J. Agr. Sci. Tech. (2022) Vol. 24(4): 951-960

Differences among *Bacillus velezensis* Strains from Biofilm Formation to Competition in Niche Determination on Plant Roots

A. Lagzian[1], R. Saberi Riseh[1*], S. Sarikhan[2], M. Moradzadeh-Eskandari[3], and P. Khodaygan[1]

ABSTRACT

Biofilm formation and rhizosphere colonization of the plants are the main infrastructures for the biological control of the plant diseases. Bacteria accumulation in the protective layer, which results from their self-production of Exopolysaccharides (EPS), is called the biofilm. The formation of these complex structures originates from the multicellular behaviors of bacteria. Various elements can play a role in these mechanisms. In this study, we examined biofilm formation, root colonization, and salt tolerance to four concentrations of NaCl in the strains of *Bacillus velezensis* (Q12, US1, and UR1). The results showed that the biofilm strength plays an important role in the efficiency of tomato root colonization. Furthermore, UR1 that had defects in producing the surfactin, iturin, and fengycin using Ultrahigh-Performance Liquid Chromatography-High Resolution Electrospray Ionization Mass Spectrometry (UHPLC-HRESIMS), was incapable of tolerance to salinity, biofilm formation, competition, and rhizosphere colonization. Confocal Laser Scanning Microscopy (CLSM) studies showed that strains US1 and Q12 differed in the biofilm strength, the position of the bacteria that are located laterally, polar, or both, and root colonization. Q12 was introduced as the best strain in all these experiments. Also, based on the findings of this and previous studies, the possibility to create the subpopulations influenced by genetic diversity in *Bacillus velezensis* strains during biofilm formation is suggested.

Keywords: Electroporation, GFP, Rhizobacteria, Root colonization.

INTRODUCTION

Rhizobacteria are root-associated bacteria that coexist with the plant in symbiotic relationships (Cain *et al*., 2011; Kevin, 2003). Various biocontrol mechanisms by rhizobacteria have been identified so far including antibiosis, competition, Induced Systemic Resistance (ISR), protective biofilm formation (Ruiu, 2020).

To achieve the beneficial effects of PGPR (Plant Growth Promoting Rhizobacter), they should provide the minimum population density around the roots (Das and Dkhar, 2011; Lugtenberg and Kamilova, 2009). Therefore, efficient rhizosphere colonization is an essential step to ensure the advancement of growth and safety of plant (Bertin *et al*., 2003).

A biofilm is a community of adhesive bacterial cells, which are maintained and protected by an extracellular matrix. The major components of matrix that are produced in *Bacilli* are EPS and TasA,

[1] Department of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Islamic Republic of Iran.
[2] Molecular Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Islamic Republic of Iran.
[3] Department of Plant Protection Research, Khorasan Razavi Agricultural and Natural Resources Research and Education Center (AREEO), Mashhad, Islamic Republic of Iran.
*Corresponding author; e-mail: r.saberi@vru.ac.ir

which are obtained via expressing *eps* (A-O) operon and *tapA-sipW-tasA* operon, respectively (Kovács and Dragoš, 2019; Branda *et al*., 2005; Branda *et al*., 2001).

Among the biocontrol agents, the lipopeptide derivatives in *Bacillus* sp., which are called fengycin, iturin, and surfactin, are characterized by variability in the peptide chains and fatty acids. In addition to controlling the plant diseases by antifungal and antibacterial properties, they could induce the resistance (Zaman and Toth, 2013; Henry *et al*., 2011) and help to form biofilm and colonize of plant roots (Zeriouh *et al*., 2014; Beauregard *et al*., 2013).

In the evolution of bacilli, it was observed that the highest matrix production causes the stronger biofilm, such as the wrinkled phenotype, which is accompanied via colonies of rough and spreader, and in the smooth morphotype lowermost matrix production that is downscale to the ancestor. In a complex structure, the position of cells is determined by the matrix (Kovács and Dragoš, 2019; Kim *et al*., 2014; Xavier and Foster, 2007). Some studies showed that *Bacillus subtilis* strains that form biofilm also compete against competitors to gain their ecological niche by root colonization (Luo *et al*., 2015; Bais *et al*., 2004; Ahimou *et al*., 2000).

Surfactin has more antibacterial properties than antifungals; nevertheless, it can have synergistic effects on iturin (Deravel *et al*., 2014). Surfactin is known as a prerequisite for signaling the pathway to form a biofilm (Bais *et al*., 2004). Thus, surfactin is necessary for the biofilm development and roots colonization (Xu *et al*., 2019; Zeriouh *et al*., 2014; Bais *et al*., 2004). Furthermore, cyclic lipopeptides mycosubtilin and bacillomycin D, which belong to the iturin family, facilitate biofilm development and colonization of plant roots (Xu *et al*., 2013; Ongena and Jacques, 2008).

In previous studies, *Bacillus velezensis* strains Q12, US1, and UR1 were different in controlling the fungi and bacteria plant pathogens *in vitro* and *in vivo*. Moreover, studies showed that they differed in the production or non-production of bioactive lipopeptides such as surfactin, fengycin, and iturin. In this study, we aimed to survey these strains from the perspective of the possibility of biofilm formation and colonization of tomato roots using a labeled strain with Green Fluorescent Protein (GFP). This experiment will be helpful in introducing the strains as biofertilizers.

MATERIALS AND METHODS

Microorganisms and Plasmids

Regarding our previous studies, we obtained *Bacillus velezensis* strains Q12, US1, and UR1 from among 500 bacterial isolates of *Solanaceae* family rhizosphere, and *Pseudomonas chlororaphis* VUPf5 from the peach rhizosphere (Lagzian *et al*. 2021). They are the best strains to control many bacterial and fungal pathogens of plants. Since some gram-negative bacteria are good colonizers, especially *P. chlororaphis* VUPf5 (in many of our previous experiments) that had high capabilities for bio-control of plant pathogens, it was selected as a control in these experiments. For long-term storage, a loop of overnight culture bacteria was suspended and homogenized in the microtubes containing 65% rich LB (Lysogeny Broth, Carl Roth, Germany) and 35% glycerol, then, stored at -80°C. To investigate tomato root colonization, strain UR1 was labeled by plasmid pTB603 (pNW33n-hyGFP) of *Escherichia coli*. The plasmid extraction was carried out by High Pure Plasmid Isolation Kit (Merck, Roche, Germany).

Electroporation Assay

From an overnight culture of strain UR1 on a plate, we took a single colony and transferred it into 2 mL liquid LB media, and incubated it on a shaker at 30℃, 220 rpm, for 16 hours. Then, the bacteria suspension (1 mL) was inoculated into 100 mL LB media. The growth of bacteria was detected by the measurement of OD600

using spectrophotometer until it reached 0.2-0.3, which was checked every 30 minutes. The next step included cell wall-weakening treatment by adding sterile glycine solution 3% (w/v) and located for 1 hour at 30℃, 220 rpm. All steps were cold and performed on the ice from this stage. Then, 16 mL ice-cold electroporation buffer (Sucrose 272 mmol L^{-1}, $MgCl_2$ 0.5 mmol L^{-1}, K_2Hpo_4 0.5 mmol L^{-1},, KH_2po_4 0.5 mmol L^{-1}) was used to prepare the electrocompetent cells, after cooling down cells on ice (15 minutes) and centrifuging at 2,700 rpm at 4℃ for 15 minutes and discarding the supernatant. We repeated this step three times, then, added electroporation buffer and saved the tubes at -80℃. Afterwards, 1.0 µg of plasmid pTB603 and 100 µL of the competent cells (chloramphenicol resistant) were mixed and incubated on the ice for 10 minutes. Then, 100 µL of the final suspension was added to the electroporation cuvette. Electroporation was carried out by the 25 µF, 200Ω and from 1.5 to 3.0kV and using Micro Pulser Electroporator (Bio-Rad Ltd., Richmond, USA). Furthermore, the contents of cuvettes were quickly transferred to 2 mL LB media and incubated at 30℃, 220 rpm for 2 hours, then, spread on the petri dishes containing chloramphenicol 10 µg mL^{-1} and were incubated at 30℃ for 24-48 hours. Producing the GFP per transformant was tested using the fluorescence stereomicroscope (Infinitive F200 PRO, TECAN), (Peng *et al*., 2009).

Artificial Soil

In an experiment, to study the colonization on tomato roots, we used a hydroponic medium substrate instead of soil. Dropping of a sterile solution of sodium alginate and phytagel (1:4, ratio) into the stirrer solution $CaCl_2$ 2% created the spherical gel beads, which were then saturated with the nutrient solution TSB 3% for 24 hours. Finally, we drained and used inside glass tubes for planting tomato seedlings (Ma *et al*., 2019).

Root Colonization Assay

Seed surface sterilization is an important step using sodium hypochlorite solution 2 % (w/v), which was discarded; then, we used Ethanol 70%, shook for 1 minute and discarded the solution. The seeds were rinsed four times by sterile MiliQ water and every time centrifuged for1 min at 11,000 rpm. The seeds were located on MS agar (2.2 g L^{-1}+Agar 1%) plates (Murashige and Skoog, Sigma). The sealed plates were left at 25℃ for 2-4 days, allowing enough space among the seeds. Then, they were placed at a growth chamber at 22℃, 17 hours light every day, for 3 days. Afterwards, the seedlings were placed in microtubes containing 24 hours culture of bacteria that were diluted to OD 0.3. Then, they were transferred to glass tubes containing artificial soil and incubated at 21℃, 16 hours light per day, for 3 days (Dragoš *et al*., 2018). Afterwards, the roots were rinsed four times and fixed on the glass slides for imaging by CLSM. Fluorescent reporter incitement was carried out for GFP (488 nm). The emitted fluorescence was performed at 490-543 nm for green fluorescence. Images were analyzed with ImageJ by Fiji software.

Bacterial Biofilm Formation

Bacterial strains were cultured into LB media and incubated for 17 hours at 30°C at 200 rpm. To investigate the pellicle growth, 20 µL of the bacterial suspension was inoculated into MSgg media, located inside a 24-well plate (Fan *et al*., 2017; Branda *et al*., 2001). Pellicle biofilms were incubated at 30°C and imaged after 3 days.

Salt Tolerance Assay

The tolerance of biocontrol strains to different amounts of salt was measured in vitro. Four levels of NaCl, i.e. 0, 1, 5, and 10%, were added to the tubes containing LB

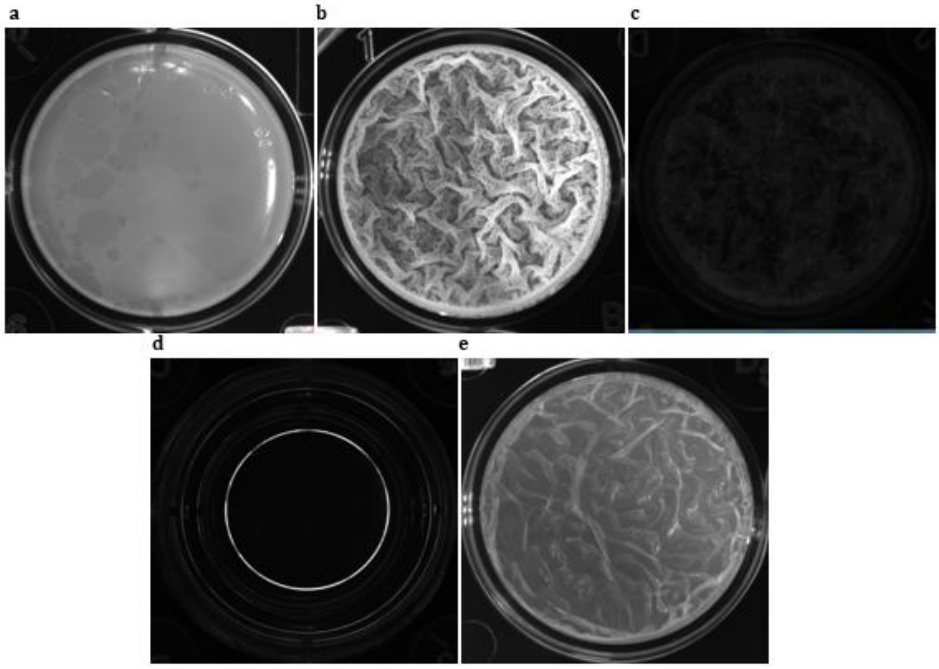

Figure 1. Biofilm formation on the pellicle (MSgg media). Treatments were imaged after 3 days using the stereomicroscope: **(a)** *B. velezensis* UR1, (**b)** *B. velezensis* Q12, (**c)** *B. velezensis* US1, (**d)** Negative control, (e) *P. chlororaphis* VUPf5, and (**f)** *B. velezensis* UR1.

media. From an overnight culture of bacteria, 10^8 CFU was inoculated into each tube; as a blank, for each salt concentration, the same amount of LB was added without bacteria. The tubes were incubated at 30°C, 220 rpm for 24 hours. Bacterial populations were measured by OD 600 nm using a spectrophotometer. Samples had 3 replications, and the experiment was repeated twice.

RESULTS AND DISCUSSION

Biofilm Formation and Root Colonization

B. velezensis Q12 created a strong biofilm on the pellicle. The biofilm structure in strain US1 was seen with some weak points, while in strain UR1, biofilm structure was not observed. Strain VUPf5 was also able to create a biofilm (Figure 1).

Confocal Laser Scanning Microscopy was used for the visualization of root colonization trial and the bacteria replacement arrangement on the tomato root. The complex structures of biofilm were well seen in *Bacillus velezensis* Q12 on the roots. Moreover, bacterial connections in strain US1 were reduced and delayed in biofilm formation on roots so that the bacterial connections were made only by the cellular poles, while Q12 colonized the complex structure and tangled coil around the root tip of the tomato. Cellular polar and lateral connections were well seen in its biofilm matrix. These findings were consistent with the biofilm robust structure of *B. velezensis* Q12 on the pellicle (Figure 2). In previous reports, a significant relationship was seen between lateral and polar flagella mutants in Gram-negative *Aeromonas* spp with adhesion levels and biofilm formation on surfaces. Every flagellar mutant indicated a reduced capability to form biofilms (Kirov *et al*., 2004). Since *Bacilli* have peritrichous flagella (Rigolet *et al*., 2019; Fujii *et al*., 2008), the question arises whether the lack of lateral cell attachment may be related to the dysfunction of lateral flagella in US1 on the root. Weak points on the pellicle in the strain US1 may be due to the lack of extracellular matrix production in several monoculture cells. Martin *et al.* (2020) showed that genetic diversity in matrix gene

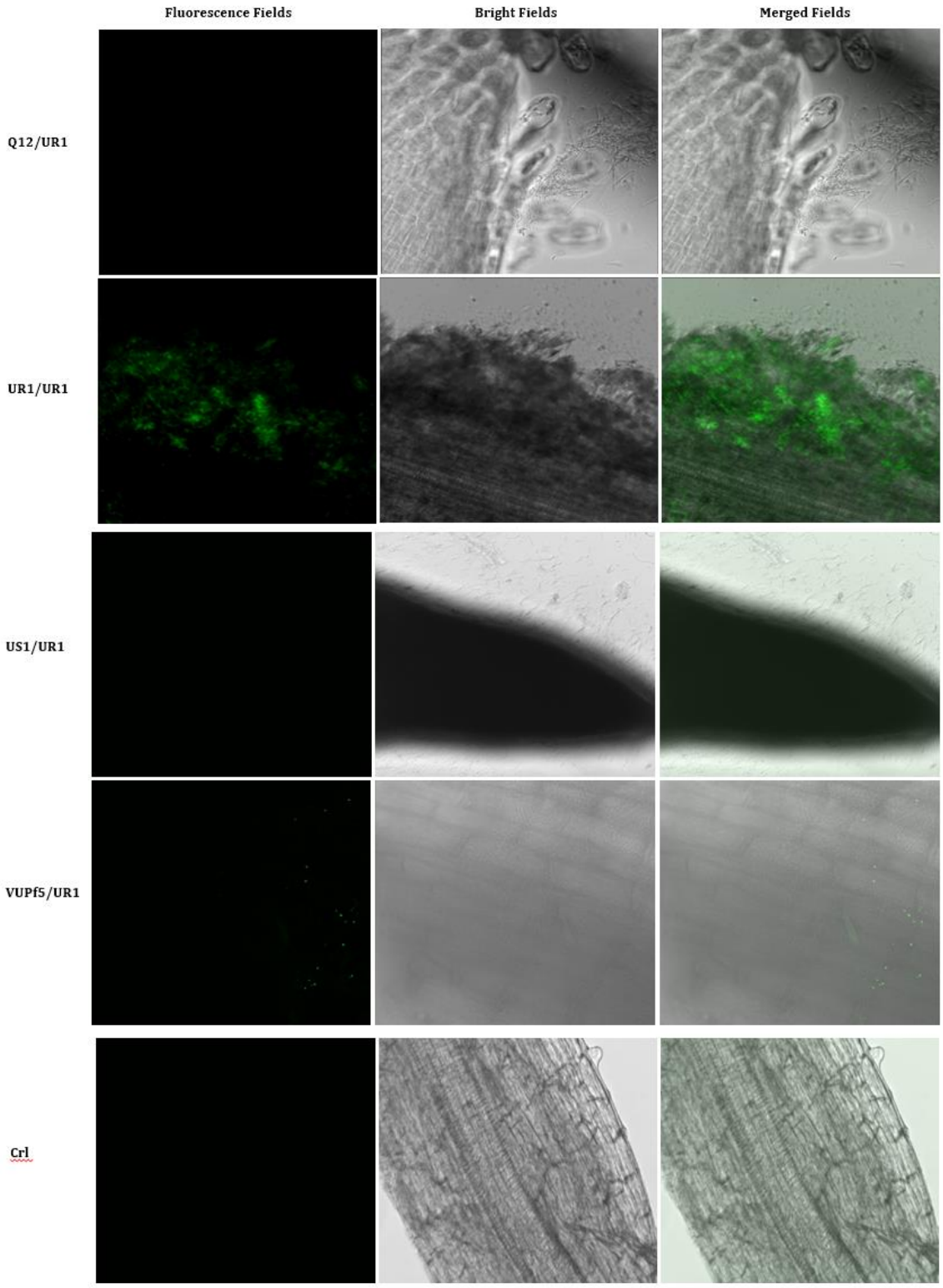

Figure 2. Investigation of the root tip colonization of tomato by mixed bacterial treatments. Crl: Negative Control.

expression in the *Bacillus subtilis* strains leads to the heterogeneous populations. Since the production of these proteins is costly, the subpopulations of bacteria tend to cheat. These genetic changes can be called morphotypes with weak points in the pellicle biofilm structure (Otto *et al*., 2020).

In co-culture treatments, the competition between UR1 with other strains in the colonization of Solanaceae roots showed the inefficiency of UR1 compared to its counterparts' strains, where no fluorescent was detected in its mixed treatments with Q12 and US1. Moreover, a limited amount of fluorescent was detected in the treatment UR1+VUPf5 (Figure 2). Strains *B. velezensis* are known as good root colonizers (Al-Ali *et al*., 2017). Based on our previous results, bioactive cyclic lipopeptides such as surfactin, fengycin, and iturin were detected in US1 and Q12 using UHPLC-HRESIMS. These secondary metabolites are not produced in the treatment UR1. Mutations in the *sfp* gene showed that this gene is required for the synthesis of lipopeptides and polyketides (McLoon *et al*., 2011). Cyclic lipopeptides surfactin, fengycin, and iturin are not only involved in plant health by antifungal and antibacterial activity, but are also essential for biofilm formation, competition for ecological niche, and colonization of rhizosphere (Zeriouh *et al*., 2014; Beauregard *et al*., 2013; Bais *et al*., 2004). Some authors believe that bacillomycin L and surfactin in *B. subtilis* 916 synergistically, caused biocontrol of rice sheath blight via antifungal acting, facilitating the formation of biofilms and increasing the abilities of plant colonization (Luo *et al*., 2015). Non-producing surfactin in *B. velezensis* FZB42 showed a difference in the rhizosphere colonization and mutations *eps*$^{-}$ not able to colonize tomato roots and to form a biofilm (Al-Ali *et al*., 2017). UR1 is not only weak to control the fungal and bacterial pathogens, but also failed to form biofilms on the pellicle. Furthermore, it showed poor competition to occupy the root surface compared to strong strains Q12, US1, and VUPf5. The mutations in genes *sfp*, *epsC*, *swrA*, and *degQ* in *B. subtilis* 3610 led to the loss of disease control and root colonization. Moreover, this bacterium was unable to form biofilm. These four genes and gene *spo0F* were located in a large plasmid (Fan *et al*., 2018; McLoon *et al*., 2011). In *Bacillus velezensis* Δ*sfp* mutants, the biosynthesis of wall teichoic acid-synthesizing protein, GgaA, was reduced 4-fold. This deficiency also delayed the formation of biofilm and reduced the colonization of cucumber root (Xu *et al*., 2019).

Salt Tolerance Assay

Salinity tolerance was surveyed at four levels of 0, 1, 5, and 10% of NaCl that were added to the LB medium. Like others, UR1 showed better growth with the addition of 1% of salt. Furthermore, population growth in strain UR1 in normal media, 1% and 5% was much higher than other strains. It is possible that due to mutations in some genes, the bacterium does not expend energy on the production of secondary metabolites, so, it can reproduce more. Strain Q12 could grow at 10% salt and strain US1 grew slightly but UR1 did not grow at this salt concentration (Figure 3). It was shown that the exoenzyme levansucrase helps *Gluconacetobacter diazotrophicus* tolerate excessive salt and sucrose concentrations, drought, and biofilm formation. The levan could protect versus stress, which are affected by living or non-living agents and contribute to the biofilm organization (Velázquez-Hernández *et al*., 2011). Salinity tension greatly stimulates the biosynthesis of levansucrase in *Bacillus* sp. two-component system DegS/DegU that plays a key role in the salt stress (Kunst and Rapoport, 1995). Due to the large and extensive changes in UR1, it seems that mutation or deletions have occurred in several genes and the main systems.

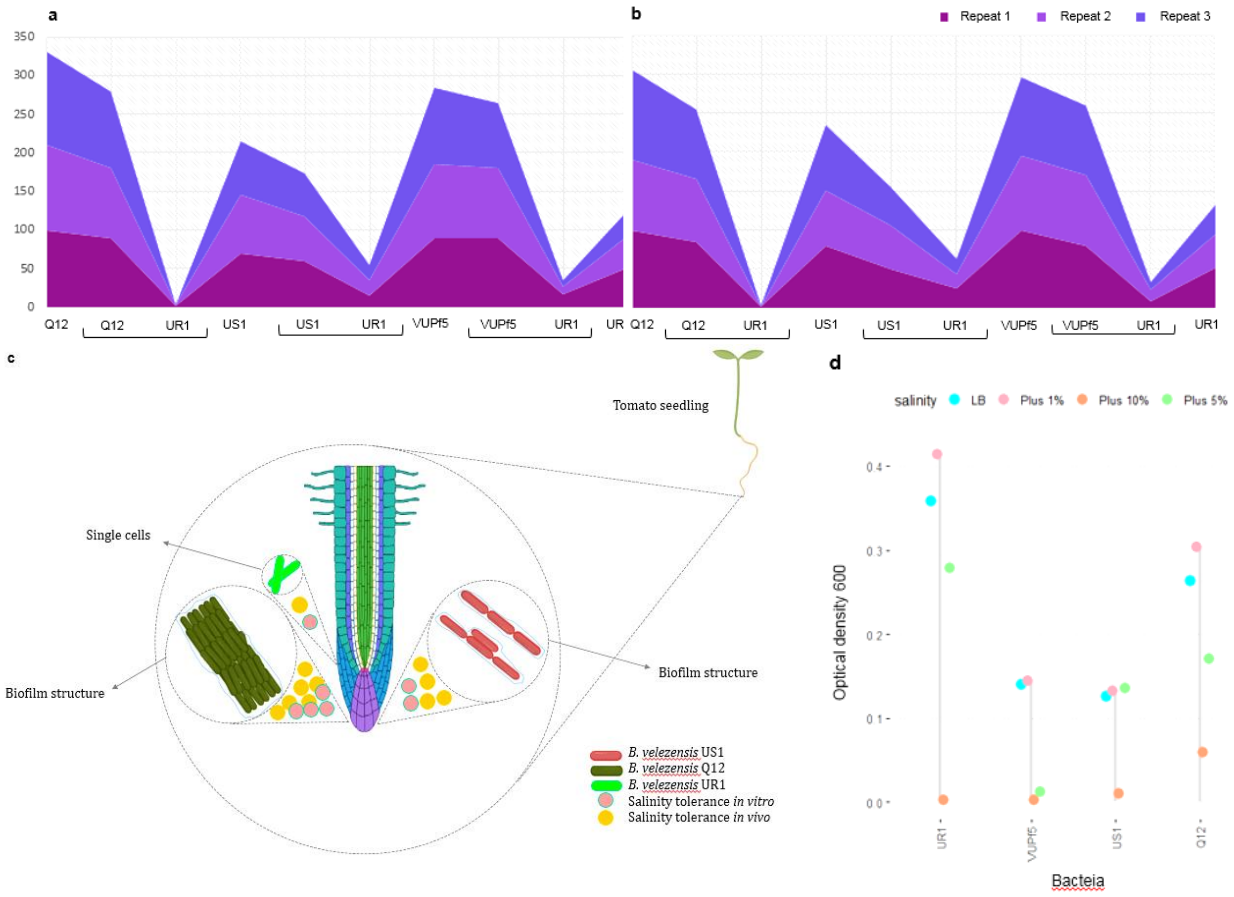

Figure 3. The population of each bacterium in separate and mixture treatments obtained from tomato root dilution series after two days and repeating the experiments two times (**a** and **b**), (**c)** Schematic of how the bacteria are located around the tomato root, and the tolerance of the bacteria to the amount of NaCl around the root, and (**d)** Bacterial populations at different NaCl concentrations.

ACKNOWLEDGEMENTS

The authors appreciate Vali-e-Asr University of Rafsanjan to provide the research funding, and

from Dr. Sara Gharouni-Kardani at Plant Protection Research Department, Khorasan Razavi Agricultural and Natural Resources Research and Education Center. AREEO, Mashhad, Iran; for her research help.

REFERENCES

1. Ahimou, F., Jacques, P. and Deleu, M. 2000. Surfactin and Iturin A Effects on *Bacillus subtilis* Surface Hydrophobicity. *Enzyme Microb. Technol*., **27(10):** 749–754.
2. Al-Ali, A., Deravel, J., Krier, F., Béchet, M., Ongena, M. and Jacques, P. 2017. Biofilm Formation Is Determinant in Tomato Rhizosphere Colonization by *Bacillus velezensis* FZB42. *Environ. Sci. Pollut. Res*., **25(30):** 29910-29920.
3. Bais, H. P., Fall, R. and Vivanco, J. M. 2004. Biocontrol of *Bacillus subtilis* against Infection of Arabidopsis Roots by *Pseudomonas syringae* Is Facilitated by Biofilm Formation and Surfactin Production. *Plant Physiol*., **134(1):** 307–319.
4. Beauregard, P. B., Chai, Y., Vlamakis, H., Losick, R. and Kolter, R. 2013. *Bacillus subtilis* Biofilm Induction by Plant Polysaccharides. *PNAS USA*, **110(17):** E1621-E1630.

5. Bertin, C., Yang, X. and Weston, L.A. 2003. The Role of Root Exudates and Allelochemicals in the Rhizosphere. *Plant Soil*, **256:** 67–83.
6. Branda, S. S., González-Pastor, J. E., Ben-Yehuda, S., Losick, R. and Kolter, R. 2001. Fruiting Body Formation by *Bacillus subtilis*. *PNAS USA*, **98(20):** 11621–11626.
7. Branda, S. S., Vik, S., Friedman, L. and Kolter, R. 2005. Biofilms: The Matrix Revisited. *Trends Microbiol*, **13(1):** 20 –26.
8. Cain, M. L., Bowman, W. D. and Hacker, S. D. 2011. Ecology. Chapter 16: "*Change in Communities".* Sinauer Associates, Oxford University Press, Sunderland, MA, PP. 359–362.
9. Das, B. B. and Dkhar, M. S. 2011. Rhizosphere Microbial Populations and Physicochemical Properties as Affected by Organic and Inorganic Farming Practices. *Am Eurasian J. Agric. Environ. Sci.*, **10(2):** 140–150.
10. Deravel, J., Lemiere, S., Coutte, F., Krier, F., Van, H. N., Bechet, M. and Jacques, P. 2014. Mycosubtilin and Surfactin Are Efficient, Low Ecotoxicity Molecules for the Biocontrol of Lettuce Downy Mildew. *Appl. Microbiol. Biotechnol*., **98(14):** 6255–6264.
11. Dragoš, A., Kiesewalter, H., Martin, M., Hsu, C. Y., Hartmann, R., Wechsler, T., Eriksen, C., Brix, S., Drescher, K., Stanley-Wall, N., Kümmerli, R. and Kovács, Á. T. 2018. Division of Labor during Biofilm Matrix Production. *Curr. Biol*., **28(12):** 1903-1913. e5.
12. Fan, B., Blom, J., Klenk, H. P. and Borriss, R. 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "Operational Group *B. amyloliquefaciens*" within the *B. subtilis* Species Complex. *Front. Microbiol*., **8(22)**. 1-15
13. Fan, B., Wang, C., Song, X., Ding, X., Wu, L., Wu, H., Gao, X. and Borriss, R. 2018. *Bacillus velezensis* FZB42 in 2018: The Gram-Positive Model Strain for Plant Growth Promotion and Biocontrol. *Front. Microbiol*., **9(2491)**.
14. Fujii, M., Shibata, S. and Aizawa, S-I. 2008. Polar, Peritrichous, and Lateral Flagella Belong to Three Distinguishable Flagellar Families. *J. Mol. Biol*., **379(2):** 273–283.
15. Henry, G., Deleu, M., Thonart, P. and Ongena, M. 2011. The Bacterial Lipopeptide Surfactin Targets the Lipid Fraction of the Plant Plasma Membrane to Trigger Immune-Related Defense Responses. *Cell. Microbiol*., **13(11):** 1824–1837.
16. Kevin, V. J. 2003. Plant Growth Promoting Rhizobacteria as Biofertilizers. *Plant Soil*, **255(2):** 571–586
17. Kim, W., Racimo, F., Schluter, J., Levy, S. B. and Foster, K. R. 2014. Importance of Positioning for Microbial Evolution. *PNAS USA*, **111(16):** E1639-E1647.
18. Kirov, S. M., Castrisios, M. and Shaw, J. G. 2004. *Aeromonas flagella* (Polar and Lateral) Are Enterocyte Adhesins that Contribute to Biofilm Formation on Surfaces. *Infect. Immun.*, **72(4):** 1939–1945.
19. Kovács, Á. T. and Dragoš, A. 2019. Evolved Biofilm: Review on the Experimental Evolution Studies of *Bacillus subtilis* Pellicles. *J. Mol. Biol*., **431(23):** 4749-4759
20. Kunst, F. and Rapoport, G. 1995. Salt Stress is an Environmental Signal Affecting Degradative Enzyme Synthesis in *Bacillus subtilis*. *J. Bacteriol.*, **177(9):** 2403–2407.
21. Lagzian, A and Saberi Riseh, R. 2021. Genome Mining Conformance to Metabolite Profile of Bacillus Strains to Control Potato Pathogens. *Appl. Microbiol. Biot*. In press.
22. Lugtenberg, B. and Kamilova, F. 2009. Plant-Growth-Promoting Rhizobacteria. *Annu. Rev. Microbiol*., **63:** 541–556.
23. Luo, C., Zhou, H., Zou, J., Wang, X., Zhang, R., Xiang, Y. and Chen, Z. 2015. Bacillomycin L and Surfactin Contribute Synergistically to the Phenotypic Features of *Bacillus subtilis* 916 and the Biocontrol of Rice Sheath Blight Induced by *Rhizoctonia solani*. *Appl. Microbiol. Biotechnol*., **99(4):** 1897-1910.
24. Martin, M., Dragoš, A., Otto, S. B. Schäfer, D., Brix, S., Maróti, G. and Kovács, Á. T. 2020. Cheaters Shape the Evolution of Phenotypic Heterogeneity in *Bacillus subtilis* Biofilms. *ISME,* **14:** 2302–2312.
25. Ma, L., Shi, Y., Siemianowski, O., Yuan, B., Egner, T. K., Mirnezami, S. V., Lind, K. R., Ganapathysubramanian, B., Venditti, V. and Cademartiri, L. 2019. Hydrogel-Based Transparent Soils for Root Phenotyping *in Vivo*. *PNAS USA*, **116(22):** 11063-11068.
26. McLoon, A. L., Guttenplan, S. B., Kearns, D. B., Kolter, R. and Losick, R. 2011. Tracing the Domestication of a Biofilm-Forming Bacterium. *J. Bacteriol*., **193(8):** 2027-2034.

27. Ongena, M. and Jacques, P. 2008. *Bacillus* Lipopeptides: Versatile Weapons for Plant Disease Biocontrol. *Trends Microbiol.*, **16(3):** 115–125.
28. Otto, S. B., Martin, M., Schäfer, D., Hartmann, R., Drescher, K., Brix, S., Dragoš, A. and Kovács, Á. T. 2020. Privatization of Biofilm Matrix in Structurally Heterogeneous Biofilms. *Msystems*, **5(4):** e00425-20.
29. Peng, D., Luo, Y., Guo, S., Zeng, H., Ju, S., Yu, Z. and Sun, M. 2009. Elaboration of an Eelectroporation Protocol for Large Plasmids and Wild-Type Strains of *Bacillus thuringiensis*. *J. Appl. Microbiol.*, **106(6):** 1849-1858.
30. Rigolet, A. 2019. Molecular Crosstalk between Plant Beneficial Rhizobacteria: Identification of *Pseudomonas* Compounds Influencing the Growth and Antimicrobial Potential of *Bacillus velezensis***.** Master's Thesis, Liège University Library, University of Liège.
31. Ruiu, L. 2020. Plant-Growth-Promoting Bacteria (PGPB) against Insects and Other Agricultural Pests. *Agron.*, **10(861)**.
32. Velázquez-Hernández, M. L., Baizabal-Aguirre, V. M. and Cruz-Vázquez, F. 2011. *Gluconacetobacter diazotrophicus* Levansucrase Is Involved in Tolerance to NaCl, Sucrose and Desiccation, and in Biofilm Formation. *Arch. Microbiol.*, **193 (2):** 137-149.
33. Xavier, J. and Foster, K. R. 2007. Cooperation and Conflict in Microbial Biofilms. *PNAS USA*, **104(3):** 876–881.
34. Xu, Z., Shao, J., Li, B., Yan, X., Shen, Q. and Zhang, R. 2013. Contribution of Bacillomycin D in *Bacillus amyloliquefaciens* SQR9 to Antifungal Activity and Biofilm Formation. *Appl. Environ. Microbiol.*, **79(3):** 808–815.
35. Xu, Z., Zhang, H., Sun, X., Liu, Y., Yan, W., Xun, W., Shen, Q. and Zhang, R. 2019. *Bacillus velezensis* Wall Teichoic Acids Are Required for Biofilm Formation and Root Colonization. *Appl. Environ. Microbiol.*, **85(5):** e02116- e02118.
36. Zaman, M. and Toth, I. 2013. Immunostimulation by Synthetic Lipopeptide Based Vaccine Candidates: Structure–Activity Relationships. *Front. Immunol.*, **4:** 318.
37. Zeriouh, H., de Vicente, A., Pérez-García, A. and Romero, D. 2014. Surfactin Triggers Biofilm Formation of *Bacillus subtilis* in Melon Phylloplane and Contributes to the Biocontrol Activity. *Environ. Microbiol.*, **16(7):** 2196–2211.

از تشکیل بیوفیلم تا رقابت در تعیین نیچ *Bacillus velezensis* تفاوت بین سویه‌های اکولوژیکی روی ریشه گیاه

آ. لگزیان، ر. صابری‌ریسه، س. ساری‌خان، م. مرادزاده اسکندری، و پ. خدایگان

چکیده

تشکیل بیوفیلم و کلونیزاسیون ریزوسفر گیاهان، زیرساخت های اصلی کنترل بیولوژیک بیماری‌های گیاهی است. تجمع باکتری‌ها در لایه محافظی که از تولید اگزوپلی ساکارید (EPS) خود باکتری حاصل می شود، بیوفیلم نامیده می شود. تشکیل این ساختارهای پیچیده از رفتارهای چند سلولی باکتری‌ها ناشی می‌گردد. عناصر مختلفی می‌توانند در این مکانیسم‌ها نقش داشته باشند. در این مطالعه، تشکیل بیوفیلم، کلونیزاسیون ریشه و تحمل به نمک (NaCl)، در چهار غلظت، در سویه‌های *Bacillus velezensis*(Q12، US1 و UR1) مورد بررسی قرار گرفت. نتایج نشان داد که قدرت

تشکیل بیوفیلم نقش مهمی در کارایی کلونیزاسیون ریشه گوجه‌فرنگی دارد. علاوه بر این، UR1که در تولید سورفکتین، ایتورین و فنجایسین با استفاده از کروماتوگرافی مایع با عملکرد فوق‌العاده بالا – اسپکترومتری جرمی یونیزاسیون الکترواسپری با وضوح بالا نقص داشت، قادر به تحمل شوری، تشکیل بیوفیلم، رقابت و کلونیزاسیون در ریزوسفر نبود. مطالعات میکروسکوپ اسکن لیزری کنفوکال نشان داد که سویه های US1 و Q12 در قدرت توسعه بیوفیلم، نحوه قرارگیری باکتری‌ها به صورت جانبی، قطبی و یا هر دو، و کلونیزاسیون ریشه متفاوت هستند. Q12 به‌عنوان بهترین سویه در تمام این آزمایشات معرفی شد. بر اساس یافته‌های این مطالعه و مطالعات پیشین، امکان ایجاد زیرجمعیت‌هایی تحت تأثیر تنوع ژنتیکی در سویه‌های *Bacillus velezensis* در طی تشکیل بیوفیلم پیشنهاد می‌شود.