Int. J. Forest, Soil and Erosion, 2013 3 (4):141-145
ISSN 2251-6387

Research Paper

Acyl homoserin lactone mimic compounds from plants excite quorum sensing related behaviors in *Chromobacterium violaceum* CV026 and *Pectobacterium carotovorum*

Esmaeil Mahmoudi[1*], Safoura Tarzaban[2], Pejman khodaygan[2], Abbas Abed[3]

1. Corresponding Author: Department of Plant Protection, College of Agriculture, Islamic Azad University, Khorasgan Branch, Isfahan, Iran. e.mahmoudi@khuisf.ac.ir
2. Department of Plant Pathology, College of Agriculture, Vali-e-Asr University, Rafsanjan, Iran
3. Department of Environmental Chemistry, College of Science, Islamic Azad University, Khorasgan Branch, Isfahan, Iran

Abstract: Quorum sensing is bacterial cell to cell communication with production of diffusible signal molecules such as acyl-homoserine lactones (AHLs) which regulates the virulence of many pathogenic bacteria. Some plants secrete substances that mimic the activity of the *N*-acyl-l-homoserine lactone signal molecules in bacteria. Among the thirty five plant species that tested in this research, the QS induction activity was observed in the leaves and stem extracts of lettuce (*Lactuca sativa*), Clover (*Trifolium repens*), kidney bean (*Phaseolus vulgare*) and fenugreek (*Trigonella foenum-graecum*) which induced violacein production in *Chromobacterium violaceum* CV026. Further, the outcome of the present investigation reveals that fenugreek extract strongly interferes with acyl homoserine lactone (AHL) regulated physiological function in *Pectobacterium carotovorum*. It promoted production of virulence factors and powerfully increased tissue maceration on potato tubers at low concentration of the bacterium (10^3 cfu). It is a new finding that fenugreek, kidney bean, clover and lettuce contain substances which mimic the activity of AHL signal molecules and stimulate AHL related quorum sensing in these bacteria. The AHL signal-mimic compounds could prove to be important in determining the outcome of interactions between higher plants and a diversity of pathogenic, symbiotic, and saprophytic bacteria.

Keywords: Fenugreek, Quorum sensing, violacein induction, *Pectobacterium carotovorum*

Introduction

Quorum sensing involves the regulation of gene expression in response to small, diffusible signal molecules (so called "autoinducer" AI) that move in and out through the cell membrane and between cells. When the concentration of signal molecules reaches a threshold, the bacterial population as a whole alters gene expression, which may be involved in pathogenesis, biofilm formation, bioluminescence, and production of antibiotics or the modulation of association with higher organisms (Fuqua and Greenberg, 2002). It is suggested that quorum sensing (QS) regulatory systems ensure that pathogenic traits are only expressed when the bacterial population density is high enough to overwhelm the host. This phenomenon is being operated through a wide range of signal molecules including Oligopeptides (5–10 amino acid cyclic thiolactone), N-acyl homoserine lactones (AHLs), Furanosyl borate (Autoinducer-2, AI-2), Hydroxyl-palmitic acid methylester, and Methyl dodecanoic acid (Dong and Zhang, 2005; Mc Dougald *et al.,* 2007). The two most widely studied QS signals are AHLs produced by more than 70 species of Gram-negative bacteria, which diffuse across the cell membrane and bind to regulatory proteins within the cell and peptide based QS system in Gram-positive bacteria, which operate through membrane bound receptor histidine kinases (Waters and Bassler, 2005; Amara *et al.,* 2011; Galloway *et al.,* 2011).

Bacterial pathogens and symbionts of both animals and plants require quorum sensing to colonize and invade their hosts (Loh *et al.,* 2002). In view of the bacterial dependence on quorum sensing for infection of hosts, it makes good evolutionary sense that eukaryotes have acquired the ability to recognize and respond to bacterial quorum sensing signals (Telford *et al.,* 1998; Mathesius *et al.,* 2003; Smith and Iglewski, 2003) and the ability to actively interfere with bacterial quorum sensing through the production of compounds that mimic/inhibit the bacterial signals (Bauer and Teplitski, 2001). Numerous examples of biomimicry QS compounds exist in nature (Teplitski *et al.,* 2011). The model legume *Medicago truncatula* can detect nanomolar to micromolar concentrations of AHLs and responds by producing over 150 proteins as well as secreting QS signal mimics that potentially interfere with bacterial QS. Other plants have been shown to produce AHL mimics, possibly to manipulate the microbial rhizosphere population. Seedlings of various plant species and exudates from pea seedling induce swarming in *Serratia liquefaciens* and activate of several AHL reporter systems, in addition to inhibiting QS-regulated phenotypes in *C. violaceum* (Teplitski *et al.,* 2000).

An important recent study has demonstrated that halogenated furanones produced by the marine red algae *Delisea pulchra* are able to disrupt AHL-regulated behaviors in *Serratia liquefaciens* and other bacterial species (Givskov *et al.,* 1996). The furanones are structurally similar to AHLs, and inhibit the quorum sensing related behaviors by binding competitively to the AHL receptor protein (Manefield *et al.,* 2002). The concentration of furanone mimics at the algal surface was found to be effective in disrupting colonization of the algal thalli by gram-negative bacteria (Dworjanyn *et al.,* 1999; Kjelleberg and Steinberg, 2002). The discovery of AHL signal-mimic compounds in an alga raises the possibility that higher plants might also synthesize and secrete compounds that mimic the activity of bacterial AHL signal compounds. The secretion of AHL mimic compounds could have important effects on bacterial colonization and infection of host plants, and might be of considerable medical and agricultural interest.

In addition to the halogenated furanones of *D. pulchra*, varieties of bacteria and eukaryotes have been shown to produce diketopiperazines (cyclic dipeptides) that can act as AHL mimics to affect quorum sensing-regulated behaviors in bacteria (Holden *et al.,* 1999). More recently, various higher plants including pea seedlings, garlic, *Medigo sativa*, vanilla, carrot (*Daucus*

carota), chamomile (*Matricaria* sp.), water lily (*Nymphaea* sp.), various peppers (*Capsicum* spp.), *Scorzonera sandrasica*, and *Tremella fuciformis* were also shown to secrete AHL signal mimic substances and have anti-QS activity (Teplitski *et al.,* 2000; Gao *et al.,* 2003; Persson *et al.,* 2005). Some of the AHL mimic compounds from plants stimulate quorum sensing-regulated responses in bacteria, in contrast to the halogenated furanones from *D. pulchra*, which all act to inhibit them.

Pectobacterium carotovorum is a plant pathogenic bacterium responsible for diseases characterized by a maceration of the tissues, such as the black leg disease of potato, or the soft rot disease of various plants, including cabbage, fritillaria, chili, celery, lettuce, carrot, melon. Production of virulence factors in *Pectobacterium* (maceration enzymes, harpin and carbapenem antibiotic) are controlled by NAHL-dependent QS system that relies upon 3-oxo hexanoyl- N-homoserine lactone (3-oxoC6-HSL) or octanoyl homoserine lactone (C8-HSL) as the main signals (Barnard and Salmond, 2007; Mahmoudi *et al.,* 2011). There is main interest in plant pathology, to find natural or synthetic compounds active in small quantities that are capable of interfering with quorum sensing in pathogenic bacteria in order to disrupt their pathogenicity/virulence factor production. Bacterial diseases are much more difficult to control than fungal diseases due to lack of effective and benign plant protecting products. Chemical molecules that target quorum sensing have been proposed as an antivirulence strategy that could be used in control of bacterial diseases. Using quorum sensing as a target for controlling and handling detrimental infections caused by human, animal, and plant pathogens is potentially an attractive strategy (Cirou *et al.,* 2009; Mahmoudi *et al.,* 2012).

This study was conducted to investigate the production of AHL mimic substances which can induce QS regulation behavior in bioreporter strain by thirty five plant species from different families and to evaluate the effect of them on virulence of *Pectobacterium carotovorum* in potato tubers.

Martial mad Methods

Plant materials and extraction

Leave and stem of the thirty five plants species (Table 1) were used in this research. Seeds of plants were sterilized using active sodium hypochlorite solution (5%) for 30 minutes, and washed twice with sterilized water. Plant seeds were grown in a sterilized peat substrate under greenhouse conditions (28 °C, with 50-70% humidity) with a 16:8 light–dark photoperiod. Plants were taken for extraction after 5-7 weeks (depend on growth rate of plants), the roots were cut and the aerial parts of the plants were washed several times using sterile water. Twenty-five grams of fresh plant material were then frozen with liquid nitrogen and ground using mortar. Produced fine powder was suspended in the ethyl acetate after acidifying with 0.1% glacial acetic acid and mixed for 30 minutes. The plant residues were removed using paper filter (Whatman paper) and the extracts were evaporated under vacuum evaporator at room temperature. Finally, the resultant residues were resuspended in 1ml acidified ethyl acetate and were stored at -20 °C for further analysis.

Violacein induction in *Chromobacterium violaceum* CV026 by plant extracts

Production of quorum sensing related violacein in CV026 was assessed in two methods:

1- In disc-diffusion assay, 50 µL of C. *violaceum* CV026 was streaked on LB-Agar medium and sterilized discs (6 mm diameter) containing 20 µL of each extract was placed on the plates. The plates were then incubated overnight at 30°C and QS induction was detected as purple colony of bioreporter strain grown around the discs. To ensure the sterility of the extracts and to minimize any introduction of exogenous QS induction compounds, extracts were sterilized using 0.45 µm membrane and were tested for microbial contamination before the violacein induction assay by streaking onto Luria Bertani agar (LB) plates and incubation at 37 °C for overnight.

2- Leaves and stems of plants were surface disinfected in a 5% sodium hypochlorite solution for 10 min and thoroughly crushed in sterilized mortar. One gram of plant residues were placed on LB medium that inoculated with 50 µL of CV026 strain. The plates were incubated at 30 °C for 48 hours. These were positively controlled by added 5 mg L^{-1} of C6-HSL to LB medium as exogenous QS signal in bioreporter strain.

Crude extract in the ethyl acetate of quorum sensing inducing plants were separated by tin layer chromatography on C_{18}-reversed phase plate (Sigma Aldrich, Inc., St. Louis, Mo., USA). 20 µL of each extract was spotted on plate and 5 µL of C6-HSL (5mg L^{-1}) was used as positive control. The plate was developed with a solvent system of methanol-water (60:40, vol/vol). After development, the solvent was evaporated, and the dried plates were overlaid with a culture of the biosensors bacteria as described by Shaw *et al.* (1997) and McClean *et al.* (1997).

Anti quorum sensing assay of plant extracts

The disc-diffusion assay was used to detect anti-QS activity of the plant extracts by means of the double layer culture plates. 10 ml LB-Agar medium was overlaid with 10 ml semi-solid LB (0.5% agar) containing 50 µL of C. *violaceum* CV026 supplemented with C6-HSL (5 mg L^{-1}). 20 µL of each extract was loaded onto sterile disks (6 mm diameter). Discs were air dried and were then transferred in triplicates onto *C. violaceum* CV026 inoculated Luria Bertani plates which were then incubated at 30 °C for 24 h after which results were recorded. Quorum sensing inhibition was detected as a colorless zone around the disk, where viable cells, indicative of growth but QS-inhibition, were also present. Measurements were made from the outer edge of the discs to the edge of the zones of anti-QS inhibition.

Soft rot induction of quorum sensing interfering plants on potato tuber

In this assay, effects of quorum sensing inducing plants on virulence of *Pectobacterium carotovorum* at low concentrations of the bacterium were evaluated. Potato tubers cv. Agria was surface sterilized with 10% sodium hypochlorite for 2 min, rinsed with tap water and air-dried. In the middle of each tuber, one puncture wound (6 mm in diameter and 3 mm deep) were made. After wounding, potatoes were inoculated with 50 µL of *P. carotovorum* (1×10^6, 1×10^5 and 1×10^3 cfu) and 50 µL of Fenugreek extract. The control wounds were inoculated with 50 µL of *P. carotovorum* (1×10^6, 1×10^5 and 1×10^3 cfu) alone and 20 µL of each extracts. Four potato tubers were used for each combination of treatments. The potato tubers were incubated at 37 °C in

moist chamber (80% humidity). Three days after incubation, the tubers were cut in the middle and the results were assessed by visual inspection and the disease severity was estimated by the progression of maceration.

Results and Discussions

Plants have been interacted with bacteria for many years and can respond to their behaviors. One of the biological behaviors of bacteria is making cell to cell communication which regulates genes expression in bacteria. To this, signal molecules are being used to make relationships and targeted social behaviors in response to environmental stimulations. It has been determined that some plants produce compounds which can mimic behaviors of signal molecules of bacteria and cause incitation or suppression of QS depending behaviors (Bauer and Teplitski, 2001).

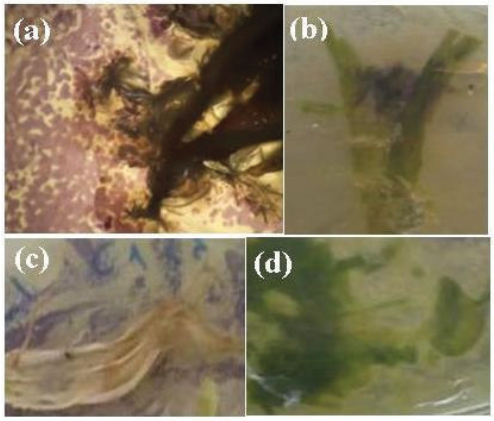

Figure 1. Induction of quorum sensing in *Chromobacterium violaceum* CV026 by plant materials. Crushed plant materials were placed on surface of LB medium inoculated with CV026 strain. Stimulation of QS was detected as growth purple colony of biosensor strain around plant residues. (a), Fenugreek; (b), Lettuce; (c) Kidney bean.

In order to determine plants contained AHLs and/or AHL mimics, leaves stems and roots of thirty five plant species were tested as described in above. Adding 20 µL of plant extracts to paper discs on media led to weak production of pigment in *C. violaceum* CV026 and only kidney bean extract could stimulate CV026 bacterium system to produce violet color. Although, the amount of pigment production was very low and only some of colonies near to discs were became purple (data not presented). The reason was very low concentration of AHL mimic molecules in extract or non appropriate solvent (ethyl acetate) for compounds of studied plants. The intensity of pigment production was higher in CV026 by kidney bean plant in crushing plant material test and putting them directly on media (Fig 1). In this method, all plant tissues and its extract were given to indicator bacterium directly and there was a high concentration of AHL similar molecules in bacterium environment which caused increase in pigment production of CV026 colonies. There are no any inhibition of bacterial growth was observed around the crushed plant. The induction of violacein synthesis in areas adjacent to the plant material was similar to that caused by the addition of C6-HSL to a lawn on CV026 medium. Addition to kidney bean, other plants like lettuce, Clover and fenugreek could also cause QS system induction for biosensor strain in crushing method (Fig 1). None of these two plants could stimulate pigment production in bacterial colonies in extracting method which can may be because of too low concentration of them in extract which to be perceived by QS system of CV026 bacterium. TLC separation of AHL similar compounds in plants extract was done too. There was only one bacterium recognizable spot on plate in extract of lettuce, clover, kidney bean, and fenugreek plants which was too small and pale. Five micro liter of C_6-HSL solution (5 mgL^{-1}) was used as control treatment which caused a big obvious spot on TLC plate (Fig 2).

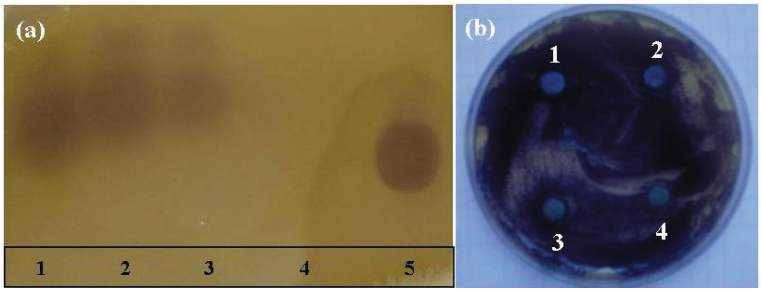

Figure 2. (a) Purification of AHL like components and detection from vegetative phase of Fenugreek, Lettuce and Bean plants (left to right) extract. 15 µL of AHL mimic molecules which were purified by organic extraction with ethyl acetate of tested plants and 5 µL of C6-AHL (5 mgL^{-1}) as positive control were separated on C_{18} silica plate with a solvent system of methanol-water (60:40, vol/vol). The plate was then overlaid with AHL-biosensor Chromobacterium violaceum CV026 strain. The violet spots represent production of bioluminescence as a result of the presence of molecules that are able to induce the AHL biosensor. Lane 1, Fenugreek extract; 2, lettuce extract; 3, bean extract; 4, ethyl acetate; 5, C6-AHL. (b) Anti-quorum sensing activity of tested plant using *C. violaceum* CV026. Anti- QS assay was performed using 20 µL of ethyl acetate extract of (1) Fenugreek, (2) Lettuce, and (3) Kidney bean. Also 20 µL of ethyl acetate (4) was used as control.

Many eukaryotic organisms are able to produce and secrete compounds that mimic the quorum sensing signals of bacteria and thus affect the behavior of associated bacteria (Keshavan *et al.,* 2005). The halogenated furanones of marine alga, *Delisea pulchra*, were observed to share structural similarity with bacterial AHLs and were shown to strongly inhibit quorum-sensing-regulated behaviors in a variety of bacterial species (Manefield *et al.,* 2002). Moreover, higher plants, such as pea, *M. truncatula*, soybean, and tomato, were also shown to produce substances that appear to mimic the activities of acyl homoserine lactone and have specific effects on quorum-sensing-regulated behaviors in bacteria. The chemical structure of AHL mimic compounds are not well identified to now, and most of them have QS inhibitory activity that interfered QS related behaviors in studied bacteria. However, the tested plants in this research showed QS incitation activity and stimulate violacein production in biosensore CV026. There are few reports on QS excitation activity of plants. Vegetative phase of rice (*Oryza sativa*) contains molecules which were sensitive to *aiiA* lactonase and was stimulated quorum sensing related functions in bioreporter strains (Degrassi *et al.,* 2007).

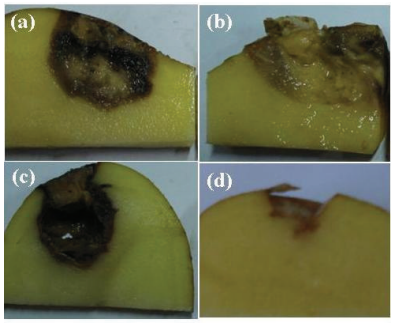

Figure 3. The effect of the AHL mimic compounds from Fenugreek on the virulence *Pectobacterium carotovorum* on potato tuber. Development of tissue maceration and soft rot disease on potato were increased after added the trigonella extract into bacterial suspension. (a) 50 µL of bacterial suspension (10^6 cfu) alone; (b) 50 µL of bacterial suspension (10^6 cfu) with 20 µL of plant extract; (c) 50 µL of bacterial suspension (10^3 cfu) with 20 µL of plant extract; d, 50 µL of distilled water as control.

The ability of QS system suppression was measured too in this study for studied plants. As it was expected and according to figure 2, none of the mentioned plants extracts could suppress QS system in presence of C_6-HSL. Actually, these plants had only molecules effective in simulating and inducing quorum sensing system and have caused activity of QS dependent behaviors in indicator bacteria via AHL competition. Previous studies have shown that the extract of some plants have some compounds which connect to LuxR receptive protein in competition with AHL molecule, decompose receptive protein and finally turning off QS in target bacterium (Manefield *et al.,* 2002). However, for tested plants in this study, no any interfere of QS system was observed. *Pectobacterium carotovorum* uses QS system for producing soft rot enzymes on hosts (Mahmoudi *et al.,* 2012). At the present research, the ability of QS exciting plants in pathogenicity of *P. carotovorum* was studied at different concentrations of bacterium. Adding 20 µL of extract to bacterial suspension (with concentrations 10^3 and 10^5 cfu) caused similar severity of signs in potato tubers (Fig 3). On the other hand, injecting these concentrations with plant extract showed higher soft rot symptom in proportion to pure injection of them on tubers. As shown in figure 3, adding extract containing AHL mimic compounds to bacterium suspension caused increase in pathogenicity of *P. carotovorum*. In other words, *P. carotovorum* can also identify AHL mimic molecules of plants like indicator bacterium. Increase in concentration of bacterial AHL molecules (because of bacterial multiplication), and AHL similar molecules (because of adding plant extract) in bacteria environment caused over expression of cell wall degradation enzymes and this caused increase in soft rot symptom in potato tuber, although the population of bacteria (cell density) was 10^1 and 10^3 times less than control (10^6 cfu).

Conclusion

Bacteria have been always one of the important pathogens of animals and plants and bactericide poisons and antibiotics have been used highly for controlling or decreasing bacterial diseases which have been led to resistant strains production. Researchers have tried constantly to solve the problem. Quorum sensing interfere is one of the new strategy for bacterial diseases management which can affect future of dealing with bacteria, in human or plants. Plants, as host of many pathogen bacteria, saprophyte or epiphyte, have been considered continuously by plant protection researchers. Existence of imitator compounds of bacteria signal molecules either in exciting and induction form or in destruction and suppression form can be an appropriate platform for improving chemical control methods based on environment protection and using effective compounds of plants (Rasmuseen and Givskov, 2006). The chemical nature of the active AHL mimic compounds from plants and their effect on bacterial QS could be one of the intrinsic plants defense mechanism against pathogenic invades. Using AHL analogues as the antagonists to interfere with QS dependent bacteria might use as a strategy in controlling in bacterial pathogens. Although, using herbal compounds with QS system induction properties increase pathogenicity of plant pathogen bacteria (as shown in this study). However, these compounds can increase production of antibiotic or QS dependent secondary metabolites in useful bacteria and biological control agents which can use these compounds to increase antagonistic ability of them. However, these studies are in early laboratory stages now and future studies can focus on about the chemical structure, mechanisms and extant effects of plant compounds to reach these goals.

Acknowledgment

We are thankful for the funds (project no. 13901125-341) provided by the Research Council of the Khorasgan (Isfahan) Branch, Islamic Azad University, IR. Also many thanks to Dr. Vittorio Venturi (International Center for Genetic Engineering and

Biotechnology, Padriciano, Trieste, Italy) for providing the biosensore strain CV026 and for all help provided by Dr. Najafi, Research Vice President of Khorasgan Branch- IAU, Iran.

References

Amara N, Krom B P, Kaufmann G F, Meijler M M (2011). Macromolecular inhibition of quorum sensing: enzymes, antibodies, and beyond. Chem. Rev. 111: 195–208.

Barnard A M, Salmond G P (2007). Quorum sensing in Erwinia species. Ana. Bioana. Chem. 387: 415-23.

Bauer W D, Teplitski M (2001). Can plants manipulate bacterial quorum sensing? Aust. J. Plant Physiol. 28: 913-921.

Cirou A, Uroz S, Chapelle E, Latour X, Orange N, Faure D, Dessaux Y (2009). Quorum sensing as a target for novel biocontrol strategies directed at Pectobacterium. In: Gisi U., I. Chet, and M.L. Gullino, editors. Recent Developments in Management of Plant Diseases, Dordrecht, Springer, p, 121–31.

Degrassi G, Devescovi G, Solis R, Steindler L, Venturi V (2007). Oryza sativa rice plants contain molecules that activate different quorum-sensing N -acyl homoserine lactone biosensors and are sensitive to the specific AiiA lactonase. FEMS Microbiol. Lett. 269: 213–220.

Dong Y H, Zhang L H (2005). Quorum sensing and quorum-quenching enzymes. J. Microbiol. 43: 101-109.

Dworjanyn S A, deNys R, Steinberg P D 1999. Localization and surface quantification of secondary metabolites in the red alga Delisea pulchra. Mar. Biol. 133: 727-736.

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

Galloway W R J D, Hodgkinson J T, Bowden S D, Welch M, Spring D R (2011). Quorum Sensing in Gram-Negative Bacteria: Small-Molecule Modulation of AHL and AI-2 Quorum Sensing Pathways. Chem. Rev. 111: 28–67.

Gao M, Teplitski M, Robinson J B, Bauer W D (2003). Production of substances by Medicago truncatula that affect bacterial quorum sensing. Mol. Plant -Microbe Interact., 16: 827–834.

Givskov M, deNys R, Manefield M, Gram L, Maximilien R, Eberl L (1996). Eukaryotic interference with homoserine lactone-mediated prokaryotic signaling. J. Bacteriol. 178: 6618–6622.

Holden M T, Ram C S, deNys R, Stead P, Bainton N J, Hill PJ (1999). Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from Pseudomonas aeruginosa and other gram-negative bacteria. Mol. Microbiol. 33: 1254–66.

Keshavan N D, Chowdhary P K, Haines D C, González J E (2005). L-canavanine made by Medicao sativa interferes with quorum sensing in Sinorhizobium meliloti. J. Bacteriol. 187: 8427–8436.

Kjelleberg S, Steinberg P (2002). Defenses against bacterial colonization of marine plants. In: Phyllosphere Microbiology. Lindow, SE, Poinar E, Elliott V, eds. American Phytopathological Society Press, St. Paul, MN, USA.

Loh J, Pierson E A, Pierson L S, Stacey G, Chatterjee A (2002). Quorum sensing in plant-associated bacteria. Curr. Opin. Plant Biol. 5: 285–290.

Mahmoudi E, Hasanzadeh N, Sayed-Tabatabaei B E, Venturi V (2011). Virulence attenuation of Pectobacterium carotovorum using N-acylhomoserin lactone degrading bacteria isolated from potato rhizosphere. Plant Pathol. J. 27: 242-248.

Mahmoudi E, Naderi D, Venturi V (2012). AiiA lactonase disrupts N-Acylhomoserine Lactone and attenuates quorum sensing related virulence in Pectobacterium carotovorum EMPCC. Ann. Microbiol. DOI.10.1007/s13213-021-0521-9.

Manefield M, Rasmussen T B, Henzter M, Andersen J B, Steinberg P, Kjelleberg S, Givskov M (2002). Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Microbiology. 148: 1119-27.

Mathesius U, Mulders S, Gao M S, 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. Nat. Acad. Sci. USA. 100: 1444-1449.

McClean K H, Winson M K, Fish L, Taylor A, Chhabra S R, Camara M, Dayykin M, Lamb J H, Swift S, Bycroft B W, Stewart G S A B, Williams P (1997). Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acyl homoserine lactones. Microbiology. 143: 3703–3711.

McDougald D, Rice S A, Kjelleberg S (2007). Bacterial quorum sensing and interference by naturally occurring biomimics. Analyt. Bioanalyt. Chem. 387: 445–453.

Persson T, Thomas H H, Thomas B R, Mette E S, Givskov M, Nielsen J (2005). Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic. Org. Biomol. Chem. 3: 253-62.

Shaw P D, Ping G, Daly S L, Cha C, Cronan J E, Rinehart K L, Farrand S K (1997). Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin layer chromatography. Biochem. J. 94: 6036–6041.

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

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.

Teplitski M, Mathesius U, Rambaugh K P (2011). Perception and degradation of N-acyl homoserine lactone quorum sensing signals by mammalian and plant cells. Chem. Rev. 111: 100–116.

Teplitski M, Robinson J B, Bauer W D (2000). Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol. Plant-Microbe Interact. 13: 637-648.

Waters C M, Bassler B L (2005). Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell Develop. Biol. 21: 319–346.