

# GRAIN-RESIDING ENDOPHYTIC BACTERIUM *Paenibacillus polymyxa* P 6.3 POSSESSES GROWTH- PROMOTING ACTIVITY AND PROTECT WHEAT GRAIN FROM PATHOGENIC EFFECT OF *PSEUDOMONAS SYRINGAE*

D. A. Shustyk, J. M. Yumyna, P. P. Zelena, J. V. Faidiuk

Taras Shevchenko National University, Ukraine, Kyiv

E-mail: [dasashustik@gmail.com](mailto:dasashustik@gmail.com)

Received 25.07.2022

Revised 17.08.2022

Accepted 31.08.2022

**Aim.** To examine the effect of endophytic bacteria *Paenibacillus polymyxa* P 6.3, which was isolated from grains of winter wheat variety Podolyanka, on the germination of wheat grains after the exposure *Pseudomonas syringae*.

**Methods.** Growth-promoting and biocontrol activity of *P. polymyxa* P 6.3 were examined using roll method. Standardized wheat grains were soaked in a suspension of 24 h culture of *P. polymyxa* P 6.3 for 12 h, control grains were soaked in sterile distilled H<sub>2</sub>O. After soaking, both pre-treated and control grains (of 25 pcs) were put into rolls. In three days, half of both pre-treated and control grains were exposed to phytopathogen *P. syringae*, and germination was continued. The lengths of coleoptile and main root were measured on the 7th day of the experiment. Results were expressed as  $M \pm m$ . Differences were considered significant at  $P \leq 0.05$ .

**Results.** Treatment wheat grains with *P. polymyxa* P 6.3 resulted in increased growth of coleoptile and main root in all three varieties. Most prominent effect was registered in Favorytka variety. After the exposure to phytopathogenic pseudomonads, slowing down of the growth of coleoptile and main root occurred in all wheat varieties. Highest susceptibility to *P. syringae* pathogenic effect was registered in Holikovs'ka variety. Pre-treatment of wheat grains with endophytic bacteria abrogated growth-inhibiting effects of *P. syringae*.

**Conclusion.** Endophytic bacteria *P. polymyxa* P 6.3 exerts a growth-stimulating effect on wheat germination and a protective effect against *P. syringae*. The plant growth promoting potential and antagonistic activity make strain P 6.3 a promising biocontrol agent and growth stimulator as a biofertilizer.

**Key words:** endophytes, wheat, *Paenibacillus polymyxa*, *Pseudomonas syringae*, roll method.

Plant Probiotic Microorganisms (PPM) are useful, plant-associated microorganisms that provide a promising alternative to chemical fertilizers and pesticides as biofertilizers and biostimulants [1]. Particular attention is focused on endophytic bacteria as a group of PPM. A wide variety of endophytes with probiotic properties makes them a powerful tool in agroindustry. It is known that endophytic bacteria promote plant growth and nutrient uptake, and also have a biocontrol activity [2, 3]. Their employment

as bioinoculants is a promising practice in multitudinous parts of the world [4], but the best strategy for their application in agriculture is still unknown [5]. Since wheat is a significant food crop in Ukraine, the selection of endophytic composition and development of agrobiotechnological approaches based on them could be a new ecological way to achieve a high-quality wheat crop.

To examine the effect of endophytic bacteria *Paenibacillus polymyxa* P 6.3, which was isolated from grains of winter wheat

variety Podolyanka, on the germination of wheat grains after the exposure *Pseudomonas syringae*.

### Material and Methods

Three wheat varieties of Ukrainian selection were used in the study: two winter wheat varieties (*Triticum aestivum* L.) — Favorytka and Chyhyrynka, and one spring wheat variety (*Triticum dicoccum* Schuebl) — Holikovs'ka. Growth-promoting and biocontrol activity of *P. polymyxa* P 6.3 were examined using roll method [6]. Standardized wheat grains were soaked in a suspension of 24 h culture of *P. polymyxa* P 6.3 for 12 h, control grains were soaked in sterile distilled H<sub>2</sub>O. After soaking, both pre-treated and control grains (of 25 pcs) were put into rolls. In three days, half of both pre-treated and control grains were exposed to phytopathogen *P. syringae*, and germination was continued. The lengths of coleoptile and main root were measured on the 7<sup>th</sup> day of the experiment. Results were expressed as  $M \pm m$ . Differences were considered significant at  $P \leq 0.05$ .

### Results and Discussion

Treatment wheat grains with *P. polymyxa* P 6.3 resulted in increased growth of coleoptile and main root in all three varieties (Fig. A, B). Most prominent effect was registered in Favorytka variety: length of coleoptile has

risen by 24%, and length of main root — by 40% as compared to untreated control. Less pronounced effect was observed in Chyhyrynka variety. After the exposure to phytopathogenic pseudomonads, slowing down of the growth of coleoptile and main root occurred in all wheat varieties. Highest receptivity to *P. syringae* pathogenic effect was registered in Holikovs'ka variety: the length of coleoptile was 12% lower, and the length of main root — 30% lower than those in control untreated grains. Surprisingly, treatment with phytopathogenic bacteria led to the increase of main root length in Favorytka grains, and did not affect germination in Chyhyrynka variety. Pre-treatment of wheat grains with endophytic bacteria abrogated growth-inhibiting effects of *P. syringae*: values of lengths of coleoptile and main root in exposed grains did not differ significantly from these values in corresponding untreated control.

In this study, we found that treatment of wheat grains with endophytic bacteria, which we previously isolated from wheat variety with high resistance to causative agent of basal glume rot and leaf blight — *P. syringae* [7], stimulates growth of coleoptile and main root in three other wheat varieties of domestic selection, and abolishes negative effect of phytopathogen on grain germination. These growth-promoting and preventive effects slightly differed between examined varieties. According to literature data, wheat resistance to bacterial diseases is reached when frost

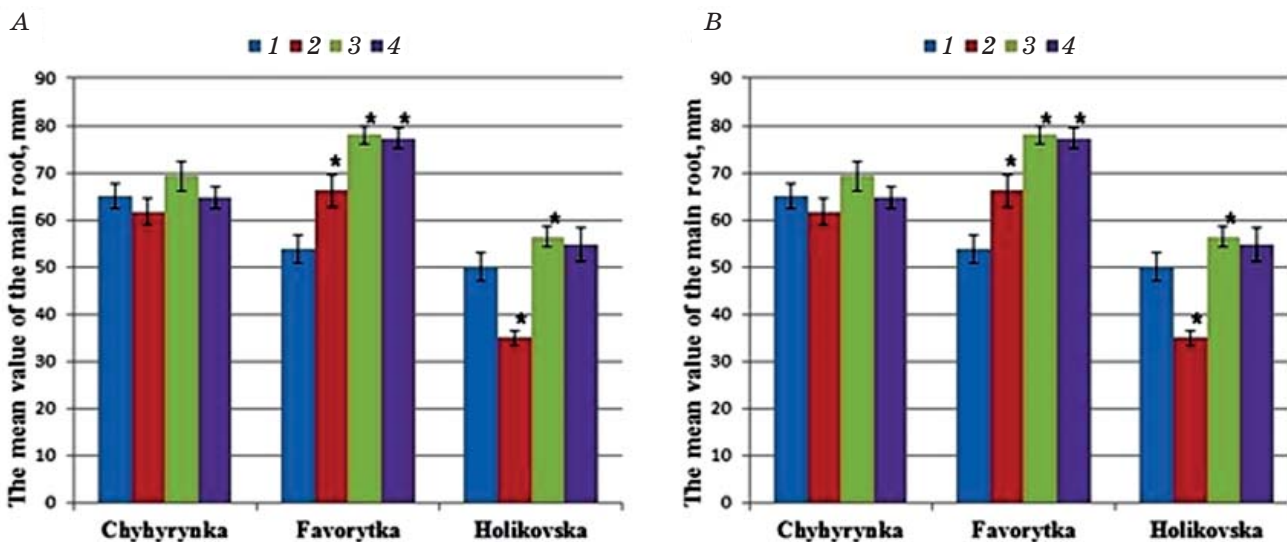


Fig. Effect of *P. polymyxa* P 6.3 on coleoptile (A) and main root growth (B) in wheat grain: 1 — control; 2 — seedlings infected with phytopathogen; 3 — grains pre-treated with *P. polymyxa* P 6.3; 4 — grains pre-treated with the investigated endophyte followed by infection of seedlings with phytopathogens,  $M \pm m$ ,  $n = 25$ ,  $*P \leq 0.05$  compared to control

tolerance and disease-resistance genes are combined in the same genetic background [8]. All three varieties are characterized by high winter hardiness and high resistance to fungal diseases. Nevertheless, high growth-promoting and phytopathogen-protecting effects of endophytic bacteria were observed in winter wheat variety Favorytka and spring wheat variety Holikovs'ka with different vulnerability to *P. syringae*. One can suppose the existence of additional determinant of high receptivity of wheat grains to positive effects of external endophytic bacteria - their own endophytic community, which governs and maintains defense responses in host plant, and probably positively perceives foreign endophytic bacterium. However, our assumption needs in further experimental validation.

### REFERENCES

1. Vandenberghe L., Garcia L., Rodrigues C., Candido Camara M., Pereira G., Oliveira J., Socol C. Potential applications of plant probiotic microorganisms in agriculture and forestry. *AIMS Microbiology*. 2017, 3(3), 629–648. <https://doi.org/10.3934/microbiol.2017.3.629>
2. Afzal I., Shinwari Z., Sikandar S., Shahzad S. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiological Research*. 2019, 221, 36–49. <https://doi.org/10.1016/j.micres.2019.02.001>
3. Ali MA., Lou Y., Hafeez R., L, X., Hossain A., Xie T., Lin L., Li B., Yin Y., Yan J., An Q. Functional Analysis and Genome Mining Reveal High Potential of Biocontrol and Plant Growth Promotion in Nodule-Inhabiting Bacteria Within *Paenibacillus polymyxa* Complex. *Front. Microbiol.* 2021, 11:618601. <https://doi.org/10.3389/fmicb.2020.618601>
4. Marag P., Suman A. Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and composite maize (*Zea mays* L.). *Microbiological Research*. 2018, 214, 101–113. <https://doi.org/10.1016/j.micres.2018.05.016>
5. Le Cocq K., Gurr S., Hirsch P., Mauchline T. Exploitation of endophytes for sustainable agricultural intensification. *Molecular Plant Pathology*. 2017, 18(3), 469–473. <https://doi.org/10.1111/mpp.12483>
6. DSTU 4138-2002. Methods for determining quality. Seeds of agricultural crops. Kyiv: Derzhspozhyvstandart Ukrainy. 2003. [in Ukrainian].
7. Pastoshchuk A., Yumyna Y., Zelena P., Nudha V., Yanovska V., Kovalenko M., Taran N., Patyka V., Skivka L. Beneficial traits of grain-resided endophytic communities in wheat with different sensitivity to *Pseudomonas syringae*. *Reg. Mech. Biosyst.* 2021, 12(3), 498–505. <https://doi.org/10.15421/022168>
8. Andersen E. J., Nepal M. P., Purinton J. M., Nelson D., Mermigka G., Sarris P. F. Wheat Disease Resistance Genes and Their Diversification Through Integrated Domain Fusions. *Front. Genet.* 2020, 11, 898. <https://doi.org/10.3389/fgene.2020.00898>

### Conclusion

Endophytic bacteria *P. polymyxa* P 6.3, depending on the individual characteristics of varieties, have a growth-stimulating effect on wheat germination and a protective effect against *P. syringae*. The plant growth promoting potential and antagonistic activity make strain P 6.3 a promising biocontrol agent and growth stimulator as a biofertilizer.

### Funding source

The study was supported by a project funded by the Ministry of Education and Science of Ukraine (State Registration No. 16KF036-07).