Mol. Cells *33*, 53-59, January 31, 2012
DOI/10.1007/s10059-012-2208-2

Molecules and Cells

Interspecies Signaling through QscR, a Quorum Receptor of *Pseudomonas aeruginosa*

Changwan Ha[1], Seong Joon Park[1], Su-Jin Im, Su-Jin Park, and Joon-Hee Lee*

The QS machinery of *Pseudomonas aeruginosa*, an opportunistic human pathogen, consists of three acyl-homoserine lactone (acyl-HSL) signaling systems, LasR-I, RhlR-I, and QscR. QscR, known as an orphan receptor and a repressor of other QS systems, operates its own regulon using *N*-3-oxododecanoyl HSL (3OC12), which is synthesized by LasI, as its signal. In this study, we addressed the role of QscR in interspecies communication. We found that QscR auto-activates its own transcription in the presence of 3OC12. In a single population of *P. aeruginosa*, where 3OC12 is the sole signal available for QscR, the QscR regulon is activated by 3OC12 produced by the LasI-R system. However, the broad signal specificity of QscR allowed it to respond to a non-*P. aeruginosa* signal, such as *N*-decanoyl HSL (C10) and *N*-3-hydroxydecanoyl HSL (3OHC10), which preferentially activated QscR to LasR. The signal extracts from *Pseudomonas fluorescens* and *Burkholeria vietnamiensis* also preferentially activated QscR. These non-*P. aeruginosa* signals activated QscR more strongly than 3OC12, the authentic *P. aeruginosa* signal. Since a variety of acyl-HSLs are produced in the multi-species habitat of nature, our study provides a clue for the particular situation that allows QscR to secede from the conventional QS cascade in mixed microbial community.

INTRODUCTION

Bacterial communication through signal molecules allows cells to coordinate group activity, which ensures stronger and more efficient ways to survive in a hostile environment than the individual activity. A well-known mechanism for bacterial communication is quorum sensing (QS). QS controls the expression of a variety of virulence genes in many bacteria, and one of the most intensively studied organisms with QS is *Pseudomonas aeruginosa*, an opportunistic human pathogen (Smith and Iglewski, 2003). *P. aeruginosa* produces two acyl-homoserine lactone (acyl-HSL) QS signals, *N*-3-oxododecanoyl HSL (3OC12) and *N*-butanoyl HSL (C4). C4 is produced by an acyl-HSL synthase, RhlI, and its receptor is RhlR; the *rhlI* and *rhlR* genes are adjacent to each other (Fuqua and Greenberg, 2002). 3OC12, generated by LasI, has two receptors, LasR and QscR (Fuqua and Greenberg, 2002; Lee et al., 2006; Lequette et al., 2006). Like *rhlI* and *rhlR*, the *lasI* and *lasR* genes are adjacent to each other, but the *qscR* gene is independently located as an orphan receptor (Fuqua, 2006; Fuqua and Greenberg, 2002; Venturi, 2006). Recent results showed that OscR is also a transcription factor involved in QS signaling of *P. aeruginosa* like LasR and RhlR; it operates its own regulon using 3OC12 as its signal (Fuqua, 2006; Lee et al., 2006; Lequette et al., 2006).

Two major QS systems, LasI-R and RhlI-R, are regulated in a hierarchical cascade where LasR activates the transcription of *rhlR* and *rhlI* genes (Daniels et al., 2004; Fuqua and Greenberg, 2002), and early studies of QscR function suggested that QscR has a negative effect on some genes of other QS systems (Chugani et al., 2001; Ledgham et al., 2003). In this close interrelation, the LasI-R, RhlI-R, and QscR systems control expression of several hundred *P. aeruginosa* genes (Lequette et al., 2006; Schuster et al., 2003). However, it is not clear yet whether or not the QscR system is regulated by other QS systems in this interrelated QS cascade, although it has been reported that *qscR* expression is not under the control of LasR (Ledgham et al., 2003). The regulation network of the QS genes by three QS systems is complicated and many may be controlled indirectly (Hentzer et al., 2003; Schuster and Greenberg, 2006; Wagner et al., 2003).

While the two 3OC12-receptors, LasR and QscR, share the same signal, 3OC12, they differ fundamentally in the peculiarity and specificity of the signal binding. QscR binds to 3OC12 weakly compared with LasR and has broader signal specificity than LasR (Lee et al., 2006). QscR responds to *N*-3-oxodecanoyl HSL (3OC10), *N*-decanoyl HSL (C10) and *N*-dodecanoyl HSL (C12) better than it does to 3OC12 (Lee et al., 2006). These molecules are not expected to be produced in a single pure culture of *P. aeruginosa*. However, in a mixed bacterial population, these signals could come from other bacteria that coexist with *P. aeruginosa*. This suggests that QscR could be differentially activated from LasR in a certain environmental situations. Since the previous study showed that QscR represses a number of genes controlled by LasR and RhlR (Chugani et al., 2001; Ledgham et al., 2003), the preferential activation of QscR might affect the virulence of *P. aeruginosa*.

In this paper, we investigated whether or not QscR and LasR could be differentially activated based on their distinct signal

Department of Pharmacy, College of Pharmacy, Pusan National University, Busan 609-735, Korea, [1]These authors contributed equally to this work.
*Correspondence: joonhee@pusan.ac.kr

Received April 19, 2011; revised October 21, 2011; accepted October 24, 2011; published online January 3, 2012

Keywords: interspecies signaling, LasR, *Pseudomonas aeruginosa*, QscR, quorum

specificity. Our results showed that some non-*P. aeruginosa* signals were able to preferentially activate QscR to LasR and the activated QscR could boost its own expression. This particular route of the activation of QscR regulon suggests that QS regulation can be tuned by the composition of species in a multi-species community.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions

The strains and plasmids we used in this study are described in Table 1. *P. aeruginosa*, *Pseudomonas fluorescens, Burkholderia vietnamiensis* and *Escherichia coli* strains were grown in Luria-Bertani (LB) broth containing 50 mM 3-(*N*-morpholino) propanesulphonic acid (MOPS), pH 7.0, at 37°C with vigorous shaking. Growth was monitored as the optical density at 600 nm (OD_{600}). Antibiotics were used at the following concentrations: ampicillin, 50 μg/ml; carbenicillin, 150 μg/ml; gentamicin, 12.5 μg/ml (for *E. coli*) or 100 μg/ml (for *P. aeruginosa*). Various concentrations of synthetic acyl-HSLs (Sigma) and L-arabinose (0.4%) were added where indicated.

To create the *qscR* promoter-*lacZ* reporter plasmids with or without QscRbox (pJL111 and pJL112), the fragments from -509 to +41 (for pJL111) and from -466 to +41 (for pJL112) relative to the *qscR* translation start codon were cloned into *Xba*I/*Hin*dIII site of pQF50. To construct pJL102, a QscRbox-deleted version of pJL101 (Lee et al., 2006), a fragment from –275 to +39 relative to the PA1897 translation start codon was cloned into *Sal*I/*Sma*I site of pQF50. pJL102 was exactly the same as pJL101 except that half of QscRbox was deleted. To construct the RhlR expression plasmid, pJN105R, we used the same expression plasmid, pJN105, which was used for pJN105L and pJN105Q (Lee et al., 2006). The *rhlR* ORF was amplified with the primers containing *Eco*RI and *Xba*I sites and cloned into *EcoR*I-*Xba*I-digested pJN105. The sizes, orientations and integrity of all constructs were confirmed by restriction patterns and DNA sequencing.

RNA isolation and real-time PCR analysis

For the real-time PCR analysis, cells were inoculated into fresh medium from seed culture (initial OD_{600} = 0.01) and cultivated; samples were collected at the indicated growth points for RNA extraction. The sampled cells (about 2×10^9) were directly mixed with RNA Protect Bacteria reagent (Qiagen) to stabilize the RNA and disrupted by lysozyme treatment and sonication. RNA was purified by RNeasy mini columns (Qiagen) and contaminated DNA was removed by the on-column DNase I (Qiagen) and RQ1 DNase I (Promega) digestion. DNase I was removed by purification in another RNeasy column. The purity and integrity of RNA was confirmed by agarose gel electrophoresis and the absence of genomic DNA contamination was confirmed by the lack of amplification in PCR reactions with primers to a ribosomal protein gene, *rplU*. For cDNA synthesis, 12 μg of purified RNA was annealed with 750 ng of semi-random decamer primers of 75% G+C content [5′-$(NS)_5$-3′], which was mixed with 750 U of Superscript II reverse transcriptase (Life Technologies), 1 × 1st strand buffer, 10 mM DTT, 0.5 mM dNTPs, and 1U RNaseIN (Ambion) in 30 μl reaction volume and incubated at 25°C for 10 min, 37°C for 1 h, 42°C for 1 h, and then 70°C for 10 min (for the inactivation of enzyme). After RNA removal by alkaline hydrolysis, final cDNA products were purified by the Qiaquick PCR purification kit (Qiagen) and qualified on agarose gel. For the real-time PCR analysis, primers for PA1897, *qscR*, and *nadB* (PA0761) genes were designed using Primer Express software (Taqman). Each real-time PCR reaction included 1 ng of cDNA as template, 300 nM primers, and 1× SYBR green PCR amplification master mix (Applied Biosystems) in 25 μl, and was performed in 96-well optical plates with a real-time PCR machine (Applied Biosystems Model 7000). PCR conditions were as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C (denaturation step), and 1 min at 60°C (annealing and extension steps). A fixed amount of genomic DNA (0.001, 0.01, 0.1, 1, 10, 100 ng) was co-quantified as a standard and the expression level of *nadB* was used as an internal control for normalization.

β-galactosidase activity assay

The β-galactosidase activity of reporter strains was measured by using a Galacto-Light Plus™ kit (Tropix) as described elsewhere (Choi et al., 2011; Lee et al., 2006). To prepare the *E. coli* reporter strains, *E. coli* DH5α cells were transformed with two compatible plasmids, one of the regulator-expressing plasmids (pJN105L, pJN105R, or pJN105Q) and one of the promoter-*lacZ* fusion plasmids (pJL101, pJL102, pJL111, pJL112, or pSC11) in pairs as indicated. For the β-galactosidase assay, the reporter *E. coli* cells were inoculated into fresh medium at an initial OD_{600} = 0.04 and grown to $OD_{600} \approx 0.3$. Then, 0.4% L-arabinose and the synthetic signals or signal extracts were added at the indicated concentration for 2 h, and β-galactosidase activity was measured using a Galacto-Light Plus™ kit and the recommended procedure. The *P. aeruginosa* reporter strains were prepared by transforming PAO-MW1 cells with the promoter fusion plasmid (pJL101 or pSC11). The reporter cells were inoculated into fresh medium at an initial OD_{600} = 0.04 from seed culture and cultivated at 37°C with vigorous shaking. Cells were taken at the indicated OD and β-galactosidase activity was assayed by a Galacto-Light Plus™ kit. β-Galactosidase activity was presented in luminescence per OD_{600}.

Extraction of QS signals

To extract whole acyl-HSLs from *P. fluorescens 2-79* and *B. vietnamiensis* strains, 20 ml of each cell culture at OD_{600} = 3.0 was extracted twice with acidified ethyl acetate; the extract was evaporated to dryness and dissolved in 2 ml of acidified ethyl acetate. Because the signal extract was concentrated from 20 ml to 2 ml, we defined this concentration as 10×.

RESULTS

QscR auto-activates its own expression

We investigated whether or not the expression of the *qscR* gene is under the control of QS regulation. We tested this using the recombinant *E. coli* system (Lee et al., 2006), where each of LasR, RhlR, or QscR cloned downstream of arabinose-inducible promoter was introduced for expression in one plasmid and the *qscR* promoter-*lacZ* fusion was co-introduced as a target promoter in the other compatible plasmid (Table 1). Unlike *rhlR*, which is activated by LasR (Fuqua and Greenberg, 2002), *qscR* was activated by QscR, its own product in the presence of 3OC12 (Fig. 1). This demonstrated that QscR auto-activates its own expression. *qscR* is divergently oriented from PA1897, a QscR regulon gene (Lee et al., 2006), and the length of the intergenic region between *qscR* and PA1897 is 761 bp. Since only one QscR-binding sequence (QscRbox) was found within the intergenic region, we suspected that QscR activates two genes on the single QscRbox. The reporter fusion assay with QscRbox deletion showed that QscR activates two genes bidirectionally on the single binding site, whereas the activating strength differed greatly depending on the direction (Fig. 2). Similar bidirectional activation by single binding was

Table 1. Strains and Plasmids used in this study

Strains, Plasmids	Genotype	Reference
P. aeruginosa		
PAO1	Wild type prototroph	Pearson et al. (1997)
PAO *lasR rhlR*	*lasR, rhlR* double mutant of PAO1,	Schuster et al. (2003)
PAO-MW1	*lasI, rhlI* double mutant of PAO1 (Whiteley et al., 1999)	
P. fluorescens		
2-79	A type of *P. fluorescens*	Khan et al. (2005)
B. vietnamiensis		
G4	A type of *B. vietnamiensis*	Conway and Greenberg (2002)
E. coli		
DH5α	*supE44* Δ*lac*U169 (*ϕ*80*lacZ*ΔM15)	Sambrook et al. (1989)
	hsdR17 recA1 endA1 gyrA96 thi-1 relA1	
Plasmids		
pQF50	Broad-host-range *lacZ* transcriptional fusion vector, Ap^R	Farinha and Kropinski (1990)
pJL101	Broad-host-range PA1897-*lacZ* reporter in pQF50, Ap^R	Lee et al. (2006)
pJL102	QscRbox deletion of pJL101, Ap^R	This work
pJL111	Broad-host-range *qscR-lacZ* reporter in pQF50, Ap^R	This work
pJL112	QscRbox deletion of pJL111, Ap^R	This work
pSC11	Broad-host-range *lasI-lacZ* reporter in pQF50, Ap^R	Chugani et al. (2001)
pJN105	*araC*-pBAD cassette cloned in pBBR1MCS-5, Gm^R	Newman and Fuqua (1999)
pJN105Q	*qscR* in pJN105, Gm^R	Lee et al. (2006)
pJN105L	*lasR* in pJN105, Gm^R	Lee et al. (2006)
pJN105R	*rhlR* in pJN105, Gm^R	Schuster and Greenberg (2007)

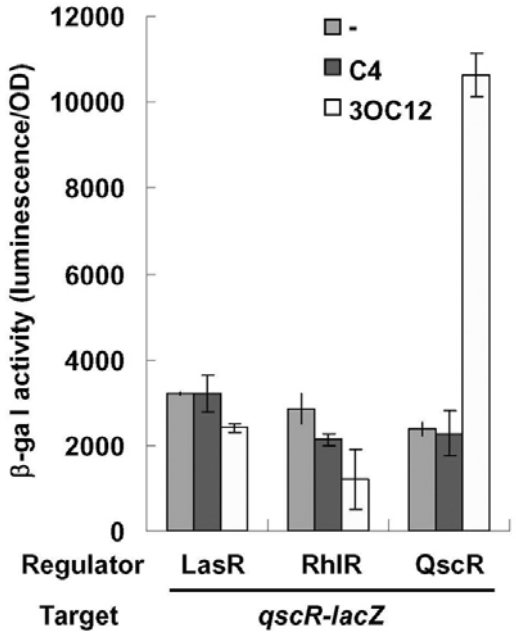

Fig. 1. Autoregulation of *qscR* gene by QscR. Either the LasR-, RhlR-, or QscR-expressing plasmid (pJN105L, pJN105R, and pJN105Q, respectively) was co-transformed into *E. coli* DH5α cells together with the *qscR* promoter-*lacZ* fusion plasmids (pJL111) in pairs. The recombinant cells were grown to $OD_{600} \approx 0.3$ and the indicated signals and 0.4% L-arabinose were added. After further cultivation for 2 h with vigorous shaking, β-galactosidase activity was measured.

observed on the LasR-binding site of the *lasI-rsaL* intergenic region (Schuster et al., 2004) and on the presumed RhlR-binding site of the *phzM-phzA1* intergenic region (Schuster et al., 2003), where only one putative RhlR-binding sequence was found (Schuster et al., 2004; Whiteley and Greenberg, 2001).

QscR can be activated independently of LasR, insofar as the signal is present

Since the auto-activation of the *qscR* expression could boost the QscR pathway, we assumed that the QscR regulon might be activated by a low concentration of signals. Early experiments showed that the *lasR* mutation reduced the expression of LasI and the production of 3OC12 ($\approx$ 7 nM) (Pearson et al., 1995). When we examined the expression of PA1897 in *lasR*$^-$ *rhlR*$^-$ double mutant (PAO *lasR rhlR*), while the low level production of 3OC12 could induce PA1897 transcription only at late stationary phase, it was greatly enhanced by supplementing 3OC12 (Fig. 3A). When we investigated whether or not the *qscR* expression itself could be enhanced by the exogenous signal in *lasR*$^-$ *rhlR*$^-$ double mutant, the *qscR* expression was also enhanced by the exogenous addition of 3OC12 like PA1897 (Fig. 3B). This suggested that QscR can activate its regulon genes independently of LasR or RhlR, insofar that the signal capable of activating it is supplied.

Non-self signals from other species can activate QscR regulon

Since QscR uses 3OC12 as a signal, QscR regulon is co-activated together with the LasR regulon in a single culture of *P. aeruginosa*, where self-signaling is the sole signal source. In contrast, in a mixed population of multiple species, *P. aeruginosa* can be exposed to non-self signals from other species.

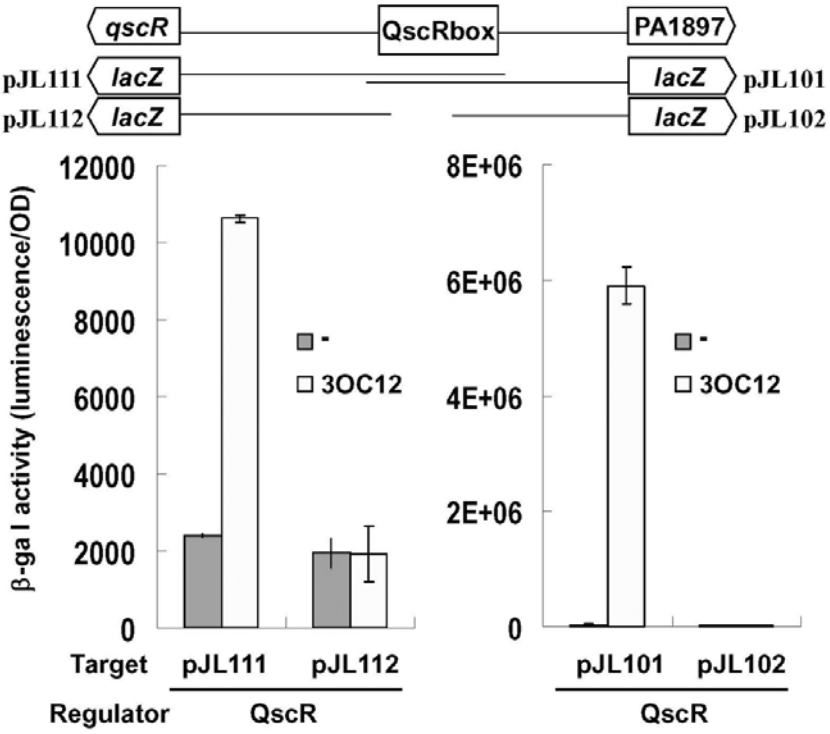

Fig. 2. Bidirectional activation of *qscR* and PA1897 on the single QscR-binding site (QscRbox). The PA1897 promoter-*lacZ* fusion (pJL101) and the *qscR* promoter-*lacZ* fusion (pJL111) and their QscRbox-deleted versions (pJL102 and pJL112, respectively) were co-transformed into *E. coli* DH5α with pJN105Q (QscR-expressing plasmid). After cultivation to $OD_{600} \approx 0.3$, addition of 3OC12 (2 μM) and 0.4% L-arabinose, and 2-hour incubation with vigorous shaking, β-galactosidase activity was measured.

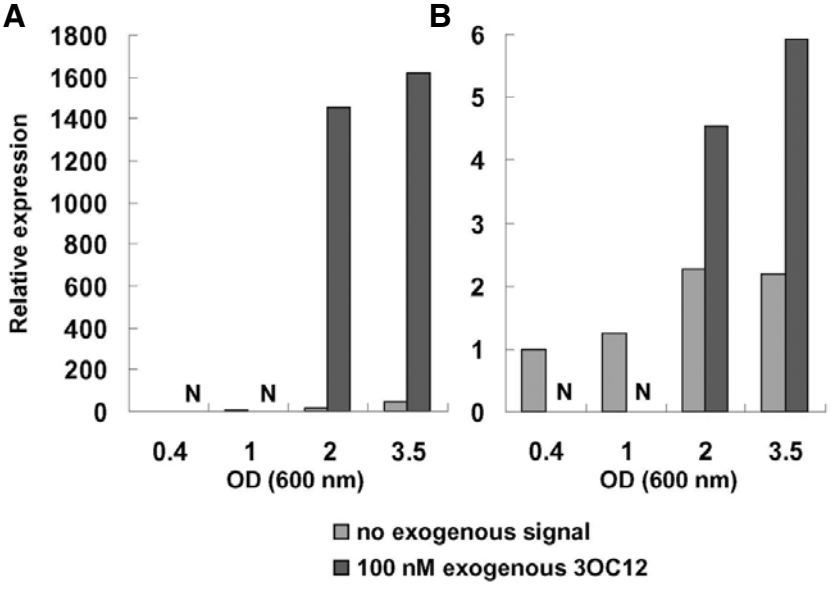

Fig. 3. *las/rhl*-independent activation of QscR regulon. The expression of PA1897 (A) and *qscR* (B) along the growth was measured in *lasR*⁻ *rhlR*⁻ double mutant strain (PAO *lasR rhlR*) by real-time PCR analysis. For the measurement of the induced level, 100 nM 3OC12 was exogenously added into the culture. N, not measured.

QscR has broader signal specificity than LasR and can respond to some non-self signals, such as C10 and C12, which *P. aeruginosa* doesn't produce (Lee et al., 2006). Some bacteria that have a similar habitat as *P. aeruginosa* in nature have been suggested to produce these signal molecules (Conway and Greenberg, 2002; Khan et al., 2005; Mattiuzzo et al., 2011). One of them, *P. fluorescens 2-79*, has been known to produce many acyl-HSL, such as *N*-3-hydroxyhexanoyl HSL (3OHC6), *N*-hexanoyl HSL (C6), *N*-3-hydroxyheptanoyl HSL (3OHC7), *N*-3-hydroxyoctanoyl HSL (3OHC8), *N*-octanoyl HSL (C8), and *N*-3-hydroxydecanoyl HSL (3OHC10), but not 3OC12 and C4, the *P. aeruginosa* signals (Khan et al., 2005). To see the interspecies communication between *P. aeruginosa* and *P. fluorescens*, we extracted the whole signals from *P. fluorescens* and added them to recombinant *E. coli* that harbored QscR expressing plasmid and PA1897-*lacZ* fusion. The whole mixture of *P. fluorescens* signals was able to activate QscR (Fig. 4A). In that the previous study showed that QscR responds to 3OC10 and C10 (Lee et al., 2006), it seemed that 3OHC10 of *P. fluorescens* signals might be the most probable signal to activate QscR.

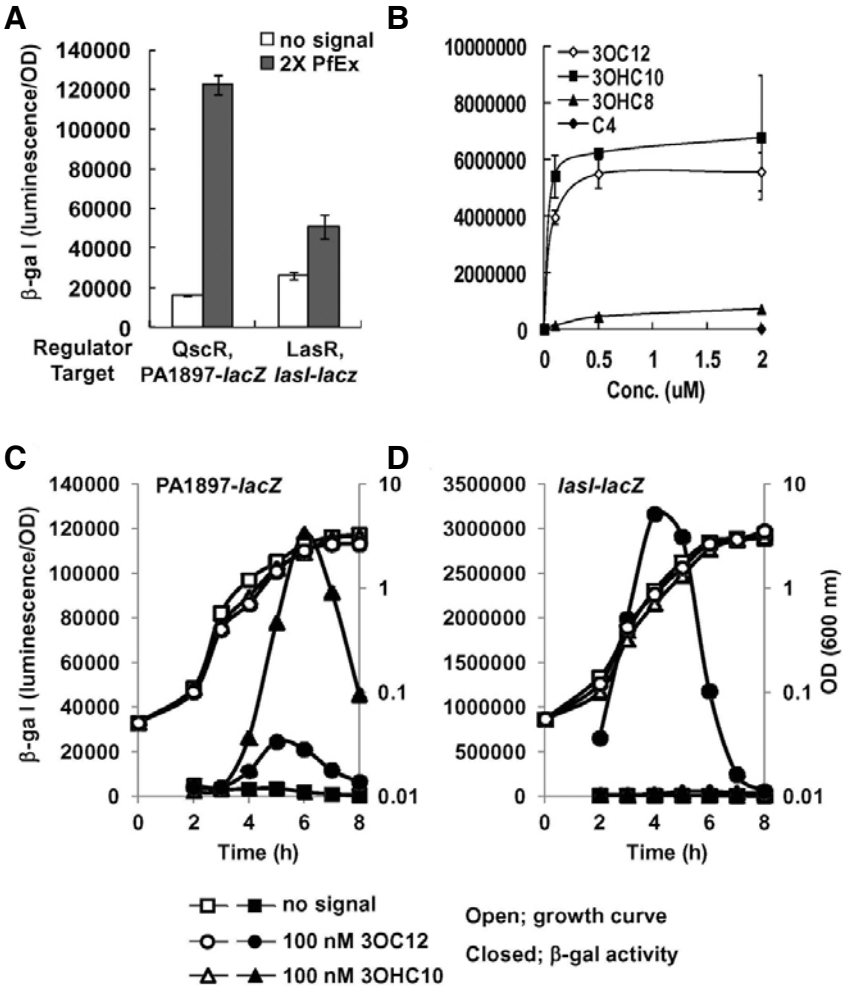

Fig. 4. Signals from *fluorescens 2-79* and 3OHC10 activate QscR preferentially to LasR. (A) pJN105Q (for QscR expression) and pJL101 (PA1897-*lacZ*), or pJN105L (for LasR expression) and pSC11 (*lasI-lacZ*) were co-transformed into *E. coli* DH5α in pairs. This recombinant *E. coli* strain was grown to OD_{600} ≈ 0.3 and the whole signal extract of *P. fluorescens 2-79* (PfEx) was treated at 2× concentration together with 0.4% L-arabinose. After 2-hour incubation with vigorous shaking, β-galactosidase activity was measured. (B) *E. coli* reporter cells harboring pJN105Q and pJL101 were treated with synthetic 3OHC10 and 3OHC8 at various concentrations. In (C) and (D), the *P. aeruginosa* signal mutant strain (PAO-MW1) carrying pJL101 (C) or pSC11 (D) was grown with 100 nM 3OHC10. Cells were sampled at regular intervals throughout the growth, and β-galactosidase activity and OD_{600} were measured. These experiments were performed at least three times and the most representative results were presented.

When we used synthetic 3OHC10 instead of whole signal extract, 3OHC10 activated QscR as strongly as 3OC12 did (Fig. 4B). To see this effect in *P. aeruginosa*, we introduced either PA1897 promoter- or *lasI* promoter-*lacZ* fusion into signal mutant strain (PAO-MW1) to monitor the activity of QscR and LasR, respectively. When we treated the PAO-MW1 cells harboring the PA1897-*lacZ* or *lasI-lacZ* fusion with 3OHC10, 3OHC10 strongly activated QscR but failed to significantly activate LasR (Figs. 4C and 4D). Interestingly, 3OHC10 activated QscR much more strongly than 3OC12 did (Fig. 4C).

Another bacterium, *Burkholderia vietnamiensis*, has also been reported to produce several acyl-HSLs including C8, C10, and C12, but not C4 and 3OC12 (Conway and Greenberg, 2002). We extracted the whole signals from *B. vietnamiensis* and tested their ability to activate LasR and QscR using the recombinant *E. coli* reporter. The whole signal mixture of *B. vietnamiensis* was able to preferentially activate QscR (Fig. 5A). Since the most abundant acyl-HSL species of *B. vietnamiensis* was C10 and QscR was reported to respond to C10 more strongly than to 3OC12 (Conway and Greenberg, 2002; Lee et al., 2006), we investigated the effect of synthetic C10 on the LasR and QscR activities in PAO-MW1 strain harboring PA1897-*lacZ* or *lasI-lacZ* fusion as in Figs. 4C and 4D. As shown in Figs. 5B and 5C, 100 nM C10 activated only QscR selectively. In comparison with 3OC12, the whole signal extract of *B. vietnamiensis* activated QscR much more strongly than 100 nM 3OC12 (Fig. 6A). In the same condition, it activated LasR less than 3OC12 (Fig. 6B). These results demonstrate that *P. aeruginosa* can sense non-*P. aeruginosa* signals through QscR, and the non-*P. aeruginosa* QS signals produced by other species, such as 3OHC10 or C10, can activate QscR preferentially.

DISCUSSION

The first study on QscR showed that it represses the activation of some LasR- and RhlR-dependent QS genes (Chugani et al., 2001). While this repression by QscR was suggested to be achieved in a direct fashion through heterodimer formation with LasR or RhlR (Ledgham et al., 2003), an independent study suggested that QscR could influence the expression of many genes in an indirect manner, presumably through the functions of QscR-regulon genes (Lee et al., 2006; Lequette et al., 2006). To operate its regulon, QscR requires the cognate signal. In pure culture, *P. aeruginosa* cells themselves are the sole signal source and the available signals are 3OC12 and C4. 3OC12 is a triggering signal that initiates the QS response and commonly activates both LasR and QscR. Since QscR is presumed to be expressed at lower levels and later than LasR (Oinuma and Greenberg, 2011), the QS signaling should be led by LasR in pure culture conditions. Most information about *P. aeruginosa*

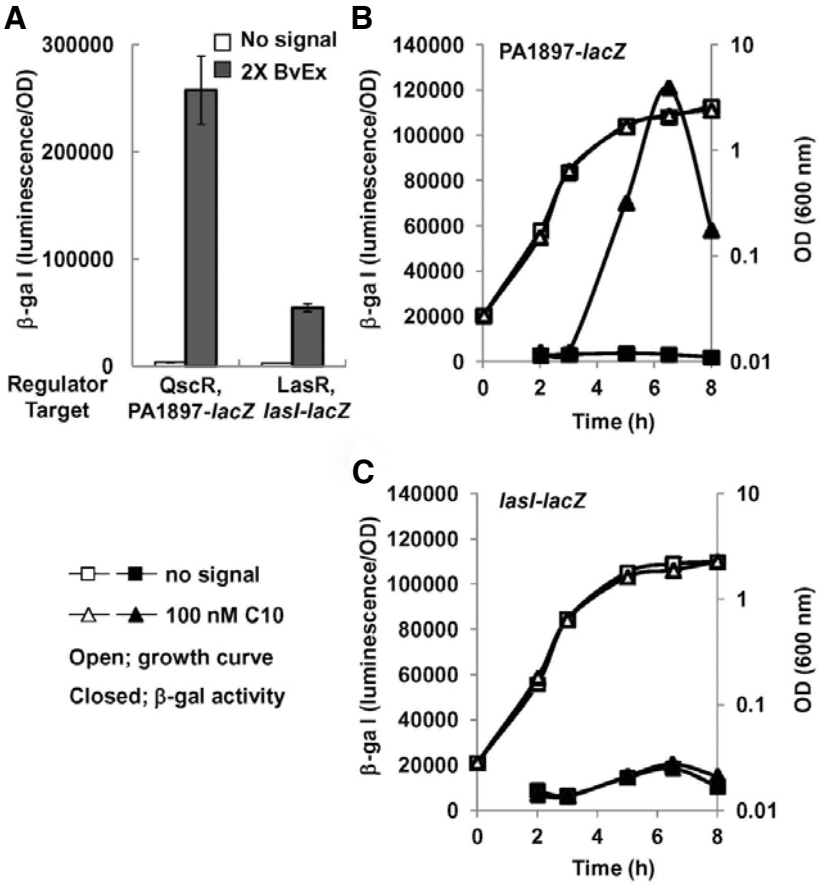

Fig. 5. Preferential activation of QscR by *B. vietnamiensis* signal extract and C10. (A) as in Fig. 4A, the *E. coli* reporter cells harboring pJN105Q and pJL101 or pJN105L and pSC11 were grown to $OD_{600} \approx 0.3$ and the whole signal extract of *B. vietnamiensis* (BvEx) was treated at 2 × concentration together with 0.4% L-arabinose. After 2-hour incubation with vigorous shaking, β-galactosidase activity was measured. As in Figs. 4C and 4D, the PAO-MW1 cells carrying either pJL101 (B) or pSC11 (C) were grown with 100 nM C10. Cells were sampled at regular intervals throughout the growth, and β-galactosidase activity and OD_{600} were measured. These experiments were performed at least three times and the most representative results were presented.

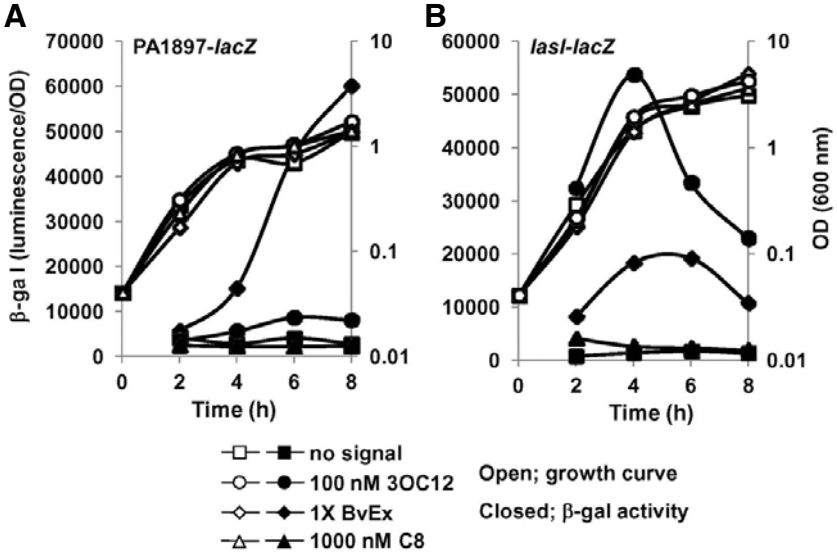

Fig. 6. Heterologous signals from *B. vietnamiensis* activate QscR more strongly than 3OC12, an authentic *P. aeruginosa* signal. The PAO-MW1 cells carrying either pJL101 (A) or pSC11 (B) were grown with BvEx, 1 μM C8, or 100 nM 3OC12. Cells are sampled at regular intervals throughout the growth, and β-galactosidase activity and OD_{600} were measured. These experiments were performed at least three times and the most representative results were presented.

QS signaling has been obtained from this situation. However, a fundamental difference of QscR from LasR is the broader signal specificity, suggesting that QscR might respond to signals produced by other bacteria that co-exist with *P. aeruginosa* (Lee et al., 2006; Oinuma and Greenberg, 2011). In this study, we showed that QscR auto-activates its own expression (Figs. 1 and 3), which suggested a situation where conventional QS of *P. aeruginosa* can be altered by a multi-species habitat. In a mixed bacterial population, some non-*P. aeruginosa* signals derived from other species can preferentially activate QscR. For example, some bacteria including *P. fluorescens*, *B. vietnamiensis*, and *Roseobacter gallaeciencis* have been reported

to produce signals such as C10, C12, and 3OHC10, which can preferentially activate QscR to LasR (Venturi et al., 2004; Wagner-Dobler et al., 2005). Since QscR can auto-activate its expression in the presence of its signal, the QscR regulon may be turned on independently of the LasR-I system. The earlier and stronger activation of QscR may antagonize the conventional QS signaling pathway led by LasR because QscR represses some genes of LasR and RhlR regulons (Chugani et al., 2001; Lequette et al., 2006). As the functions of LasR and RhlR regulons are related to the production of virulence factors, the strong and LasR-I-independent activation of QscR may reconcile cells with accordable neighbors. This means that *Pseudomonas* can differently betray its humor depending on its neighbors, by sensing them through QscR.

Although QscR has been known as an orphan quorum receptor, QscR is not a real orphan because it has a cognate signal and it may be oriented to sense a broader spectrum of signals. Similarly, other orphan receptors may function as ports for foreign signals in mixed populations and define the particular relationship among different species. This may be an important role for orphan receptors yet to be unraveled. Extensive genome sequencing of bacteria showed the existence of orphan receptors is quite common. This implies that the QS mechanism needs to be studied in terms of the mixed population in a natural habitat.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant number; 2010-0006622). This work was also supported by a National Research Foundation of Korea Grant funded by the Korean Government (2010-0015901).

REFERENCES

Choi, Y., Park, H.Y., Park, S.J., Kim, S.K., Ha, C., Im, S.J., and Lee, J.H. (2011). Growth phase-differential quorum sensing regulation of anthranilate metabolism in *Pseudomonas aeruginosa*. Mol. Cells *32*, 57-65.

Chugani, S.A., Whiteley, M., Lee, K.M., D'Argenio, D., Manoil, C., and Greenberg, E.P. (2001). QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA *98*, 2752-2757.

Conway, B.A., and Greenberg, E.P. (2002). Quorum-sensing signals and quorum-sensing genes in Burkholderia vietnamiensis. J. Bacteriol. *184*, 1187-1191.

Daniels, R., Vanderleyden, J., and Michiels, J. (2004). Quorum sensing and swarming migration in bacteria. FEMS Microbiol. Rev. *28*, 261-289.

Farinha, M.A., and Kropinski, A.M. (1990). Construction of broad-host-range plasmid vectors for easy visible selection and analysis of promoters. J. Bacteriol. *172*, 3496-3499.

Fuqua, C. (2006). The QscR quorum-sensing regulon of *Pseudomonas aeruginosa*: an orphan claims its identity. J. Bacteriol. *188*, 3169-3171.

Fuqua, C., and Greenberg, E.P. (2002). Listening in on bacteria: acyl-homoserine lactone signalling. Nat. Rev. Mol. Cell. Biol. *3*, 685-695.

Hentzer, M., Wu, H., Andersen, J.B., Riedel, K., Rasmussen, T.B., Bagge, N., Kumar, N., Schembri, M.A., Song, Z., Kristoffersen, P., et al. (2003). Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J. *22*, 3803-3815.

Khan, S.R., Mavrodi, D.V., Jog, G.J., Suga, H., Thomashow, L.S., and Farrand, S.K. (2005). Activation of the phz operon of Pseudomonas fluorescens 2-79 requires the LuxR homolog PhzR, N-(3-OH-Hexanoyl)-L-homoserine lactone produced by the LuxI homolog PhzI, and a cis-acting phz box. J. Bacteriol. *187*, 6517-6527.

Ledgham, F., Ventre, I., Soscia, C., Foglino, M., Sturgis, J.N., and Lazdunski, A. (2003). Interactions of the quorum sensing regulator QscR: interaction with itself and the other regulators of *Pseudomonas aeruginosa* LasR and RhlR. Mol. Microbiol. *48*, 199-210.

Lee, J.H., Lequette, Y., and Greenberg, E.P. (2006). Activity of purified QscR, a Pseudomonas aeruginosa orphan quorum-sensing transcription factor. Mol. Microbiol. *59*, 602-609.

Lequette, Y., Lee, J.H., Ledgham, F., Lazdunski, A., and Greenberg, E.P. (2006). A distinct QscR regulon in the *Pseudomonas aeruginosa* quorum-sensing circuit. J. Bacteriol. *188*, 3365-3370.

Mattiuzzo, M., Bertani, I., Ferluga, S., Cabrio, L., Bigirimana, J., Guarnaccia, C., Pongor, S., Maraite, H., and Venturi, V. (2011). The plant pathogen *Pseudomonas fuscovaginae* contains two conserved quorum sensing systems involved in virulence and negatively regulated by RsaL and the novel regulator RsaM. Environ. Microbiol. *13*, 145-162.

Newman, J.R., and Fuqua, C. (1999). Broad-host-range expression vectors that carry the L-arabinose-inducible *Escherichia coli araBAD* promoter and the araC regulator. Gene *227*, 197-203.

Oinuma, K., and Greenberg, E.P. (2011). Acyl-homoserine lactone binding to and stability of the orphan *Pseudomonas aeruginosa* quorum-sensing signal receptor QscR. J. Bacteriol. *193*, 421-428.

Pearson, J.P., Passador, L., Iglewski, B.H., and Greenberg, E.P. (1995). A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA *92*, 1490-1494.

Pearson, J.P., Pesci, E.C., and Iglewski, B.H. (1997). Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J. Bacteriol. *179*, 5756-5767.

Sambrook, J.E., Fritsch, F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, N. Y., USA: Cold Spring Harbor Laboratory Press)

Schuster, M., and Greenberg, E.P. (2006). A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. Int. J. Med. Microbiol. *296*, 73-81.

Schuster, M., and Greenberg, E.P. (2007). Early activation of quorum sensing in *Pseudomonas aeruginosa* reveals the architecture of a complex regulon. BMC Genomics *8*, 287.

Schuster, M., Lostroh, C.P., Ogi, T., and Greenberg, E.P. (2003). Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. J. Bacteriol. *185*, 2066-2079.

Schuster, M., Urbanowski, M.L., and Greenberg, E.P. (2004). Promoter specificity in *Pseudomonas aeruginosa* quorum sensing revealed by DNA binding of purified LasR. Proc. Natl. Acad. Sci. USA *101*, 15833-15839.

Smith, R.S., and Iglewski, B.H. (2003). *P. aeruginosa* quorum-sensing systems and virulence. Curr. Opin. Microbiol. *6*, 56-60.

Venturi, V. (2006). Regulation of quorum sensing in Pseudomonas. FEMS Microbiol. Rev. *30*, 274-291.

Venturi, V., Friscina, A., Bertani, I., Devescovi, G., and Aguilar, C. (2004). Quorum sensing in the *Burkholderia cepacia* complex. Res. Microbiol. *155*, 238-244.

Wagner, V.E., Bushnell, D., Passador, L., Brooks, A.I., and Iglewski, B.H. (2003). Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. J. Bacteriol. *185*, 2080-2095.

Wagner-Dobler, I., Thiel, V., Eberl, L., Allgaier, M., Bodor, A., Meyer, S., Ebner, S., Hennig, A., Pukall, R., and Schulz, S. (2005). Discovery of complex mixtures of novel long-chain quorum sensing signals in free-living and host-associated marine alphaproteobacteria. Chembiochem *6*, 2195-2206.

Whiteley, M., and Greenberg, E.P. (2001). Promoter specificity elements in *Pseudomonas aeruginosa* quorum-sensing-controlled genes. J. Bacteriol. *183*, 5529-5534.

Whiteley, M., Lee, K.M., and Greenberg, E.P. (1999). Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA *96*, 13904-13909.