aeruginosa* culture fluids was determined by elastin congo-red assays as detailed previously (19).

P. aeruginosa is exposed to antibiotics during the treatment of mucus infections, such as CF-infected airway epithelium (27), where *P. aeruginosa* coexists with *B. cenocepacia* and other C8-HSL-producing bacteria (7, 26). We hypothesized that the addition of C8-HSL would influence the antibiotic resistance of *P. aeruginosa*. To test this hypothesis, *P. aeruginosa* PAO1 and TNP1001 MexAB-OprM efflux pump-deficient mutants were subjected to the modified cup test and the MIC test with several antibiotics as described above. MexAB-OprM efflux pumps are a well-studied mechanism for antibiotic resistance in *P. aeruginosa* (11, 22). It has been reported that the MexAB-OprM efflux pump is also involved in transport of AHL signal molecules (4, 20).

Although C8-HSL had no effect on resistance to gentamicin or aztreonam (data not shown), it promoted the resistance of TNP1001 to ofloxacin as demonstrated both in the modified cup test and in the MIC test (Table 2 and Fig. 1A). As shown in Fig. 1B, the effect of C8-HSL on strain TNP1001 was dose dependent, with as little as 5 μM weakly promoting ofloxacin resistance. On the other hand, the ofloxacin resistance of strain PAO1 was not influenced by C8-HSL (Table 2 and Fig. 1A). Similar results were observed with strains PS1090 and PS1107, *rhlI/lasI* mutant derivatives of strains PAO1 and TNP1001, respectively, indicating that C8-HSL

Table 2. MIC of the *Pseudomonas aeruginosa* strains[†]

Relevant properties		Minimal Ofloxacin concentration to inhibit cell growth in mixed agar ($\mu\text{g mL}^{-1}$)			
		Control	+C4-HSL	+C8-HSL	+3-oxo-C12HSL
PAO1	Wild-type	0.8	0.8	0.8	0.8
PS1090	$\Delta rhII\Delta lasI$	0.8	0.8	0.8	0.8
PS1091	$\Delta rhIRI\Delta lasRI$	0.8	0.8	0.8	0.8
TNP1001	$\Delta mexAB-oprM$	0.1	0.1	0.2	0.1
PS1107	$\Delta mexAB-oprM \Delta rhII\Delta lasI$	0.1	0.1	0.2	0.1
PS1108	$\Delta mexAB-oprM \Delta rhIRI\Delta lasRI$	0.1	0.1	0.2	0.1
PS1104	$\Delta mexAB-oprM \Delta qscR$	0.1	0.1	0.2	0.1

[†] LB-agar supplemented with 50 mM MOPS (pH 7.0).

Plates containing 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 or 25.6 $\mu\text{g mL}^{-1}$ of ofloxacin were used for the MIC test.

Acyl-homoserine-lactones were added at a final concentration of 50 μM .

Fig. 1. Effect of C8-HSL on ofloxacin resistance. (A) Panels show the results of the modified cup method. Each plate contained *Pseudomonas aeruginosa* PAO1, PS1090, PS1091, TNP1001, PS1107, PS1108 and PS1104, as indicated. Arrows indicate where a cylinder filled with C4-HSL (BHL), 3-oxo-C12-HSL (OdHL), C8-HSL (OHL), or DMSO (DMSO) was placed. AHLs were adjusted to 1 mM and added to the cylinders. DMSO (10%) was used as a control. Concentrations of ofloxacin in the plates are indicated under the panels. (B) Ofloxacin MIC of strain TNP1001 at various concentrations of C8-HSL. C8-HSL was added at 50, 10, 5 and 1 μM . DMSO (0.5%) was used as a control. Plates containing 0.025, 0.035, 0.05, 0.07, 0.1, 0.14 or 0.2 $\mu\text{g mL}^{-1}$ of ofloxacin were used in the MIC test.

can modulate the ofloxacin resistance of strain TNP1001 without the synergistic effect of other AHL signals (Table 2 and Fig. 1A).

Both the modified cup test and the MIC test showed that the ofloxacin resistance of strain TNP1001 was promoted by C8-HSL, but not C4-HSL or 3-oxo-C12-HSL (Table 2 and Fig. 1A). This result was unexpected because C8-HSL shares structural similarity with C4-HSL and 3-oxo-C12-HSL, implying that the effect of C8-HSL was not achieved via the *rhl* or *las* QS circuits. The results of the modified cup test and MIC tests revealed that the ofloxacin resistance of an *rhlRI/lasRI*-deficient derivative (PS1108) of strain TNP1001 was also promoted by C8-HSL, showing that neither RhIR nor LasR was essential to ofloxacin resistance induced by C8-HSL in strain TNP1001. The result is consistent with the finding of Riedel *et al.* (23) that C8-HSL is unable to trigger

rhl and *las* QS systems.

So through which pathway does C8-HSL act to promote the ofloxacin resistance of strain TNP1001? The *qscR* gene is a *luxR* homologue that has no corresponding *luxI* homologue, and the QscR protein exhibits a more relaxed specificity to AHLs than does LasR (10). *qscR* regulates many *P. aeruginosa* genes and C8-HSL has been shown to activate *qscR*-dependent regulation (10). However, as shown by both the modified cup method and MIC test, ofloxacin resistance in strain PS1104 (a TNP1001 derivative deficient in *qscR*) was still promoted by C8-HSL (Table 2 and Fig. 1A), showing that a functional *qscR* gene was not essential. Further investigation is required to elucidate the exact pathway involved in the induction of ofloxacin resistance by C8-HSL in strain TNP1001.

Given that C8-HSL modulated antibiotic resistance, we

Fig. 2. Effect of C8-HSL on pyocyanin production. (A) Pyocyanin production by strain TNP1001 or PS1104 measured as A_{520} in chloroform extracts of culture medium supplemented with C8-HSL (C8), C4-HSL (C4), 3-oxo-C12-HSL (3oC12), or DMSO as a control (Control). Cells were grown in LB medium for 15 h. AHLs were added at a concentration of 50 μM and DMSO was added at a concentration of 0.5%. (B) Pyocyanin production in strain TNP1001 at various C8-HSL concentrations taking pyocyanin production in TNP1001 grown with 0.5% DMSO as 100%. Values indicate the means of triplicate experiments and error bars show the standard deviations.

investigated its effect on other phenotypes involved in *P. aeruginosa*'s pathogenicity. Phenazine compounds produced by fluorescent *Pseudomonas* species have been suggested to be involved in virulence in human and animal hosts (12), and pyocyanin is one of the best-characterized phenazine compounds produced by *P. aeruginosa* (16). To further examine the effect of C8-HSL on the behavior of *P. aeruginosa* strains, we addressed whether exogenous C8-HSL influences pyocyanin production. C4-HSL, C8-HSL, or 3-oxo-C12-HSL was added to the liquid culture medium of strain PAO1 or strain TNP1001 ($\Delta mexAB-oprM$), and pyocyanin production was measured. The results showed that C8-HSL repressed pyocyanin production in TNP1001 (Fig. 2A). As PAO1 produced no pyocyanin in this study, we were unable to determine whether the repressive effect of C8-HSL was specific to the strains with a defective MexAB-OprM efflux pump. As shown in Fig. 2B, the effect on strain TNP1001 was dose dependent, with as little as 1 μM of C8-HSL weakly repressing pyocyanin production. The filtered supernatant of strain TNP1001 was incubated with or without 50 μM of C8-HSL for 24 h, and almost no difference in pyocyanin concentration was found between the supernatant with C8-HSL and that without (data not shown), showing that C8-HSL did not accelerate the degradation of pyocyanin.

The strain PS1107, an *rhl/lasI* derivative of TNP1001, produced no pyocyanin. Therefore, it is unclear if C8-HSL could modulate pyocyanin production in strain TNP1001 without the synergistic effect of other AHL signals.

As shown in Fig. 2A, the effect of C4-HSL on pyocyanin production in strain TNP1001 was the opposite to that of C8-HSL while 3-oxo-C12-HSL had almost no effect. This result is consistent with previous studies that revealed pyocyanin production to be under the regulation of *rhl*-QS system (1). Because the TNP1001 mutant lacking functional *rhl* and *las* QS circuits (PS1108: $\Delta mexAB-oprM\Delta rhlRI\Delta lasRI$) did not produce pyocyanin, it remained unclear whether the *rhl* and *las* QS circuits are essential in the repression of pyocyanin production in TNP1001 by C8-HSL. On the other hand, pyocyanin production in PS1104 ($\Delta mexAB-oprM\Delta qscR$) was also repressed by C8-HSL and enhanced by C4-HSL (Fig. 2A), showing that a functional *qscR* gene was not essential. Further investigation is required to elucidate the exact mechanism involved in the repression of pyocyanin production by C8-HSL.

Next, we addressed whether exogenous C8-HSL influences elastase production by *P. aeruginosa*. Elastase production is another factor involved in virulence in human and animal hosts and reported to be under the control of the QS system (19). C8-HSL was added to the liquid culture medium of strain PAO1 or TNP1001 ($\Delta mexAB-oprM$), and elastase production was measured. However, the results showed that C8-HSL had no effect on elastase production in PAO1 and TNP1001 (Data not shown).

In this study, C8-HSL was shown to influence ofloxacin resistance and pyocyanin production in *P. aeruginosa* MexAB-OprM efflux pump-deficient strains.

In our study, C8-HSL enhanced the ofloxacin resistance of the MexAB-OprM-deficient mutants but not the wild-type strain. Why then did only the mutants respond to C8-HSL? One explanation is that, in the wild-type strain, the influence of C8-HSL on ofloxacin resistance was not detectable because the effect of the MexAB-OprM efflux pump was more significant. Another possible explanation is that the MexAB-OprM pump regulated the cellular response to C8-HSL. While C4-HSL, AHL with a short N-acyl side chain, is freely diffusible, 3-oxo-C12 HSL, a more hydrophobic AHL with a longer N-acyl side chain, requires functional MexAB-OprM pump for its transport (20). Therefore, it is possible that the MexAB-OprM efflux pump is involved in transporting C8-HSL, which possesses a longer N-acyl side chain than C4-HSL.

Another important question is via which pathway C8-HSL enhanced the ofloxacin resistance of the MexAB-OprM-deficient mutants. Our results showed that the effect of C8-HSL on the ofloxacin resistance of strain TNP1001 was different from that of C4-HSL and 3-oxo-C12-HSL. It was also found that the *rhlR* and *lasR* quorum sensing receptor genes and *qscR* gene were not essential to the effect on ofloxacin resistance by C8-HSL, supporting the above results. In light of this, it is highly possible that C8-HSL induced changes in ofloxacin resistance independent of the *rhl/las* QS systems or *qscR*. In addition to *lasR*, *rhlR* and *qscR*, *P. aeruginosa* possesses an orphan *luxR*-homolog, PA1136 (10). Therefore, PA1136 could be a candidate for the C8-HSL receptor

responsible for modulating ofloxacin resistance.

Our results also showed that C8-HSL affected pyocyanin production in strain TNP1001, suggesting multiple roles of C8-HSL in artificial MexAB-OprM efflux pump-deficient mutants. However, elastase production was not affected by C8-HSL, indicating that not all QS-controlled virulence-factor production was influenced by C8-HSL. Although we have ruled out the involvement of *qscR* in the repression of pyocyanin production (Fig. 2A), we were unable to determine whether the *rhl/las* QS system is essential. Therefore further study is required to unveil the exact mechanism involved.

We must note that the effect of C8-HSL was only found in artificial MexAB-OprM efflux pump-deficient mutant, and the magnitude of the effect was relatively small. However, our study showed that exogenous C8-HSL, the role and effect of which in *P. aeruginosa* have not yet been elucidated, could have actual influences on *P. aeruginosa* MexAB-OprM efflux pump-defective strains. These results could provide a new perspective on the diversity of cell-cell signaling pathways of *P. aeruginosa*.

Acknowledgements

This study was partially supported by grants to N. N. from the Industrial Technology Research Grant Program 2004 from the New Energy and Industrial Technology Development Organization (NEDO), Japan.

References

- Brint, J.M., and D.E. Ohman. 1995. 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 auto-inducer-responsive LuxR-LuxI family. *J. Bacteriol.* 177:7155–7163.
- Cox, C.D. 1986. Role of pyocyanin in the acquisition of iron from transferrin. *Infect. Immun.* 52:263–270.
- Erickson, D.L., R. Endersby, A. Kirkham, K. Stuber, D.D. Vollman, H.R. Rabin, I. Mitchell, and D.G. Storey. 2002. *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. *Infect. Immun.* 70:1783–1790.
- Evans, K., L. Passador, R. Srikumar, E. Tsang, J. Nezezon, and K. Poole. 1998. Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 180:5443–5447.
- Gambello, M.J., S. Kaye, and B.H. Iglewski. 1993. LasR of *Pseudomonas aeruginosa* is a transcriptional activator of the alkaline protease gene (*apr*) and an enhancer of exotoxin A expression. *Infect. Immun.* 61:1180–1184.
- Girard, G., and G.V. Bloemberg. 2008. Central role of quorum sensing in regulating the production of pathogenicity factors in *Pseudomonas aeruginosa*. *Future Microbiol.* 3:97–106.
- Govan, J.R., and V. Deretic. 1996. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol. Rev.* 60:539–574.
- Heurlier, K., V. Dénervaud, and D. Hass. 2006. Impact of quorum sensing on fitness of *Pseudomonas aeruginosa*. *Int. J. Med. Microbiol.* 296:93–102.
- Latifi, A., M.K. Winson, M. Foglino, B.W. Bycroft, G.S. Stewart, A. Lazdunski, and P. Williams. 1995. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. *Mol. Microbiol.* 17:333–343.
- Lee, J.H., Y. Lequette, and E.P. Greenberg. 2006. Activity of purified QscR, a *Pseudomonas aeruginosa* orphan quorum-sensing transcription factor. *Mol. Microbiol.* 59:602–609.
- Li, X.Z., H. Nikaido, and K. Poole. 1995. Role of *mexA-mexB-oprM* in antibiotic efflux in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 39:1948–1953.
- Mahajan-Miklos, S., M.W. Tan, L.G. Rahme, and F.M. Ausubel. 1999. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*-*Caenorhabditis elegans* pathogenesis model. *Cell* 96:47–56.
- Maseda, H., H. Yoneyama, and T. Nakae. 2000. Assignment of the substrate-selective subunits of the MexEF-OprN multidrug efflux pump of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 44:658–664.
- Maseda, H., K. Saito, A. Nakajima, and T. Nakae. 2000. Variation of the *mexT* gene, a regulator of the MexEF-oprN efflux pump expression in wild-type strains of *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* 192:107–112.
- Maseda, H., I. Sawada, K. Saito, H. Uchiyama, T. Nakae, and N. Nomura. 2004. Enhancement of the *mexAB-oprM* efflux pump expression by a quorum-sensing autoinducer and its cancellation by a regulator, MexT, of the *mexEF-oprN* efflux pump operon in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 48:1320–1328.
- Mavrodi, D.V., R.F. Bonsall, S.M. Delaney, M.J. Soule, G. Phillips, and L.S. Thomashow. 2001. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* 183:6454–6465.
- Ochsner, U.A., and J. Reiser. 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:6424–6428.
- Pearson, J.P., L. Passador, B.H. Iglewski, and E.P. Greenberg. 1995. A second *N*-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:1490–1494.
- Pearson, J.P., E.C. Pesci, and B.H. Iglewski. 1997. Roles of *Pseudomonas aeruginosa* *las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.* 179:5756–5767.
- Pearson, J.P., C. Van Delden, and B.H. Iglewski. 1999. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J. Bacteriol.* 181:1203–1210.
- Pesci, E.C., J.P. Pearson, P.C. Seed, and B.H. Iglewski. 1997. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179:3127–3132.
- Poole, K. 2001. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. *J. Mol. Microbiol. Biotechnol.* 3:255–264.
- Riedel, K., M. Hentzer, O. Geisenberger, *et al.* 2001. *N*-Acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* 147:3249–3262.
- Sambrook, J., and D.W. Russell. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory Press, New York, USA.
- Schuster, M., C.P. Lostroh, T. Ogi, and E.P. Greenberg. 2003. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J. Bacteriol.* 185:2066–2079.
- Singh, P.K., A.L. Schaefer, M.R. Parsek, T.O. Moninger, M.J. Welsh, and E.P. Greenberg. 2000. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 407:762–764.
- Taccetti, G., S. Campana, A.S. Neri, V. Boni, and F. Festini. 2008. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis. *J. Chemother.* 20:166–169.
- Taga, M., and B.L. Bassler. 2003. Chemical communication among bacteria. *Proc. Natl. Acad. Sci. USA* 100:14549–14554.
- Willcox, M.D., H. Zhu, T.C. Conibear, E.B. Hume, M. Givskov, S. Kjelleberg, and S.A. Rice. 2008. Role of quorum sensing by *Pseudomonas aeruginosa* in microbial keratitis and cystic fibrosis. *Microbiology* 154:2184–2194.