Received: 7 April 2019 | Revised: 20 June 2019 | Accepted: 16 July 2019

DOI: 10.1002/jobm.201900202

RESEARCH PAPER

Journal of Basic Microbiology

Occurrence of diversified *N*-acyl homoserine lactone mediated biofilm-forming bacteria in rice rhizoplane

Viveka Balasundararajan | **Balachandar Dananjeyan**

Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India

Correspondence
Dananjeyan Balachandar, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 641003, India.
Email: dbalu@tnau.ac.in

Funding information
Ministry of Human Resource Development, New Delhi, India, Grant/Award Number: F. No. 5-5/2014 – TS VII

Abstract

Quorum sensing (QS)-mediated biofilm-forming rhizobacteria are indispensable due to their competitiveness in the crop rhizosphere. In the present work, we have reported on the occurrence of diversified bacterial species capable of producing *N*-acyl homoserine lactone (AHL) as the QS signal in the roots of a rice plant grown under field conditions. The AHL-producing bacteria were directly isolated from the rice root by the biosensor reporter (*Chromobacterium violaceum* CV026) overlay method and characterized for biofilm production by the microtiter plate method. A total of 48 QS-positive bacterial isolates were purified from different aged (7, 20, 24, 26, and 36 days) rice seedlings. The in vitro biofilm production and genetic diversity as revealed by BOX-PCR fingerprinting showed high variability among the isolates. Most of the best biofilm-forming isolates produced a *N*-butyryl DL-homoserine lactone (a C4-AHL type) signal in the medium. The 16S ribosomal RNA (rRNA) gene sequence of these putative elite isolates identified that they were close to *Aeromonas hydrophila* (QS7-4; QS36-2), *A. enteropelongenes* (QS20-8), *A. veronii* (QS36-3), *Enterobacter* sp. (QS20-11), *Klebsiella pneumoniae* (QS24-6), *Kosakonia cowanii* (QS24-21), *Providentia rettigeri* (QS24-2), *Sphingomonas aquatilis* (QS24-17), and *Pseudomonas sihuiensis* (QS24-20). These strains profusely colonized the rice root upon inoculation and formed biofilms on the surface of the root under gnotobiotic conditions. Developing inoculants from these strains would ensure competitive colonization on the rhizoplane of the crop through their biofilm and thereby improve plant growth and health.

KEYWORDS

biofilm, *N*-acyl homoserine lactone, plant-associated bacteria, quorum sensing, rice

1 | INTRODUCTION

The soil microbiome and its role in soil and plant health are well known [1]. However, root-associated microorganisms, recently designated as rhizo-microbiome, have not yet fully exploited for the improvement of nutrient supply and for protection of crops against biotic and abiotic stresses [2]. The soil microorganisms, which are attracted by the root chemicals migrate and proliferate in the soil around the root regions or on the surface of the root, and this process is known as rhizosphere colonization. This event is the primary step for the soil microorganisms either to cause disease or to benefit the plant they colonize [3]. When the beneficial bacteria colonize the rhizosphere on the plant root, they normally promote plant growth through several mechanisms and also suppress or eliminate the

pathogens in the vicinity of the root region [4]. Hence, the competitive exclusion of pathogens is directly linked to the colonizing ability of the beneficial bacteria in the rhizosphere. In fact, the colonizing ability of these bacteria is also essential to provide maximum benefits to the crop plant [5]. Therefore, plant-growth-promoting rhizobacteria (PGPR) with biofilm-forming capability upon root colonization of a plant always are more expedient than those strains without biofilms [6].

The biofilm-forming capability of pathogenic bacteria has been well studied rather than beneficial and commensal organisms that are associated with plants. *Agrobacterium tumifaciens*, *Burkolderia glumae*, *Dickeya solani*, *Erwinina carotovora*, *E. stewartii*, *Pantoea stewartii, Ralstonia solanacearum*, *Pseudomonas syringae*, *Xanthomonas* spp., and *Xylella fastidiosa* are the biofilm-forming plant pathogens reported so far [7]. Similarly, *Ensifer* spp., *Rhizobium* spp., *Sinorhizobium meliloti*, *P. aeruginosa*, *P. putida*, *P. fluorescens*, *P. chlororaphis*, *Azotobacter vinelandii*, and *Azospirillum lipoferum* are the biofilm-forming beneficial rhizobacteria found in agricultural crops [6]. Quorum sensing (QS) signals are key players in the cell aggregation and biofilm formation in these bacteria [8]. The Gram-positive bacteria produce peptide derivatives such as autoinducing peptides as QS signal molecule, while most of the plant-associated Gram-negative bacteria use fatty acid derivatives such as *N*-acyl homoserine lactone (AHL) for this [9]. LuxI (AHL synthase, I protein) and LuxR (transcriptional regulator, R protein) are involved in AHL-mediated cell to cell communication, and regulate the biofilm formation in these bacteria [10].

Rice is the staple food for most Asian counties. Improvement of nutrient uptake and resistance against drought and diseases could help to enhance rice growth and sustain the yield [11]. Use of microbial resources for sustainable rice production is gaining importance, as they can mitigate the use of synthetic agrochemicals so as to ensure environmental safety [12]. Developing an inoculant with QS-mediated biofilm formation may increase the association between the PGPR and root and thereby improving the crop health. Hence, biofilm-forming plant-growth-promoting bacteria would be a future strategy to improve their efficiency. In the present investigation, we have assessed the genetic diversity of such rhizobacteria in the rice root under field conditions and reported on their biofilm-forming capability. We have used a new and simple method developed in our laboratory to isolate QS-mediated biofilm-forming bacteria [13].

2 | MATERIALS AND METHODS

2.1 | Bacterial culture and media

Chromobacterium violaceum (CV026), the AHL biosensor reporter strain, grown in Luria Bertani (LB) medium at 30°C was used for the isolation of QS-positive rhizobacteria from the rice root [14]. *C. violaceum* ATCC12472 (QS-positive standard strain) was grown on LB medium at 30°C. *Escherichia coli* DH5α used as a negative control for QS assay was routinely grown on LB medium at 37°C.

2.2 | Assessment of occurrence of QS-positive rhizobacteria from rice root using *C. violaceum* as a biosensor

The occurrence and diversity of QS-positive biofilm-forming rhizobacteria in the rice root was assessed at different stages of the rice plant grown under field conditions by following the method developed in our laboratory [13]. For this, the 7, 9, 20, 24, 26, and 36 days old rice seedlings (Cultivar Co51) were collected periodically from Wetlands Farm, Tamil Nadu Agricultural University, Coimbatore. After careful removal of soil adhered on the surface of the root followed by a gentle rinse in sterile water, the roots were cut off and placed in a 100 mm^2 sterile Petri dish. Nearly 50 ml of LB agar seeded with 1 ml of CV026 grown in LB broth at 30°C for 18 h ($\approx 10^8$ cells/ml) was poured over the root at a lukewarm temperature (~50°C). The plates were incubated at 30°C for 1–2 days and observed for violacein pigment production near the root. The non-pigmented root-associated bacteria surrounded by pigmented CV026 indicate the presence of AHL synthesized by the isolates. Such colonies were carefully picked by a sterile toothpick and streaked on new LB agar medium.

2.3 | Authentication of QS-positive isolates

Freshly grown rice root-associated isolates and the biosensor reporter strain CV026 were streaked parallel to each other on Petri plates containing LB agar and incubated at 30°C. *C. violaceum* ATCC12472 was used as a positive control. The plates were observed after 24 and 48 h for violacein production by CV026 due to AHL production by the isolate.

2.4 | Biofilm assay

All the bacterial isolates were assessed for biofilm formation in a 96-well titer plate [15] and the biofilm production quantified with Crystal violet staining as

described by O'Toole [16]. The time-course data (4, 6, 8, and 12 days) on biofilm population (optical density [OD] at 550 nm) and the ratio between biofilm and planktonic population of the isolates (A_{550}/A_{660}) were used to identify the potential biofilm-forming rhizobacteria using principal component analysis (PCA) [17].

2.5 | Identification of AHL by potential QS-positive isolates

The potential biofilm-forming QS-positive isolates were further characterized for *N*-acyl-L-homoserine lactone with gas chromatography/mass spectrometry (GC/MS) using the method described by Cataldi et al. [18].

2.6 | Genetic and phylogenic analysis

All the 48 QS-positive rhizobacterial isolates from the rice root were assessed for molecular fingerprints using the BOX filament polymerase chain reaction [PCR] (BOX-PCR) [19]. The presence or absence of bands in the fingerprint profile was imported to PRIMER 6 statistical software (Plymouth Routines in Multivariate Ecological Research, version 6.1.13; PRIMER-E, Plymouth, UK) and the similarity matrix was computed using the Bray–Curtis coefficient [20]. The relativity of the isolates was visualized using a nonmetric multidimensional scaling (MDS) plot and hierarchical cluster analysis (HCA) dendrogram.

All the potential QS-positive biofilm-forming bacterial strains were identified with a 16S ribosomal RNA (rRNA) gene sequence [21]. The 16S rRNA gene of the strains was amplified as described elsewhere using FD1 and RP2 primers [21] and sequenced in both directions by using an Applied Biosystems automated sequencer (Bioserve, Hyderabad, India). The identity of the 16S rRNA gene sequence was established by performing a similarity search against the GenBank database (http://www.ncbi.nih.gov/BLAST). The sequences obtained for the isolates were deposited in GenBank with the accession numbers of MK649834 (QS7-4), MK649835 (QS20-8), MK649836 (QS20-11), MK649837 (QS24-2), MK649838 (QS24-6), MK649839 (QS24-17), MK649840 (QS24-20), MK649841 (QS24-21), MK649842 (QS36-2), and MK649843 (QS36-3).

2.7 | Colonizing ability of the isolates on rice root

Dehusked rice seeds (Cultivar Co51) were surface-sterilized with sodium hypochlorite (5% available chlorine) for 10 min, followed by a rinse with sterile water five times and then soaked overnight in darkness. Then, the seeds were treated with 2% ketoconazole to avoid fungal contaminations followed by gentle washing with sterile water three times. The seeds were then transferred to a sterile Petri plate and incubated at room temperature in the dark for the next 24 h. The uniformly sprouted seeds were placed over Murashige and Skoog medium with 0.5% Phytagel™ (Sigma-Aldrich) in long culture tubes (30 cm height × 2.5 cm diameter) at one seed per tube. The rice culturing was done in a plant-growth chamber with 12-h light (200 mole·m^{-2}·s^{-1}) at 28°C and maintained for 20 days. All the elite biofilm-forming rhizobacterial strains were grown overnight in LB broth at 30°C. The cells were harvested by centrifugation and washed twice in 10 mM $MgCl_2$ and resuspended in the same. This suspension was inoculated (20 µl per seed) at the hypocotyl region of 5 days old seedlings at OD_{660} of 0.05 (10^8 cfu/ml), whereas uninoculated controls were maintained simultaneously. *Azotobacter chroococcum* AC1 (biofilm-forming PGPR strain) and *Enterobacter cloacae* ZSB14 (nonbiofilm-forming PGPR strain) served as standards to compare colonizing efficiency. The colonization and biofilm formation on the surface of the root were visualized and photographed on the third day after inoculation.

3 | RESULTS

3.1 | AHL-producing bacterial isolates from rice rhizoplane

A total of 56 bacterial isolates showing positivity for QS activity through AHL production were isolated from the rhizoplane of rice seedlings collected from the field-grown crop. White colonies appearing from the rice root that were surrounded by the purple-colored reporter strain, CV026 were considered as possible QS-positive bacterial isolates. The age of the seedlings had an influence on the number of QS bacteria isolated from the rhizoplane. The 7-days-old seedlings had four isolates; 9 days had 2 colonies; 20 days had 14 colonies; 24 days had 25 colonies; 26 days had 5; and 36-days-old rice had 6 QS-positive colonies in their rhizoplane region. All the bacterial isolates were purified and designated as "QS-day-isolate number" from QS-7-1 to QS-36-6. Among the 56 QS-bacterial isolates screened for AHL production using CV026 as the reporter strain, QS7-1, QS7-3, QS9-2, QS20-1, QS20-4, QS20-7, QS20-8, QS20-10, QS20-11, QS20-12, QS20-13, QS20-14, QS24-5, QS24-10, QS24-13, QS24-14, QS24-15, QS24-16, QS24-17, QS24-18, QS24-20, QS24-21, QS24-23, QS24-24, QS24-25, QS26-1, QS26-2, QS26-4, QS26-5, QS36-2, QS36-3, and QS36-6 showed AHL production within 24 h of incubation, turning the CV026 colony into purple pigmentation. The isolates QS7-2, QS7-4, QS20-2, QS20-5, QS20-6, QS20-7, QS20-9, QS24-1, QS24-2, QS24-4, QS24-6,

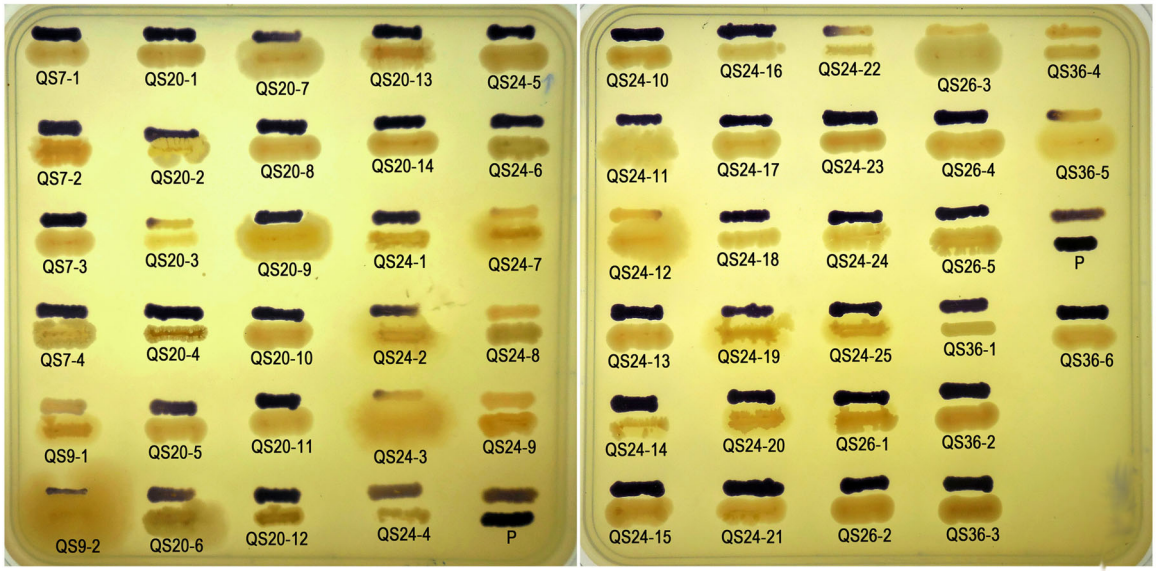

FIGURE 1 Authentication of the QS-positive isolates of rice rhizoplane by parallel streaking with the reporter strain, *Chromobacterium violaceum* (Cv026) in LB agar. QS7-1 to QS36-6, QS-positive strains from rice root. LB, Luria Bertani; P, positive control (*C. violaceum* ATCC12472); QS, quorum sensing

QS24-11, QS24-19, QS24-22, QS36-1, QS36-5, and positive control ATCC12472 produced AHL after 48 h of incubation and pigmented the CV026 strain in the parallel streak assay (Figure 1).

3.2 | Screening potential biofilm-forming isolates

PCA was performed on biofilm formation (A_{550}) and the biofilm/planktonic ratio (A_{550}/A_{660}) data to identify the potential biofilm formers. The PCA plot showing the variables and observations are presented as Figure 2. The PCA explained 64.97% cumulative variability due to biofilm-forming capability and its ratio with the planktonic population. The principal component (PC1) adds 41.05% variability, while PC2 adds an additional 23.92% to the total cumulative variability. All the assessed variables used for PCA were orthogonally positioned in the PC1 and PC2 positive quadrant (left-hand top) and the PC1 positive and PC2 negative quadrant (left-hand bottom) of the plot (Figure 2a). Corresponding to these two plots, all the potential biofilm-forming QS isolates

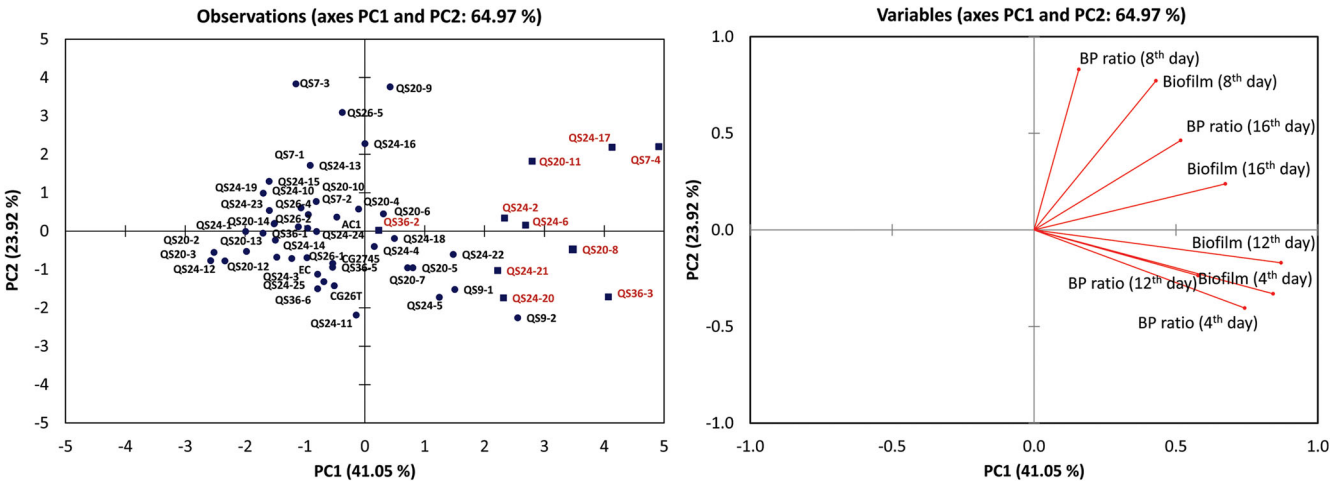

FIGURE 2 Principal component analysis plots relating the biofilm data and QS-bacterial isolates. (a) Scoring plot showing the positions of QS isolates. (b) Loading plot showing orthogonal positions of variables (biofilm and biofilm/planktonic ratio). Values in parentheses indicate the days after inoculation. Mean of three replicates was plotted. The % variance explained by each component (PC1 and PC2) is given in parentheses in axes. The higher biofilm-forming strains positioned with the variables in the scoring plot are highlighted with □ and red-colored. PC, principal component; QS, quorum sensing

were positioned in the "high" (left-hand top) and "moderate" (left-hand bottom) quadrants of the PCA plot (Figure 2b). The QS bacteria with less-biofilm are negatively correlated with the variables and positioned in the "low" quadrant, which is negative for both PCs. The isolates QS20-8, QS20-11, QS24-6, QS24-17, and QS36-3 showed the highest biofilm formation on all the days of observation. QS7-4 was observed to form more biofilm during 8th, 12th, and 16th day, whereas QS24-2, QS24-20, and QS24-21 showed more biofilm on the later stages, that is, the 12th and 16th day. QS36-2 formed more biofilm on the 12th day than other cultures.

3.3 | Genetic diversity of QS bacteria

The BOXA1 primer targeting BOX repetitive element of an isolate showed discriminative amplicons for the QS-bacterial isolates. The agarose gel electrophoresis of the BOX fingerprint showed that a range of 100–1,200 base pairs sized with 5–8 amplicons per isolate was noticed (Figure 3a). The nonmetric MDS plot using the Bray–Curtis similarity index of BOX fingerprinting discriminated the QS isolates with a similarity ranging from 30% to 100%. The QS isolates were evenly positioned in the MDS plot without any pattern and the two-dimensional stress of 0.06 showed the reliability of the plot (Figure 3b). The HCA dendrogram clustered identical and most similar isolates into groups (Figure 3c). QS20-4 and QS20-13; QS24-16 and QS24-18 had 100% similarity, and are genetically identical isolates. The wild and mutant strains of *C. violaceum*, QS24-15 and QS24-17 had 90% genetic similarity with reference to BOX fingerprinting. If 80% similarity is used as the cut off for clustering, only five clusters of isolates remain namely, Cluster I consists of QS24-3, QS24-4, QS24-16, QS24-18; Cluster II with QS20-12, QS20-11, QS20-3, QS20-4, QS20-13; Cluster III with QS20-14 and QS24-4; Cluster IV containing QS24-15 and QS24-17; and Cluster V with QS24-14 and QS36-2 (Figure 2c), while the rest of the isolates were unique and unclustered.

3.4 | Identification of AHL produced by elite QS-bacterial isolates

The GC/MS analysis of the culture extract of most of the elite QS-bacterial isolates (QS7-4, QS20-8, QS20-11, QS24-2, QS24-6, QS24-17, QS24-20, QS24-21, and QS36-2) identified the AHL as *N*-butyryl L-homoserine lactone (retention time between 12.17 and 12.23 min; *m/z* 171; 92.0–96.0 purity). The isolate QS36-3 produced *N*-Decanoyl-DL-homoserine lactone (12.14 min; *m/z* 255; 93.4 purity).

3.5 | Phylogeny of elite strains

All the potential AHL-mediated biofilm-forming bacteria were identified based on their 16S rRNA gene sequence

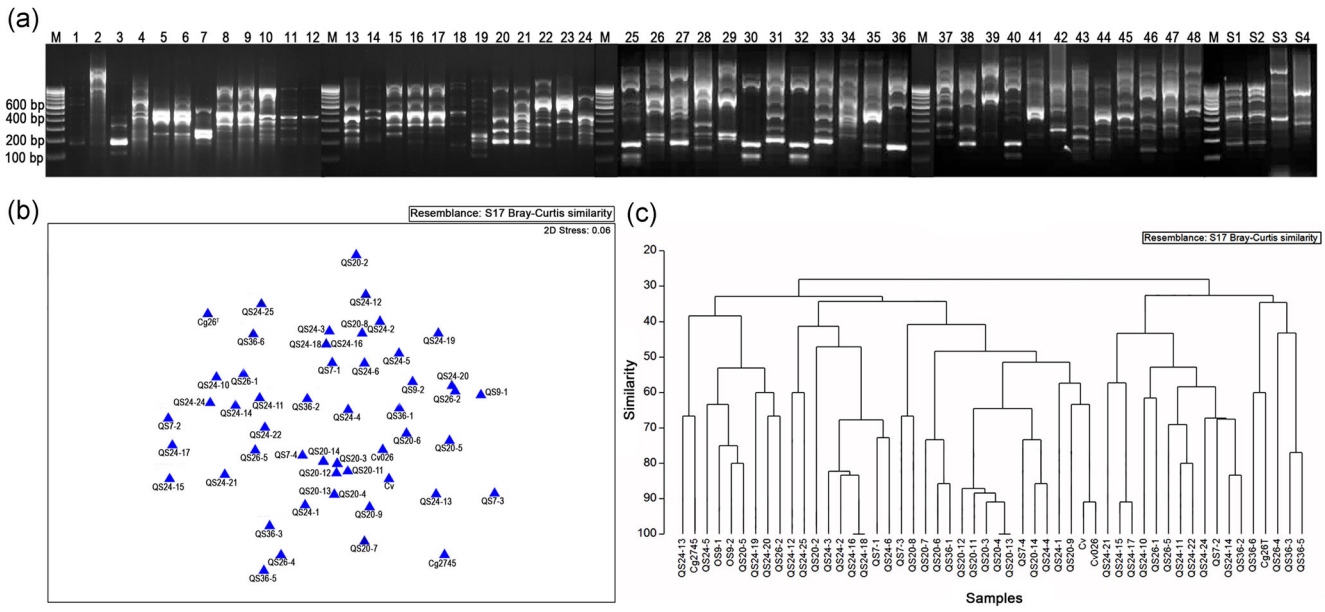

FIGURE 3 (a) BOX-PCR fingerprint profiles of QS-positive isolates of rice rhizosphere. Lanes, M–100 bp DNA ladder; 1–48, QS-positive isolates (QS7-1 to QS36-6); S1, *Corynebacterium glutamicum* ATCC13032; S2, *C. glutamicum* ATCC13869; S3, *Chromobacterium violaceum* cv026; S4, *C. violaceum* ATCC12472. Nonmetric MDS plot (b) and HCA dendrogram (c) constructed from BOX-PCR fingerprint data of QS-positive isolates and standard strains. The similarity matrix using the Bray–Curtis coefficient was applied for both the analyses in Primer 6.0 software. bp, base pair; MDS, multidimensional scaling; PCR, polymerase chain reaction; QS, quorum sensing

homology with the nucleotide database using the BLAST search. The QS7-4 is close to *Aeromonas hydrophila* with 100% sequence homology, whereas QS20-8, QS36-2, and QS36-3 belong to *A. enteropelongenes*, *A. hydrophila*, and *A. veronii*, respectively. QS20-11 was phylogenetically close to *E. cloacae* (99% homology); whereas QS24-2 with *Providentia rettigeri* (99% homology); QS24-6 with *Klebsiella pneumoniae*; QS24-17 with 99% homology to *Sphingomonas aquatilis*; QS24-20 with *Pseudomonas sihuiensis*; and QS24-21 with 99% homology with *Kosakonia cowanii*.

3.6 | Colonizing ability of the strains

The root colonization and biofilm-forming capability of QS-bacterial strains differed (Figure 4). A clear and visible biofilm on the root surface of rice was observed on the third day in QS20-11, QS24-6, QS24-20, QS36-2, and *A. chroococcum* strain Ac1 inoculated rice roots, whereas the least response was noticed in QS7-4, QS20-8, QS24-17. The non-biofilm PGPR strain (ZSB14) did not show any visual biofilm on the rice root.

4 | DISCUSSION

The plant-associated bacteria use QS signals for biofilm-mediated colonization as well as for the production of antagonistic compounds to avoid competition from other microbial communities [22]. QS increases the cell density of a strain and further tends to produce extracellular polymeric substances in which cells are embedded. This structure known as the biofilm is a microhabitat of that strain in which the cells live in with maximum protection [23]. Hence, biofilm-forming PGPR strains have better space and nutritional benefits than the nonbiofilm-forming strains and subsequently elicit more benefits to the colonizing plant species [5,6]. Obviously, developing the biofilm-forming microbial inoculants as biofertilizers and biocontrol agents could make a great contribution to sustainable agriculture [6]. With this background, we have shown through this study that the rice root harbors diversified QS-mediated biofilm-forming bacteria, which can be explored for plant and soil health.

In our previous work, we have developed a straightforward method for isolation of QS-positive rhizobacteria from the root. In that method, the QS-positive bacteria

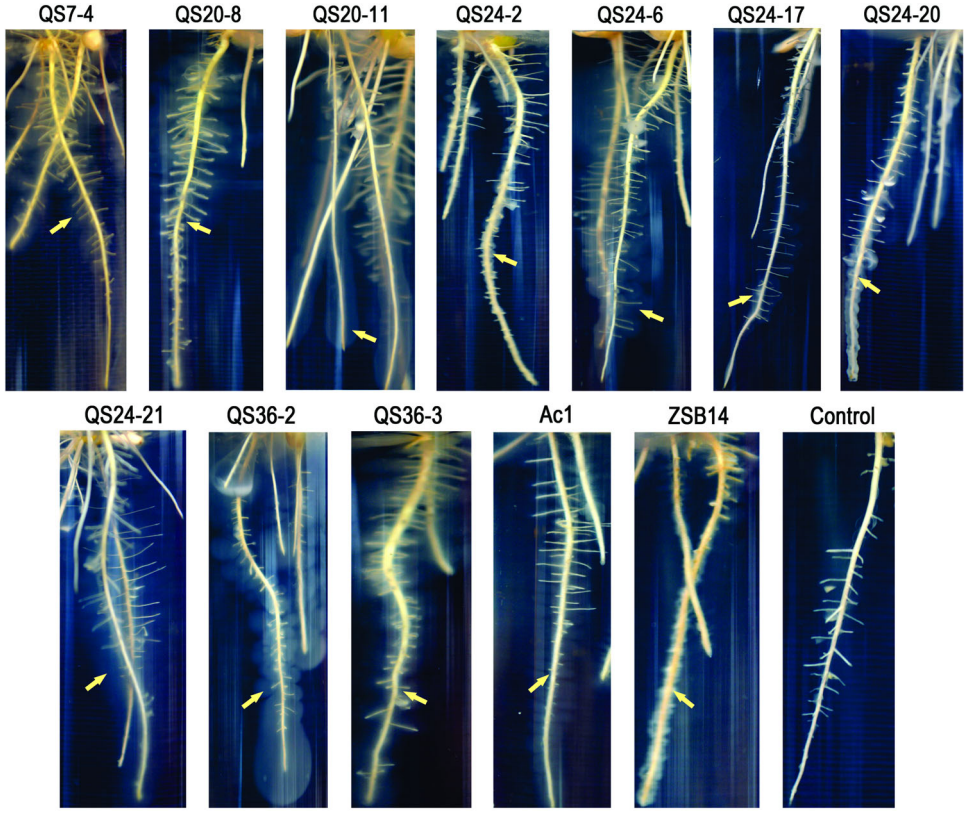

FIGURE 4 Biofilm formation by elite QS-bacterial isolates on the root surface of rice seedlings (Co51) under gnotobiotic conditions. All the elite strains were inoculated at the hypocotyl region of the 5-day-old seedlings and imaged after 3 days of incubation. QS7-4 to QS36-3: Elite QS-bacterial strains. Ac1, *Azotobacter chroococcum* strain Ac1; ZSB14, *Enterobacter cloacae* strain ZSB14; control, uninoculated control. Biofilmed growth of inoculated strain on rice root indicated with arrow mark. QS, quorum sensing

adhering on the surface of the root could be detected by the change of color of biosensor reporter strain (*C. violaceum* CV026) overlaid in an agar medium [13]. In the present work, we have adopted that method to assess the genetic diversity of QS-positive bacteria in rice rhizoplane, when grown under field conditions. We have focused on the AHL alone and omitted other signal molecules, as the AHL-based QS is predominant among rhizobacteria [8]. From a field-grown rice root (sampled between 7 and 36 days old), a total of 48 QS-positive strains could be isolated and authenticated using the newly developed method. The in vitro assay also confirmed their biofilm-producing capability and showed a difference among the isolates. Similarly, the genetic diversity as revealed by BOX-PCR fingerprints also showed significant variability among the QS-positive isolates. These findings suggest that the QS bacteria colonizing the rice root differ across the time course of seedling growth. There is no correlation between the age of the seedling and the genetic diversity of QS-bacterial isolates, which means that no specific bacterial community is colonizing the rice seedling.

Among the AHL-mediated biofilm-forming isolates, efficient isolates were further characterized by the type of AHL produced. Most of the isolates (QS7-4, QS20-8, QS20-11, QS24-2, QS24-6, QS24-17, QS24-20, QS24-21, and QS36-2) produced C4-HSL, *N*-butyryl *L*-homoserine lactone. This type of AHL was reported earlier in plant-associated bacteria such as *A. veronii* [24]; *A. hydrophila* [13,25]; *A. caviae* [13,26]; *Enterobacter asburiae* [27]; *P. aeruginosa* [28,29]; *P. sihuiensis* [13]. The phylogeny of the elite biofilm-forming bacteria reported in the present study belongs to species of *Aeromonas* (*A. hydrophila*, *A. enteropelongenes*, *A. veronii*), *E. cloacae*, *Providentia rettgeri*, *K. pneumoniae*, *S. aquatilis*, *P. sihuiensis*, and *K. cowanii*. All these strains also proved their ability to colonize the rice root better than the well known biofilm-producing biofertilizer (*A. chroococcum*). The preliminary gnotobiotic experiment on rice seedling revealed that the biofilm formation and root colonization of these strains varied. Most of the strains covered the entire root within 3 days of inoculation and the mantle of bacterial growth on the surface of the rice root is visible. The in vitro experiments conducted with our isolates (data not shown) as well as previous reports [5,6] suggest that these biofilm-forming rhizobacteria can increase the availability of phosphorus, potassium, and zinc by solubilization, produce siderophores and indole-3-acetic acid, thereby benefiting crop growth.

The species that are capable of producing biofilms through QS signaling reported to be found in the rice root surface were reported previously in various studies. PGPR strains of *Aeromonas* were isolated from sugarcane [30]. The occurrence of biofilm-forming *Aeromonas* spp. on the root surface of rice was already reported in our previous work [13]. However, the biofilm-forming capability and QS regulation have been well studied for pathogenic isolates [31–33]. Similarly, several species of *Klebsiella* including *K. pneumoniae, K. oxytoca*, and *K. planticola* have been reported for their presence on the root surface of several plant species [34]. The plant-growth-promoting *K. pneumoniae* isolated from the surface of rice root uses biofilm-related proteins to colonize the surface of nonbiological planes [35]. One of the isolates possessing potential biofilm-forming capability belongs to the species *S. aquatilis* (QS24-17). *Sphingomonas* belonging to α-proteobacteria, is a strictly aerobic bacterium, distinguished from other related genera by their sphingolipids [36]. This is a soil-dwelling bacterium often reported with plant association. This bacterium was isolated from various tissue parts of rice [37]. Enya et al. [38] isolated four strains of *Sphingomonas* with biocontrol potential against tomato diseases and also observed AHL synthesis. Similarly, the AHL-mediated biofilm formation of both pathogenic and plant-growth-promoting *Enterobacter* spp. and *Pseudomonas* spp. has also been documented [39]. In the current work, *K. cowanii*, a reclassified species of *Enterobacter* [40] was also reported to be present in rice root. *Kosakonia* spp. found in clinical samples, soil and tree species and several species promotes plant growth by nitrogen fixation [41,42]. We also found *P. rettgeri* (QS24-2) as one of the best biofilm-forming rhizobacteria from the surface of the rice root. Though *P. rettgeri* is recognized as a pathogen to humans [43], recently a strain with plant-growth-promoting traits was isolated from lichens [44]. However, cell to cell communication via QS in pathogenic strains of *Providentia* has already been reported [45].

To conclude, we showed that rice root recruits diversified soil-dwelling bacteria that are capable of producing biofilms through AHL-mediated QS. These bacteria may be commensal, pathogenic, or beneficial to the rice crop. However, by careful examination of their plant-growth-promoting traits and virulence, it could be possible to develop a biofilm-forming inoculant for providing nutrients and for biotic and abiotic stress mitigation.

ACKNOWLEDGMENTS

Financial support from the Ministry of Human Resource Development, New Delhi, India through the Center of Excellence in Frontier Areas of Science and Technology on Microbes to Feed the World: Plant–microbe interaction to boost the agricultural production (F. No. 5-5/2014-TS VII) to conduct this investigation is acknowledged.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

ORCID

Balachandar Dananjeyan http://orcid.org/0000-0002-4434-6316

REFERENCES

[1] Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol. 2009;63:541-56.
[2] Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev. 2013;37:634-63.
[3] Benizri E, Baudoin E, Guckert A. Root colonization by inoculated plant growth-promoting rhizobacteria. BioControl Sci Techn. 2001;11:557-74.
[4] Beneduzi A, Ambrosini A, Passaglia LMP. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol. 2012;35:1044-51.
[5] Timmusk S, Behers L, Muthoni J, Muraya A, Aronsson AC. Perspectives and challenges of microbial application for crop improvement. Front Plant Sci. 2017;8:49.
[6] Velmourougane K, Prasanna R, Saxena AK. Agriculturally important microbial biofilms: present status and future prospects. J Basic Microbiol. 2017;57:548-73.
[7] Sibanda S, Moleleki LN, Shyntum DY, Coutinho TA. Quorum sensing in Gram-negative plant pathogenic bacteria. In: Kimatu JN, editor. Advances in plant pathology. London, UK: IntechOpen; 2018. p. 67-89.
[8] Loh J, Pierson EA, Pierson LS, Stacey G, Chatterjee A. Quorum sensing in plant-associated bacteria. Curr Opin Plant Biol. 2002;5:285-90.
[9] Papenfort K, Bassler BL. Quorum sensing signal–response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14:576-88.
[10] Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol. 2005;21:319-46.
[11] Bhullar GS. Sustainable rice production. In: Lichtfouse E, Goyal A, editors. Sustainable agriculture reviews: cereals. Cham: Springer International Publishing; 2015. p. 107-21.
[12] Naher UA, Othman R, Panhwar QA, Ismail MR. Biofertilizer for sustainable rice production and reduction of environmental pollution. In: Hakeem KR, editor. Crop production and global environmental issues. Cham: Springer International Publishing; 2015. p. 283-91.
[13] Begum JF, Tamilarasi M, Pushpakanth P, Balachandar D. A simple method for direct isolation of N-acyl-L-homoserine lactone mediated biofilm-forming rhizobacteria from roots. J Microbiol Meth. 2019;156:34-9.
[14] McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, et al. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acyl homoserine lactones. Microbiology. 1997;143:3703-11.
[15] Pierce CG, Uppuluri P, Tristan AR, Wormley FL Jr, Mowat E, Ramage G, et al. A simple and reproducible 96 well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. Nat Protoc. 2008;3:1494-500.
[16] O'Toole GA. Microtiter dish biofilm formation assay. J Visualized Exp. 2011;47:2437.
[17] Wold S, Esbensen K, Geladi P. Principal component analysis. Chemom Intell Lab Syst. 1987;2:37-52.
[18] Cataldi TRI, Bianco G, Frommberger M, Schmitt-Kopplin P. Direct analysis of selected N-acyl-L-homoserine lactones by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2004;18:1341-4.
[19] Versalovic J, Schneider M, de Bruijn FJ, Lupski JR. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Method Mol Cell Biol. 1994; 5:25-40.
[20] Clarke KR. Non-parametric multivariate analyses of changes in community structure. Aust Ecol. 1993;18:117-43.
[21] Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173: 697-703.
[22] Pieterse CMJ, de Jonge R, Berendsen RL. The soil-borne supremacy. Trends Plant Sci. 2016;21:171-3.
[23] Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nat Rev Microbiol. 2016;14:563-75.
[24] Zhao D, Lyu F, Liu S, Zhang J, Ding Y, Chen W, et al. Involvement of bacterial quorum sensing signals in spoilage potential of *Aeromonas veronii* bv. *veronii* isolated from fermented surimi. J Food Biochem. 2018;42e12487.
[25] Yang Y, Zhou M, Hardwidge PR, Cui H, Zhu G. Isolation and characterization of N-acyl homoserine lactone-producing bacteria from cattle rumen and swine intestines. Front Cell Infect Microbiol. 2018;8:155.
[26] Lim YL, Ee R, Yin WF, Chan KG. Quorum sensing activity of *Aeromonas caviae* strain YL12, a bacterium isolated from compost. Sensors. 2014;14:7026-40.
[27] Lau Y, Sulaiman J, Chen J, Yin WF, Chan KG. Quorum sensing activity of *Enterobacter asburiae* isolated from lettuce leaves. Sensors. 2013;13:14189-99.
[28] Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in Gram-negative bacteria: A signaling mechanism involved in associations with higher organisms. PNAS. 2000;97:8789-93.
[29] Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature. 2000;407:762-4.
[30] Morgado González A, Espinosa Victoria D, Gómez Merino FC. Efficiency of plant growth promoting rhizobacteria (PGPR) in sugarcane. Terra Latinoamericana. 2015;33:321-30.
[31] Du H, Pang M, Dong Y, Wu Y. Identification and characterization of an *Aeromonas hydrophila* oligopeptidase eene *pepF* negatively related to biofilm formation. Front Microbiol. 2016;7:1497.

[32] Ponnusamy K, Paul D, Kweon JH. Inhibition of quorum sensing mechanism and *Aeromonas hydrophila* biofilm formation by vanillin. Environ Eng Sci. 2009;26:1359-63.
[33] Swift S, Karlyshev AV, Fish L, Durant EL, Winson MK, Chhabra SR, et al. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida*: identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J Bacteriol. 1997;179:5271-81.
[34] Ladha JK, Barraquio WL, Watanabe I. Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella planticola* associated with rice plants. Can J Microbiol. 1983;29:1301-8.
[35] Liu Y, Wang H, Sun X, Yang H, Wang Y, Song W. Study on mechanisms of colonization of nitrogen-fixing PGPB, *Klebsiella pneumoniae* NG14 on the root surface of rice and the formation of biofilm. Curr Microbiol. 2011;62:1113-22.
[36] Yabuuchi E, Yano I, Oyaizu H, Hashimoto Y, Ezaki T, Yamamoto H. Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb, nov., and two genospecies of the genus *Sphingomonas*. Microbiol Immunol. 1990;34:99-119.
[37] Kim H, Nishiyama M, Kunito T, Seeno K, Kawahara K, Murakami K, et al. High population of *Sphingomonas* species on plant surface. J Appl Microbiol. 1998;85:731-6.
[38] Enya J, Shinohara H, Yoshida S, Tsukiboshi T, Negishi H, Suyama K, et al. Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. Microb Ecol. 2007;53:524-36.
[39] Danhorn T, Fuqua C. Biofilm formation by plant-associated bacteria. Annu Rev Microbiol. 2007;61:401-22.
[40] Inoue K, Sugiyama K, Kosako Y, Sakazaki R, Yamai S. *Enterobacter cowanii* sp. nov., a new species of the family enterobacteriaceae. Curr Microbiol. 2000;41:417-20.
[41] Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): Proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii, E. radicincitans, E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis, E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. Syst Appl Microbiol. 2013;36:309-19.
[42] Kämpfer P, McInroy JA, Doijad S, Chakraborty T, Glaeser SP. *Kosakonia pseudosacchari* sp. nov., an endophyte of *Zea mays*. Syst Appl Microbiol. 2016;39:1-7.
[43] Yoh M, Matsuyama J, Ohnishi M, Takagi K. Importance of *Providencia* species as a major cause of travellers' diarrhoea. J Med Microbiol. 2005;54:1077-82.
[44] Swamy CT, Gayathri D, Devaraja TN, Bandekar M, D'Souza DE, Meena RM. Plant growth promoting potential and phylogenetic characteristics of a lichenized nitrogen fixing bacterium, *Enterobacter cloacae*. J Basic Microbiol. 2016;56:1369-79.
[45] Rather PN, Ding X, Baca-DeLancey RR, Siddiqui S. *Providencia stuartii* genes activated by cell-to-cell signaling and identification of a gene required for production or activity of an extracellular factor. J Bacteriol. 1999;181:7185-91.

How to cite this article: Balasundararajan V, Dananjeyan B. Occurrence of diversified *N*-acyl homoserine lactone mediated biofilm-forming bacteria in rice rhizoplane. *J Basic Microbiol*. 2019; 1-9. https://doi.org/10.1002/jobm.201900202