Tomsk State University Journal of Biology. 2013. № 3 (23). P. 19–37

БИОТЕХНОЛОГИЯ И МИКРОБИОЛОГИЯ

UDK 579.64:631.46

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EFFECTIVENESS OF APPLYING BACTERIA *Pseudomonas* sp., STRAIN B-6798, FOR ANTI-PHYTOPATHOGENIC PROTECTION OF CROPS IN WESTERN SIBERIA

Biological method of plant protection from phytopathogens is based on applying microbial antagonists of phytopathogens. Today, domestic and foreign markets are represented by a number of bacteria-based formulations; nevertheless, more effective plant protection agents are being searched. The present work examines a possibility of applying formaldehyde-resistant bacteria Pseudomonas sp., strain B-6798, as a potential biofungicide. Different methods for estimation of antifungal activity of the strain were used, including the method linked with inhibition of phytopathogenic fungi by bacteria-antagonists. Growth-promoting activity of bacteria was assessed both in laboratory and field experiments. As the test objects were used the following crops: wheat, oats, maize, potato and fiber flax. Treatment of seeds with Pseudomonas sp., strain B-6798, reduced the overall infestation of cereal crops by seed infection agents by 12–36% due to decreasing the percentage of seeds affected by Helminthosporium and Fusarium root rot causing agents. Bacterization of potato tubers reduced late blight in the period of growing season by 45–50%, rhizoctonia blight and common scab by 40–70% during storage. The optimum concentrations of the applied bacteria for plant protection were within 10^4–10^7 CFU/ml. It was established that under the activity of antagonistic bacteria in the laboratory conditions shoot length was increased in 1.5–3.5 times, dry biomass – 1.5–5 times and total root system length – 1.7–3.0 times. According to the results of field experiments, bacterization contributed to increasing the yield of cereal crops and potato by 10–40%, depending on the species and variety.

Key words: *antifungal activity; bacteria-antagonists; biofungicide; growth-promoting activity; phytopathogen.*

Introduction

At the present time, most part of microbial formulations is based on rhizosphere bacteria related to genus *Pseudomonas*. The domestic microbial industry produces numerous biological fungicides using these bacteria, including the following trademarks: such *Pseudomonas*-based formulations as Pseudobacterin 2™, Agat-25K, Planriz and others [1, 3]. Many formulations based on different

species of *Pseudomonas* genus are known worldwide: Dagger G, Conquer, Victus, BioCoat, Blue Circle, Intercept [4–5]. Wide utilization of *Pseudomonas* spp. bacteria in biotechnology is linked with multifunctional properties of these bacteria, together with direct inhibition of phytopathogenic microorganisms and as a result of the synthesis of siderophores, cyanide, antibiotics, plant growth stimulation, vitamins, phosphate mobilization and nitrogen fixation [1–5]. Despite a wide range of already developed and applied microbial fungicides, there is a constant search for new strains capable of providing more stable positive effects on plant growth and development and inhibiting phytopathogens [2]. The first experiments linked with estimation of antifungal properties of bacteria *Pseudomonas* sp., strain B-6798, demonstrated high antagonistic activity of this strain to the most economically important pathogenic fungi. The antagonist is capable of active growth and multiplication in the rhizosphere of agricultural plants. The first data served as a reason for more detailed investigation of useful properties of the new bacterial strain [6–7]. It is known that a stimulating effect on the growth and development of plant vegetative mass is based on the fact that *Pseudomonas* are capable of synthesizing various growth regulators (indole-3-acetic acid – IAA, gibberellins) and vitamins [1–8]. According to Ju.A. Gushchina and I.F. Glovatskaya [9], bacteria of the strain B-6798 can secrete auxin substances to the environment, particularly, IAA in the quantity of 0.8 mg/ml.

The purpose of the present work was connected with further study and summary of the results on the properties of formaldehyde-utilizing *Pseudomonas* sp., strain B-6798, as a plant growth stimulator and a potential inhibitor of phytopathogenic fungi affecting various crops.

Material and methods

Research objects

The strain of *Pseudomonas* sp., strain B-6798, was isolated from active silt in purifying construction of Tomsk petrochemical industrial complex. These are facultative methylotrophic bacteria capable of utilizing formaldehyde as a part of poor mineral media as the only source of carbon and energy in the concentration of 250 mmol.

Fusarium oxysporum, F. oxysporum f.sp. *lini*, *F. oxysporum* f.sp. *gladioli*, *Bipolaris sorokiniana* and *Rhizoctonia solani* were examined as test objects for assessing antagonistic bacterial activity.

Evaluation of *Pseudomonas* sp., strain B-6798, growth-stimulating activity, was realized basing on the seeds of the following crops: Moldavskaya-215 maize variety (the average seed weight – 0.5 g, total germination – 75%), Tulunskaya-12 wheat variety (0.03 g, 97%), Narymsky-943 oats variety (0.05g, 93%), Tomsky-10 fiber flax variety, as well as potato tubers of Fresco, Zhukovsky early ripening, Nevsky and Lugovskoy varieties.

Media and cultivation. Bacteria *Pseudomonas* sp., strain B-6798, were cultivated in 50ml of the minimal M9 medium [10] with formaldehyde (2–4 g/l) in

a 250-ml retort. Microorganisms were incubated at 30±0.5°C in thermostat until the quantity was established in the limit from 1 to $9*10^9$ CFU/ml. The quantity control was performed by direct counting of cells in a Goryaev's chamber and by inoculation in accordance with the limiting-dilution method on the solid medium with a further count of CFU.

20-% wort agar (4–6°B) was used for phytopathogenic fungi incubation.

Investigation of Fungistatic Properties

Investigation of Kinetics of Phytopathogenic Fungi Growth Inhibition by Bacteria

Analyses of antifungal activity of the strain in laboratory experiments were conducted using both standard method of co-seeding of bacteria-antagonists together with phytopathogenic fungi located on the surface of the medium with a further evaluation of mycelium development inhibition and the method of determining kinetics of phytopathogenic fungi growth inhibition by bacteria-antagonists [6]. The second method elaborated in our laboratory assessed the fungistatic effect according to reduction in the growth rate of fungal colonies on solid medium with different bacteria concentrations, using standardized inoculation procedure.

Investigation of the influence of bacterization on seeds contaminated by phytopathogens. During the experiment, the seeds, previously disinfected with 70% ethanol solution, were soaked in bacterial suspension with concentration $1–6 \times 10^6$ CFU/ml for 30 minutes. Control seeds were soaked in sterilized water. The presence of fungistatic effect with experimental strain bacteria was estimated according to reduction in seed infestation by seed infection agents when conducting a phytopathological analysis in sterile absorbent paper rolls 7 days after the setting up of the experiment [11].

Fungistatic effect assessment in field experiments was carried out according to disease incidence and index with plants treated with bacteria in comparison with plants without bacterization [11–12]. The severity of cereal crops infection with root and foot rots was estimated according to the degree of browning of the stem base and plant root system, selected among the control and experimental variants. A rating scale was used to determine plant disease index [11–12]. Root rots were examined three times during the growing season: I – the phase of germination, II – tillering and III – flowering. To evaluate the effect of the bacterial preparation on potato blight development during the growing season, inspections of bacterized and control plants were organised. The development of blight affection of plants was analyzed four times: I – during series closing, II – during budding, III – during full blossoming and IV – before top-killing. Potato late blight was estimated in percentage terms on a generally accepted scale [12]. Disease incidence and index were determined by standard methods [11–12].

Disease incidence is a number of diseased plants in percent to total number of plants observed. Disease incidence was calculated as follows:

$$Incidence = \frac{Total\ number\ of\ diseased\ plants}{Total\ number\ of\ plants\ observed} \times 100.$$

Disease index is a measure of disease damage assessment which was calculated in percent as follows:

$$Index = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + (4 \times e)}{(a + b + c + d + e)} \times \frac{100}{4},$$

a, b, c, d, e – the number of tillers examined which fall into the categories 0, 1, 2, 3 and 4, respectively

To assess bacterial influence on a phytosanitary condition of the new crop during the storage period, a number of phytopathological analyses of potato tubers treated with bacteria and control plants were conducted. At least 100 tubers from each variant were selected three times during the whole period of storage. The tubers were washed, examined and cut in order to identify external and internal symptoms of diseases.

Investigation of stimulating activity

Investigation of the influence of concentration of bacterial suspension on plant growth and development in vegetation experiments

In the experiments, a model of small terrestrial artificial systems was created, consisting of three parts: a sterile substrate, used to fill vegetation vessels, host-plant and bacterial strains at concentrations of 10^1–10^9 CFU/ml. Pre-germinated and disinfected seeds of cereal crops and fiber flax were placed in test tubes with sand (12.5 cm^3); one-centimeter long disinfected potato shoots were put in a container with sterile soil and evenly moistened with sterile Knop's solution. Inoculation was carried out by 0.1 ml of bacterial suspension at different concentrations. Plants were grown in a climate camera: 12-hour light period, illumination intensity – 3 000 lux and temperature ±22±1°C. The experiment was carried out in three replications with 35–40 plants per each variant. The experiments being terminated, the following parameters were measured: the main root length (for maize), total root system length (for oats and wheat), the number of second-order roots and plant dry biomass. For each potato plant was measured the wet vegetation mass, the number of leaves and the number of tubers together with their weight.

Investigation of the influence of bacterial culture on plant growth and development in field experiments

Field experiments lasted for 6 growing seasons. The soil in the experiments was gray, forest, podzolized and medium-loam with low humus content (4–5%) as well as a thin humus accumulation horizon (20–25 cm).

Maize seeds were planted at a distance of 10–15 cm from each other, wheat and oats were sown at 600 seeds/m^2, and potatoes were planted at 42000 tubers/ha. Bacterisation of seeds was conducted with the help of pre-planting humidification treatment by bacterial culture with a titre of no less than 10^6 CFU/ml.

Potato tubers were soaked in bacterial suspension of the same concentration for 30 minutes. Seeds and tubers treated with water in the same manner as seeds in the test variants were used as control.

In the period of the experiment, the length of the maximum potato shoot was measured in each plant in all variants during a month since sprouting. The number of stems and the intensity of plant flowering were also analyzed during the experiments. Small sheaves of other plants were selected uniformly over the whole area of the test variant. Then, the length and dry biomass of the selected plants were measured; the quantity of grains per filled ear, the number of reproductive stems and the weight of 1 000 grains were examined in cereal plants during the phase of full ripeness.

Processing experimental results

The data obtained during the experiments were processed by means of StatSoft STATISTICA 6.0. The data are presented as a mean with confidence interval, using Student's t-test for 95% significance level. Statistical significance of the obtained results of seed phytopathological expertise, the incidence of root rots and foliar infections in field experiments were assessed by comparing sampling fractions, using Student's t-test for the probabilities 25–75% inclusive and using F-test for other probability values. Growth rates of fungal colonies on solid medium were determined by linear regression analysis. The data on parameters of plant growth and development in field experiments were compared by non-parametric Mann-Whitney test ($p < 0.05$) [13].

Results and discussion

Antifungal activity of Pseudomonas sp., strain B-6798, in model experiments

In vitro experiments, the strain showed a high antagonistic activity against a number of fungal pathogens. The bacteria facilitated reduction in the growth rate of fungal colonies on solid media in Petri dishes within the limits of 5–8 times in comparison with control (fig. 1). In control, fungal colonies grew on a bacteria-free medium. It was experimentally established that the magnitude of fungistatic effect depended on the type of phytopathogens and the concentration of bacteria in dishes. Fungus *Bipolaris sorokiniana* was the most sensitive to antagonistic bacteria and *F. oxysporum* f.sp. *lini* – the least.

The ratio of antagonist concentration to the level of disease development inhibition is very important for development and application of microbial fungicides.

G.M. Raaijmakers et al [14] demonstrated that there was a ‘‘threshold” level of antagonist concentration, below which a slight decrease in bacteria concentration, expressed as a logarithm (log_{10}-dose), results in a significant modification of inhibition of the development of fungi pathogens. Our experiments detected no threshold effects in relation to the growth rate of phytopathogenic fungal colonies. In order to describe kinetics of fungal colonies growth inhibition by the studied bacteria, we used N.D. Ierusalimsky modified equation [6]. Fungal colonies growth inhibition was already fixed at bacteria concentration 10^2 cells/ml in the inoculum. As bacteria concentrations in Petri dishes increased, the growth rate of fungal colonies tended to reach the limit value, not equal to zero, i.e. no total absolute fungal growth inhibition by bacteria was observed. In all the studied relationships between fungi and bacteria, concentration of 10^7–10^8 cells/ml is enough to reach the smallest limit value of fungal growth rate.

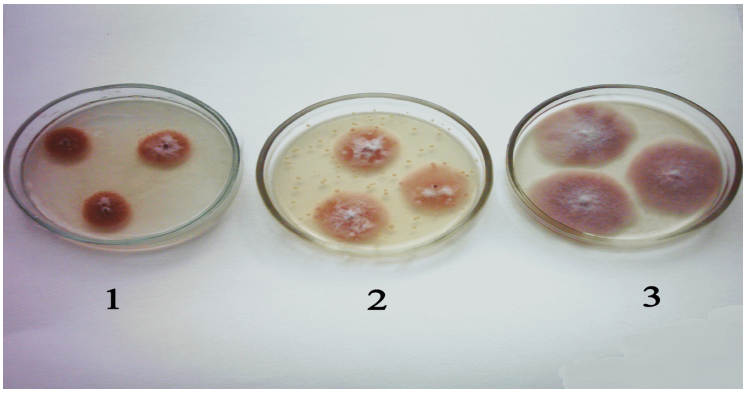

Fig. 1. *Fusarium oxysporum* fungal colonies in the experiment with bacteria *Pseudomonas,* strain B-6798, on the sixth day of the experiment
Note. 1 – concentration of bacterial suspension 10^4 cells/ml; 2 – concentration 10^2 cells/ml; 3 – control (without bacteria)

In the mechanism of fungal growth inhibition by the studied bacteria, a crucial role is, apparently, played by *Pseudomonas* sp., strain B-6798, producing yellow-green fluorescent pigments – siderophores. Siderophores are chelators, highly specific to Fe^{3+}, which are synthesized and used in an extracellular way in the conditions of low iron content [15]. The siderophores of fluorescent pseudomonades have different chemical structure and, having affinity for iron, form stable complexes together with it; thereby, they successfully compete for this element with siderophores of pathogenic fungi, which have a lower iron binding constant [8, 16]. Siderophore inhibition mechanism are testified by experimental data, showing reduction in fungistatic effect when increasing Fe^{3+} salts in the medium [17]. As it is known, the antagonism of pseudomonades against phytopathogens is largely conditioned by competition for iron and is only effective at low iron content in soil [8, 15, 18–21].

The influence of bacterization on seeds to infection agents

Phytopathological examination of bacterized seeds of wheat and oats revealed an antagonistic effect of bacteria on seed infection agents *in planta* model conditions.

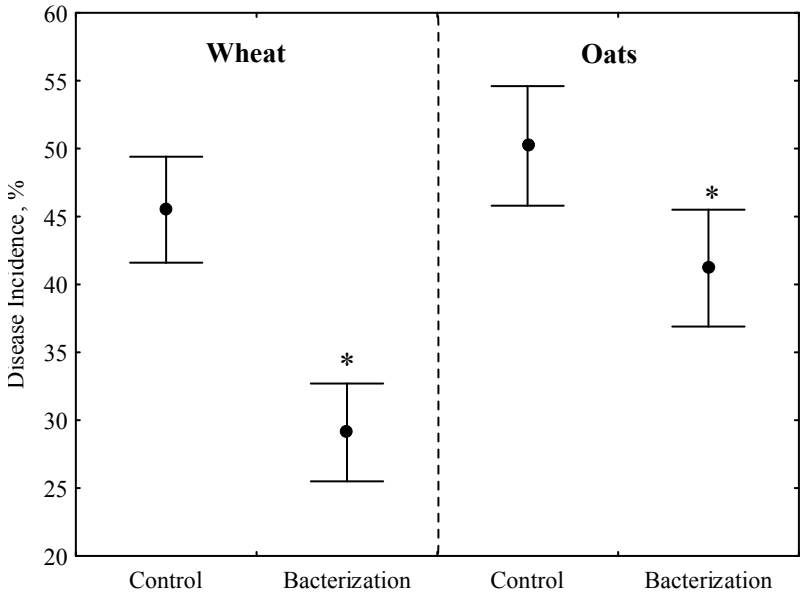

Fig. 2. Total infestation of wheat seeds by seed infection agents during seed bacterization
Note. * – statistically significant difference from the control variant ($p < 0.05$)

As it can be seen from Figure 2, a decrease in total infestation of the studied crops by seed infection agents is observed by 36% and 18% for wheat and oats correspondingly ($p < 0.05$), in comparison with control. The greatest antagonistic effect was seen on *Fusarium* and *Bipolaris* fungi, which are known to be the most noxious among root rot pathogens [22–23]. Wheat and oats seed infestation by a complex of Fusarium and Helminthosporium infection during bacterization is less than control (4.6 times for wheat and 15 times for oats).

Thus, the obtained data prove that the experimental *Pseudomonas* strain has antifungal properties, demonstrated earlier *in vitro* experiments without any vegetation component.

The influence of bacteria on plant infestation by fungal infections in field experiments

Basing on the results obtained in the laboratory conditions, the effectiveness of bacterial antagonist for prevention of plant roots infestation was investigated. Root rots of wheat and oats were examined 4 times during the growing season. Over all the counts, root rot index decreased as a result of seed bacterization (fig. 3).

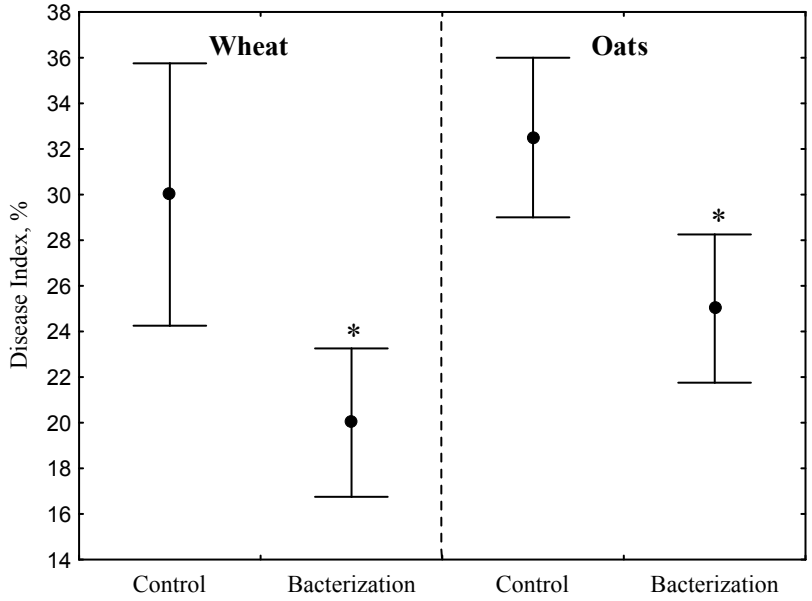

Fig. 3. Root rot index during seed bacterization in the phase of ear formation

A plant under favourable conditions and due to a balanced nutrient supply generates immunity providing resistance to pathogens [24]. Apparently, bacterization may result in systemic acquired resistance induction in plants owing to an increased production of jasmonates and salycylates [25]. So, the analysis of plant infestation by such foliar infections as rust, powdery mildew, Septoria disease, late blight and others can be indicative of a possibility of systemic resistance formation in host-plants under the influence of the studied bacterial strain.

The analysis of data on field observations, obtained during ear formation phase showed a decrease in foliar infections development in bacterized plants ($p < 0.05$). Thus, Septoria disease index decreased 2.3 times and rust – 3 times in comparison with control.

Antifungal activity of bacteria was also demonstrated when analyzing potato late blight in field experiments during the growing season. Figure 4 shows late blight index on potato leaves.

All the counts revealed a decrease in the disease incidence on bacterized plants throughout the growing season. By the end of vegetation, late blight index on bacterized plants had reached 13–22%, whereas this index was considerably higher (25–37%) on the control area (fig. 4). When analyzing the data on late blight index on Lugovskoy variety, a tendency to reduction in plant disease incidence and index was observed ($p > 0.05$). This is due to the fact that this variety is late blight resistant and late blight is not widely dispersed on this variety, even in control.

Plant systemic resistance formation during bacterization can be also demonstrated by the results of a phytopathological analysis of wheat seeds (fig. 5).

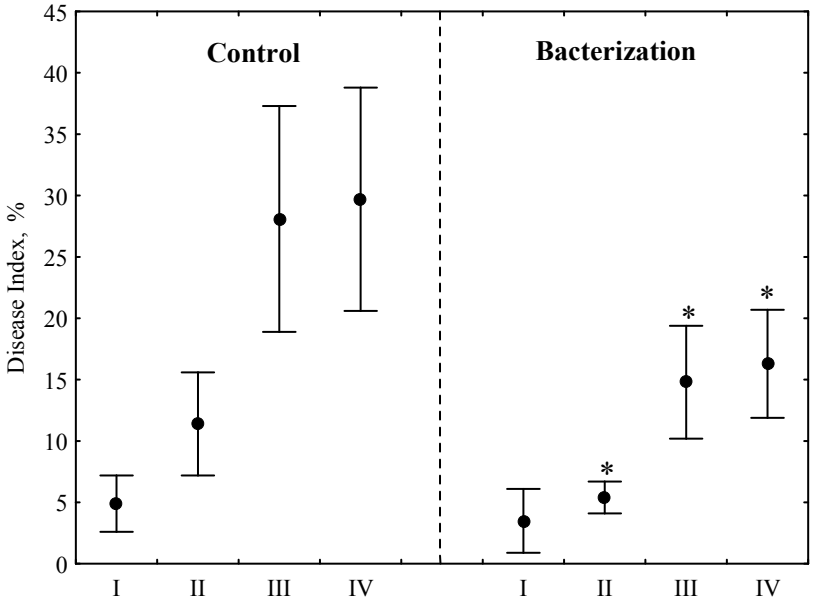

Fig. 4. Late blight index on potato leaves, Nevsky variety, in a field experiment
Note. I, II, III, IV – plant development phases

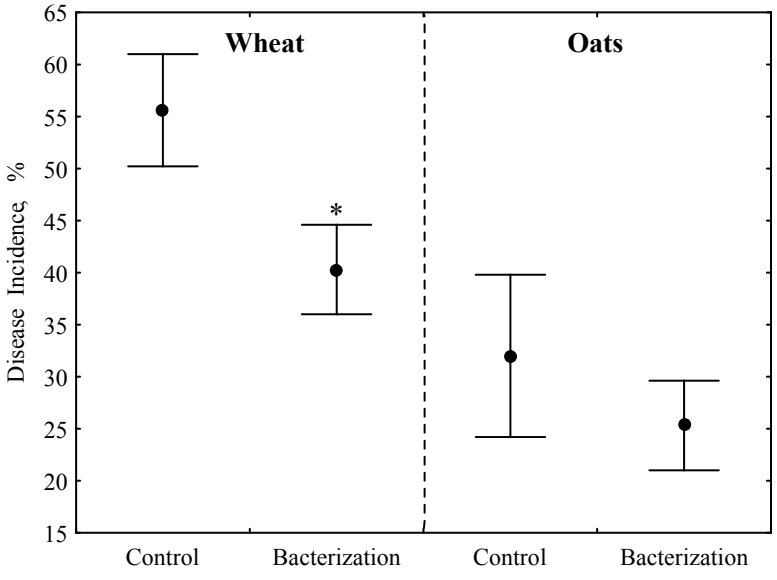

Fig. 5. Total infestation of wheat seeds, collected from control
and the areas with bacterized seeds in a field experiment

According to the given data, the grains of bacterized plants are less affected by seed infection agents, by 28% and 21% for wheat and oats correspondingly times in comparison with control.

It is established that crop yield and tuber disease development during storage depend directly on potato disease severity in the growing season. The data on the development of diseases on potato tubers of new harvest yield are demonstrated in Figure 6.

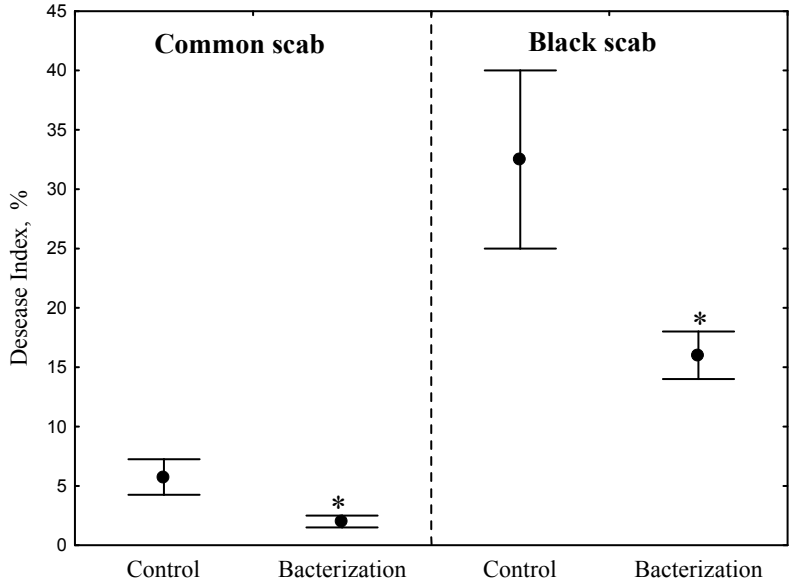

Fig. 6. The development of diseases on potato tubers, the case of Zhukovsky early variety

It should be noted that bacterial treatment of tubers reduced the disease incidence of formed tubers during storage ($p<0.05$): the development of common scab – 2 times and black scab – 4 times, compared with control. The average reduction in common scab and black scab development during bacterization was 40–50% and 50–75%, correspondingly. Disease index reduction was primarily affected by a decrease in disease intensity and, to a lesser degree, the number of infected tubers. A pre-plant seed tuber treatment and further plant spraying by formulations during vegetation reduce population density of pathogens on new crop tubers both by means of decreasing population density of phytopathogens in soil and increasing plant resistance to these microorganisms [26].

Thus, antifungal activity of bacteria can be effectively proved *in vitro* and *in planta* laboratory experiments. However, certain instability of positive effects must be considered while applying bacterial strains in field conditions, associated with the type of soil, the cultivated crop as well as prevailing climatic conditions. When analyzing the data on soil fertility and creating specifications for applying

formulations for each agro-climatic zone and crop, seed bacterization may, undoubtedly, influence sanitation of cereal and other crops in a positive way.

Growth-promoting activity of bacteria Pseudomonas sp., strain B-6798, in model experiments

As it has already been mentioned, bacteria *Pseudomonas* are widely known as plant growth stimulants that increase productivity and affect plant growth dynamics through mobilizing sparingly soluble compounds and discharging a number of plant hormones [1–3, 6–8]. Our experiments demonstrated a positive impact of the studied strain on the growth and development of maize, wheat, oats, potato and fiber flax. In laboratory experiments, when assessing plant growth rate during bacterization, it was shown that kinetic parameters of plant growth vary differently under the influence of bacteria. An increase in the initial shoot growth rate within one or two days ($p < 0.05$) was observed in the experiments with maize in the presence of bacteria, that is why the plants in experimental variants have a greater length by the moment of experiment completion despite a further shoot growth rate that does not show a significant statistical difference from control. Furthermore, acceleration of the shoot root growth rate was fixed for maize.

Potato treatment with bacterial culture had a positive impact on different plant growth and development parameters: plant growth rate augmented in comparison with untreated plants ($p < 0.05$); in the variant with bacterization, the finite plant length increased 1.3 times; plant mass, the number of leaves and the number and weight of tubers increased as well; the phase of tuber formation began earlier than control.

There are a number of investigations devoted to studying the dependence of growth-promoting effects from bacteria concentration in the inoculum. As a rule, bacteria are applied within 10^5–10^7 cells/ml [2, 27–29]. However, in these investigations there is no satisfactory justification for the concentration of the used inoculum. Model experiments with plant treatment with bacterial cultures of different titre (0–10^9 cells/seed) made it possible to reveal concentrations which were optimal for plant growth and development. The table gives data on optimal concentrations of bacterial inoculum, the maximum value of the measured parameters of growth and development of certain crops and their increase in comparison with control.

As table 1 shows, bacterization has a positive effect on various parameters of plant development, increasing them 1.15–5 times depending on a host-plant type and the measured parameter in comparison with control. Of all the studied crops, maize turned out to be the most responsive to bacterization, whereas oats was affected the least. Optimal bacteria concentrations appeared to be 10^4–10^6 cells/ml.

L.A. Somova [30] also specifies in her work that the application of bacteria *Ps. putida*, with abundance for wheat within 10^4–10^8 cells/ml, stimulated seed germination and increased the shoot length; while the number of bacteria over 10^9 cells/ml

inhibited seed germination, and the number of bacteria less than 10^3–10^4 cells/ml made a subtle influence on germination. According to the aforementioned author, the use of *P. fluorescens* stimulated wheat seed germination within a narrower range of concentrations: 10^5–10^7 cells/ml. M.Lacher et al [31] point out in their work that significant root system stimulation was observed at rape seed germination on solid medium in Petri dishes with various concentrations of *Phyllobacterium* ($3 \times 10^7 – 3 \times 10^8$ UFC/ml). Besides, the effect of stimulation and the number of bacterial cells, found on the root surface, increased together with the applied concentration.

Table 1

Optimal concentrations of bacterial culture *Pseudomonas* sp., strain B-6798, on agricultural plants in model experiments

Crop	Parameter	Optimal concentration, cells/ml	Maximum value	Comparison with control
Maize	Main root length (mm)	10^5	100.40 ± 7.60*	2.8
	Number of secondary roots (pcs.)	10^6	17.00 ± 2.00*	2.1
	Dry biomass (g)	10^6	0.55 ± 0.03*	5.0
Wheat	Total root length (mm)	10^4	294.30 ± 55.20*	1.6
	Dry biomass (g)	10^4	0.30 ± 0.04*	2.1
Oats	Total root length (мм)	10^3	121.70 ± 12.10 *	2.2
	Dry biomass (g)	10^6	0.05 ± 0.02 *	1.4
Potato	Plant length (mm)	10^6	359.90 ± 18.30	1.15
	Dry biomass (g)	10^6	6.56 ± 1.74 *	1.3
	Formed tuber weight (g)	10^6	2.04 ± 0.37 *	3.6

Most papers that we have analyzed do not attach any importance to bacterial titre and use simultaneously different bacteria concentrations; their comparisons of the effect of bacterial impact on plants are to be examined and defined more precisely.

Thus, on the basis of the obtained data, it appears that the concentration of cells for the optimum zone of each bacterial species can vary. Moreover, the optimum concentration of the applied bacteria also depends directly on the species of a host-plant, but to a lesser degree.

The influence of bacterization on plant growth and development in field experiments

Field experiments allowed improving the influence of *Pseudomonas* sp., strain B-6798, on plant growth and development under agrocoenosis conditions. In gen-

eral, a positive impact of seed bacterization on plant development was discovered. According to the obtained data, plant bacterization accelerated the growth of all the studied host-plants. Depending on a host-plant species, the length of plants in the variants with bacterization by *Pseudomonas* sp.,strain B-6798, exceeded the length of control plants since full germination phase, though, sometimes, the effect of stimulation showed itself during later phases. An increase in plant length during bacterization is, apparently, associated both with direct growth stimulation caused by plant hormones discharged by bacteria and indirect stimulation caused by a decrease in a degree of plant infestation by pathogens, including reduction in root rot index and incidence.

Figure 7 presents the lengths of maize and oats in different variants of field experiments.

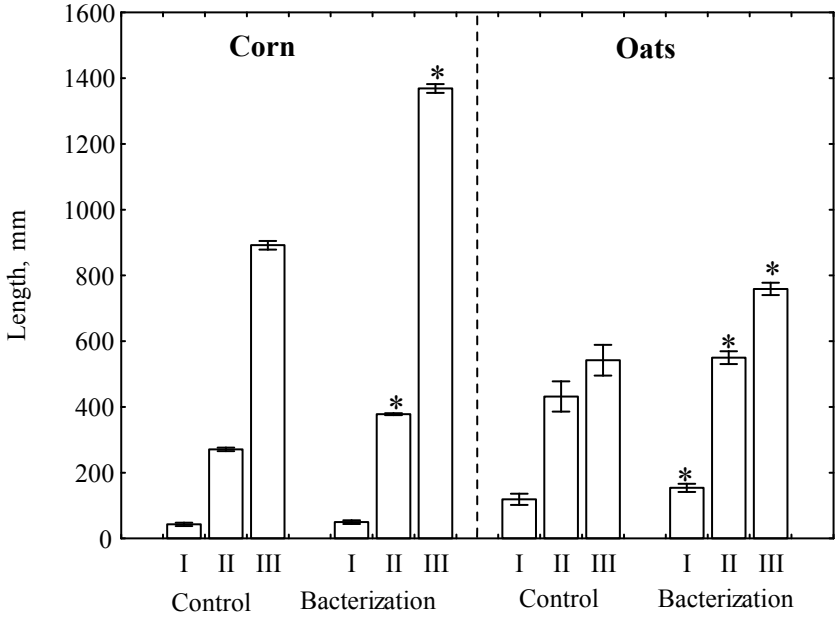

Fig. 7. The length of maize and oats in different variants of the field experiment
Note. I–III – plant development phases (for maize: I – phase 2–3 leaves, II – phase 6–8 leaves, III – panicle formation phase; for oats: I – phase 2–3 leaves, II – tillering phase, III – ear formation phase)

Table 2 shows the data on final measurements of the length of plants and absolutely dry biomass of some crops during bacterization and their comparison with plant development parameters in control.

It was also found out that cereal crops had an increase in the number of reproductive stems during bacterization by *Pseudomonas* sp., strain B-6798, and plants underwent development phases faster, coming into bloom in a shorter time.

For fiber flax there was an increase in the number of seed cases, plant stem diameter and its technical length by 15–50%. The treatment of potato tubers by *Pseudomonas* sp., strain B-6798, resulted in seed vigour and tuber formation acceleration.

Table 2

Plant development parameters during bacterization in field experiments

Crop	Parameter	Value	Comparison with control
Oats	Plant length (cm)	75.9 ± 4.2*	1.4
	Dry biomass (gr)	2.12 ± 0.13*	1.4
Maize	Plant length (cm)	136.9 ± 13.4*	1.5
	Dry biomass (gr)	146.9 ± 3.0*	1.6
Wheat	Plant length (cm)	82.1 ± 2.2*	1.4
	Dry biomass (gr)	2.33 ± 0.14*	1.6
Potato	Plant length (cm)	52.2 ± 8.2*	1.4
Fibre flax	Plant length (cm)	71.2 ± 6.2*	1.4

As a rule, plant bacterization by the studied strain *Pseudomonas* sp., strain B-6798, led to an increase in crop yield during field experiments. The absence of statistically significant differences from the control variant in a number of growing seasons is explained by unfavorable climatic conditions for plant growing (drought or excessive soil moistening). An extremely high soil temperature had the most unfavorable impact on growth-promoting properties of bacteria during their introduction. According to literature data, optimal temperature for successful plant rhizosphere colonization by bacteria is +16…+18°C, at higher temperatures bacteria from the seed surface, where they were spread during seed treatment before sowing in the soil, do not colonize growing roots [26]. However, even during growing seasons with the most unfavourable conditions for efficient host-plant rhizosphere colonization, no inhibition of plant growth and development or a crucial reduction in the yield of bacterized crops were observed.

Bacterization did not only directly increase crop yield, but also affected significantly the structure of host-plant crops. The crop structure in field experiments is exemplified by wheat and potato crop yields in the tables (3–4) and in Figure 8 below.

Table 3

Wheat yield and crop structure in field experiments

Variant	Crop yield, dt/ha	Crop structure		
		Average number of grains per ear, pcs	Weight of 1000 grains, g	Number of reproductive stems per m^2, pcs
Control	25.1 ± 1.6	15.0 ± 1.7	31.7 ± 0.1	527 ± 13
Bacterization	30.6 ± 1.3*	17.0 ± 2.0	34.1 ± 0.1*	527 ± 10

Table 4

Potato yield and crop formation in field experiments

Variant	Number of tubers, pcs	Weight of tubers, g	Number of tubers, pcs	Weight of tubers, g	Number of tubers, pcs	Weight of tubers, g	Crop yield, dt/ha
	Initial blossom		Ceasing to flower		Harvesting		
Nevsky variety							
Control	6.00 ±5.00	6.63 ±3.1	5.0±1.5	191.0 ±99.4	7.0±1.0	458.0 ±48.0	37.8 ±4.3
Bacterization	6.00 ±2.00	15.57 ±5.6 *	7.0±3.0	228.4 ±49.6	7.3±1.0	476.0 ±41.0	40.5 ±3.5
Lugovskoy variety							
Control	2.0±2.0	21.98 ±20.2	5.0±2.2	172.0 ±37.6	4.0±1.0	388.5 ±48.0	48.9± 4.9
Bacterization	5.00 ±2.3*	54.95 ±9.7*	6.0±2.1	263.2 ±107.8	4.5±1.0	412.0 ±51.0	67.8 ±5.3*

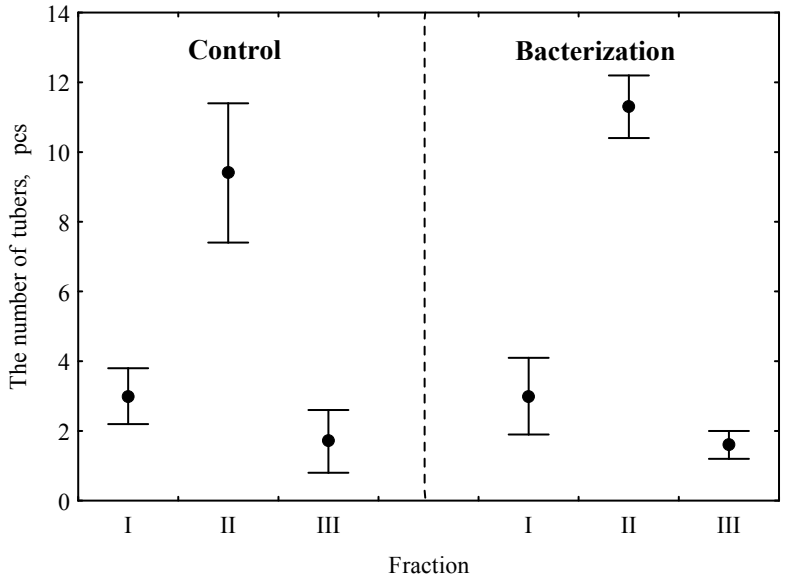

Fig. 8. The structure of potato crop yield, Lugovskoy variety, in field experiments
Note. I – small-sized tubers (up to 50 g); II – average-sized tubers (from 50 to 120 g); III – large-sized tubers (over 120 g)

The positive influence of bacterization was traced during the whole period of crop formation but it was at early stages that its stimulating effect was the best. The analysis of potato crop formation showed an increase in the weight of tubers: for Nevsky variety – 2.2 times, for Lugovskoy variety – 2.5 times and for the number of tubers – 2.8 times in comparison with control. Thereafter, the identified differences can be leveled under conditions which are favorable for

crop formation (see table 4). In case of unfavourable climatic conditions, earlier and more successful crop formation of bacterized plants results in its significant increase in comparison with control at the moment of harvesting. A more rapid passage through plant development phases during bacterization is also important: for wheat and oats a phase of full ripeness began earlier than for plants without bacterization.

Conclusion

On the basis of experimental data, it can be concluded that bacteria *Pseudomonas* sp., strain B-6798, show a high antagonistic activity against fungal pathogens of cereal root rot in Western Siberia. Antifungal activity was detected both in field and *in vitro* and in *planta* laboratory experiments; *in vitro* experiments the strain was active against such fungi as *Fusarium sp.* and *Bipolaris sorokiniana.* The treatment of cereal seeds by bacteria *in planta* reduced the overall incidence of seed infection agents. In field experiments, the use of bacteria reduced the development of root rots and leaf infection manifestation; decreased the overall infestation of new seeds by seed infection pathogens. Bacterization of potato tubers had a positive impact on reducing late blight in the growing season and rhizoctonia blight and common scab during storage. One of the factors of effective activity against phytopathogenic fungi is production of special pigments-siderophores by bacterial culture.

Besides antifungal activity, bacteria *Pseudomonas* sp., strain B-6798, are able to stimulate plant growth and development. In laboratory experiments, utilization of bacteria promoted plant growth and the length and quantity of roots of maize, wheat and oats, increased plant mass, the number of leaves and the number and weight of potato tubers. In field experiments, bacteria also had a positive effect on various plant development parameters: an increase in the number of reproductive stems (cereal crops), the number of seed cases, the diameter of the plant stem and technical length (fiber flax), the area of the leaf blade (cucumber) and tuber formation (potato). Plants of all the crops passed development phases faster, which is important when an earlier crop is formed under unfavorable weather conditions. The success of bacterization was established to depend on the type of host-plants and its variety: of all the studied crops, maize turned out to be the most responsive to bacterization and oats- the least. However, it is necessary to take into consideration certain instability of positive effects when applying bacterial strains under agrocoenosis conditions, associated with the type of soil, cultivated crop and prevailing weather conditions. At the same time, bacterization may influence soil and seed sanitation in a good way. The optimum concentration of the applied bacteria of the examined strain is 10^4–10^7 cells/ml, depending on the crop species and variety.

Thus, the conducted field and model experiments showed effectiveness of applying bacteria *Pseudomonas* sp., strain B-6798, as a producer of formulation

aimed at stimulating plant growth and development, decreasing the development of late blight, cereal root rots, cereal septoria leaf blotch and flax Fusarium wilt, as well as increasing crop yield in Tomsk oblast. The advantage of the isolated and studied strain *Pseudomonas* sp., strain B-6798, is demonstrated by the fact that the presence of formaldehyde in the environment, as the only source of energy and carbon, contributed both to maintaining the stability of the producer's properties and protecting the potential formulation on the basis of this strain from contamination. In the used concentrations, formaldehyde has no additional phytotoxic or fungicidal effect. These properties of the strain can be useful for economically sound biofungicide production on its basis.

The authors thank Dr. Margarita Shternshis (Novosibirsk State Agrarian University, Novosibirsk, Russia) and Dr. Vladimir Gouli (University of Vermont, Burlington, Vermont, USA) for valuable advice, constructive remarks and helpful comments.

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Received July 15, 2013

Вестник Томского государственного университета. Биология. 2013. № 3 (23). С. 19–37

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ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ БАКТЕРИЙ *Pseudomonas* sp. В-6798 ДЛЯ ЗАЩИТЫ СЕЛЬСКОХОЗЯЙСТВЕННЫХ КУЛЬТУР ОТ ФИТОПАТОГЕНОВ В УСЛОВИЯХ ЗАПАДНОЙ СИБИРИ

Биологический метод защиты растений от фитопатогенов основан на применении бактерий – антагонистов фитопатогенов. На современных отечественном и зарубежном рынках существует ряд биопрепаратов на их основе, но при этом продолжается поиск более эффективных агентов защиты растений. В работе исследована возможность применения формальдегидрезистентных бактерий Pseudomonas sp. B-6798 в качестве потенциальной основы биофунгицида. Для учета антифунгальной активности штамма использовались различные методики, в том числе разработанный авторами метод определения кинетики ингибирования роста фитопатогенных грибов бактериями-антагонистами. Оценка ростостимулирующей активности бактерий проводилась как в лабораторных, так и в полевых экспериментах. В качестве тест-объектов использованы следующие сельскохозяйственные культуры: пшеница, овес, кукуруза, картофель и лен-долгунец. Установлено, что обработка семян бактериями Pseudomonas sp. B-6798 снижает общую пораженность зерновых возбудителями семенных инфекций на 12–36% за счет уменьшения процента семян, пораженных гельминтоспориозно-фузариозными возбудителями корневых гнилей. Бактеризация клубней и растений положительно сказывается на снижении развития заболеваний картофеля: фитофтороза в вегетационный период – на 45–50%, ризоктониоза и парши обыкновенной в период хранения – на 40–70%. Оптимальные для развития растений концентрации бактерий находятся в пределах 10^4–10^7 КОЕ/мл. Показано, что под действием бактерий в лабораторных тестах происходит увеличение длины проростка в 1,5–3,5 раза, сухой биомассы – 1,5–5 раз, суммарной длины корневой системы – 1,7–3,0 раза. Согласно результатам полевых испытаний, бактеризация способствует увеличению урожайности зерновых культур и картофеля на 10–40% в зависимости от вида и сорта.

Ключевые слова: *антифунгальная активность; бактерии-антагонисты; биофунгицид; ростостимулирующая активность; фитопатоген.*

Поступила в редакцию 15.07.2013 г.