Interaction of *Pseudostellaria heterophylla* with Quorum Sensing and Quorum Quenching Bacteria Mediated by Root Exudates in a Consecutive Monoculture System

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Many plant-pathogenic bacteria are dependent on quorum sensing (QS) to evoke disease. In this study, the population of QS and quorum quenching (QQ) bacteria was analyzed in a consecutive monoculture system of *Pseudostellaria heterophylla*. The isolated QS strains were identified as *Serratia marcescens* with *SurIR*-type QS system and exhibited a significant increase over the years of monoculture. Only one QQ strain was isolated from newly planted soil sample and was identified as *Bacillus thuringiensis*, which secreted lactonase to degrade QS signal molecules. Inoculation of *S. marcescens* to *P. heterophylla* root could rapidly cause wilt disease, which was alleviated by *B. thuringiensis*. Furthermore, the expression of lactonase encoded by the *aiiA* gene in *S. marcescens* resulted in reduction of its pathogenicity, implying that the toxic effect of *S. marcescens* on the seedlings was QS-regulated. Meanwhile, excess lactonase in *S. marcescens* led to reduction in antibacterial substances, exoenzymes, and swarming motility, which might contribute to pathogenesis on the seedlings. Root exudates and root tuber extracts of *P. heterophylla* significantly promoted the growth of *S. marcescens*, whereas a slight increase of *B. thuringiensis* was observed in both samples. These results demonstrated that QS-regulated behaviors in *S. marcescens* mediated by root exudates played an important role in replanting diseases of *P. heterophylla*.

**Keywords:** *P. heterophylla*, consecutive monoculture, quorum sensing, quorum quenching, root exudates, interaction

**Introduction**

*Pseudostellaria heterophylla* is a traditional medicinal plant used in China for over 3,000 years [21, 42]. It contains polysaccharides, ginseng saponins, flavonoids, cyclic peptides, amino acids, and trace elements [16, 38]. The root tuber of *P. heterophylla* can be used to treat splenic asthenia, anorexia, lassitude, weakness, and palpitation [19, 36]. Owing receiving to increasing market demand, *P. heterophylla* cultivation is recently receiving much attention [35]. High-quality *P. heterophylla* is mainly obtained from the ZheRong region of Fujian Province in southern China because of the suitable soil and climate conditions [38]. However, its cultivation suffers from serious consecutive monoculture problems, resulting in declined yield and quality [21, 38, 42]. Successive cropping for 2 years could lead to more than 30% reduced biomass of the plant tuberous roots [20].

Autotoxicity of root exudates excreted by plants is one major cause for consecutive monoculture problems [6, 18, 37]. Autotoxicity is an intraspecific allelopathy phenomenon, common in monocropping systems, where the plants inhibit their own growth through release of autotoxic chemicals...
Many plant-pathogenic bacteria contained the proportionally increase with the increase of cell numbers their population density. This communication system is to regulate their related gene expression depending on employed quorum sensing (QS) to cause disease [15, 23]. Mycelial growth, sporulation, and toxin production of found that a phenolic acid mixture could efficiently promote biochemical processes in the soil [14, 31]. The attracting specific microbial species, and influence many root exudates could shape rhizospheric microbiology by deterring or communities in the rhizosphere of cucumber and promote the rapid growth of soil-borne pathogens [17]. Wu et al. [39] found that a phenolic acid mixture could efficiently promote mycelial growth, sporulation, and toxin production of Fusarium oxysporum, one of the most important fungal pathogens, and lead to increased replanting disease incidence of R. glutinosa. In previous studies, four phenolic acids (benzoic acid, myristic acid, cinnamic acid, and 2-butenolic acid) have been identified as potential autotoxic chemicals, and exhibited partially autotoxic effects of P. heterophylla [21, 22]. Furthermore, these phenolic acids released by P. heterophylla root could be utilized efficiently by three specific pathogens (Fusarium oxysporum, Talaromyces helicus, and Kosakonia sacchari) from the soil or tissue of P. heterophylla, which resulted in increased replanting disease incidence [38, 42]. These findings implied that replanting diseases of P. heterophylla might result from its interaction with soil pathogenic organisms mediated by root exudates from the consecutive monoculture system of P. heterophylla.

Recent studies showed that many plant-pathogenic bacteria employed quorum sensing (QS) to cause disease [15, 23, 34]. QS is a communication system that is used by bacteria to regulate their related gene expression depending on their population density. This communication system is mediated by a process of chemical signal molecules, which proportionally increase with the increase of cell numbers [33]. Many plant-pathogenic bacteria contained the LuxIR-type QS system. The LuxI gene encodes signal molecule synthase to synthesize acylhomoserine lactones (AHLs), which comprise a homoserine lactone ring with different lengths of fatty acyl side-chain [3], whereas the receptor protein of AHLs is produced by the LuxR gene. During the bacterial growth process, AHLs produced by signal molecule synthase could diffuse freely into the suspending medium and bind with the corresponding receptor proteins by the LuxR gene when the AHL concentration reaches a threshold, and thus trigger the transcription and expression of QS-regulated genes, including biofilm formation, virulence production, exoenzyme secretion, etc. [4]. Previous studies showed that the cultivable bacteria in rhizospheric soil contained 10–20% AHL-producing bacteria, suggesting AHL-producing bacteria might play an important role during the plant growth process [10, 11, 29]. In addition, some bacteria termed “quorum quenching” (QQ) were also found to degrade AHLs by secreting exoenzymes such as lactonase [9, 15]. D’Angelo-Picard et al. [10, 11] analyzed the population of the AHL-degrading bacteria in soil and tobacco rhizosphere. Their results showed that 5–10% of the cultivable bacterial populations in both samples exhibited the abilities of degrading AHLs. These findings indicated that QS and QQ bacteria existed widely in the soil and rhizospheric environment, and the imbalance of their population might be vital for the plant growth [10–12, 26].

In this study, the population of QS and QQ bacteria in rhizosphere soils of P. heterophylla with different years of monoculture was analyzed using the reporter strain Chromobacterium violaceum CV026. Thirty-two QS strains and one QQ strain were isolated and identified as Serratia marcescens and Bacillus thuringiensis, respectively. Subsequently, the SwrIR genes of the QS system in S. marcescens and the aiiA gene encoding lactonase, a degrading enzyme of QS signal molecules, in B. thuringiensis were confirmed. Furthermore, we assessed the stimulatory effect of root exudates and root tuber extracts on the growth of S. marcescens and B. thuringiensis and their relationships with consecutive monoculture problems. Finally, S. marcescens with overexpression of AiiA enzyme was developed and used to reveal the underlying mechanism of replanting diseases based on the interaction of P. heterophylla with QS and QQ bacteria mediated by root exudates. This paper provides new insight into understanding the chemoeutrophical process of host-pathogen interactions in the consecutive monocultural problem of P. heterophylla.

Materials and Methods

Isolation of QS and QQ Bacteria in the Rhizosphere Soil of P. heterophylla

The rhizosphere soil samples of P. heterophylla were collected from both newly planted and replanted fields at the harvesting time, and the adjacent uncultivated field soil sample was used as a control. The soil samples of three conditions (uncultivated soil, newly planted soil, and replanted soil) were randomly chosen and analyzed. Five grams of three fresh samples was resuspended in 50 ml of sterile 0.8% NaCl by very vigorous shaking for 10 min, and the resulting suspension was serially diluted. Appropriate
dilutions were spread on the plate of LB medium for isolation of total cultivable bacteria. All the plates were incubated at 30°C for 24 h. Among the plates showing the lowest number of colonies, 300 strains (100 per soil sample) were randomly collected, purified twice, and individually tested for their ability to produce and degrade QS signaling molecule. The screening of QS bacteria from three soil samples was carried out using the biosensor Chromobacterium violaceum CV026 [7, 10]. In brief, the isolated strains were cultured overnight in TY medium (0.5% tryptone, 0.3% yeast extract, and 6 mM CaCl2). The culture broth (1 ml) after filtration sterilization was transferred into a well of a sterile 384-well plate, where 10 µl of overnight culture of the reporter strain CV026 was inoculated and incubated at 28°C for 24 h. Purple pigmentation indicated that the isolated strain could produce AHLs of QS signal molecules. For screening of QQ bacteria, 10 µl of overnight cultures of the isolated strains were transferred into the wells of a sterile 384-well plate containing 990 µl of liquid TY medium supplemented with 10 µM of N-hexanoyl homoserine lactone (C6-HSL) and incubated at 30°C for 36 h. To determine AHL-degrading activity, the culture broths from each well was centrifuged to remove the bacterial cells, and the remaining C6-HSL in the supernatant was detected using the biosensor strain CV026 as indicated above. The obtained QS and QQ bacteria were identified based on their physiological and biochemical characteristics as well as the 16S rDNA sequences as previously described [40].

Identification of the Genes Involved in QS and QQ Bacteria

Based on their physiological and biochemical characteristics as well as the 16S rDNA sequences, 32 QS bacteria and one QQ bacteria were identified as S. marcescens and B. thuringiensis respectively. The genes of QS system in the isolated S. marcescens were amplified by PCR with 10 pairs of primers designed according to the reported gene sequences of the QS system (SrnIR, SpIIIR, SpIIR, SmIIR, and SuvIR) from the genus Serratia (Table 1). The gene encoding lactonase in B. thuringiensis was amplified by PCR using the primers aiiA1/2 according to the reported B. thuringiensis aiiA sequence (Table 1). These PCR-amplification products were purified and subjected to commercial sequencing. The obtained sequence data were analyzed with the BLAST program on the GenBank website.

Assessment of the Pathogenicity of S. marcescens and the Biocontrol Potential of B. thuringiensis

S. marcescens and B. thuringiensis were inoculated into LB medium and cultured at 30°C for 12 h. The OD600 of the cultures were adjusted to 1 by dilution with sterile distilled water. The effects of S. marcescens and B. thuringiensis on the growth of P. heterophylla were tested in pots by exogenous addition of diluted cultures (inoculated 2 cm away from the seedling root). The assessment of B. thuringiensis against S. marcescens was performed by adding the diluted cultures of different proportions (S. marcescens:B. thuringiensis, 2:3, 1:1, 7:3, 4:1, and 9:1), which were inoculated 2 cm away from the seedling root. Equal amounts of LB medium and E. coli were used as the controls. Each treatment had two replicates.

Development of S. marcescens with Overexpressed aiiA and Its Effect on P. heterophylla Growth

The aiiA gene was amplified by PCR with the B. thuringiensis genomic DNA as template using the primers aiiA3/4 (Table 1). The PCR-amplification product was digested by BamHI and EcoRI, and then ligated into the constitutive expression vector pPbudK at the BamHI and EcoRI sites, generating the vector pPbudK-aiiA [41]. The recombinant vector pPbudK-aiiA was transformed into wild S. marcescens by electroporation. The resulting recombinant strain was designated as S. marcescens/pPbudK-aiiA. The ability of degrading signal molecules by S. marcescens/pPbudK-aiiA was evaluated against the biosensor strain CV026 [7], and wild-type S. marcescens was used as the control. The effect of S. marcescens/pPbudK-aiiA on the growth of P. heterophylla was tested in pots by exogenous addition of diluted cultures into the seedling root as indicated above. Equal amounts of LB medium were used as the control.

Bioassays of Antibacterial Activity, Exoenzymes, and Motility

The assays of antibacterial activity by S. marcescens and S. marcescens/pPbudK-aiiA were carried out on agar plates as described previously [41]. Activity of pectate lyase and cellulase was analyzed on agar plates containing the substrates as described previously [1]. Motility was assessed on tryptone swarm agar plates (10 g/l bacto tryptone, 5 g/l NaCl, and 3 g/l agar) [10]. Overnight bacteria cultures were adjusted to an OD600 of 0.5, and 5 µl of diluted cultures was spotted on the center of the plates, which was incubated at 30°C. The bacteria motility was observed after growth for 48 h.

Preparation of P. heterophylla Root Exudates and Root Extract

A series of tissue culture seedlings of P. heterophylla were incubated under sterile condition using MS medium supplemented with 0.05 mg/l 1-naphthylacetic acid and 2.5 mg/l 6-benzylaminopurine. Each group included 10 replicates in 25 ml of MS medium and 20 tissue culture plantlets. A total of 50 ml of methanol was added to each group medium. After ultrasonic processing and incubation for 30 min, the extracting mixtures were centrifuged and filtered through a 0.45 µm membrane to remove insoluble matter. The filtrate was evaporated to dryness using a rotary evaporator. The obtained root exudate extract was stored at −20°C for further experiments. For preparation of P. heterophylla root extracts, the harvested root tuber from the ZheRong region of Fujian Province in southern China was rinsed to remove the soil and sand with at least 10 volumes of distilled water. Root tubers were then cut into small pieces and pulverized. The crushed roots were transferred to 50 ml centrifuge tubes containing 40 ml of deionized water and disrupted by sonication for 30 min at 4°C. The resulting plant cell suspension was then centrifuged at 10,000 ×g for 10 min, and filtered using glass filters (1.2 µm) and membranes (0.45 µm). Finally, the clear supernatant was evaporated to dryness using a...
rotary evaporator. The obtained root tuber extracts were stored at -20°C.

**Effects of Root Exudates on the Growth of* S. marcescens* and *B. thuringiensis***

The seeds of *S. marcescens* and *B. thuringiensis* (5% (v/v)) were inoculated into 10 ml culture tubes with 5 ml of M9 medium containing 0, 5, 25, and 50 mg/l of root exudates extract. The cultures were incubated at 30°C in a rotary shaker for 24 h. The growth of *S. marcescens* and *B. thuringiensis* was determined from the optical density at 600 nm using a spectrophotometer (UV-1800, Mapada). All the experiments were performed in parallel triplicate tests.

**Effects of Root Extract on the Growth of* S. marcescens* and *B. thuringiensis***

The seeds of *S. marcescens* and *B. thuringiensis* (5% (v/v)) were inoculated into 10 ml culture tubes with 5 ml of M9 medium, where root extract was added at different concentrations of 0, 0.18, 1.8, and 18 g/l. The cultures were incubated at 30°C in a rotary

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**Table 1. Bacterial strains, plasmids, and primers used in this study.**

<table>
<thead>
<tr>
<th>Strains, plasmids, or primers</th>
<th>Genotype, properties, or sequences</th>
<th>Source or reference</th>
</tr>
</thead>
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<tr>
<td><strong>Strains</strong></td>
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<tr>
<td><em>Serratia marcescens</em></td>
<td>Wild; isolation from rhizospheric soil of <em>P. heterophylla</em></td>
<td>This study</td>
</tr>
<tr>
<td><em>S. marcescens/pPbudK-aiiA</em></td>
<td><em>S. marcescens</em> harboring the expression vector of pPbudK-aiiA</td>
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</tr>
<tr>
<td><em>Bacillus thuringiensis</em></td>
<td>Wild; isolation from rhizospheric soil of <em>P. heterophylla</em></td>
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<td><strong>Plasmids</strong></td>
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<td>pPbudK</td>
<td>Km'; constitutive expression vector of <em>S. marcescens</em></td>
<td>[34]</td>
</tr>
<tr>
<td>pPbudK-aiiA</td>
<td>Km'; pPbudK vector carrying the aiiA gene from <em>B. thuringiensis</em></td>
<td>This study</td>
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shaker for 24 h with three replicates. The growth of *S. marcescens* and *B. thuringiensis* was determined as mentioned above.

**Results**

**Isolation and Identification of QS and QQ Bacteria**

The soil samples of three conditions from *P. heterophylla* rhizosphere were collected from a *P. heterophylla* manufacturing location and used to analyze the population of the QS and QQ bacteria. As shown in Fig. 1, 300 colonies from three soil samples (100 colonies for each sample) were randomly selected for testing their abilities of producing or degrading signal molecules. Thirty-two bacteria exhibited the ability to produce AHL and led to violacein production by the biosensor CV026 strain (Fig. 1A). Among these isolates, 5, 12, and 15 colonies were obtained from uncultivated soil, newly planted soil, and replanted soil, respectively, which indicated that the number of QS bacteria increased significantly over the years of monoculture under consecutive monoculture conditions. However, only one QQ bacteria, isolated from newly planted soil, exhibited the ability of degrading AHL (Fig. 1B). A detailed physiological characteristics analysis and biochemical tests showed that all the isolated QS bacteria were gram-negative, facultatively anaerobic, motile, non-spore-forming, rod-shaped, VP-positive, oxidase-negative and strongly catalase-positive. These isolates were classified as a member of the genus *Serratia*. The 16S rDNA analysis indicated that the isolates of QS bacteria belonged to the genus of *Serratia* and shared more than 98% identity with that of *S. marcescens*. According to the morphological and biochemical properties, the isolated QQ strain was gram-positive, motile, rod-shaped, spore-forming, VP-positive, catalase-positive, and oxidase-positive, and classified as a member of *Bacillus*. 16S rDNA sequencing showed that the QQ isolate was a strain of *Bacillus*. Sequence alignment by the BLAST program revealed a consistently high identity (more than 98% identity) with species of *B. thuringiensis*.

![Screening of quorum sensing (QS) and quorum quenching (QQ) bacteria in uncultivated soil, newly planted soil, and replant soil of *P. heterophylla* using the biosensor strain *C. violaceum* CV026, where 100 colonies of each condition were randomly selected from the plates.](image)

(A) Screening of QS strains (purple pigmentation represented QS strain); (B) screening of QQ strains (no purple pigmentation represented QQ strain); 1, negative control; 2, positive control.
Effects of Root Exudates and Root Extracts on the Growth of \textit{S. marcescens} and \textit{B. thuringiensis}

Compared with the control, a significant increase in the growth of \textit{S. marcescens} was observed after addition of root exudates at concentrations ranging from 5 to 50 mg/l (Fig. 2A). The addition of root exudates with the final concentration of 50 mg/l led to the maximum biomass of \textit{S. marcescens} with the increase of 45.4%. The growth of \textit{B. thuringiensis} also showed a slight increase by root exudates of \textit{P. heterophylla} at concentrations of 5 and 25 mg/l. However, growth inhibition of \textit{B. thuringiensis} could be found when root exudates of 50 mg/l were added into the M9 medium. The effects of \textit{P. heterophylla} root tuber extract on the growth of \textit{S. marcescens} and \textit{B. thuringiensis} was also determined. As shown in Fig. 2B, root tuber extract could efficiently promote the growth of both the strains. A similar growth trend of \textit{S. marcescens} and \textit{B. thuringiensis} could be observed by root tuber extracts at concentrations of 0.18 and 1.8 g/l. However, the addition of 18 g/l root tuber extract in the medium resulted in a significant growth difference between \textit{S. marcescens} and \textit{B. thuringiensis}. A 1-fold increase of \textit{S. marcescens} growth was obtained when compared with that in the presence of 1.8 g/l root tuber extract, whereas only 7% increase was determined for the growth of \textit{B. thuringiensis} under the same condition. These results demonstrated that the root exudates and root tuber extract of \textit{P. heterophylla} provided adequate nutrients for the growth of \textit{S. marcescens}, and led to rapid pathogen proliferation during the consecutive monoculture process of \textit{P. heterophylla}.

Identification of the Genes Involved in QS and QQ Bacteria

According to the above results, the isolated QS and QQ strains were identified as \textit{S. marcescens} and \textit{B. thuringiensis}, respectively. In previous studies, the QS system in the genus \textit{Serratia} showed diversity, and QS \textit{luxIR} homologs, including \textit{SmaIR}, \textit{SpnIR}, \textit{SplIR}, \textit{SprIR}, and \textit{SwrIR} from the genus \textit{Serratia} have been reported [32]. To confirm the genes in the QS system of the 32 isolates, 10 pairs of primers were designed and used to amplify the QS \textit{luxIR} homologs using the genomic DNA of the 32 isolates. The sequencing results showed that these isolated strains only contained the \textit{SwrIR} genes (GenBank Accession No. EKF66792.1 and AAO38761.1) of the QS system (Fig. S1), which have been reported in \textit{S. marcescens} MG1. The isolated QQ strain was identified as \textit{B. thuringiensis}, which has shown the ability of effectively degrading AHLs through secreting lactonase encoded by the \textit{aiiA} gene (GenBank Accession No. ANS49085.1) in previous studies [12, 15]. Thus, PCR amplification was used to confirm the \textit{aiiA} gene in the isolated QQ strain with the primers \textit{aiiA1}/2 based on the reported \textit{aiiA} gene sequence (Fig. S1). Sequence alignment indicated that the amplified fragment from the isolated QQ strain shared 99% identity with the reported \textit{aiiA} gene of \textit{B. thuringiensis}. Based on the findings above, the isolated QS strains should belong to \textit{S. marcescens} with the \textit{SwrIR}-type QS system, whereas the QQ strain should be classified as \textit{B. thuringiensis}, which secreted lactonase encoded by the \textit{aiiA} gene to degrade signal molecules of QS.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Effects of root exudates (A) and root tuber extracts (B) on the growth of \textit{S. marcescens} and \textit{B. thuringiensis}.}
\end{figure}
Assessment of the Pathogenicity of *S. marcescens* and the Biocontrol Potential of *B. thuringiensis*

As shown in Figs. 3 and S2, the purified isolate of *S. marcescens* rapidly caused root rot and wilt disease on the seedlings of *P. heterophylla* in the pots with sterilized soils. The pathogenic strain of *S. marcescens* could be isolated from the leaf of the infected plant and was verified to be the same strain based on 16S rDNA sequencing (Fig. S3). This demonstrated that the isolated QS strain of *S. marcescens* invaded roots to cause root rot and wilt disease. On the contrary, *B. thuringiensis* and the control strain *E. coli* showed no pathogenicity on the seedlings, which implied that *B. thuringiensis* was not a pathogenic strain under the consecutive monoculture system of *P. heterophylla*.

The effect of the isolated QQ strain *B. thuringiensis* on the pathogenicity of *S. marcescens* was evaluated. The seedlings of *P. heterophylla* treated with the mixed strains of *S. marcescens* and *B. thuringiensis* at the ratios of 7:3, 4:1, and 9:1 soon developed disease symptoms, and withered and died. The disease symptom could be alleviated by treating the seedlings of *P. heterophylla* with a mixture of equal amounts of both strains. No disease symptoms could be observed during the entire experiment period using the treatment of the mixed strains at the ratio of 2:3. These results showed that exogenous addition of QQ strain *B. thuringiensis* could efficiently alleviate plant infection by the QS strain *S. marcescens*, whereas the imbalances between these two strains could result in increase of replanting disease incidence in consecutive monoculture.

**Fig. 3.** Assessment of the pathogenicity of isolated *S. marcescens* and the biocontrol potential of *B. thuringiensis.*

*S. marcescens* and *B. thuringiensis* were inoculated around 2 cm away from the seedling root of *P. heterophylla*. Equal amounts of LB medium and *E. coli* DH5α were added as the blank control and strain control. The seedlings of *P. heterophylla* were cultivated for 25 days. BT, *B. thuringiensis*; SM, *S. marcescens*. 

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Interaction of *P. heterophylla* with QS and QQ Bacteria

December 2016 | Vol. 26 | No. 12
Effect of *S. marcescens* with Overexpressed *aiiA* on *P. heterophylla* Growth

The above results showed that the pathogenicity of *S. marcescens* on the seedlings of *P. heterophylla* could be alleviated by *B. thuringiensis*. However, it is not clear whether alleviation of the *S. marcescens* pathogenicity is related to degradation of signal molecules by *B. thuringiensis*. To confirm the mechanism, *S. marcescens* with overexpression of *aiiA* was developed using the constitutive expression vector pBbudK. As shown in Fig. 4A, violacein production by the biosensor strain CV026 could be observed in the presence of wild *S. marcescens*, whereas excess *aiiA* in *S. marcescens* abolished violacein production by the biosensor strain CV026, implying that signal molecule AHLs of the QS system were degraded in the recombinant strain. Furthermore, *S. marcescens* with overexpression of *aiiA* was used to treat the seedlings of *P. heterophylla* in the pots with sterilized soil. The seedling of *P. heterophylla* grew well and exhibited no disease symptoms during the entire cultivation process (Fig. 4B). These results showed that *S. marcescens* pathogenicity on the *P. heterophylla* seedlings was regulated by the bacterial QS system.

Bioassays of Antibacterial Activity, Exoenzymes, and Motility

The phenotypes of antibacterial substances production, exoenzymes secretion, and swarming motility were tested using plate assays to assess the possible mechanism of *P. heterophylla* infection by *S. marcescens*. Antibacterial experiment indicated that the growth of *B. thuringiensis* and *E. coli* was inhibited obviously by the fermentative broth of *S. marcescens* (Fig. 5A). No growth inhibition for *B. thuringiensis* and *E. coli* was observed in the presence of the fermentative broth by *S. marcescens* with overexpressed *aiiA*. Furthermore, *S. marcescens* with overexpressed *aiiA* showed reductions in exoenzymes secretion (pectate lyase, cellulase, and xylanase) and swarming motility when compared with wild strain (Figs. 5B–5E). These findings provided some useful evidence for explaining the increase of the population of *S. marcescens* and the possible invasion mechanism in consecutive monoculture of *P. heterophylla*.

Discussion

The consecutive monoculture problem, also known as soil sickness or replanting disease, results from many factors such as soil nutrient imbalance, autotoxin generation, and the change of soil microbial community structure [42]. Our previous studies showed the reduction of beneficial microorganisms and the increase of pathogenic microorganisms under the consecutive monoculture system of *P. heterophylla* by the T-RFLP method [20]. However, the toxic mechanism

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**Fig. 4.** Construction of *S. marcescens* with overexpression of *aiiA* from the isolated *B. thuringiensis* using the constitutive vector pBudK (A) and its effect on the growth of *P. heterophylla* seedlings (B). *S. marcescens*/*pPbudK-aiiA* was inoculated around 2 cm away from the seedling root of *P. heterophylla* and the seedlings were cultivated for 25 days. An equal amount of LB medium was added as the blank control.

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by these pathogenic microorganisms on the growth of *P. heterophylla* still remains unknown. Recent studies showed that many plant-pathogenic bacteria widely existing in soil and rhizospheric environments could employ the QS system to infect plants by sensing the signal molecules [10, 11]. Meanwhile, some microorganisms termed “quorum quenching” in nature could alleviate the pathogenicity of QS bacteria by producing degrading enzymes or the structure analogs of signal molecules [15]. It was not known whether a similar phenomenon exists in the consecutive monoculture system of *P. heterophylla*. In this study, we attempted to analyze the population of QS and QQ bacteria in the consecutive monoculture system of *P. heterophylla*. In this study, the number of QS bacteria was 5, 12, and 15 from 100 colonies of each sample, exhibiting obvious increase over the years of monoculture, whereas only one QQ strain was obtained from the newly planted sample of *P. heterophylla* (Fig. 1). These isolated strains were identified based on their physiological and biochemical characteristics as well as the 16S rDNA sequences. The QQ strain was classified as *B. thuringiensis*. Surprisingly, the 32 QS strains were identified as *S. marcescens*. Furthermore, the genes of the QS system in the 32 strains were amplified and sequenced. Sequence alignment indicated that the amplified genes from the 32 strains shared high identity of 99% with the *swrIR* genes of the QS system in *S. marcescens* MG1. Therefore, these 32 isolates should be from the same parent strain. As expected, the *aiiA* gene encoding lactonase could be amplified from the QQ strain *B. thuringiensis*, implying that *B. thuringiensis* silenced the AHL molecules by secreting the degrading enzyme lactonase.

The significant increase of *S. marcescens* over the years of monoculture suggested that rhisospheric soil of *P. heterophylla* might provide a suitable environment and adequate nutrient for its proliferation. As shown in Fig. 2, root exudates and root tuber extracts of *P. heterophylla* could efficiently promote the growth of *S. marcescens*, whereas a slight increase for the growth of *B. thuringiensis* was observed. In addition, *S. marcescens* produced antibacterial substances to inhibit or kill the gram-negative (such as *E. coli*) and gram-positive (such as *B. thuringiensis*) strains (Fig. 5A), and further became dominant in rhisospheric soil of *P. heterophylla*. The

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**Fig. 5.** Bioassays of antibacterial activity, exoenzymes, and motility by *S. marcescens* (WT) and *S. marcescens*/*pPbudK-aiiA* (AIIA). (A) Antibacterial activity for *E. coli* (the upper part) and *B. thuringiensis* (the lower part); (B) pectate lyase production; (C) cellulase production; (D) xylanase production; E, swarming motility.
validation of *S. marcescens* as an important soil-borne pathogen indicated that root rot and wilt disease could be caused by this strain. Of note is that the pathogenicity of *S. marcescens* on *P. heterophylla* seedling could be alleviated by *B. thuringiensis*. Therefore, it might be worthwhile to develop *B. thuringiensis* as a biocontrol strain against the pathogenicity of *S. marcescens* on *P. heterophylla*.

The present study also demonstrated the regulatory mechanism of *B. thuringiensis* against the pathogenicity of *S. marcescens* on the *P. heterophylla* seedling. The expression of the aiiA gene from *B. thuringiensis* in *S. marcescens* efficiently degraded signal molecule AHLLs of the QS system and led to reduced pathogenicity on the seedling (Fig. 4). The results demonstrated that *S. marcescens* was dependent on the QS system to evoke disease. The SavIR genes of the QS system in *S. marcescens* MG1 from liquefied plant tissue have been shown to regulate some phenotypes such as swarming motility, biofilm formation, exoenzymes, and carbapenem production, which contributes to pathogenesis of the host [32]. The ability of swarming motility for survival of soil pathogens is important since it is helpful for the movement of pathogens to a suitable environment where the pathogens can utilize nutrients and proliferate rapidly. In addition, *S. marcescens* also produced antibacterial substances such as carbapenem to ensure competition superiority in the rhizospheric environment by inhibiting or killing other surrounding microorganisms. The secretion of exoenzymes is necessary for plant-pathogenic bacteria to infect the plant host. Soft rot pectobacteria are amongst the better characterized groups of phytopathogens dependent on AHL-QS for infection [15]. In several pectobacteria, QS has been reported to regulate the production of plant cell wall-degrading enzymes such as pectate lyase and cellulase [3]. We used the plate method to assay the antibacterial activity, exoenzyme secretion, and swarming motility of *S. marcescens* and *S. marcescens* with overexpressed aiiA (Fig. 5). The results showed the wild *S. marcescens* exhibited antibacterial activity against *B. thuringiensis* and *E. coli*, which was defective in *S. marcescens* with overexpressed aiiA. Obvious reductions in exoenzyme production and swarming motility by *S. marcescens* with overexpressed aiiA were also observed when compared with those of wild *S. marcescens*. Our results demonstrated that the QS system regulated the antibacterial substance production, exoenzyme secretion, and swarming motility in *S. marcescens*. These traits might contribute to pathogenesis in the *P. heterophylla* seedlings.

Considering the important role of QS bacteria in consecutive monoculture of *P. heterophylla*, some feasible strategies, including *B. thuringiensis* fertilizer, QS inhibitors, and transgenic *P. heterophylla* with AiiA protein, might be used to treat the replant diseases of *P. heterophylla*. *B. thuringiensis* fertilizer as a QQ agent could efficiently alleviate the toxic effect of QS bacteria on *P. heterophylla* as shown in our results. In nature, many plants such as pea seedlings, *Curcuma longa*, garlic, vanilla, and *Terminalia catappa* produce QS inhibitors, which are used as QS receptors to block communication among the QS bacteria [5, 8, 24, 25, 30]. Previous studies also showed that some plants expressing the aiiA gene could enhance resistance to the toxic effects of QS bacteria [12,13]. Ban et al. [2] developed transgenic *Amorphophallus konjac* with overexpression of aiiA, which efficiently enhanced resistance to soft rot disease [2]. In future, these strategies might provide alternative methods for alleviating replanting diseases.

In conclusion, we analyzed and identified 32 QS strains of *S. marcescens* and one QQ strain of *B. thuringiensis* in the consecutive monoculture system of *P. heterophylla*. The QS strain of *S. marcescens* exhibited a toxic effect on *P. heterophylla* seedlings, which were alleviated by the QQ strain *B. thuringiensis*. Furthermore, the toxic effect was dependent on the SavIR QS system of *S. marcescens*, while *B. thuringiensis* could secret lactonase to degrade QS signal molecules produced by *S. marcescens* and alleviate the toxic effect. These results demonstrated that QS-regulated behaviors in *S. marcescens* mediated by root exudates play an important role in replanting diseases of *P. heterophylla*.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (No. 31301858 and No. 31501694), the Research Fund for the Doctoral Program of Higher Education of China (No. 20133515120011), the KU Research Professor program of Konkuk University, and 2015 KU Brain Pool fellowship of Konkuk University.

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