Characterization of Biofilm Structures Produced by Some Plant Growth Promoting Bacteria and Their Colonization on Plant Roots

Tacettin Çiftçi
Ahi Evran University
Murat Çınarlı
Ahi Evran University
İbrahim Erdoğan (ibrahim.erdogan@ahievran.edu.tr)
Ahi Evran University

Research Article

Keywords: Biofilm, PGPR, Raman Spectroscopy, FT-IR, SEM

Posted Date: May 30th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2962504/v1

Abstract

Plant growth promoting rhizobacteria (PGPR) increase plant root development and provide plants with maximum nutrient uptake opportunities for better growth. Besides promoting growth, PGPRs have a number of functions including stress tolerance (heat, drought, salinity and disease), bioremediation of heavy metals, biodegradation of complex toxic organic compounds, biocontrol agents and biofilm formation. The interaction between various plant growth promoting rhizobacteria was mainly investigated under the planktonic growth mode. In-depth investigation of biofilm development on root surface and rhizosphere colonization is needed to improve understanding of plant-microorganism interaction. In this study; The biofilm structures formed by *Bacillus drentensis*, *Bacillus mojavensis*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Arthrobacter nitroguajacolicus* bacteria, which have plant growth promoting properties, were characterized by Raman and FT-IR Spectroscopy. In addition, the biofilm formation capacity of these bacteria on polystyrene surfaces was determined by the crystal violet test, and the colonization of *Bacillus drentensis*, *Pseudomonas fluorescens* and *Arthrobacter nitroguajacolicus* bacteria on *Arabidopsis thaliana* Columbia ecotype roots was determined by Scanning Electron Microscope (SEM) analysis.

1. Introduction

In order to meet the food needs of the growing human population, it is confronted with a number of challenges, including increased demand for agriculture and animal husbandry, sustainable agricultural production, the use of limited resources (such as fertile lands), and the elimination of severe environmental problems caused by traditional agricultural practices. These issues can be mitigated by using organic fertilizers containing microorganisms instead of chemical fertilizers to boost food yield, as well as by discovering and utilizing local soil microbes for this purpose (Küçük 2019). Beneficial bacteria colonized on plant roots, known as plant growth promoting rhizobacteria (PGPR), are regarded one of the major groups that exhibit a variety of beneficial effects in improving plant growth (Lugtenberg et al. 2001). Bacteria that promote plant growth; it should have characteristics such as settling in the soil around the roots, developing in competition with other microorganisms in the soil, replicating, surviving, and supporting plant growth and development (W 1994). Beneficial PGPRs linked with plant root surfaces are known to contribute to increased plant yield (Ryu et al. 2004). The mechanisms by which various PGPRs promote plant growth have been thoroughly investigated, both directly (production of plant hormones, nitrogen fixation, phosphate solubilization, and iron cleavage) and indirectly (antibiotics and lytic enzymes, induced resistance, HCN production, and competition) (Kloepper et al. 1980; Ahmad et al. 2008; Bernard 2012). Microbial cells and extracellular polymeric materials such as polysaccharides, lipids, proteins, genetic materials, humic-like compounds, and EPS (Extracellular Polymeric Substances) combine to create biofilm (Costerton et al. 1995). EPS allows microorganisms to bind irreversibly to any surface and protects them from environmental stress conditions (Vu et al. 2009; Keleştemur et al. 2018). The type of the microorganism influences the composition of EPS. Gram-negative bacteria produce EPS from neutral and polyanionic polysaccharides, whereas gram-positive bacteria produce cationic

polysaccharides. Environmental variables such as pH, temperature, oxygen and nitrogen levels, as well as surface qualities, on the other hand, influence the composition of a biofilm (Costerton et al. 1981; Ahimou et al. 2007).

In this study; Biofilm structures formed by *Bacillus drentensis*, *Bacillus mojavensis*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Arthrobacter nitroguajacolicus* bacteria, which have plant growth promoting properties, were characterized by Raman and FT-IR Spectroscopy. In addition, the biofilm formation capacity of these bacteria on polystyrene surfaces was determined by the crystal violet test, and the colonization of *Bacillus drentensis*, *Pseudomonas fluorescens* and *Arthrobacter nitroguajacolicus* bacteria on *Arabidopsis thaliana* Columbia ecotype roots was determined by SEM (Scanning Electron Microscopy) analysis.

2. Materials and Methods

2.1. Microorganisms

Bacillus mojavensis, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus drentensis* and *Arthrobacter nitroguajacolicus* strains isolated from Ankara province soils and classified according to 16s rDNA sequence results were obtained from Ankara University Faculty of Science Biology Department Prokaryote Genetics Laboratory stock cultures to be used in our study (Özdoğan et al. 2022). In the first stage, strains were grown in Nutrient Broth (NB) medium at 30°C at 150 rpm shaking speed for 18–24 hours. During the study, all cultures were stored in sterile microfuge tubes with 60% glycerol at -20°C and − 80°C.

2.2. Determination of Biofilm Forming Capacity of the Strains

Biofilm formation trials and optimization were carried out in a biofilm reactor with continuous nutrient flow. First of all, optimum biofilm production capacities of bacterial biofilms were determined on polystyrene surfaces. In this method, the isolates included in the experiment were inoculated at 1% in Nutrient Broth (NB) medium and activated for 18 hours in a shaking incubator at 37 ^{0}C. Then, bacterial cultures were adjusted to OD595 = 0.2 and 30 µL of these dilutions were transferred to 96-well microtitration plate wells containing 100 µL of NB. Plates were incubated at 20°C for 24, 48 and 72 hours (Vestby et al. 2009). At the end of the incubation period, the wells were washed by applying physiological saline 3 times and the planktonic cells were removed in this way. The fixation of the biofilm structure formed by 10 minutes incubation was carried out by adding 130 µL of 95% (v/v) methanol to the wells that were dried at room temperature. At the end of the incubation period, methanol was removed and these media were left to dry. Then, 130 µL of 1% crystal violet was added to the wells to stain the biofilm structure and incubated for 30 minutes. At the end of this period, the wells were washed 3 times with distilled water and then 130 µL of 33% (v/v) glacial acetic acid was added to dissolve the dye bound to the biofilm structure and kept at room temperature for 45 minutes (Stepanovic et al. 2000). This

experiment was carried out in 3 parallel and 2 repetitions. Biofilm production amounts were determined by measuring the dye density with an Elisa reader in OD595 and subtracting the values obtained from the wells containing only NB used as a control from the average of the obtained values.

2.3. Bacterial Inoculation and Fixation for SEM (Scanning Electron Microscopy)

Bacterial strains were grown to late log phase in 100 ml flasks at 30°C. After 2 weeks of plant growth, the plant roots were inoculated by soaking in 10 ml of diluted bacterial cultures and allowed to incubate for 2 hours. After the incubation, the media were withdrawn from the tubes and 0.5% glutaraldehyde-4% paraformaldehyde fixation solution was added to the tubes and left to incubate for 24 hours. After 24 hours of incubation, the roots were washed with PBS (Phosphate Buffered Saline) and then the fixation process was completed by passing through 20%, 30%, 50%, 70%, 90% and 100% alcohol series and the samples were prepared for SEM (Vaikuntapu et al. 2014). As a result of the fixation procedures, bacterial colonization in the roots was visualized with the Zeiss Evo 40 scanning electron microscope (SEM) located at Ankara University Nuclear Sciences Institute.

2.4. Analysis of Biofilm Structures by FT-IR Spectroscopy

FT-IR spectra were collected in a transflection mode with the use of a Nicolet 6700 FT-IR spectrometer over the range 4000–400 cm^{-1}. Each spectrum represented 16 scans taken at a resolution of 4 cm^{-1} with an optimal signal-to-noise ratio. For a given material, a final spectrum was obtained using OMNIC software.

2.5. Analysis of Biofilm Structures by Raman Spectroscopy

Raman spectra were recorded with the use of a DXR Confocal Raman Microscope (JASCO NRS4500). The excitation laser wavelength was 532 nm and the output power was set at 9.1 mW. The spectra were recorded in the 2200–200 cm^{-1} spectral range with 4 cm^{-1} of Raman shift resolution. A 25 µm pinhole aperture and exposure time of 15 s with 15 exposures per point with 20x objective were used. The microscope was equipped with a CCD Camera (DU420_OE). All data processing and image assembly was performed using Spectra Manager software.

3. RESULTS

3.1. FT-IR Spectra of Biofilm Structures

In the IR spectra of the samples (Fig. 1), broad bands around 3200–3300 cm^{-1} indicate the presence of amide A. The bands appearing in the region of 2800–3000 cm^{-1} are the bands belonging to the CH, CH_2 and CH_3 symmetrical and asymmetric vibrations of the methylene groups known as characteristic for the lipids condensed in the bacterial cell walls and membranes. The highest intensity of this band was determined for P5-1. It is thought that the reason for this may depend on the hydrocarbon chain length

and the chemical structure of the polar head group of membrane lipids. The Amid I and Amid II bands are sensitive to changes in the secondary structures of proteins. The characteristic absorbance bands for amide I and amide II are around 1500–1700 cm^{-1}. In the IR spectra, amide 1 bands were observed in the range of 1636 cm^{-1} and amide II bands were observed in the range of 1544–1553 cm^{-1} for the samples. In the literature, around 1200 – 950 cm^{-1} is known as the sugar zone. Bands in the range of 1148–1152 cm^{-1} in the IR spectra of the samples indicate the presence of oligosaccharides. Weak bands in the 1097–1125 cm^{-1} region support the presence of carbohydrates, phosphates/phospholipids and nucleic acids. The bands of the β-glucan bonds of the samples were observed in the range of 1052–1083 cm^{-1}.

3.2. Raman Spectra of Biofilm Structures

In the Raman spectra of the samples (Fig. 2), Amide I and Amide II bands appeared in the region of 1700 – 1600 cm^{-1} and 1600 – 1500 cm^{-1}, respectively. Raman spectra of lipids, which indicate the presence of a hydrocarbon chain, are mainly detected in three spectroscopic regions: 1500–1400 cm^{-1}, 1300 – 1250 cm^{-1} and 1200 – 1050 cm^{-1}. The bands observed around 1300–1400 cm^{-1} in Raman spectra belong to CH_2 groups originating from protein structure in bacterial biofilm samples. The highest intensity of the bands around 1450 cm^{-1}, which is thought to belong to saturated lipids, was observed for K4-1 and the lowest for KH3-1. Differences in the intensity of this band indicate changes in lipid amounts and bacterial biofilm compositions during maturation in terms of saturation of lipid fatty acids. The most specific bands for glucans are those centered at ~ 750 cm^{-1}. These peaks were observed in the Raman spectra of the samples. The presence of bands observed at 880–980, 1055 and 1075 cm^{-1} in Gram-positive cells may be due to a combination of the vibrational modes of the bacterial cell walls of rhamnose, galactose and glucose. The peaks observed at 1125 cm^{-1} are glucose peaks in accordance with the literature. Raman bands at ~ 1380 cm^{-1} often indicate the presence of a polyanionic polysaccharide in the bacterial biofilm matrix. The emergence of these bands in the Raman spectra of the samples confirms this situation.

3.3. Biofilm Formation Amounts on Polystyrene Surfaces

As a result of the analysis, it was determined that the bacteria reached the highest biofilm production capacity at the end of the 24th hour. It was determined that the strain with the highest biofilm forming capacity was *B. mojavensis*, followed by *P. putida*, *P. fluorescens*, *B. drentensis* and *A. nitroguajacolicus* strains, respectively.

3.4. Screening Colonization of Bacteria on Plant Roots (SEM)

As a result of SEM analysis (Fig. 4–6), it was determined that all three bacterial species colonized mainly by forming biofilms at the root tips. It was determined that bacteria accumulate and colonize in the intercellular spaces outside the vascular cylinder. Bacterial colonization in the roots; It has been stated that it is more common in the region where cell division and growth is more and in the root cap, compared

to the mature root region and root hairs. They also reported that Paenibacillus polymyxa forms a biofilm around the root tip of the plant and this biofilm form plays a role in biocontrol and increasing tolerance to drought (Timmusk et al. 2005).

4. Discussion

In this study; Biofilm structures formed by bacterial strains with plant growth promoting properties were analyzed, colonization and biofilm structures in plant roots were visualized with SEM, and biofilm characterizations were performed with FT-IR and Raman Spectroscopy. As a result of the analysis, it was determined that the bacteria reached the highest biofilm production capacity at the end of the 24th hour. It was determined that the strains with the highest biofilm forming capacity were *B. mojavensis* and *P. putida*, followed by *P. fluorescens*, *B. drentensis* and *A.nitroguajacolicus* strains, respectively. As a result of our SEM analysis, it was observed that bacterial colonization mostly took place around the root tip. These findings were in parallel with the literature data. The properties of the root surface vary along the length of the roots and biofilm formation is affected by the compounds and nutrients released by the roots in different regions. Bacterial colonization in the roots; It has been stated that it is more common in the region where cell division and growth is more and in the root cap, compared to the mature root region and root hairs. They also reported that Paenibacillus polymyxa forms a biofilm around the root tip of the plant and this biofilm form plays a role in biocontrol and increasing tolerance to drought. (Timmusk et al. 2005). Studies have indicated that bio-fertilizer biofilms focusing on the agricultural sector may have significant potential benefits. Such products can protect the plant not only against various environmental and soil-borne diseases, but also against abiotic stresses such as salinity, drought, inorganic and organic pollutants, and potentially increase crop productivity (Malusá et al. 2012). One of the most important factors determining bacterial colonization in the plant root is root exudation (Weller and Thomashow 1994; Walker et al. 2004). Plant roots release a wide variety of compounds into the rhizosphere. Exudation is heterogeneous in time and space. It also differs along the longitudinal axis from the base to the root tip, where the metabolic activity changes (Gilroy and Jones 2000). The root cap is a source of hydrated polysaccharides shed from the root tip. These polysaccharides form mucilage, which lubricates the root during passage through the medium. Apoplastic sucrose can also spread from the root tip due to the concentration gradient. Sucrose does not leak from mature root segments because diffusion of this compound is blocked by the suberized endodermal cell layer (Jaeger et al. 1999). Microorganisms that prefer this type of nutrition can be attracted by these rich food sources. Some PGPRs produce hydrolytic enzymes such as chitinases and glucanases against soil-borne bacterial and fungal pathogens to cleave fungal cell walls, elicit defensive responses in plants, and confer resistance to subsequent infection by pathogenic bacteria, fungi and viruses (Manjula and Podile 2001) or it forms biofilms on root surfaces that can protect roots (O'Toole and Kolter 1998). *Pseudomonas* and *Bacillus* spp. are the most important PGPRs colonizing roots in various plants. Members of this group have a widespread distribution in soil, are effective colonizers of the rhizosphere, and produce several types of metabolites that are inhibitory to a wide variety of pathogens in plants (Sunita Rangarajan et al. 2003). Many other root-colonizing strains of PGPR have also been found to have antifungal properties against a range of pathogens in the soil.

Studies show the importance of PGPR and biofilm formation, which is an important component of plant-microorganism interactions, and its effect on plant health and soil fertility. The viability of PGPR is a promising sustainable and environmentally friendly approach to achieve sustainable productivity of soil and plant growth. This approach includes soil structure formation, decomposition of organic matter, recycling of essential elements, mineral nutrients, production of numerous plant growth regulators, degradation of organic pollutants, stimulation of root growth, biological control of soil and seed-borne plant pathogens and promotion of changes in vegetation, thawing, etc. It inspires a broad use of PGPR that can lead to a reduction in the need for agrochemicals (fertilizers and pesticides) for better soil fertility through mechanisms. Rhizobacterial biofilms that promote plant growth have been shown to play a fundamental role in futuristic farming approaches such as biofertilizers, plant growth accelerators and biocontrollers for plant health. Beneficial biofilms attached to plant roots of some crops have been shown in studies to aid in nutrient cycling as well as biological control of pests and diseases, ultimately improving productivity and crop yields. Moreover, rhizobial polysaccharides are very important in promoting plant growth, act as an active signaling molecule during beneficial interactions, and provide a defensive response during the infection process (Parada et al. 2006). Raman spectroscopy and FT-IR analyzes are methods that are widely used in biofilm characterization due to the fact that biological samples are produced quickly, simply and without damaging the biofilm structure, and there is no need for sample preparation. In this study, detailed analyzes and characterization of bacterial biofilm structures were investigated by Raman and FTIR analyzes. The highest intensity was determined for P5-1 in the bands of methylene groups (CH, CH2 and CH3), which are characteristic for the lipids concentrated on the cell wall and membranes in the IR spectra of the examined bacterial biofilms. When the Raman spectra were examined, it was determined that the bands of saturated lipids were at the highest intensity in K4-1, and the lowest intensity was observed in KH3-1. This shows the changes in lipid amounts during the biofilm maturation stage. It is thought that lipids, together with polysaccharides, increase the viscosity of the biofilm and increase the effect of binding to the surface (Gieroba et al. 2020). Different amounts of protein bands were found in the Raman and FT-IR peaks of the bacteria examined. The surface structure of bacterial cells, such as the concentration of lipopolysaccharide and the presence of proteins in the surface membrane, are important factors affecting biofilm formation in plant roots. In recent studies, a 900 kDa cell surface protein called LapA (Large adhesion protein A) has been discovered and has been reported to have important effects on the colonization of *P. fluorescens* bacteria (Hinsa et al. 2003). Similarly, *P. putida* KT2440 LapA homologues were observed to be involved in seed adhesion during competitive root colonization and seed coating. As a result, this protein acts as a biofertilizer and supports plant growth (Espinosa-Urgel et al. 2002). LapA, which has domains that are used to attach to the surface, is especially involved in the biofilm formation of Gram-positive bacteria. Similarly, other Ca2 + binding protein domains and hemolysins are frequently involved in host-cell interactions (Hinsa et al. 2003). A series of agglutinins called Rap (Rhizobium adhesion proteins) localized at the cell poles of *Rhizobium leguminosarum* are thought to bind Ca2 + and are suggested to play a key role in the attachment of rhizobial cells to plant tissues (Ausmees et al. 2001). In both FT-IR and Raman analyzes, specific bands were observed indicating the presence of oligosaccharides and polysaccharides, consistent with the literature. In particular, the role of polysaccharides in the structure of PGPR-EPS in

promoting plant growth has been revealed in previous studies. Some PGPR-EPS (Plant Growth Promoting Bacteria-Extracellular Polymeric Substance) ie EPSs produced by PGPRs can bind cations including Na+, which plays a role in reducing salinity stress by reducing the available Na + content that the plant takes from the soil (Upadhyay et al. 2012). Co-grafting of biofilmed vaccines can be used to successfully establish beneficial microorganisms introduced in plants for biological control of diseases (Jayasinghearachchi and Seneviratne 2006). However, few studies have been conducted on the biofilm properties of plant growth promoting bacteria, and this area has been overlooked until now. It is stated that this integrated biofilm biofertilizer approach to support the growth of plants will increase soil fertility as well as more sustainable agriculture. In this tstudy; biofilm structures formed by bacterial strains with plant growth promoting properties were analyzed, colonization and biofilm structures in plant roots were visualized with SEM, and biofilm characterizations were performed with FT-IR and Raman Spectroscopy. When the findings were compared with the literature data, it was determined that these bacterial strains had the necessary characteristics to be used as biofertilizer with biofilm. There is a need to investigate the biofertilizer efficiency of these bacterial strains with future greenhouse plant trials.

Declarations

Acknowledgements

The authors thank to Prof Mustafa Akçelik who is head of Ankara University Prokaryote Genetic Laboratory to give opportunity carriying out biofilm assays in his lab.

Author Contributions

TÇ: Conducted experiments, wrote original draft. MÇ: Conducted and interpretted spectroscopy experiments. İE: Conducted experiments, reviewed & edited manuscript, supervision and funding acquisition.

Funding

We are grateful for the support of the Kırşehir Ahi Evran University Scientific Research Projects Unit [Grant numbers; ZRT.A4.20.002 and ZRT.A4.21.036].

Availability of data

All 16s rDNA sequences are publicly available from the NCBI under the accession number of MW228104, MW737658, MW217268, MW241065 and MW228157.

Experimental research and feld studies on plants were performed according to institutional, national, and international guidelines and legislation.

Competing interests

The authors declare no competing interests.

References

1. H.S. Jayasinghearachchi and G. Seneviratne (2006) A Mushroom-fungus Helps Improve Endophytic Colonization of Tomato by Pseudomonas fluorescens Through Biofilm Formation. Research J of Microbiology 1:83–89. https://doi.org/10.3923/jm.2006.83.89
2. Ahimou F, Semmens MJ, Haugstad G, Novak PJ (2007) Effect of protein, polysaccharide, and oxygen concentration profiles on biofilm cohesiveness. Applied and environmental microbiology 73:2905–2910
3. Ahmad F, Ahmad I, Khan MS (2008) Screening of Free-Living Rhizospheric Bacteria for Their Multiple Plant Growth Promoting Activities. Microbiological Research 163:173–181
4. Ausmees N, Jacobsson K, Lindberg M (2001) A unipolarly located, cell-surface-associated agglutinin, RapA, belongs to a family of Rhizobium-adhering proteins (Rap) in Rhizobium leguminosarum bv. trifolii The GenBank accession numbers for the sequences reported in this paper are AF265222, AF265223, AF315809 and AF315810. Microbiology 147:549–559. https://doi.org/10.1099/00221287-147-3-549
5. Bernard RG (2012) Plant Growth-Promoting Bacteria: Mechanisms and Applications. Scientifica
6. Costerton JW, Irvin RT, Cheng KJ (1981) The bacterial glycocalyx in nature and disease. Annual Reviews in Microbiology 35:299–324
7. Costerton JW, Lewandowski Z, Caldwell DE, et al (1995) Microbial biofilms. Annual review of microbiology 49:711–745
8. Espinosa-Urgel M., Kolter R., Ramos J-L. (2002) Root colonization by Pseudomonas putida: love at first sight. Microbiology 148:341–343. https://doi.org/10.1099/00221287-148-2-341
9. Gieroba B, Krysa M, Wojtowicz K, et al (2020) The FT-IR and Raman Spectroscopies as Tools for Biofilm Characterization Created by Cariogenic Streptococci. IJMS 21:3811. https://doi.org/10.3390/ijms21113811
10. Gilroy S, Jones DL (2000) Through form to function: root hair development and nutrient uptake. Trends in Plant Science 5:56–60. https://doi.org/10.1016/S1360-1385(99)01551-4
11. Hinsa SM, Espinosa-Urgel M, Ramos JL, O'Toole GA (2003) Transition from reversible to irreversible attachment during biofilm formation by Pseudomonas fluorescens WCS365 requires an ABC transporter and a large secreted protein: P. fluorescens biofilm formation. Molecular Microbiology 49:905–918. https://doi.org/10.1046/j.1365-2958.2003.03615.x
12. Jaeger CH, Lindow SE, Miller W, et al (1999) Mapping of Sugar and Amino Acid Availability in Soil around Roots with Bacterial Sensors of Sucrose and Tryptophan. Appl Environ Microbiol 65:2685–2690. https://doi.org/10.1128/AEM.65.6.2685-2690.1999
13. Keleştemur S, Avci E, Çulha M (2018) Raman and Surface-Enhanced Raman Scattering for Biofilm Characterization. Chemosensors 6:5
14. Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhancing Plant Growth by Siderophores Produced by Plant Growth-Promoting Rhizobacteria. Nature 286:885–886

15. Küçük Ç (2019) Bitki Probiyotik Bakteriler: Bitkiler Üzerindeki Rolleri ve Uygulamalar. International Journal of Life Sciences and Biotechnology 2:1–15
16. Lugtenberg BJJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by Pseudomonas. Annual Reviews Phytopathology Journal 39:461–490
17. Malusá E, Sas-Paszt L, Ciesielska J (2012) Technologies for Beneficial Microorganisms Inocula Used as Biofertilizers. The Scientific World Journal 2012:1–12. https://doi.org/10.1100/2012/491206
18. Manjula K, Podile AR (2001) Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF 1. Can J Microbiol 47:618–625. https://doi.org/10.1139/w01-057
19. O'Toole GA, Kolter R (1998) Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Molecular Microbiology 28:449–461. https://doi.org/10.1046/j.1365-2958.1998.00797.x
20. Özdoğan DK, Akçelik N, Akçelik M (2022) Genetic diversity and characterization of plant growth-promoting effects of bacteria isolated from rhizospheric soils. Current Microbiology 79:132
21. Parada M, Vinardell JM, Ollero FJ, et al (2006) *Sinorhizobium fredii* HH103 Mutants Affected in Capsular Polysaccharide (KPS) are Impaired for Nodulation with Soybean and *Cajanus cajan*. MPMI 19:43–52. https://doi.org/10.1094/MPMI-19-0043
22. Ryu CM, Farag MA, Hu CH, et al (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. Plant physiology 134:1017–1026
23. Stepanovic S, D. V, I. D, et al (2000) A modi ed microtiter-plate test for quanti cation of staphylococcal biofilm formation". J Microbiol Methods 40:175–179
24. Sunita Rangarajan, Saleena LM, Vasudevan P, Nair S (2003) [No title found]. Plant and Soil 251:73–82. https://doi.org/10.1023/A:1022950811520
25. Timmusk S, Grantcharova N, Wagner EGH (2005) Paenibacillus polymyxa invades plant roots and forms biofilms. Applied and environmental microbiology 71:7292–7300
26. Upadhyay SK, Singh JS, Saxena AK, Singh DP (2012) Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions: Effect of rhizobacteria on wheat in saline conditions. Plant Biology 14:605–611. https://doi.org/10.1111/j.1438-8677.2011.00533.x
27. Vaikuntapu PR, Dutta S, Samudrala RB, et al (2014) Preferential promotion of Lycopersicon esculentum (Tomato) growth by plant growth promoting bacteria associated with tomato. Indian journal of microbiology 54:403–412
28. Vestby LK, T. M, S. L, et al (2009) Biofilm forming abilities of Salmonella are correlated with persistence in fish meal and feed factories". BMC Vet Res 5:20–26
29. Vu B, Chen M, Crawford RJ, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535–2554
30. W KJ (1994) Plant growth promoting rhizobacteria (other systems. Azospirullum/Plant Associations, Boca Raton, FL, USA

31. Walker TS, Bais HP, Déziel E, et al (2004) *Pseudomonas aeruginosa* -Plant Root Interactions. Pathogenicity, Biofilm Formation, and Root Exudation. Plant Physiology 134:320–331. https://doi.org/10.1104/pp.103.027888
32. Weller DM, Thomashow LS (1994) Current Challenges in Introducing Beneficial Microorganisms into the Rhizosphere. In: O'Gara F, Dowling DN, Boesten B (eds) Molecular Ecology of Rhizosphere Microorganisms. Wiley-VCH Verlag GmbH, Weinheim, Germany, pp 1–18

Figures

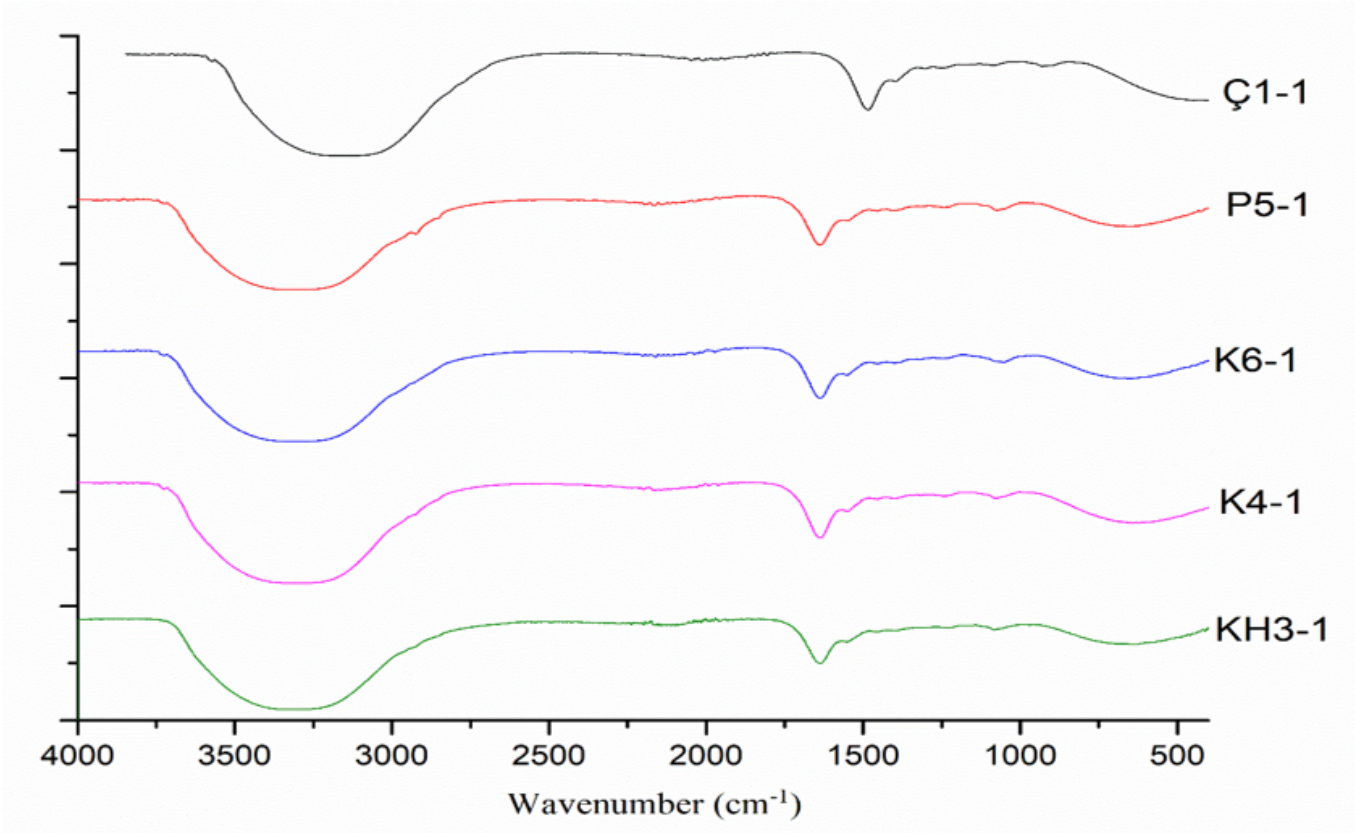

Figure 1

IR spectra of the samples (Ç1-1: *P.putida*, P5-1: *B. mojavensis*, K6-1: *B. drentensis*, K4-1: *P. fluorescens*, KH3-1: *A. nitroguajacolicus*)

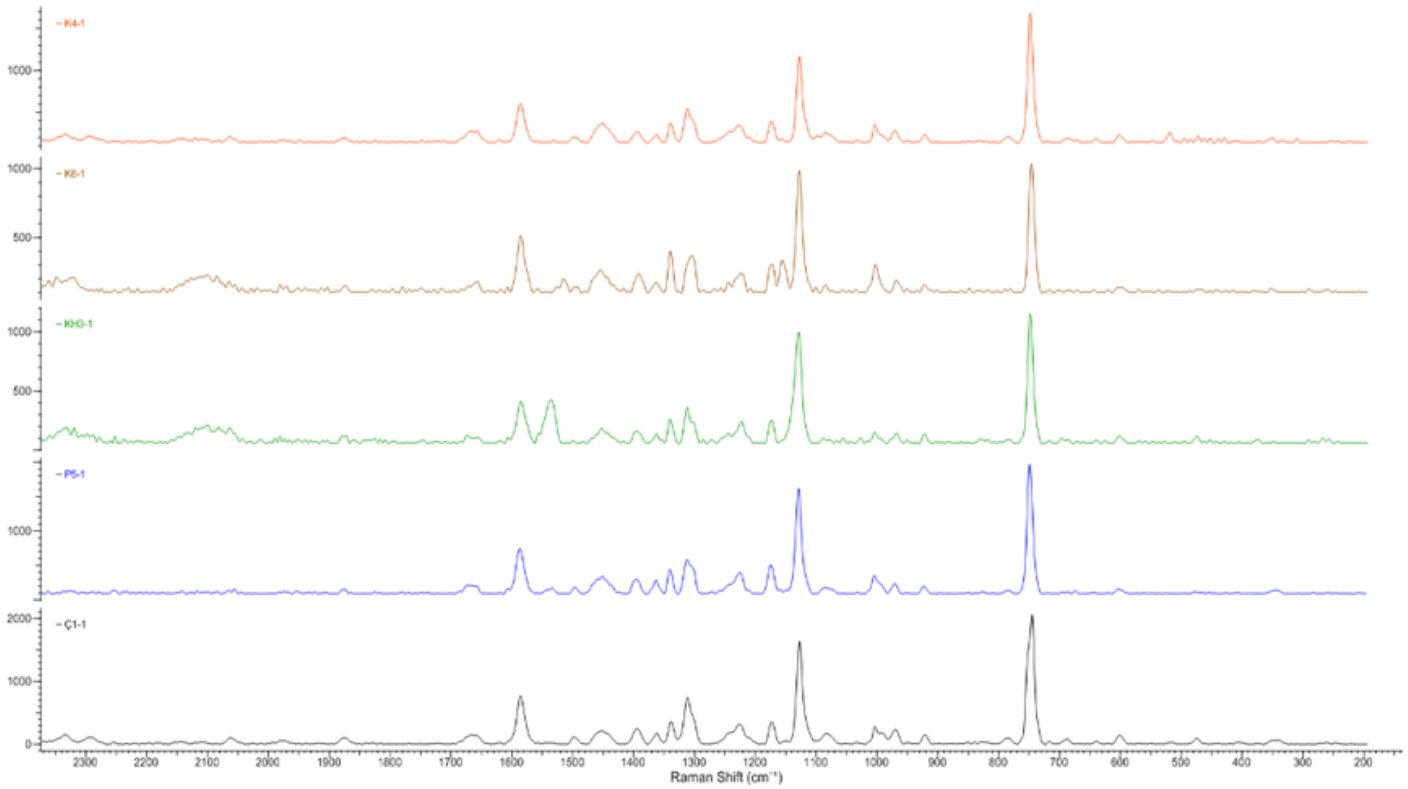

Figure 2

Raman spectra of the samples (Ç1-1: *P.putida*, P5-1: *B. mojavensis*, K6-1: *B. drentensis*, K4-1: *P. fluorescens*, KH3-1: *A. nitroguajacolicus*)

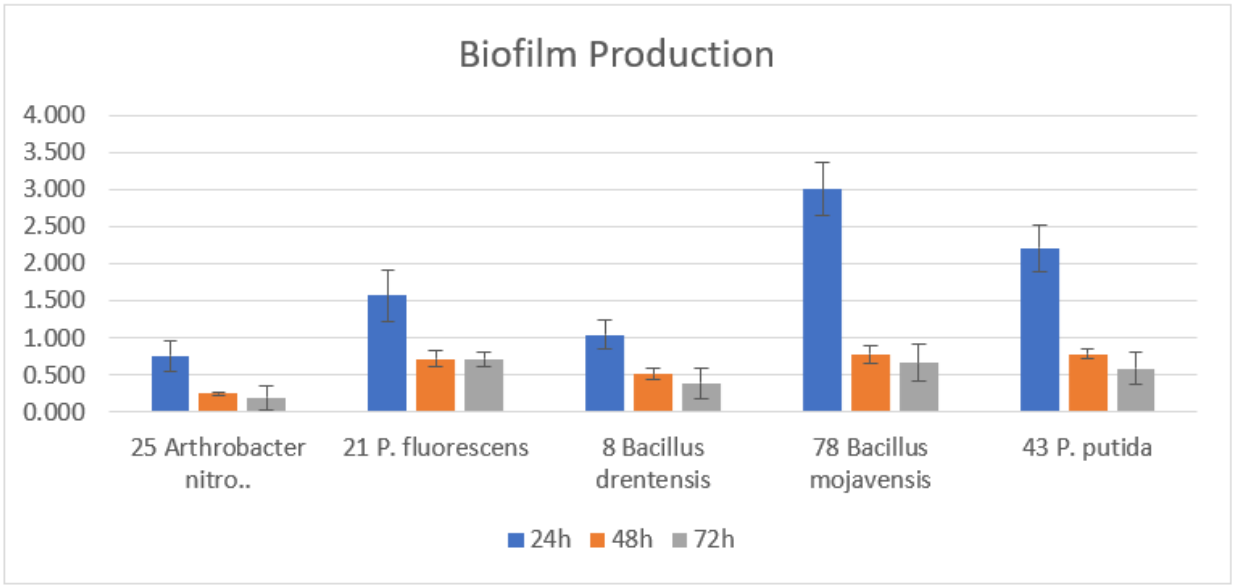

Figure 3

Biofilm formation amounts of *Bacillus drentensis*, *Bacillus mojavensis*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Arthrobacter nitroguajacolicus* bacteria.

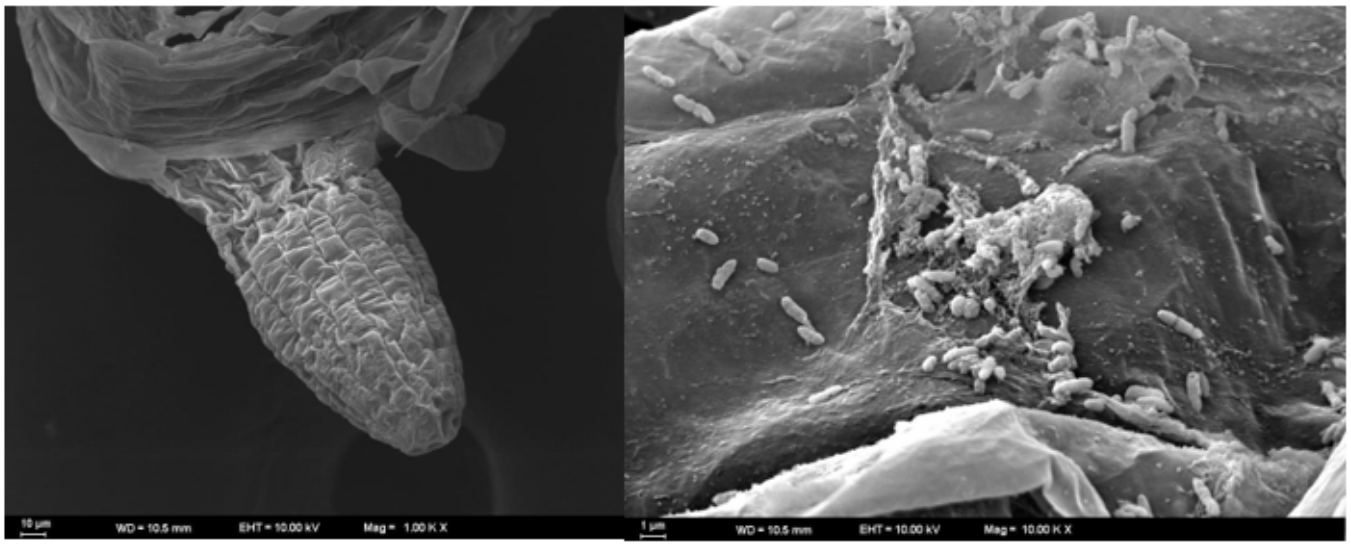

Figure 4

SEM image of the colonization of *B. drentensis* bacteria in the root of the *A. thaliana* Columbia ecotype.

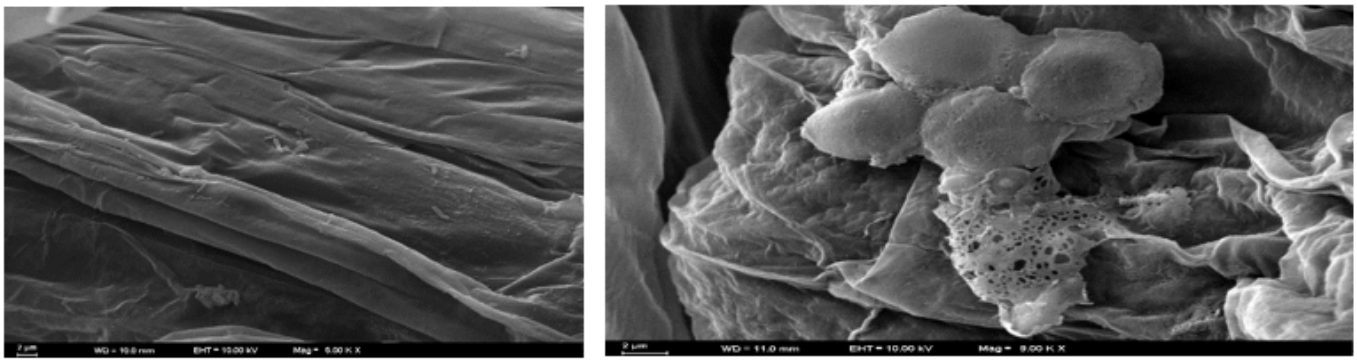

Figure 5

SEM image of the colonization of *P. fluorescens* bacteria in the root of the *A. thaliana Columbia* ecotype.

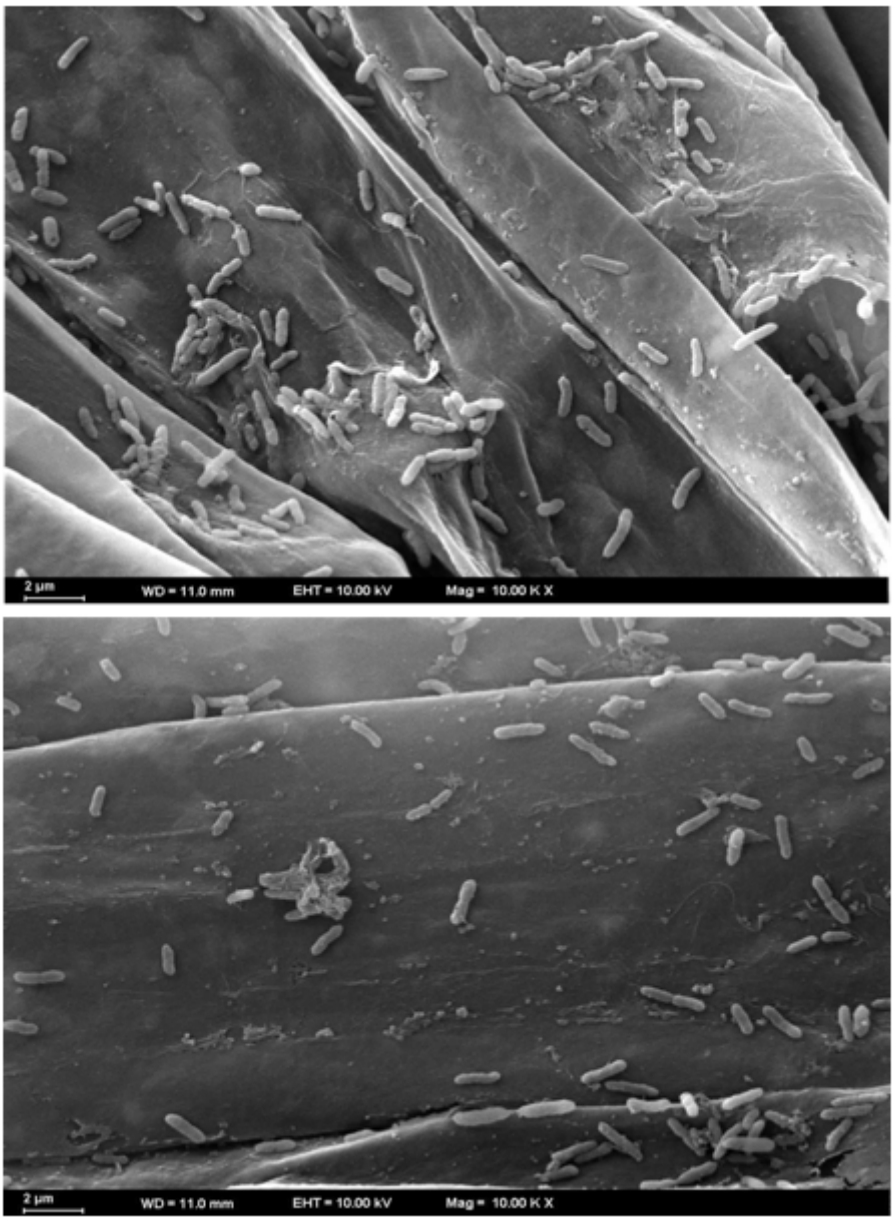

Figure 6

SEM image of the colonization of *A. nitroguajacolicus* bacteria in the root of the *A. thaliana Columbia*ecotype.