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# Identification of the effectiveness of associative rhizobacteria in spring wheat cultivation

L.E. Kolesnikov[1,*], A.A. Belimov[2], E.Y. Kudryavtseva[3], B.A. Hassan[4] and Yu.R. Kolesnikova[3]

[1]Saint-Petersburg State Agrarian University, Faculty of Agrotechnologies, Soil science and Ecology, Department of Plant Protection and Quarantine, Petersburgskoe Shosse, 2, RU196601, St. Petersburg - Pushkin, Russia
[2]Federal State Budgetary Scientific Institution "All-Russian Research Institute for Agricultural Microbiology", Laboratory of rhizosphere microflora, sh. Podbelskogo, 3, RU196608, St. Petersburg, Pushkin-8, Russia
[3]Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), genetic resources of wheat department, plant introduction department, Bolshaya Morskaya Str. 42–44, RU190000, St. Petersburg, Russia
[4]Ministry of Agriculture, Agricultural Research Office, Abo-Ghraib, St. Al-Zaytun, H. IQ10081, Baghdad, Iraq
[*]Correspondence: kleon9@yandex.ru

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**Abstract.** The maximum increase in wheat yield (by 67% to the control), associated with a decrease in the root rot development by 19%, an increase in the productive bushiness by 18%, the spike weight by 26%, in the grains number per spike by 8% was noted when using the *Bacillus subtilis* strain 124-11; the strain effect on leaf diseases was insignificant (2–5%). The plants differed in the maximum changes (to control) in the total bushiness by 59%, the plants vegetative part weight by 27%, the flag leaf area by 21%, the pre-flag leaf area by 28%, the roots numbers and weight by 20% and 62%. After plants treatments with the *Pseudomonas fluorescens* strain SPB2137, the wheat maturation period was reduced by 9% (to the control), wheat yield increased by 58% due to a decrease in the development of root rot and septoria by 18%, the yellow rust pustules area by 44%; the productive bushiness and plant height increased by 25% and 19%, the plant vegetative weight by 21%, the spike length by 4%. The most expressed protective and growth-stimulating effect was shown by the *Sphingomonas* sp. K1B, which caused a maximum decrease (to the control) in the root rot and yellow rust development by 22% and 7%, the strips length by 22%, the pustules number in the strip by 29%, brown rust by 10%, septoria by 11%. Wheat plants were characterized by a large number and length of roots by 17% and 13%, root weight by 49%, a maximum increase in the nodal roots number and length by 15% and 17%; total bushiness by 34.5%; a maximum increase in plant vegetative weight by 37%; the spike length by 3%.

**Key words:** associative rhizobacteria, biocontrol of phytopathogens, productivity, soft wheat, yield structure.

## INTRODUCTION

The bacteria that stimulate plant growth (Plant Growth Promoting Bacteria - PGPB) relate to different groups, with most species belonging to the genera*, Azospirillum, Bacillus, Enterobacter, Gluconacetobacter, Paenibacillus, Pseudomonas, Rhizobium, Streptomyces* and *Agrobacterium* (Kumar et al., 2015; Kalantari et al., 2018; Tabande et al., 2020). Stimulating effects of the majority of PGPB are traditionally associated with three main mechanisms: phytohormones production, an increasing nutrients availability and water for crops, and plant protection from diseases (Asaf et al., 2017a).

One of the most studied phytohormones, found in a large number of metabolites of microorganisms - is auxins (Tsavkelova et al., 2006). Auxins synthesis is a process which depends on carbon and nitrogen sources, temperature, pH, and tryptophan presence in soils (Mohite, 2013). The application of *Bacillus amyloliquefaciens* S-134 with the ability of secreting indolyl-3-acetic acid in an amount of 26 mcg mL, could stimulate wheat growth and gave an increase in yield by 34% (Raheem et al., 2018).

One of the key elements of antagonistic mechanisms of PGPB activity, is the synthesis of biologically active compounds of various nature, such as antibiotics, lytic enzymes, siderophores and etc. (Sharma et al., 2009; Naseri & Younesi, 2021). A large and diverse group of antibiotics that are effective against phytopathogenic microorganisms is produced by spore-forming gram-positive bacteria *Bacillus subtilis*. One of the first antibiotics isolated from culture fluid of *Bacillus subtilis* was subtilin (Housusright, 1948), which is a short peptide, then lipopeptide antibiotics of several classes were isolated from various strains of *B. subtilis*: subsporins (Loeffler et al., 1986), bacillomycins L and D (Peypoux et al., 1987), phengicins (Loeffler et al., 1986) and others, and also $Fe^{3+}$ siderophores were identified (Hofemeister et al., 2004).

The study of the genus *Pseudomonas* as typical representatives of the rhizosphere microflora aroused great interest for researchers. In addition to its antagonistic abilities against phytopathogenic fungi (Naseri, 2019), the genus *Pseudomonas* exhibits other interesting properties: improving phosphorus nutrition for plants (Satyaprakash et al., 2017), synthesizing plant growth stimulators (Selvakumar et al., 2011; Pham et al., 2017), producing siderophores responsible for iron transport (Trapet et al., 2016), as well as substances responsible for inducing resistance to phytopathogens (Strunnikova et al., 2007; Pieterse et al., 2014). Pseudomonads, as typical soil bacteria, are able to synthesize a whole complex of antibiotics. The best studied antibiotics are phenazines (Briard et al., 2015), phloroglucins (Kidarsa et al., 2011), as well as pyoluteorin (Hu HBO et al., 2005) and pyrrolnitrin (Park et al., 2011). The protective action of PGPB-based biopreparation is also explained by the presence of the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase), which reduces the concentration of ethylene phytohormone in plants (Nadeem et al., 2013).

Inoculation of wheat seeds with a pseudomonas-based preparation leads to increase root and stem growths, increase germination energy thus, enhancing yield amount, especially under the circumstances of low doses of phosphorous fertilizers (Ali et al., 2011). Wheat seeds treatment with *Pseudomonas putida* 108 strain combined with of 50% phosphorus application caused an increase in wheat yield by 37% (Zabihi et al., 2011), and with *Pseudomonas fluorescens* Pf strain - by 16% (Naiman et al., 2009).

There is little information in the literature about rhizospheric bacteria belonging to the genus *Sphingomonas*. The strain *Sphingomonas spiritivorum* 38-22 had a high growth-stimulating activity, which provided an increase in yield of winter wheat at the level of 21% (Pukhaev et al., 2009). The *Sphingomonas* S11 strain had a greater antagonistic activity against eight *Fusarium* strains that cause wheat diseases (Wachowska et al., 2013a). Treating soybean with *Sphingomonas* sp. LK11 significantly increased plant height and biomass, photosynthetic pigments, glutathione, amino acids (proline, glycine, and glutamate) and primary sugars, compared to control plants (Asaf et al., 2017b).

The scientific novelty of this work consists in a comprehensive assessment of the impact of associative rhizobacteria strains (*Bacillus subtilis* 124-11, *Pseudomonas fluorescens* SPB2137 and *Sphingomonas* sp. K1B) on a wide range of indicators that characterize morphological characteristics of plants, grain yield and wheat resistance to the most dangerous diseases, namely root rot, powdery mildew, brown and yellow rust.

The purpose of the research is to obtain the data that indicate the possibility of developing an environmentally friendly technology for wheat cultivation, which provides an increase in its productivity and a decrease in the pathogens' harmfulness, with reducing the cost of plant protection measures.

## MATERIALS AND METHODS

The place of experimental work is Laboratory of Rhizosphere Microflora of the All-Russian Research Institute of Agricultural Microbiology (ARRIAM, Saint Petersburg) and Department of Plant Protection and Quarantine of Saint-Petersburg State Agrarian University SPbGAU (Saint Petersburg). The effectiveness of associative rhizobacteria strains on Triticum aestivum cultivars study was carried out in the experimental field of Federal research centre «The N.I.Vavilov All-Russian Institute of Plant Genetic Resources» (VIR) from 2017 to 2019 (Fig. 1).

**Figure 1.** Spring wheat sowing in the experimental field of the VIR, 2019.

**Bacterial samples.** The object of the study was the strains *Bacillus subtilis* 124-11 (growth inhibitor of phytopathogenic fungi according to unpublished data of Laboratory of Rhizosphere Microflora of the ARRIAM), *Sphingomonas* sp. K1B (hyper-producer of auxins according to unpublished data of Laboratory of Rhizosphere Microflora of the ARRIAM) and *Pseudomonas fluorescens* SPB2137 (producer of auxins, contains ACC deaminase, growth inhibitor of phytopathogenic fungi (Kravchenko et al., 2003). Strains were obtained from the Russian Collection of Agricultural Microorganisms (All-Russia Research Institute for Agricultural Microbiology, Saint-Petersburg), and information on their properties have not been published.

**Wheat experiment.** Plant material of the study were *Triticum aestivum* cultivars Trizo, k-64981 and Sudarynya, k-66407. In the field experiment, seeds were inoculated and sprayed with the strains *Bacillus subtilis* 124-11, *Sphingomonas* sp. K1B and *Pseudomonas fluorescens* SPB2137. For this purpose, strains were grown for two days on a Potato Dextrose Broth (P6685, Sigma-Aldrich, USA). Then, seeds were dipped with a suspension of bacteria ($10^8$ cells $mL^{-1}$) at the rate of 2 mL suspension per 10 g seeds and kept for an hour as previously described (Kozhemyakov &d Tikhonovich, 1998). Prophylactic spraying of plants with a culture liquid of bacteria ($10^9$ cells $mL^{-1}$) was carried out in the phases of stem extension and the beginning of flowering.

The experiments were arranged on a randomized complete block designed with four replicates. For one variant of the experiment, plot area was 1.0 $m^2$, treatments for plots in replicates were arranged systematically. The experiment samples was sown manually on plots in an ordinary way of sowing with a distance between rows of 15 cm and the distance between seeds in a row was 1–2 cm. The seeding depth was 5–6 cm.

Wheat productivity was studied in the phases: development of the germ shoot (stage 3-leaves), earing-flowering and maturation according to a set of indicators that characterize morphological characteristics and yield structure (Kolesnikov et al., 2019). In the ear-flowering phase, a complex of plant indicators: productive and total bushiness (pieces), plant phase (score, according to the Zadok's Scale (Zadoks, 1974) flag and pre-flag leaf area ($cm^2$), plant height (cm), spike length (cm), spikelets number per spike (pieces), spike weight (g) was studied. In addition, number and length of roots (main embryonic root, embryonic coleoptile and roots) extending from the epicotyl were calculated. Number and length of nodal roots, root weight, plants vegetative part weight were taken into account. In the maturation phase (stage of full ripeness), structure of wheat yield was studied according to the following indicators: spikelets number per spike, pieces; spike length, cm; weight of an spike with grain; grains number per spike, pieces; grains weight per spike, 1,000 grains weight. The potential (biological) yield of a single wheat plant was calculated in accordance with data about reproductive tillering and grain weight per an spike of one plant (Kolesnikov, Kremenevskaya et al., 2020; Kolesnikov, Novikova et al., 2020).

**Analysis of the development of wheat diseases.** Assessment of plants damage degree caused by root rot disease was carried out in laboratory in the phases of tillering (complete tillering) and earing-flowering in accordance with generally accepted scale (Popov, 2011). The flag and pre-flag leaves damage intensity caused by powdery mildew (*Blumeria graminis* Speer.), was calculated according to the generally accepted indicator- conditional degree of plant damage (Geshele, 1978), as well as additional indicators - number and area of spots with plaque. Affection of wheat flag and pre-flag leaves by the causative agent of brown rust (*Puccinia recondita* Rob. ex Desm. f. sp. tritici Eriks.) was taken into account on the R. F. Peterson scale (Geshele, 1978). As additional phytopathological parameters, pustules number per leaf and pustule area were used.

The wheat damage intensity caused by yellow rust pathogen was evaluated according to the generally accepted Manners scale, and, also, the pathogenesis indicators were used: pustules number (total per leaf), number of stripes with pustules, length of stripes with pustules, pustule area and their number in the strip.

The size of infectious structures of pathogens formed on leaves during pathogenesis (spots, pustules, etc.) was determined using an ocular micrometer. The values of pustules and spots with plaque area were calculated on the assumption of their elliptical shape (Kolesnikov, Kremenevskaya et al., 2020; Kolesnikov, Novikova et al., 2020).

**Statistical analyses.** The algorithm for statistical processing of field experiment data was based on the creation of an electronic database, first in Microsoft Excel spreadsheets, then in IBM SPSS Statistics software platform was utilized. Methods of parametric statistics based on calculation of mean and standard errors (SEM), 95% confidence intervals, and the Student's *t-test* were used in the calculations. In addition, methods of ANOVA using the Scheffe test to compare and verify the likeness of sample variances were applied (Lemeshko & Ponomarenko, 2006).

## RESULTS AND DISCUSSION

At the first stage of study, wheat productivity indicators were compared in the experimental variants: when plants were treated with associative rhizobacteria strains and without treatment (control group).

Yield is an integral feature that depends on the values of wheat productivity and the grains weight per one spike. Table 1 shows the data of multivariate analysis of wheat yield variance from inoculants, wheat cultivars, replicates, years. A significant effect of the inoculants and the years of research on wheat yield was revealed. A significant change in the wheat yield was defined from the interaction of Inoculant * Year factors was determined* Wheat cultivar* Replicate.

**Table 1.** Multivariate analysis of variance wheat yield, the 2017–2019

| Source | Type III Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 1,601.24 | 55 | 29.11 | 16.40 | 0.000 |
| Intercept | 2,179.83 | 1 | 2,179.83 | 1,228.26 | 0.000 |
| Inoculant | 138.81 | 3 | 46.27 | 26.07 | 0.000 |
| Year | 901.55 | 2 | 450.78 | 254.00 | 0.000 |
| Wheat cultivar | 5.70 | 1 | 5.70 | 3.21 | 0.074 |
| Replicate | 8.03 | 2 | 4.02 | 2.26 | 0.105 |
| Inoculant * Year | 143.72 | 6 | 23.95 | 13.50 | 0.000 |
| Inoculant* Wheat cultivar | 16.64 | 3 | 5.55 | 3.13 | 0.025 |
| Inoculant* Replicate | 53.53 | 6 | 8.92 | 5.03 | 0.000 |
| Year * Wheat cultivar | 35.51 | 2 | 17.76 | 10.00 | 0.000 |
| Year* Replicate | 14.41 | 2 | 7.21 | 4.06 | 0.018 |
| Wheat cultivar* Replicate | 21.70 | 2 | 10.85 | 6.11 | 0.002 |
| Inoculant* Year * Wheat cultivar | 60.22 | 5 | 12.04 | 6.79 | 0.000 |
| Inoculant * Year * Replicate | 17.24 | 6 | 2.87 | 1.62 | 0.139 |
| Inoculant* Wheat cultivar* Replicate | 42.02 | 6 | 7.00 | 3.95 | 0.001 |
| Year * Wheat cultivar* Replicate | 18.67 | 2 | 9.33 | 5.26 | 0.005 |
| Inoculant * Year* Wheat cultivar* Replicate | 54.56 | 6 | 9.09 | 5.12 | 0.000 |
| Error | 990.30 | 558 | 1.77 | | |
| Total | 6,077.10 | 614 | | | |
| Corrected Total | 2,591.53 | 613 | | | |
| R Squared = 0.62 | | | | | |

Based on the calculation of 95% confidence intervals for average statistically significant differences in wheat yield in the experimental variants were revealed in 2018 and 2019. In 2017, the wheat yield changed insignificantly. The greatest impact on wheat yield in 2019 was exerted by *B. subtilis* 124-11 and *Ps. fluorescens* SPB2137 (Fig. 2). When using *B. subtilis* 124-11, yield of wheat cultivars Sudarynya, k-66407 and Trizo, k-64981 in 2019 significantly increased ($P < 0.05$) in comparison with the control by 50% - *t*-test = 3.8 (on average for the period 2017–2019 - by 88%, *t-test* = 4.7) and by 52% - *t*-test = 3.4 (2017–2019 - by 46%, *t*-test = 2.7), respectively. With the application of *Ps. fluorescens* SPB2137 in 2019, there was a significant increase ($P < 0.05$) in the yield of Sudarynya, k-66407 cultivar by 95% - *t*-test = 5.7 (on average for the period 2017–2019 - by 122%, *t*-test = 5.3). While the yield of Trizo, k-64981 cultivar in 2019 was not significantly affected by this strain (in 2019, the yield increased by 5%, for the period 2017–2019 - by 9%).

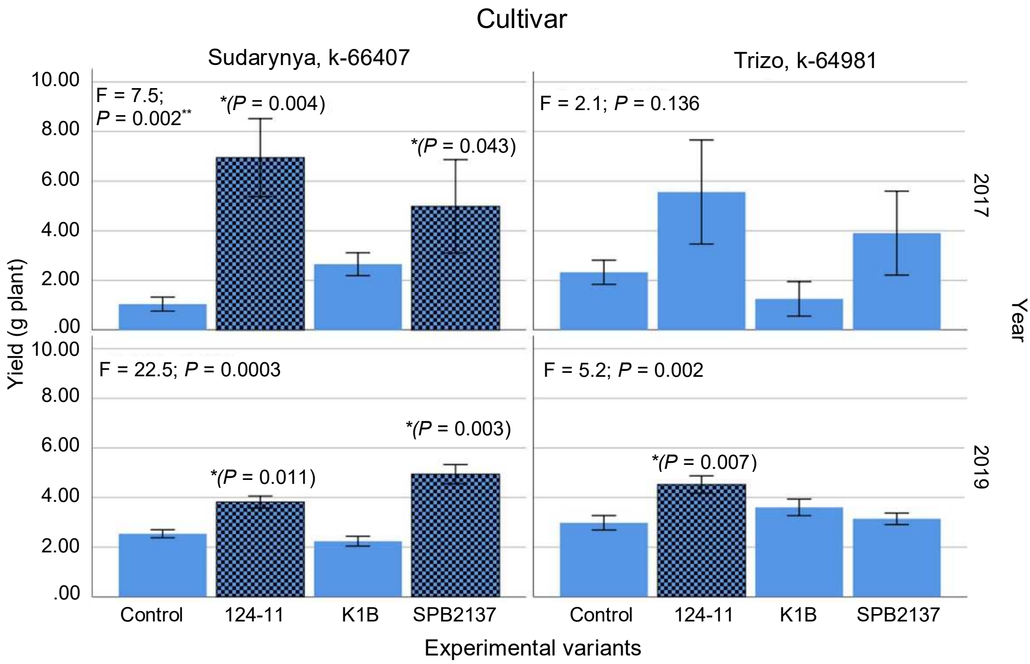


**Figure 2.** Changes in wheat yield of cultivars Sudarynya, k-66407 and Trizo, k-64981 when using associative rhizobacteria, the 2017 and 2019. Inoculation treatments: Control - water, 124-11 – *B. subtilis* 124-11, K1B – *Sphingomonas* sp. K1B, SPB2137 – *Ps. fluorescens* SPB2137. Vertical line – standard error of mean; * – significant values of the indicator, different from the control, according to the Scheffe criterion at a certain significance level; F – Fisher criterion according to the single-factor analysis of variance.

Figs. 3, 4 summarize data on biological yield of soft wheat, averaged over above-mentioned wheat cultivars and calculated based on the results of field experiment in 2019, also for the period 2017–2019. Using strains of *B. subtilis* 124-11 and *Ps. fluorescens* SPB2137 showed a statistically significant increase in wheat yield in 2019 at $P < 0.05$ by 51% (*t*-test = 5.1) and 45% (*t*-test = 4.2), and for the time 2017–2019 - by 67% (*t*-test = 5.2) and 58% (*t*-test = 4.6), respectively.

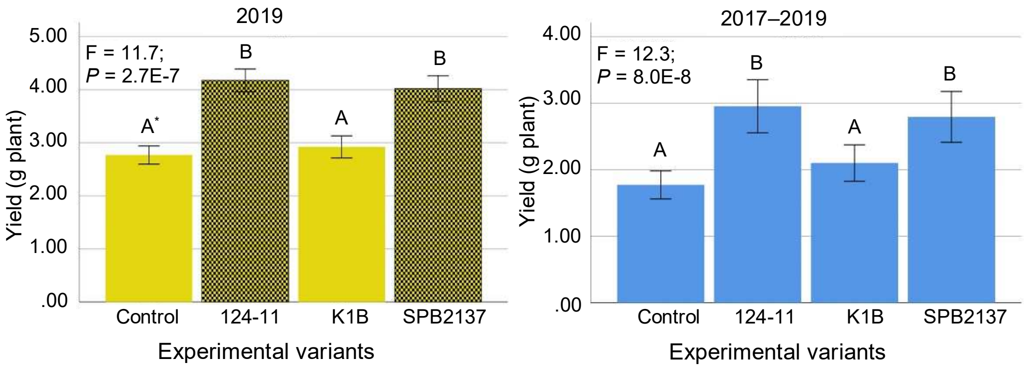


**Figure 3.** Average yield of soft wheat when using associative rhizobacteria, the 2017–2019. Variants of inoculation: Control – water, 124-11 – *B. subtilis* 124-11, K1B – *Sphingomonas* sp. K1B, SPB2137 – *Ps. fluorescens* SPB2137. The graphs show the average values of the indicators and 95% confidence intervals, * the same letters mark the values of the indicator that are not significantly different.

In 2019, the use of *B. subtilis* 124-11 strain caused an increase in the values comparing with the control ($P < 0.05$) in the indicators: rate of plant development in the phases of ontogenesis (by 11%; *t*-test = 2.2), plant height (by 22%; *t*-test = 2.8), the number of roots (by 25%; *t*-test = 2.7), pre-flag leaf area (by 31%; *t*-test = 3.3). In the experimental variant, where *Sphingomonas* sp. K1B was applied, an increase in the values of following indicators ($P < 0.05$) were noticed: rate of plant development in the phases of ontogenesis (by 11%; *t*-test = 2.6), plant height (by 16%; *t*-test = 2.2), roots number (by 23%; *t*-test = 2.9), root length (35%; *t*-test = 3.7), nodal root length (21%; *t*-test = 2.8). The use of *Ps. fluorescens* SPB2137 influenced plant development rate in the phases of ontogenesis (by 12%; *t*-test = 3.0), plant height (by 24%; *t-test* = 3.8), number of roots (by 20%; *t-test* = 2.8), pre-flag leaf area (by 29%; *t*-test = 3.3).

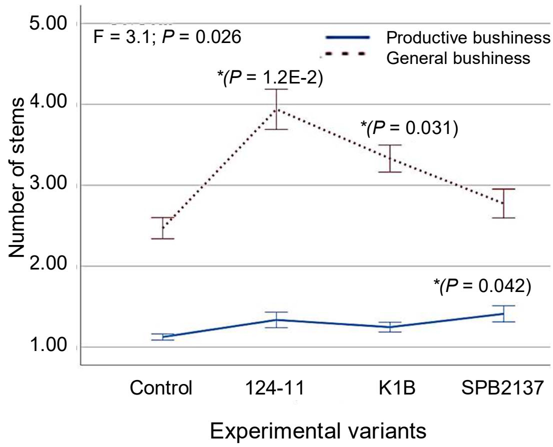


**Figure 4.** Total and productive bushiness of soft wheat when using associative rhizobacteria, the 2017–2019. Variants of inoculation: Control – water, 124-11 –*B. subtilis* 124-11, K1B – *Sphingomonas* sp. K1B, SPB2137 – *Ps. fluorescens* SPB2137. Vertical line – standard error of mean; * – significant values of the indicator, different from the control, according to the Scheffe criterion at a certain significance level; F – Fisher criterion according to the single-factor analysis of variance.

The strains of associative bacteria had the greatest impact on the wheat productive and total bushiness (Fig. 4). In particular, for the period 2017–2019, it was noticed that the application of *B. subtilis* 124-11 and *Sphingomonas* sp. K1B strains resulted in a significant increase in the total bushiness at $P < 0.05$ by 59% (*t*-test = 5.2) and 35% (*t*-test = 4.1). Using *Ps. fluorescens* SPB2137 led to an increase in the productive bushiness by 25% (*t*-test = 2.6). Strains of *B. subtilis* 124-11 and *Sphingomonas* sp. K1B did not significantly affect the productive bushiness (the change to the control was 18% and 10%, respectively, $P > 0.05$).

For the period 2017–2019, a significant effect of the studied strains ($P < 0.05$) on the increase in the plants vegetative weight was noted (*B. subtilis* 124-11 - by 27%; *t*-test = 2.6, *Sphingomonas* sp. K1B - by 37%; *t*-test = 3.1, *Ps. fluorescens* SPB2137 - by 21%; *t*-test = 2.3).

The greatest influence on grain number per spike increasing by 8% (*t*-test = 2.5), compared with control, for the period 2017–2019 was exerted by *B. subtilis* 124-1 strain. In the variant of the experiment, where the strain *Sphingomonas* sp. K1B was tested, a decrease in grain number per spike by 12% was noticed (*t-test* = 3.9).

Fig. 5 shows the positive changes ($P > 0.05$) and significantly positive changes ($P < 0.05$) in the values of wheat cultivars productivity indicators (Sudarynya, k-66407 and Trizo, k-64981) when using strains of associative rhizobacteria comparing with the control. In 2019, the greatest number of significant positive changes in productivity indicators (32%) was registered in the variant of experiment where *Ps. fluorescens* SPB2137 strain was used on the Sudarynya, k-66407 cultivar. Also, *B. subtilis* 124-11 strain had highest efficiency in relation to the productivity of Trizo, k-64981 cultivar (22% of significant positive changes in productivity indicators).

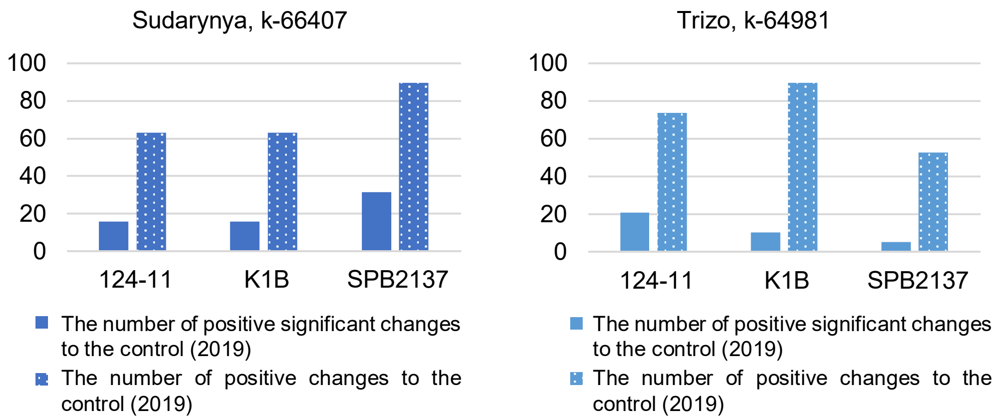


**Figure 5.** The number of changes (%) in the values of wheat productivity indicators when using strains of associative rhizobacteria comparing with control. 2019 Inoculation variants: 124-11 – *B. subtilis* 124-11, K1B – *Sphingomonas* sp. K1B, SPB2137 – *Ps. fluorescens* SPB2137.

For the period 2017–2019, the greatest number of significant positive changes in productivity indicators (47% of the total number of indicators) were registered in the experimental variants where *Sphingomonas* sp. K1B and *Ps. fluorescens* SPB2137 strains were used (Fig. 6).

At the second stage of study, influences of associative rhizobacteria strains on wheat pathogens development intensity were studied.

In 2019, the Trizo, k-64981 and Sudarynya, k-66407 cultivars were almost equally affected by root rot (*Rg* = 40%). The fungus *Bipolaris sorokiniana* (Sacc.) Shoem as the causative agent of wheat helminthosporium root rot, was identified by microscopic analysis.

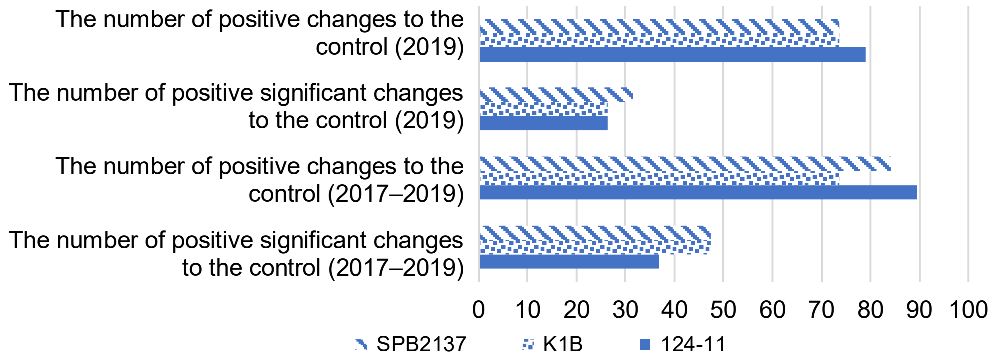


**Figure 6.** The number of changes (%) in the values of productivity indicators of two wheat cultivars (Sudarynya, k-66407 and Trizo, k-64981) when using strains of associative rhizobacteria comparing with control, the 2017–2019. Inoculation variants: 124-11 – *B. subtilis* 124-11, K1B – *Sphingomonas* sp. K1B, SPB2137 – *Ps. fluorescens* SPB2137.

In 2019, the most pronounced statistically significant decrease in disease development $P < 0.05$ was recorded on Sudarynya, k-66407 cultivar in the experimental variants when using *B. subtilis* 124-11 - by 21%, *t-test* = 6.6 and *Ps. fluorescens* SPB2137 - by 27%, *t-test* = 2.5.

The results of a comparative analysis of root rot development for the period 2017–2019 in the experiment variants with using of strains of associative rhizobacteria and without using (control), on average on two wheat cultivars (Trizo, k-64981 and Sudarynya, k-66407) are shown in Fig. 7.

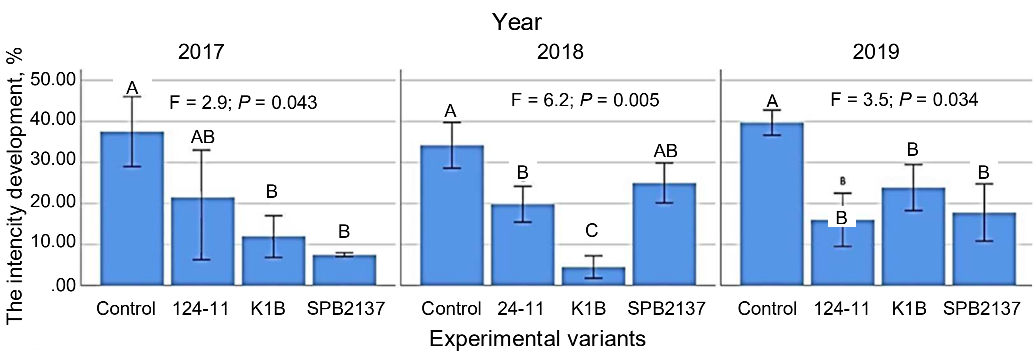


**Figure 7.** Development of wheat root rot in experimental variants with using associated rhizobacteria strains and without using (control) on wheat cultivars (Trizo, k-64981 and Sudarynya, k-66407) 2017–2019. Variants of inoculation: Control – water, 124-11 – *B. subtilis* 124-11, K1B – *Sphingomonas* sp. K1B, SPB2137 – *Ps. fluorescens* SPB2137. The graphs show average values of the indicator and 95% confidence intervals; * – the same letters mark values of the indicator that are not significantly different.

In accordance with the Fischer criterion (F), the strongest differences in the experimental variants were revealed in 2018. The *Sphingomonas* sp. K1B had the greatest effectiveness against wheat root rot, in 2018 it caused the disease development significant decreasing - by 86.8% compared to the control (from 34.2 ± 5.6% - in the control to 4.5 ± 2.7%). In 2017 and 2019, in this experimental variant, the root rot development decreased by 68.0% and 39.8%, respectively. The greatest decrease in the disease development - by 80% compared to the control, when using the *Pseudomonas fluorescens* SPB2137 was revealed in 2017, and in the variant with the 124-11 *Bacillus subtilis* strain - in 2019 (by 59.6%).

Powdery mildew is one of the most common and harmful diseases in wheat, causing significant losses in its yield. The causative agent of this disease is the microscopic fungus *Blumeria graminis (DC.) Speer f.* sp. *tritici* March (Fig. 8).

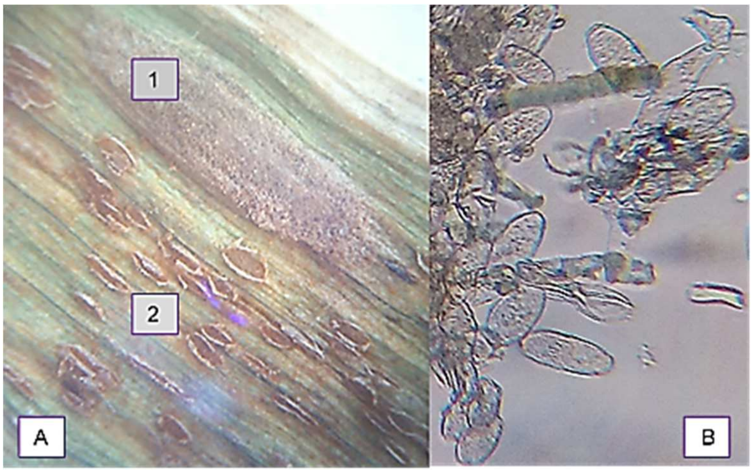


**Figure 8.** Symptoms of mixed infection on leaves of Trizo wheat cultivar, k-64981: A – spots with plaque of powdery mildew (1) and uredopustule of brown rust (2), an increase of 16X; B – conidia of the causative agent of powdery mildew, an increase of 800X (orig.).

In 2019, only *Sphingomonas* sp. K1B strain had an insignificant effectiveness in reducing intensity development of powdery mildew. In this variant of experiment, the decrease in disease development was 6% and the number of spots with plaque decreased by 27%. However, for the period 2017–2019, a significant decrease in the area of spots with plaque of powdery mildew after the treatments of Sudarynya, k-66407 cultivar was marked (with *Sphingomonas* sp. K1B. - by 58%, *t*-test = 2.3; with *Ps. fluorescens* SPB2137 - by 60%, *t*-test = 2.3; with *B. subtilis* 124-11 - by 64%; *t*-test = 2.5).

The causative agent of wheat brown rust, *Puccinia recondita* Rob. ex Desm f. sp. tritici, which causes the formation of many uredopustules on leaves during the growing season (Fig. 10). In 2019, the most significant plants damage caused by brown rust was registered on the Trizo cultivar, k-64981 ($R_b$ = 28.8 ± 6.9%, pustules number $N_p$ = 295.8 ± 126.3). The intensity of disease affection of wheat cultivar Sudarynya, k-66407 was: $R_b$ = 6.9 ± 1.8%, pustules number $N_p$ = 48.0 ± 20. A small decrease in disease development from 7% to 8% comparing with a control, was recorded in the experimental variants where strains of *B. subtilis* 124-11, *Sphingomonas* sp. K1B. and

*Ps. fluorescens* SPB2137 where used. While for the period 2017–2019, a significant decrease in brown rust development on average for both wheat cultivars when using *Sphingomonas* sp. K1B strain (by 10%, *t-test* = 2.6), in comparison with the control, was revealed.

The international scientific name of the causative agent of yellow rust is *Puccinia striiformis West. f.* sp. *tritici*. In 2019, the most significant damage caused by yellow rust was registered on Sudarynya, k-66407 cultivar ($R_b = 21.7 \pm 6.6\%$, pustules number $N_p = 857.2 \pm 242.5$). The intensity of disease affection of Trizo, k-64981 cultivar was: $R_b = 8.0 \pm 4.0\%$, pustules number $N_p = 242.0 \pm 122.4$. The greatest decrease in the wheat damage by yellow rust in comparison with the control was registered in Sudarynya, k-66407 cultivar when using *Sphingomonas* sp. K1B strain (unreliable for disease development at $P > 0.05$ - by 12%; strips number - by 13% and reliable at $P < 0.05$, pustules number in the strip - by 34%, *t*-test = 2.7; pustules number per leaf - by 35%, *t*-test = 3.0). No symptoms of yellow rust were detected in 2019 after the treatments by *B. subtilis* 124-11 on Trizo, k-64981 cultivar. Using *Ps. fluorescens* SPB2137 on Trizo, k-64981cultivar caused a slight ($P > 0.05$) decrease in the disease development intensity by 7%, the stripes number by 13%, and a significant decrease ($P < 0.05$) in the stripe length by 50% (*t*-test = 2.2), the pustules number per stripe by 75%, (*t*-test = 2.5) the pustules number per leaf - by 80% (*t*-test = 2.7). On average, for the above-mentioned cultivars for the period 2017–2019, the strain *Sphingomonas* sp. K1B had the highest efficiency against yellow rust, the use of which led to a decrease in the strip length at $P < 0.05$ by 22% (*t*-test = 2.6), the pustules number per strip by 29% (*t*-test = 2.5). A significant decrease in the pustule area by 44% ($t = 4.7$) was marked in the experiment variant with the using *Ps. fluorescens* SPB2137.

## CONCLUSIONS

The greatest potential yield of wheat in 2019 was revealed in the experimental variants with using *B. subtilis* 124-11 strains ($Y_r = 4.2 \pm 0.2$ g plant, when recalculated per 1 ha $Y = 6.19 \pm 0.33$ t ha) and *Ps. fluorescens* SPB213 ($Y_r = 4.0 \pm 0.2$ g plant, $Y = 5.9 \pm 0.4$ t ha), and for the period 2017–2019 - when using *B. subtilis* 124-11 ($Y_r = 3.0 \pm 0.2$ g plant, $Y = 6.2 \pm 0.3$ t ha). At the same time, in 2019, the maximum number of significant positive changes in productivity indicators was recorded when using *Ps. fluorescens* SPB2137 (32%), and for the period 2017–2019 when using *Sphingomonas* sp. K1B and *Ps. fluorescens* SPB2137 (47%). For the period 2017–2019, it was noticed that only with the application of *Ps. fluorescens* SPB2137 revealed a significant increase in the productive bushiness by 25% (*t*-test = 2.6, $P < 0.05$). Although, the treatments with *B. subtilis* 124-11 and *Sphingomonas* sp. K1B strains did not significantly affect the productive bushiness, but had a protective effect against the pathogens of wheat, particularly helminthosporium root rot. The maximum decrease in the disease development intensity by 26% was registered in 2017–2019 on Trizo k-64981 cultivar when using *Sphingomonas* sp. K1B. The strain of *Sphingomonas* sp. K1B showed the greatest effectiveness against the complex of leaf diseases.

The rhizobacteria high efficiency revealed in our research caused due to their growth-stimulating effect on plants and an increase in their adaptive potential to environmental factors, which is consistent with the results of studies presented in a number of scientific papers in this field (Araujo et al., 1994; Belimov et al., 2014;

Hashem et al., 2019; Naseri, 2019). The antagonistic activity of rhizobacteria against phytopathogenic fungi, its dependence on environmental factors and application methods, as well as the bacterium ability to cause induced plant resistance are widely discussed in various works (Araujo et al., 1994; Matzen et al., 2019; Wachowska et al., 2013b). Perhaps, the high efficiency of our rhizobacteria in wheat cultivation is associated with their combined application during sowing and throughout the entire growing season, as well as the diseases development inhibiting by plants preventive spraying before the first signs of disease development appearance.

The obtained data indicate the possibility of more effective cultivation and wheat protection from diseases when using bacterial strains (*B. subtilis* 124-11, *Ps. fluorescens* SPB2137, *Sphingomonas* sp. K1B), which can increase the wheat yield and its resistance to the main pathogens.

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## REFERENCES

Ali, S.Z., Sandhya, V., Grover, M., Linga, V.R. & Bandi, V. 2011. Effect of inoculation with a thermotolerant plant growth promoting *Pseudomonas putida* strain AKMP7 on growth of wheat (*Triticum* spp.) under heat stress. *Journal of Plant Interactions* **6**, 239–246. doi:10.1080/17429145.2010.545147

Araujo, M.A.V., Mendonça-Hagler, L., Hagler, A. & van Elsas, J. 1994. Survival of genetically modified Pseudomonas fluorescens introduced into subtropical soil microcosms. *FEMS Microbiology Ecology* **13**, 205–216. 10.1111/j.1574-6941.1994.tb00067.x.

Asaf, S., Khan, A., Khan, M., Imran, Q., Yun, B.-W. & Lee, I-J. 2017a. Osmoprotective functions conferred to soybean plants via inoculation with *Sphingomonas* sp. LK11 and exogenous trehalose. *Microbiological Research* **205**, 135–145. doi: 10.1016/j.micres.2017.08.009

Asaf, S., Khan, M.A., Khan, A.L., Waqas, M., Shahzad, R., Kim, Ah-Yeong, Kang, Sang-Mo & Lee, In-Jung. 2017b. Bacterial endophytes from arid land plants regulate endogenous hormone content and promote growth in crop plants: an example of *Sphingomonas* sp. and *Serratia marcescens*. *Journal of Plant Interactions* **12**(1), 31–38. doi.org/10.1080/17429145.2016.1274060

Belimov, A.A., Dodd, I.C., Safronova, V.I., Dumova, V.A., Shaposhnikov, A.I., Ladatko, A.G., & Davies, W.J. 2014. Abscisic acid metabolizing rhizobacteria decrease ABA concentrations in planta and alter plant growth. *Plant physiology and biochemistry* PPB **74**, 84–91. doi:10.1016/j.plaphy.2013.10.032

Briard, B., Bomme, P., Lechner, Beatrix, E., Mislin, Gaëtan, L.A., Lair, V., Prévost, Marie-Christine, Latgé, Jean-Paul, Haas, H. & Beauvais, A. 2015. *Pseudomonas aeruginosa* manipulates redox and iron homeostasis of its microbiota partner *Aspergillus fumigatus* via phenazines. *Scientific reports* **5**, 8220. doi: 10.1038/srep08220

Geshele, E.E. 1978. Fundamentals of phytopathological assessment in plant breeding. Moscow, Kolos, pp. 53 (in Russian).

Hashem, A., Tabassum, B., Abd, A. & Elsayed, F. 2019. Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences* **26**(6), 1291–1297. 10.1016/j.sjbs.2019.05.004

Hofemeister, J., Conrad, B., Adler, B., Hofemeister, B., Feesche, J., Kucheryava, N., Steinborn, G., Franke, P., Grammel, N., Zwintscher, A., Leenders, F., Hitzeroth, G. & Vater, J. 2004. Genetic analysis of the biosynthesis of non-ribosomal peptide-and polyketide-like antibiotics, iron uptake and biofilm formation by *Bacillus subtilis* A1/3. *Molecular Genetics and Genomics* **272**(4), 363–378. doi: 10.1007/s00438-004-1056

Housusright, D.R., Henry, J.R. & Berkman, S.A. 1948. Microbiological method for the assay of subtilling. *Journal of Bacteriology* **55**(4), 545–550.

Hu, H., Xu, Y., Chen, F., Zhang, X.H. & Hu, B.K. 2005. Isolation and characterization of a new fluorescent Pseudomonas strain that produces both phenazine 1-carboxylic acid and pyoluteorin. *Journal of microbiology and biotechnology* **15**(1), 86–90.

Kalantari, S., Marefat, A., Naseri, B. & Hemmati, R. 2018. Improvement of bean yield and Fusarium root rot biocontrol using mixtures of *Bacillus, Pseudomonas* and *Rhizobium*// *Tropical Plant Pathology* **43**, 499–505. https://doi.org/10.1007/s40858-018-0252-y

Kidarsa, T.A., Goebel, N.C., Zabriskie, T.M. & Loper, J.E. 2011. Phloroglucinol mediates cross-talk between the pyoluteorin and 2, 4-diacetylphloroglucinol biosynthetic pathways in *Pseudomonas fluorescens* Pf-5. *Molecular microbiology* **81**(2), 395–414. doi: 10.1111/j.1365-2958.2011.07697.x

Kolesnikov, L.E., Belimov, A.A. & Dones, P.M. 2019. The biological effectiveness of the associative rhizobacteria strains on soft wheat. *Proceedings of the St. Petersburg State Agrarian University* **1**(54), 57–64 (in Russian). doi: 10.24411/2078-1318-2019-11057

Kolesnikov, L.E., Kremenevskaya, M.I., Razumova, I.E., Kolesnikova, Yu.R., Ambulatova, E.V & Yazeva, E.O. 2020. The biological basis for the use of protein growth stimulant made from cattle split for wheat foliar feeding and disease suppression. *Agronomy Research* **18**(S2), 1336–1349 doi.org/10.15159/AR.20.082

Kolesnikov, L.E., Novikova, I.I., Popova, E.V, Priyatkin, N.S., Zuev, E.V., Kolesnikova, Yu.R., & Solodyannikov, M.D. 2020. The effectiveness of biopreparations in soft wheat cultivation and the quality assessment of the grain by the digital x-ray imaging. *Agronomy Research* **18**(4), 2436–2448. doi.org/10.15159/AR.20.206

Kozhemyakov, A.P. & Tikhonovich, I.A. 1998. Use of legume inoculants and biopreparations with complex action in agriculture. *Proceedings of the Russian Academy of Agricultural Sciences* **6**, 7−10.

Kravchenko, L.V., Azarova, T.S., Leonova-Erko, E.I., Shaposhnikov, A.I., Makarova, N.M. & Tikhonovich, I.A. 2003. Root exudates of tomato plants and their effect on the growth and antifungal activity of pseudomonas strains. *Microbiology* **72**(1), 37–41.

Kumar, A., Bahadur, I., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Singh, D.K. & Dixit, J. 2015. Does a plant growth-promoting rhizobacteria enhance agricultural sustainability. *Journal of Pure and Applied Microbiology* **9**(1), 715–724.

Lemeshko, B.Yu. & Ponomarenko, V.M. 2006. Investigation of statistical distributions of the Scheffe criterion under the laws of observation errors that differ from normal. 2006. *Proceedings of the VIII International Conference 'Actual problems of Electronic Instrumentation' APEP-2006* **(6)**, 87–90.

Loeffler, W., Tschen, Johannes S.-M., Vanittanakom, N. & Kugler, M. 1986. Antifungal Effects of Bacilysin and Fengymycin from *Bacillus subtilis* F-29-3 A Comparison with Activities of Other Bacillus Antibiotics. *Journal of Phytopathology* **115**(3), 204–213. doi: 10.1111/j.1439-0434.1986.tb00878.x

Matzen, N., Heick, T. & Jørgensen, L. 2019. Control of powdery mildew (*Blumeria graminis* spp.) in cereals by Serenade®ASO (*Bacillus amyloliquefaciens* (former subtilis) strain QST 713). *Biological Control*. **139**, 104067. 10.1016/j.biocontrol.2019.104067

Mohite, B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of soil science and plant nutrition* **13**(3), 638–649.

Nadeem, S.M., Zahir, Z.A., Naveed, M. & Nawaz, S. 2013. Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions. *Annals of Microbiology* **63**(1), 225–232. doi.org/10.1007/s13213-012-0465-0

Naiman, A.D., Latronico, A. & Garcia de Salamone, I.E. 2009. Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact on the production and culturable rhizosphere microflora. *European Journal of Soil Biology* **45**(1), 4–51. doi.org/10.1016/j.ejsobi.2008.11.001

Naseri, B. 2019. Legume root rot control through soil management for sustainable agriculture. In: Meena, R.S., Kumar, S., Bohra, J.S., Jat, M.L. (Eds.) *Sustainable Management of Soil and Environment,* pp. 217–258. doi:10.1007/978-981-13-8832-3

Naseri, B. & Younesi, H. 2021. Beneficial microbes in biocontrol of root rots in bean crops: A meta-analysis (1990–2020). *Physiological and Molecular Plant Pathology* **116**. doi.org/101712. 10.1016/j.pmpp.2021.101712

Park, J.Y., Oh, S.A., Anderson, A.J., Neiswender, J., Kim, J.-C. & Kim, Y.C. 2011. Production of the antifungal compounds phenazine and pyrrolnitrin from *Pseudomonas chlororaphis* O6 is differentially regulated by glucose. *Letters in applied microbiology* **52**(5), 532–537. doi.org/10.1111/j.1472-765X.2011.03036.x

Peypoux, F., Pommier, M.T., Das, B.C., Bessonn, F., Delcambe, L. & Michel, G. 1984. Structures of bacillomycin D and bacillomycin L peptidolipid antibiotics from *Bacillus subtilis*. *The Journal of antibiotics* **77**(12), 1600–1604.

Pham, V.T., Rediers, H., Ghequire, M.G., Nguyen, H.H., De Mot, R., Vanderleyden, J. & Spaepen, S. 2017. The plant growth-promoting effect of the nitrogen-fixing endophyte *Pseudomonas stutzeri* A15. *Archives of microbiology* **199**(3), 513–517. Doi 10.1007/s00203-016-1332-3

Pieterse, Corné M.J., Zamioudis, C., Berendsen, Roeland, L., David, M.W., Van Wees, Saskia C.M. & Bakker, Peter A.H.M. 2014. Induced systemic resistance by beneficial microbes. *Annual review of phytopathology* **52**, 347–375.

Popov, Yu.V. 2011. Method for the estimation of the root rots development in cereals. *Plant protection and quarantine* **8**, 45–47 (in Russian).

Pukhaev, A.R., Farniev, A.T. & Kozhemyakov, A.P. 2009. Efficiency of new associative rhizobacteria strains at winter wheat sowings. *Zemledelie* **8**, 40–41 (in Russian).

Raheem, A., Shaposhnikov, A., Belimov, A.A., Dodd, I.C. & Ali, Basharat. 2018. Auxin production by rhizobacteria was associated with improved yield of wheat (*Triticum aestivum* L.) under drought stress. *Archives of Agronomy and Soil Science* **64**(4), 574–587. doi:10.1080/03650340.2017.1362105

Satyaprakash, M., Nikitha, T., Reddi, E.U.B., Sadhana, B. & Vani, S. Satya. 2017. Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. *International Journal of Current Microbiology and Applied Science* **6**(4), 2133–2144. doi: 10.20546/ijcmas.2017.604.251

Selvakumar, G., Joshi, P., Suyal, P., Mishra, P., Joshi, G., Bisht, J.K., Bhatt, J. & Gupta, H.S. 2011. *Pseudomonas lurida* M2RH3 (MTCC 9245), a psychrotolerant bacterium from the Uttarakhand Himalayas, solubilizes phosphate and promotes wheat seedling growth. *World Journal of Microbiology and Biotechnology* **27**, 1129–1135. doi:10.1007/S11274-010-0559-4

Sharma, R.R., Singh, D. & Singh, R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological control* **50**(3), 205–221.

Strunnikova, O.K., Shakhnazarova, V.Yu., Vishnevskaya, N.A., Chebotar, V. & Tikhonovich, I.A. 2007. Development and relations of *Fusarium culmorum* and *Pseudomonas fluorescens* in soil. *Microbiology* **76**(5), 596–602. doi:10.1134/S002626170705013X

Tabande, L. & Naseri, B. 2020. How strongly is rhizobial nodulation associated with bean cropping system? *Journal of Plant Protection Research* **60**, 176–184. doi: 10.24425/jppr.2020.133307

Trapet, P., Laure, A., Agnès, K., Stéphanie, P., Sylvie, C., Christian, C., Sylvie, M., Philippe, L., David, W. & Angélique, Besson-Bard. 2016. The *Pseudomonas fluorescens* siderophore pyoverdine weakens *Arabidopsis thaliana* defense in favour of growth in iron-deficient conditions. *Plant physiology* **171**(1), 675–93. doi: 10.1104/pp.15.01537

Tsavkelova, E.A., Klimova, S.Yu., Cherdyntseva, T.A. & Netrusov, A.I. 2006. Microbial producers of plant growth stimulators and their practical use: a review. *Applied biochemistry and microbiology* **42**(2), 117–126. doi: 10.1134/S0003683806020013 (in Russian).

Wachowska, U., Jedryczka, M., Irzykowski, W. & Glowacka, K. 2013a. Use of *Aureobasidium pullulansto* control fusarium head blight on winter wheat ears caused by *Fusarium culmorum*. *Communications in agricultural and applied biological sciences* **78**(3), 545–549.

Wachowska, U., Irzykowski, W., Jedryczka, M., Stasiulewicz-Paluch, A. & Glowacka, K. 2013b. Biological control of winter wheat pathogens with the use of antagonistic Sphingomonas bacteria under greenhouse conditions. *Biocontrol Science and Technology* **23**, 1110–1122. 10.1080/09583157.2013.812185

Zabihi, H., Savaghebi, G.R., Khavazi, K., Ganjali, A. & Miransari, M. 2010. Pseudomonas bacteria and phosphorous fertilization, affecting wheat (Triticumaestivum L.) yield and P uptake under greenhouse and field conditions. *Acta Physiologiae Plantarum* **33**, 145–152. doi:10.1007/s11738-010-0531-9

Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A Decimal Code for the Growth Stages of Cereals. *Weed Research* **14**, 415–421.