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Quorum-sensing antagonist (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone influences siderophore biosynthesis in *Pseudomonas putida* and *Pseudomonas aeruginosa*

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Abstract Siderophore synthesis of *Pseudomonas putida* F1 was found to be regulated by quorum sensing since normalized siderophore production (per cell) increased 4.2-fold with cell density after the cells entered middle exponential phase; similarly, normalized siderophore concentrations in *Pseudomonas aeruginosa* JB2 increased 28-fold, and a 5.5-fold increase was seen for *P. aeruginosa* PAO1. Further evidence of the link between quorum sensing and siderophore synthesis of *P. putida* F1 was that the quorum-sensing-disrupter (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone (furanone) from the marine red alga *Delisea pulchra* was found to inhibit the formation of the siderophore produced by *P. putida* F1 in a concentration-dependent manner, with 57% siderophore synthesis repressed by 100 μg/ml furanone. In contrast, this furanone did not affect the siderophore synthesis of *Burkholderia cepacia* G4 at 20–40 μg/ml, and stimulated siderophore synthesis of *P. aeruginosa* JB2 2.5- to 3.7-fold at 20–100 μg/ml. Similarly, 100 μg/ml furanone stimulated siderophore synthesis in *P. aeruginosa* PAO1 about 3.5-fold. The furanone appears to interact with the quorum-sensing machinery of *P. aeruginosa* PAO1 since it stimulates less siderophore synthesis in the *P. aeruginosa qscR* quorum-sensing mutant (QscR is a negative regulator of LasI, an acylated homoserine lactone synthase).

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Introduction

Quorum sensing is the regulation of bacterial gene expression based on production of, and response to, secreted autoinducers (AIs), whose concentrations are proportional to the cell population (Bassler 1999; Miller and Bassler 2001). When cell density is high, the binding of AIs to receptors will induce the genes for phenotypes such as biofilm formation (Davies et al. 1998), swarming motility (Eberl et al. 1996), recombinant protein production (DeLisa et al. 2001), and competence development (Lazazzera and Grossman 1998). *Pseudomonas aeruginosa* produces two quorum-sensing signals: *N*-(3-oxododecanoyl)-L-homoserine lactone (synthesized by LasI and sensed by LasR) and *N*-butanoyl-L-homoserine lactone (synthesized by RhlI and sensed by RhlR) (Whitehead et al. 2001). *Pseudomonas putida* (Elasri et al. 2001) and *Burkholderia cepacia* (Lewenza et al. 1999) have also been shown to produce acylated homoserine lactones (AHLs).

In iron-limited environments, bacteria produce iron-binding chelators, known as siderophores, for iron uptake and transfer into the cell (Moat and Foster 1995); over 500 siderophores have been identified (Lamont et al. 2002). *P. aeruginosa* produces two siderophores, pyochelin (Cox 1980) and pyoverdine (Cox and Adams 1985). *P. putida* A1 has also been shown to produce a pyoverdine-class siderophore, although the peptide chain is different from that in the siderophore of *P. aeruginosa* (Boopathi and Rao 1999). *B. cepacia* strains produce four different types of siderophores, including salicylic acid, cepabactin, pyochelin, and ornibactins (Darling et al. 1998). It has been shown that siderophore synthesis may be regulated by quorum sensing (Stintzi et al. 1998). *P. aeruginosa lasI* and *lasR* mutants both exhibit a 2-fold decrease in pyoverdine synthesis and addition of the quorum-sensing signal, AHL, restored siderophore production (Stintzi et al. 1998).

Several natural brominated furanones (produced by the red marine alga *Delisea pulchra*), and their synthetic derivatives, inhibit AHL-mediated multicellular behavior

(Rice et al. 1999) such as the swarming motility of *Proteus mirabilis* (Gram et al. 1996) and *Serratia liquefaciens* (Givskov et al. 1996) as well as biofilm formation of *Escherichia coli* (Ren et al. 2001) and *P. aeruginosa* (Hentzer et al. 2002, 2003). Furanones interrupt AHL quorum sensing by competing with AHL for binding to the receptor (Manefield et al. 1999). Hentzer et al. (2003) found, using DNA microarrays, that 80% of the *P. aeruginosa* PAO1 genes repressed by the synthetic 4-bromo-5-(bromomethylene)-2(*5H*)-furanone were also induced by AHL (also known as AI-1). In addition, we have found (Ren et al. 2001) that natural (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone (henceforth furanone) interrupts AI-2 quorum sensing based on the borate diester signal (a common quorum sensing system used for interspecies communication) (Bassler 1999). Using DNA microarrays, we found that 79% of the *E. coli* genes repressed by furanone were also induced by AI-2 (Ren et al. 2004). Hence, furanone is a quorum-sensing antagonist since it inhibits both AI-1 (Hentzer et al. 2003) and AI-2 genes (Ren et al. 2004) and phenotypes.

In this study, siderophore concentrations in *P. putida* F1 were found to be quorum-sensing-related since cell-density-normalized siderophore concentrations increased with the cell population, and they were inhibited by the quorum sensing antagonist, plant-derived furanone in a concentration-dependent manner. In contrast, siderophore synthesis in both *P. aeruginosa* PAO1 and *P. aeruginosa* JB2 was stimulated by furanone. To investigate the mechanism of induction of siderophore synthesis, a *P. aeruginosa* PAO1 *qscR* mutant was studied for siderophore synthesis (*qscR* encodes a repressor of the *P. aeruginosa* quorum-sensing signal producer LasI; Chugani et al. 2001).

Materials and methods

Bacterial strains, culture media, and growth rate

P. putida F1 (Spain et al. 1989), *P. aeruginosa* JB2 (Hickey and Focht 1990), *P. aeruginosa* PAO1 (Holloway 1969), and *B. cepacia* G4 (Shields et al. 1989) were used to study the effect of furanone on siderophore synthesis. *P. aeruginosa* PAOR3 (*qscR* mutant of PAO1) and PAOR3/pKL9 carrying *qscR* under control of the *tac* promoter were used to study the effect of the quorum sensing repressor on siderophore synthesis (Chugani et al. 2001). Minimal standard succinate medium (Meyer and Abdallah 1978), which consists of 6 g/l K_2HPO_4, 3 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$, 0.2 g/l $MgSO_4{\cdot}7H_2O$, and 4 g/l succinic acid, was used for measuring siderophore synthesis. The optical density (OD) at 600 nm was used to measure the cell density, and the specific growth rate was calculated based on the linear part of the natural logarithm of OD_{600} versus time (OD_{600} from 0.01 to 0.4).

Furanone synthesis

(*5Z*)-4-Bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone of *D. pulchra* (see Fig. 1a; inset) was synthesized as described previously (Beechan and Sims 1979; Manny et al. 1997; Ren and Wood 2004). The furanone was dissolved in 95% ethanol to 14.9 mg/ml and stored at 4°C.

Spectrophotometric siderophore assay

This assay was adapted from the protocol of Stintzi et al. (1998). The absorption spectrum of *Pseudomonas* siderophores has a peak at a specific wavelength, such as 400 nm for pyoverdine produced by *P. fluorescens* (Meyer and Abdallah 1978) and 405 nm for pyoverdine produced by *P. aeruginosa* PAO1 (Stintzi et al. 1998). In this study, the absorbance spectrum (200–800 nm, DU 640 spectrophotometer; Beckman Coulter, Fullerton, Calif.) of supernatants for *P. putida* F1, *P. aeruginosa* PAO1, and *B. cepacia* G4 also showed a peak at 405 nm, and the peak for *P. aeruginosa* JB2 was 400 nm. Hence, OD at 405 nm was used to measure the siderophore concentrations of *P. putida* F1 and *B. cepacia* G4, as well as that of *P. aeruginosa* PAO1, PAOR3, and PAOR3/pKL9; siderophore synthesis of *P. aeruginosa* JB2 was measured by OD at 400 nm. *P. putida* F1, *B. cepacia* G4, and the *P. aeruginosa* strains were grown in standard succinate medium overnight, then the cells were diluted 1:250 in fresh standard succinate medium containing furanone at 0, 20, 40, or 100 μg/ml. The same amount of ethanol was added to all samples to eliminate solvent effects. One milliliter culture was centrifuged for 1 min at 20,000 *g* in a microcentrifuge tube (Spectrafuge 16M; Labnet International, Edison, N.J.), and the supernatant was then used to determine the siderophore concentration. For the siderophore assay of *P. aeruginosa* PAO1, PAOR3, and PAOR3/pKL9, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added to each culture because the *qscR* gene on plasmid pKL9 is under the control of the *tac* promoter (Chugani et al. 2001). Furanone and IPTG were both added upon inoculation of the cells. Each experiment was conducted in duplicate and averaged data are shown with one standard deviation.

Siderophore iron-binding assay

The iron-binding assay was modified from a previously reported protocol in which the binding of Fe^{3+} to siderophore increases the absorbance at 450 nm, and the siderophore is released from the complex by EDTA (Meyer and Abdallah 1978). Thus, $FeCl_3$ (3.25 mM) was added to the *P. putida* F1 supernatant at pH 7.0 followed by the addition of EDTA (0.5 M at pH 7.0), and the absorbance at 450 nm was monitored after each addition.

Purification of the siderophore from *P. putida* F1 supernatants

To confirm the presence of the putative siderophore of *P. putida* F1, the siderophore was purified from the supernatant as described previously (Meyer and Abdallah 1978). The *P. putida* culture was grown for 40 h in standard succinate medium at 30°C with shaking at 120 rpm (Boopathi and Rao 1999), and the pH was maintained from 7 to 7.3 to prevent pigment decomposition (Meyer and Abdallah 1978). Upon harvesting the supernatant, $FeCl_3$ was added to a final concentration of 3.57 mM to transform the putative siderophore to a stable Fe^{3+} complex to facilitate purification. 8-Hydroxyquinoline (5% w/v in chloroform) was used to withdraw Fe^{3+} from the siderophore complex by chelation, leaving the iron-free pigment, which was then further purified by following the reported protocol (Meyer and Abdallah 1978). The purified iron-free pigment was lyophilized, dissolved in 0.1 M sodium acetate buffer (pH 5.3), and the absorbance spectra were measured at 200–500 nm before and after the addition of $FeCl_3$ solution and EDTA solution.

Results

Furanone inhibits siderophore synthesis of *P. putida* F1 with no effect on general growth

In the absence of furanone, *P. putida* F1 was found to produce siderophore. Specific siderophore production (normalized by cell density OD_{405}/OD_{600}) decreased in the first 5.5 h (OD 0–0.5) after inoculation and then increased steadily 4.2-fold with cell density suggesting the siderophore synthesis is quorum-sensing-related (Fig. 1a). The cultures without furanone showed a fluorescent green color 5–6 h after inoculation, and the green color was found to accumulate with increasing cell density, consistent with the previous report that *Pseudomonas* strains produce yellow-green fluorescent siderophores (Meyer and Abdallah 1978).

To study the effect of furanone on siderophore synthesis of *P. putida* F1, furanone at different concentrations (0, 20, 40, and 100 μg/ml) was added upon inoculation of the cells, and the time course of cell growth and siderophore production was measured for 10 h. From 5.5 h to 9.5 h after inoculation (OD 0.5–1.0), the addition of furanone decreased the specific siderophore concentration by 1.7-fold at 20 μg/ml and 40 μg/ml, and 100 μg/ml furanone inhibited the siderophore production 2.3-fold (Fig. 1a). Furanone with concentrations up to 100 μg/ml had no effect on growth of *P. putida* F1 (specific growth rate μ=1.20±0.02 h^{-1} without furanone, 1.11±0.01 h^{-1} at 20 μg/ml, 1.02±0.04 h^{-1} at 40 μg/ml, and 1.05±0.26 h^{-1} at 100 μg/ml).

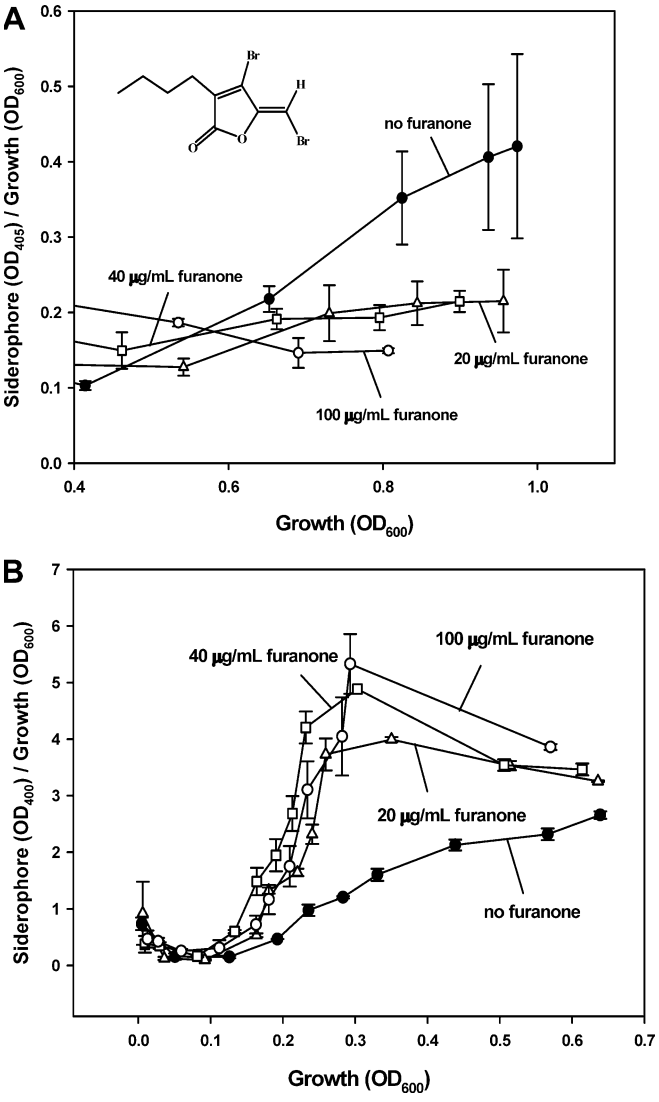

Fig. 1 Cell-normalized siderophore synthesis (siderophore yield/growth) of **a** *Pseudomonas putida* F1 and **b** *Pseudomonas aeruginosa* JB2 with different concentrations of furanone (μg/ml): ● no furanone, ▵ 20, □ 40, ○ 100. *Inset* in **a** indicates the structure of (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone from *Delisea pulchra* [*Z* indicates that the stereoisomer (double bond at carbon 5 of the furanone ring) is Z (zusammen) form, and *5H* indicates a derivative of the basic 2(*5H*) furanone]. Data were collected every hour. *Error bars* 1 SD

Furanone does not interfere with the siderophore measurement

The addition of furanone to *P. putida* F1 cultures caused a color change (the supernatants became more yellowish and turned a slight orange color in the presence of 100 μg/ml furanone). Hence, it was investigated if the color change was caused by direct interaction between the furanone and siderophore and if it influenced siderophore measurement by affecting the OD reading at 405 nm. Furanone was added to the siderophore-containing, cell-free supernatant and the spectrum from 200 nm to 800 nm was measured (Fig. 2). The addition of furanone caused a slight absorbance change at 450–550 nm and this was the

reason for the yellowish color, which is due to absorption of green color at these wavelengths; however, the absorbance at 405 nm was the same for samples with 0, 100, or 200 μg/ml furanone (all contain the same amount of ethanol). Although a possible physical interaction between furanone and siderophore cannot be ruled out, the consistent OD_{405} reading ensures the correct measurement of siderophore concentration with different concentrations of furanone (up to 200 μg/ml). Hence, the lower OD_{405} reading for the samples incubated with furanone was the result of lower siderophore concentrations in the presence of furanone.

Furanone induces siderophore synthesis with *P. aeruginosa* JB2 and *P. aeruginosa* PAO1

In the absence of furanone, cell-normalized production of siderophore in *P. aeruginosa* was about ten times greater than that of *P. putida* F1, and increased 28-fold for JB2 (Fig. 1b) and 5.5-fold for PAO1 (Fig. 3) indicating siderophore synthesis is quorum-sensing-regulated in these strains. It has been reported that the mutation in the quorum sensing genes *luxI* or *luxR* leads to a 2-fold decrease in siderophore production for *P. aeruginosa* PAO1 (Stintzi et al. 1998). Previously, synthetic furanones having structures similar to that of the furanone in this study were shown to inhibit the quorum sensing of *P. aeruginosa* PAO1 (Hentzer et al. 2002, 2003) and to reduce siderophore synthesis by a factor of 10 (Hentzer et al. 2003). Therefore, the furanone used in this study was expected to repress siderophore production in *P. aeruginosa*. However, furanone was found to stimulate siderophore production in *P. aeruginosa* JB2 since 20–100 μg/ml furanone caused a 2.5- to 3.7-fold increase in siderophore synthesis (Fig. 1b). As OD_{405} was not changed by furanone for *P. putida* F1, furanone did not have any significant effect on siderophore measurement of *P. aeruginosa* JB2 with OD_{400} since the spectra were unaltered at this wavelength (data not shown). Furanone has no effect on growth of *P. aeruginosa* JB2 (μ=0.45 h^{-1} with 0, 20, 40, and 100 μg/ml furanone).

To corroborate the induction of siderophore production in *P. aeruginosa* JB2, we studied the effect of furanone on another *P. aeruginosa* strain, PAO1; the furanone induced siderophore production by 3.5-fold at 100 μg/ml (Fig. 3). Similar to siderophore measurement for *P. putida* F1 and *P. aeruginosa* JB2, furanone did not show significant interference with siderophore measurement for *P. aeruginosa* PAO1 with OD_{405} since the spectra did not change at this wavelength (data not shown).

qscR mutation and siderophore production

The reason for the stimulation of siderophore production of *P. aeruginosa* JB2 and PAO1 by furanone is not clear. However, it is possible that the furanone used in this study repressed some negative regulator of quorum sensing and therefore increased siderophore production. QscR is a repressor of the *P. aeruginosa* autoinducer synthase LasI (subject to quorum sensing regulation), and the *qscR* mutant produces *N*-(3-oxododecanoyl)-L-homoserine lactone constitutively (Chugani et al. 2001). Hence, we hypothesized that furanone repressed the *qscR* gene and therefore induced more AI synthesis, which led to an increase in siderophore concentrations. To test this hypothesis, a *qscR* mutant (PAOR3) was tested for its siderophore synthesis in the absence of furanone and compared to the wild type strain, *P. aeruginosa* PAO1, as well as a *qscR* mutant containing plasmid pKL9, which carries the *qscR* gene under the control of the *tac* promoter (Chugani et al. 2001). However, there was no apparent difference in siderophore production among the wild type PAO1, *qscR* mutant PAOR3, and PAOR3 carrying pKL9 to complement the *qscR* gene (Fig. 3) as all three strains

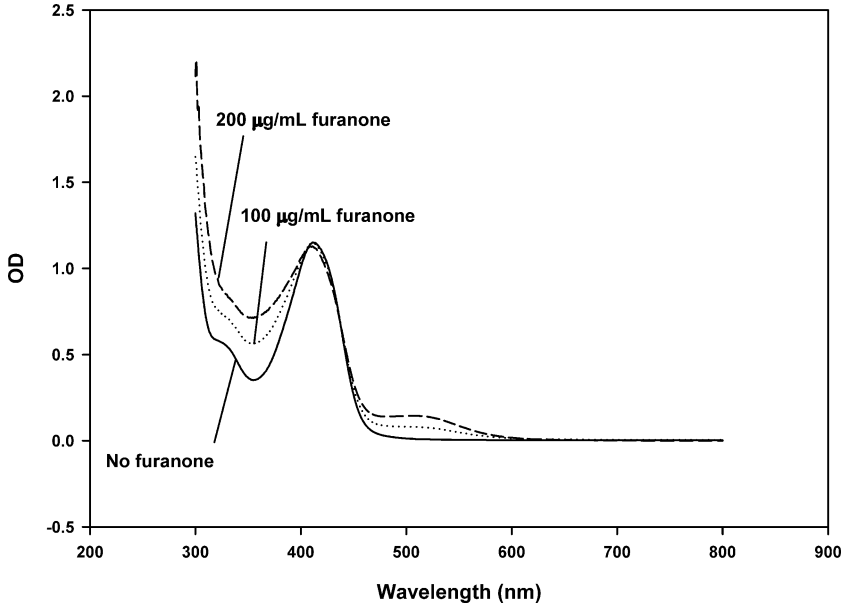

Fig. 2 Effect of furanone on the UV spectrum of *P. putida* F1 siderophore. *Solid line* No furanone, *dashed line* 20 μg/ml furanone , *dotted line* 100 μg/ml furanone

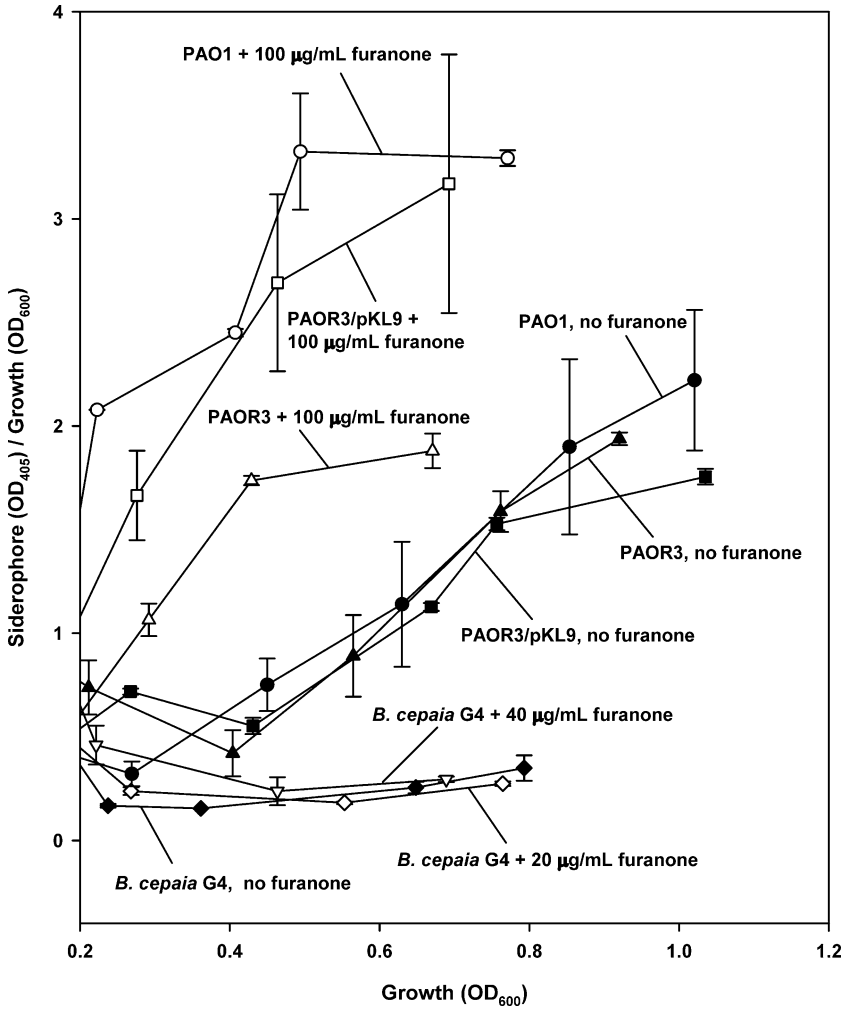

Fig. 3 Cell-normalized siderophore synthesis (siderophore yield/growth) of *P. aeruginosa* PAO1, its *qscR* mutants, and *Burkholderia cepacia* G4 with different concentrations of furanone. ● PAO1 + no furanone, ○ PAO1 +100 µg/ml furanone, ▲ PAOR3 + no furanone, △ PAOR3 +100 µg/ml furanone, ■ PAOR3/pKL9 + no furanone, □ PAOR3/pKL9 +100 µg/ml furanone , ◆ G4 + no furanone, ◊ G4 +20 µg/ml furanone, ▽ G4 +40 µg/ml furanone). The sampling time was about 1 h between each two data points. *Error bars* 1 SD

showed quorum-sensing-related (cell-concentration-dependent) siderophore synthesis.

To study the possible interaction between furanone and QscR further, furanone at 100 µg/ml was added upon inoculation of *P. aeruginosa* PAOR3 and PAOR3/pKL9. As with the wild type strain, furanone induced siderophore synthesis with these two strains. However, there was less induction in the *qscR* mutant PAOR3 (about 2-fold) than the wild type strain PAO1 (3.5-fold) (Fig. 3). When the furanone at 100 µg/ml was added to the PAOR3 carrying pKL9 to complement the *qscR* gene, induction was restored to about 3-fold, similar to that of the wild type strain PAO1 (3.5-fold) (Fig. 3).

Furanone had no effect on the growth of the above *P. aeruginosa* strains except that it slightly decreased the growth of *P. aeruginosa* PAOR3 by 16% at 100 µg/ml (μ=0.84±0.06 h^{-1} without furanone and 0.81±0.08 h^{-1} with 100 µg/ml furanone for *P. aeruginosa* PAO1, 0.84 ±0.06 h^{-1} without furanone and 0.70±0.05 h^{-1} with 100 µg/ml furanone for *P. aeruginosa* PAOR3, and 0.85 ±0.06 h^{-1} without furanone and 0.84±0.21 h^{-1} with 100 µg/ml furanone for *P. aeruginosa* PAOR3/pKL9). Hence, furanone is not toxic to the *P. aeruginosa* strains used in this study.

Furanone has no effect on siderophore synthesis of *B. cepacia* G4

To investigate if furanone interferes with siderophore synthesis by other bacteria, *B. cepacia* G4 was grown with different concentrations of furanone and siderophore concentrations were measured. Interestingly, furanone did not exhibit any significant effect on siderophore synthesis (Fig. 3) and, in the absence of furanone, siderophore synthesis was not closely linked to cell density, indicating that it may not be regulated significantly by quorum sensing. Similar to the other stains tested above, furanone has no effect on growth of *B. cepacia* G4 (μ about 0.9 h^{-1} with 0, 20, or 40 µg/ml furanone). Hence, furanone has different effects on siderophore synthesis in these different bacterial species.

Absorbance spectra of purified siderophore and the interaction between *P. putida* F1 supernatants and iron

To show that *P. putida* F1 produces siderophore, an iron-binding assay was conducted for both supernatants and purified siderophore. The absorbance of the supernatant at

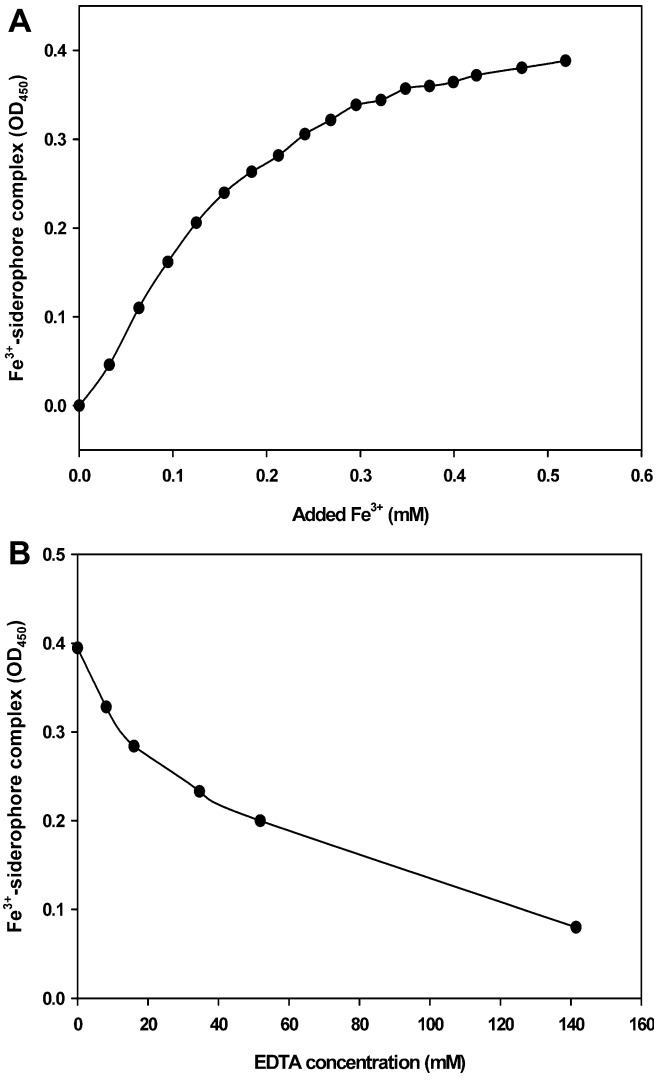

Fig. 4 Change of absorbance at 450 nm by **a** adding Fe^{3+} to *P. putida* F1 supernatants containing siderophore and **b** adding EDTA to the Fe^{3+}-siderophore complex

450 nm increased with the addition of Fe^{3+} and decreased with the addition of EDTA as expected (Fig. 4) based on the behavior of the pyoverdine-like siderophore from *P. fluorescens* (Meyer and Abdallah 1978). The culture was yellow-green at the time it was harvested but changed to brown-red immediately upon addition of $FeCl_3$. During purification of the siderophore, the addition of 8-hydroxyquinoline changed the color of the Fe^{3+}-pigment-containing supernatant from brown-red to yellow-green, which is the characteristic color of pyoverdine (Meyer and Abdallah 1978). The absorbance spectrum of the purified free pigment (dissolved in 0.1 M sodium acetate, pH 5.3) showed one peak in the ultraviolet range at 235 nm with a shoulder at 255 nm and another peak in the visible range at 380 nm with shoulders at 400 nm and 365 nm; this spectrum is very similar to that of *P. fluorescens* pyoverdine (Meyer and Abdallah 1978). With the addition of $FeCl_3$, the peak at 380 nm shifted to 396 nm and the absorbance at 396 nm increased accordingly, while the absorbance spectra in the ultraviolet region showed less change, which is also in agreement with the reported properties of pyoverdine (Meyer and Abdallah 1978). The iron-binding assay using the putative siderophore purified from the *P. putida* F1 supernatant showed results similar to those from unpurified supernatant (data not shown). These results strongly suggest that *P. putida* F1 produces a pyoverdine-like siderophore. Note that the absorption spectrum of the purified *P. putida* F1 siderophore (dissolved in 0.1 M sodium acetate at pH 5.3) has a peak at 380 nm, while the absorption spectrum of the unpurified *P. putida* F1 supernatant (pH around 9) showed a peak at about 405 nm (Fig. 2). The difference in the location of this peak is due to the different pH environment. Meyer and Abdallah (1978) have reported that the absorption of pyoverdine in the visible range was pH dependent, and the position of the peak in this range shifted from 380 nm at pH ≤5.0 to 402 nm at pH 7 and to 410 nm at pH 10. Thus, the different position of the peak in the visible range of the putative siderophore from *P. putida* F1 supernatant also supports the notion that *P. putida* F1 might produce a pyoverdine-like siderophore.

Discussion

In this paper, we show clearly that siderophore synthesis of *P. putida* F1, *P. aeruginosa* JB2, and *P. aeruginosa* PAO1 is growth dependent. It is also shown that the quorum-sensing antagonist furanone represses siderophore synthesis of *P. putida* F1 in a concentration-dependent manner, and this furanone induces siderophore synthesis in both *P. aeruginosa* JB2 and *P. aeruginosa* PAO1. Since the furanone caused less induction of siderophore synthesis in the *qscR* mutant of *P. aeruginosa* PAO1 than the wild type strain, and since full induction was restored by the plasmid-borne *qscR* (Fig. 3), it appears that furanone induced siderophore synthesis through interaction with *qscR* gene or its product QscR. Because the mutation of *qscR* did not cause a significant change in siderophore synthesis (Fig. 3), the *qscR* gene may not control siderophore synthesis directly. Since furanone also induced siderophore synthesis in the *qscR* mutant, there may be other factors controlling siderophore synthesis and subject to the interaction with furanone.

It is interesting that the quorum-sensing antagonist furanone was found to have opposite effects on siderophore production in these *Pseudomonas* bacteria. Siderophore synthesis in *B. cepacia* was found previously to be connected with quorum sensing; however, inactivation of *cepR*, a *lasR* homolog, led to increased siderophore synthesis (Lewenza et al. 1999). Hence, it appears that quorum sensing plays different roles in controlling siderophore production. It is also possible that additional, as yet unidentified, quorum sensing systems may also play a role in siderophore production.

Among the different *Pseudomonas* bacteria that colonize the roots of plants, some are beneficial and some are deleterious or indifferent (Boopathi and Rao 1999). In

iron-limiting environments, the ability to produce siderophore may be critically important for survival. Hence, it is possible that plants may produce chemicals like furanone to selectively stimulate and repress siderophore production and therefore influence the growth of favorable bacteria in the rhizosphere. In the present study, furanone from marine red alga has selective effects on siderophore synthesis. Further study on the effects of furanone may help discover better antagonists for inhibiting plant pathogens or promoting symbiotic bacteria. Since recent reports indicate that by controlling siderophore concentrations, biofilms may be inhibited (Banin and Greenberg 2003), and since pyoverdine concentrations in *P. aeruginosa* regulate at least three virulence factors (Lamont et al. 2002), furanone holds potential to combat disease.

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