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NOTE

Inhibition of quorum sensing in *Pseudomonas aeruginosa* by two herbal essential oils from *Apiaceae* family

Ehsan Sepahi[1], Saeed Tarighi[2*], Farajollah Shahriari Ahmadi[1], and Abdolreza Bagheri[1]

[1]Department of Biotechnology, [2]Laboratory of Phytopathology, Department of Crop Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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Ferula (*Ferula asafoetida* L.) and Dorema (*Dorema aucheri* Bioss.) both from Apiaceae family were tested for their anti-quorum sensing (QS) activity against *Pseudomonas aeruginosa*. Both essential oils exhibited anti-QS activity at 25 μg/ml of concenteration. At this concenteration Ferula fully abolished and Dorema reduced the violacein production by *C. violaceum*. Pyocyanin, pyoverdine, elastase and biofilm production were decreased in Ferula oil treatments. Dorema oil reduced pyoverdine and elastase production, while pyocyanin and biofilm production were not affacted. Expresion analysis of QS-dependent genes confirmed our phenotypic data. Our data introduced native Dorema and Ferula plants as novel QS and virulence inhibitors.

Keywords: quorum sensing, *N*-acyl-homoserine lactones, *Pseudomonas aeruginosa*, *Dorema aucheri*, *Ferula assafoetida*, quorum quenching, virulence

Ferula [*Ferula asafoetida* L. (Apiaceae)] is an herbaceous plant with a wide distribution in arid climates including Iran, Afghanistan and India. This plant is widely used to alleviate bacterial infections in western part of Iran. Dorema [*Dorema aucheri* Boiss. (Apiaceae)] is a large umbelliferous herb that is also growing in western mountains of Iran and in Persian called kandale koohi. Medicinally, the roots of *D. aucheri* have been used to treat microbial infections. This species is the first umbelliferous plant found to produce flavonoids which well known as antimicrobial agents (Wollenweber, 1988; Cushnie and Lamb, 2005). Due to their use in treatment of microbial infections, it is possible that these plants might be possess anti-quorum sensing (QS) properties.

*For correspondence. E-mail: starighi@um.ac.ir; Tel.: +98-511-8805815; Fax: +98-511-8788875

Pseudomonas aeruginosa is an opportunistic pathogen in diverse hosts including plants, mammals, insects, and nematodes (Govan and Deretic, 1996; Tan *et al*., 1999; Jander *et al*., 2000). Three main QS systems in *P. aeruginosa* are *las*, *rhl*, and *pqs* systems which influence around 6% of all the genes in the complete genome sequenced *P. aeruginosa* (Stover *et al*., 2000; Hentzer *et al*., 2003; Bredenbruch *et al*., 2006; Diggle *et al*., 2007). Most of these genes provide bacterial population with distinctive density-dependent behaviors such as exopolysaccharide synthesis, biofilm formation, swarming motility, pigment, and antibiotic production (Schonewille *et al*., 2012). Owing to the importance of QS during bacterial pathogenesis, interfering with this mechanism is being considered as a rational strategy to attenuate their virulence (Adonizio *et al*., 2006; Tarighi and Taheri, 2011). Likewise the strategy to treat bacterial diseases with antibiotics is nowadays seriously threatened by the spread of drug-tolerant strains, which cause persistent infections, often involving the formation of highly resistant biofilms. The production of biofilm and other pathologically significant virulence factors were strictly interrelated with the expression of the QS alarm one, therefore the discovery of anti-pathogenic drugs that are capable of interfering with the bacterial communication system, without inducing lethal effects can disrupt the pathogenicity process (Ghysels *et al*., 2004; Otto, 2004). QS inhibitory compounds, unlike conventional antibiotics, do not kill or inhibit microbial growth and are less likely to impose a selective pressure for the development of drug resistant bacteria (Hong *et al*., 2012). Although different plant metabolites have been identified to treat microbial infections (Shahid *et al*., 2009), but their mode of action is not clearly understood (Bjarnsholt *et al*., 2005; Khan *et al*., 2009; Szabó *et al*., 2010). This article reports the anti-quorum sensing activity of the two essential oils from Apiaceae family on which there are no previous studies.

The *P. aeruginosa* PAO1 (ATTC 15692) was grown at 37°C in Luria-Bertani (LB) broth or on LB agar plates, iron poor casamino acids (CAA) medium (Difco Laboratories) or *Pseudomonas* agar medium (Difco Laboratories). For every experiment 25 μg/ml of each essential oil were added to suitable media.

The air-dried foliar parts of plant (250 g) were subjected to hydro-distillation for 3 h using a clevenger apparatus, according to the method recommended by the European Pharmacopoeia. The essential oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4°C until further analysis (Aminifard and Mohammadi, 2013).

Table 1. List of primers used in this study

Sequence (5′→3′)	Primers
GCTCTCTGAATCGCTGGAA	*rhlI-F*
GCGATCACGCCCTTGCGAA	*rhlI-R*
GTGCGGCGCGAAGAGTTCGAT	*lasI-F*
GTGGCGCTCGATGCCGATCTT	*lasI-R*
GTGCGACCTTCACCTGTCCGT	*antA-F*
GTGCGCTGAAGAGCACGCTGT	*antA-R*
GTGCGTTGAACGACTCGCCTT	*pqsA-F*
GTGGCCGAGGCTCCGCTGAA	*pqsA-R*
ATGGAAATGCTGACCTTAGG	*oprL-F*
TGAGCCCAGGACTGCTCGT	*oprL-R*

Pyocyanin was visualized by growing the bacteria in LB medium or on P-agar for 48 h. Pyocyanin and elastase production were measured as described previously (Rust *et al.*, 1994; Mavrodi *et al.*, 2001). For the detection of pyoverdine, strains were grown in liquid CAA medium and the absorption of the filter sterilized supernatants were measured at 405 nm (Wei *et al.*, 2011). All experiments were done in triplicate.

Rapid detection of *N*-acyl homoserine lactones (AHL)s in filter sterilized (0.2-µm-pore-size filters) culture supernatants was done by AHL reporter plate bioassays using *Chromobacterium violaceum* CV026 (McClean *et al.*, 1997).

Total RNA was isolated by using the RNeasy kit from QIAGEN. Reverse transcription was performed using 1 µg of total RNA and the first-strand cDNA synthesis kit from GE Healthcare as indicated by the manufacturer. A 100 ng of first-strand cDNA in a total volume of 25 µl were used to assess the effect of oils on expression of the selected target genes (Table 1). PCR reactions contained 100 nmol of each primer and the PCR master mix. After heating samples to 95°C for 30 min, 25 cycles were performed, consisting of 95°C for 1 min, 54°C for 45 sec and 72°C for 50 sec. The primers *oprL*-F and *oprL*-R were used for normalization controls (Tarighi *et al.*, 2008).

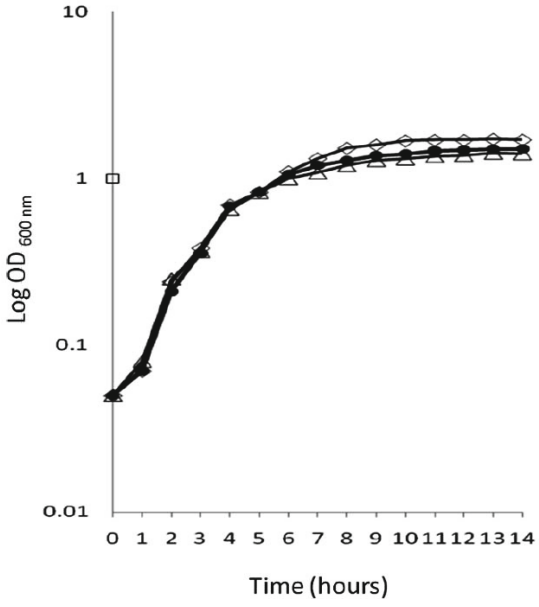

Fig. 1. Growth of *P. aeruginosa* in LB broth media with Ferula oil (triangle), Dorema oil (lozenge), and without oils (black circle). The data represent the mean values of experiments performed in triplicate. Y axis, indicate logarithm of cell density based on absorbance measurement at 600 nm. X axis, shows the times (h) of absorbance measurement.

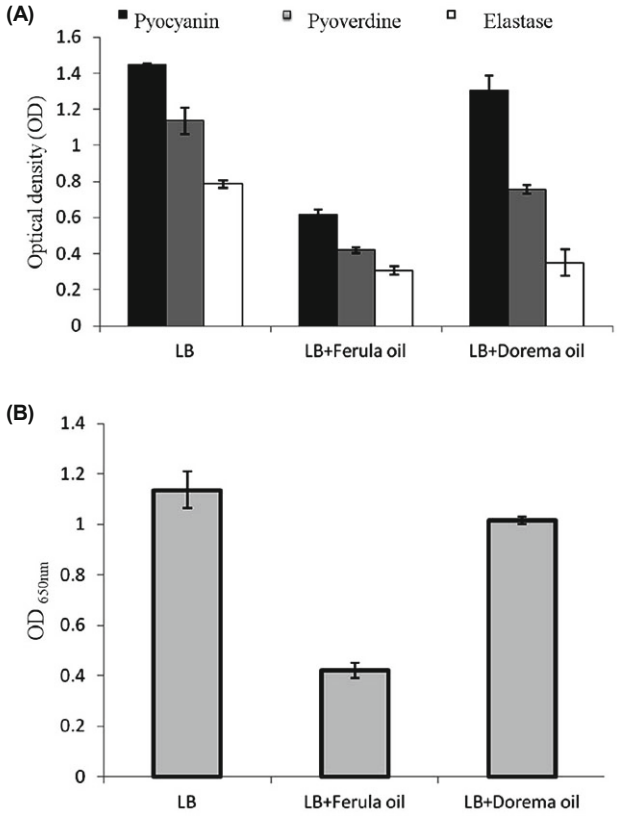

Fig. 2. (A) Influence of Ferula and Dorema oils on pyocyanin, pyoverdine and elastase production. Samples for each assay were taken when cells had reached to the stationary phase. Measurments are based on the changes in optical density as mentioned in Methods. All of the experiments were repeated at least three times with similar results. Black, gray and white bars indicates pyocyanin, pyoverdin and elastase production. Y axis shows the optical density (OD) at 520 nm for pyocyanin, 405 nm for pyoverdin, and 495 nm for elastase measurements. Results presented are from three experiments that consisted of 3 replicates per treatment. Vertical bars represent the standard error of the mean ($P \leq 0.05$). (B) Biofilm production were qunatified as the measurment of crystal violet at OD_{650nm}.

When different native plant essential oils were screened for their inhibition activity on *P. aeruginosa* elastase production, two oils were identified with the most potent anti-elastase activity rather than antimicrobial properties. *P. aeruginosa* elastase is believed to play a major role in pathogenesis via host tissue degradation (Morihara and Homma, 1985; Kharazmi, 1989). The growth rate of *P. aeruginosa* in the presence of essential oils was assayed in shake flasks. Ferula and Dorema oils did not have any inhibitory effect on *P. aeruginosa* growth at 25 µg/ml concentration (Fig. 1). Infections caused by *P. aeruginosa* had been seen with high prevalence in the world due to multi-antibiotic resistance. In most cases the antibiotic resistance variants of *P. aeruginosa* were found to be more adherent and virulent (Proctor *et al.*, 2006; Wei

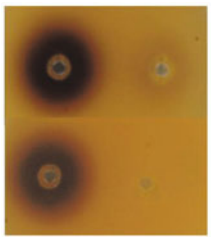

Fig. 3. LB-agar plate bioassay of HSL production using *C. violaceum* CV026. Right in prescence and left in absence of the essential oils.

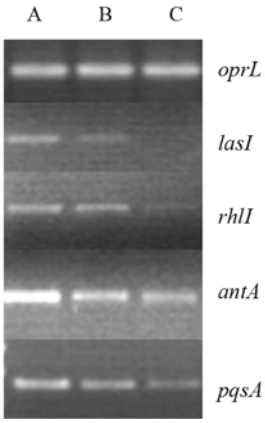

Fig. 4. RT-PCR analysis of the genes involved in QS system of *P. aeruginosa*. The gene expression was evaluated in absence of essential oils (A), in presence of Dorema oil (B), and in presence of Ferula oil (C).

et al., 2011). Therefore, identification of substances which don't kill bacteria but reduce their virulence motivated most of researchers. QS is a target for this purpose since most of virulence factors in *P. aeruginosa* are under control of QS. *P. aeruginosa* has three QS systems, namely *las*, *rhl*, and *pqs*. The *las* and *rhl* systems are LuxI/LuxR homologues of LasI/LasR and of RhlI/RhlR, respectively (Dekimpe and Deziel, 2009). Our data demonstrated that Dorema and Ferula oils decreased both the elastase activity of *P. aeruginosa* PAO1 and the transcriptional activation of *lasI*, which indicated that these two oils inhibited the *las* system (Figs. 2A and 4). The *rhlI* gene slightly repressed in the presence of Ferula oil and it was not affected in response to Dorema oil treatment (Fig. 4). The elastase production is generally thought to be under the control of *lasI-lasR* system (Gambello and Iglewski, 1991); however, *rhlI-rhlR* also controls elastase activity to a lesser extent (Brint and Ohman, 1995; Pearson *et al*., 1997).

Pseudomonas aeruginosa pyoverdin production is one of the QS-controlled factors and is mainly regulated by *las* system (Stintzi *et al*., 1998; Li *et al*., 2013). The drastically inhibition of pyoverdin production by both examined oils indicated that the *las* system was inhibited (Fig. 2A). The production of pyoverdin might be indirectly affected by pH or iron concentration changes in culture media (Jacques *et al*., 2003). Therefore we checked the pH solution of our culture media throughout the experiments. No changes in pH were observed. The furanones, which are well known QS inhibitors, have previously been shown contradictory results on pyoverdin production. A [(5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone] from *Delisea pulchra* increased the production of pyoverdin (Reimmann *et al*., 1997). Whereas, furanone C-30, a synthetic derivative of a compound from *D. pulchra*, conferred a 90% reduction in pyoverdin levels (Hentzer *et al*., 2003).

The ferula oil reduced also the pyocyanin production of PAO1, whereas transcriptional activation of *pqsA* were not affected, suggesting inhibition of pyocyanin production might be outside of the *pqs* system (Figs. 2A and 5). PQS signaling is associated with the AHL based QS systems, involved in regulation of virulence factors production, particularly phenazine, pyocyanin, hydrogen cyanide and in autolysis of *P. aeruginosa* colonies (D'Argenio *et al*., 2002). In a previous study, *antA* is repressed by LasR, a QS regulator during log phase and activated by RhlR, another QS regulator in a late stationary phase, whereas *pqs* operon for PQS synthesis is activated by LasR in the log phase and repressed by RhlR (Choi, 2011). Meanwhile our results indicated that the expression of *antA,* which is involved in degradation of anthranilate (PQS precursor) (Gallagher *et al*., 2002; Kim *et al*., 2012), was highly reduced in the presence of both essential oils (Fig. 4). It was discovered that the *phnA* mutant of *P. aeruginosa* was deficient in production of the virulence factor pyocyanin while the *trpE* mutant was not (Essar *et al*., 1990a, 1990b). Other researches clarified that PhnAB generated anthranilate for pyocyanin production, but later

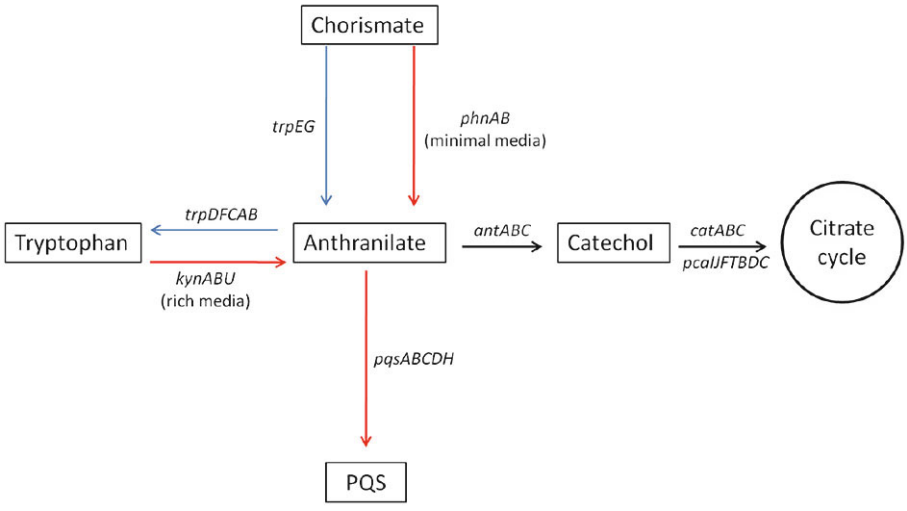

Fig. 5. Schematic overview of anthranilate biosynthesis and degradation. The red arrows indicate the genes involved in PQS biosynthesis via anthranilate. The blue arrows show the genetic context of tryptophan biosynthesis pathway.

works determined that anthranilate is instead a precursor for PQS production, which is required to induce genes for pyocyanin biosynthesis (Calfee *et al*., 2001; Mavrodi *et al*., 2001; Gallagher *et al*., 2002). Therefore, we speculated that the accumulation of anthranilate by downregulation of *antA* gene could be used in biosynthesis of tryptophan, owing to the expression of *pqsA* gene was not affected (Fig. 4). Farrow and Pesci (2007) indicated that the anthranilate derived from the action of the TrpEG is mainly necessary for tryptophan biosynthesis while anthranilate derived from tryptophan degradation through the kynurenine pathway in rich media would be used for PQS production and in minimal media the PhnAB anthranilate synthase supplies the required anthranilate for PQS production (Fig. 5).

Disruption of the QS system with furanones has also been shown to inhibit biofilm growth (Diggle *et al*., 2007). Previous work with garlic and *Delisea pulchra* furanones showed a qualitative change in biofilm morphology and a reduction in thickness; however, these analyses were not quantified (Stover *et al*., 2000; Rasmussen *et al*., 2005; Diggle *et al*., 2007). Adonizo *et al*. (2008) demonstrated significant decrease in biofilm formation in the presence of *Callistemon viminalis*, *Quercus virginiana*, *Tetrazygia bicolor*, and *Bucida buceras* plant extracts. But there was a significant increase in biofilm formation compared to that of the control when strain PAO1 was grown in the presence of *F. asafoetida* (Fig. 2B). *D. aucheri* slightly decreased biofilm formation. *Quercus virginiana* extract has been indicated no correlation between biofilm formation and the production of pyocyanin, pyoverdin and elastase (Davies *et al*., 1998; Adonizo *et al*., 2008). Therefore, the biofilm production should be partially controled by QS and other factors including physical media condition can affect biofilm production.

In *P. aeruginosa*, the amount of virulence factors production including pyoverdine, pyocyanin, elastase and biofilm are generally thought to act in concert with the production of quorum sensing signal molecules (QSSMs). We determined the effect of tested essential oils on the production of homoserin lactones (HSL) of *P. aeruginosa* by assessing the ability of bacterial supernatants in induction of *C. violaceum* CV026 to violacein production (Fig. 3). Control assays indicated that ferula oil fully inhibited the production of HSLs in *P. aeruginosa*. Dorema oil treatment decreased the production of HSLs in *P. aeruginosa*. QS inhibition assays and repression of *P.aeruginosa* virulence factors production suggested that QS circuits were targeted by the tested essential oils. However, these observations didn't exclude other targets of the plant essential oils. RT-PCR analysis offered the ability to reveal essential oil target specity by monitoring changes in transcript accumulation (Fig. 4).

The redundant and autoregulatory nature of the QS system is quite convoluted. This fact, together with the complex plant essential oil compounds, prevented us from precisely linking QS gene expression, the AHL level, and virulence factor production. Although the mechanism of action of these plant essential oils is a complex problem, there was an overall inhibition of the QS system with Dorema and Ferula oils. Therefore, we supposed that multiple chemicals in these oils might be associated with distinct effects on different aspects of the QS system. The second explanation is that the effect is not directly on *las-rhl* system but, rather, on a more global QS regulator, such as Vfr (Albus *et al*., 1997) or GacA (Reimmann *et al*., 1997). Our data indicated that Dorema and Ferula oils had anti-quorum sensing activity, a finding which might be correlated with the herb's use in traditional medicine. Through further research, it might be possible to isolate therapeutic molecules and developed the compounds responsible for QS inhibition activity.

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References

Adonizio, A.L., Downum, K., Bennett, B.C., and Mathee, K. 2006. Anti-quorum sensing activity of medicinal plants in southern Florida. *J. Ethnopharma.* **105**, 427–435.

Adonizio, A., Kong, K.F., and Mathee, K. 2008. Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. *Antimicrob. Agents Chemother.* **52**, 98–203.

Albus, A.M., Pesci, E.C., Runyen-Janecky, L.J., West, S.E., and Iglewski, B.H. 1997. Vfr controls quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* **179**, 3928–3935.

Aminifard, M.H. and Mohammadi, S. 2013. Essential oils to control *Botrytis cinerea in vitro* and *in vivo* on plum fruits. *J. Sci. Food Agric*. **93**, 348–353.

Bjarnsholt, T., Jensen, P.O., Rasmussen, T.B., Christophersen, L., Calum, H., Hentzer, M., Hougen, H.P., Rygaard, J., Moser, C., Eberl, L., and *et al.* 2005. Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. *Microbiology* **151**, 3873–3880.

Bredenbruch, F., Geffers, R., Nimtz, M., Buer, J., and Hussler, S. 2006. The *Pseudomonas aeruginosa* quinolone signal (PQS) has an iron-chelating activity. *Environ. Microbiol.* **8**, 1318–1329.

Brint, J.M. and Ohman, D.E. 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *J. Bacteriol.* **177**, 7155–7163.

Calfee, M.W., Coleman, J.P., and Pesci, E.C. 2001. Interference with *Pseudomonas* quinolone signal synthesis inhibits virulence factor expression by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* **98**, 11633–11637.

Choi, Y. 2011. Growth phase-differential quorum sensing regulation of anthranilate metabolism in *Pseudomonas aeruginosa*. *Mol. Cells* **32**, 57–65.

Cushnie, T.P. and Lamb, A.J. 2005. Antimicrobial activity of flavonoids. *Inter. J. Antimicrob. Agents* **26**, 343–356.

D'Argenio, D.A., Calfee, M.W., Rainey, P.B., and Pesci, E.C. 2002. Autolysis and autoaggregation in *Pseudomonas aeruginosa* colony morphology mutants. *J. Bacteriol.* **184**, 6481–6489.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., and Greenberg, E.P. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280**, 295–298.

Dekimpe, V. and Déziel, E. 2009. Revisiting the quorum-sensing hierarchy in *Pseudomonas aeruginosa*: the transcriptional regulator RhlR regulates LasR-specific factors. *Microbiology* **155**, 712–723.

Diggle, S.P., Matthijs, S., Wright ,V.J., Fletcher, M.P., Chhabra, S.R., Lamont, I.L., Kong, X., Hider, R.C., Cornelis, P., Cámara, M., *et al.* 2007. The *Pseudomonas aeruginosa* 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. *Chem. Biolog.* **14**, 87.

Essar, D., Eberly, L., Hadero, A., and Crawford, I. 1990a. Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. *J. Bacteriol.* **172**, 884–900.

Essar, D.W., Eberly, L., Han, C.Y., and Crawford, I.P. 1990b. DNA sequences and characterization of four early genes of the tryptophan pathway in *Pseudomonas aeruginosa*. *J. Bacteriol.* **172**, 853–866.

Farrow, J.M. and Pesci, E.C. 2007. Two distinct pathways supply anthranilate as a precursor of the *Pseudomonas* quinolone signal. *J. Bacteriol.* **189**, 3425–3433.

Gallagher, L.A., McKnight, S.L., Kuznetsova, M.S., Pesci, E.C., and Manoil, C. 2002. Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa. J. Bacteriol.* **184**, 6472–6480.

Gambello, M.J. and Iglewski, B.H. 1991. Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. *J. Bacteriol.* **173**, 3000–3009.

Ghysels, B., Dieu, B.T., Beatson, S.A., Pirnay, J.P., Ochsner, U.A., Vasil, M.L., and Cornelis, P. 2004. FpvB, an alternative type I ferripyoverdine receptor of *Pseudomonas aeruginosa*. *Microbiology* **150**, 1671–1680.

Govan, J.R. and Deretic, V. 1996. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol. Rev.* **60**, 539–574.

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

Hong, K.W., Koh, C.L., Sam, C.K., Yin, W.F., and Chan, K.G. 2012. Quorum quenching revisited from signal decays to signaling confusion. *Sensors* **12**, 4661–4696.

Jacques, P., Ongena, M., Bernard, F., Fuchs, R., Budzikiewicz, H., and Thonart, P. 2003. Fluorescent *Pseudomonas* mainly produce the dihydroform of pyoverdine at low specific growth rate. *Lett. Appl. Microbiol.* **36**, 259–262.

Jander, G., Rahme, L.G., and Ausubel, F.M. 2000. Positive correlation between virulence of *Pseudomonas aeruginosa* mutants in mice and insects. *J. Bacteriol.* **182**, 3843–3845.

Khan, M.S., Zahin, M., Hasan, S., Husain, F.M., and Ahmad, I. 2009. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett. Appl. Microbiol.* **49**, 354–360.

Kharazmi, A. 1989. Interactions of *Pseudomonas aeruginosa* proteases with the cells of the immune system. *Anti. Chem.* **42**, 42.

Kim, S.K., Im, S.J., Yeom, D.H., and Lee, J.H. 2012. AntR-mediated bidirectional activation of *antA* and *antR*, anthranilate degradative genes in *Pseudomonas aeruginosa*. *Gene* **505**, 146–152.

Li, K., Xu, C., Jin, Y., Sun, Z., Liu, C., Shi, J., Chen, G., Chen, R., Jin, S., and Wu, W. 2013. SuhB is a regulator of multiple virulence genes and essential for pathogenesis of *Pseudomonas aeruginosa*. *MBio.* **29**, 419–428.

Mavrodi, D.V., Bonsall, R.F., Delaney, S.M., Soule, M.J., Phillips, G., and Thomashow, L.S. 2001. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* **183**, 6454–6465.

McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R., Camara, M., Daykin, M., Lamb, J.H., Swift, S., Bycroft, B.W., *et al.* 1997. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology* **143**, 3703–3711.

Morihara, K. and Homma, J. 1985. *Pseudomonas* proteases, pp. 41–79. Bacterial enzymes and virulence. CRC Press, Inc., Boca Raton, Fla, USA.

Otto, M. 2004. Quorum-sensing control in *Staphylococci* a target for antimicrobial drug therapy? *FEMS Microbiol. Lett.* **241**, 135–141.

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

Proctor, R.A., VonEiff, C., Kahl, B.C., Becker, K., McNamara, P., Herrmann, M., and Peters, G. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat. Rev. Microbiol.* **4**, 295–305.

Rasmussen, T.B., Bjarnsholt, T., Skindersoe, M.E., Hentzer, M., Kristoffersen, P., Köte, M., Nielsen, J., Eberl, L., and Givskov, M. 2005. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J. Bacteriol.* **187**, 1799–1814.

Reimmann, C., Beyeler, M., Latifi, A., Winteler, H., Foglino, M., Lazdunski, A., and Haas, D. 1997. The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer N-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. *Mol. Microbiol.* **24**, 309–319.

Rust, L., Messing, C.R., and Iglewski, B.H. 1994. Elastase assays. *Meth. Enzym.* **235**, 554–562.

Schonewille, E., Nesse L.L., Hauck, R., Windhorst, D., Hafez, H.M., and Vestby, L.K. 2012. Biofilm building capacity of *Salmonella enterica* strains from the poultry farm environment. *FEMS Immunol. Med. Microbiol.* **65**, 360–365.

Shahid, M., Shahzad, A., Sobia, F., Sahai, A., Tripathi, T., Singh, A., Khan, H.M., and Umesh, T. 2009. Plant natural products as a potential source for antibacterial agents: Recent trends. *Anti-infect. Agents Med. Chem.* **8**, 211–225.

Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J., Brinkman, F.S., Hufnagle, W.O., Kowalik, D.J., Lagrou, M., *et al.* 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**, 959–964.

Stintzi, A., Evans, K., Meyer, J.M., and Poole, K. 1998. Quorum-sensing and siderophore biosynthesis in *Pseudomonas aeruginosa*: *lasR/lasI* mutants exhibit reduced pyoverdine biosynthesis. *FEMS Microbiol. Lett.* **166**, 341–345.

Szabó, M.A., Varga, G.Z., Hohmann, J., Schelz, Z., Szegedi, E., Amaral, L., and Molnár, J. 2010. Inhibition of quorum-sensing signals by essential oils. *Phytoth. Res.* **24**, 782–786.

Tan, M.W., Mahajan-Miklos, S., and Ausubel, F.M. 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl. Acad. Sci. USA* **96**, 715–720.

Tarighi, S. and Taheri, P. 2011. Different aspects of bacterial communication signals. *World J. Microbiol. Biotechnol.* **27**, 1267–1280.

Tarighi, S., Wei, Q., Cámara, M., Williams, P., Fletcher, M.P., Kajander, T., and Cornelis, P. 2008. The PA4204 gene encodes a periplasmic gluconolactonase (PpgL) which is important for fitness of *Pseudomonas aeruginosa. Microbiology* **154**, 2979–2990.

Wei, Q., Tarighi, S., Dötsch, A., Häussler, S., Müsken, M., Wright, V.J., Cámara, M., Williams, P., Haenen, S., Boerjan, B., *et al.* 2011. Phenotypic and genome-wide analysis of an antibiotic-resistant small colony variant (SCV) of *Pseudomonas aeruginosa. PLoS One* **6**, 1–14.

Wollenweber, E. 1988. Occurrence of flavonoid aglycones in medicinal plants. *Prog. Clin. Biol. Res.* **280**, 45–55.