J. Agr. Sci. Tech. (2018) Vol. 20: 609-619

Effectiveness of the *Bacillus* sp. SP-A9 Strain as a Biological Control Agent for Spring Wheat (*Triticum aestivum* L.)

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ABSTRACT

The SP-A9 strain of *Bacillus* sp., which is most closely related to *Bacillus subtilis*, demonstrated excellent antifungal properties in laboratory analyses. The percentage of inhibition in the dual culture test was similar for all investigated phytopathogens (*Fusarium culmorum*, *F. oxysporum* and *Monographella nivalis*) at approximately 46%. The analyzed strain was found to be cellulolytic and strongly chitinolytic, and its biochemical properties indicate that it easily adapted to various environmental conditions. The strain's sporulation ability and high proliferation rate in acidic, alkaline, and highly saline environments (9% NaCl) further confirmed its adaptability to adverse conditions. In a pot experiment, the basic biometric parameters of spring wheat grain inoculated with *Bacillus* sp. SP-A9 were not modified, but a significant increase in grain yield was observed (by 18% in soil contaminated with *F. culmorum* and by 19% in soil contaminated with *F. oxysporum*). The increase in yield was correlated with the number of wheat plants, which suggests that the analyzed strain minimized the pathogen-induced inhibition of plant growth. *Bacillus* sp. SP-A9 can reduce economic losses resulting from diseases caused by fungi of the genus *Fusarium* and contribute to reduced use of crop protection chemicals, thus minimizing environmental pollution.

Keywords: Antifungal properties, Environmental pollution, *Fusarium* spp, Phytopathogens.

INTRODUCTION

Crop protection is one of the key factors that determine yield and its market value. High-input production systems promote the spread of plant diseases and necessitate the use of crop protection agents, which contribute to the degradation of natural and anthropogenic environments. Chemical substances can be replaced with biological control agents involving selected microorganisms that have beneficial effects on the agricultural ecosystem and improve the quantity and quality of crops.

Bacterial genera that support plant growth and are used in biological protection of crops against phytopathogens and pests include *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* (Bhattacharyya and Jha, 2012; Mendes *et al*., 2012; Ahemad and Kibret, 2014; Przemieniecki *et al*., 2015; Unalmis *et al*., 2015).

Fungi of the genus *Fusarium* are widespread pathogens on all continents, excluding permafrost regions (Przemieniecki

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[DOR: 20.1001.1.16807073.2018.20.3.15.1]

et al., 2014a), and they significantly reduce crop yields. *Fusarium* fungi produce mycotoxins which contaminate cereal grains and pose a health threat for animals and humans. *Fusarium culmorum* causes Fusarium crown rot and Fusarium Head Blight (FHB) in wheat, whereas *F. oxysporum* infects wheat seedlings and decreases the number of emerged wheat plants (Ittu *et al*., 2010; Cornea *et al*., 2013; Grosu *et al*., 2015)

Biological protection agents exert a much smaller influence on the environment than chemical substances that accompany intensive agricultural production. Reduced use of chemical agents can also minimize pathogens' resistance to fungicides. Bacteria of the genus *Bacillus* are a popular biological protection agent. They produce antibiotics which eliminate phytopathogens, and they are capable of surviving in unfavorable environments in the form of spores (Zhao *et al*., 2014). *Bacillus* bacteria also produce lytic enzymes such as chitinase and cellulase which degrade cell walls in phytopathogens (Ashwini and Srividya, 2013). Bacteria of the genus *Bacillus* are also resistant to extreme temperatures, pH, and osmotic conditions, which provides *Bacillus* species with a significant competitive advantage over other microorganisms (Islam *et al*., 2012; Kumar *et al*., 2014).

The aim of this study was to evaluate the antagonistic properties of the strain against *F. culmorum* and *F. oxysporum* and to demonstrate the ability of the tested bacterial strain to increase the crop of spring wheat.

MATERIALS AND METHODS

Identification of the Analyzed Strain

Rhizosphere samples were collected from a rye field in spring. The samples were transported to a laboratory under sterile conditions. Ten g of soil were combined with 90 mL of saline (0.85% NaCl) solution; the mixture was shaken at 180 rpm for 30 minutes and subjected to serial dilutions. Bacteria were cultured on tryptic soy agar (TSA, Merck Millipore), and a colony characteristic of the genus *Bacillus* was preserved in sterilized glycerol diluted 2:3 with water at -80°C.

Bacteria were cultivated overnight on nutrient agar at 30°C. After incubation, the total DNA of the pure strain was isolated with the use of the Genomic Mini AX Bacteria Spin kit (A&A Biotechnology).

The *16S rRNA* sequence was amplified with 27F (AGAGTTTGATCCTGGCTCAG) and 1,492 (GGTTACCTTGTTACGACTT) primers developed by Lane (1991). The PCR reaction was carried out with 20 ng of DNA, 2.5 µL of 10X Buffer (A&A Biotechnology, Poland), 0.25 mM of dNTPs (A&A Biotechnology, Poland), 0.2 µM of each primer (IBB, Poland) and 1 U of RUN polymerase (A&A Biotechnology, Poland). Final sample volume was 25 µL. Amplification was carried out in the Mastercycler®Nexus thermocycler (Eppendorf, Germany) in the following steps: initial denaturation at 95°C for 3 minutes, 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.30 minutes, with final extension at 72°C for 7 minutes.

The obtained PCR products were purified with the use of the Clean-Up kit (A&A Biotechnology, Poland). They were sequenced in the ABI Prism® 3130 genetic analyzer (Applied Biosystem, USA) with the BrightDye Terminator Cycle Sequencing Kit (Nimagen, Netherlands).

The obtained sequence was deposited in GenBank under accession number KF499272, and it was compared with other sequences with the use of the BLAST algorithm. The following sequences, previously analyzed by Kadyan *et al*. (2013), were selected for comparison: JX129227, JX144691, JX144693, JX144696, JX144697, JX144698, JX144701, JX144704, JX144706, JX144709, JX144712, JX144713, JX144715, JX155763, JX155764, JX155768, JX155769 and KC012993.

[DOR: 20.1001.1.16807073.2018.20.3.15.1]

A phylogenetic tree showing evolutionary relationships was developed in the Clustal W program which is built into MEGA 5.2 software. The analyzed sequences were 1,342 bp in length.

Morphological Traits

The analyzed strain's ability to thrive in environments characterized by various pH and salinity was tested in 5 mL of tryptic soy broth (TSB, Merck Millipore, Germany). Culture media had a pH of 5, 7 and 9, with NaCl concentrations of 0, 1, 5 and 9%. The sporulation test was performed on solid TSA (Merck Millipore, Germany) which was used to culture 0.1 mL of bacterial suspension that had been incubated at 80°C for 15 minutes. Incubation was carried out at 30°C. The effect of temperature on colony growth was analyzed on TSB at 41, 30, 22, and 4°C.

Regardless of incubation temperature, all treatments were cultured for 48 h with constant stirring. After incubation, the increase in bacterial mass on TSB was determined with a spectrophotometer (Nanodrop 2000c; Thermo Fisher Scientific, USA) by measuring Optical Density (OD_{600}) and comparing the results with the bacteria-free control medium. Each TSB medium was inoculated with 50 µL of the input material, i.e. an overnight culture of bacteria with the density of 1×10^8 CFU (Colony Forming Units) mL^{-1}.

Biochemical Parameters

Biochemical parameters were evaluated in qualitative analyses using API® 20NE and API® ZYM kits (Biomerieux, France) in accordance with the manufacturer's instructions. Tests were incubated at optimal temperature for the genus *Bacillus*.

Cellulose Degradation Test

The cellulase production test was conducted on a solid medium [K_2HPO_4 1.2 g, KH_2PO_4 0.5 g, $MgSO_4$ $7H_2O$ 0.5 g, CarboxyMethyl Cellulose (CMC) 5 g, gelatin 2 g, agar 15 g per liter H_2O]. The hydrolysis of CMC was described with the use of the method proposed by Hankin and Anagnostakis (1977). For cellulase activity, the detecting culture of the testing bacteria was streaked on the medium. Then, the culture was incubated for 3 days at 30°C. After incubation, the surface of the culture medium was rinsed with 1% aqueous solution of hexadecyltrimethylammonium bromide. The presence of a clearance zone was a positive result.

Chitin Degradation Test

The chitin degradation test was performed on the minimal salt medium (K_2HPO_4 1.2 g, KH_2PO_4 0.5 g, $MgSO_4$ $7H_2O$ 0.5 g, colloidal chitin 10 g, agar 15 g per liter H_2O). Colloidal chitin was obtained from pulverized crab shells according to the modified method described by Roberts and Selitrennikoff (1988). Ten g of chitin were combined with 100 mL of concentrated sulfuric acid, and the mixture was shaken for 2 hours. The mixture was quickly stirred, combined with 1,000 mL of ice-cold 95% ethanol and left to stand overnight at room temperature. Colloids were isolated by transferring chitin to paper filters and rinsing with tap water until it reached pH 5. Chitin was sterilized in an autoclave and stored at 4°C. For chitinase activity, the detecting culture of testing bacteria was streaked on the medium. Then, the culture was incubated for 3 days at 30°C. The presence of a clearance zone was a positive result.

Dual Culture Method

The inhibition of pathogenic fungi by *Bacillus* spp. was analyzed by the method described by Przemieniecki *et al*. (2104b) with minor modifications. Mycelial discs with a diameter of 5 mm were cut out from 14-day cultures of *Fusarium oxysporum*, *F.*

[DOR: 20.1001.1.16807073.2018.20.3.15.1]

culmorum and *Monographella nivalis* and were placed in the central part of a plate containing potato dextrose agar (PDA, DB Difco, USA). The analyzed bacteria were cultured on both sides of the plate, at a distance of 3 cm from mycelial discs.

All plates were cultured at 28°C for 5 days. The results were read, and the plates were incubated for another 5 days in a high humid environment to observe the behavior of mycelia (Przemieniecki, 2014b).

The percentage of inhibition was calculated according to the following formula:

% Of inhibition = [1 - (Fungal growth/Control without bacteria)]×100.

Pot Experiment

For the pot experiment, we used kernels of spring wheat *cv.* Bombona that were sterilized in 1% sodium hypochlorite and dried in a laminar flow cabinet. The test group was 50 g of kernels inoculated by submerging for 5 min with the bacterial culture suspension of density of 5×10^8 CFU mL^{-1} combined with sterile: deionized water, CMC (1% w/v) and silicon dioxide (10% w/v). The control group had the same number of *cv.* Bombona wheat kernels, not inoculated with the tested *Bacillus* sp. SP-A9 strains. All kernels, were sown at a depth of 2 cm in 4 pots filled with 8 kg of soil, with 24 kernels per pot. Before sowing of kernels, each pot was inoculated using 50 mL of a *F.* culmorum or *F.* oxysporum conidial suspension (10^7 conidia mL^{-1}), and 5 cm of soil was mixed to a depth of 5 cm. Soil parameters are given in Table 1. Wheat was harvested at the fully ripe stage (BBCH 89). The basic parameters of wheat stems and spikes, the weight of spikes and roots, and the dry matter content and weight of grain were determined to calculate the basic biometric parameters of spring wheat (Table 2) in comparison with the control group.

Table 1. Basic soil parameters in the pot experiment.

Parameter	Value
Soil texture	Loamy sand
pH (in KCl)	5.8
Total nitrogen (%)	0.013
P_2O_5 phosphate (mg 100 g^{-1} soil)	24.5
K_2O phosphate (mg 100 g^{-1} soil)	20.0
Magnesium (mg 100 g^{-1} soil)	4.5

Statistical analysis

All analyses were performed in three replications. Data were processed using Duncan's test (P= 0.05) in the Statistical 10 program (ANOVA).

RESULTS

In the group of 99 sequences with 100% nucleotide similarity, 57 sequences were indicative of *Bacillus subtilis*. After the elimination of 32 sequences of unidentified microorganisms and members of the genus *Bacillus* that were not identified to species level, 85% of the sequences belonged to *Bacillus subtilis*. A phylogenetic analysis revealed that *Bacillus* sp. SP-A9 was most closely related to *Bacillus subtilis* (*B. subtilis* subsp. *spizizenii*) and *B. licheniformis* (Figure 1).

An analysis of growth potential revealed that *Bacillus* sp. SP-A9 tolerated various

Table 2. Selected biometric parameters.

Soil pollutant	Treatment	No. of spikes per pot	Stem length (cm)	1000 Kernel weight (g)	Grain yield (g pot^{-1})
Fusarium culmorum	Inoculated	21.3a	44.672b	32.813a	6.167a
	Not inoculated	15.7b	46.967a	32.893a	5.228b
Fusarium oxysporum	Inoculated	18.7a	43.634b	33.013a	5.963a
	Not inoculated	15.0a	47.322a	32.487a	5.005b

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Table 3. Biochemical characterization.

	Biochemical properties	Result[a]
Enzymatic Activity	Alkaline phosphatase	-
	Acid phosphatase	-
	Esterase (C4)	+
	Ester lipase (C8)	(+)
	Lipase (C14)	(+)
	Leucine arylamidase	-
	Valine arylamidase	-
	Cystine arylamidase	-
	Trypsin	-
	α-Chymotrypsin	-
	Naphthol-AS-BI phosphohydrolase	+
	α-Galactosidase	-
	β-Galactosidase	+
	β-Glucoronidase	-
	α-Glucosidase	-
	β-Glucosidase	+
	N-Acetyl-β-glucosaminidase	-
	α-Mannosidase	-
	α-Fructosidase	-
	Arginine dihydrolase	-
	Urease	-
	Protease	+
Assimilation	*D*-Glucose	+
	L-Arabinose	+
	D-Mannose	+
	D-Mannitol	+
	N-Acetylglucosamine	+
	D-Maltose	+
	Potassium gluconate	+
	Capric acid	-
	Adipic acid	(+)
	Malic acid	-
	Trisodium citrate	-
	Phenylacetic acid	-
Reduction of nitrate to nitrite		(+)
Indole production		-
D-Glucose fermentation		-

[a] +: Positive result, (+): Weak positive result, -: Negative result.

environmental conditions. Bacterial cell density was similar in pH 5 to 9, but it increased with a rise in the NaCl dose. The greatest variations were observed in response to temperature. The optimal temperature for the proliferation of *Bacillus* sp. SP-A9 was 30°C (second bar from the top, marked pH 7), whereas lower growth rates were noted at both higher (40°C) and lower (22°C) temperatures. Further observations (data not presented) indicated that OD_{600} values for the above temperatures were equalized after the third day of incubation, and they were determined in the range of 0.8-1.1. Bacterial growth was not observed at 4°C (Figure 2).

The key biochemical properties of the analyzed bacterial strain (Table 3) include the ability to decompose esters, the activity of proteases, *β*-galactosidase and *β*-glucosidase, and the ability to assimilate sugars such as *D*-glucose, *L*-arabinose, *D*-mannose, *D*-mannitol, *D*-maltose and *N*-acetylglucosamine. The only organic acids assimilated by *Bacillus* sp. SP-A9 were adipic acid and potassium gluconate, a derivative of gluconic acid. The analyzed strain was characterized by low denitrification potential.

The results of cellulase and chitinase production tests were positive. The analyzed bacterial strain rapidly proliferated on a culture medium containing colloidal chitin.

In the dual culture test, *Bacillus* sp. SP-A9 was an antagonist of all three analyzed species of phytopathogenic fungi. The percentage of inhibition was estimated at 50% in all cases (Figure 3), and prolonged storage under more humid conditions did not contribute to further mycelial growth on the culture medium. Proliferation of aerial mycelium was observed only in the proximity of the mycelial disc.

The results of the pot experiment (Table 2) demonstrated that the tested strain effectively inhibited the growth of both fungal species of the genus *Fusarium*. The number of wheat spikes increased in each inoculated treatment, which directly increased grain yield per pot. Grain yield was 18 and 19% higher in inoculated treatments contaminated with *F. culmorum* and *F. oxysporum*, respectively. Inoculation did not influence 1,000 kernel weight. Both control treatments were characterized by somewhat longer stems and smaller spikes.

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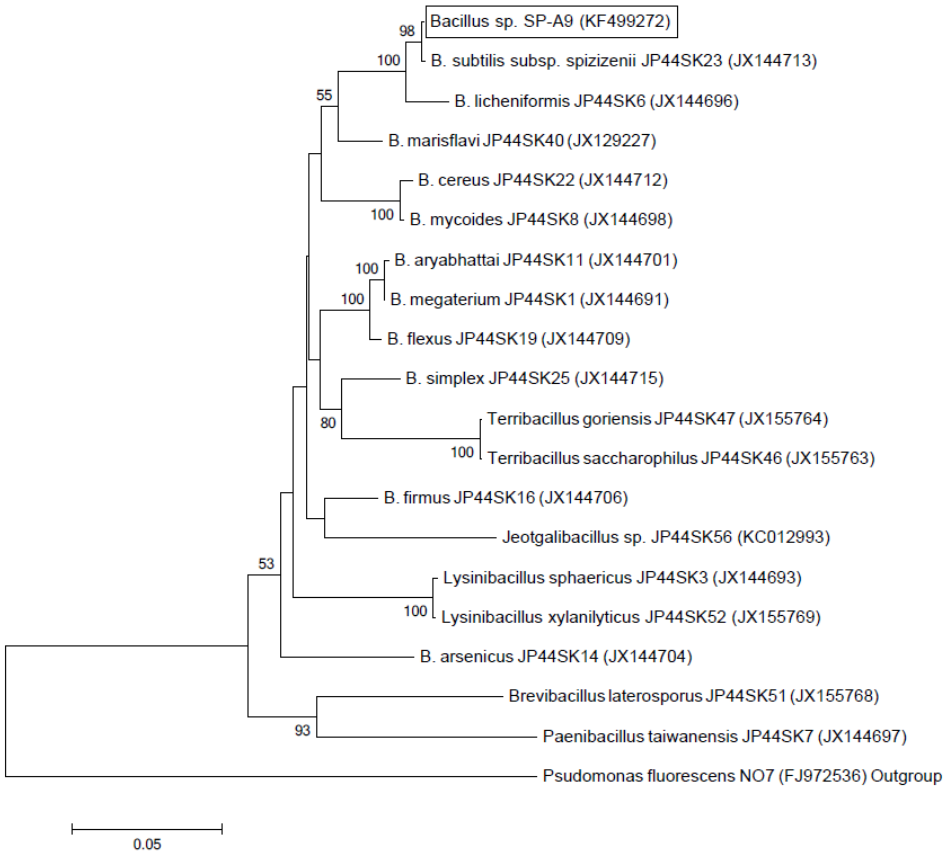

Figure 1. A phylogenetic tree developed with the use of the neighbor joining method illustrating the evolutionary relationships between the analyzed strain *vs.* other bacterial species of the genus *Bacillus* and closely related taxa. The tree was developed based on a fragment of the 16S *rRNA* gene, with 1,000 replications. Bootstrap support values greater or equal to 50% are shown for branches in the tree.

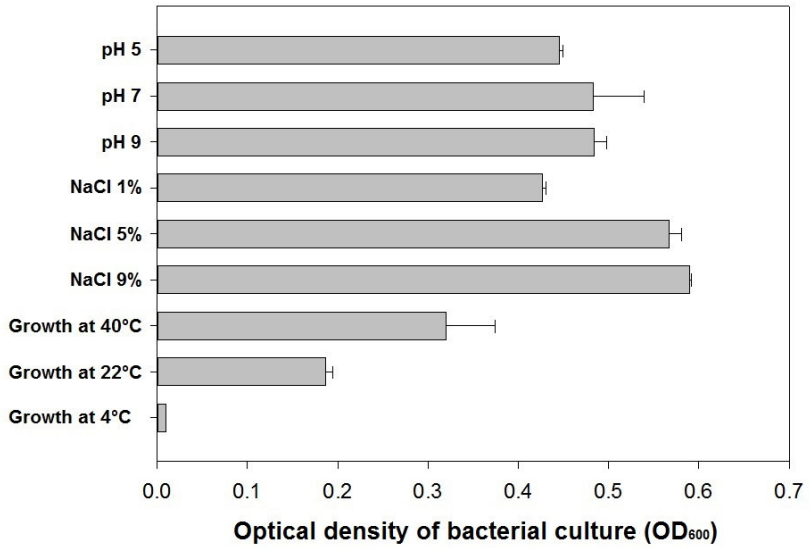

Figure 2. A qualitative analysis of selected growth parameters measured after 24 hours of incubation. From the top, each bars show the growth of tested bacteria in a medium in different: pH (1-3 bars), salinity (4-6 bars) and temperature of incubation (bars 7-9).

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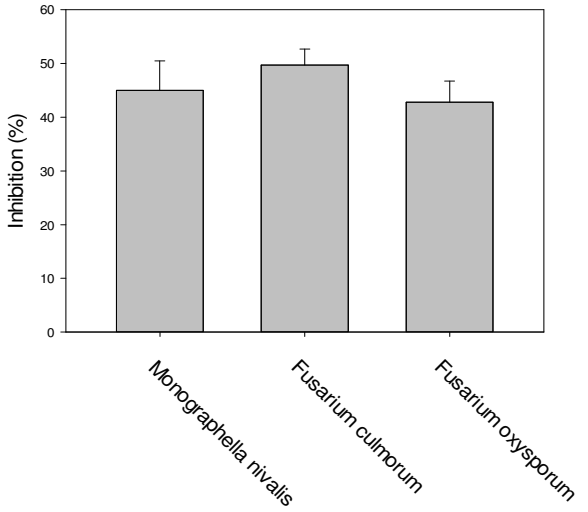

Figure 3. The ability of the *Bacillus* SP-A9 strain to inhibit the proliferation of pathogenic fungi.

DISCUSSION

Bacteria of the genus *Bacillus,* which enhance plant growth and protect plants against phytopathogens, are often isolated from the environment. The described *Bacillus* species of such properties are: *Bacillus simpex* (Schwartz *et al*., 2013), *B. amyloliquefaciens* (Idris *et al*., 2007), *B. pumilus* (Benhamou *et al*., 1996), *B. licheniformis* (Probanza *et al*., 2001), *B. thuringiensis* (Bai *et al*., 2003), *B. cereus* (Handelsman *et al*., 1990), *B. megaterium* (López-Bucio *et al*., 2007) and *Bacillus subtilis* (Ashwini and Srividya, 2013).

The genus *Bacillus* is characterized by moderate genetic variation, described mainly by the analysis of the 16S *rRNA* gene sequence. We confirmed that *Bacillus* sp. SP-A9 strain is closely related to the strains described as plant pathogens antagonistic, like: *B. licheniformis*, *B. marisflavi*, *B. cereus* and *B. mycoides*. Those results are in agreement with a phylogenetic analysis performed by Huang *et al*. (2012). The cited authors did not observe differences in the 16S rRNA sequence between selected strains of *B. amyloliquefaciens, B. megaterium* and *B. subtilis*, and the above was confirmed by an analysis of the KF499272 sequence. Rooney *et al*. (2009) performed a MultiLocus Sequence Analysis (MLSA) of five housekeeping genes where *B. subtilis* was clearly subdivided into *B. subtilis* subsp. *subtilis*, *B. subtilis* subsp. *inaquosorum* and *B. subtilis* subsp. *spizizenii*. Our tested strain exhibited the highest similarity to *B. subtilis* subsp. *spizizenii*, witch, in combination with mentioned 16S rRNA analysis, allows us to consider an SP-A9 strain as belonging to the species of *B. subtilis.*

The analyzed bacterial strain demonstrated a wide range of biochemical activities which are useful in preventing the spread of plant pathogens and indirectly allow plants to grow in more favorable environmental conditions. *Bacillus* sp. SP-A9 was capable of hydrolyzing esters and proteins and degrading chitin and cellulose which favors it in the environmental resources competition with other microorganisms, including fungi. Other bacterial species were described to have a similar qualities like those in the study of Rekha *et al*. (2007), the *Bacillus subtilis* CC-pg104 and *P. putida* CC-FR2-4 that both exhibited extensive metabolic diversity. However, the *P. putida* CC-FR2-4, despite having broader range of

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biochemical activities than *B. subtilis* CC-pg104, it turned out to be less effective as a plant promoter in the 21-day experiment. The tested *Bacillus* sp. SP-A9 strain exhibited both wide range of biochemical activities and plant growth promotion abilities, which makes it a good candidate to be used as a form of biological control of the wheat crops against *Fusarium* species.

Ashwini and Srividya (2013) isolated a strain of *Bacillus subtilis* which, similar to the strain analyzed in this study, degraded cellulose and chitin. The dual culture test revealed that the percentage of inhibition of *Fusarium* fungi was similar to that noted for *Bacillus* sp. SP-A9, reaching 41% for *F. solani* and 40% for *F. oxysporum*. The cited authors focused on chili pathogens which were inhibited more than 50%. The percentage of inhibition of *Colletotrichum gloeosporioides* was determined at 57%, which indicates that bacteria of the genus *Bacillus* are versatile biological control agents. The chili seed inoculation test delivered highly satisfactory results. Only 1% of non-inoculated seeds germinated under exposure to the analyzed pathogen and 78% of seeds were infected, whereas in the treatment where seeds were inoculated with *Bacillus subtilis,* 85% of seeds germinated and only 13% were infected. The *Fusarium* inhibition efficacy of the tested *Bacillus* strain is also high similar to described level.

Patil *et al*. (2015) demonstrated that two species of *Bacillus subtilis* and *Paenibacillus polymyxa* produced chitinases, *β*-1,3-glucanase, protease, and cellulase. Catalase activity and the ability to produce siderophores, Fe^{3+} chelating agents, were also observed in *Bacillus subtilis*. In laboratory analyses, both species were antagonistic against phytopathogens such as *Alternaria alternata*, *Aspergillus flavus*, *Botrytis cinerea*, *Colletotrichum acutatum*, *Fusarium oxysporum* f. sp. *ciceri*, *Fusarium oxysporum* f. sp. *pisi*, *Fusarium graminearum*, *Verticillium dahlia,* and *Xanthomonas compestris*. Traits characteristic of Plant Growth-Promoting Bacteria (PGPB), including the ability to produce ammonia and solubilize phosphates, were observed in at least one of the bacteria examined by authors.

Zhao *et al*. (2014) isolated the *Bacillus subtilis* SG6 strain which demonstrated strong antifungal activity against *Fusarium graminearum*. The percentage of inhibition in the dual culture test was determined at 88%, and inhibition of sporulation and DeOxyNivalenol (DON) production in additional tests reached 96% and 100%, respectively. In a field experiment, crop spraying with a suspension of the analyzed bacterial strain also produced satisfactory results. In comparison, the examined strain decreased Disease Incidence (DI) by 73% and the incidence of Fusarium Head Blight (FHB) by 78%, and it was more effective as inhibitor of pathogens than carbendazim. Spraying with the bacterial suspension did not affect 1,000 kernel weight, but it led to an approximately 71% reduction in DON content, which was similar to the result noted for carbendazim.

Compared with bacteria isolated from the rhizosphere of winter wheat, the *Bacillus* sp. SP-A9 strain was a much more effective antagonist of *F. culmorum*, *F. oxysporum,* and *Monographella nivali* at the optimal temperature for saprotrophs, but its antagonistic potential has not been investigated at lower temperatures. The examined strain was significantly more effective in degrading chitin than any bacterial strain isolated from the rhizosphere of winter wheat (Przemieniecki *et al*., 2014b).

In the pot experiment, inoculation with the *Bacillus* sp. SP-A9 strain did not influence the weight or the remaining biometric parameters of wheat grain, and similar results were reported by Zhao *et al*. (2014). Despite the above, bacterial inoculation minimized the adverse effects of pathogens and contributed to plant emergence in the pot experiment. The analyzed strain does not have the characteristic traits of Plant Growth Promoting Rhizobacteria (PGPR) and does not contribute directly to plant growth. But,

[DOR: 20.1001.1.16807073.2018.20.3.15.1]

when used as a grain inoculant and, probably, when sprayed on plants, it effectively prevents diseases caused by *Fusarium* fungi and minimizes losses in food production.

The obtained results indicate that *Bacillus subtilis* SP-A9 can effectively be used for fertilization in organic cultivation of spring crops and in warm climates. As the results, in the case of strong *Fusarium* pressure, the inoculation of kernels can increase the yield by nearly 20%, which is especially important for cereals tillage monoculture and intensive agricultural production. Moreover, it is naturally present in the environment and, due to its ability to adjust to a wide range of environmental conditions and antifungal properties, once introduced, it will most likely colonize to the soil and, to some extent, prevent the future *Fusarium* infections of wheat.

ACKNOWLEDGEMENTS

This work was financed by the Ministry of Science and Higher Education of Poland, research project No. 20.610.016-300.

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[DOR: 20.1001.1.16807073.2018.20.3.15.1]

کارآمد بودن ریسه SP-A9 از گونه *Bacillus* sp. به عنوان عامل کنترل بیولوژیکی در گندم بهاره (*Triticum aestivum* L.)

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چکیده

در آزمون های آزمایشگاهی، ریسه SP-A9 از گونه Bacillus sp. که بسیار به Bacillus subtilis نزدیک است، خواص ضد-قارچی بسیار خوبی نشان داد. در آزمون دو-کشتی، درصد بازدارندگی (inhibition) برای همه فیتوپاتوژن ها (شاملFusarium culmorum ، F. oxysporum، وMonographella nivalis) مشابه بود. تجزیه ریسه مزبور آشکار ساخت که این ریسه سلولوزدوست و به شدت کیتینولیتیک بود و خواص بیوشیمیایی آن حاکی بود که به آسانی با محیط های مختلف سازگار می شود. توانایی هاگ آوری این ریسه و نرخ بالای افزویش (proliferation) آن در شرایط اسیدی، قلیایی و محیط های بسیار شور(9% NaCl) نیز تاکید بیشتری بود بر سازگازی آن با شرایط نامساعد. در یک آزمایش گلدانی، پارامترهای اصلی بیومتریک گندم پاییزه که با Bacillus sp. SP-A9مایه کوبی شده بود، دگرگونی نشان نداد، ولی افزایش معناداری در عملکرد مشاهده شد (18٪ در خاک آلوده به F. culmorumو 19٪ در خاک آلوده به F. oxysporum). افزایش عملکرد با تعداد بوته های گندم همبستگی داشت، و این امر حاکی از آن بود که ریسه مزبور بازداندگی رشد گیاه توسط عامل بیماریزا را به حد اقل می رساند. بنا بر این می توان گفت که Bacillus sp. SP-A9 قادر است تا خسارت های اقتصادی ناشی از قارچ های جنس فوزاریوم را کم کند و به کاهش مصرف مواد شیمیایی حافظ گیاه بیانجامد و در نتیجه آلودگی محیط را کمینه سازد.

[DOR: 20.1001.1.16807073.2018.20.3.15.1]