Pseudomonas aeruginosa Las quorum sensing autoinducer suppresses growth and biofilm production in *Legionella* species

Soichiro Kimura,¹ Kazuhiro Tateda,¹ Yoshikazu Ishii,¹ Manabu Horikawa,² Shinichi Miyairi,³ Naomasa Gotoh,⁴ Masaji Ishiguro² and Keizo Yamaguchi¹

Correspondence

Soichiro Kimura

kimsou@med.toho-u.ac.jp

¹Department of Microbiology and Infectious Diseases, Toho University Faculty of Medicine, Ota-ku, Tokyo 143-8540, Japan

²Suntory Institute for Bioorganic Research, Mishima-gun, Osaka 618-8503, Japan

³Laboratory of Bio-organic Chemistry, College of Pharmacy, Nihon University, Funabashi, Chiba 274-8555, Japan

⁴Department of Microbiology, Kyoto Pharmaceutical University, Yamashina, Kyoto 607-8414, Japan

Bacteria commonly communicate with each other by a cell-to-cell signalling mechanism known as quorum sensing (QS). Recent studies have shown that the Las QS autoinducer *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C₁₂-HSL) of *Pseudomonas aeruginosa* performs a variety of functions not only in intraspecies communication, but also in interspecies and interkingdom interactions. In this study, we report the effects of *Pseudomonas* 3-oxo-C₁₂-HSL on the growth and suppression of virulence factors in other bacterial species that frequently co-exist with *Ps. aeruginosa* in nature. It was found that 3-oxo-C₁₂-HSL, but not its analogues, suppressed the growth of *Legionella pneumophila* in a dose-dependent manner. However, 3-oxo-C₁₂-HSL did not exhibit a growth-suppressive effect on *Serratia marcescens*, *Proteus mirabilis*, *Escherichia coli*, *Alcaligenes faecalis* and *Stenotrophomonas maltophilia*. A concentration of 50 µM 3-oxo-C₁₂-HSL completely inhibited the growth of *L. pneumophila*. Additionally, a significant suppression of biofilm formation was demonstrated in *L. pneumophila* exposed to 3-oxo-C₁₂-HSL. Our results suggest that the *Pseudomonas* QS autoinducer 3-oxo-C₁₂-HSL exerts both bacteriostatic and virulence factor-suppressive activities on *L. pneumophila* alone.

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INTRODUCTION

Bacteria respond and adapt to a constantly changing environment because they have to compete with other micro-organisms for nutrients. To sense their own environmental conditions, bacteria utilize quorum sensing (QS) systems that operate via small molecules known as autoinducers (Miller & Bassler, 2001). These systems regulate the expression of a number of genes synchronously across the bacterial population. Gram-negative bacteria utilize lipid-based molecules, termed acylhomoserine lactones (AHLs) to communicate with each other, whereas Gram-positive bacteria employ peptide-based molecules, termed autoinducing peptides (Federle & Bassler, 2003; Fuqua & Greenberg, 2002). Autoinducer-2 has been found in a variety of bacteria and is considered to be a universal QS molecule (Xavier & Bassler, 2003). When an autoinducer

accumulates to the threshold concentration in a population-density manner, the expression triggers induction or repression of certain sets of genes that co-ordinate the behaviour of the bacterial population, including the expression of virulence factors. The mechanisms of QS have been extensively characterized in the pathogenic bacterium *Pseudomonas aeruginosa*, an important causative agent of nosocomial respiratory and urinary tract infections (Willcox *et al.*, 2008). *Ps. aeruginosa* produces several virulence factors, including LasB elastase, rhamnolipids, pyocyanin, lipase and hydrogen cyanide that contribute to its pathogenesis (Winstanley & Fothergill, 2009). The *las*- and *rhl*-QS systems regulate the production of these virulence factors via *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C₁₂-HSL) and *N*-butanoyl-L-homoserine lactone (C₄-HSL), respectively.

In general, AHLs bind to their receptor proteins (e.g. LasR, RhlR and LuxR) to regulate many bacterial behaviours, including attacking host cells. On the other hand, recent studies have revealed that AHLs directly induce the

Abbreviations: 3-oxo-C₁₂-HSL, *N*-(3-oxododecanoyl)-L-homoserine lactone; AHL, acylhomoserine lactone; C₄-HSL, *N*-butanoyl-L-homoserine lactone; QS, quorum sensing.

transcriptional activation of IL-8 or apoptosis of host cells (Smith *et al.*, 2001; Tateda *et al.*, 2003b). Other researchers have also reported that 3-oxo-C₁₂-HSL modulates cytokine production or chemotaxis of host cells (Shiner *et al.*, 2005; Telford *et al.*, 1998). Interestingly, it has been reported that in *Staphylococcus aureus*, 3-oxo-C₁₂-HSL not only inhibits growth but also the expression of a number of virulence genes (Qazi *et al.*, 2006). These results indicate that 3-oxo-C₁₂-HSL produced by *Ps. aeruginosa* plays a role not only in intraspecies communication, but also in interspecies or interkingdom interactions. Little is known about the interactions between Gram-negative bacteria in response to AHL signal molecules. In this study, we demonstrated the effects of 3-oxo-C₁₂-HSL produced by *Ps. aeruginosa* on the growth and expression of virulence factors in *Legionella* species, a Gram-negative bacterial species that frequently exists in freshwater habitats together with *Ps. aeruginosa* (Exner *et al.*, 2005; Sharma *et al.*, 2003).

METHODS

Bacterial strains and culture media. Bacterial strains used in this study are described in Table 1. Luria–Bertani (LB) medium (Difco) was used for cultivating *Ps. aeruginosa*, *Alcaligenes faecalis*, *Proteus mirabilis*, *Stenotrophomonas maltophilia*, *Serratia marcescens* and *Escherichia coli* (Berg *et al.*, 1999; Chang *et al.*, 2005; Lin *et al.*, 2000). These organisms were first grown on LB agar medium. A single colony was transferred to LB broth medium and incubated at 37 °C with constant shaking. Clinical isolates of *Legionella pneumophila*, *Legionella bozemanii*, *Legionella gormanii* and *Legionella micdadei*

were grown over 3–4 days at 37 °C on buffered charcoal yeast extract (BCYE) agar supplemented with L-cysteine and ferric nitrates (Byrne & Swanson, 1998). A single colony was transferred to buffered yeast extract broth and incubated overnight at 37 °C with constant shaking (Byrne & Swanson, 1998). The bacterial suspension was transferred to fresh buffered yeast extract broth in serial fivefold dilutions, and incubated overnight under the same conditions as those described above. The concentration of bacteria in a 1 cm path-length cuvette was determined by measuring the optical density at 660 nm (DU-640; Beckman). After treatment with AHLs, the viability of the bacterial suspension was confirmed by plating on BCYE agar. The number of viable bacteria was determined on BCYE agar after 4 days incubation.

Synthetic AHLs and their analogues. We synthesized 3-oxo-C₁₂-HSL, C₄-HSL, and several other HSL analogues using the method of Horikawa *et al.* (2006). The synthesized 3-oxo-C₁₂-HSL and C₄-HSL were structurally and functionally identical to the natural molecules produced by *Ps. aeruginosa*. The purity of each batch of HSLs and their analogues was confirmed by HPLC and ¹H NMR experiments.

Quantitative analysis of biofilm production. The effect of biofilm formation of *L. pneumophila* on 3-oxo-C₁₂-HSL was measured by using the method of Hindre *et al.* (2008). Briefly, overnight cultures of *L. pneumophila* were resuspended in fresh BCYE broth with or without 3-oxo-C₁₂-HSL in 96-well tissue culture plates (Becton Dickinson). After incubation at 25 °C, the biofilms on the plates were stained with 0.3% crystal violet solution. The plates were rinsed to remove planktonic cells, and the biofilm was then quantified by solubilizing the dye in ethanol and measuring the absorbance at 595 nm. The values presented are the results of six replicate experiments.

RNA isolation and quantitative PCR analysis of *L. pneumophila*. Total RNA from *L. pneumophila* was purified using an RNeasy Mini kit with RNAProtect Bacteria Reagent (Qiagen), according to

Table 1. Bacterial strains and primers used in this study

Strain or primer	Description	Source or reference
<i>Pseudomonas aeruginosa</i>	ATCC 15692 (PAO1)	
<i>Legionella pneumophila</i>		
Suzuki	Clinical isolate, highly pathogenic bacterium	Tateda <i>et al.</i> (2003a)
SG1	Clinical isolate, serogroup 1	This study
SG3	Clinical isolate, serogroup 3	This study
SG4	Clinical isolate, serogroup 4	This study
SG5	Clinical isolate, serogroup 5	This study
SG6	Clinical isolate, serogroup 6	This study
<i>Legionella bozemanii</i>	Clinical isolate	This study
<i>Legionella gormanii</i>	Clinical isolate	This study
<i>Legionella micdadei</i>	Clinical isolate	This study
<i>Alcaligenes faecalis</i>	Isolated from aquatic environment	This study
<i>Proteus mirabilis</i>	Clinical isolate	This study
<i>Stenotrophomonas maltophilia</i>	Isolated from aquatic environment	This study
<i>Escherichia coli</i>	Clinical isolate	This study
<i>Serratia marcescens</i>	Clinical isolate	This study
Primer	Sequence (5'–3')	Source or reference
16S rRNA for	TGAGTTTCCCAAGTTGTCC	This study
16S rRNA rev	ACGGCAGCATGTCTAGCTT	This study
<i>lqsR</i> for	GGCATGAATAGCAGCCAAAT	This study
<i>lqsR</i> rev	CAAAAACGCTGTGAAGTGA	This study

the manufacturer's instructions. To remove potential DNA contaminants, RNA was treated with RNase-free DNase (Qiagen). The concentration of RNA was determined by UV spectrophotometry using a DU 640 spectrophotometer (Beckman). Quantitative PCR (qPCR) was carried out by first synthesizing cDNA from 1 µg of a total RNA sample using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). The cDNA product was then diluted 1:9 with water, and 5 µl was used as template with 10 µM each qPCR primer (Table 1) in a PCR performed with SYBR GreenER qPCR SuperMix (Invitrogen), according to the manufacturer's instructions.

RESULTS

3-oxo-C₁₂-HSL exerts a bacteriostatic effect against *L. pneumophila*

It has been reported that *Ps. aeruginosa* 3-oxo-C₁₂-HSL exerts several activities in mammalian cells, plant cells, fungi and Gram-positive bacteria (Shiner *et al.*, 2005). We evaluated the effects of synthetic 3-oxo-C₁₂-HSL on the growth of several Gram-negative organisms, namely *L. pneumophila*, *Pr. mirabilis*, *A. faecalis*, *St. maltophilia*, *E. coli* and *Se. marcescens*; all these bacterial species frequently co-exist with *Ps. aeruginosa* in nature. As shown in Fig. 1, the growth of *Pr. mirabilis*, *A. faecalis*, *St. maltophilia*, *E. coli* and *Se. marcescens* was not affected by synthetic 3-oxo-C₁₂-HSL. However, the growth of *L. pneumophila* was suppressed by 50 µM 3-oxo-C₁₂-HSL. After 24 h, light-microscopic examination revealed that the morphology of the suppressed *L. pneumophila* cells was normal (data not shown). Furthermore, the growth of the treated *L. pneumophila* cells was normal on BCYE agar. This suggests that synthetic 3-oxo-C₁₂-HSL exerts a bacteriostatic effect against *L. pneumophila*.

Growth of *L. pneumophila* is affected by 3-oxo-C₁₂-HSL, but not by C₄-HSL

Although it is well known that *Ps. aeruginosa* produces two homoserine lactones, namely, 3-oxo-C₁₂-HSL and C₄-HSL, this bacterium has been reported to produce small

amounts of other types of AHLs in culture medium (Winson *et al.*, 1995). We evaluated whether these AHLs also exhibited a growth-suppressive effect on *L. pneumophila*. As shown in Fig. 2(a), synthetic C₄-, C₅- and C₇-HSL did not affect the growth of *L. pneumophila*, whereas both 3-oxo-C₁₂- and 3-oxo-C₁₀-HSL did suppress growth of *L. pneumophila*. Moreover, 3-oxo-C₁₂-HSL inhibited the growth of *L. pneumophila* more effectively than 3-oxo-C₁₀-HSL. As shown in Fig. 2(b), the growth of *L. pneumophila* was not affected by C₄-HSL at 100 µM. On the other hand, 3-oxo-C₁₂-HSL suppressed the growth of *L. pneumophila* in a dose-dependent manner. At 50 µM concentration, 3-oxo-C₁₂-HSL completely inhibited the growth of *L. pneumophila*.

Transient suppression of growth of *L. pneumophila*

Bacteria pre-treated with 3-oxo-C₁₂-HSL were used to determine whether growth suppression using 3-oxo-C₁₂-HSL is a transient response. *L. pneumophila* cells treated with 3-oxo-C₁₂-HSL after 20 h incubation were collected, washed and resuspended in fresh culture broth with or without 3-oxo-C₁₂-HSL, prior to determination of their growth. Regardless of the pre-treatment with 3-oxo-C₁₂-HSL, the growth of *L. pneumophila* fully recovered in the absence of 3-oxo-C₁₂-HSL (Fig. 3).

3-oxo-C₁₂-HSL decreases biofilm formation and *lqsR* transcription in *L. pneumophila*

We assessed whether 3-oxo-C₁₂-HSL stimulated biofilm formation in *L. pneumophila*. As shown in Fig. 4, biofilm formation was not affected by the presence or absence of 50 µM 3-oxo-C₁₂-HSL after incubation for 3 days. At day 5 and day 7, however, 3-oxo-C₁₂-HSL significantly suppressed *L. pneumophila* biofilm formation. AHL also suppressed growth of *L. pneumophila* (data not shown). This result suggests that growth inhibition of *L. pneumophila* by

Fig. 1. Growth of the organisms treated with (white bars) or without (black bars) 50 µM 3-oxo-C₁₂-HSL. Organisms were incubated overnight at 37 °C with constant shaking. The concentration of bacteria in the broth was determined by measuring optical density at 660 nm.

Fig. 2. Effect of AHLs on the growth of *L. pneumophila*. (a) Each AHL was added to BCYE medium at a final concentration of 50 μM. The organisms were incubated overnight at 37 °C with constant shaking. Bacterial growth in the broth was determined by measuring the optical density at 660 nm. (b) *L. pneumophila* was grown in BCYE medium with constant shaking. 3-oxo-C₁₂-HSL was added to the medium at a final concentration of 0 (●), 2 (△), 5 (◇), 20 (□) or 50 μM (○). The insert shows the effect of C₄-HSL on the growth at the same concentrations.

3-oxo-C₁₂-HSL results in biofilm suppression as well. We also assessed whether 3-oxo-C₁₂-HSL stimulated expression of the QS gene in *L. pneumophila*. Total RNA was extracted from *L. pneumophila* treated with 50 μM 3-oxo-C₁₂-HSL for 2, 7 and 28 h. By using qPCR, we found that the expression of the *lqsR* genes was significantly reduced by 3-oxo-C₁₂-HSL (Fig. 5). This result suggests that the expression of the QS gene of *L. pneumophila* was also stimulated by the QS signal of *Ps. aeruginosa*.

Fig. 3. Transient suppression of growth of *L. pneumophila*. *L. pneumophila* was grown in BCYE medium with (●) or without (○) 50 μM 3-oxo-C₁₂-HSL at 37 °C with constant shaking. The bacteria treated with 3-oxo-C₁₂-HSL were collected and washed, and resuspended in BCYE medium with (▲) or without (△) 50 μM 3-oxo-C₁₂-HSL.

DISCUSSION

Following characterization of the QS system in the marine, luminescent bacterium *Vibrio fischeri*, this system of communication between species via autoinducers has been found in many bacterial species (Whitehead *et al.*, 2001; Williams *et al.*, 2007). Recently, however, it has been

Fig. 4. Suppression of *L. pneumophila* biofilm production by 3-oxo-C₁₂-HSL. *L. pneumophila* biofilm production was estimated by crystal violet staining measured by absorbance at 590 nm. Overnight cultures of *L. pneumophila* were resuspended in BCYE broth with (left) or without (right) 3-oxo-C₁₂-HSL. After incubations for 2 h (black bars), 3 h (dark grey bars), 5 h (light grey bars) or 7 days (white bars) at 25 °C, biofilms produced in the plate were stained with 0.3% crystal violet solution, rinsed and crystal violet incorporation into the biofilm was monitored by using a microtitre plate reader. The bars represent the mean value from six different wells.

Fig. 5. Decreased expression of the QS gene (*lqsR*) in *L. pneumophila*. Total RNA was extracted from *L. pneumophila* treated with or without 50 μ M 3-oxo-C₁₂-HSL for 2, 7 and 28 h. The figure shows expression of *lqsR* relative to control gene (16S rRNA) expression.

proposed that autoinducers, such as 3-oxo-C₁₂-HSL, are directly used as a tool for signalling between pathogens and their host. Our previous study demonstrated that 3-oxo-C₁₂-HSL could accelerate apoptosis in the neutrophil and monocytic cell lines U-937 and P388D1, but not in the epithelial cell lines CCL-185 and HEp-2 (Tateda *et al.*, 2003b). Other researchers have also reported the immunomodulatory properties of 3-oxo-C₁₂-HSL in mammalian cells (Smith *et al.*, 2001; Telford *et al.*, 1998). In plants, proteome analysis revealed that AHLs from both symbiotic (*Sinorhizobium meliloti*) and pathogenic (*Ps. aeruginosa*) bacteria influence protein expression of *Medicago truncatula* (Mathesius *et al.*, 2003). These results suggest that bacterial autoinducers, in particular 3-oxo-C₁₂-HSL, may play a role not only in intraspecies communication, but also in interkingdom sensing and response. In this study, we demonstrated that the *Ps. aeruginosa* QS molecule 3-oxo-C₁₂-HSL (but not C₄-HSL) exerts both bacteriostatic and biofilm formation-suppressing activities against *Legionella* spp., but not against other bacteria that we tested. It has been reported that naturally occurring tetramic acid efficiently chelates a variety of metal cations (Kaufmann *et al.*, 2005). Furthermore, these authors suggested that 3-oxo-C₁₂-HSL and its tetramic acid degradation product [3-(1-hydroxydecylidene)-5-(2-hydroxyethyl) pyrrolidine-2,4-dione] may be potent antibacterial agents against Gram-positive bacteria. To examine the possible effects against Gram-negative bacteria such as *L. pneumophila*, we tested whether the growth of *L. pneumophila* is inhibited by 3-oxo-C₁₂-HSL in the presence or absence of ferric nitrate. However, 3-oxo-C₁₂-HSL did not act as a chelating agent in *L. pneumophila* (data not shown). These results suggest a novel role for 3-oxo-C₁₂-HSL in interspecies competition between two Gram-negative bacterial organisms – *Ps. aeruginosa* and *Legionella* spp.

Legionella spp. persists in biofilms, which are widespread not only in natural water environments, but also on medical and

dental devices (Atlas, 1999). When inhaled, *Legionella* spp. can replicate within alveolar macrophages of the human lung, causing Legionnaires' disease. Many studies focusing on the disinfection of *Legionella* spp. have been published. There are two major treatments to disinfect *Legionella* spp., the thermal or the chemical method (Cachafeiro *et al.*, 2007; Kim *et al.*, 2002), and the use of copper/silver ionization systems is becoming popular. Ions released into the water result in permeability changes in cellular membranes that cause denaturing of protein and subsequent cellular lysis (Cachafeiro *et al.*, 2007). We have shown in this study that the *Pseudomonas* autoinducer 3-oxo-C₁₂-HSL possessed bacteriostatic activity and biofilm-suppressing activity in *L. pneumophila*. The results suggest that 3-oxo-C₁₂-HSL or analogues of 3-oxo-C₁₂-HSL may have the potential to limit the severity of Legionnaires' disease.

AHLs bind to autoinducer-dependent transcriptional activator proteins, such as LuxR and LasR, and then modulate the activities of target genes. Although the genomes of three strains of *L. pneumophila* were completely sequenced in 2004 (Cazalet *et al.*, 2004; Chien *et al.*, 2004), no classical QS system, such as *las*- or *rhl*-QS, was detected (Molofsky & Swanson, 2004). It is also thought autoinducer-2 plays a role in interspecies interactions (Federle & Bassler, 2003). However, a LuxS/autoinducer-2 QS system has not yet been detected in *L. pneumophila* (Molofsky & Swanson, 2004). A number of Gram-negative bacteria possess both genes encoding signal synthase and signal receptor on their chromosome. However, some species of Gram-negative bacteria, such as *E. coli*, *Salmonella enterica* serovar Typhimurium and *Shigella flexneri*, produce a signal receptor of the LuxR family, termed SdiA, but not a corresponding signal-generating enzyme. The recent discovery of the crystal structure of SdiA revealed that this LuxR-type detection molecule may act as a detector of autoinducers produced by other bacterial species (Yao *et al.*, 2006). We screened the *L. pneumophila* genome but failed to find an SdiA homologue (data not shown). More recently, it has been discovered that *L. pneumophila* possess a novel QS system (*lqsA*, *lqsR*, *lqsS*) that differs from classical QS systems (Spirig *et al.*, 2008; Tiaden *et al.*, 2007). The autoinducer produced by *L. pneumophila* was identified as 3-hydroxytridecan-4-one, which is not AHL-based. However, the relationship between 3-oxo-C₁₂-HSL and this new QS system has not yet been elucidated. Therefore, further studies are required to determine the detailed mechanism of this function.

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REFERENCES

Atlas, R. M. (1999). *Legionella*: from environmental habitats to disease pathology, detection and control. *Environ Microbiol* 1, 283–293.

- Berg, G., Roskot, N. & Smalla, K. (1999). Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. *J Clin Microbiol* 37, 3594–3600.
- Byrne, B. & Swanson, M. S. (1998). Expression of *Legionella pneumophila* virulence traits in response to growth conditions. *Infect Immun* 66, 3029–3034.
- Cachafeiro, S. P., Naveira, I. M. & Garcia, I. G. (2007). Is copper–silver ionisation safe and effective in controlling legionella? *J Hosp Infect* 67, 209–216.
- Cazalet, C., Rusniok, C., Brüggemann, H., Zidane, N., Magnier, A., Ma, L., Tichit, M., Jarraud, S., Bouchier, C. & other authors (2004). Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nat Genet* 36, 1165–1173.
- Chang, H. C., Chen, C. R., Lin, J. W., Shen, G. H., Chang, K. M., Tseng, Y. H. & Weng, S. F. (2005). Isolation and characterization of novel giant *Stenotrophomonas maltophilia* phage phiSMA5. *Appl Environ Microbiol* 71, 1387–1393.
- Chien, M., Morozova, I., Shi, S., Sheng, H., Chen, J., Gomez, S. M., Asamani, G., Hill, K., Nuara, J. & other authors (2004). The genomic sequence of the accidental pathogen *Legionella pneumophila*. *Science* 305, 1966–1968.
- Exner, M., Kramer, A., Lajoie, L., Gebel, J., Engelhart, S. & Hartemann, P. (2005). Prevention and control of health care-associated waterborne infections in health care facilities. *Am J Infect Control* 33, S26–S40.
- Federle, M. J. & Bassler, B. L. (2003). Interspecies communication in bacteria. *J Clin Invest* 112, 1291–1299.
- Fuqua, C. & Greenberg, E. P. (2002). Listening in on bacteria: acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* 3, 685–695.
- Hindre, T., Brüggemann, H., Buchrieser, C. & Hechard, Y. (2008). Transcriptional profiling of *Legionella pneumophila* biofilm cells and the influence of iron on biofilm formation. *Microbiology* 154, 30–41.
- Horikawa, M., Tateda, K., Tuzuki, E., Ishii, Y., Ueda, C., Takabatake, T., Miyairi, S., Yamaguchi, K. & Ishiguro, M. (2006). Synthesis of *Pseudomonas* quorum-sensing autoinducer analogs and structural entities required for induction of apoptosis in macrophages. *Bioorg Med Chem Lett* 16, 2130–2133.
- Kaufmann, G. F., Sartorio, R., Lee, S. H., Rogers, C. J., Meijler, M. M., Moss, J. A., Clapham, B., Brogan, A. P., Dickerson, T. J. & Janda, K. D. (2005). Revisiting quorum sensing: discovery of additional chemical and biological functions for 3-oxo-*N*-acylhomoserine lactones. *Proc Natl Acad Sci U S A* 102, 309–314.
- Kim, B. R., Anderson, J. E., Mueller, S. A., Gaines, W. A. & Kendall, A. M. (2002). Literature review – efficacy of various disinfectants against *Legionella* in water systems. *Water Res* 36, 4433–4444.
- Lin, M., Smalla, K., Heuer, H. & van Elsas, J. D. (2000). Effect of an *Alcaligenes faecalis* inoculant strain on bacterial communities in flooded soil microcosms planted with rice seedlings. *Appl Soil Ecol* 15, 211–225.
- Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-Anolles, G., Rolfe, B. G. & Bauer, W. D. (2003). Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc Natl Acad Sci U S A* 100, 1444–1449.
- Miller, M. B. & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annu Rev Microbiol* 55, 165–199.
- Molofsky, A. B. & Swanson, M. S. (2004). Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. *Mol Microbiol* 53, 29–40.
- Oazi, S., Middleton, B., Muharram, S. H., Cockayne, A., Hill, P., O'Shea, P., Chhabra, S. R., Camara, M. & Williams, P. (2006). *N*-Acylhomoserine lactones antagonize virulence gene expression and quorum sensing in *Staphylococcus aureus*. *Infect Immun* 74, 910–919.
- Sharma, S., Sachdeva, P. & Virdi, J. S. (2003). Emerging water-borne pathogens. *Appl Microbiol Biotechnol* 61, 424–428.
- Shiner, E. K., Rumbaugh, K. P. & Williams, S. C. (2005). Inter-kingdom signaling: deciphering the language of acyl homoserine lactones. *FEMS Microbiol Rev* 29, 935–947.
- Smith, R. S., Fedyk, E. R., Springer, T. A., Mukaida, N., Iglewski, B. H. & Phipps, R. P. (2001). IL-8 production in human lung fibroblasts and epithelial cells activated by the *Pseudomonas* autoinducer *N*-3-oxododecanoyl homoserine lactone is transcriptionally regulated by NF-kappa B and activator protein-2. *J Immunol* 167, 366–374.
- Spirig, T., Tiaden, A., Kiefer, P., Buchrieser, C., Vorholt, J. A. & Hilbi, H. (2008). The *Legionella* autoinducer synthase LqsA produces an α -hydroxyketone signaling molecule. *J Biol Chem* 283, 18113–18123.
- Tateda, K., Deng, J. C., Moore, T. A., Newstead, M. W., Paine, R., III, Kobayashi, N., Yamaguchi, K. & Standiford, T. J. (2003a). Hyperoxia mediates acute lung injury and increased lethality in murine *Legionella* pneumonia: the role of apoptosis. *J Immunol* 170, 4209–4216.
- Tateda, K., Ishii, Y., Horikawa, M., Matsumoto, T., Miyairi, S., Pechere, J. C., Standiford, T. J., Ishiguro, M. & Yamaguchi, K. (2003b). The *Pseudomonas aeruginosa* autoinducer *N*-3-oxododecanoyl homoserine lactone accelerates apoptosis in macrophages and neutrophils. *Infect Immun* 71, 5785–5793.
- Telford, G., Wheeler, D., Williams, P., Tomkins, P. T., Appleby, P., Sewell, H., Stewart, G. S., Bycroft, B. W. & Pritchard, D. I. (1998). The *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. *Infect Immun* 66, 36–42.
- Tiaden, A., Spirig, T., Weber, S. S., Brüggemann, H., Bosshard, R., Buchrieser, C. & Hilbi, H. (2007). The *Legionella pneumophila* response regulator LqsR promotes host cell interactions as an element of the virulence regulatory network controlled by RpoS and LetA. *Cell Microbiol* 9, 2903–2920.
- Whitehead, N. A., Barnard, A. M., Slater, H., Simpson, N. J. & Salmond, G. P. (2001). Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol Rev* 25, 365–404.
- Willcox, M. D., Zhu, H., Conibear, T. C., Hume, E. B., Givskov, M., Kjelleberg, S. & Rice, S. A. (2008). Role of quorum sensing by *Pseudomonas aeruginosa* in microbial keratitis and cystic fibrosis. *Microbiology* 154, 2184–2194.
- Williams, P., Winzer, K., Chan, W. C. & Camara, M. (2007). Look who's talking: communication and quorum sensing in the bacterial world. *Phil Trans R Soc B* 362, 1119–1134.
- Winston, M. K., Camara, M., Latifi, A., Foglino, M., Chhabra, S. R., Daykin, M., Bally, M., Chapon, V., Salmond, G. P. & other authors (1995). Multiple *N*-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 92, 9427–9431.
- Winstanley, C. & Fothergill, J. L. (2009). The role of quorum sensing in chronic cystic fibrosis *Pseudomonas aeruginosa* infections. *FEMS Microbiol Lett* 290, 1–9.
- Xavier, K. B. & Bassler, B. L. (2003). LuxS quorum sensing: more than just a numbers game. *Curr Opin Microbiol* 6, 191–197.
- Yao, Y., Martinez-Yamout, M. A., Dickerson, T. J., Brogan, A. P., Wright, P. E. & Dyson, H. J. (2006). Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. *J Mol Biol* 355, 262–273.

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