Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Endophytic ability of *Bacillus mojavensis* Ps17 to colonize various agronomic crop varieties

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ABSTRACT

Using endophytic microorganisms as biological agents is gaining particular interest due to their multiple properties. In this study, we evaluated the ability of the endophytic bacterium, *Bacillus mojavensis* PS17 (Russian patent RU2737208C1) isolated from surface-sterilized wheat seeds, to colonize different agronomic crops. The plant colonization ability was studied in pot experiments containing a mixture (1:1) of the garden and bulk soil moistened with distilled water to 60% of its water-holding capacity. The bacterium culture was applied on non-sterile seed as vegetative cells diluted in PBS buffer to an optical density of 0.1 at 595 nm. As a marker, a spontaneous rifampicin-mutant (15 µg mL^{-1}) of the studied strain was used. The plants were grown in a laboratory condition. After 3 weeks, their roots and shoot were surface-sterilized and plated onto LB medium agar amended with Rifampicin and nystatin. For the realities of results, bacterium grown on a selective medium was compared with their parent strain using fingerprinting BOX-PCR. The obtained results demonstrated that *B. mojavensis* PS17 can reside in tissues of all plants tested without causing any harm to plant growth and development, making it a good candidate for using as an endophyte biological agent for plant protection.

Keywords: Microbes, Biological agents, Agronomic crop varieties, Endophytic microorganisms, Agronomic crops, Parent strain.
Article type: Research Article.

INTRODUCTION

Using microorganisms as fertilizers, stimulants, and protective agents is considered a promising environmentally friendly method to reduce the negative environmental impact of agricultural practices. Biological agents are currently formulated based on their active substances derived from microbial cells, on cell microbes with or without their metabolites (cell-based formulation, powder bioformulations), etc. When applied as a cell-based formulation, they act as endophytes or epiphytes. As endophytes, they colonize and live in plant interior tissues without causing any dysfunction. Endophytes are considered as an interesting group of bacteria associated with plants due to their ability to exhibit multidimensional interactions within the host (Khare *et al.* 2018; Dewi *et al.* 2023), such as the secretion of various biological metabolites serving as biocontrol agents, antimicrobial agents, and phytohormones, the induction of the plant's immune system and promotion of nutrient uptakes from the soil (Sturz *et al.* 2000; Shen *et al.* 2019; Kloepper & Ryu 2006). Moreover, in plants, pathogens, and endophytic bacteria usually compete in the same nutritional niche, which makes endophytic microorganisms a suitable method for pathogenic control and crop performance improvement (Hallmann *et al.* 1997; Senthilkumar *et al.*

2011). Nevertheless, to produce these beneficial effects on the host plant, some endophytic bacteria should have the ability to conquer and minimize the presence of other native microorganisms in host plants, which can affect their distribution. Thus, the endophytic ability to colonize non-host plants is related not only to various factors, including the type of plant tissue, plant genotype, biotic and abiotic environmental conditions, but also to microbial communities present in plants. Based on the above mentioned, this study aimed to evaluate the ability of endophytic *B. mojavensis* PS17 to colonize most agronomic crops.

MATERIALS AND METHODS

The endophytic bacterial *B. mojavensis* PS17 (Russian patent RU2737208C1) and seeds used in this study were provided by the Centre for Agroecological Research of Kazan Agrarian State University, Kazan, Russia. The ability evaluation of *B. mojavensis* to colonize plant tissues of different crops (Table 1) was conducted under laboratory conditions, based on the presence of *B. mojavensis* PS17 in plant tissues without causing any harm. So, *B. mojavensis* PS17 spontaneous rifampicin-mutants (Rif) were used as a marker.

Table 1. Plants used in this study.

Plant varieties
Emmer, *T. dicoccum*
Spring wheat, *T. aestivum*
Soybeans, *Glycine max*
Barley, *Hordeum vulgare*
Pea, *Pisum sativum*

The bacterial cell suspension was prepared from an overnight culture of *B. mojavensis* PS17 (Rif) grown in LB medium, three times washed, and diluted with PBS buffer to an optical density of 0.5 at 595 nm. Non-sterile seeds were inoculated in a cell suspension for 25 min and dried in a laminar flow hood. For control, seeds were inoculated with sterile tap water. Further, seeds were sown into pots containing a mixture (1:1) of the garden and bulk soil moistened with distilled water to 60% of its water-holding capacity. Pots were then placed in the growth chamber with an average temperature of 26 ºC, 70% humidity, and 16: 8h day-night light cycle. Pots were watered with tap water at a frequency of 2 times/day. After 3 weeks of cultivation, plant roots and shoots were analysed for colonization ability. So that, plant roots and shoots were surface-sterilized according to (Simon *et al.* 1996) , plated onto a Lysogeny broth medium [LB (g L^{-1}): 10 g, tryptone 5 g, yeast extract 5 g, NaCl 20 g, agar] amended with rifampicin (100 ug mL^{-1}) and nystatin. Plates were then overnight incubated at 28 ºC ± 1. The bacteria were analysed based on their ability to grow in a selective medium. For further confirmation of the presence of *B. mojavensis* PS17 (Rif) on selective medium, a comparative genomic analysis was also carried out using Repetitive Extragenic Palindromic Sequences BOX-PCR. Total DNA genomes from bacteria were isolated using Trizol reagent, according to the manufacturer's protocol. BOX-PCR was carried in a 25-mL reaction mixture containing 5 mL 10 × PCR buffer, 10 uL mixture of deoxynucleotide triphosphates (dNTP), 1 U Taq. DNA polymerase (Evrogen, Russia), 5 µL DNA matrix, 0.4 uL primer BOXA1R (5'-CTACGGCAAGGCGACGCTGACG-3'). The amplification reaction consisted of initial denaturation at 94 °C for 5 min, followed by 35 denaturation cycles at 94 °C for 1 min, annealing at 42 °C for 1 min, elongation at 72 ° C for 2 min, with final elongation at 72 °C for 10 min. PCR products were fractionated by electrophoresis in 2.3% agarose gel in 1× TBE buffer. The gel was stained with ethidium bromide. 1 Kb DNA ladder (Eurogen, Russia) was used as a marker of molecular weight. Gel visualization was performed using the Gel Doc EZ system (Bio-Rad, USA).

RESULTS AND DISCUSSION

Microorganisms used as a biocontrol in agriculture were primarily selected for their ability to secrete compounds with high antimicrobial activities against phytopathogens causing yield losses. In our previous work, we demonstrated that *B. mojavensis* PS17 can inhibit the growth of most plant pathogens such as *Fusarium* spp., *Verticillium* spp., etc (Diabankana *et al.* 2021). Successful and early plant colonization by endophytes is a key factor for beneficial plant-microbe interactions in which the obtained mutualism can lead to the direct activation of resistance systems, such as induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Kandel *et al.* 2017; Kumar *et al.* 2020).

Therefore, the ability of *B. mojavensis* PS17 to colonize plant tissues of most agronomic crops was evaluated. The results are presented in Figs. 1 - 2. As it can be observed, the growth of inoculated bacteria was observed in all groups in which plants were pretreated with bacterial suspension of *B. mojavensis* PS17 (Rif). This suggests that *B. mojavensis* PS17 can colonize many

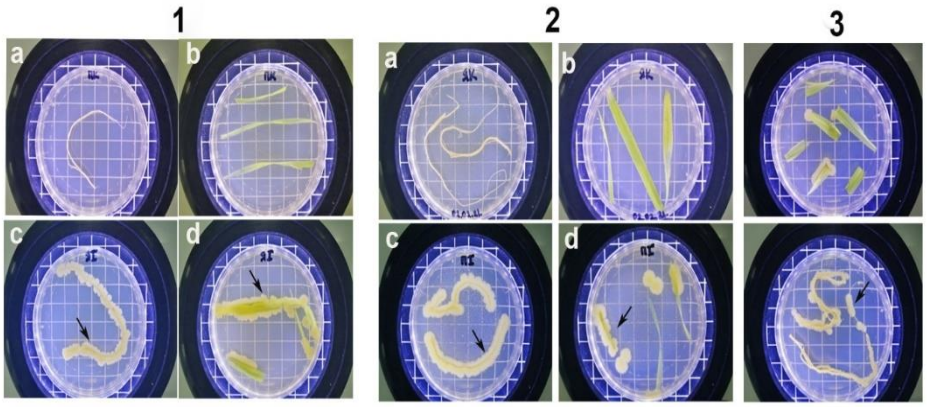

Fig. 1. The colonization ability of *B. mojavensis* PS17 (Rif) on wheat (1), barley (2), and emmer (3) grown on selective LB medium agar. Arrows indicate bacterial growth; a, b: as shoot and root plants from the control group (without treatment) and (c, d) from the tested group.

non-host plants. The ability of endophyte bacteria isolated from one host plant to colonize other specific and non-specific non-host plants has been reported by previous studies. *Herbaspirillum seropedicae*, for example, has been reported to act as an endophyte for a variety of agronomic crops including maize, sorghum, sugarcane, and other Gramineae plants (Baldani *et al.* 1986; Olivares *et al.* 1996). Similar results were also reported by (Nowak *et al.* 1995; Compant *et al.* 2005). In these studies, the endophytic bacteria *Burkholderia* sp. isolated from onion (*Allium cepa* L.) has shown the ability to colonize grapes, potatoes, and other vegetables.

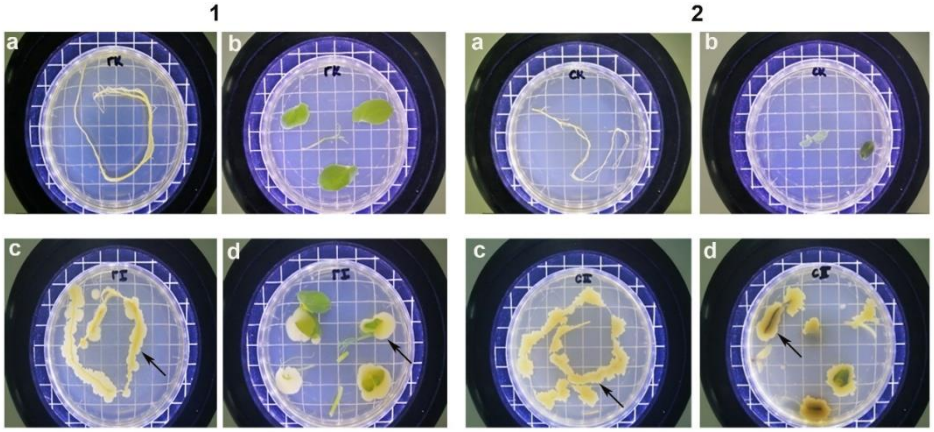

Fig. 2. The colonization ability of *B. mojavensis* PS17 (Rif) on peas (1) and soybean (2) grown on selective LB medium agar. Arrows indicate bacterial growth; (a, b) as shoot and root plants from the control group (without treatment) and (c, d) from the tested group.

Since non-sterile seeds were used, an additional test was carried out based on the genetic similarity between these bacteria grown on a selective medium and the parent strain of *B. mojavensis* PS17 (Rif). In our case, a BOX-PCR fingerprint was used. The result is represented in Fig. 3. BOX-PCR analysis showed that all the isolates produced identical amplified fragments as compared to their parent strain of *B. mojavensis* PS17 (Rif). This suggests that the isolates genetically belong to the same strain, i.e., *B. mojavensis* PS17 (Rif).

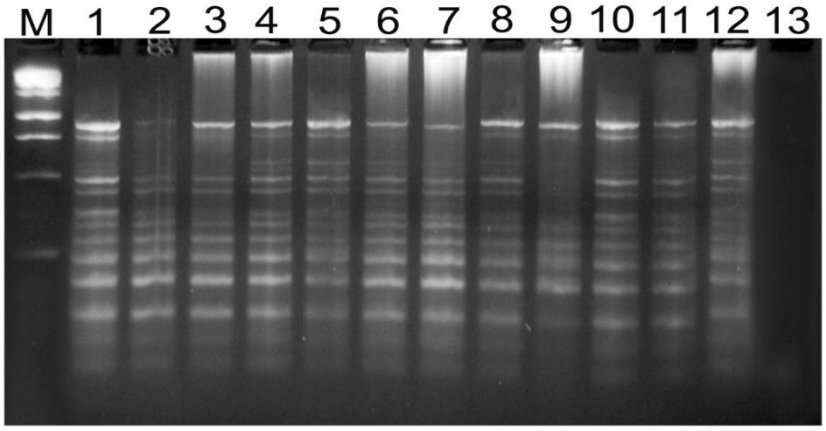

Fig. 3. Electrophoresis of BOX-PCR products of bacterial strains genome isolated from plants. BOX DNA fragments generated by bacterial strains from the shoot and root of spring wheat (1- 2), soybeans (3- 4), barley (5- 6), peas (7- 8), emmer (9- 10), positive control as *B. mojavensis* PS17 (Rif) (11- 12), and negative control (13).

SUMMARY

In this study, we demonstrated that *B. mojavensis* PS17 can colonize different closely and non-closely related host plant species of most agronomic crops. Moreover, field trials are needed to be evaluated, since, in natural environmental conditions, many factors can affect their distribution and transmission to the next plant generation.

CONCLUSIONS

Seeds that carry future plants and all the elements necessary for their germination can be considered as cargo carried by freighter. Seed-borne endophytes can be taken into account as the engine of a given cargo ship since several mechanisms involved in plant cycle development depend on their mutualism. The team of authors expresses their deep gratitude to the Kazan State Agrarian University, the center of agroecological research in conducting research.

ACKNOWLEDGEMENTS

This study has been supported by the Kazan Federal University Strategic Academic Leadership Program.

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