Modulation of Quorum Sensing in Acyl-homoserine Lactone-Producing or -Degrading Tobacco Plants Leads to Alteration of Induced Systemic Resistance Elicited by the Rhizobacterium *Serratia marcescens* 90-166

Choong-Min Ryu1,2, Hye Kyung Choi3, Chi-Ho Lee1, John F. Murphy3, Jung-Kee Lee4 and Joseph W. Kloepper3*

1Molecular Phytobacteriology Laboratory, Systems and Synthetic Biology Research Center, KRIBB, Daejeon 305-806, Korea
2Biosystems and Bioengineering Program, University of Science and Technology (UST), Daejeon 305-350, Korea
3Department of Entomology and Plant Pathology, Auburn University, AL 36849-5409, USA
4Department of Biomedicinal Science & Biotechnology, Paichai University, Seo-Gu, Daejeon 302-735, Korea

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Numerous root-associated bacteria (rhizobacteria) are known to elicit induced systemic resistance (ISR) in plants. Bacterial cell-density-dependent quorum sensing (QS) is thought to be important for ISR. Here, we investigated the role of QS in the ISR elicited by the rhizobacterium, *Serratia marcescens* strain 90–166, in tobacco. Since *S. marcescens* 90–166 produces at least three QS signals, QS-mediated ISR in strain 90–166 has been difficult to understand. Therefore, we investigated the ISR capacity of two transgenic tobacco (*Nicotiana tabacum*) plants that contained either bacterial acyl-homoserine lactone-producing (AHL) or -degrading (AiiA) genes in conjunction with *S. marcescens* 90–166 to induce resistance against bacterial and viral pathogens. Root application of *S. marcescens* 90–166 increased ISR to the bacterial pathogens, *Pectobacterium carotovorum* subsp. *carotovorum* and *Pseudomonas syringae* pv. *tabaci*, in AHL plants and decreased ISR in AiiA plants. In contrast, ISR to *Cucumber mosaic virus* was reduced in AHL plants treated with *S. marcescens* 90–166 but enhanced in AiiA plants. Taken together, these data indicate that QS-dependent ISR is elicited by *S. marcescens* 90–166 in a pathogen-dependent manner. This study provides insight into QS-dependent ISR in tobacco elicited by *S. marcescens* 90–166.

**Keywords**: AiiA, N-Acyl homoserine lactone, plant-growth promoting rhizobacteria, quorum sensing

Induced systemic resistance (ISR) is the resistance of plants to disease induced by root-associated bacteria (rhizobacteria), also referred to as plant-growth promoting rhizobacteria (PGPR) (Kloepper et al., 1992). Diverse bacterial determinants have been proposed to play a role in the elicitation of ISR (Kloepper et al., 2004). These include bacterial surface components, such as flagellin, lipopolysaccharides, and exopolysaccharides, and bacterial metabolites, such as siderophores and antibiotics (De Vleesschauwer and Hofte, 2009). In addition to these bacterial determinants, it has also been reported that volatile organic compounds (VOCs), including acetoin and 2,3-butanediol, emitted by *Bacillus subtilis* GB03 activate an ISR pathway in *Arabidopsis* seedlings inoculated with *Pectobacterium carotovorum* (syn. *Erwinia carotovora* subsp. *carotovora*) (Ryu et al., 2004a).

ISR can be triggered by acyl-homoserine lactones (AHLs), which are quorum sensing (QS) molecules that control the expression of various genes to regulate a variety of physiological functions in various Gram-negative bacteria in a cell density-dependent manner (Miller and Bassler 2001; Schuhegger et al., 2006). The application of *Serratia liquefaciens* MG1 (a PGPR strain that produces two different AHLs) to the roots of tomato (*Solanum lycopersicum* L.) plants resulted in ISR to the leaf fungal pathogen, *Alternaria alternata*, but an AHL-null mutant of *S. liquefaciens* MG1 treatment reduced elicitation of ISR (Schuhegger et al., 2006). In the same pathosystem, *Pseudomonas putida* F117 (an AHL non-producing mutant) was less effective than its wild type, *P. putida* IsoF (a PGPR strain that produces four different AHLs), at eliciting an ISR response. These indicate that bacterial QS molecules contribute to the capacity of *P. putida* IsoF to elicit ISR responses.

The findings of these previous studies led us to ask the following questions about the role of AHLs in eliciting ISR responses:

1) Are single AHLs or combinations of AHLs responsible for eliciting ISR responses when a bacterium produces more than one AHL?

2) Can ISR be indirectly elicited by production of secondary metabolite(s) regulated by QS rather than elicited via AHL production by PGPR?

*Corresponding author.
Phone) +1-334-844-1950, FAX) +1-334-844-5067
E-mail) kloepjw@auburn.edu
3) What is the plant response to AHLs?

In answer to the first question, many studies report that direct application of several AHLs comprising different acyl lengths resulted in different plant responses. The addition of 10 µM N-hexanoyl (C6) homoserine lactone (HHL) and N-butanoyl (C4) homoserine lactone to the roots of tomato plants was enough to increase the induction of defense gene expression (Schuhegger et al., 2006). A recent study evaluated the defense responses of Arabidopsis and barley (Hordeum vulgare L.) to a variety of AHLs ranging from C4 to C14 in length (Schikora et al., 2011).

In answer to the second question, many studies show that bacterial secondary metabolites regulated by QS act to ranging from C4 to C14 in length (Schikora et al., 2011). A recent study evaluated the defense responses of Arabidopsis and barley (Hordeum vulgare L.) to a variety of AHLs ranging from C4 to C14 in length (Schikora et al., 2011).

In answer to the second question, many studies show that bacterial secondary metabolites regulated by QS act to increase ISR. The PGPR strain, S. plicymuthica HRO-C48, secretes a strong antibiotic, pyrrolnitrin, via QS regulation, and protects cucumber seedlings against infection by the soil-borne pathogen, Pythium aphanidermatum, and tomato plants against the foliar pathogen, Botrytis cinerea (Pang et al., 2009). Indirect evidence for the role of secondary metabolites in eliciting ISR also exists for P. aeruginosa PAO1 and S. plicymuthica RVH1 (Moons et al., 2011; Reimann et al., 2002; Whiteley et al., 1999). Also, pyocyanin from P. aeruginosa 7NSK2 has been defined as a bacterial determinant of ISR in tomato (Audenaert et al., 2002). During fermentation of glucose, S. plicymuthica, like many other bacterial species, is able to switch from producing mixed acids to producing natural chemicals such as acetoin and 2,3-butanediol to avoid the lethal acidification of its environment. N-(3-oxo-hexanoyl)-L-homoserine lactone (OHL) plays an important role in acetoin and 2,3-butanediol production (Moons et al., 2011).

In answer to the third question, the first demonstration of plant responses to bacterial QS signals was the description of extensive proteome changes in a plant in response to physiological concentrations of AHL (Mathesius et al., 2003). The levels of over 150 proteins in the model legume, Medicago truncatula. were altered in response to 3-oxo-C12-HSL or 3-oxo-C16:1-HSL, with the response depending on the concentration and identity of the AHLs, suggesting that plants can differentiate between QS signals from different bacteria. In addition, AHL treatment affected diverse metabolites such as phenol containing chemicals secreted by the roots, including the AHL mimics (Mathesius et al., 2003; Teplitski et al., 2000). Recently, treatment of Arabidopsis roots with o xo-C14-HSL was shown to promote stronger activation of the mitogen-activated protein kinases AtMPK3 and AtMPK6 and the defense-related transcription factors WRKY22 and WRKY29 (Schikora et al., 2011). These results suggest that long chain AHLs, such as C12, C14 and C16, can efficiently activate plant resistance. S. marcescens 90–166 is a well-known PGPR strain. When applied to seeds or roots, it results in ISR to a broad spectrum of foliar pathogens, including bacterial, fungal, and viral pathogens (Kloeper and Ryu, 2006). The salicylic acid (SA) non-producing mutant of S. marcescens 90–166 retained ISR to anthracnose in cucumber and to Cucumber mosaic virus (CMV) in Arabidopsis, while the iron-chelation enzyme null mutant abolished ISR capacity against anthracnose in cucumber but had no effect against CMV in Arabidopsis (Press et al., 2001; Ryu et al., 2004b). These data indicate that a specific bacterial determinant can be involved in inducing pathogen-specific resistance, or that more than one bacterial determinant can be involved in ISR induction for each pathosystem. More interestingly, an ISR-negative mini-Tn5phoA mutant of S. marcescens 90–166 reduced AHL production (Wilson et al., 1997). This result led us to investigate whether QS-dependent bacterial traits of S. marcescens 90–166 contribute to the elicitation of ISR against diverse pathogens. Studies of the role of QS-regulated ISR-related traits are limited due to a lack of genetic tools to manipulate S. marcescens 90–166 to generate AHL-negative mutants, or to obtain genome information. To obtain clear evidence about the role of QS-regulated traits that influence ISR, null mutants of all three AHL-producing genes in S. marcescens 90–166 would be required.

In the current study, we established an alternative plant-based system that included two transgenic tobacco plants harboring genes for either AHL degradation (AiiA) or AHL production (AHL). The tobacco plants allowed us to investigate the role of bacterial AHL-based QS molecules on ISR to bacterial and viral pathogens.

Materials and Methods

Plant and PGPR preparation. The AHL-producing transgenic tobacco (AHL tobacco), which was constructed by transforming bacterial yehl that targeted to the chloroplasts, was provided by Dr. Rupert G. Fray, Nottingham University, UK (Fray et al., 1999). The AiiA transgenic tobacco, which was created by transformation of the acyl-homoserine lactonase (AiiA) gene from Bacillus sp., was provided by Dr. Jian-Hui Zhang, National University of Singapore (Dong et al., 2001). Seeds of tobacco (Nicotiana tabacum L.) cvs. wild types Samsun NN and GX3 and their transgenic plants, AHL and AiiA plants, respectively, were surface-disinfested by soaking in 70% ethanol for 2 min followed by 1% sodium hypochlorite for 30 min. The seeds were rinsed thoroughly in sterile distilled water (SDW) and transferred to a commercial potting mix, Nongwoo (Nongwoo, Daejeon, S. Korea), without sterilization. Plants were grown in a controlled-environment growth room at 25 ± 2°C under fluorescent light (a 12 h/12 h day/night cycle, with a light intensity of approximately 7,000 lux). S. marcescens 90–166 was grown in tryptic soy agar (TSA;