Modulation of Inter-kingdom Communication by PhcBSR Quorum Sensing System in *Ralstonia solanacearum* Phylotype I Strain GMI1000

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**INTRODUCTION**

*Ralstonia solanacearum* is a notorious soil-borne pathogen that causes lethal bacterial wilt of many plants around the world (Hayward, 1991). The pathogen encodes numerous virulence determinants, including extracellular polysaccharide (EPS), cell wall-degrading enzymes (CWDE), chemotaxis system, and secretion systems, which are collectively contribute to its virulence (Denny and Ryel, 1991; Arlat et al., 1992; Yao and Allen, 2006; Genin and Denny, 2012). Previous studies have outlined the sophisticated regulatory mechanisms that control the production of virulence factors in *R. solanacearum*. Among them, PhcA is a LysR family transcriptional regulator (Brumbley et al., 1993), which is located at the center of the complex regulatory network, and can
directly or indirectly regulate the genes involved in production of EPS and other virulence factors (Huang et al., 1995). Along with bacterial proliferation, PhcA activity is regulated by accumulated quorum sensing (QS) signal 3-hydroxy-palmitic acid methyl ester (3-OH-PAME) or (R)-methyl 3-hydroxymyristate (3-OH-MAME), which is encoded by phcB (Flavier et al., 1997; Kai et al., 2015). Consequently, PhcA directs the production of EPS, CWDE, and other virulence factors in a population density dependent manner. Evidence indicates that a two component system, encoded by phcS and phcR in the same operon as phcB, is involved in detection and response to the QS signal 3-OH-PAME (Clough et al., 1997).

_Ralstonia solanacearum_ species complex is well known not only for their ability to infect a unusually broad range of host plants, but also for their wide geographic distributions and capability to live and compete for versatile and diverse habitats (Salanoubat et al., 2002; Alvarez et al., 2010). Involvement of secondary metabolites in interspecies and inter-kingdom signaling and interference between _R. solanacearum_ and the other organisms in competition for environmental niches has recently attracted much attention. Genome sequencing analysis and genetic studies show that _R. solanacearum_ complex has the potential to produce an array of secondary metabolites. For example, ralfurane is known to contribute to the potential to produce an array of secondary metabolites. Ralfuranone is known to contribute to the ability to infect a unusually broad range of host plants, but also for their wide geographic distributions and capability to live and compete for versatile and diverse habitats (Salanoubat et al., 2002; Alvarez et al., 2010). Involvement of secondary metabolites in interspecies and inter-kingdom signaling and interference between _R. solanacearum_ and the other organisms in competition for environmental niches has recently attracted much attention. Genome sequencing analysis and genetic studies show that _R. solanacearum_ complex has the potential to produce an array of secondary metabolites. For example, ralfurane is known to contribute to the potential to produce an array of secondary metabolites. Ralfuranone is known to contribute to the potential to produce an array of secondary metabolites.

**Materials and Methods**

**Bacterial and Fungal Strains, Plasmids, and Media**

The plasmids and _R. solanacearum_ strains used in this study are listed in Table 1. _Escherichia coli_ strain DH5α (Invitrogen, Carlsbad, CA, United States) was used as a host in gene cloning and vector construction. _R. solanacearum_ strains were maintained at 30°C in casamino acid-peptone-glucose (CPG) plates (Hendrick and Sequeira, 1984), and cultured in CPG broth for testing CWDE activities (Yap et al., 2005), or on minimal medium (MM) agar plate for screening transformants after tri-parental mating (Hussain et al., 2008). _E. coli_ was grown at 37°C in LB medium. Antibiotics were added at the following final concentrations (μg/L): kanamycin, Km (50), gentamicin, Gm (50), and rifampicin, Rif (50). The fungal strains used in this study were maintained in PDA medium unless otherwise stated.

**In-Frame Deletion and Complementation**

The phcB, rmyA, rmyB, and rmyAB deletion mutants were generated by amplifying two DNA fragments flanking their coding sequences (using primers as described in Table 2), the 859 bp gentamicin resistance gene sequence (Gen) was added between the left and right fragments of phcB or rmyA by using the overlap primers, the left and right DNA fragments of rmyB were ligated without adding the Gen sequence. The two/three fragments were fused using the primer pair L1/R2.

**TABLE 1 | List of the bacterial and fungal strains and plasmids used in this study.**

| Strain/Plasmid | Relevant characteristics | Source |
|----------------|--------------------------|--------|
| **R. solanacearum** | | |
| GM1000 | Phylotype I, wild-type, Rif | Salanoubat et al., 2002; ATCC® BAA1114™ |
| ΔphcB | phcB deletion mutant (Gm, Rif) | This study |
| ΔphcB(phcB) | phcB complement (Gm, Km, Rif) | This study |
| ΔrmyA | rmyA deletion mutant (Gm, Rif) | This study |
| ΔrmyB | rmyB (Rif) mutant | This study |
| ΔrmyAB | rmyB double mutant (Gm, Rif) | This study |
| **E. coli** | | |
| DH5α | λ-φ80d lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17 supE44 thi-1 gyrA resA1 | Invitrogen |
| **Fungi** | | |
| XJJ2 | F. oxysporum f. cubense | Li et al., 2014 |
| Ss17 (MAT-1) | Pair of mating strains of S. scitamineum | Yan et al., 2016 |
| Ss18(MAT-2) | | |
| **Plasmids** | | |
| px18mobscacB | Km, suicide and narrow-broad-host vector | Schäfer et al., 1994 |
| pBBR1MCS2 | Km, broad-host-range cloning vector | Kovach et al., 1994 |
| pRK2013 | Km | Ditta et al., 1980 |
| pMD18T | T vector; Amp | TAKARA |
**Co-culture Experiments and Microscopy Examination of Chlamydospore Formation**

Preparation of conidial suspensions of the FOC strain XJZ2 was performed as described (Spraker et al., 2016). For co-culture experiments, *R. solanacearum* strain GMI1000 and the fungal pathogen were plated as previously described and incubated for 8 days at 28°C. When the colonies met each other, the fungi mycelia of the interaction zone were harvested and examined for FOC chlamydospore formation by using ZEISS Observer Z1 microscopy. For testing bacterial interaction with *S. scitamineum*, the MAT-1 and MAT-2 (Yan et al., 2016) mating mixture of *S. scitamineum* was grown on PDA plates for observation of hyphal growth. To observe the dose effect of strain GMI1000 on inhibition of *S. scitamineum*, the GMI1000 (A) was cultured 24 h on the CPG plate before adding GMI1000 (B), other *R. solanacearum* mutants (Ditta et al., 1980). Mutants were selected on CPG plate containing 10% sucrose, antibiotics Gm and Rif, and then confirmed by PCR and DNA sequencing. To construct complementary strains, the DNA fragment containing 235 bp promoter sequence and ORF of *phcB* was amplified using primers *phcB*-CF and *phcB*-CR (Table 2). The purified PCR products were digested by required restriction enzymes, and then purified again prior to ligation with the same enzymes digested expression vector pBBR1MCS2 (Kovach et al., 1995). The ligation products were transformed into *E. coli* DH5α competent cells, and the transformants were selected on LB plate containing Km. Triparental mating was performed as above description, and the complemented strains were selected on MM plates supplemented with Km and Rif, and confirmed by PCR product analysis. The gel electrophoresis results of the mutants and control are provided in Supplementary Information File 1 (SI 1).

**RNA Preparation and Quantitative Real-Time PCR (qRT-PCR) Analysis**

Cultures of *R. solanacearum* strains in CPG broth (OD$_{600}$ = 1.5) were centrifuged and total RNA were isolated by using RNAasy Mini Kit (QIAGEN, Hilden, Germany). The contaminated genomic DNA were removed by treating with Dnasel (Takara, Dalian, China) at 37°C for 1 h, and was confirmed by PCR using the 16S primer pair and visualized on an agarose gel. The qRT-PCR experiments were performed with SuperReal PreMix Color SYBR Green, 2X, (TIANGEN BIOTECH CO. LTD, Beijing, China) on QuantStudio 6 Flex (applied biosystems by life technologies, Carlsbad, CA, United States) following the user's guide from the manufacturer. The PCR conditions were as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 15 s and 55°C for 31 s, and then the melting curve analysis was carried out to determine the specificity of PCR products. The cDNA samples of each treatment were replicated three times, and the
absolute value of $-\Delta \Delta Ct = -(\Delta Ct_1 - \Delta Ct_2)$ were calculated as described in the $2^{-\Delta \Delta Ct}$ method (Livak and Schmittgen, 2001).

**LC/MS Analysis of Ralsolamycin**

To prepare ralsolamycin extracts, bacteria were inoculated in 1 litre CPG broth and cultured at 28°C for 24 h with shaking at 150 rpm (OD$_{600} = 1.5$). Bacterial supernatants were collected by centrifugation at 10,000 rpm for 10 min, mixed with an equal volume of ethyl acetate with shaking. The upper organic phase was collected and air dried, and the residues were dissolved in methanol for further analysis. LC/MS analysis was conducted using a Waters LC-MS system (Waters, MA, United States) with an ACQUITY UPLC system coupled to the Waters Q-ToF Premier high resolution mass spectrometer. An ACQUITY UPLC BEH C18 column (2.1 mm × 50 mm) was used for chromatography analysis. Solution A was composed of 0.01% formic acid in water; solution B was composed of 0.01% formic acid in CH$_3$OH. A linear gradient elution from 10 to 100% B over 10 min at 0.4 ml/min, 100% B over 3 min and re-equilibrated with 10% B for an additional 3 min. The injection volume was 1 μl. The entire column elute was introduced into the Q-Tof mass spectrometer. Ion detection was achieved in ESI mode using a source capillary voltage of 1.5 kV, source temperature of 120°C, desolvation temperature of 350°C, cone gas flow of 50 L/h (N$_2$), and desolvation gas flow of 600 L/h (N$_2$). Presence of the peak in the bacterial extract showing the accurate mass of ralsolamycin (m/z 1291.7142) was identified, and the corresponding peak areas from wild-type and mutant were compared and calculated.

**RESULTS**

**Expression of the rmy Genes and Production of Ralsolamycin Are Controlled by the PhcBSR QS System**

To evaluate the role of the PhcBSR QS system in regulation of the rmy genes expression and ralsolamycin production, the QS signal synthase gene phcB deletion mutant was constructed in the genetic background of *R. solanacearum* strain GMI1000 by deletion of its coding sequence. The resulting strain ΔphcB was non-mucoid and nearly avirulent with weak cellulose activity, but was highly motile and with an increased polygalacturonase activity as previously reported (Flavier et al., 1997). The expression levels of rmyA and rmyB in ΔphcB were determined and compared to its parental strain GMI1000. In the ΔphcB, the rmyA transcription was drastically decreased by about 24-fold (Welch t-test, p-value < 3.64e-4; Figure 1), and the gene rmyB transcription was decreased by around 4.6-fold (Welch t-test, p-value < 3.02e-3; Figure 1). Meanwhile, deletion of phcB also resulted in decreasing the expression level of RSp0638 and RSp0639 by approximately 6- and 7-fold, respectively (Welch t-test, p-value < 2.32e-3 and 2.08e-3, respectively; Figure 1), which are the two neighbor genes of the rmy operon. In trans expression of a wild-type phcB gene in ΔphcB restored their expression to the wild-type levels. Consistent with the results of transcriptional assay, LC-MS analysis of the ethyl acetate extracts from wide-type strain GMI1000 and ΔphcB showed that ralsolamycin production in ΔphcB was decreased by about 81-fold compared with the wide-type strain (Welch t-test, p-value < 6.86e-5; Figure 2).

**Cell Density Affects the Expression Level of the rmy Genes**

Given that bacterial QS systems modulate target gene expression in a population density dependent manner, the transcriptional levels of rmyA and rmyB in strain GMI1000 was then determined at different growth stages of bacterial cells. The results showed that the transcripts level of rmyA and rmyB in strain GMI1000 at a high cell density (∼10$^8$ CFU/ml) was increased by about 2.3- and 4.8-fold, respectively (Welch t-test, p-value < 3.22e-3 and 2.18e-3, respectively; Figure 3), compared with those at a low cell density (∼10$^6$ CFU/ml).
FIGURE 3 | Quantitative Real-Time PCR analyses of rmyA and rmyB at high and low cell density. The results were calculated as $-\Delta \Delta Ct = (\Delta Ct_{\text{high cell density}} - \Delta Ct_{\text{low cell density}})$. Three biological repeats (independent cultures) and three technical repeats were done to calculate and compare the values. All statistics were presented as means ± SE.

Mutation of phcB Attenuates the Bacterial Induction Activity on FOC Chlamydospore Formation and Inhibitory Ability on S. scitamineum Growth

Interaction of *R. solanacearum* strain GMI1000 and fungal pathogen FOC strain XJZ2 were examined by spotting them side by side in the same plate as shown in Figure 4. After 8 days of co-culture, distinct zones of fungal inhibition were formed in the area containing *R. solanacearum* wide-type strain GMI1000. We also detected the diameter of XJZ2 colony co-cultured with GMI1000 and its derivatives to quantify the inhibition effect, result demonstrated that the XJZ2 colony co-cultured with wide-type strain GMI1000 with the smallest diameter. In comparison to GMI1000, the reduction of phcB deletion mutant inhibition effect reached significant level (Welch t-test, $p$-value = 0.02; Figure 5). In addition, the chlamydospores of strain XJZ2 were routinely found in the interaction zones with wide type strain GMI1000 Supplementary Information File 2 (SI 2), whereas in the area containing phcB deletion mutant ΔphcB, chlamydospores were hardly found. Complementation of the mutant ΔphcB with a wild-type phcB gene restored the bacterial induction ability on chlamydospore formation Supplementary Information File 2 (SI 2). When the mutant ΔrmyA, or ΔrmyB, or the double deletion mutant ΔrmyAB was co-cultured with the fungal strain XJZ2, the chlamydospore was also hardly found within their interaction zones Supplementary Information File 2 (SI 2).

Distinct inhibition zones were also found between wide-type strain GMI1000 and the sugarcane fungal pathogen *S. scitamineum* (Figure 6). The colony of strain GMI1000 inoculated 1 day earlier generated a more obvious inhibition zones than the bacterial colony inoculated at the same time with the fungal strain, suggesting a clear dosage effect of the inhibitory compound(s) produced by strain GMI1000. The mutant ΔphcB could also inhibit the growth of *S. scitamineum*, but was weaker than the wide-type. In contrast, deletion of rmyA, rmyB, or...
rmyAB completely abolished the growth inhibition ability on S. scitamineum, as the hyph of S. scitamineum could grow around or cover the colonies of these rmy gene deletion mutants, and the E. coli as a control showed no inhibition effect on the fungi.

DISCUSSION

Quorum sensing system is a widely conserved mechanism of bacterial cell-cell communications, which acts in a coordinated manner, and the individual bacteria can benefit from group behavior in competition for survive and persistence in nature (Fuqua et al., 1994; Von-Bodman et al., 2003). In R. solanacearum, the PhcBSR QS system serves as a master regulator to regulate most of the traits needed for infection and virulence (Denny, 1995, 2000; Schell, 2000). As an inter-species communication signal associated with competition for environmental niches, we are curious whether ralsolamycin biosynthesis is regulated by QS mechanisms. In this study, we provide sufficient genetic and chemical evidences that the two rmy genes associated with ralsolamycin biosynthesis and production in R. solanacearum are positively regulated by the PhcBSR QS system. During the course of bacteria–fungi interaction, we also showed that deletion of phcB attenuated the bacterial induction activity on chlamydospore development in fungal organisms, and reduced the inhibition activity to fungi.

It was predicted that up to five genes are involved in biosynthesis of ralsolamycin biosynthesis and transport, including rmyA, rmyB, RSp0638, RSp0639, and RSp0640 (Spraker et al., 2016). Although the later three genes are not in the same operon as rmyA and rmyB, their protein characteristics, and the similar QS-dependent expression pattern as the rmy genes unveiled in this study seem to support that these genes are functionally related probably, for deleting phcB also resulted in significant reduction in the transcripts levels of the two genes (RSp0638 and RSp0639) next to the rmy cluster.

Comparing with the NRPs that have been intensively investigated (Stachelhaus and Marahiel, 1995; Marahiel et al., 1997), the rmy genes in R. solanacearum GMI1000 are highly related to the syringomycin synthetase gene, which is required for the production of syringomycin in P. syringae.
Another two NRPs product nunamycin and nunapeptin isolated from *P. fluorescens* In5 are the key biocontrol components against *Rhizoctonia solani* (Michelsen et al., 2015). A BLAST search found that RmyA and RmyB are also similar to AmbB and AmbE associated with iQS signal biosynthesis in human pathogen *P. aeruginosa* (Lee et al., 2013), with over 74% coverage and 35% identity at amino acids level. For the phosphate depletion is a host stress signal commonly encountered by invading pathogen *P. aeruginosa* (Lee et al., 2013), which activates the iQS system. Moreover, evidence reveals that EfpR is a novel key component of the complex regulatory network of the *R. solanacearum* cell, tightly linking the bacterial metabolism to virulence in response to multiple environmental signals (Perrier et al., 2016). Accordingly, whether there is any stress or environmental signal will involve in modulating the ralsolamycin production is still worth for further research.

*Ralstonia solanacearum* is known to wide distributed and persisted in soils for remarkably long periods (Alvarez et al., 2010), and the soil is a heterogeneous and complex microcosm replete with inter-organismal interactions, thus, the *R. solanacearum* will encounter a diversity of other soil microbes. The FOC and *S. scitamineum* are two spread worldwide and causes considerable yield losses and reduction in their host plants (Singh et al., 2004; O’Donnell et al., 2009). Based on our results, the ralsolamycin functioned in the crosstalk between *R. solanacearum* and the two tested fungi. On one side, the *R. solanacearum* can use the ralsolamycin to antagonize the fungi; on the other hand, which can also induce the fungi around to formate chlamydospores significantly, together with the fact that *R. solanacearum* can enter the endofungal lifestyle with chlamydospores (Spraker et al., 2016), which is a novel persistence mechanism for bacterial survival in the harsh environments. Moreover, we also want to determine whether the ralsolamycin can affect the sexual matting of *S. scitamineum*, result showed that except for the antagonization on *S. scitamineum*, the ralsolamycin does not inhibit the sexual matting. It was noteworthy that there was still weak inhibition effects on the fungi after deleting the rmy genes, thus, maybe some other metabolisms are also with the antagonization effect on the fungi. Obviously, the ralsolamycin plays a major role. It has been demonstrated that the “trade-off” existed between virulence factor production and bacterial proliferation is controlled by PhcBSR system dependent regulatory protein PhcA, a phcA mutant has an expanded metabolic versatility to metabolize up to 17 substrates and proliferation ability more than the wild-type (Peyraud et al., 2016). Rather paradoxically, our results indicate that the production of ralsolamycin is decreased dramatically when phcB is inactive. Hence, we can hypothesize that the *R. solanacearum* can compete for a regnant niche and enhance survival ability for the partnering fungus under the modulation of PhcBSR QS system, and it shall be one kind of active regulatory mechanism to adapt to the harsh environment when the phcB is active, which may also contribute to understand why the *R. solanacearum* species complex could share such a broad ecological range with various host plants and soil cohabitants.

**CONCLUSION**

We have demonstrated that the biosynthesis of inter-kingdom communication signal ralsolamycin is regulated by the phcB dependent QS system. Significantly, the findings from this study unveiled a link between QS and inter-kingdom communication. Further studies are required to understand the molecular mechanisms and signaling pathways that govern the QS-dependent expression of the rmy genes and ralsolamycin.

**AUTHOR CONTRIBUTIONS**

PL, YD, and L-HZ designed the experiments and wrote the paper; WY, JY, YC, JZ, and ML helped to obtain the mutants; SF and SS helped to perform the LC-MS analysis; YD and L-HZ revised the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2017.01172/full#supplementary-material
Denny, T. P. (1995). Involvement of bacterial polysaccharides in plant pathogenesis. *Annu. Rev. Phytopathol.* 33, 173–197. doi: 10.1146/annurev.py.33.090195.001133

Denny, T. P. (2000). *Ralstonia solanacearum*: a plant pathogen in touch with its host. *Trends Microbiol.* 8, 486–489. doi: 10.1016/S0966-842X(00)01460-6

Denny, T. P., and Ryl, B. S. (1991). Genetic evidence that extracellular polysaccharide is a virulence factor of *Pseudomonas solanacearum*. *Mol. Plant Microbe Interact.* 4, 198–206. doi: 10.1094/MPMI-4-198

Ditta, G., Stanfield, S., Corbin, D., and Helinski, D. R. (1980). Broad host range cloning vectors. *Front. Microbiol.* 8, 2651–2674. doi: 10.1016/cr960029e

Michelsen, C. F., Watrous, J., Glaring, M. A., Kersten, R., Koyama, N., Dorrestein, P. C., et al. (2015). Nonribosomal peptides, key biocontrol components for *Pseudomonas* flavescens InS, isolated from a Greenlandic suppressive soil. *mBio* 6, e00799-15. doi: 10.1128/mBio.00799-15

O’Donnell, K., Guedian, C., Sink, S., Johnston, P. R., Crous, P. W., and Glenn, A., et al. (2009). A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genet. Biol.* 46, 936–948. doi: 10.1016/j.fgb.2008.08.006

Perrier, A., Peyraud, R., Rengel, D., Barlet, X., Lucasson, E., Gouzy, J., et al. (2016). Enhanced in planta fitness through adaptive mutations in EfpR, a dual regulator of virulence and metabolic functions in the plant pathogen *Ralstonia solanacearum*. *PLoS Pathog.* 12:e1006044. doi: 10.1371/journal.ppat.1006044

Peyraud, R., Cottret, L., Marmiesse, L., Gouzy, J., and Genin, S. (2016). A resource allocation trade-off between virulence and proliferation drives metabolic versatility in the plant pathogen *Ralstonia solanacearum*. *PLoS Pathog.* 12:e1005939. doi: 10.1371/journal.ppat.1005939

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Amlat, M., et al. (2002). Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* 415, 497–502. doi: 10.1038/415497a

Singh, N., Somai, B. M., and Pillay, D. (2004). Smut disease assessment by PCR and microscopy in inoculated tissue cultured sugarcane cultivars. *Plant Sci.** 167, 987–994. doi: 10.1016/j.plantsci.2004.05.006

Spraker, J. E., Sanchez, L. M., Lowe, T. M., Dorrestein, P. C., and Keller, N. P. (2016). *Ralstonia solanacearum* lipopeptide induces chlamydomosome development in fungi and facilitates bacterial entry into fungal tissues. *ISME J.* 10, 2317–2330. doi: 10.1038/ismej.2016.32

Stachelhaus, T., and Marahiel, M. A. (1995). Modular structure of genes encoding multifunctional peptide synthetases required for non-ribosomal peptide synthesis. *FEBS Microbiol. Lett.* 125, 3–14. doi: 10.1111/j.1574-6968.1995.tb07328.x

Yin, J., and Allen, C. (2006). Chemotaxis is required for virulence and competitive fitness of the bacterial wilt pathogen *Ralstonia solanacearum*. *J. Bacteriol.* 188, 3697–3708. doi: 10.1128/JB.188.9.3697-3708.2006

Yap, M. N., Yang, C. H., Barak, J. D., Jahn, C. E., and Charkowski, A. O. (2005). The *Erwinia chrysanthemi* type III secretion system is required for multicellular behavior. *J. Bacteriol.* 187, 639–648. doi: 10.1128/JB.187.2.639-648.2005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.