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Bacterial endophytes of Trans-Ili Alatau region's plants as promising components of a microbial preparation for agricultural use*

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In this study, 382 isolates of bacterial endophytes from tissues of plants grown in the foothills and piedmont plains of the Trans-Ili Alatau were isolated. It was found that certain strains actively produce indole-3-acetic acid (IAA) in a medium containing L-tryptophan. Among the strains studied, 26 strains (66%) showed a positive response to production of IAA. Bacteria synthesized IAA in the range of 18.6±1.1 to 82.4±2.3 μg/ml. IAA was synthesized more actively by *Streptosporangium* sp. KK1 (44.1μg/ml), *Rhodococcus* sp. KK 2 (42.5 μg/ml), *Streptomyces tendae* KK3 (44.9 μg/ml) strains. The most active auxin's producer is a *Jeotgalicoccus halotolerans* BAK1 strain, whose total level of IAA production reached 82.4 μg/ml. Such strains as *Streptomyces griseorubiginosis* KK4, *Streptomyces* sp. KK5 and *Jeotgalicoccus halotolerans* BAK1 were found to have a significant stimulating effect which was reflected in the increase of the length of the roots of soybean and barley. As a result, 8 promising strains with fungicidal, growth-stimulating, phosphorus dissolving and enzymatic activities were selected for the further development of highly microbiological preparations for crop research.

Key words: bacterial endophytes, growth-stimulating effect, plant growth-promoting bacteria, 3-indolylacetic acid, barley, soybean

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INTRODUCTION

Currently, the associations of plants and useful microorganisms attract scientists with the view of their possible practical application in ecologically oriented crop farming. Worldwide experience is armed with a number of biological preparations based on useful strains of bacterial endophytes (Berg *et al*., 2005; Ryan *et al*., 2008; Germaine *et al*., 2006; Chebotar *et al*., 2015; Lodewyckx *et al,* 2002). Bacterial endophytes inhabiting inner tissues of healthy plants that are not generating morphological changes and are not harmful for the host have been widely explored (Zinniel *et al*., 2002; Schulz *et al*., 2006). Over 300 thousand of existing plants turn up as hosts of one or more endophytes (Strobel *et a*l., 2004) and many researchers find the maintaining of endophytic cenosis as a universal property of all plants. For example, endophytic microorganisms were found in tissues and seeds of primary agricultural crops such as rice (Baldani *et al*., 2000; Okunishi *et al.,* 2005), maize (McInroy *et al*., 1995; Rijavec *et al.,* 2007; Szilagyi-Zecchin *et al*., 2014), cotton (Misaghi *et al.,* 1990), potato (Sturz *et al*., 1998; Krechel *et al*., 2002), sugarcane (Rennie *et al.,* 1982), wheat (Larran*et al*., 2002), soybean (Impulilitti *et al*., 2011), and others.

It was shown that inoculation of non-leguminous plants by endophytic rhizobacteria is able to significantly increase the plant productivity and product quality (Jasim*et al*., 2014; Okon*et al*., 1994; Weller, 1988). In some cases, the use of biological products from endophytic microbes helps to protect plants from diseases, thus replacing chemical pesticides (Sun *et al*., 2008).

Little is known about the endophytic microorganisms of wild plants (Rosenblueth *et al*., 2006; Brooks, 1994). It was indicated that inoculation of non-leguminous plants with endophytic rhizobacteria can significantly increase crop yield and quality of production (Jasim *et al*., 2014; Okon, 1994). In some cases, an application of biological preparations including endophytic microorganisms makes it possible to protect plants from diseases in a way that substitutes chemical pesticides (Weller, 1988).

Flora of the Trans-Ili Alatau region includes more than 1 000 species. Of the great number of useful plants, there is a large group of crops (over 80 species): *Kobresia capilliformis, Carex stenocarpa, Festuca kryloviana, Poa alpina, Poa pratensis, Dáctylis glomeráta, Brachypodium pinnatum,* and species of clover, lathyrus, and pea. Among herbs there are – angelica, juniper, sagebrush. Medicinal plant are very widespread as well, such as: tansy, yarrow, mother and stepmother, rose hips, zhoster, valerian, juniper, dandelion, plantain and other (Lodewyckx *et al*., 2002; Zinniel *et al*., 2002; Kasana et *al*., 2008). Studies on the detection and study of endophytic microorganisms in endemic plants of Trans-Ili Alatau were not conducted. Based on this, the aim of this work was the selection of endophytic bacteria from plants grown in the foothills and piedmont plains of the Trans-Ili Alatau and the study of the taxonomic composition of the bacterial populations, as well as the selection of promising strains of the complex agronomic properties to further the creation of preparations for crop production. This study may allow the provision of new and beneficial endophytic bacteria among a variety of plants in this ecosystem.

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Abbreviations: IAA, indole-3-acetic acid; TSA, Tryptic Soy Agar; PDA, Potato Dextrose Agar; NBRIP, National Botanical Research Institute's phosphate growth medium

MATERIALS AND METHODS

Isolation of bacterial endophytes. Endophytic bacterial strains were defined as isolates and were obtained from various tissues of such plants as *Festuca pratensis*, *Festuca kryloviana*, *Artemisia lercheana*, *Artemisia annua*, *Xanthium strumarium*, *Póa alpína*, *Poa praténsi*s, *Melilotus officinalis*, *Salvia deserta,* ,*Chenopodium botrys* and *Glycyrrhiza glabra.* The plants were collected from May to September of the years 2013 and 2014, during expeditions to the foothills and piedmont plains of the Trans-Ili Alatau. Three geographically remote points located at the distance of 5 kilometers apart were selected for the plant collection. Samples of each plant's species were collected in four places located 50 m from each other. Each extracted plant was placed in a sterile plastic bag and delivered to the laboratory for further study.

Fresh samples of 5–10 plants with total weight of about 5 g were washed with sterile solution of NaCl for the removal of extraneous particles and plant residues; damaged plants were not used. Samples were surface disinfected *in* the following way: 70% ethanol for one minute, 2.5% sodium hypochlorite for 4 minutes, 70% ethanol for 30 seconds. To remove the disinfectant, the plants were rinsed three times in two consecutive washes of sterile distilled water. The plants were then dissected into small pieces and macerated using a sterile mortar and pestle. Tissue extracts were incubated at 28°C for 3 hours for full release of endophytic microorganisms from the host's tissues. To prove the disinfection protocol was successful, aliquots of sterile water used at final rinsing were spread on the TSA medium and incubated at 28°C for 15 days. For further study, morphologically different colonies were used.

Study of culture and morphological features. Properties of bacterial isolates that had grown on PDA agar medium, such as color and shape of colonies, size, texture profile and the edge of the colonies were evaluated. During microscopic observations (MOTIC BA 300 microscope), Gram-staining of bacteria, the presence and location of the spores, and other features were noted.

Study of enzymatic activity. A medium with casein was used for studying of the impact of protease activity. Bacterial strains were inoculated by injection onto the surface of the medium. For detection of zones dissolving casein, after incubation period the plates were overlayed with 10 ml of 10% trichloroacetic acid solution.

To determine the activity of the amylase, a medium with starch was used. Bacterial strains were inoculated by injection onto the surface of the medium and incubated for 2 days at 28°C. After incubation period, the Petri dishes were filled with a dilute Lugol solution (a solution of elemental iodine and potassium iodide in water) for the manifestation of starch dissolution zones.

In order to study the lipase activity, a mineral medium with 0.05% bromothymol blue was used. The medium was dispensed into Petri dishes and after solidification, on the surface of the medium 1 ml of sterile sunflower oil was added. Bacterial strains were inoculated by injection into the medium. The lipase activity of bacterial strains was assessed based on the indicator's color change from blue to yellow, which indicates hydrolysis of fats into fatty acids.

To study the cellulase activity, a modified technique was used (Kasana *et al.,* 2008). Mineral medium with addition of microcrystalline cellulose was dispensed into Petri dishes, and the center of the plate was inoculated with bacterial strain by injection. To identify areas of microcrystalline cellulose dissolution, the plates were over layered with a dilute Lugol solution.

Study of the ability of isolates to dissolve poorly soluble inorganic phosphorus compounds. To study the phosphate-mobilizing activity, the following medium was used: (glucose 10.0 g /l, asparagine 1.0 g /l, K_2SO_4 0.2 g /l, $MgSO_4$ 0.2 g /l, corn steep liquor 0.02 g /l, agar 20.0 g /l; pH 6.8) (Mehta *et al*., 2001; Rodriguez *et al*., 1999). As insoluble phosphates, such salts as $Ca_3(PO_4)_2$ were added to the medium at a concentration of 5 g/l as the sole source of phosphorus. The appearance of the transparent zones (halos) in the case of dissolved phosphate was scored for 7–10 days. The phosphate mobilizing microbial activity was evaluated by the diameter of the halo regions. Ability of endophytic microorganisms to dissolve inorganic phosphate was also assessed by incubating in NBRIP (National Botanical Research Institute's phosphate growth medium) medium (glucose 10.0 g/l, $MgCl_2 \times 6H_2O$ 5.0 g/l, $MgSO_4 \times 7H_2O$ 0.25 g/l, KCl 0.2 g/l, $(NH_4)_2SO_4$ 0.1 g/l, inorganic phosphate, $Ca_3(PO_4)_2$ 5.0 g/l, pH 7.0). For a quantitative analysis, the NBRIP medium was added with additional 0.025 g of bromophenol blue. Bacteria were incubated for 2 to 3 days in this broth at 30°C. The optical density of the culture supernatant was obtained after centrifugation at 5000 rpm for 20 min. Measurement of the color indicator was performed with a spectrophotometer set at 600 nm.

Study of fungicidal activity. The fungicidal activity was tested on 4 strains of pathogenic fungi: *Fusarium graminearum, Alternaria alternate, Phytophtora infestans, Botrytis cinerea* and *Cladosporium sp.* (strains were obtained from the collection of the Institute of Microbiology and Virology of the RK) using the method of "agar blocks." Strains of endophytic micromycetes were grown in agar medium tubes for 5 days. An aqueous suspension with a conidia titer of 10^6/ml was prepared, 0.1ml was plated onto the surface of the PDA lawn and grown for 4–5 days, after which the pitch of the formed blocks 8 mm in diameter were excised. Phytopathogenic fungi were cultured in an agar medium for 5-7 days. Cultures grown from aqueous suspensions were prepared with a titer of 10^4 conidia/ml, and 0.1 ml of the suspension was plated on a PDA lawn surface in Petri dishes. Blocks with endophyte micromycetes cultures were placed on the surface of the Petri dishes inoculated with phytopathogenic fungi. An antagonistic activity was judged by the lack of growth of the phytopathogenic zone (Netrusov *et al.,* 2005).

Study of the ability of isolates to produce IAA on a medium with L-tryptophan. This was determined colorimetrically by using the Salkowski reagent (Gordon, 1950). Bacterial isolates were cultured for 2–3 days at 28°C in the liquid PDA medium supplemented with 2 mM L-tryptophan. Then, 1 ml of the culture was inoculated into microcentrifuge tubes and centrifuged for 2 minutes at 14000 rpm. 0.5 ml of the supernatant was inoculated into fresh tubes, and then 0.5 ml of the Salkowski reagent was added. The tubes were incubated for 10 min at room temperature, and the change in color intensity from pale pink to magenta-saturated allowed for assessment of the production level of the IAA and its derivatives by the bacterial isolates (Glickmann *et al.,* 1995).

The study of growth-promoting activity conducted by moistening the seeds. The studied strains of bacteria were cultured for 3–5 days at 28°C on a stationary liquid PDA medium. To perform bioassays soy-

Table 1. Number of endophytic bacteria in different parts of plants (CFU cells/g of plant tissue).

Host plant	PDA			TSA		
	Leaves (103)	Root (103)	Stem (103)	Leaves (103)	Root (103)	Stem (103)
Festuca pratensis	15.3 (0.6)**	173.8 (5.1)**	51.4 (1.7)**	10.8 (0.3)**	121.4 (5.1)**	32.3 (1.1)**
Festuca kryloviana	8.1 (0.7)**	174.9 (5.1)**	24.5 (1.3)**	6.7 (0.6)**	125.9 (5.9)**	17.4 (1.3)**
Artemisia lercheana	4.4 (0.7)**	179.4 (4.2)**	42.7 (1.1)***	5.2 (0.1)**	136.4 (4.8)**	13.6 (0.1)*
Artemisia annua	29.1 (0.3)*	203.4 (7.1)**	58.6 (2.7)**	17,4 (0.2)***	145.9 (3.6)**	39.7 (0.2)**
Xanthium strumarium	8.6 (0.1)**	176.6 (5.8)**	37.4 (3.1)**	6.7 (0.1)**	117.6 (1.1)**	21.4 (1,2)**
Póa alpína	3.7 (0.05)**	147.9 (3.4)**	19.5 (1.3)*	2.4 (0.1)**	127.3 (1.3)**	17.8 (1.1)**
Poa praténsis	8.7 (0.6)**	164.2 (3.9)**	36.8 (1.3)***	6.4 (0.3)**	129.4 (3.1)**	15.3 (0.6)**
Melilotusofficinalis	8.8 (0.3)**	267.7 (3.4)**	38.1 (2.0)**	18.7 (1.4)**	128.3 (2.2)*	31.4 (2.0)**
Salvia deserta	25.7 (2.9)**	212.4 (3.7)*	41.4 (3.5)**	56.4 (2.7)**	156.3 (4.4)**	44.7 (1.3)**
Acbillea millefolium	9.5 (0.5)**	181.6 (5.2)**	34.5 (1.7)***	6.6 (0.4)**	145.8 (3.2)**	21.9 (1.2)**
Chenopodium botrys	4.4 (0.7)**	108.9 (4.4)**	96.4 (5.9)***	2.6 (0.4)**	79.8 (4.4)**	39.6 (0.4)**
Glycyrrhiza glabra	3.6 (0.3)**	152.6 (3.6)**	49.5 (3.1)**	2.7 (0.6)**	137.9 (3.4)**	26.4 (0.2)**

*P<0.05; **P<0.01; ***P<0.001 compared with the control group

bean seeds (*Glycine max*), the Almaty variety, and barley *(Hordeum vulgare*) of the Arna grade, were used. A protocol was optimized for seed surface sterilization to completely suppress all epiphytic microflora of the seeds, but preserving a viable state of the seed embryo: seed is incubated for 2 min in 70% ethanol, washed in a sterile tap water and washed twice with 30% sodium hypochlorite Na for 5 min, and then washed in sterile tap water. For quality control of surface sterilization, 20 seeds were laid out on the surface of nutrient agar and incubated for 48 hours at 28°C. In the absence of bacterial growth around the seeds, the degree of their sterility was determined. The studied strains of bacteria were cultured for 3–5 days at 28°C in a stationary liquid PDA medium. Sterilized seeds were soaked in the resulting suspension of bacterial cells for 30 minutes, and then under the experimental *in vitro* conditions the seeds were placed in pots filled with a mixture of sand and sawdust. All pots were moistened with an equal amount of tap water. Seeds were germinated for 14 days. For each option, 25 seeds were used in triplicate. In the control experiment, the seeds were soaked in sterile saline. Biometric studies were conducted: the number of germinated seeds, the length of the stems and roots in the experiment and control were taken into account. Determination of germination was carried out for 7 days and was expressed as a percentage of germinated normal seeds out of the total number of seeds taken for germination.

Determination of colonizing activity using the method of gnotobiotic systems. Toidentify the ability of the strains of endophytic bacteria for active colonization of higher plants, we used the method of plant inoculation and cultivation of plants under sterile conditions of gnotobiotic systems. Objects of the study were such crops as soybean (*Glycine max*) of the Almaty cultivar, and barley (*Hordeum vulgare)* of the Arna cultivar. To determine the number of endophytic bacteria introduced in to roots, the plants were sterilized according to the protocol used to isolate endophytic bacteria. The washed roots were then suspended in physiological saline, and the number of microorganisms was counted on the PDA solid medium (34–35). As control, the *Gordonia rubripertincta* L-RP20 strain was used (Mikolasch *et al*, 2015).

RESULTS

Isolation of endophytic bacteria

In the development of microbial preparations of complex action for increasing crop yield and tolerance, it is preferable to join various features of the microsymbionts, such as protection against phytopathogens, increased efficiency in phosphate mobilization and production of different enzymes. A search of prospective isolates with complex biological activity was conducted among endophytic bacteria isolated from plants grown in the foothills and piedmont plains of the Trans-Ili Alatau.

Various substances were used in surface sterilization of plants to remove epiphytic microorganisms before the endophyte's isolation, such as a water solution of sodium hypochlorite, ethanol, hydrogen peroxide and others.

The sterilizing agent was screened for optimization of the protocol of plant surface sterilization. Hydrogen peroxide, ethanol, sodium hypochlorite and various commercial products based on it, as well as chlorinated products, were used. Removing of commercial products based on sodium hypochlorite and chlorine-based products during the washing off was problematical and their remnants caused the death of allocated bacteria during subsequent manipulations.

High efficiency of plant surface disinfection, completeness and speed of removal of the agent in the subsequent washing steps, and the absence of any degradation products that can have an impact on the development of bacteria were characteristic of hydrogen peroxide, ethanol and sodium hypochlorite. The protocol for surface plant sterilization, allowing to completely suppress the entire surface microflora, but to keep the cells of bacterial endophytes that inhabit the internal plant tissues in a viable state, was modified.

The number of microorganisms isolated on nutrient medium from interior tissues of plant roots, stems and leaves ranged from 10^3 to 10^5CFU/g of plant tissue (Table 1).

The number of bacteria in leaves was lower in comparison to bacteria isolated fromroots and stems. Regardless of the plant species, the number of isolated bac-

Table 2. Component composition of bacterial communities of endophytic bacteria of plants selected in the foothills and piedmont plains of the Trans-Ili Alatau.

Plant	Number of isolates				
	Total	Gram+ bacteria	Gram- bacteria	Actino bacteria	Spore-forming bacteria
Festuca pratensis	29	11	18	6	4
Festuca kryloviana	34	13	21	8	3
Artemisia lercheana	31	12	19	5	2
Artemisia annua	27	9	18	4	3
Xanthium strumarium	35	14	21	6	3
Póa alpína	32	12	20	5	4
Poa praténsis	31	11	20	4	3
Melilotusofficinalis	29	9	20	3	3
Salvia deserta	28	9	19	3	3
Acbillea millefolium	36	12	24	6	2
Chenopodium botrys	32	10	22	4	4
Glycyrrhiza glabra	38	13	25	7	4
Total	382	135	247	61	38

teria was equal, and only the numbers for *Artemisia annua* and *Salvia deserta* were higher.

Endophytes are microorganisms which live inside healthy plant tissues and they are found in most of plants. Endophytes include various bacteria, actinobacteria and fungi; they can be isolated from wild or cultured plants. Mostly bacteria are isolated from plants. Endophytic bacteria are a source of biologically active substances. It is known that plants infected by endophytes are healthier than plants with no endophytes.

Three hundred eighty two isolates of cultivated forms of endophytic bacteria were isolated from various healthy parts of plants, such as leaves, stems and roots (Table 2), 60% of them were isolated from roots, and more than 15% from leaves. Most of the isolated bacteria were Gram-negative (more than 60%). Among the isolates, 38 were Gram-positive spore-forming bacteria of the *Bacillus* genera (Table 2). Similar results were reported by other authors, for example 150 micromycetes and 71 actinomycetes were isolated from internal tissues of wood plants (Caruso *et al.,* 2000), and 78 bacteria and 142 fungi were isolated from aerial and underground parts of various medical plants (Jalgaonwala *et al.,* 2010).

Characterization of the bacterial endophytes

Plants can be considered as complex micro-ecosystems, which serve as ahabitat for a variety of microorganisms, both free-living and endophytic, i.e. those which populate the inner tissues of plants; they are able to stimulate the growth of plants and are called PGPR-microorganisms (Antoun *et al.,* 2005). Endophytes, when compared to free-living PGPK, are increasingly restricted in the manifestation of metabolic activity. It is very likely that, penetrating into the plant tissues, endophytes fall under adverse conditions, as they are ex-

Table 3. Number of isolates with enzymatic activity.

Plant	Number of isolates				
	Activity				
	Proteinase	Amylase	Lipase	Cellulase	Solubilization of calcium orthophosphate
Festuca pratensis	2	4	3	6	4
Festuca kryloviana	2	3	3	8	3
Artemisia lercheana	5	6	6	6	5
Artemisia annua	2	2	4	4	3
Xanthium strumarium	6	8	7	5	8
Póa alpína	2	3	5	5	4
Poa praténsis	7	7	9	6	6
Melilotus officinalis	2	3	5	3	3
Salvia deserta	3	2	5	3	3
Acbillea millefolium	2	3	3	6	2
Chenopodium botrys	3	4	4	4	4
Glycyrrhiza glabra	6	8	9	7	9
Total	42	53	63	66	54

Table 4. Number of isolates with antifungal activity.

Plant	Number of isolates				
	Fusarium graminearum	*Alternaria alternate*	*Phytophtora infestans*	*Botrytis cinerea*	*Cladosporium* sp.
Festuca pratensis	6	4	6	3	2
Festuca kryloviana	8	3	8	3	3
Artemisia lercheana	5	6	8	6	6
Artemisia annua	4	2	4	4	2
Xanthium strumarium	7	8	5	7	8
Póa alpína	5	3	5	5	3
Poa praténsis	11	7	6	9	7
Melilotus officinalis	3	3	3	5	3
Salvia deserta	3	2	3	5	2
Acbillea millefolium	6	3	6	3	3
Chenopodium botrys	4	4	4	4	4
Glycyrrhiza glabra	7	8	7	9	6
Total	64	50	69	63	49

posed to the protective systems of the host plant (Bacon *et al.,* 2006). However, what attracts the researchers is the ability of bacteria not only to stimulates the growth of plants, but also their ability to increase the availability of sparingly soluble phosphate, which is considered to be one of their most important properties and the fundamental factor in the use of promising microorganisms for the creation of the so-called bio-fertilizers (Takuria *et al.,* 2004; Plassard *et al.,* 2010). Also, in the development of microbial preparations of complex action to improve the productivity and sustainability of legumes, a preferred strategy is to join the microsymbiont properties protecting against plant pathogens and their ability to synthesize hydrolytic enzymes.

The results of the biochemical analysis are summarized in Table 3.

Characteristics of the enzymatic activity of bacterial isolates testified that they possess quite active hydrolytic enzymes. Some researchers believe that the endophytic bacteria actively penetrate into the plant tissue using hydrolytic enzymes, such as the cellulase and pectinase (Hallmann *et al.,* 1997; Castro *et al.,* 2014).

Isolates producing the most active proteases, amylases, lipases, and cellulases were identified. Most isolates capable to produce hydrolytic enzymes were isolated from the roots of plants. Furthermore, the largest number of isolates with enzymatic activity was isolated from the following plants: *Artemisia lerchean, Xanthium strumarium, Poapraténsis* and *Glycyrrhiza glabra*. The release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability (Rodriguez *et al.,* 1999). The use of phosphate-solubilizing bacteria as inoculants simultaneously increases the phosphorus uptake by the plant and the crop yield (Mehta *et al.,* 2001). Bacteria belonging to the *Bacillus, Pseudomonas, Serratia, Enterobacter* genera are reported to solubilize the insoluble phosphate and aid in plant growth (Frey-Klett*et al.,* 2005; Hameeda *et al.,* 2008.). Among 382 isolates, 54 showed phosphate-solubilizing activity by forming distinct zones on the NBRIP agar. As a result, only 8 most active isolates were selected after screening for ability to dissolve calcium orthophosphate.

Study of fungicidal activity of more than 382 bacterial isolates allocated from tissues of the plants, revealed that the proportion of the strains capable to suppress development of the phytopathogenic fungi is quite high, and is about 60% of the total number of bacteria (Table 4).

As a result, a number of strains characterized by the active fungicidal properties were selected. These strains were able to actively suppress all pathogenic fungi of different taxonomic position that were being tested (*Fusarium graminearum, Alternaria alternate, Phytophtora infestans, Botrytis cinerea* and *Cladosporium sp.*). Several isolates were characterized by acomplex activity; they had a pronounced fungicidal effect and ability to dissolve calcium orthophosphate as well.

Another criterion for screening of strains was assessment of their ability to produce plant hormones and stimulate crop growth. Visual evaluation of the quality of the reaction showed that some isolates rather actively produced IAA in the medium supplemented with L-tryptophan (Table 5). The largest number of isolates was obtained from licorice's root.

Endophytic microorganisms are potential producers of IAA and this may be the reason for growth stimulation of certain plants in the case of colonization. The ability of IAA production by different species of *Pseudomonas*

Table 5. Synthesis of auxins by endophytic isolates (in the medium supplemented with L-tryptophan)

Strains	IAA, μg/ml	Source of isolation
KK6	36,6 (2,1)*	*Glycyrrhiza glabra,* root
KK7	33,2 (2,7)*	*Glycyrrhiza glabra,* root
KK5	25,1 (2,3)**	*Glycyrrhiza glabra,* root
KK 2	42,5 (2,4)*	*Poa praténsis,* stem
KK 1	44,1 (2,3)**	*Glycyrrhiza glabra,* root
BAK 1	82,4 (3,1)**	*Artemisia annua,* root
KK4	25,2 (2,1)*	*Poa praténsis,* stem
KK3	44,9 (2,4)**	*Glycyrrhiza glabra,* root

*$P<0.01$; **$P<0.001$ compared with the control group

Table 6. Plant growth-promoting effect of endophytic bacteria in relation to crop plants.

Strains	Length of stem/root, mm	
	Glycine max	*Hordeum vulgare*
KK6	92.0 (0.7)***/58.1(0.5)**	105.1 (0.6)**/43.1 (0.5)***
KK7	99.6 (1.2)***/48.1 (0.8)***	102.1 (0.7)***/46.1 (0.8)***
KK5	85.9 (0.2)***/38.1 (0.8)**	104.9 (0.9)***/108.8 (0.7)**
KK 2	57.9 (0.5)***/30.1 (1.1)***	84.9 (0.07)***/45.5 (0.03)***
KK 1	62.1 (0.7)***/50.1 (1.1)***	80.5 (0.7)***/52.0(0.2)***
BAK 1	70.3 (1.1)***/40.9 (0.9)**	105.1 (0.7)*/98.3 (0.03)***
KK4	77.1 (0.2)**/45.1 (0.7)***	121.0 (0.8)***/48.9 (0.3)***
KK3	72.4.1 (1.3)*/49.9 (1.1)***	109.9 (0.8)***/49.1 (0.07)*
Control	47.2 (1.5)***/30.5 (1.1)***	75.3 (1.1)***/30.2 (1.8)***

*$P<0.05$; **$P<0.01$; ***$P<0.001$ compared with the control group

was reported by many authors (Hardoim *et al.,* 2008; Kamwal, 2009).

It was equally important to assess the growth-promoting effect of the interaction of cultures of bacteria with seedlings of crop plants. Thus, a phytotest was conducted with *Glycine max* (Almaty variety) seedlings and *Hordeum vulgare* (Arna variety), which showed that a diluted culture fluid of 8 strains of the investigated bacteria was able to stimulate the growth of plants *in vitro* (Table 6). Especially high stimulatory effect were demonstrated for the KK5, KK6, KK7, BAK 1 and KK3 strains, which caused a significant increase in the length of the roots of the plants when compared to the control variants without treatment.

Screening of isolates with a complex of valuable properties allowed to find several promising strains of endophytic bacteria isolated from plants that grow in the foothills and piedmont plains of the Trans-Ili Alatau.

Initial identification revealed a wide variety of the isolated microorganisms. The dominant groups among the studied bacteria were microorganisms of the *Pseudomonas, Rhodococcus* and *Streptomyces* genera. It should be mentioned that the *Jeotgalicoccus* genus members were also present among the studied isolates.

The tested strains were well adapted in the roots of barley and soybean. The maximum survival values in the roots of barley and soybean were obtained for *Jeotgalicoccus halotolerans*. BAK 1, and *Streptosporangium sp.* KK 1. The number of the remaining strainswas high and comparable with colonization activity of the control strain (Table 8).

Bacteria with high antagonistic potential have been identified as representatives of the *Pseudomonas* and *Agromyces* genera. These genera of bacteria have already recommended themselves as good biocontrol and growth-stimulating agents, and are widely used in agricultural microbiology to create a broad spectrum of biological products (Okon *et al.,* 1994; Ruby *et al.,* 2011; Eckert *et al.,* 2001).

Originally, we wanted to address the question of the ability of bacterial strains to successfully colonize the rhizosphere of higher plants with agricultural importance. This problem is very important because it is important to know how the strains with increased growth-stimulating and antagonistic potential against harmful pathogens under the "higher plant - bacteria" conditions are effective. The use of gnotobiotic systems allowed to answer this key question (Kobayashi *et al.,* 2000; Zinniel *et al.,* 2002).

The tested strains well adapted in the roots of barley and soybean. The maximum values of survival in the roots of barley and soybeans were recorded for *Jeotgalicoccus halotolerans* BAK 1, and *Streptosporangium sp.* KK 1. The relative values of the number of these bacteria in the roots, when compared to the rhizosphere, were increased. The number of the remaining strains was high and comparable with that of colonization activity of the control strain (Table 8).

Table 7. Taxonomic affiliation and physiological properties of the most prospective strains of endophytic bacteria

Strains	Fungicidal activity	Enzyme activity	Solubilization of phosphates $Ca_3(PO_4)_2$)	Synthesis of auxins (in the presence of L-trypthophan)
Agromyces albus KK6	+++	L(+) P(++) A(+++)	+++	++
Pseudomonas oleovorus KK7	+++	L(+) P(++) A(+++)	+++	++
Streptomyces sp.KK5	–	L(+) A(++) P(++)	+++	+
Rhodococcus sp. KK 2	–	L(+) A(++) P(++)	–	++
Streptosporangium sp. KK 1	–	C(++) P(+)	–	++
Jeotgalicoccus halotolerans BAK 1	+++	L(+) P(++) A(+++)	+++	+++
Streptomyces griseorubiginosis KK4	–	L(++) P(+)	–	+
Streptomyces tendae KK3	++	L(+) A(++) P(++)	++	++

Notes: indication: –, absence of activity; +, poorly defined; ++, well defined; +++, strongly expressed; enzymatic activity: P, protease; A, amylase; L, lipase; C, cellulase

Table 8. Colonization ability of eight bacterial strains isolated from plants.

Strains	Number of bacteria. 10^5 CFU/sm of barley roots		Number of bacteria. 10^5 CFU/sm of soybean
	rhizosphere	root	roots
Agromyces albus KK6	15.3 (0.6)*	7.2 (0.4)*	3.6 (0.3)**
Pseudomonas oleovorus KK7	18.1 (0.7)**	9.8 (0.3)**	6.6 (0.4)***
Streptomyces sp. KK5	14.4 (0.7)**	6.7 (0.6)**	5.2 (0.1)**
Rhodococcus sp. KK2	19.1 (0.3)**	7.4 (0.2)*	5.1 (0.2)**
Streptosporangium sp. KK1	18.6 (0.1)**	6.7 (0.1)**	7.4 (0.2)***
Jeotgalicoccus sp. BAK1	25.7 (2.9)***	8.4 (0.1)**	6.7 (0.1)*
Streptomyces griseorubiginosis KK4	18.7 (0.6)***	6.4 (0.3)***	9.1 (0.3)**
Streptomyces sp. KK3	11.8 (2.1)**	6.4 (2.0)**	8.6 (0.1)***
Gordonia rubripertincta L-RP20 (control strain)	9.7 (0.05)**	3.4 (2.7)**	2.4 (0.1)*

*P<0.05; **P<0.01; ***P<0.001 compared with the control group

It was found that the isolated strains are promising in their ability to actively colonize the roots of crops, while their number is comparable with the number of the control strain.

DISCUSSION

It was found by seeding on solid nutrient media, that the tissues of plants grown in the foothills and piedmont plains of Trans-Ili Alatau allocated 382 different bacterial isolates, while the dominant taxonomic groups associated with these plants of different geographical origin are representatives of *Pseudomonadaceae* (whose number is up to 40 % of the total heterotrophic microflora), there are subdominant component families such as

Actinomycetales and Corynebacteriaceae. A significant portion of isolates possessed a set of properties (up to 30% of the strains were demonstrating a pronounced fungicidal effect, while some strains were capable of producing IAA), which may provide the biocontrol and growth-stimulating effects in the process of symbiosis between plants and endophytic microorganisms inhabiting their tissues. In this work, 8 promising strains of the *Pseudomonas, Agromyces, Rhodococcus, Streptomyces, Streptosporangium, Jeotgalicoccus* genera were selected with fungicidal, growth-stimulating, phosphorus dissolving, and enzymatic activities. These strains may be used for further creation of highly microbiological preparations for plant growing. A highly antagonistic potential of these promising strains against the causative agents of fungal diseases in plants was demonstrated. The selected strains also displayed a pronounced growth-stimulating effect on the crops.. Thus, the results obtained in this work show the high potential of the use of endophytic bacteria, isolated from tissues of plants grown in the foothills and piedmont plains of the Trans-Ili Alatau, in the establishment of microbiological preparations for agricultural production.

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