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ORIGINAL ARTICLE

Acyl-homoserine lactone-dependent eavesdropping promotes competition in a laboratory co-culture model

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Many *Proteobacteria* use acyl-homoserine lactone (AHL)-mediated quorum sensing to activate the production of antibiotics at high cell density. Extracellular factors like antibiotics can be considered public goods shared by individuals within a group. Quorum-sensing control of antibiotic production may be important for protecting a niche or competing for limited resources in mixed bacterial communities. To begin to investigate the role of quorum sensing in interspecies competition, we developed a dual-species co-culture model using the soil saprophytes *Burkholderia thailandensis* (*Bt*) and *Chromobacterium violaceum* (*Cv*). These bacteria require quorum sensing to activate the production of antimicrobial factors that inhibit growth of the other species. We demonstrate that quorum-sensing-dependent antimicrobials can provide a competitive advantage to either *Bt* or *Cv* by inhibiting growth of the other species in co-culture. Although the quorum-sensing signals differ for each species, we show that the promiscuous signal receptor encoded by *Cv* can sense signals produced by *Bt*, and that this ability to eavesdrop on *Bt* can provide *Cv* an advantage in certain situations. We use an *in silico* approach to investigate the effect of eavesdropping in competition, and show conditions where early activation of antibiotic production resulting from eavesdropping can promote competitiveness. Our work supports the idea that quorum sensing is important for interspecies competition and that promiscuous signal receptors allow eavesdropping on competitors in mixed microbial habitats.
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Introduction

Quorum sensing affords bacteria the ability to control the expression of specific genes in a cell density-dependent manner (Fuqua *et al.*, 1994, 2001; Bassler, 2002; Waters and Bassler, 2005). Many species of *Proteobacteria* use small molecules, acylated homoserine lactones (AHLs), as quorum-sensing signals. AHLs are produced by LuxI family synthases, and specifically interact with cytoplasmic LuxR family transcription factors to influence gene expression. AHL specificity is defined by the nature of the acyl side group. AHLs can diffuse through lipid bilayers and thus can move out of and into cells by diffusion. Because of the signal diffusibility, AHLs must reach a critical environmental concentration before they cause changes in gene expression. It is common that the AHL synthase gene is among the genes activated, creating a positive feedback loop that results in increased production of signal (Engebrecht *et al.*, 1983; Seed *et al.*, 1995; Latifi *et al.*, 1996; Duerkop *et al.*, 2009; Stauff and Bassler, 2011). Thus, AHL signaling can coordinate population-wide changes in a cell-density-dependent manner.

Quorum-sensing-regulated genes are predominated by those required for the production of shared 'public goods', such as secreted or excreted factors. One commonly occurring example is antimicrobials. Quorum-controlled antimicrobials have been described in many saprophytic *Proteobacteria* including *Erwinia carotovora* (Bainton *et al.*, 1992), *Pseudomonas aeruginosa* (Kownatzki *et al.*, 1987; Bainton *et al.*, 1992; Gallagher and Manoil, 2001; Ran *et al.*, 2003; Schuster and Greenberg, 2006), *Burkholderia thailandensis* (*Bt*) (Duerkop *et al.*, 2009) and *Chromobacterium violaceum* (*Cv*) (Latifi *et al.*, 1995; McClean *et al.*, 1997). Although some groups have proposed that antimicrobial activity of

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secondary metabolites is a side effect and the primary function of these compounds is as signals (Davies *et al.*, 2006; Yim *et al.*, 2007), the classic view is that they are used for competition with other strains or species in multi-species environments. This classic view suggests that quorum sensing may be important for interspecies competition. Quorum sensing is best understood in the context of virulence, and few studies have addressed its importance in competition (Mazzola *et al.*, 1992; Moons *et al.*, 2005, 2006; An *et al.*, 2006). The advantage of using quorum sensing to control the production of antimicrobials is unknown, but it may allow a population to coordinate delivery of a sudden killing dose that deprives competitors of the ability to adapt during exposure to subinhibitory antimicrobial concentrations (Hibbing *et al.*, 2010, D An and M Parsek, unpublished). Quorum sensing may also defer production of an antimicrobial to minimize the metabolic cost of production.

We are interested in the connection between quorum sensing and production of antibiotics, and specifically whether quorum-sensing-controlled antibiotics are important for interspecies competition. Thus, we developed a dual-bacterial species model with two soil saprophytes, *Bt* and *Cv*. Although it is not unlikely that these species coexist in nature, we selected this pair of bacteria because we have a base of knowledge about their quorum-sensing systems, about quorum-sensing control of antibiotic synthesis and because these species exhibit similar laboratory growth characteristics. The *Bt* genome encodes three LuxR–LuxI pairs. The BtaI1–R1 pair produces and responds to octanoyl-HSL (C8-HSL). Little is known about the genes controlled by this system, but it facilitates clumping under some conditions (Chandler *et al.*, 2009). BtaI3 is a 3-hydroxy-octanoyl-HSL synthase, but little is known about BtaI3–R3 (Chandler *et al.*, 2009). Finally, BtaR2–I2 senses and produces 3-hydroxy-octanoyl-HSL and 3-hydroxy-decanoyl-HSL (Duerkop *et al.*, 2009). The BtaR2–I2 system activates *btaI2* and a set of genes responsible for the production of a family of hydrophilic antibiotics, the bactobolins, that have activity against a broad range of bacterial species (Duerkop *et al.*, 2009; Seyedsayamdost *et al.*, 2010; Carr *et al.*, 2011) including *Cv* (see below). The most potent of these is bactobolin A (Carr *et al.*, 2011).

Cv has a single AHL circuit, the CviR–CviI quorum-sensing system. This circuit activates genes required for the production of a purple pigment called violacein and related compounds that have broad-spectrum antimicrobial activity (McClean *et al.*, 1997). We found that *Bt* is resistant to purified violacein, but shows sensitivity to other quorum-sensing-dependent factors produced by *Cv*. The CviI-produced AHL signal is hexanoyl-HSL (C6-HSL), and although CviR is a C6-HSL-responsive transcription factor, it is promiscuous and also responds to a number of different AHL signals (McClean *et al.*, 1997; Swem *et al.*, 2009). This promiscuity may allow *Cv* to eavesdrop on other AHL-producing species. There are now a number of examples of *Proteobacteria* with promiscuous LuxR homologs (Pierson *et al.*, 1998; Riedel *et al.*, 2001; Steidle *et al.*, 2001; Venturi *et al.*, 2004; Dulla and Lindow, 2009; Ahlgren *et al.*, 2011; Hosni *et al.*, 2011). It is not known if AHL receptor promiscuity provides any advantage over more signal-specific receptors.

We report here that quorum-sensing-dependent production of antimicrobials can provide a competitive advantage to either *Bt* or *Cv* by inhibiting growth of the other species in co-culture. We also present evidence that although *Bt* and *Cv* produce different AHLs, the promiscuous signal receptor of *Cv* can sense *Bt* signals, and that this ability to eavesdrop on *Bt* can provide a competitive advantage to *Cv*. We describe a mathematical model of our dual species system and use this model to show that eavesdropping can promote fitness during competition as long as the population can produce sufficient antibiotic to kill the competitor. Our results support the idea that quorum sensing is important for interspecies competition and that promiscuous signal receptors promote fitness in some situations by enabling eavesdropping on AHLs produced by competitors.

Materials and methods

Bacterial strains and growth

Strains and plasmids are described in the Supplementary Text and Supplementary Table S1. All bacteria were grown in Luria–Bertani (LB) broth containing morpholinepropanesulfonic acid (50 mM; pH 7). Bactobolin A was generously supplied by Jon Clardy (Seyedsayamdost *et al.*, 2010) and dissolved in filter-sterilized water. Synthetic C6-HSL and purified violacein were purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in acidified ethyl acetate ($0.1\,ml\,l^{-1}$ glacial acetic acid) or in dimethylformamide, respectively. AHLs were prepared from the *Bt* bactobolin$^-$ strain BD20 by extracting stationary-phase (OD_{600} 8–10) culture fluid with two equal volumes of acidified ethyl acetate and drying to completion under a stream of nitrogen gas. The dried extracts were dissolved in volumes of media equivalent to the volumes from which they were extracted. The extracts did not affect growth of *Bt* or *Cv*. Extracts similarly prepared from cultures of an AHL$^-$, bactobolin$^-$ double mutant had no effect on the outcome of co-culture experiments. Co-cultures and cultures for AHL preparation were grown at 30 °C. All other growth was at 30 °C for *Cv* and 37 °C for *Bt*. Pure cultures and co-cultures containing visibly aggregated cells of *Cv* were dispersed by homogenization or water-bath sonication before plating for viable counts. Gentamicin was used at $10\,\mu g\,ml^{-1}$ (*Cv* and

Escherichia coli) or 100 μg ml^{-1} (*Bt*) and trimethoprim was used at 100 μg ml^{-1}. For selection of *Bt* and *Cv* transconjugants, gentamicin was at 10 μg ml^{-1} and trimethoprim was at 100 μg ml^{-1}.

Antimicrobial susceptibility testing

We determined the minimum inhibitory concentration of bactobolin or violacein using a protocol modified from the 2003 guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Inocula were prepared from logarithmic-phase cultures and suspended to 5×10^6 cells in 1 ml morpholinepropanesulfonic acid-buffered LB containing dilutions of antibiotic compounds. The minimum inhibitory concentration was defined as the lowest concentration (μg ml^{-1}) that prevented visible growth of bacteria after 24 h. To assess susceptibility to cell culture fluid, bacteria were similarly suspended in a broth with 10% (*Bt*) or 75% (*Cv*) (vol vol^{-1}) filtered fluid from stationary-phase cultures grown for 24 or 16 h, respectively. Culture fluid was filtered through a 0.22-μm pore-size membrane and tested immediately. Fluid from cultures of *Cv* was diluted into 4 × concentrated LB to a 1 × final LB concentration. *Cv* and *Bt* were treated for 24 and 10 h, respectively, before plating for viability. All antimicrobial susceptibility testing was at 30 °C with shaking.

Co-culture experiments

To inoculate co-cultures, pure cultures were grown to mid-logarithmic phase, subcultured to fresh medium at an optical density at 600 nm (OD_{600}) 0.05 and grown an additional 3 h before combining at the appropriate ratios in 10 ml (Figures 1 and 2) or 20 ml (Figures 3 and 4) of medium in 125-ml culture flasks. The initial OD_{600} of the co-culture was 0.05 ($2–4 \times 10^7$ cells per ml) for *Bt* and 0.005 ($2–4 \times 10^6$ cells per ml) for *Cv*. Co-cultures were incubated with shaking at 250 r.p.m. Colony-forming units (CFUs) of each species were determined by using differential antibiotic selection on LB agar plates. *Bt* was selected with gentamicin and *Cv* was selected with trimethoprim.

Results

Antibiotic sensitivities

As a first step in developing our binary culture model, we needed to test the sensitivity of *Cv* to bactobolin and the sensitivity of *Bt* to violacein. Thus, we used purified antibiotics to determine the minimum inhibitory concentrations. The minimum inhibitory concentration of bactobolin A for *Cv* was 8 μg ml^{-1}, and at concentrations exceeding 8 μg ml^{-1}, *Cv* was killed during treatment (data not shown). This bactobolin was estimated to be at 5.3 μg ml^{-1} in pure *Bt* culture fluid in growth conditions similar to those we use (Seyedsayamdost *et al*., 2010). *Bt* produces at least seven other bactobolin compounds (Seyedsayamdost *et al*., 2010; Carr *et al*., 2011). To test if *Bt*-produced bactobolins in cell culture fluid are sufficient to kill *Cv*, we assessed *Cv* viability after treatment with filtered fluid from a stationary-phase (OD_{600} 8–10) *Bt* culture. After treatment with 10% (vol vol^{-1}) culture fluid from a wild-type *Bt* culture diluted into fresh broth, we were unable to recover viable *Cv*. After similar treatment with 10% (vol vol^{-1}) culture fluid from a *Bt* bactobolin-defective mutant (*btaK*$^-$)

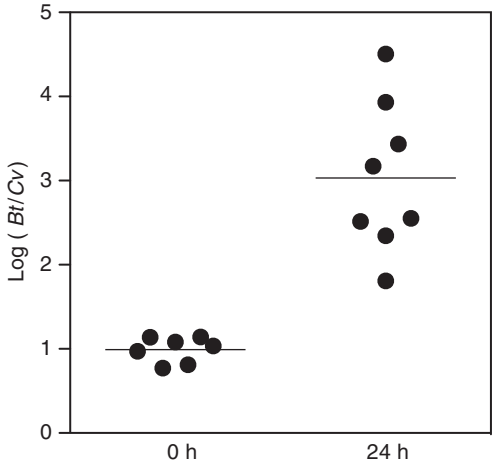

Figure 1 *B. thailandensis–C. violaceum* competition. Initial cell densities were $2–4 \times 10^7$ *B. thailandensis* (*Bt*) cells per ml and $2–4 \times 10^6$ *C. violaceum* (*Cv*) cells per ml. The initial and final cell densities of *Bt* and *Cv* were determined for each independent experiment by selective plating and colony counts. Each data point represents the log-transformed average of the ratios of the two species from duplicate measurements of an independent co-culture experiment. The lines represent the mean of all of the experiments in each set.

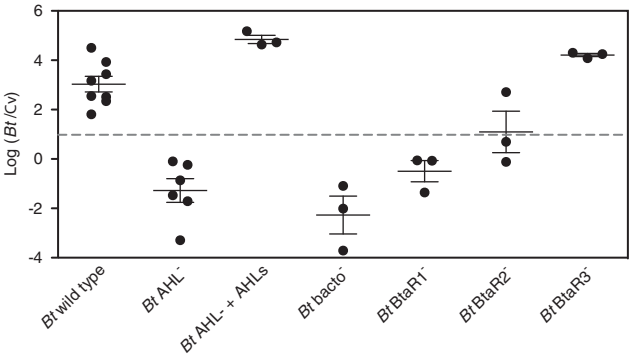

Figure 2 Competition in co-cultures of wild-type *C. violaceum* (*Cv*) and wild-type or mutant *B. thailandensis* (*Bt*) strains. The dashed line indicates the starting 10:1 ratio of *Bt* to *Cv*. The ratio of *Bt* to *Cv* after 24 h was determined by selective plating and colony counts. The co-culture results with wild-type *Bt* are also shown in Figure 1 and the final average CFU of each species is also partially represented in Table 3. *Bt* AHLs were extracted from culture fluid of a *Bt* bactobolin mutant (see Materials and methods) and added to culture medium. The solid lines represent means for each group. The vertical bars show the standard error of the mean for each group.

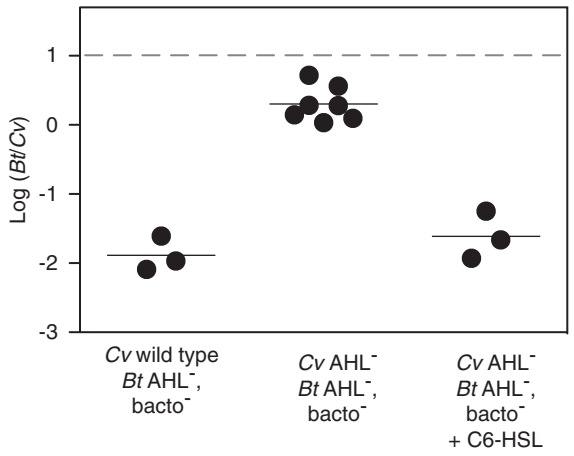

Figure 3 Co-cultures of the *C. violaceum* (*Cv*) wild-type Cv017 or the AHL mutant Cv026 and the *B. thailandensis* (*Bt*) competition-impaired AHL, bactobolin double mutant JBT125. The dashed line shows the initial ratio of *Bt* to *Cv*. After 24 h, the ratio of *Bt* to *Cv* was determined by colony counts on selective agar. Co-cultures were grown in 20 ml medium. C6-HSL was added before inoculation where indicated (250 nM final concentration). The solid lines represent the means of each group.

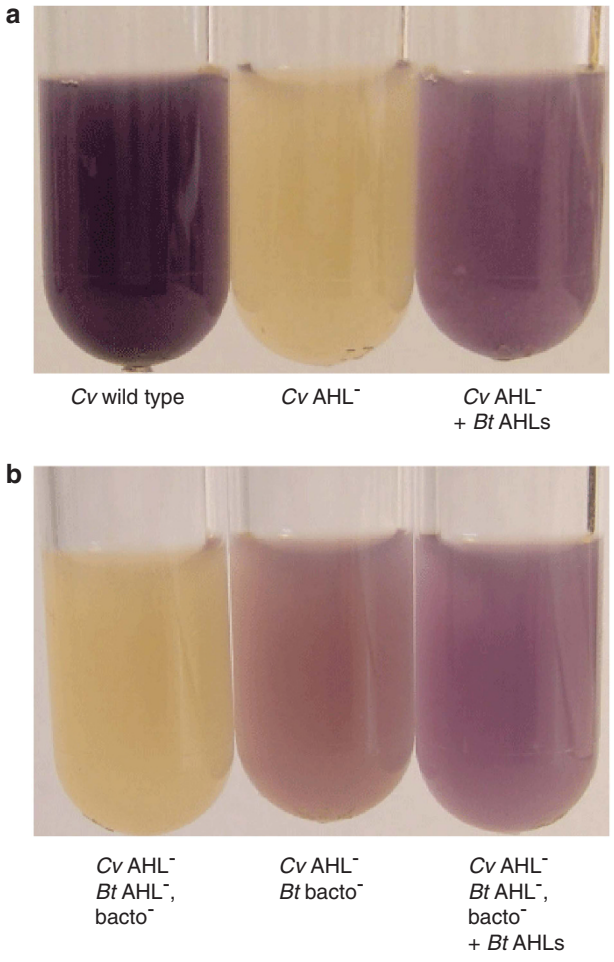

Figure 4 *C. violaceum* (*Cv*) quorum sensing is activated by *B. thailandensis* (*Bt*) AHLs. Quorum-sensing activation is indicated by the *Cv* quorum-sensing-dependent purple pigment, violacein, in stationary-phase cultures. (**a**) *Cv* wild-type (Cv017) and the AHL mutant (Cv026) with or without added *Bt* AHLs. (**b**) Co-cultures of the *Cv* AHL mutant and *Bt* strains as indicated (AHL mutant JBT112; AHL, bactobolin double mutant JBT125). AHLs were extracted from stationary-phase cultures of *Bt* BD20, a bactobolin mutant.

or in broth alone, *Cv* grew to 2–3 $\times 10^9$ CFU per ml (Table 1). Our results show that stationary-phase *Bt* cultures produce sufficient bactobolins to kill *Cv*.

Bt was resistant to violacein at the highest concentration tested, 125 μg ml^{-1} (data not shown), which is in excess of amounts produced by *Cv* (Tobie, 1935; Strong, 1944). *Cv* codes for other putative antimicrobial factors, including phenazines and hydrogen cyanide (Brazilian National Genome Project Consortium, 2003). To test whether *Cv* produces quorum-sensing-dependent antimicrobials with activity against *Bt*, we incubated *Bt* with filtered fluid from *Cv* wild-type or mutant stationary-phase cultures (OD_{600} 4–5). After 10 h, *Bt* grew modestly to 3×10^8 in the presence of wild-type *Cv* culture fluid, but grew to 2×10^9 in the presence of fluid from the AHL synthesis mutant (Table 2). This indicates that *Cv* quorum sensing regulates production of extracellular factors that inhibit growth of *Bt*, and that this inhibition is not due to violacein alone.

The Bt–Cv *co-culture model*

In pure culture, the doubling times of all *Bt* strains were 60 min ± 5% and *Cv* strains were 48 min ± 5% (see Supplementary Table S2), and both species reached densities of about 3×10^9 cells per ml in early stationary phase. Because of the modest growth-rate discrepancy, we used an inoculum of 2–4 $\times 10^7$ *Bt* per ml and 2–4 $\times 10^6$ *Cv* per ml in our co-culture experiments. Wild-type *Bt* outcompetes wild-type *Cv*, increasing in relative abundance by about 100-fold in 24 h (Figure 1). To study the competition further, we enumerated bacteria during logarithmic, early stationary and late stationary growth phases. In logarithmic and early stationary

Table 1 Sensitivity of *C. violaceum* (*Cv*) strains to *B. thailandensis* (*Bt*) culture fluid

Bt *culture fluid tested*[a]	Cv *(CFU per ml)*[b]	
	Wild type	*AHL*$^-$
Wild type	<100	<100
AHL$^-$	3×10^9	2×10^9
Bactobolin$^-$	2×10^9	2×10^9
No added culture fluid	2×10^9	1×10^9

[a]Sensitivity was assessed by growing *Cv* in the presence of filtered culture fluid from stationary-phase (24 h) *Bt* cultures as described in the Materials and methods. The *Bt* AHL (*btaI1*, *I2*, *I3*) mutant JBT125 and the bactobolin (*btaK*) mutant BD20 were used. The *Cv* AHL (*cviI*) mutant Cv026 was used. Experiments were carried out in duplicate and in all cases the ranges did not exceed 10%.
[b]*Bt* cell culture fluid was added to a final concentration of 10% (vol vol^{-1}) in 90% (vol vol^{-1}) in 1 ml Luria–Bertani-morpholinepropanesulfonic acid broth.

Table 2 Sensitivity of *B. thailandensis* (*Bt*) strains to *C. violaceum* (*Cv*) culture fluid

Cv *culture fluid tested*[a]	Bt *(CFU per ml)*[b]		
	Wild type	*AHL*$^-$	*Bactobolin*$^-$
Wild type	3×10^8	2×10^8	3×10^8
AHL$^-$	2×10^9	2×10^9	1×10^9
No added culture fluid	7×10^9	8×10^9	8×10^9

[a]Sensitivity was assessed by growing *Bt* in the presence of filtered culture fluid from stationary-phase (16 h) *Cv* cultures as described in the Materials and methods. The *Bt* AHL (*btaI1*, *I2*, *I3*) mutant JBT125 and the bactobolin (*btaK*) mutant BD20 were used. The *Cv* AHL (*cviI*) mutant Cv026 was used. Experiments were carried out in duplicate and in all cases the ranges did not exceed 10%.
[b]*Cv* cell culture fluid was added to a final concentration of 75% (vol vol^{-1}) in 25% (vol vol^{-1}) concentrated Luria–Bertani-morpholinepropanesulfonic acid broth in 1 ml.

phase, both species reached densities in co-culture that were identical to the densities in pure culture ($2–5 \times 10^9$ cells per ml). However, the final densities of both species in late stationary phase (24 h) was lower in co-culture than in pure culture (Table 3). The final cell density of *Cv* decreased over three logs from 5×10^9 cells per ml in early stationary phase to 1×10^6 cells per ml at 24 h. There was no significant decrease in *Cv* density in pure culture (Table 3). The final density of *Bt* was 10-fold lower in co-culture than in pure culture (Table 2). Our results are consistent with the hypothesis that both species produce quorum-sensing-controlled antimicrobials during stationary phase that inhibit growth of or kill the other species.

Quorum-sensing-controlled bactobolin synthesis promotes Bt *competitiveness in binary culture*

To test the hypothesis that quorum sensing promotes *Bt* competitiveness in co-culture, we assessed competition with a *Bt* AHL mutant and wild-type *Cv*. We also assessed the competitiveness of a *Bt* bactobolin mutant. In co-culture conditions where wild-type *Bt* had a robust competitive advantage, either the *Bt* AHL or bactobolin mutant were outcompeted by *Cv* (Figure 2). We could rescue competitiveness of the AHL mutant by supplementing our co-cultures with *Bt* AHLs that were obtained by ethyl acetate extraction of culture fluid from a stationary-phase (OD_{600} 8–10) *Bt* bactobolin mutant (Materials and methods). These results demonstrate that quorum sensing and quorum-sensing-dependent bactobolin production are critical for the competitive success of *Bt* in our co-culture model.

Bactobolin production is controlled by the BtaI2–R2 quorum-sensing system (Duerkop *et al.*, 2009). Next, we assessed the importance of BtaI2–R2 and each of the other two *Bt* quorum-sensing systems, BtaI1–R1 and BtaI3–R3, to the competitiveness of *Bt* in our co-culture model. For this, we used *Bt* strains harboring individual deletions in

Table 3 Final yields of *B. thailandensis* (*Bt*) and *C. violaceum* (*Cv*) in pure culture and co-culture

Strain(s)	*Final growth yield (CFU per ml)*[a,b]	
	Bt	Cv
Pure culture		
Bt wild type	$1.4\ (\pm 0.7) \times 10^{10}$	
Bt AHL$^-$	$1.0\ (\pm 0.9) \times 10^{10}$	
Cv wild type		$9.9\ (\pm 8.4) \times 10^8$
Co-culture (with wild-type Cv)[c]		
Bt wild type	$1.3\ (\pm 0.8) \times 10^9$	$1.4\ (\pm 2.0) \times 10^6$
Bt AHL$^-$	$2.3\ (\pm 2.6) \times 10^8$	$2.1\ (\pm 1.2) \times 10^9$
Bt bactobolin$^-$	$1.0\ (\pm 1.5) \times 10^8$	$2.7\ (\pm 0.5) \times 10^9$

[a]The values are the means of at least three independent experiments with ranges indicated within parantheses. The *Bt* AHL (*btaI1*, *I2*, *I3*) mutant JBT125 and the bactobolin (*btaK*) mutant BD20 were used.
[b]The growth yield in early stationary phase (9 h) of *Bt* and *Cv* in pure and co-culture was $1–3 \times 10^9$.
[c]Co-culture data from individual experiments are also represented in Figure 1.

each of the AHL receptor genes *btaR1*, *btaR2* or *btaR3* (Figure 2). Not surprisingly, the *btaR2* mutant competed poorly with *Cv*. Results were similar to those with the bactobolin mutant and the AHL synthesis mutant. The outcome with the *btaR3* mutant was identical to wild type, indicating that BtaR3 is not important for competition in our model. The *btaR1* mutant showed an intermediate ability to compete with *Cv*, suggesting that this regulator may be important for the production of bactobolin or production of other factors that enhance competition or bactobolin activity. In support of the former, we found that expression of a bactobolin *btaK-lacZ* transcriptional fusion is delayed in a *btaR1* mutant (data not shown), suggesting that BtaR1 may advance the production of bactobolin. We also tested the competitiveness of strains with individual mutations in each of the AHL synthase genes. All three individual AHL synthase mutants outcompeted *Cv* with results similar to competitions with wild-type *Bt* (data not shown). These findings suggest that the AHL synthases have overlapping abilities to induce expression of bactobolin. This is supported by our previous finding that BtaR2 can respond to both 3-hydroxy-octanoyl-HSL and 3-hydroxy-decanoyl-HSL, which are produced by the BtaI3 and BtaI2 synthases, respectively (Duerkop *et al.*, 2009).

Quorum sensing can promote competitiveness of Cv

Our results indicate that *Cv* also produces quorum-sensing-dependent antimicrobial factors that inhibit growth of *Bt* (Table 1). Thus, we hypothesized that quorum sensing promotes competitiveness of *Cv* as it does for *Bt*. To address this, we compared the competitiveness of the *Cv* wild-type and AHL mutant strains in co-culture with *Bt*. We modified

our experiment to give wild-type *Cv* a competitive advantage by using a competition-defective *Bt* AHL, bactobolin double mutant, and we increased the co-culture volume to 20 ml because we observed that this further improves *Cv* competitiveness for reasons that are unknown (data not shown). In these conditions, wild-type *Cv* strongly outcompeted the *Bt* mutant, whereas the *Cv* AHL mutant barely outcompeted the *Bt* mutant (Figure 3). Competitiveness could be restored to the *Cv* AHL mutant by the addition of C6-HSL (the AHL produced by *Cv*) (Figure 3). These results show that quorum sensing can promote the competitiveness of *Cv*. Because violacein does not have any antimicrobial activity against *Bt*, we note that this is not due to violacein, but must be caused by as-yet undefined quorum-sensing-dependent factors.

Cv *can sense and respond to Bt AHLs*

The *Cv* AHL receptor CviR can be activated by a range of AHLs including at least one of the AHLs produced by *Bt*, C8-HSL (McClean *et al.*, 1997; Swem *et al.*, 2009). We hypothesized that *Bt* AHLs can activate the *Cv* quorum-sensing receptor CviR and that this promotes competitiveness of *Cv* in co-culture with *Bt*. We first tested whether a pure culture of *Cv* can sense and respond to *Bt* AHLs; these AHLs were ethyl acetate extracted and concentrated from stationary-phase (OD_{600} 8–10) culture fluid and added to *Cv* cultures to match concentrations in the culture from which they were extracted. As a read-out for quorum-sensing activation, we followed the purple pigment violacein. The *Cv* AHL mutant is not pigmented, but pigmentation can be restored by supplementing the culture medium with *Bt* AHL extracts (Figure 4a). This result shows that *Cv* can sense and respond to physiological levels of *Bt* AHLs.

Next, we tested whether the *Cv* AHL mutant can respond to *Bt* AHLs during co-culture growth. Because *Cv* is killed by *Bt*-produced bactobolin in co-culture (Table 1), we used the *Bt* bactobolin mutant BD20 for these experiments (Figure 4b). When in co-culture with a *Bt* AHL, bactobolin double mutant, the *Cv* AHL mutant did not turn purple. However, in co-culture with the AHL-producing *Bt* bactobolin mutant BD20, or with exogenously supplied *Bt* AHLs, the co-culture turned purple. This finding indicates that the *Cv* CviR responds to *Bt* AHLs. We conclude that *Bt* AHLs are cues that alter the behavior of *Cv*, although they did not evolve for that purpose (Keller and Surette, 2006). In our experiment, the *Cv* AHL synthase mutant can eavesdrop on *Bt*.

Eavesdropping promotes competitiveness of Cv

To determine whether eavesdropping can influence competitiveness of *Cv*, we enumerated *Bt* and *Cv* in co-cultures (Figure 5). As in our previous experiments, we grew the *Cv* AHL mutant with the *Bt* bactobolin mutant or an AHL, bactobolin double mutant. The *Cv* AHL mutant was more competitive with the *Bt* bactobolin mutant than it was with the double mutant. As a control, we added *Bt* AHLs to the co-culture with the *Bt* double mutant and observed that this improved the competitiveness of *Cv*. These results suggest that eavesdropping on *Bt* AHLs promotes *Cv* competitiveness. As an additional control, we tested whether the *Cv* AHL receptor CviR is required for eavesdropping. To address this, we constructed a *Cv* AHL synthase, receptor double mutant. We found that CviR is required for the competitive advantage provided to *Cv* by eavesdropping on *Bt* AHLs (Figure 5).

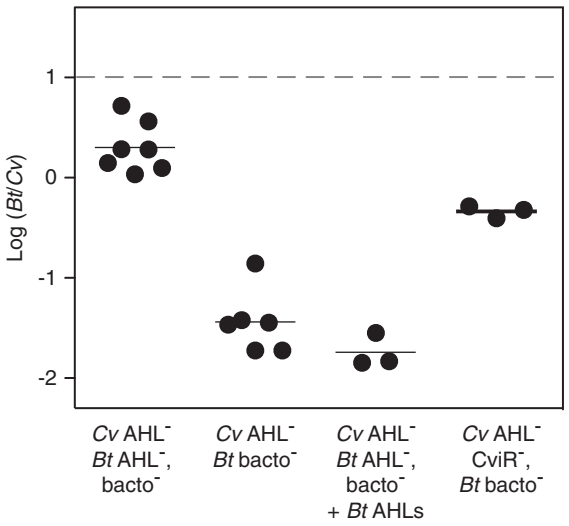

Figure 5 Eavesdropping promotes competitiveness of *Cv* in co-cultures with a *B. Bt* bactobolin mutant. After 24 h of co-culture, the ratio of *Bt* to *Cv* was determined. Co-cultures of the *Cv* AHL mutant (Cv026), or the *Cv* AHL synthase, receptor double mutant (Cv026R) and the *Bt* strains as indicated and described in Figure 4 legend. Co-cultures were grown in 20 ml volumes. The dashed line indicates the initial ratio of *Bt* to *Cv*. The solid lines represent the means for each group. AHLs were obtained as described for Figure 4.

An in silico *eavesdropping model*

Our experimental approach has limitations and with the conditions we used, we could not observe an effect of eavesdropping with wild-type strains (data not shown). However, we suspect there may be conditions where eavesdropping provides an advantage to wild-type *Cv*. This may be as the population nears the critical density required for quorum-sensing activation. At this density, AHLs produced by a nearby competitor may cause early activation of quorum-sensing-dependent antibiotics and would improve competitiveness of the eavesdropping microbe.

To explore this hypothesis further, we developed a mathematical model of our binary culture system (see Supplementary Text and Supplementary Table S3). The model accounts for two wild-type species

that produce antibiotics in response to AHL signals in a well-mixed environment, similar to species *Bt* and *Cv* in our experimental system. *In silico*, the antibiotic produced by each species has equal killing efficiency towards the competing species, but no influence on the producing species. The two species in our *in silico* model also have identical growth rates, rates of antibiotic and AHL production, and antibiotic-production costs. However, as we observed experimentally, in some conditions one species (which we refer to here as species C) can eavesdrop on the other (species B). In the *in silico* model, we assume that antibiotic production accelerates once the inducer reaches a critical threshold concentration. However, antibiotic-production rates eventually level off as AHL concentrations exceed the quorum-sensing threshold. We use several different activation thresholds in our analysis.

Our *in silico* model has a bistable dynamic where one species completely dominates under most conditions. In the absence of eavesdropping, the outcome favors the species that is numerically dominant at the beginning (Figure 6). When we vary the activation thresholds for antibiotic production (by varying K_B and K_C of B and C, respectively, see Supplementary Text and Supplementary Table S3), there is an optimal value ($K^{optimal}$) where one species can dominate the other; if we fix K_B at this value, B can dominate C at any value of K_C (other than when K_C was equal to $K^{optimal}$), and the same is true for C if K_C is set at $K^{optimal}$ (see Supplementary Figure S1). For every set of parameter values we explored, we find that $K^{optimal}$ is greater than zero. Thus, waiting until a population reaches a quorum provides a fitness benefit for antibiotic-producing bacteria.

We then investigated eavesdropping in our *in silico* model when species B and C had identical thresholds above (high), equal to (optimal) and below (low) the optimal threshold. At a relatively high threshold, eavesdropping provided a distinct advantage to C by allowing it to invade B from lower starting frequencies (Figure 6a), supporting our initial hypothesis. However, with an optimal or low threshold, eavesdropping was disadvantageous (Figures 6b and c). We posit that in the latter two cases, the eavesdropping population activates production of antibiotic too early to accumulate a sufficient killing dose and antibiotic production is an ineffective metabolic burden. To test this hypothesis, we kept the same conditions as in Figure 6c and increased the toxicity of the antibiotic of both species. In these conditions, eavesdropping provides an advantage (Figure 6d), supporting our hypothesis. Furthermore, eavesdropping is also advantageous if the antibiotic cost is decreased (Supplementary Figures S2 and S3). However, these changes in toxicity and cost alter the optimal threshold (Supplementary Figure S1B and data not shown), effectively resetting the system so that antibiotic production is induced after the optimal threshold is achieved. Thus, eavesdropping-dependent early production of antibiotics promotes competition in a population that has already reached a sufficient density to produce a killing dose.

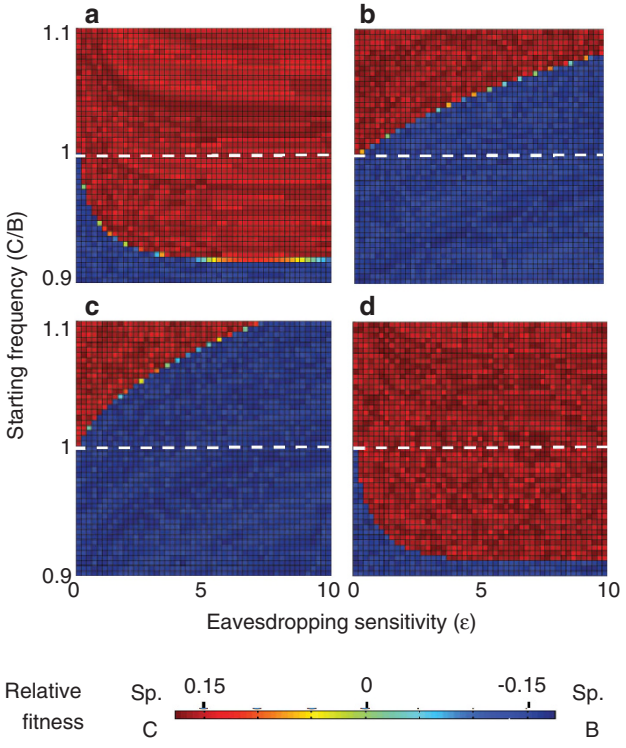

Figure 6 *In silico* modeling. Our model accounts for two species with quorum-sensing-controlled antibiotics, similar to our experimental model of *Bt* and *Cv*. As in our experimental model, our *in silico* model accounts for two species (B and C) that produce antibiotics in a density-dependent manner. In our model, species C can eavesdrop on species B (see Supplementary Text). We show relative fitness of each species as a function of the initial ratio (C/B) and the eavesdropping sensitivity (ε) of C. The fitness of C relative to B was measured using the log relative fitness measure given in Wu *et al.* (2006) and is indicated by the color spectrum on the far right. (**a**) The inducer concentration required for production of antibiotic (activation threshold, K_B and K_C) is relatively high for both species (0.01, see text). (**b**) The activation threshold is lower (0.003898) and corresponds to an optimal threshold for each species that gives it an advantage over the other species regardless of the other species' threshold. (**c**) Both species have an activation threshold lower than the optimal threshold (0.003). (**d**) The same parameters were used as in (**c**); however, the antibiotic toxicity is raised 10-fold. This changes the optimal activation threshold to 0.001113, which is below the activation threshold value used (0.003) (see Supplementary Figure S1).

Discussion

We have developed a dual-species competition model with two soil saprophytes, *Bt* and *Cv*, which both use quorum sensing to control production of antimicrobial factors. We show that both of these species can gain a competitive advantage over the other with success dependent on quorum sensing. The advantage of quorum-sensing control of antimicrobials has also been shown in other laboratory

co-culture models (Moons *et al.*, 2005, 2006; An *et al.*, 2006). The previous reports, together with the results reported here, support the idea that quorum-sensing regulation is important in multi-species competition. Our results indicate that competitiveness of *Bt* relies on the *btaI2-R2*-controlled antibiotic bactobolin and *Cv* uses as-yet unidentified quorum-sensing-dependent factors for competition. The bactobolin biosynthetic genes and *btaI2-R2* are encoded within a large (120-kb) DNA element that is absent from a close relative, the host-adapted pathogen *Burkholderia mallei*. That this element is retained in *Bt* supports the view that *btaI2-R2* and bactobolin are important for competition during saprophytic growth.

Why do bacteria use quorum sensing to regulate antibiotic production? Our *in silico* model provides some possible clues. The results indicate that when antibiotic production is costly, early production slows population growth without effectively killing the competitor. Thus, quorum sensing defers the cost of antibiotic production until a sufficient killing dose can be delivered. We do not include in our model the additional possibility that sublethal concentrations of antibiotics may induce in the competitor an adaptation to higher concentrations of antibiotic. Both of these possibilities can be further explored with our experimental co-culture model. An alternative hypothesis is that deferred production may also protect the producing population against the emergence of non-producing cheaters. Cheaters can exploit public goods producers by utilizing the available goods without incurring the cost of their production. In a recent study by Xavier *et al.* (2011), delayed production of an exploitable public good, surfactant, protected the producing population against the emergence of cheaters. This strategy maximized growth of the producing population, thereby increasing its ability to compete with cheaters. Quorum-sensing regulation may similarly promote competitiveness with non-producing cheaters.

Our experimental model also showed that cross-species AHL activation of the *Cv* broad-specificity AHL receptor can promote the competitiveness of *Cv* (Figure 5). In addition to *Cv*, there are several other species with broad-specificity AHL receptors and these are also saprophytes: *E. carotovora* (ExpR2)(Sjoblom *et al.*, 2006); *P. aeruginosa* (QscR)(Lee *et al.*, 2006); and receptors encoded by two species of *Bradyrhizobium* (BraR and BjaR) (Ahlgren *et al.*, 2011; Lindemann *et al.*, 2011). ExpR2 and QscR are both orphan receptors without a cognate AHL synthase gene (Cui *et al.*, 2006; Fuqua, 2006; Sjoblom *et al.*, 2006). The potential role of each of these receptors in competition has not been determined. AHL receptor specificity can be easily altered by single amino-acid changes (Collins *et al.*, 2005; Hawkins *et al.*, 2007; Chen *et al.*, 2011; Lintz *et al.*, 2011), suggesting that AHL recognition may be very adaptable in nature. In contrast to these broad-specificity AHL receptors, the receptor of the squid symbiont *Vibrio fischeri* is quite specific for its cognate AHL (Visick and Ruby, 1999). *V. fischeri* activates quorum-sensing-dependent functions when it is at high cell densities in its squid host; in this environment it rarely encounters other bacterial species (Visick and McFall-Ngai, 2000). Thus, AHL receptors may evolve broad signal specificity in specific environments where eavesdropping might be of use, although the role of these receptors in inter-species competition and eavesdropping requires further study.

In the conditions of our experimental model, eavesdropping did not provide an observable fitness advantage to wild-type strains during competition. However in another study, AHLs produced by epiphytic bacteria on plant leaves altered the quorum-sensing-regulated virulence phenotype of a wild-type *Pseudomonas syringae* strain (Dulla and Lindow, 2009), suggesting that wild-type strains can be responsive to AHLs from other species in natural environments. Our co-culture model may provide a limited view of the possible interactions between species in nature, for example, Dulla and Lindow 2009) identified several epiphytic species that produce 10-fold more AHL than their laboratory *P. syringae* strain. High-level signal producers may play a significant role in cross-species induction.

Our mathematical model allowed a simple assessment of the costs and benefits of eavesdropping between competing wild-type strains. For the model, we made the basic assumption that detection of exogenous AHLs can cause early quorum-sensing-dependent activation of antibiotic genes. We have observed this experimentally in *Bt* with a transcriptional fusion to the bactobolin biosynthetic gene *btaK* (data not shown), but it is more difficult to address with *Cv* because we do not yet know what quorum-controlled genes are involved in competition, and during early logarithmic phase the activity of the antimicrobials is too low for our methods of detection. The *in silico* model indicates that eavesdropping can promote competition in certain conditions where production of antibiotic occurs relatively late during growth. However, eavesdropping can also be detrimental if the activation threshold is relatively low. We observed similar results in other variations of this model (data not shown). Our results suggest that receptors would evolve broad specificity only in particular circumstances where eavesdropping is beneficial. Our bias is that specificity is the more evolved trait and that highly specific receptors likely arose from receptors with less specificity.

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References

Ahlgren NA, Harwood CS, Schaefer AL, Giraud E, Greenberg EP. (2011). Aryl-homoserine lactone quorum sensing in stem-nodulating photosynthetic bradyrhizobia. *Proc Natl Acad Sci USA* **108**: 7183–7188.

An D, Danhorn T, Fuqua C, Parsek MR. (2006). Quorum sensing and motility mediate interactions between *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* in biofilm cocultures. *Proc Natl Acad Sci USA* **103**: 3828–3833.

Bainton NJ, Bycroft BW, Chhabra SR, Stead P, Gledhill L, Hill PJ *et al.* (1992). A general role for the *lux* autoinducer in bacterial cell signalling: control of antibiotic biosynthesis in *Erwinia*. *Gene* **116**: 87–91.

Bassler BL. (2002). Small talk. Cell-to-cell communication in bacteria. *Cell* **109**: 421–424.

Brazilian National Genome Project Consortium (2003). The complete genome sequence of *Chromobacterium violaceum* reveals remarkable and exploitable bacterial adaptability. *Proc Natl Acad Sci USA* **100**: 11660–11665.

Carr G, Seyedsayamdost MR, Chandler JR, Greenberg EP, Clardy J. (2011). Sources of diversity in bactobolin biosynthesis by *Burkholderia thailandensis* E264. *Org Lett* **13**: 3048–3051.

Chandler JR, Duerkop BA, Hinz A, West TE, Herman JP, Churchill ME *et al.* (2009). Mutational analysis of *Burkholderia thailandensis* quorum sensing and self-aggregation. *J Bacteriol* **191**: 5901–5909.

Chen G, Swem LR, Swem DL, Stauff DL, O'Loughlin CT, Jeffrey PD *et al.* (2011). A strategy for antagonizing quorum sensing. *Mol Cell* **42**: 199–209.

Collins CH, Arnold FH, Leadbetter JR. (2005). Directed evolution of *Vibrio fischeri* LuxR for increased sensitivity to a broad spectrum of acyl-homoserine lactones. *Mol Microbiol* **55**: 712–723.

Cui Y, Chatterjee A, Hasegawa H, Chatterjee AK. (2006). *Erwinia carotovora* subspecies produce duplicate variants of ExpR, LuxR homologs that activate *rsmA* transcription but differ in their interactions with *N*-acylhomoserine lactone signals. *J Bacteriol* **188**: 4715–4726.

Davies J, Spiegelman GB, Yim G. (2006). The world of subinhibitory antibiotic concentrations. *Curr Opin Microbiol* **9**: 445–453.

Duerkop BA, Varga J, Chandler JR, Peterson SB, Herman JP, Churchill ME *et al.* (2009). Quorum-sensing control of antibiotic synthesis in *Burkholderia thailandensis*. *J Bacteriol* **191**: 3909–3918.

Dulla GF, Lindow SE. (2009). Acyl-homoserine lactone-mediated cross talk among epiphytic bacteria modulates behavior of *Pseudomonas syringae* on leaves. *ISME J* **3**: 825–834.

Engebrecht J, Nealson K, Silverman M. (1983). Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* **32**: 773–781.

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

Fuqua C, Parsek MR, Greenberg EP. (2001). Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu Rev Genet* **35**: 439–468.

Fuqua WC, Winans SC, Greenberg EP. (1994). Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* **176**: 269–275.

Gallagher LA, Manoil C. (2001). *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. *J Bacteriol* **183**: 6207–6214.

Hawkins AC, Arnold FH, Stuermer R, Hauer B, Leadbetter JR. (2007). Directed evolution of *Vibrio fischeri* LuxR for improved response to butanoyl-homoserine lactone. *Appl Environ Microbiol* **73**: 5775–5781.

Hibbing ME, Fuqua C, Parsek MR, Peterson SB. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* **8**: 15–25.

Hosni T, Moretti C, Devescovi G, Suarez-Moreno ZR, Fatmi MB, Guarnaccia C *et al.* (2011). Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease. *ISME J* **5**: 1857–1870.

Keller L, Surette MG. (2006). Communication in bacteria: an ecological and evolutionary perspective. *Nat Rev Microbiol* **4**: 249–258.

Kownatzki R, Tummler B, Doring G. (1987). Rhamnolipid of *Pseudomonas aeruginosa* in sputum of cystic fibrosis patients. *Lancet* **1**: 1026–1027.

Latifi A, Foglino M, Tanaka K, Williams P, Lazdunski A. (1996). A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol Microbiol* **21**: 1137–1146.

Latifi A, Winson MK, Foglino M, Bycroft BW, Stewart GS, Lazdunski A *et al.* (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 JH, Lequette Y, Greenberg EP. (2006). Activity of purified QscR, a *Pseudomonas aeruginosa* orphan quorum-sensing transcription factor. *Mol Microbiol* **59**: 602–609.

Lindemann A, Pessi G, Schaefer AL, Mattmann ME, Christensen QH, Kessler A *et al.* (2011). Isovaleryl-homoserine lactone, an unusual branched-chain quorum-sensing signal from the soybean symbiont *Bradyrhizobium japonicum*. *Proc Natl Acad Sci USA* **108**: 16765–16770.

Lintz MJ, Oinuma K, Wysoczynski CL, Greenberg EP, Churchill ME. (2011). Crystal structure of QscR, a *Pseudomonas aeruginosa* quorum sensing signal receptor. *Proc Natl Acad Sci USA* **108**: 15763–15768.

Mazzola M, Cook RJ, Thomashow LS, Weller DM, Pierson LS III. (1992). Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl Environ Microbiol* **58**: 2616–2624.

McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M *et al.* (1997). Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of

N-acylhomoserine lactones. *Microbiology* **143** (Part 12) 3703–11.

Moons P, Van Houdt R, Aertsen A, Vanoirbeek K, Engelborghs Y, Michiels CW. (2006). Role of quorum sensing and antimicrobial component production by *Serratia plymuthica* in formation of biofilms, including mixed biofilms with *Escherichia coli*. *Appl Environ Microbiol* **72**: 7294–7300.

Moons P, Van Houdt R, Aertsen A, Vanoirbeek K, Michiels CW. (2005). Quorum sensing dependent production of antimicrobial component influences establishment of *E. coli* in dual species biofilms with *Serratia plymuthica*. *Commun Agric Appl Biol Sci* **70**: 195–198.

Pierson EA, Wood DW, Cannon JA, Blachere FM, Pierson LS 3rd. (1998). Interpopulation signaling via *N*-acyl-homoserine lactones among bacteria in the wheat rhizosphere. *Mol Plant Microbe Interact* **11**: 1078–1084.

Ran H, Hassett DJ, Lau GW. (2003). Human targets of *Pseudomonas aeruginosa* pyocyanin. *Proc Natl Acad Sci USA* **100**: 14315–14320.

Riedel K, Hentzer M, Geisenberger O, Huber B, Steidle A, Wu H *et al.* (2001). *N*-acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* **147**: 3249–3262.

Schuster M, Greenberg EP. (2006). A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *Int J Med Microbiol* **296**: 273–81.

Seed PC, Passador L, Iglewski BH. (1995). Activation of the *Pseudomonas aeruginosa lasI* gene by LasR and the *Pseudomonas* autoinducer PAI: an autoinduction regulatory hierarchy. *J Bacteriol* **177**: 654–659.

Seyedsayamdost MR, Chandler JR, Blodgett JA, Lima PS, Duerkop BA, Oinuma K *et al.* (2010). Quorum-sensing-regulated bactobolin production by *Burkholderia thailandensis* E264. *Org Lett* **12**: 716–719.

Sjoblom S, Brader G, Koch G, Palva ET. (2006). Cooperation of two distinct ExpR regulators controls quorum sensing specificity and virulence in the plant pathogen *Erwinia carotovora*. *Mol Microbiol* **60**: 1474–1489.

Stauff DL, Bassler BL. (2011). Quorum sensing in *Chromobacterium violaceum*: DNA recognition and gene regulation by the CviR receptor. *J Bacteriol* **193**: 3871–3878.

Steidle A, Sigl K, Schuhegger R, Ihring A, Schmid M, Gantner S *et al.* (2001). Visualization of *N*-acylhomoserine lactone-mediated cell–cell communication between bacteria colonizing the tomato rhizosphere. *Appl Environ Microbiol* **67**: 5761–5770.

Strong FM. (1944). Isolation of violacein. *Science* **100**: 287.

Swem LR, Swem DL, O'Loughlin CT, Gatmaitan R, Zhao B, Ulrich SM *et al.* (2009). A quorum-sensing antagonist targets both membrane-bound and cytoplasmic receptors and controls bacterial pathogenicity. *Mol Cell* **35**: 143–153.

Tobie WC. (1935). The pigment of *Bacillus violaceus*: I. The production, extraction, and purification of violacein. *J Bacteriol* **29**: 223–227.

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

Visick KL, Ruby EG. (1999). The emergent properties of quorum sensing: consequences to bacteria of autoinducer signaling in their natural environment. In: Winans GMDaSC (ed.) *Cell–Cell Signaling in Bacteria*. ASM Press: Washington, DC, pp 333–352.

Visick KL, McFall-Ngai MJ. (2000). An exclusive contract: specificity in the *Vibrio fischeri*–Euprymna scolopes partnership. *J Bacteriol* **182**: 1779–1787.

Waters CM, Bassler BL. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* **21**: 319–346.

Wu H, Huang Y, Dykes C, Liu D, Ma J, Perelson AS *et al.* (2006). Modeling and estimation of replication fitness of human immunodeficiency virus type 1 *in vitro* experiments by using a growth competition assay. *J Virol* **80**: 2380–2389.

Xavier JB, Kim W, Foster KR. (2011). A molecular mechanism that stabilizes cooperative secretions in *Pseudomonas aeruginosa*. *Mol Microbiol* **79**: 166–179.

Yim G, Wang HH, Davies J. (2007). Antibiotics as signalling molecules. *Philos Trans R Soc Lond Ser B* **362**: 1195–1200.

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