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Ferenc F. BAGI[1], Renata M. ILIČIĆ[1], Đina S. KONSTANTIN[1], Nemanja S. PAVKOVIĆ[1], Predrag M. MILOVANOVIĆ[2], Tatjana Č. POPOVIĆ-MILOVANOVIĆ[3]*

[1] University of Novi Sad, Faculty of Agriculture,
Trg Dositeja Obradovića 8, Novi Sad 21102, Serbia
[2] Agrounik d.o.o.,
Krnješevačka bb, Šimanovci 22310, Serbia
[3] Institute for Plant Protection and Environment,
Teodora Drajzera 9, Belgrade 11040, Serbia

IN VITRO EFFECT OF *BACILLUS* SPP. ON *ALTERNARIA ALTERNATA* INFECTING WHEAT

ABSTRACT: Species of the genus *Alternaria* are significant wheat contaminants during production, transport and storage, requiring biocontrol measures which typically rely on the bacteria from the *Bacillus* genera. As these are among the most beneficial and exploited biocontrol agents, in this study, the inhibitory activity of indigenous *Bacillus* spp. was assessed against the *Alternaria alternata* isolate originating from the wheat seed. Two of the fifteen *Bacillus* spp. included in the study showed the inhibitory effect. Specifically, 25.0−55.0% inhibition of *A. alternata* growth was achieved when the isolate coded as NB11 was applied in 10^6–10^9 cells mL^{-1} concentrations. On the other hand, when applied in 10^7–10^9 cells mL^{-1} concentrations, the isolate coded as NB16 inhibited *A. alternata* growth by 35.2−51.1%, but was ineffective at lower concentrations. Thus, these *in vitro* assays indicate that both *Bacillus* spp. (NB11 and NB16) isolated from the wheat rhizosphere can be applied in practice in the control of *A. alternata*.

KEYWORDS: wheat, pathogen, biological control

INTRODUCTION

Cereals are highly important for human consumption due to which they are the most prevalent culture. They form the base of the food pyramid in both

* Corresponding author. E-mail: ferenc.bagi@polj.edu.rs

developed countries as well as countries in transition (Brkić et al., 2021). In Serbia, wheat production volume is second only to corn, while globally wheat is the third most widely produced grain (Janić Hajnal et al., 2014). Although there are many species of wheat, which make up the genus *Triticum*, common wheat (*T. aestivum*) is the most widespread.

From seed germination to harvest, wheat can be attacked by a number of fungi, which can significantly reduce the yield and crop quality under certain weather conditions (Perelló & Larrán, 2013). Due to evident climate changes and global warming and their adverse influence on the agricultural environment, occurrences of mycotoxigenic fungi on wheat are becoming more frequent. This not only exerts a negative impact on the safety and quality of food products, but also inevitably leads to large economic losses (Janić Hajnal et al., 2014).

Phytopathogenic fungi from the genus *Alternaria* are significant contaminants of cereals that—in addition to producing mycotoxins and causing damage to the field—can cause product spoilage during processing, transport and storage. As a result, diseases caused by *Alternaria* sp. are among the most common and most researched crop diseases throughout the world (Perelló & Larrán, 2013). Among the *Alternaria* species, *A. alternata* is considered the most dominant mycotoxin-producing species associated with black spot, early blight disease and stem canker in cereal grains (Kumar et al., 2022). Although the *Alternaria* species produces more than 70 secondary metabolites, only a few phytotoxins have been chemically characterised and reported to act as mycotoxins in humans and animals (Janić Hajnal et al., 2014). Under favourable conditions, the most important mycotoxins produced by *A. alternata* are alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TA) (Somma et al., 2019). The occurrence of these metabolites in food represents a serious global problem for human and animal health due to their toxic effects.

To mitigate this issue, fungicides are usually utilised in agricultural practice, whereby different chemical fungicides are adopted as a means of wheat pathogen control (Casa et al., 2012). However, integrated pest management programs (IPMs) are being increasingly employed in the agricultural sector, with the emphasis on the use of biological products as an environmentally friendly pathogen control strategy. In this context, *Bacillus* species have been shown to be promising biological control agents (Berić et al., 2012; Gond et al., 2015; Marković et al., 2020, 2023; Jelušić et al., 2021; Iličić et al., 2022; Soliman et al., 2023). They exhibit multiple modes of action and produce a wide range of biologically active compounds with antifungal potential, making them suitable for controlling different diseases.

Guided by this evidence, the aim of this study was to utilise *in vitro* assays in order to determine the biocontrol potential of indigenous *Bacillus* species isolated from wheat rhizosphere in the control of *A. alternata*.

MATERIAL AND METHODS

Isolation of antagonistic bacteria

Isolation of the potential antagonistic bacteria was performed from the soil samples collected in the wheat rhizosphere (locality Rimski Šančevi) according to the procedure described by Berić et al. (2012). *Bacillus*-like colonies that developed after 24^h incubation at 30 °C were selected, purified and maintained in sterile LB (Luria Bertani) glycerol at -20 °C. Fifteen isolates were subsequently subjected to morphological and biochemical tests (Gram staining, spore formation, catalase production, glucose fermentation, starch hydrolysis and growth on 5% NaCl).

Antagonistic activity of the selected Bacillus species on the Alternaria alternata isolates from wheat

One isolate of *A. alternata* (SOR1IIIZA1) originating from wheat seed in Vojvodina Province was used in testing. This isolate was grown at 25 °C on PDA (Potato-Dextrose Agar) in Petri plates (ø 90 mm) for 10 days.

Antagonistic activity of the fifteen selected *Bacillus* spp. isolates (coded as NB1-9 and NB11-16) against the *A. alternata* was determined using the dual culture method. Agar discs (ø 7 mm) of the tested fungi pathogen isolate were placed in the centre of PDA plates (ø 90 mm). Drops (8 µl in volume) of bacterial suspensions of appropriate *Bacillus* spp. isolate adjusted to 10^6, 10^7, 10^8 and 10^9 cells mL^{-1} were placed on four sides near the edge of the Petri plates. Petri dishes were kept at a constant temperature (25 °C) for seven days. The assay was set in three replications and sterile distilled water served as a control treatment. All experiments were performed twice.

In order to quantify the antagonistic potential of *Bacillus* spp. isolates, the pathogen growth area was measured after 7 days of incubation at 25 °C, and the percent of inhibition was calculated using the formula given by Zarrin et al. (2009). The results were subjected to analysis of variance (ANOVA), whereby Duncan's Multiple Range Test ($p < 0.01$) was performed for assessing the differences between means related to different treatments.

RESULTS AND DISCUSSION

While all wheat pathogens have the capacity to significantly reduce yield quantity and seed quality, fungi from the *Alternaria* genus are particularly harmful as they can compromise food safety due to their toxin-producing potential (Bagi et al., 2022). Consequently, in wheat production, seed and plant treatments are regularly implemented during the vegetation phase. However, there is increased concern about the application of synthetic pesticides, prompting

research into less harmful alternatives (Moumni et al., 2023). In this context, focus is primarily given to the implementation of beneficial microorganisms, such as biological control agents from *Bacillus* genera, which have been used in experimental tests on a wide range of economically important crops (Slama et al., 2019; Wang et al., 2021; Rabbee et al., 2022).

This study contributes to this research endeavour by evaluating the antagonistic potential of 15 *Bacillus* isolates from the wheat rhizosphere, which were selected as promising biological control agents in the control of *A. alternata* originating from wheat. The antagonistic activity testing results indicated that two *Bacillus* spp. (coded as NB11 and NB16) exhibit a strong inhibitory effect against wheat pathogen *A. alternata*. Based on the results obtained using the dual culture method, isolate NB11 achieved 25.0−55.0% inhibition of *A. alternata* growth, depending on the applied concentration (Table 1). As shown in Figure 1, this *Bacillus* spp. isolate showed antagonistic potential against *A. alternata* at the lowest tested concentration (10^6 cells mL^{-1}) with a rated inhibition of 25.0%, which increased to 38.9%, 47.2% and 55.0% at 10^7, 10^8 and 10^9 cells mL^{-1}, respectively. On the other hand, the *Bacillus* isolate coded as NB16 was ineffective at 10^6 cells mL^{-1}, but when applied at the three higher concentrations it inhibited *A. alternata* growth by 35.2%, 43.7% and 51.1%, respectively (Table 2).

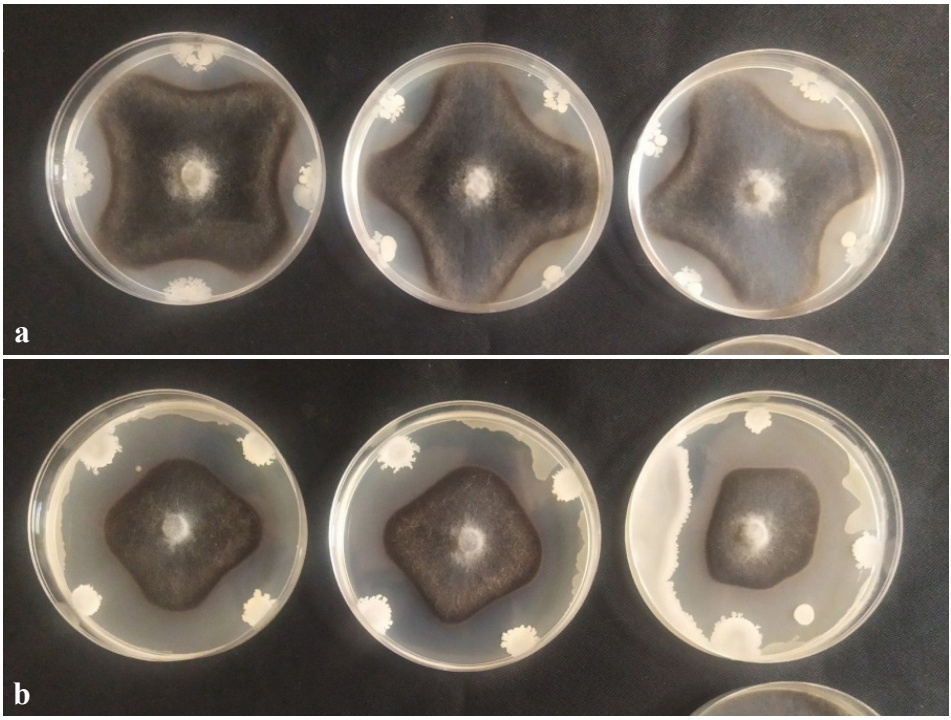

Figure 1. Antagonistic activity of the *Bacillus* isolate NB11 against *A. alternata* originating from wheat seed in dual culture, in two of the four tested concentrations: (a) 10^6 cells mL^{-1} and (b) and 10^9 cells mL^{-1}.

Table 1. The inhibitory effect of the *Bacillus* isolate NB11 on the pathogenic fungi *A. alternata*

Treatment	Concentration (cells mL^{-1})	Mycelium growth (diameter in mm)			Mean (mm)	Inhibition (%)	Sd
		Replications					
		I	II	III			
Bacillus NB11	10^6	65	67.5	70	67.5^c	**25.0**	2.04
	10^7	50	55	60	55.0^b	**38.9**	4.08
	10^8	45	50	47.5	47.5^{ab}	**47.2**	2.04
	10^9	45	39	37.5	40.5^a	**55.0**	3.24
Negative control	–	90	90	90	90.0^d	**0**	0

Sd – standard deviation; *Mean values of inhibition zone diameters are shown. Values followed by the same letter are not significantly different ($p < 0.01$) according to Duncan's multiple range test results.

Table 2. The inhibitory effects of the *Bacillus* isolate NB16 on the pathogenic fungi *A. alternata*

Treatment	Concentration (cells mL^{-1})	Mycelium growth (diameter in mm)			Mean (mm)	Inhibition (%)	Sd
		Replications					
		I	II	III			
Bacillus NB16	10^6	90	90	90	90.0^d	**0**	0
	10^7	55	60	60	58.3^c	**35.2**	2.35
	10^8	51	52	49	50.7^b	**43.7**	1.24
	10^9	45	45	42	44.0^a	**51.1**	1.41
Negative control	–	90	90	90	90.0^d	**0**	0

Sd – standard deviation; *Mean values of inhibition zone diameters are shown. Values followed by the same letter are not significantly different ($p < 0.01$) according to Duncan's multiple range test results.

The inhibitory activity of *Bacillus* species against different phytopathogens has been demonstrated in numerous *in vitro*, *in vivo* and *in planta* trials, providing evidence in support of their potential use as biocontrol agents and plant growth promoters (Slama et al., 2019; Brzezinska et al., 2020; Wang et al., 2021; Soliman et al., 2023). *Bacillus* spp. produces a variety of biologically active compounds involved in the biocontrol of plant pathogens (Prasad et al., 2023). For example, Abdelmoteleb et al. (2017) reported that *B. subtilis* produce three mucolytic enzymes which cause damage to the hypha of *A. alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Macrophomina* sp. and *Sclerotium rolfesii*. More recently, Brzezinska et al. (2020) conducted a study on *B. subtilis* and *Bacillus* sp. and demonstrated that chitinases inhibited the growth of *A. alternata* and *Fusarium oxysporum*. In the same study, the isolates showed the ability to produce a broad range of biological substances that promote plant growth.

The present study shows that two indigenous isolates of *Bacillus* have a strong biocontrol potential and could be used to control *Alternaria* disease in wheat. Further research should thus incorporate *in vitro* and *in planta* testing to determine their efficacy and potential use as biocontrol agents and plant growth promoters.

CONCLUSION

The results obtained as a part of this work indicate that the applied treatments based on two indigenous *Bacillus* spp. are effective in *A. alternata* control and should be subjected to further trials on wheat in different stages of development.

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REFERENCES

Abdelmoteleb A, Troncoso-Rojas R, Gonzalez-Soto T, González-Mendoza D (2017): Antifungical activity of autochthonous *Bacillus subtilis* isolated from *Prosopis juliflora* against phytopathogenic fungi. *Mycobiology* 45: 385–391.

Bagi F, Iličić R, Savić Z, Jeftić R, Orbović B, Suman M, Tóth B, Berényi A, Popović T (2022): Toxigenic potential of *Alternaria* species from cereals. *Matica Srpska J. Nat. Sci.* 142: 39–45.

Berić T, Kojić M, Stanković S, Topisirović Lj, Degrassi G, Myers M, Venturi V, Fira Dj (2012): Antimicrobial activity of *Bacillus* sp. natural isolates and their potential use in the biocontrol of phytopathogenic bacteria. *Food Technol. Biotechnol*. 50: 25–31.

Brkić D, Bošnir J, Jajetić A, Prskalo I, Šabarić J, Kuharić Ž, Pavlek Ž, Benić M, Racz A (2021): Žitarice i mlinski proizvodi kao dobar izvor magnezija u svakodnevnoj prehrani. *J. Appl. Health Sci.* 7: 71–86.

Brzezinska MS, Kalwasińska A, Świątczak J, Żero K, Jankiewicz U (2020): Exploring the properties of chitinolytic *Bacillus* isolates for the pathogens biological control. *Microb. Pathog.* 148: 104462.

Casa RT, Kuhnem JPR, Bogo A, Belani ANM, Bolzan JM, Oliveira FS, Blum MMC (2012): Survey, survival and control of *Alternaria alternata* in wheat seeds. *Rev. Bras. Sementes*. 34: 358–365.

Gond SK, Bergen MS, Torres MS, White JFJr (2015): Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. *Microbiol. Res.* 172: 79–87.

Iličić R, Popović T, Jelušić A, Bagi F, Trkulja N, Živković I, Stanković S (2022): Biocontrol ability of *Bacillus halotolerans* against stone fruit pathogens. *4th International Symposium: Modern Trends in Agricultural Production, Rural Development, Agro-economy, Cooperatives and Environmental Protection*, 29–30. June, Vrnjačka Banja, Serbia. Book of Proceedings, 170–179.

Janić Hajnal E, Orčić D, Mastilović J, Milovanović I, Kos J (2014): The choice of preparation method for the determination of *Alternaria* toxins from wheat samples by LC-MS/MS. *Food Feed Res*. 4: 131–138.

Jelušić A, Popović T, Dimkić I, Mitrović P, Peeters K, Višnjevec AM, Tavzes Č, Stanković S Berić T (2021): Changes in the winter oilseed rape microbiome affected by *Xanthomonas campestris* pv. *campestris* and biocontrol potential of the indigenous *Bacillus* and *Pseudomonas* isolates. *Biol. Control*. 160: 104695.

Kumar PS, Nattudurai G, Islam VIH, Ignacimuthu S (2022): The effects of some essential oils on *Alternaria alternata*, a post-harvest phyto-pathogenic fungus in wheat by disrupting ergosterol biosynthesis. *Phytoparasitica* 50: 513–525.

Marković S, Popović Milovanović T, Jelušić A, Iličić R, Medić O, Berić T, Stanković S (2023): Biological control of major pathogenic bacteria of potato by *Bacillus amyloliquefaciens* strains SS-12.6 and SS-38.4. *Biol. Control*. 182: 105238.

Marković S, Popović T, Jelušić A, Iličić R, Stanković S (2020): Potential of *Bacillus amyloliquefaciens* strains SS-12.6 and SS-38.4 in biological control of potato rot pathogens. *FEMS Online Conference on Microbiology,* October, Belgrade, Serbia, 28–31.

Moumni M, Brodal G, Romanazzi G (2023): Recent innovative seed treatment methods in the management of seedborne pathogens. *Food Secur.* 15: 1365–1382.

Perelló AE, Larrán S (2013): Nature and effect of *Alternaria* spp. complex from wheat grain on germination and disease transmission. *Pak. J. Bot.* 45: 1817–1824.

Prasad B, Sharma D, Kumar P, Dubey RC (2023): Biocontrol potential of *Bacillus* spp. for resilient and sustainable agricultural systems. *Physiol. Mol. Plant Pathol*. 102173.

Rabbee FM, Islam N, Baek KH (2022): Biocontrol of citrus bacterial canker caused by *Xanthomonas citri* subsp. *citri* by *Bacillus velezensis*. *Saudi. J. Biol. Sci.* 29: 2363–2371.

Slama BH, Cherif-silini H, Bouket AC, Qader M (2019): Screening for *Fusarium* antagonistic bacteria from contrasting niches designated the endophyte *Bacillus halotolerans* as plant warden against Fusarium. *Front. Microbiol*. 9: 1–24.

Soliman SA, Abdelhameed RE, Metwally RA (2023): In vivo and In vitro evaluation of the antifungal activity of the PGPR *Bacillus amyloliquefaciens* RaSh1 (MZ945930) against *Alternaria alternata* with growth promotion influences on *Capsicum annuum* L. plants. *Microb. Cell. Factories* 22: 70.

Somma S, Amatulli MT, Masiello M, Moretti A, Logrieco AF (2019): *Alternaria* species associated to wheat black point identified through a multilocus sequence approach. *Int. J. Food Microbiol.* 293: 34-43.

Wang F, Xiao J, Zhang Y, Li R, Liu L, Deng J (2021): Biocontrol ability and action mechanism of *Bacillus halotolerans* against *Botrytis cinerea* causing grey mould in postharvest strawberry fruit. *Postharvest Biol. Technol*. 174: 111456.

Zarrin F, Saleemi M, Zia M, Sultan T, Aslam M, Rehman RU, Chaudhary MF (2009): Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. *Afr. J. Biotechnol*. 8: 219–225.

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УТИЦАЈ *BACILLUS* SPP. НА ПАТОГЕНА ПШЕНИЦЕ *ALTERNARIA ALTERNATA* И *IN VITRO* УСЛОВИМА

Ференц Ф. БАГИ[1], Рената М. ИЛИЧИЋ[1], Ђина С. КОНСТАНТИН[1], Немања С. ПАВКОВИЋ[1], Предраг М. МИЛОВАНОВИЋ[2], Татјана Ч. ПОПОВИЋ МИЛОВАНОВИЋ[3]

[1] Универзитет у Новом Саду, Пољопривредни факултет, Трг Доситеја Обрадовића 8, Нови Сад 21102, Србија
[2] "Агроуник" Д.О.О., Крњешевачка ББ, Шимановци 22310, Србија
[3] Институт за заштиту биља и животну средину, Теодора Драјзера 9, Београд 11040, Србија

РЕЗИМЕ: Врсте рода *Alternaria* су значајни патогени пшенице током производње, транспорта и складиштења. Бактерије из рода *Bacillus* сматрају се једним од најкориснијих и најискоришћенијих средстава за биоконтролу. Сходно томе, у овој студији инхибиторна активност аутохтоних *Bacillus* spp. је испитана према *Alternaria alternata* изолату пореклом са семена пшенице. Два од петнаест одабраних *Bacillus* spp. су испољили инхибиторни ефекат тј. изолат NB11 постигао је инхибицију раста *A. alternata* од 25,0–55,0% у примењеним концентрацијама од 10^6 и 10^9 ћелија/ml, респективно. Изолат NB16 инхибирао је раст *A. alternata* у опсегу од 35,2–51,1% у тестираним концентрацијама од 10^7 и 10^9 ћелија/ml, респективно, али није забележена инхибиција при најнижој концентрацији од 10^6 ћелија/ml. Оба *Bacillus* spp. NB11 и NB16 изоловани из ризосфере пшенице, представљају обећавајуће агенсе биолошке контроле *A. alternata* са семена пшенице у *in vitro* тестовима.

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