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PREFACE

"I would like to see a return to trust in small farmers, in their knowledge of the land and their skills that have been accumulated over generations – helping them to find sustainable solutions to the balance of nature. Ultimately, just as countless small businesses are the mainstay of any economy, so are small farmers the backbone, lifeblood and protectors of the rural environment."

Prince Charles, concluding part of the lecture for the Howard Lecture 2008 – the Future of Food

Agricultural science and agriculture as a profession monitor and study changes occurring in this area, point out problems in agricultural practice, and find solutions. The Faculty of Agronomy in Čačak, in addition to educating students, 30y traditionally organizes the Symposium on Biotechnology (SYMBIOTECH) every year. The main goal is to acquaint the wider scientific and professional public with the results of the latest scientific research, and bring together domestic and foreign scientists in the fields of primary agricultural production, food processing, and environmental protection. We work tirelessly in pursuit of excellence.

At the 3rd International Symposium on Biotechnology, a total of 74 papers were presented in the 8 sections: Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology, Applied Chemistry, and Nutritionism.

We owe great gratitude to the **Ministry of Science, Technological Development** and **Innovation of the Republic of Serbia** and the **City of Čačak** for their traditional financial support and patronage of SYMBIOTECH25. We thank companies, entrepreneurs, stakeholders and all long-time friends of the Faculty of Agriculture for their material and organizational support.

In Čačak, March 2025

IMPROVING GERMINATION AND PROTECTION OF WHEAT SEEDS WITH NEW BACTERICAL ISOLATES FROM ALKALINE SOIL

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Abstract: The objective of this research is to investigate the application of new *Bacillus* spp. isolates from alkaline soil as potential biocontrol agents for the management of wheat pest-wireworms (*A. lineatus* larvae), diseases caused by the phytopathogenic fungi *Fusarium* spp., and their plant growth-promoting potential. Among five new *Bacillus* spp. isolates, BHC 1.3 and BHC 1.5 showed ability to suppress only mycelial growth of *F. proliferatum*. Insecticidal activity resulting in a wireworm mortality rate of 17.24% after ten days of experimentation was observed for BHC 1.5. The final percentage of seed germination was in the range of 95% - 100% with the additional highest production of indole-3-acetic acid (IAA) by BHC 1.5. The results of this study indicate that the new *Bacillus* spp. isolate may have the potential for formulating microbial inoculants effective in promoting wheat plant growth and biocontrol of soil-borne diseases and pests.

Keywords: biocontrol, *Fusarium* spp., wireworms, plant growth promoting traits, wheat seedlings.

Introduction

Wheat is one of the world's oldest and most widely cultivated food crops, playing a crucial role in ensuring global food and nutrition security. It accounts for one-fifth of the total food calories and protein consumed by the global population, supporting food security worldwide (Shiferaw et al., 2013; Voučko et al., 2025). Additionally, wheat is a key ingredient in a wide range of food products. As one of the most important staple crops, wheat holds undeniable agronomic significance, supporting the economies of many countries (Tadesse

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et al., 2016; Igrejas and Branlard, 2020). The growing global population is increasing the demand for food, highlighting the need for sustainable agricultural practices (Tilman et al., 2011). Intensive and unsustainable farming practices pose significant risks to food security, the economy, and the environment. Climate change further exacerbates these challenges, with rising temperatures, altered precipitation patterns, and more frequent extreme weather events disrupting the lifecycle and development of phytopathogens and pests, ultimately leading to reduced crop yields (Xu et al., 2010; Latz et al., 2018; Anjana et al., 2021; Paudel et al., 2023). Wheat production faces significant challenges from various diseases and pest attacks that consistently lead to yield losses. Addressing these challenges requires a multidisciplinary approach, integrating advanced technologies and investing in research and innovation (Robles-Zazueta et al., 2023).

The genus *Fusarium* is considered one of the most significant biological stressors due to its diversity and widespread distribution. These fungi cause a range of diseases, including rots, blights, wilts, and cankers, and pose a serious threat to staple crops such as wheat, with implications for the environment, livestock, and human health (Matić et al., 2020; Baard et al., 2023). Several species of *Fusarium*, including *F. graminearum*, *F. oxysporum*, *F. poae*, *F. verticillioides*, *F. solani* (Munkvold, 2003), and *F. proliferatum*, *F. culmorum* (Baard et al., 2023), are responsible for diseases in cereal crops. In addition, the production of small grains is also threatened by pests that are transmitted in the soil, such as wireworms. Wireworm *Agriotes lineatus* L. (Coleoptera: Elateridae), is a cosmopolitan soil pest which causes a significant yield losses of cereals by feeding on the germinating seeds and the belowground plant organs, killing plants directly (Barsics et al., 2013; Kozina et al., 2015; Furlan et al., 2021).

An alternative approach for protecting wheat production from diseases and pests is the use of biologically active substances (biopesticides) with the aim of reducing conventional synthetic chemicals (European commission, 2020). Microbial inoculants (biopesticides) are consisted of microorganisms which are beneficial and can be effective against plant diseases and insect pests that cause damage to agricultural crops (Thakur et al., 2020). In addition, these microorganisms often have a wide array of plant growth promoting (PGP) properties. In recent years, strains of the *Bacillus* genus have attracted attention as an alternative for simultaneous biocontrol and plant growth promotion of various crops (Zhao et al., 2014; Villa–Rodriguez et al., 2021).

The aim of this study was to evaluate the potential of new *Bacillus* spp. isolated from alkaline soil in the biocontrol of phytopathogenic fungi *F. poae, F.*

graminearum and *F. proliferatum* and pest *A. lineatus* larvae, as well as in enhancing seed germination to contribute to the plant growth promotion of wheat. Detection of biocontrol and promote plant growth traits was performed using qualitative or quantitative tests, while the seed germination test was performed in vitro through final germination.

Materials and methods

Soil sampling and analysis

The sampling of soil was done in the territory of Veliki Crljeni, Serbia. The soil was taken from the topsoil layer (0-30 cm of depth) and placed in plastic sterile bags and transferred to the laboratory in a portable refrigerator (4°C) for bacterial isolation, while for the chemical analysis the sampled soil was air-dried, crushed and passed through a sieve (≤ 2 mm) prior to the analyses in accordance with the SRPS ISO 11464:2004.

Soil acidity (pH in water and 1M KCl) was measured according to the SRPS ISO 10390:2007 and the carbonate content was determined based on the SRPS ISO 10693:2005. Plant – available phosphorus (P₂O₅) and potassium (K₂O) were analyzed by Al-method where K₂O content was determined by flame emission photometry and P₂O₅ content by spectrophotometer (Egnér et al., 1960), while the soil organic matter (SOM) content was determined following the Kotzmann method (JDPZ, 1966).

Isolation of bacteria from soil

A 10 g sample of rhizosphere soil was mixed with 90 mL of distilled water and agitated (150 rpm, 15 min) followed by a heating treatment for *Bacillus* spp. isolation (80°C, 15 min). After the suspension settled, the supernatant was subjected to a 10-fold serial dilution, from which two concentrations (10⁻⁵ to 10⁻⁶) were selected (Li et al., 2023). A 100 μ L aliquot from each dilution was spread onto Nutrient Agar (NA) plates and incubated at 28°C for 24 hours. Individual colonies were then isolated and purified through streaking on NA plates. The purified cultures were preserved at 4°C on NA slants for subsequent analysis.

Antifungal activity of bacterial isolates

Out of the bacterial properties which are significant for their antifungal potential, the ability of bacterial isolates to produce cellulase, amylase and hydrogen cyanide (HCN) was evaluated. According to Mihajlovski et al. (2015) the production of cellulase and amylase was determined by inoculating bacterial isolates on the media supplemented with 1 g L⁻¹ of carboxymethyl cellulose and starch, respectively. The ability of bacterial isolates to produce HCN was evaluated by Cyantesmo paper by following a procedure described in Knezevic et al. (2021).

The antagonistic effect of bacterial isolates against *Fusarium* species (*F. poae, F. graminearum* and *F. proliferatum*) was evaluated on potato-dextrose agar (PDA) by methode dicribed by Knežević et al. 2021.

Insecticidal activity of bacterial isolates

Ability of bacterial isolates to produce substances significant for their insecticidal effect, such as lipase and protease was determined by inoculating isolates on the medium enriched by Tween 80 and on Skim Milk Agar (SMA) (Figueira et al., 2019) and incubated (28°C, 5 days). The appearance of calcium complex precipitate or clear hallo zones around the inoculation site was considered as lipolytic or proteolytic activity, respectively.

Wireworms (*A. lineatus* larvae) were previously collected from wirewormsinfested soils. For the wireworm bioassay, the seeds of wheat were suspended in the cultures of bacterial isolates (BHC 1.1; BHC 1.2; BHC 1.3; BHC 1.4 and BHC 1.5), or in sterile water for the control sample. Seeds were then placed in Petri dishes and left to germinate for 3 days. After that, ten larvae were introduced into each Petri dish monitored daily for 10 days, and mortality data were calculated using Abbott's formula (1925).

Plant growth promoting traits of bacterial isolates

Key plant growth-promoting traits of bacterial strains, including siderophore production, indole-3-acetic acid (IAA) synthesis, and phosphate solubilization, were assessed. Siderophore production was tested on CAS agar, with a color change from blue to yellow indicating a positive result (Milagres et al., 1999). Phosphate solubilization was evaluated on Pikovskaya medium (PVK) after 7 days at 28°C, with halo formation signifying activity (Rokhbakhsh-Zamin et al.,

2011). The IAA concentration was quantified spectrophotometrically in tryptophan-supplemented broth using Salkowski reagent (Gordon and Weber, 1951).

Seed germination assay

The ability of bacterial isolates to improve the germination of wheat (*Triticum aestivum* L.) seeds was accessed in vitro on Petri dishes by using filter paper method. Ten wheat seeds were soaked in over-night bacterial culture of each isolate which showed good PGP potential in previous PGP tests and placed in Petri dishes containing wetted filer paper. For the control, bacterial inoculation of seeds was not applied. Petri dishes were kept for two weeks in a transparent sealed box at 25 °C, and final seed germination percentage (FG %) was calculated as described by Ali et al. (2015).

Results and discussion

The chemical analysis of the BHC 1 is submersed in Table 1. According to the interpretation of the results this soil sample belongs to group of alkaline, low – carbonate soils. Considering the granulometric composition of the soil it has high organic matter content. The content of plant–available phosphorus is low, wherease the content of plant–available potassium is high (Manojlović et al., 1969).

Table 1. The basic chemical analysis of the soil										
	Soil parameter									
Soil sample	pH (KCl)	pH (H ₂ O)	K2O (mg 100g ⁻¹)	P ₂ O ₅ (mg 100g ⁻¹)	CaCO ₃ %	SOM %				
BHC 1	8.10	8.62	37.1	11.0	1.40	2.71				

A total of five *Bacillus*-like isolates were isolated from alkaline soil sample BHC 1 and the isolated were coded as BHC 1.1, BHC 1.2, BHC 1.3, BHC 1.4 and BHC 1.5. Among tested three antifungal traits, none of the five isolates were capable to produce HCN. Isolates BHC 1.3 and BHC 1.5 only characterized as amylase and celullase producers (Table 2). The suppression of mycelia growth was observed for *F. proliferatum* among the three *Fusarium* fungi tested (*F. poae, F. graminearum* and *F. proliferatum*). Isolates BHC 1.5 and BHC 1.3 showed antifungal activity against *F. proliferatum* and inhibiting rate of mycelial growth

was about 20% and 15%, respectively. In the literature, various research obtained different inhibition percentage of mycelia growth of F. proliferatum. Baard et al. 2023 tested four Bacillus strains against phytopathogenic fungi, and the most inhibited strains were *B. tequilensis* strains B3 and B4 with inhibition percentages of 60.75 ± 0.21 and $64.79 \pm 0.40\%$, respectively. Bjelić et al. 2018 showed that the percentage of inhibition of F. proliferatum BL16 ranged from 4.5% to 44.3%. Among the eleven Bacillus strains tested, most of them showed inhibition of mycelial growth between 16% and 25% (Bjelić et al., 2018) which was in accordance with obtained result in our research for Bacillus isolate BHC 1.5.

Table 2. Antifungal and plant growth promoting bacterial properties									
Strain	Amylase	Cellulase	HCN	IAA* (µg mL-1)	Phosphate solubilization (mm)	Siderophore			
BHC 1.1	-	-	-	1.10±0.13	-	-			
BHC 1.2	-	-	-	0.87±0.19	-	-			
BHC 1.3	+	++	-	2.42±0.15	-	-			
BHC 1.4	-	-	-	0.55±0.12	-	-			
BHC 1.5	+++	++	-	3.19±0.20	-	18.67±0.58			

T 11.0 A

- no production; + low production; ++ moderate production; +++ high production *IAA-indole-3-acetic acid

Isolates BHC 1.1, BHC 1.2, BHC 1.3 and BHC 1.4 were not shown insecticidal activity against wireworms. After ten days all larvae were vital. Individual mortality of wireworms was recorded for BHC 1.5 and it was 17.24%. In addition, two isolates showed proteolytic activity (BHC 1.3 and BHC 1.5), but none showed lytic activity. The biocontrol of wireworms using bacteria was rarer studded and its insecticidal activity begins with lysis of epithelial cells in the midgut of insects by proteases and lipase (Kumar et al., 2021). The percentage of mortality varied from species to species, but the most studied and the most effected was *B. thuringiensis* species (Park et al., 2014).

The ability to produce IAA was obtained for all tested isolates. The highest producer was BHC 1.5 followed by BHC 1.3, BHC 1.1 BHC 1.2 and BHC 1.4 (Table 2). Among the isolates there are no phosphate solubilization bacteria. Siderophore produces was only BHC 1.5 (18.67±0.58 mm). Final seed germination percentage was in a range from 95% - 100%. The highest FG% (100%) was recorded for the isolate BHC 1.5. The impact of *Bacillus* spp. on the improving seeds germination was previously tested by other authors.

Miljaković et al. (2024) demonstrated that IAA-producing strains *B. subtilis* B43 and *B. amyloliquefaciens* B50, had the highest effect on final germination of green peas. Similarly, Song et al. (2023) indicated that *B. subtilis* HS5B5 had a positive effect on maize (*Zea mays* L.) seed germination and seedling growth. In general, these results indicate that a proper selection of *Bacillus* spp. with PGP properties offers a good solution for improving the germination rate of different crops, including cereals.

Conclusion

Among the five new *Bacillus* spp. isolates, BHC 1.5 particularly stood out for its antifungal and insecticidal activity and its positive effect on plant growth, making it a promising candidate for further development as a bio-based pest management solution.

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