Madras Agric. J., 2019; doi:10.29321/MAJ 2019.000243

RESEARCH ARTICLE

Utilization of Rhizospheric Carbon Sources by Biofilm-forming Rhizobacteria of Rice

Naveen, S.[1] and Balachandar, D.[1]
[1]Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641003

Received : 27th May, 2019
Revised : 31st May, 2019
Accepted : 31st May, 2019

ABSTRACT

Plant growth promoting rhizobacteria are of great interest in sustainable agriculture and have gained much attention in recent years. Yet, these bacteria often failed to perform their functions in the plants due to poor colonization in the rhizosphere. There are no vibrant procedures available to identify the traits responsible for the effective colonization of these PGPR strains under in vitro conditions. In the present work, twenty low molecular weight carbon compounds predominantly present in the root exudates were used to assess the differential carbon substrate utilizing profile of the PGPR strains. Further, the synthetic root exudate solution which mimics the rhizo-deposits comprising of sugars, organic acids and amino acids in a ratio of 60:35:5 % were used to assess the growth and biofilm production under in vitro condition. The results revealed that these two assays can clearly discriminate the high and low competitive strains of PGPR. Among the twelve strains, Aeromonas hydrophila QS7-4, Enterobacter sp. QS20-11 and Klebsiella pneumoniae QS24-6 were identified as the strains with high colonizing ability. Hence, developing inoculant with these traits are essential for effective colonization and for exceling maximum benefit for the agricultural productivity.

Keywords: *Biofilm, plant growth promoting rhizobacteria, differential carbon utilization.*

INTRODUCTION

Apart from mechanical support, nutrient and water uptake, plant root is the mean for complex microbial diversity in the rhizosphere through their root exudation (Kuzyakov and Blagodatskaya 2015). The root exudates and other rhizo-depositions secreted by the plant had considerable control over the microorganisms associated with it (Berendsen *et al.* 2012). Exudates comprises of monosaccharides (fructose, mannose, glucose), disaccharides (maltose), pentoses (arabinose), oligosaccharides, amino acids including asparagine, arginine, cysteine, glutamine, organic acids such as ascorbic, acetic, benzoic, ferulic, and malic acids; high molecular compounds such as auxins, flavonoids, enzymes, fatty acids, gibberellin, nucleotides, tannins, steroids, terpenoids, alkaloids, polyacetylenes, and vitamins (Gunina and Kuzyakov 2015; Hayat *et al.* 2017). Root exudation is genetically controlled and regulated process, thus it shapes the rhizospheric microbiome. The chemistry of root exudation can modulate the distinct rhizobacterial communities for different genotypes (Kristin and Miranda 2013).

In order to proliferate and establish in the rhizosphere, rhizobacteria should able to utilize the compounds of root exudates and other rhizo-deposits effectively (Dennis *et al.* 2010). The bacterial traits that are responsible for the effective colonization include motility, growth rate, differential carbon substrate utilizing efficiency and biofilm formation (Lugtenberg *et al.* 2001). Plant-growth-promoting rhizobacteria (PGPR) are the members of rhizo-microbiota of special interest, being potential resource for sustainable agriculture, as the use of them can reduce the synthetic chemical inputs and thereby ensure the environmental safety (Naher *et al.* 2014). These PGPR strains should be competitive for both space and food in the rhizosphere in order to excel their maximum benefit to the crop. A broad-spectrum carbon-utilizing and biofilm-forming PGPR are always superior to those with less choice of carbon utilization and non-biofilm formers (Velmourougane *et al.* 2017). Hence, in the present investigation, we have assessed the rice root-associated beneficial bacteria for their ability to utilize possible carbon sources present in the root exudates. We have also assessed the growth and biofilm-forming efficiency of these strains in synthetic root exudate under *in vitro* conditions. Synthetic root exudates or root exudate compound solutions are often used to assess the impact of root exudates in

*Corresponding author's e-mail: dbalu@tnau.ac.in

the structure and activity of bacterial communities in microcosm studies (Paterson *et al.* 2007; Henry *et al.* 2008). Even though, this approach cannot be a true representative of the complex rhizosphere, it could demonstrate the importance of rhizosphere competition and colonization processes (Baudoin *et al.* 2003; Henry *et al.* 2008). Through this approach, we showed that the rhizobacteria varied their growth and biofilm-forming ability and thus it can be used as one of the traits for the inoculant for agricultural purpose.

MATERIALS AND METHODS

PGPR strains

Twelve biofilm-forming and plant-growth promoting bacteria isolated from rice roots viz., *Aeromonas hydrophila* (QS7-4; QS36-2; QSRB-2; QSRB-5), *A. enteropelongenes* QS20-8, *A. veronii* QS36-3, *A. caviae* QSRB-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 (Begum *et al.* 2019) routinely grown in Luria-Bertani (LB) medium at 30°C were used for this study. The strains belonging to *Klebsiella* and *Enterobacter* genus which were considered as potential human pathogens were also included because these strains were isolated from safe environmental samples and were positive for most of the PGPR traits.

Carbon source utilization assay

The ammonium mineral salts medium (AMS) (ATCC medium number 1683) was prepared, since it contains most of the macro and micro nutrients than the other minimal media and added with twenty different carbon sources at 500 mM carbon equivalent which is equal to 1.5 % of glucose and was fixed as a standard carbon concentration. The possible carbon compounds present in the root exudates such as glucose, maltose, galactose, fructose, xylose, raffinose, arabinose, mannitol, inositol, malic acid, citric acid, oxalic acid, acetic acid, succinic acid, glucosamine, proline, alanine, tyrosine and glutamic acid were used as sole carbon source in AMS medium to screen the strains. The clear solution of the AMS broth with respective carbon sources alone were transferred to microtiter plates (225 µl per well) to reduce the interference of the deposited salts. All the biofilm-forming PGPR strains were grown in AMS with glucose (500 mM carbon) at 30°C for 24 h. The cultures were then centrifuged (5000 g, 5 min at 24°C) and the cell pellet was suspended in AMS broth with final OD_{600} to 0.5. A volume of 25 µL of each culture was inoculated to each of the carbon sources supplemented AMS medium. Three replicates were maintained with an uninoculated broth of respective carbon source served as blank. The microtiter plates were covered with a lid and incubated at 30°C in an incubator (Lab Companion, USA). The absorbance was measured at 600 nm in 7 intervals at 0.5, 4, 8, 24, 28, 32 and 46 h using the micro plate reader (SpectraMax i3X) with path check settings enabled and with blank.

The mean growth (OD_{600}) of each strain for each carbon source was accounted for cumulative growth to screen the best strain. Further, the mean growth data were imported into PRIMER 6 statistical software ((Plymouth Routines in Multivariate Ecological Research, version 6.1.13; PRIMER-E, Plymouth, UK), normalized and similarity matrix was constructed by calculating the similarities between the strains in terms of carbon utilization by Bray-Curtis coefficient (Clarke 1993). Non-metric multi-dimensional scaling (MDS) was used to ordinate the similarity data. To visualize the relationship between the strains in terms of their carbon utilization, the similarity matrix using the Bray–Curtis coefficient was also analyzed by hierarchical cluster analysis (HCA).

Growth and biofilm formation of PGPR strains in synthetic root exudate medium

Six potential carbon-substrate utilizing strains viz., QS7-4, QS20-11, QS24-2, QS24-6, QSRB-2 and QSRB-5 screened from the above experiment were used in this experiment. AMS medium supplemented with glucose (500 mM carbon) and synthetic root exudate solution (500 mM carbon) were compared.

The synthetic root exudate was prepared as follows: the sugars includes glucose, maltose, galactose, fructose, xylose, raffinose, arabinose, mannitol and inositol; organic acids includes malic acid, citric acid, oxalic acid, acetic acid and succinic acid and amino acids includes proline, alanine, tyrosine, glucosamine and glutamic acid. The percentage of sugars, organic acids and amino acids in most of the root exudates an in the ratio of 60:35:5 (%) respectively. So the carbon concentration of sugars, organic acids and amino acids was maintained in the ratio of 300:175:25 (mM) respectively and also the C:N ratio was maintained at 20:1. At first, the sugars and organic acids were added to the AMS broth and the pH was adjusted to 7 with 1N NaOH or 1N HCl and then sterilized by autoclaving. Finally, the stock amino acids which were filter sterilized with 0.22 µ membrane filters were added to the sterilized media and then used for the experiment.

The growth (OD_{600}) of each strain in AMS + glucose and AMS + synthetic root exudate was measured spectrometrically in a microtiter plate as described earlier. A quantity of 10-ml of cultures grown in AMS + glucose and AMS + synthetic root

exudate at 30°C in an incubator shaker at 100 rpm for 5, 10 and 15 days interval were harvested by centrifugation (5000 g, 5 min at 24°C), washed twice with phosphate buffer to remove the salts present in the media and dried at 60°C for 2 days. The weight of the bacterial biomass was measured and expressed as mg/l. The biofilm forming capacity of the strains was assessed in 96-well titer plate (Pierce et al., 2008) and quantified the biofilm production by crystal violet staining as described by O'Toole (2011). The time course data (5, 10 and 15 days) on biofilm population (OD at 550 nm) and the ratio between biofilm and planktonic growth of the strains (OD_{550}/OD_{660}) were used to identify the potential biofilm-forming rhizobacterial strain. The extra polymeric substance (EPS) produced by the strains during the growth in AMS + glucose and in AMS + synthetic root exudate was quantified by adopting the method as described by Aguilera *et al.* (2008).

RESULTS AND DISCUSSION

All the twelve biofilm-forming rhizobacterial strains isolated from rice root can able to grow profusely in the AMS medium supplemented with the carbon substrates, as their sole carbon sources. When glucosamine, tritonX, oxalic acid and citric acid were used as sole carbon source, none of these strains showed growth in the AMS medium. The cumulative growth of the strains in all the carbon sources was used to screen the best strain (Figure 1) and among them, *Aeromonas hydrophila* QSRB-5 and *Enterobacter* sp. QS20-11 had the highest cumulative growth followed by *A. hydrophila* QS7-4, *Providentia rettigeri* QS24-2, *Klebsiella pneumonia* QS24-6 and *A. hydrophila* QSRB-2, while rest of the strains showed relatively low growth pattern and differential carbon substrate utilization with *A. enteropelongenes* QS20-8 having the least cumulative growth.

The MDS plot relating the rhizobacerial strains based on the similarity to utilize the carbon substrates showed 80% similarity among the strains. All the biofilm-forming strains evenly positioned in the MDS plot without any pattern and the 2D stress of 0.11 showed the reliability of the plot. Since the similarity in carbon-utilization is high (>80%), the best- and poor-performing strains could not be distinguished in the plot (Figure 2A). In hierarchical cluster analysis also the best-performing strain *A. hydrophila* QSRB-5 tightly clustered with poor-performing strains *viz., A. hydrophila* QS36-2, *A. caviae* QSRB-3, *A. enteropelongenes* QS20-8 and so on. The strains *K. pneumonia* QS24-6 and *Sphingomonas aquatilis* QS24-17; *P. rettigeri* QS24-2 and *A. hydrophila* QSRB-2; *A. hydrophila* QS36-2, *A. caviae* QSRB-3 and *A. hydrophila* QSRB-5 clustered with 90% similarity, while *Enterobacter* sp. QS20-11 and *Pseudomonas sihuiensis* QS24-20 had nearly 85% similarity (Figure 2B).

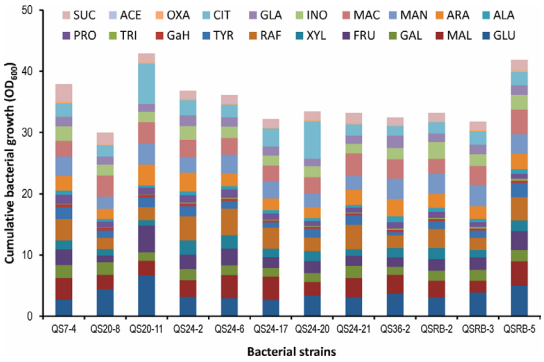

Figure 1. Cumulative growth pattern of biofilm-forming rhizobacteria of rice in different carbon substrates (500 mM) supplemented in ammonium mineral salt medium. The mean of three replicates were plotted. GLU - Glucose; MAL - Maltose; GAL - Galactose; FRU - Fructose; XUL - Xylulose; RAF - Raffinose; TYR - Tyrosine; GaH - Glucosamine; TRI - TritonX; PRO - Proline; ALA - Alanine; ARA - Arabinose; MAN - Mannitol; MAC - Malic acid; INO - Inositol; GLA - Glutamic acid; CIT - Citric acid; OXA - Oxalic acid; ACE - Acetic acid; SUC - Succinic acid. *Aeromonas hydrophila* (QS7-4; QS36-2; QSRB-2; QSRB-5), *A. enteropelongenes* QS20-8, *A. veronii* QS36-3, *A. caviae* QSRB-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.

All the six potential strains were assessed for their growth, biomass production and biofilm forming capability in the AMS medium supplemented with synthetic root exudate. The growth of these strains in synthetic root exudates was higher than glucose as standard (Figure 3). The strain *A. hydrophila* QS7-4 reached the maximum growth in terms of OD in both the media, however, in synthetic root exudate as carbon, it recorded a higher growth than glucose alone. The strains *K. pneumonia* QS24-6, *Enterobacter* sp. QS20-11 were also profusely grown in the synthetic root exudate medium. However, *A. hydrophila* QSRB-2 and *A. hydrophila* QSRB-5 could not grow in the synthetic root exudates, while progressive growth was noted in glucose as carbon source.

The dry biomass weight of the bacterial cells grown in respective both was recorded at 5, 10 and 15 days. The biomass yield of bacterial strains also significantly enhanced due to synthetic root exudates compared to glucose as carbon (Figure 4). Among the strains, *A. hydrophila* QS7-4, *K. pneumonia* QS24-6 and *Enterobacter* sp. QS20-11 had at par and higher biomass yield in AMS medium supplemented with synthetic root exudate.

P. rettigeri QS24-2, showed relatively lower yield than the above strains, while *A. hydrophila* QSRB-2 and *A. hydrophila* QSRB-5 did not produce any biomass throughout the incubation period.

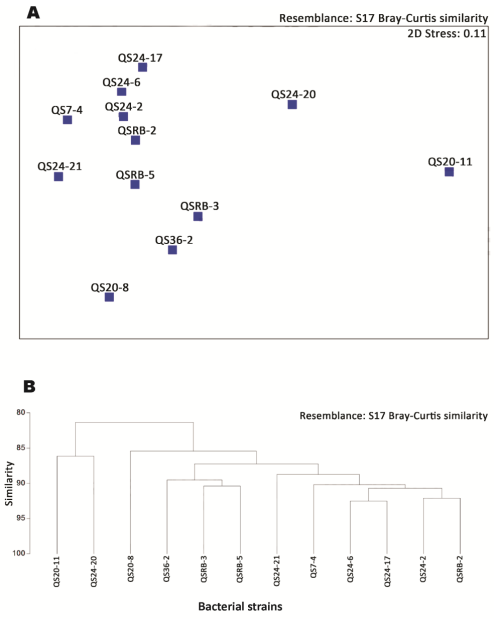

Figure 2. Non-metric MDS plot (A) and HCA dendrogram (B) constructed from differential carbon utilization data of biofilm-forming bacterial strains of rice. The similarity matrix using the Bray–Curtis coefficient was applied for both the analyses in Primer 7.0 software.

The ratio between the biofilm and planktonic population recorded in terms of A550/A660 reported the biofilm forming efficiency of these strains in the synthetic root exudate medium. There was a significant difference noticed in the biofilm forming capability of these strains between synthetic root exudate and glucose as carbon source (Figure 5). *A. hydrophila* QS7-4 had relatively high biofilm formation in the 5-days old culture in glucose, while in synthetic root exudate medium, it was 10-days old culture. Similarly, *K. pneumonia* QS24-6 also recorded the highest biofilm population on 5-days old culture in glucose medium and subsequently declined in the time course of incubation. The same strain in synthetic root exudate showed maximum biofilm population ratio in 15-days old culture.

The extra-polymeric substance production by the rhizobacterial strains as influenced by synthetic root exudate was measured in the time course of incubation. The results also indicated that the synthetic root exudate in AMS medium favoured extra polymeric substance production higher than glucose alone in most of the tested strains. A 2-fold increase of extra polymeric substance production was noticed in *K. pneumonia* QS24-6 and *A. hydrophila* QS7-4 grown in synthetic root exudate medium as compared to the same in glucose medium (Figure 6). Among the strains, *K. pneumonia* QS24-6 and *A. hydrophila* QS7-4 recorded maximum and at par EPS production followed by *Enterobacter* sp. QS20-11 and *P. rettigeri* QS24-2.

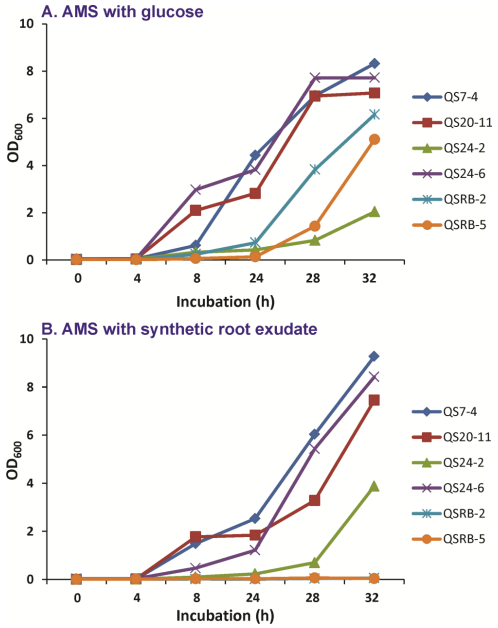

Figure 3. Growth pattern (OD600) of elite biofilm-forming bacterial strains of rice in AMS medium supplemented with glucose (500 mM) (A) and synthetic root exudate (500 mM) (B). Mean of three replicates were plotted and error bar indicates the standard error. *Aeromonas hydrophila* (QS7-4, QSRB-2; QSRB-5), *Enterobacter* sp. QS20-11, *Providentia rettigeri* QS24-2, *Klebsiella pneumoniae* QS24-6.

DISCUSSION

Considerable amounts of photosynthetically derived carbon are being released by the plant into the rhizosphere through root exudation. Thus, root exudates act as a key factor in increasing the microbial abundance and activity in the rhizosphere compared with bulk soil(Lynch and Whipps 1990). Sugars, organic acids and amino acids act as an energy source for the microorganisms, while the high molecular weight compounds such as flavonoids, siderophores and hormones regulate the microbial succession in the rhizosphere (Bais *et al.* 2006; Weisskopf *et al.* 2008). When the plant growth promoting rhizobacteria being introduced to the crop plants, they have to profusely colonize the root through their competitive ability in order to excel their benefit to the crop plants. However, several PGPR strains are being isolated, screened and assessed for their PGP activities often failed to perform its role while inoculating to the crop plants due to poor colonizing ability. Hence, along with all

the beneficial traits, the competitive colonizing ability of the inoculant has to be standardized for its persistence. Even though theoretically several traits have been so far identified for the inoculants persistence, there is no thorough investigation made so far. In the present work, we proved that the PGPR strains isolated and characterized from the rice root needs microcosm screening assay in order to assess the competitive colonizing ability of the strains.

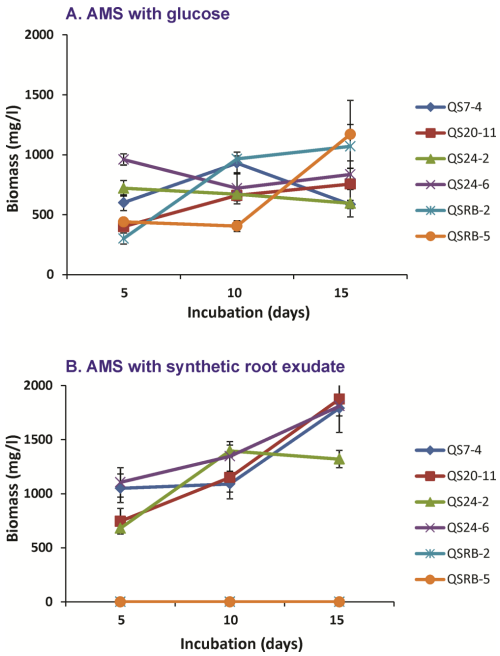

Figure 4. Biomass production of elite biofilm-forming bacterial strains of rice in AMS medium supplemented with glucose (500 mM) (A) and synthetic root exudate (500 mM) (B). Mean of three replicates were plotted and error bar indicates the standard error. *Aeromonas hydrophila* (QS7-4, QSRB-2; QSRB-5), *Enterobacter* sp. QS20-11, *Providentia rettigeri* QS24-2, *Klebsiella pneumoniae* QS24-6.

Shi *et al.* (2011) reported the model exudate solution (organic acids and sugars) stimulated the soil dehydrogenase activity and structure of active bacterial communities on an addition to the rhizosphere of *Pinus radiata*. Addition of organic acids significantly altered the richness and diversity of functional bacterial communities of soil. Likewise, Henry *et al.* (2008) also revealed that these synthetic root exudates could not stimulate the activities of plant-independent bacteria, such as nitrate reducers and denitrifiers. Thus, it is evident that the chemical compositions of root exudates are specific to the plant-associated bacterial taxa only. Campbell *et al.* (1997) used culture-independent Biolog™ plates containing different carbon sources assumed to be present in the rhizosphere to discriminate the microbial communities. The rhizobacteria should able to utilize the rhizo deposits, effectively colonize root or rhizosphere soil surface and be

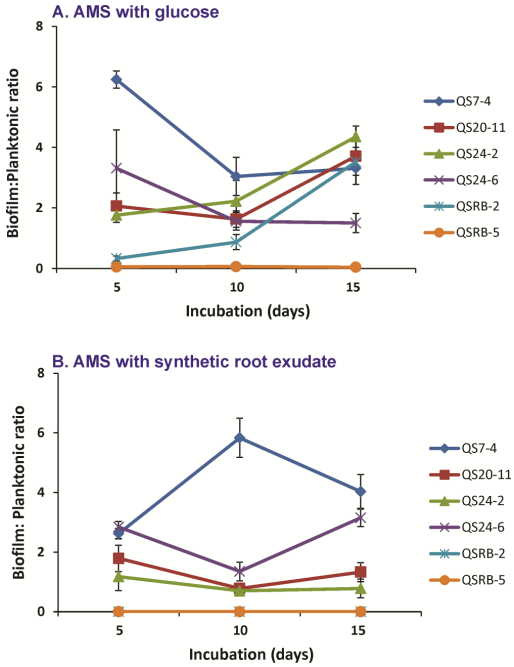

Figure 5. Biofilm:Planktonic ratio of elite biofilm-forming bacterial strains of rice in AMS medium supplemented with glucose (500 mM) (A) and synthetic root exudate (500 mM) (B). Mean of three replicates were plotted and error bar indicates the standard error. *Aeromonas hydrophila* (QS7-4, QSRB-2; QSRB-5), *Enterobacter* sp. QS20-11, *Providentia rettigeri* QS24-2, *Klebsiella pneumoniae* QS24-6.

able to compete with other organisms (Dennis *et al.* 2010). In the present investigation, we

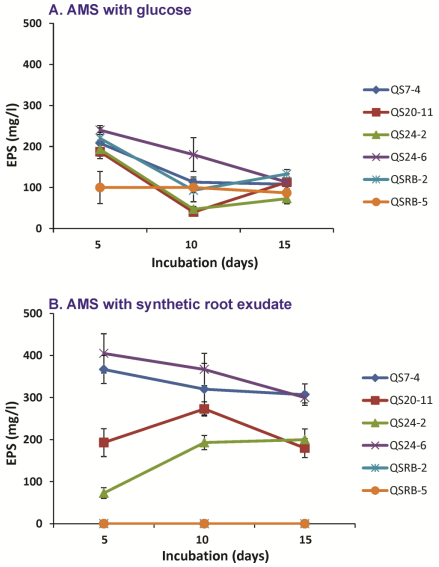

Figure 6. Extra-polymeric substances (EPS) production of elite biofilm-forming bacterial strains of rice in AMS medium supplemented with glucose (500 mM) (A) and synthetic root exudate (500 mM) (B). Mean of three replicates were plotted and error bar indicates the standard error. *Aeromonas hydrophila* (QS7-4, QSRB-2; QSRB-5), Enterobacter sp. QS20-11, Providentia rettigeri QS24-2, *Klebsiella pneumoniae* QS24-6.

outlined the procedure to be followed to assess the rhizobacterial trait for effective colonization in the crop's rhizosphere. We have developed a synthetic root exudate that more or less mimics most of the plants' rhizodeposits and screened the PGPR strains for the growth and biofilm formation. We tested twelve strains of PGPR isolated from rice root for their ability to grow in the carbon substrates that are released by the plants. Further, the synthetic root exudates allowed to discriminate the PGPR strains in terms of growth, biofilm formation and EPS production. Among the twelve strains, three PGPR viz., *Aeromonas hydrophila* QS7-4, *Enterobacter* sp. QS20-11 and *Klebsiella pneumoniae* QS24-6 effectively utilized and grown in the synthetic root exudates and produced biofilm and extra polymeric substances. Hence, it is suggested that initial screening of the PGPR strains for differential carbon substrate utilization profile and growth, biofilm and EPS production in synthetic root exudate solution are essentially needed for the inoculants. These screening procedures can improve the inoculant development by identifying the highly-competitive and rhizosphere colonizing strains.

REFERENCES

Aguilera, A., Souza-Egipsy, V., San Martín-Úriz, P. and Amils, R. 2008. Extraction of extracellular polymeric substances from extreme acidic microbial biofilms. *Appl Microbiol Biotechnol*. **78**:1079-1088.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S. and Vivanco, J. M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol*. **57**:233-266.

Baudoin, E., Benizri, E. and Guckert, A. 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biol Biochem*. **35**:1183-1192.

Begum, J. F., Tamilarasi, M., Pushpakanth, P. and Balachandar, D. 2019. A simple method for direct isolation of N-acyl-L-homoserine lactone mediated biofilm-forming rhizobacteria from roots. *J Microbiol Methods*. **156**:34-39.

Berendsen, R. L., Pieterse, C. M. and Bakker, P. A. 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci*. **17**:478-486.

Campbell, C., Grayston, S. and Hirst, D. 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. *J Microbiol Methods*. **30**:33-41.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol*. **18**:117-143.

Dennis, P. G., Miller, A. J. and Hirsch, P. R. 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiol Ecol*. **72**:313-327.

Gunina, A. and Kuzyakov, Y. 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. *Soil Biol Biochem*. **90**:87-100.

Hayat, S., Faraz, A. and Faizan, M. 2017. Root exudates: Composition and impact on plant–microbe interaction. In: Ahmad I, Husain FM (eds) Biofilms in Plant and Soil Health. Wiley-Blackwell, NJ, USA, pp 179-194.

Henry, S., Texier, S., Hallet, S., Bru, D., Dambreville, C., Cheneby, D., Bizouard, F., Germon, J. and Philippot, L. 2008. Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates. *Environ Microbiol*. **10**:3082-3092.

Kristin, A. and Miranda, H. 2013. The root microbiota— a fingerprint in the soil? *Plant Soil*. **370**:671-686.

Kuzyakov, Y. and Blagodatskaya, E. 2015. Microbial hotspots and hot moments in soil: concept & review. *Soil Biol Biochem*. **83**:184-199.

Lugtenberg, B. J., Dekkers, L. and Bloemberg, G. V. 2001. Molecular determinants of rhizosphere colonization by Pseudomonas. *Annu Rev Phytopathol*. **39**:461-490.

Lynch, J. M. and Whipps, J. M. 1990. Substrate flow in the rhizosphere. *Plant Soil*. **129**:1-10.

Naher, L., Yusuf, U. K., Ismail, A. and Hossain, K. 2014. Trichoderma spp.: a biocontrol agent for sustainable management of plant diseases. *Pak J Bot*. **46**:1489-1493.

Paterson, E., Gebbing, T., Abel, C., Sim, A. and Telfer, G. 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytol*. **173**:600-610.

Shi, S., Richardson, A. E., O'Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., Firestone, M. K. and Condron, L. M. 2011. Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiol Ecol*. **77**:600-610.

Velmourougane, K., Prasanna, R., Singh, S. B., Kumar, R. and Saha, S. 2017. Sequence of inoculation influences the nature of extracellular polymeric substances and biofilm formation in Azotobacter chroococcum and Trichoderma viride. *FEMS Microbiol Ecol*. **93**.

Weisskopf, L., Le Bayon, R.-C., Kohler, F., Page, V., Jossi, M., Gobat, J.-M., Martinoia, E. and Aragno, M. 2008. Spatio-temporal dynamics of bacterial communities associated with two plant species differing in organic acid secretion: a one-year microcosm study on lupin and wheat. *Soil Biol Biochem*. **40**:1772-1780.