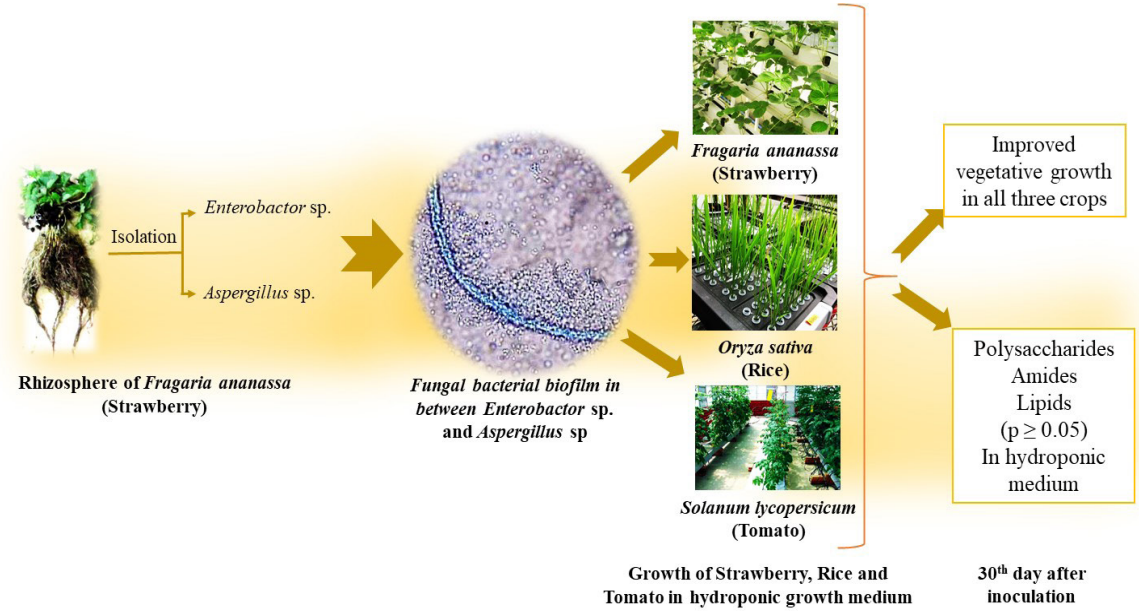
RESEARCH ARTICLE

Intimate interactions of *Enterobacter*, *Aspergillus* and *Enterobacter-Aspergillus* biofilm with strawberry, tomato and rice: early plant growth under glass house conditions

I.D. Singhalage, G. Seneviratne and H.M.S.P. Madawala



Highlights

- Strawberry rhizosphere associated *Enterobacter* and *Aspergillus* spp. were used to formulate a fungal-bacterial biofilm (FBB).
- Monocultures and FBB interact with the roots of strawberry, rice and tomato in hydroponic growth conditions.
- The FBB improved the vegetative growth of all three tested crops.

Ceylon Journal of Science 51(4) 2022: 379-387
DOI: http://doi.org/10.4038/cjs.v51i4.8055

RESEARCH ARTICLE

Intimate interactions of *Enterobacter*, *Aspergillus* and *Enterobacter-Aspergillus* biofilm with strawberry, tomato and rice: early plant growth under glass house conditions

I.D. Singhalage[1,3,*], G. Seneviratne[1] and H.M.S.P. Madawala[2]

[1]Microbial Biotechnology Unit, National Institute of Fundamental Studies, Kandy, Sri Lanka
[2]Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka
[3]Department of Science and Technology, Faculty of Applied Sciences, Uva Wellassa University of Sri Lanka, Badulla,Sri Lanka

Received: 05/07/2021; Accepted:08/11/2022

Abstract: The host specificity of biofertilizers poses many challenges at large scale production and usage. The present study investigated the versatility of a fungal-bacterial biofilmed biofertilizer (FBB) in comparison to its monoculture bacterial (BB) and fungal (FB) biofertilizers using three crops. The FBB was formulated using selected strawberry (*Fragaria ananassa*) rhizosphere-associated bacterial (*Enterobactor* sp.) and fungal (*Aspergillus* sp.) strains. Strawberry, rice and tomato were grown in hydroponics under similar conditions and the liquid biofertilizers (FBB, BB and FB) were applied at a ratio of biofertilizer: hydroponic medium in 1:90 (in volume). A control was maintained without any biofertilizers. The treatments were triplicated and arranged according to completely randomized design. The growth medium collected at different time intervals were analyzed for polysaccharides, amides and lipids by Fourier Transform Infra-red (FTIR) spectroscopy. Plant height and dry mass were recorded after a month. Data were analyzed by ANOVA and correlation. After 30 days, root-FBB interactions of all three crops observed significantly improved polysaccharides, amides and fatty acids concentrations in the hydroponic medium over the control. Total plant biomass and height of strawberry and rice were significantly ($p < 0.05$) increased with FBB over its monoculture biofilms (BB, FB) and the control. In tomato, FBB-treated seedlings showed an improved growth over their counterparts treated with monoculture biofertilizers, though the differences were not significant ($p > 0.05$). The growth of strawberry and rice showed a significant ($p < 0.05$) positive correlation with functional polysaccharides and amides, while tomato showed a similar correlation with all three functional biomolecules tested. The findings clearly indicate that the FBB developed from strawberry root-associated microbes nullify the crop specificity, and show the potential to improve growth of rice and tomato. Therefore, mixed-culture biofertilizers seem to possess a universal potential in improving crop growth in comparison to conventional monoculture biofertilizers. Further studies with other crops are imperative to confirm their potential universality.

Keywords: Biofertilizers, Host specificity, Biofilmed biofertilizers, Universality.

INTRODUCTION

Beneficial microbes and their biofilms have been used as biofertilizers in many crops. Biofertilizers are a promising alternative for chemical fertilizers in sustainable agriculture. Living microorganisms in biofertilizers bring beneficial effects on plant growth and productivity directly and/or indirectly (Saharan and Nehra, 2011). Plant Growth Promoting Rhizobacteria (PGPR) show specific interactions with their host plants, eventually leading to their better growth. Legume root - *Rhizobium* interaction is one of the well-known symbiotic interactions that show high host specificity (Bhuvaneswari *et al*., 1981). Relationship between cereal crops and *Azospirillum* is another example for crop specificity (Jagnow, 1987). Thus, specific microbial formulations need to be developed and used for different crops, posing a limitation of using biofertilizers as an alternative to chemical fertilizers. Due to this specificity, biofertilizer development and production is not always cost-effective and create many problems at application. Thus, development of non-host specific biofertilizers is the need of the hour.

The multi functionality of fungal-bacterial Biofilmed Biofertilizers (BFBFs) as plant growth promoters has been highlighted previously (Seneviratne *et al*., 2009). Microbes in BFBFs undergo profound changes in their genetic makeup, eventually leading to produce diverse metabolic compounds and activities over their planktonic mode of lives (Herath *et al*., 2013; Seneviratne *et al*., 2008). For example, a free living biofilm developed using *Bradyrhizobium japonicum* SEMIA 5019 and *Penicillium* spp. has been reported to improve soil fertility by increasing N and P mineralization and nitrogenase activity in the absence of a host plant (Seneviratne and Jayasinghearachchi, 2005). These non-host specific beneficial biofilms can play a crucial role in agriculture, because they may be effective without crop specificity, thus showing universality as biofertilizers in many crop varieties. Further, a huge knowledge gap remains that needs to be addressed to facilitate the commercialization of PGPRs (Azizoglu *et al*., 2021) and other microbial inoculants for sustainable

**Corresponding Author's Email:* darshani@uwu.ac.lk
https://orcid.org/0000-0003-0249-8634

soilless agriculture. As such, present study focused on evaluating the potential of previously developed BFBF for strawberry (Singhalage *et al.,* 2019) in other crops; rice and tomato, under hydroponic culture conditions.

MATERIALS AND METHODS

Microbial cultures

A fungal (*Aspergillus* sp.) and a bacterial (*Enterobacter* sp.) strain isolated from the rhizosphere of strawberry (*Fragaria x ananassa*) were used as starting cultures in the preparation of BFBF. The three types of biofilms, fungal (FB, *Aspergillus* sp.), bacterial (BB, *Enterobacter* sp.) and fungal-bacterial (FBB, biofilm between *Aspergillus* sp. and *Enterobacter* sp.) were developed in Biofilm Formation Medium (BFM) according to the protocol developed by National Institute of Fundamental Studies (NIFS), Sri Lanka. The exact composition of the BFM is not described here due to Intellectual Property Rights.

Hydroponic experiment

The Hoagland solution (Hoagland and Arnon, 1950) was used as the hydroponic culture medium. Strawberry runners (G1 generation), obtained from the Agricultural Research Station Seetha Eliya, were surface-sterilized by dipping in a 3% commercial chlorox solution for 3 minutes, followed by several washings in distilled water. The runner per tube then planted in plastic tubes (transparent and 50 mL volume) containing 45.0 mL of sterile culture solution with the help of a sterile mesh. After one week from planting, 500 µL of FBB, FB, and BB biofilm cultures were applied as treatments. As the control, 500 µL of sterile distilled water was used.

Rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*) seeds were surface sterilized by soaking in a commercial chlorox solution (3% w/v) for 5 minutes and then the seeds were washed thoroughly and drained several times using sterilized distilled water (Abdul-Baki, 1974). Seeds were then soaked overnight in sterilized distilled water. Rice seeds were first sown in wet paper towel in a sterile petri dish for germination and after four days, seedlings having the same length were transplanted to plastic tube (transparent and 50 mL volume) containing 45.0 mL of sterile hydroponic culture medium with the help of polyethylene plugs. One rice seedling was planted per tube. Surface sterilized tomato seeds (3-5) were sown in a plastic tube containing sterile hydroponic culture medium (45.0 mL) with the help of a sterile mesh. After five days, one healthy seedling was retained. The experimental designs including the treatments applied for rice and tomato were similar to that for strawberry.

After planting, the culture tubes were wrapped in black paper to prevent sunlight penetration. The tubes were then incubated under glass house conditions (temperature, 25 ^{o}C, and relative humidity, 67%) for a month. The level of hydroponic culture medium in the tubes was maintained throughout the experiment by adding sterile distilled water whenever necessary. Complete Randomized Design (CRD) was followed as the experimental design. Three replicates were maintained for each treatment. Plant heights were recorded at the end of the experimental period. Plants were then destructively harvested and oven-dried (60^{o}C) for a constant weight before taking the dry weights of shoot and root samples.

Microscopic features of root-microbial interactions

At the end of the experiment, pieces of root samples (having similar weight) of strawberry, rice and tomato were collected from each tube. They were mounted in lacto-phenol cotton blue stain and observed under the light microscope.

Functionality of root-microbial interaction

Sub samples (5.0 mL) of the hydroponic medium were collected from each tube at 1, 7, 14 and 30 days after the inoculation of media. The samples were centrifuged (at 6,000 rpm for 20 min; SANYO Harrier 18/80) and supernatant was filtered by syringe filtration (polysulfone, 0.45 µm filter). The filtrate (1.0 mL) was concentrated by vacuum drying (Eyela, VOS-4500) at 50 ^{o}C until it reached a final volume of ~50 µL and analyzed by FTIR spectroscopy (FTIR, Thermo Nicolet, USA). The absorbance spectra were recorded within the range of 650- 4,000 cm^{-1} having a resolution of 4 cm^{-1}. Each spectrum was produced by 256 scans. Spectra were collected and analyzed by OMINIC® software.

Data analysis

The baseline of each FTIR spectrum was corrected by using automatic baseline correction function. Peak absorbance of functional groups was observed in amide window (1,500 - 1,800 cm^{-1}), fatty acid windows (1,400 - 1,500 cm^{-1} and 2,800 - 3,000 cm^{-1}) and polysaccharide window (900 - 1,200 cm^{-1}). Differences of peak absorbance in different types of root-microbial interactions under each window were analyzed by ANOVA. Differences in plant height and dry biomasses were also analyzed by ANOVA. Treatment means under each parameter were separated by Tukey's simultaneous mean separation test. Correlation analysis was run to observe any relationships between biomass and functional polysaccharides, functional fatty acids and functional amides under each sampling time. Pearson correlation analysis was used to find out linear relations. Statistical analyses were performed using Minitab statistical package (Minitab® 16.2.1, 2010).

RESULTS AND DISCUSSION

Vegetative growth

Strawberry plant dry mass significantly ($p < 0.05$) increased with FBB compared to the other treatments and the control (Figure 1a). The plant height of strawberry supplied with FBB was also significantly ($p < 0.05$) increased over the control (Figure 1b). Rice seedlings when interacted with FBB (i.e. rice-FBB interaction) showed a significantly ($p < 0.05$) higher dry mass and height, over the other two types of interactions (root -BB and root-FB) and the control (Figure 1c and 1d). The dry mass and height of tomato grown in

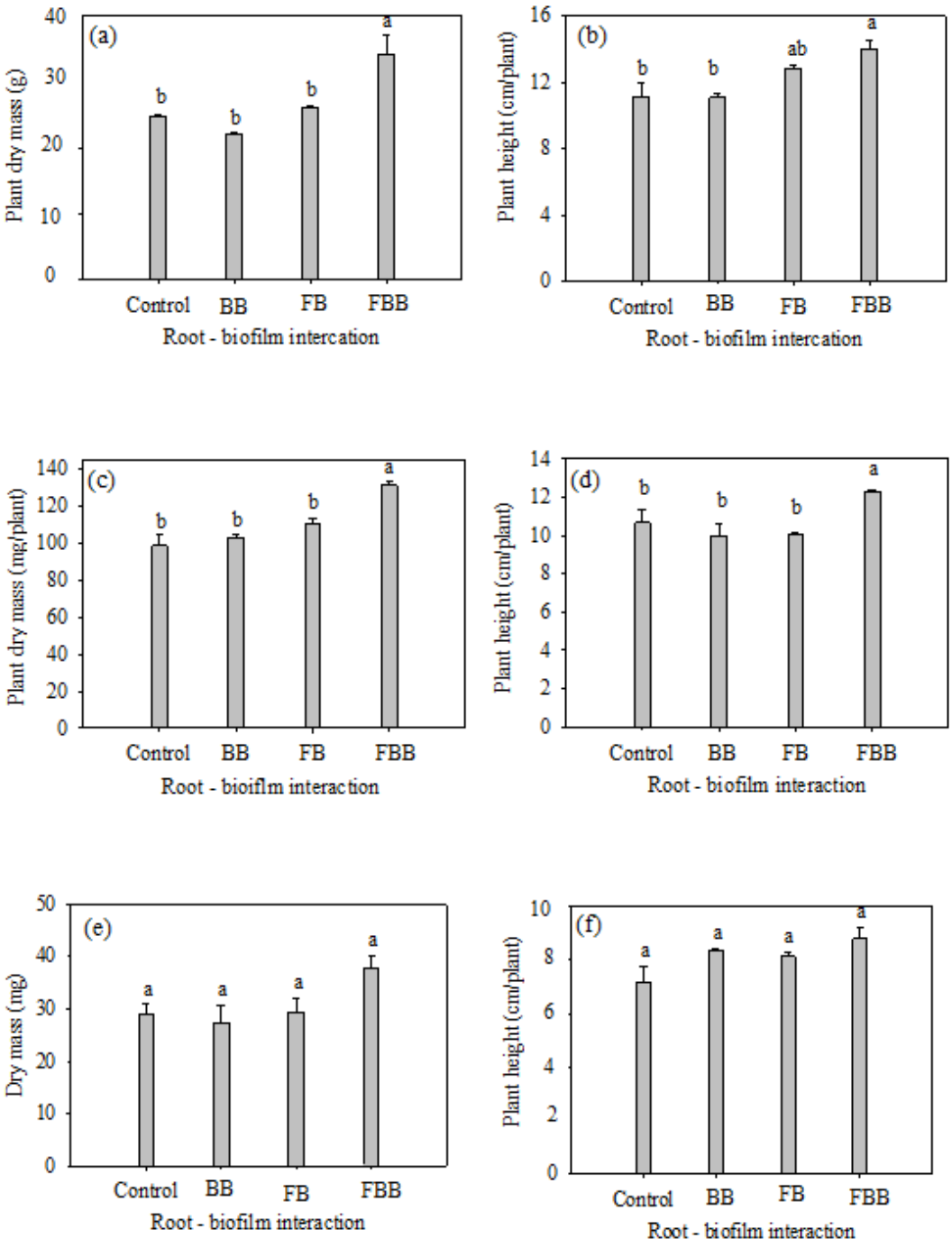


Figure 1: Dry mass and height of strawberry (a and b), rice (c and d) and tomato (e and f) plants at harvest. BB- bacterial biofilm, FB-fungal biofilm, FBB-fungal-bacterial biofilm. Treatments of the columns headed by the same letter are not significantly different at 5% probability level.

hydroponic culture were not significantly different among the treatments (Figures 1e and 1f). The monoculture based biofilms, FB and BB, were not showed significant effect on growth performances of the all three crops tested.

Previous studies confirmed that the use of monoculture-based biofertilizers in hydroponic growth medium with reduced dosage of chemical fertilizers demonstrated higher yields and vegetative growth in squash, strawberries and banana (Dagsan *et al.,* 2012; Rueda *et al*., 2016; Mia *et al*., 2010). Kalniņš *et al.* (2022) showed the bioaugmentation of bacterial consortia provided significant stimulation for the growth of *Mentha aquatica* var. *aquatica* and *Mentha aquatica* var. *litoralis* after 47 days of growth. However, the use of fungal-bacterial BFBFs in hydroponic growth medium and its potential as a biofertilizer has not been reported in the literature. The use of fungal-bacterial BFBF to improve the growth and yield of many crops such as strawberry (Singhalage *et al*., 2019), tea (Seneviratne *et al*., 2011; De Silva *et al*., 2014), rubber (Hettiarachchi *et al*., 2014), maize (Babu *et al*., 2017) and lettuce seedlings (Singhalage *et al*., 2020) have been reported previously. Present study clearly showed the potential of BFBF developed specifically for strawberry in promoting growth of strawberry and rice in hydroponic culture.

The macromolecular compounds secreted by the root system and microbes into the growth medium alter plant growth. In the present study, strawberry plant growth showed significant ($p < 0.05$) positive correlations with polysaccharides and amides (Table 1). The growth of tomato too showed significant ($p < 0.05$) positive correlations with all functional biomolecules, while rice showed significant ($p < 0.05$) positive correlations with fatty acids and amides. The polysaccharide level and dry mass of rice are inversely related. According to the literature, it has been proven that the root exudation is altered by the root associated microbiota. Chaparro *et al*. (2013) has showed strong correlations ($p<0.05$) between microbial functional genes involved in the metabolism of carbohydrates, amino acids and secondary metabolites with the corresponding compounds released by the roots at particular stages of plant development. The rhizosphere microbial community structure was also able to change the systemic root metabolomes and transcriptomes (Korenblum *et al*., 2020). On the other hand, the exudate flux of roots and its C:N:P stoichiometry reflected the plant growth rate and nutrient constraints and it mediated the plant-microbial interactions in the rhizosphere (Cardenas *et al*., 2021). Thus, the interaction between roots and microbiota play an important role to improve the nutrient availability of microenvironment around the root surface. Similarly, a positive correlation in between the macromolecular composition and the plant growth was observed in the present study.

The importance of the macromolecules during the plant growth has been shown in the literature. A noteworthy growth increment in lettuce seedlings was observed when the seeds were treated with FBB exudates containing the macromolecules such as carbohydrates, proteins and lipids (Singhalage *et al*., 2020). Polysaccharides extracted from *Spirulina platensis* increased the height of tomato and pepper plants by 20% and 30%, respectively (Elarroussi *et al*., 2016). Inoculation of wheat seedlings with exopolysaccharide-producing rhizospheric bacteria from wheat roots stimulated the growth of wheat under salinity (Ashraf *et al*., 2004). Aliphatic polyamine, spermidine that was produced by *Bacillus subtilis* OKB105 played a crucial role in plant growth (Xie *et al*., 2014). Fatty acid containing gallic (Negi *et al*., 2005) and jasmonic (Vick and Zimmerman, 1984; Farmer, 1994) acids were also reported as plant growth stimulators.

The results indicated that the vigor of strawberry and rice was improved when FBB interacted with roots. Therefore, to check the specificity of functional molecules of root-microbial interaction, a cluster analysis was performed. According to the cluster analysis, the separate clustering of the functional polysaccharides, fatty acids and amides were observed for Root-BB, Root-FB and Root-FBB interactions (Figure 2). Functional molecules of Root-FBB and Root-FB were clustered closely by showing the similar features of such exudates (Figure 2a, b and c). Functional molecules of Root-BB and the control also clustered close to each other (Figures 2a, b and c). The growth performances of the tested crops showed some similarities to the functional molecule production. That is the dry mass of strawberry, rice and tomato is highest with root-FBB interactions where it was second in root -FB interaction (Figures 1a, c, e). Root-BB interactions showed the less growth performances in comparison to the Root-FBB and Root-FB interactions.

The nutrient composition of the hydroponic culture medium with root-FBB interactions was generally higher than the control and other bipartite interactions. Thus, in the test plants (rice and strawberry), the growth was improved with the application of FBB. It has been shown that the FBBs or BFBFs developed using nitrogen fixers enhanced the plant growth of legumes as well as non-legumes (Herath *et al*., 2015). Thus, crop specificity of conventional biofertilizers, one of the constraints in popularizing biofertilizers, can be overcome by using the FBB-based biofertilizers.

Morphology of bacterial, fungal and fungal-bacterial biofilms on strawberry, rice and tomato roots in hydroponic growing media

Morphology of BB, FB and FBB attachments in strawberry, rice and tomato roots is shown in Figure 3. All biofilms showed physical interactions with respective roots. The root surfaces acted as a substratum during the biofilm attachment. The button-shaped bacterial biofilms were seen firmly attached to root surfaces. Fungal filaments were loosely colonized on roots of all the crops. FBB showed higher colonization potential over the mono-culture biofilms.

Table 1: Pearson correlation coefficients (r) between dry mass of strawberry, rice and tomato seedlings and functional parameters of the hydroponic experiment. Values within parentheses show probability levels.

Functional Macromolecules	Crop		
	Strawberry	Rice	Tomato
Polysaccharides	0.703	-0.052	0.732
	$(0.01)^{*}$	$(0.87)^{ns}$	$(0.00)^{*}$
Fatty acids	0.459	0.720	0.595
	$(0.13)^{ns}$	$(0.00)^{*}$	$(0.04)^{*}$
Amides	0.606	0.662	0.729
	$(0.03)^{*}$	$(0.01)^{*}$	$(0.00)^{*}$

*, significant at 5% probability level.
ns, not significant at 5% probability level.

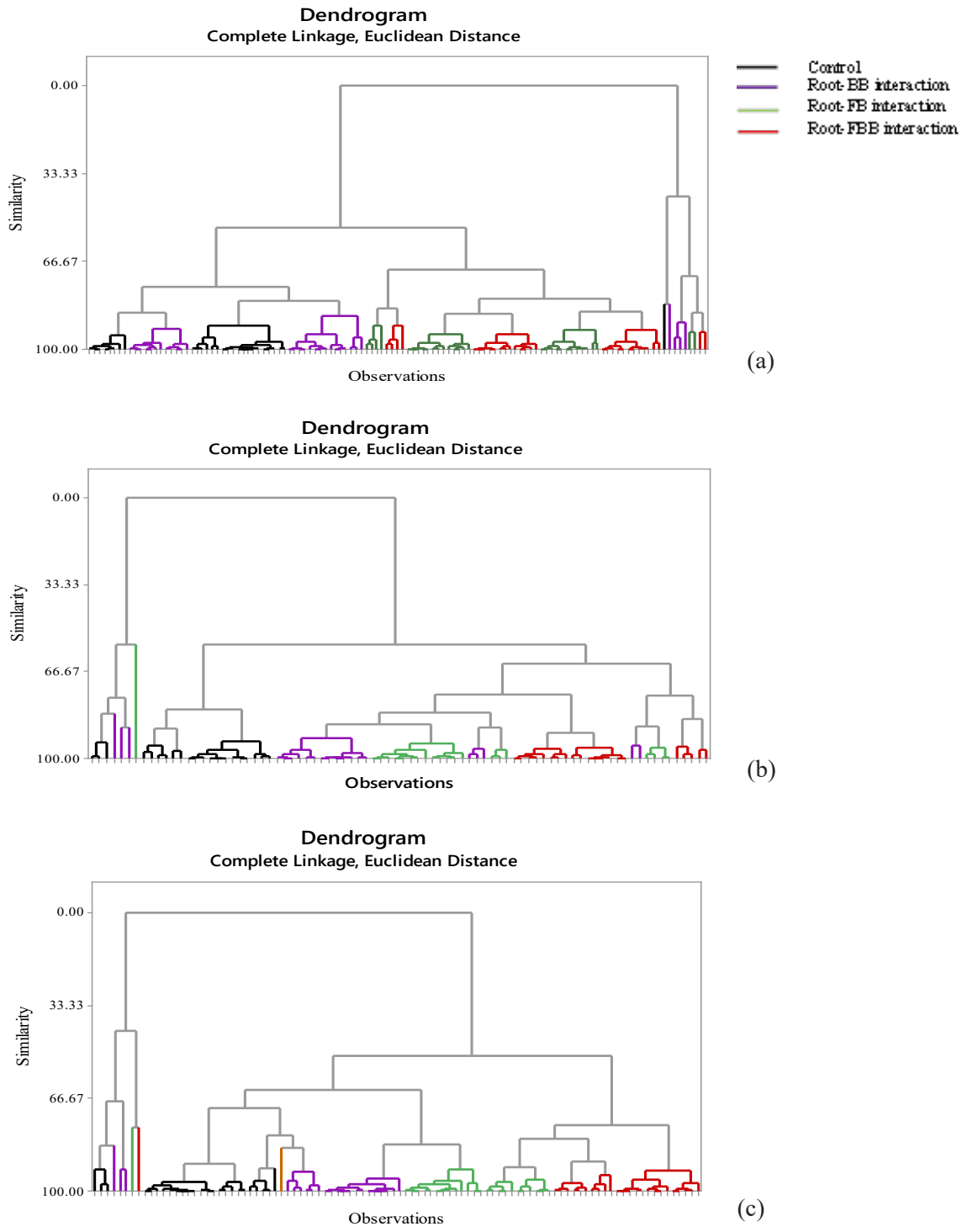


Figure 2: Dendrograms showing hierarchical cluster analysis of FTIR spectral functional groups of, (a) amide (1500 - 1800 cm-1), (b) polysaccharide (900 - 1200 cm-1), and (c) fatty acid (2800 - 3000 cm-1 and 1400 - 1500 cm-1) exudates of root - bacterial biofilm, root- fungal biofilm and root - fungal - bacterial biofilms.

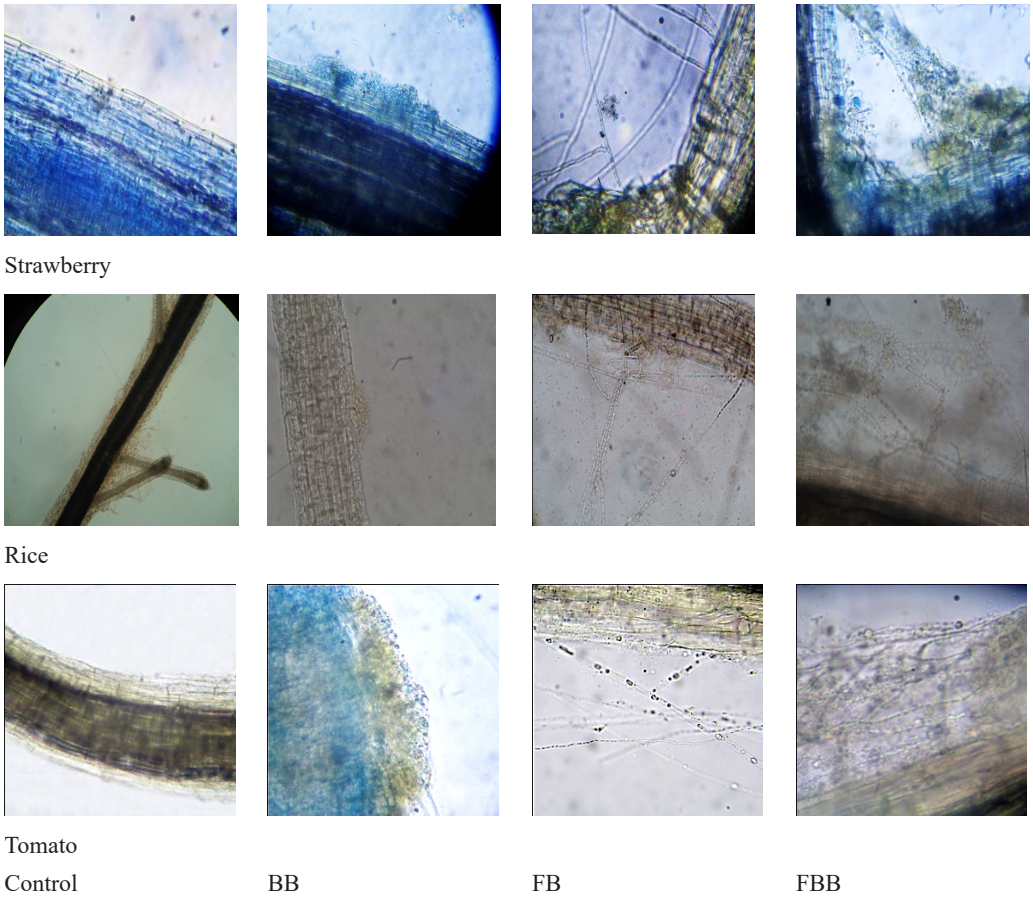


Figure 3: Morphology of attachment of bacterial biofilm (BB), fungal biofilm (FB) and fungal-bacterial biofilm (FBB) to strawberry, rice and tomato roots.

The attachment of biofilms with a particular surface depends on various properties such as surface roughness, physicochemical properties of the surface, hydrodynamics of the aqueous medium, characteristics of the medium and various properties of the cell surface (Donlan and Costerton, 2002). The solid-liquid interface between the surface (root) and the aqueous medium (hydroponic medium) provides an ideal environment for the attachment and growth of microorganisms. In the present study, attachment and detachment of microbial biofilms were observed in all culture tubes. Hence, the study organisms seem to produce attachments on strawberry, rice and tomato roots as biofilms. This attachment and detachment primarily depend on the surface charge of the biotic or abiotic substratum and microbial cells, as reported by Lower *et al*. (2000) who demonstrated attractive and repulsive forces between mineral surfaces and *Escherichia coli* biofilms at a range up to 400 nm separation. On the other hand, adhesions, lectins or lectin like proteins or carbohydrates produced by microbes also play a role in specific interactions (Ploux *et al*., 2010). Plant metabolites also elicit mechanisms for microbial attachments (Rudrappa *et al*., 2008).

Functional properties of biofilms

Plants release organic compounds such as amino acids, organic acids, sugars, phenolics, polysaccharides, fatty acids, sterols, enzymes, proteins, plant growth regulators and an array of secondary metabolites to the surrounding environment as root exudates (Badri *et al*., 2009). These compounds can act as chemotactic attractants, hence facilitate in the biofilm formation on roots (Downie, 2010). Root-biofilm interactions may alter root exudations and biosynthesis of biofilms, thus releasing new array of biomolecules to the immediate surroundings. Figure 4 shows the functional molecules such as polysaccharides, amides and fatty acids produced by root-microbial interactions under different treatments. Tomatoes produced comparatively higher levels of functional polysaccharides, amides and fatty acids over the other crops. Tomato-FBB interaction produced higher levels of functional biomolecules than the tomato-BB and tomato-FB interaction but the tomato-FBB interaction is not significant at 5% probability level. The least amounts of functional molecules were produced by strawberry root - biofilm interactions.

The tested three plant species showed the releasing of

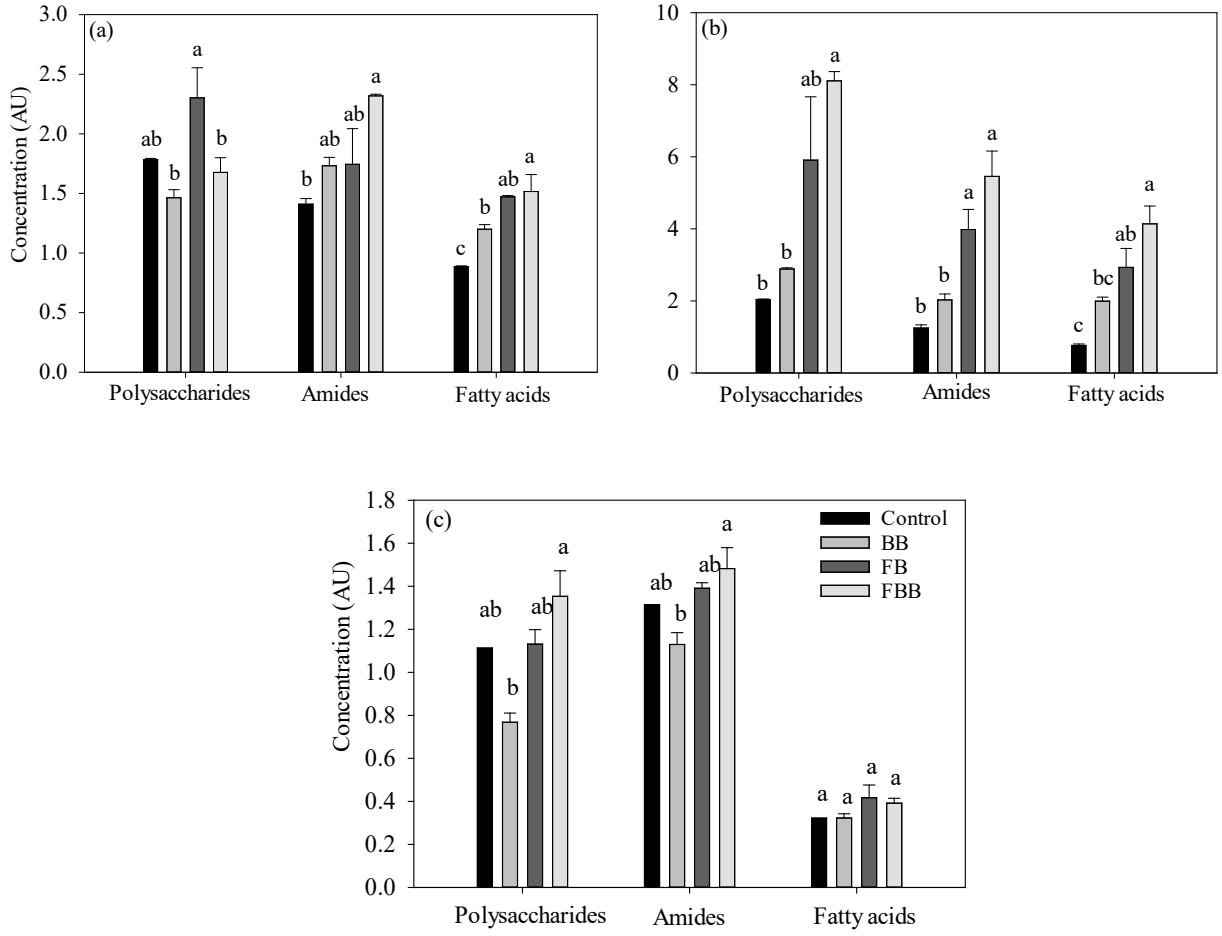


Figure 4: Concentrations of functional polysaccharides, amides and fatty acids of root-biofilm interactions, in rice (a), tomato (b) and strawberry (c). Different letters on each column indicate the significant differences ($p < 0.05$) among treatments at the same harvest. BB- bacterial biofilm, FB- fungal biofilm, FBB- fungal-bacterial biofilm.

higher amounts of functional polysaccharides and amides with the FBB interaction over the FB and BB interactions. Singhalage et al. (2018) also showed the higher efficacy of fungal-bacterial interaction for the synthesis of high level of biomolecules and it is dependent on the type of microbes in the interaction. The adhesion of biofilm to the root surface may alter the root exudation. On the other hand the same interaction could alter the functional properties of biofilm. The variations of the expression of functional molecules of tested plants could differ as they are different species. Previous studies have also attempted to explain the functional properties of biofilms. For example, fungal-bacterial interaction between *Penicillium* and *Bradyrhizobium elkanii* produced detectable quantities of monosaccharides such as fructose, ribose, arabinose and xylose (Zavahir and Seneviratne, 2007). However, the compounds were not detected in monocultures of *Penicillium* and *Bradyrhizobium elkanii*, indicating enhanced metabolic activities in mixed-cultures.

The synthesis of fatty acids-like compounds by plant roots have not been recorded in the literature. However, fungi that belong to the class Zygomycetes and certain microalgae are known to produce fatty acids (Certik and Shimizu, 1999). The improved fatty acid levels in the present study might be beneficial for plants in terms of regulation of biotic and abiotic stress mechanisms (Upchurch, 2008). Further, fatty acids secreted by microorganisms act as biosurfactants for many macromolecules, which facilitate the uptake of nutrients (Mapelli *et al*., 2008). The major role of functional amides in rhizosphere environment is their involvement in quorum sensing between rhizospheric microbes and roots (Williams, 2007). *In addition, the physical interaction between Medicago sativa* and *Sinorhizobium meliloti* Rm1021 caused a clear increase (1.5 fold increase) in the secretion of plant proteins, such as hydrolases, peptidases and peroxidases (De-la-Peña *et al*., 2008).. However, these proteins were not induced when *M. sativa* was inoculated with *Pseudomonas syringae* (De-la-Peña *et al*., 2008). Thus, biosynthesis of metabolites of root-microbial interactions depends on both plant and microbial species that are involved in the interaction.

CONCLUSION

The results confirmed that the FBB formulated using rhizospheric microorganisms (*Enterobacter* sp. and *Aspergillus* sp.) of strawberry increased the early vegetative growth of rice and strawberry in hydroponics notably. Furthermore, intimate physical interactions of studied biofilms induced the synthesis of functional polysaccharides, fatty acids and amides. Such induction

was high when FBB interacted with the roots of strawberry, tomato and rice. The BFBFs used in this study could be an interesting development for future research and commercialization efforts of BFBFs. Further research is imperative to cover other crops as well as to evaluate their yield responses under field conditions, in particular.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that the paper does not contain any conflict of interest.

ACKNOWLEDGEMENT

We acknowledge Mr. Anura Pathirana and Ms. Kumuduni Karunaratne, Chief Technical Officers of the National Institute of Fundamental Studies for their technical assistance. The study was partially funded by University Grants Commission of Sri Lanka (UGC/ICD/RG/02/2012/10).

REFERENCES

Abdul-Baki, A.A. (1974). Pitfalls in using sodium hypochlorite as a seed disinfectant in ^{14}C incorporation studies. *Plant Physiology* **53**: 768-771. doi: 10.1104/pp.53.5.768

Ashraf M., Hasnain S., Berge O. and Mahmood T. (2004). Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biology and Fertility of Soils* **40**: 157-162. doi.org/10.1007/s00374-004-0766-y.

Azizoglu, U., Yilmaz, N., Simsek, O., Ibal, J.C., Tagele, S.B. and Shin, J., (2021). The fate of plant growth-promoting rhizobacteria in soilless agriculture: future perspectives, 3 Biotech. 11, Article number: 382. doi.org/10.1007/s13205-021-02941-2.

Babu, S.V., Triveni, S., Reddy, R.S. and Sathyanarayana, J. (2017). Persistence of PSB-fungi biofilmed biofertilizer in the soils and its effect on growth and yield of maize. *International Journal of Current Microbiology and Applied Sciences* **6**(12): 1812-1821. doi.org/10.20546/ijcmas.2017.612.205

Badri, D. V., Weir, T.L., van der Lelie, D. and Vivanco, J.M. (2009). Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology* **20**: 642-650. doi.org/10.1016/j.copbio.2009.09.014

Bansal, M. and Mukerji, K.G. (1994). Positive correlations between VAM-induced changes in root exudation and mycorrhizosphere mycoflora. *Mycorrhiza* **5**: 39-44. Doi.org/10.1007/BF00204018.

Bhuvaneswari, T.V., Bhagwat, A.A. and Bauer, W.D. (1981). Transient susceptibility of root cells in four common legumes to nodulation by rhizobia. *Plant Physiology* **68**: 1144-1149.

Certik, M., Shimizu, S., 1999, Biosynthesis and regulation of microbial polyunsaturated fatty acid production, *Journal of Bioscience and Bioengineering* **87**(1): 1-14. doi: 10.1104/pp.68.5.1144

Chaparro, J.M., Badri, D.V., Bakker, M.G., Sugiyama, A., Manter, D.K. and Vivanco, J.M. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions, *PLOS ONE*, **8**(8): 10.1371/annotation/51142aed-2d94-4195-8a8a-9cb24b3c733b. https://doi.org/10.1371/annotation/51142aed-2d94-4195-8a8a-9cb24b3c733b.

Dasgan, H.Y., Aydoner, G. and Akyol, M. (2012). Use of some microorganisms as bio-fertilizers in soilless grown squash for saving chemical nutrients. *Acta Horticulturae* 155-162. doi: 10.17660/ActaHortic.2012.927.17.

De Silva, M.S.D.L., Jayasekera, A P.D.A., Seneviratne, G., Abeysekera, U.P., Premathunge, E.W.T.P., Wijesekera, S.N. (2014). Soil fertility improvement through biofilmed biofertilizers: potential for field applications in tea cultivation. *Sri Lanka Journal of Tea Science* **79**(1/2): 46-61.

De-la-Peña, C., Lei, Z., Watson, B.S., Sumner, L.W. and Vivanco, J.M. (2008). Root-microbe communication through protein secretion. *The Journal of Biological Chemistry* **283**: 25247-25255. doi: 10.1074/jbc.M801967200.

Donlan, R. M. and Costerton, J.W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical Microbiological Reviews* **15**: 167-193. doi: 10.1128/CMR.15.2.167-193.2002

Downie, J.A. (2010). The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. *FEMS Microbiological Reviews* **34**: 150-170. Doi: 10.1111/j.1574-6976.2009.00205.x.

Elarroussi, H., Elmernissi, N., Benhima, R., Kadmiri, I.M.E., Bendaou, N., Smouni, A., Wahby, I. (2016). Microalgae polysaccharides a promising plant growth biostimulant. *Journal of Algal Biomass Utilization* **7**(4) : 55-63.

Farmer, E.E. (1994). Fatty acid signalling in plants and their associated microorganisms. *Plant Molecular Biology* **26**: 1423-1437. doi:10.1007/BF00016483.

Herath, H.M.L.I., Menikdiwela, K.R., Igalavithana, A.D. and Seneviratne, G. (2015). Developed fungal-bacterial biofilms having nitrogen fixers: universal biofertilizers for legumes and non-Legumes. In *Biological Nitrogen Fixation*, 1st edn. Vol. 2, ed. F. J. de Bruijn. John Wiley and Sons, Inc, 1037-1042.

Herath, H.M.L.I., Senanayeke, D.M.N., Seneviratne. G. and Bandara, D.C. (2013). Variation of biochemical expressions of developed fungal-bacterial biofilms over their monocultures and its effect on plant growth. *Tropical Agricultural Research* **24**: 186-192.

Hettiarachchi, R.P., Dharmakeerthi, R.S., Jayakody, A.N., Seneviratne, G., de Silva, E., Gunathilake, T. and Thewarapperuma, A. (2014). Effectiveness of fungal bacterial Interactions as biofilmed biofertilizers on enhancement of root growth of *Hevea* seedlings. *Journal of Environmental Professionals Sri Lanka* **3**(2): 25-40. doi: http://dx.doi.org/10.4038/jepsl.v3i2.7844

Hoagland, D.R. and Arnon, D.I. (1950). The water-culture method of growing plants without soil. California Agricultural Experiment Station, 347 pp.

Jagnow, G. (1987). Inoculation of cereal crops and forage grasses with nitrogen-fixing rhizosphere bacteria:

possible causes of success and failure with regard to yield response - a review. *Journal of Plant Nutrition and Soil Science* **150**(6): 36-368. doi: https://doi.org/10.1002/jpln.19871500602.

Julian Cardenas, J., Santa, F. and Kaštovská, E., 2021, The exudation of surplus products links plant functional traits and plant-microbial stoichiometry. *Land*, **10**(8): 840. https://doi.org/10.3390/land10080840.

Kalniņš, M., Andersone-Ozola, U., Gudrā, D., Sieriņa, S., Fridmanis, D., Ievinsh, G. and Muter, O. (2022). Effect of bioaugmentation on the growth and rhizosphere microbiome assembly of hydroponic cultures of *Mentha aquatic*, *Ecological Genetics and Genomics*, 22:100107. https://doi.org/10.1016/j.egg.2021.100107

Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H., Meir, S., Rogachev, I. and Aharoni, A. (2020). Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling, *PNAS* **117**(7): 3874-3883. https://doi.org/10.1073/pnas.1912130117.

Lower, S.K., Tadnier, C.J. and Hochella, M.F. (2000). Measuring interfacial and adhesion forces between bacteria and mineral surfaces with biological force microscopy. *Geochimica et Cosmochimica Acta* **64**(18): 3133-3139. doi: https://doi.org/10.1016/S0016-7037(00)00430-0.

Mapelli, V., Olsson, L. and Nielsen, J. (2008). Metabolic foot printing in microbiology: methods and applications in functional genomics and biotechnology. *Cell* **26**: 490-497.doi:https://doi.org/10.1016/j.tibtech.2008.05.008.

Mia, M.A.B., Shamsuddin, Z.H.,Wahab, Z. and Marziah, M. (2010). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured *Musa* plantlets under nitrogen-free hydroponics condition. *Australian Journal of crop Science* **4**(2): 85-90.

Negi, A.S., Darokar, M.P., Chattopadhyay, S.K., Garg, A., Bhattacharya, S.K., Srivastava, V. and Khanuja, S.P.S. (2005). Synthesis of a novel plant growth promoter from Gallic acid. *Bioorganic and Medicinal Chemistry Letters* **15**: 1243-1247. doi: 10.1016/j.bmcl.2004.11.079

Ploux, L., Ponche, A. and Anselme, K. (2010). Bacteria/material interfaces: role of the material and cell wall properties. *Journal of Adhesion Science and Technology* **24:** 2165-2201. doi: https://doi.org/10.1163/016942410X511079.

Rudrappa, T., Biedrzycki, M.L. and Bais, H.P. (2008). Causes and consequences of plant-associated biofilms. *FEMS Microbiology Ecology* **64**: 153-166. doi: 10.1111/j.1574-6941.2008.00465.x.

Rueda, D., Valencia, G., Soria, N., Rueda, B.B., Manjunatha, B., Kundapur, R.R. and Selvanayagam, M. (2016). Effect of *Azospirillum* spp. and *Azotobacter* spp. on the growth and yield of strawberry (*Fragaria vesca*) in hydroponic system under different nitrogen levels. *Journal of Applied Pharmaceutical Science* **6**(1): 48-54. doi: 10.7324/JAPS.2016.600108.

Saharan, B.S. and Nehra, V. (2011). Plant growth promoting rhizobacteria: A critical review. *Life Sciences and Medicine Research* **21**:1-30.

Seneviratne, G. and Jayasinghearachchi, H.S. (2005). A rhizobial biofilm with nitrogenase activity alters nutrient availability in a soil. *Soil Biology and Biochemistry* **37**: 1975-1978.https://doi.org/10.1016/j.soilbio.2005.02.027.

Seneviratne, G., Jayasekara, A.P.D.A., De Silva, M.D.S.L., and Abeysekera, U.P. (2011). Developed microbial biofilms can restore deteriorated conventional agricultural soils. *Soil Biology and Biochemistry* **43**(5): 1059-1062. doi: https://doi.org/10.1016/j.soilbio.2011.01.026.

Seneviratne, G., Thilakaratne, R.M.M.S., Jayasekara, A.P.D.A., Seneviratne, K.A.C.N., Padmathilake, K.R.E. and de Silva, M.D.S.L. (2009). Developing beneficial microbial biofilms on roots of nonlegumes: a novel biofertilizing technique. In *Microbial Strategies for Crop Improvement*, ed. M.S. Khan *et al*. Springer-Verlag Berlin Heidelberg, 81-95.

Singhalage, I.D., Seneviratne, G., Madawala, H.M.S.P., and Manawasinghe, I.S. (2018). Characterization of structural properties of fungal-bacterial bioflms by Fourier Transform Infrared Spectroscopy. *Ceylon Journal of Science* **47**(1): 77-83. doi: http://doi.org/10.4038/cjs.v47i1.7490.

Singhalage, I.D., Seneviratne, G., Madawala, H.M.S.P. and Wijepala, P.C. (2019). Profitability of strawberry (*Fragaria ananassa*) production with biofilmed biofertilizer application. *Scientia Horticulturae* **243**: 411-413. doi: 10.1016/j.scienta.2018.08.033

Singhalage, I.D., Seneviratne, G. and Madawala, H.M.S.P. (2020). Functional heterogeneity of metabolites excreted by fungal and bacterial biofilms and their effects on seedling growth. *Ceylon Journal of Science* **49**(1): 13-19. doi: http://doi.org/10.4038/cjs.v49i1.7701.

Upchurch, R.G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnological Letters* **30**: 967-977. doi: 10.1007/s10529-008-9639-z

Vick, B.A. and Zimmerman, D.C. (1984). Biosynthesis of Jasmonic acid by several plant species. *Plant Physiology* **75**: 458-461. doi: 10.1104/pp.75.2.458.

Williams, P. (2007). Quorum sensing, communication and cross-kingdom signaling in the bacterial world. *Microbiology* **153**: 3923-3938. doi: 10.1099/mic.0.2007/012856-0.

Xie, S., Wu, H., Zang, H., Wu, L., Zhu, Q. and Gao, X. (2014). Plant growth promotion by spermidine-producing *Bacillus* subtilis OKB105 *Molecular Plant-Microbe Interactions* **27**(7): 655-663. doi: 10.1094/MPMI-01-14-0010-R

Zavahir, J. S. and Seneviratne, G. (2007). Potential of developed microbial biofilms in generating bioactive compounds. *Research Journal of Microbiol*ogy **2**(4), 397-401.