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**EXPERIMENTAL ARTICLES**

# Variety-Specific Actinomycete Complexes Associated with Barley Roots in Soddy Podzolic Soil

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**Abstract**—The root-colonizing actinomycete complexes of genotypically different barley plants grown in soddy podzolic soil were found to contain streptomycetes of the sections *Cinereus* (series *Chromogenes*, *Achromogenes*, and *Aureus*) and *Roseus* (series *Fradiae*), dominant being streptomycetes of the section *Cinereus* ser. *Chromogenes*. The abundance and diversity of soil streptomycetes in the barley rhizoplane increased in the order: var. 999-93 < var. Kumir < var. Novichok < var. 889-93. Experiments revealed functional specificity in the root-associated actinomycete complexes of different barley varieties. The actinomycete complex colonizing the barley var. 999-93 roots was distinguished by a wide range of utilizable root exudate metabolites and a low occurrence rate of antagonistic species.

*Key words*: structure of actinomycete complexes, barley rhizoplane, diversity, occurrence rate, antagonistic activity, utilization of carbon sources.

Actinomycetes are widely spread in soils and related plant substrates. These gram-negative bacteria with a complex life cycle including the mycelial and spore stages are distinguished from other prokaryotes by their ability to produce a wide range of biologically active substances, including antibiotics. Like nonmycelial bacteria, actinomycetes are able to colonize plant roots. Such colonization can be observed microscopically [1].

There is little information in the literature as to what actinomycete species colonize plant roots most frequently and what is the functional role of actinomycete–plant associations [2, 3]. In particular, little is known about the structure and the functional role of the natural root-colonizing actinomycete complexes of different plant species and varieties, while knowledge of this may aid in understanding the processes occurring in natural microbial communities and the development of advanced agricultural technologies employing the interactions of organisms in the rhizosphere of cultivated plants.

The aim of this work was to comparatively study the antagonistic activity and the trophic requirements of the root-colonizing actinomycete complexes of different barley varieties grown in soddy podzolic soil.

## MATERIALS AND METHODS

Experiments were carried out with four *Hordeum vulgare* L. barley varieties, 999-93, 889-93, Novichok, and Kumir, which were grown in acid and limed soddy podzolic soil [4]. Barley plants were grown to the panniculation stage, and the roots of ten plants of each of the barley varieties (five grown in acid soil and five grown in limed soil) were subjected to analysis. To determine the total number of actinomycetes, the roots were rid of soil by washing and homogenized in 10 ml water in a mortar. The serial dilutions of the homogenate were plated onto a sodium propionate–containing agar medium [5], which was supplemented with 1 μg/ml nalidixic acid and 50 μg/ml nystatin to suppress the growth of nonmycelial bacteria and fungi. The plates were incubated at 28°C for 2 weeks. The number of colony-forming units (CFU) was calculated per g dry wt. roots.

Actinomycete colonies were differentiated according to their cultural and morphological characteristics, and three to five representatives of each of the colonial morphotypes were isolated in pure culture. Each isolate was designated so as to know the plant from which it was isolated. The collection of the isolates amounted up to 100 strains. Preliminary screening allowed us to choose 24 isolates for further analysis. The isolates were identified to a genus level (*Streptomyces*) based on the specific morphological properties: (1) the formation of a non-septate mycelium, (2) the formation of an aerial mycelium with spores arranged in long chains, and (3) the formation of a substrate mycelium without spores. Identification of streptomycetes at the species level was performed according to the manual by Gauze *et al.* [6].

The antagonistic properties of actinomycetes were studied using the test cultures *Arthrobacter simplex* Dr. 12, *Arthrobacter mysorens* 7, and *Azospirillum lipoferum* 257 obtained from the collection of microor-

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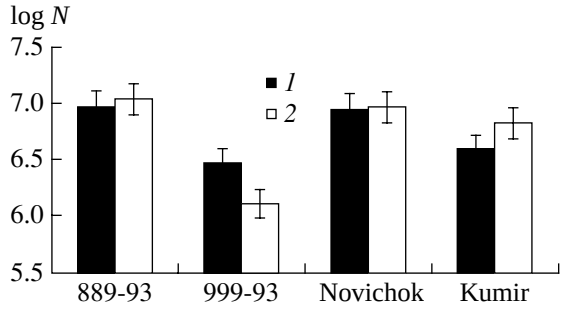


**Fig. 1.** The number (*N*) of actinomycetes in the rhizoplane of barley varieties grown in (*1*) acid and (*2*) limed soddy podzolic soil.

ganisms at the Research Institute of Agricultural Microbiology (Pushkin, St. Petersburg); *Pseudomonas aeruginosa* 381, *Pseudomonas fluorescens* 540, and *Bacillus subtilis* KC-1 obtained from the collection of microorganisms at the Research Institute of Microbiology of the Ministry of Public Health of the Russian Federation (Kirov); *Fusarium sporotrichiella* K-8999-z and *Fusarium sporotrichiella* KK-794-27 obtained from T.K. Sheshegova (Rudnitskii Research Institute of Agriculture in Northeastern Russia). To assay antagonistic activity, test bacteria and fungi were grown on RHM agar [8] and Czapek agar, respectively. Antagonistic activity was determined by the method of oat agar blocks [6, 7] by estimating the radius of the zone of inhibited growth of test cultures after 2–4 days of incubation. The tests were performed in triplicate.

The trophic requirements of actinomycetes were determined by incubating them in mineral ISP9 medium [6] supplemented with the sugars and organic acids that are typical of plant root exudates.

The structure and diversity of the root-associated actinomycete complexes was characterized by the Shannon diversity index (*H*) [9] and the occurrence rates of antagonistic species and species with different trophic requirements. The degree of similarity of the actinomycete complexes colonizing different barley varieties was assessed by cluster analysis in terms of the Sorensen similarity coefficient *Ks*.

Experimental data were statistically processed using the Excel and Statgraph software packages.

**Table 1.** The contributions of different factors to the variation of the actinomycete population in the barley rhizosphere and rhizoplane

| Substrate | Contribution of different factors to population variation, % | | | |
|---|---|---|---|---|
| | Medium | Genotype | Medium × genotype | Uncontrollable factors |
| Barley rhizosphere* | 35.4 | 29.5 | 32.1 | 3.0 |
| Barley rhizoplane | 37.0 | 36.5 | 24.7 | 1.8 |

* These data are derived from the experimental results published earlier [15].

## RESULTS AND DISCUSSION

The population density of mycelial prokaryotes in the barley rhizoplane varied from $(1.4 \pm 0.) 5 \times 10^6$ to $(1.1 \pm 0.15) \times 10^7$, depending on barley variety and soil acidity. In both types of soil (acid and limed), the barley varieties 889-93 and Novichok showed the maximum density of root-associated actinomycetes. The roots of the barley variety 999-93 were scarcely populated, especially in the limed soil (Fig. 1).

It is known that the degree of plant colonization with nonmycelial rhizobacteria is mainly determined by their motility [10] and ability to utilize the ingredients of root exudates [11–13]. The role of root exudates in the colonization of plant roots with nonmotile actinomycetes is still poorly understood. To estimate the relative contributions of the plant genotype (i.e., barley variety) and medium (more specifically, soil acidity) to the variation of the population density of actinomycetes in the barley rhizosphere and rhizoplane, the population data were subjected to bifactorial variance analysis (Table 1). The total variation of the population parameters, taken to be 100%, was divided into four components: variation due to the effect of the barley genotype (variety), variation due to the effect of medium (soil acidity), variation due to both of these factors, and variation due to uncontrollable factors (random variation or experimental error).

The factor of the barley genotype, as well as the factor of soil acidity, was responsible for about one-third of the total variation of actinomycete population in the barley rhizosphere and rhizoplane, being more significant in the rhizoplane (Table 1). In contrast, the combined contribution of these two factors (soil acidity × genotype) to the total variation of actinomycete population in the rhizoplane was lower than in the rhizosphere (Table 1). This fact can be explained by the relative constancy of pH in the plant rhizoplane [14].

The actinomycetes isolated from the barley rhizoplane using a selective medium with sodium propionate turned out to belong to the genus *Streptomyces*, although our earlier studies showed that the barley rhizoplane can be colonized by other actinomycete genera as well [15].

The rhizoplanes of all of the barley varieties studied were dominated by streptomycetes of the section *Cinereus* ser. *Chromogenes.* In addition. the rhizoplanes of the barley varieties Novichok, Kumir, and 889-93 contained streptomycetes of the section *Cinereus* series *Achromogenes* and *Aureus.* The strep-

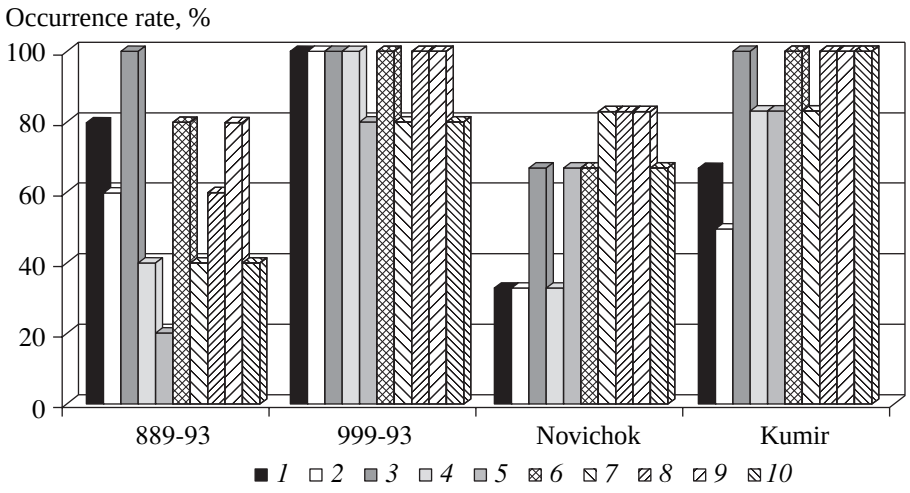


**Fig. 2.** The occurrence rates of the barley root–colonizing actinomycetes capable of utilizing (*1*) malate, (*2*) citrate, (*3*) succinate, (*4*) acetate, (*5*) oxalate, (*6*) glucose, (*7*) D-xylose, (*8*) D-fructose, (*9*) D-galactose, and (*10*) L-arabinose.

tomycetes of the section *Cinereus* ser. *Aureus* were also found in the barley var. 999-93 rhizoplane. The streptomycetes of the section *Roseus* ser. *Fradiae* were encountered, albeit at a frequency as low as 17%, only in the barley var. Kumir rhizoplane.

The diversity indices *H* of actinomycetes associated with the roots of the barley varieties Novichok, 889-93, and Kumir were almost the same and relative high (1.46, 1.37, and 1.27, respectively). In contrast, the diversity index of actinomycetes in the barley var. 999-93 rhizoplane was as low as 0.72. The diversity indices of actinomycetes colonizing the rhizoplanes of different barley varieties directly correlated with the population densities of actinomycetes in these rhizoplanes. This may be due to the fact that the abundance and diversity of actinomycetes in the barley rhizoplane depend on the variety-specific composition of root exudates.

To verify this suggestion, we compared the diversity indices of actinomycetes colonizing the roots of different barley varieties with the ranges of utilizable organic acids and sugars typical of root exudates and found that there exists an inverse correlation between the species diversity of rhizoplane-associated actinomycetes and the diversity of utilizable carbon sources. For instance, the actinomycete complex of the barley var. 999-93 was characterized by a narrow species diversity, low abundance, and a high occurrence rate (at a level of 80–100%) of species that were able to utilize a wide range of carbon sources (Fig. 2).

In contrast, the actinomycete complex colonizing the barley var. Kumir rhizoplane exhibited a high species diversity and a narrow range of utilizable carbon sources (mainly, organic acids). The occurrence rate of the actinomycete species capable of utilizing malate, citrate, acetate, oxalate, and D-xylose was considerably lower in the rhizoplane of var. Kumir than in the rhizoplane of var. 999-93. Still more demonstrative is the consideration of the properties of the actinomycete complexes of the barley varieties 889-93 and Novichok. Indeed, on the one hand, these complexes are very diverse and abundant. On the other hand, the occurrence rate of the actinomycete species capable of utilizing acetate, oxalate, D-xylose, and L-arabinose in the barley var. 889-93 rhizoplane is as low as 40% and those capable of utilizing malate, citrate, and acetate in the barley var. Novichok rhizoplane is as low as 33%.

It was of interest to investigate the ability of root-colonizing actinomycetes to produce antibiotics, which are believed to play a role in the competition of microorganisms in the rhizosphere and rhizoplane of plants [7], where many species behave as *K* strategists [16].

If antibiotic-producing actinomycetes are actually involved in the regulation of microbial communities in the plant rhizosphere, their antagonistic properties must depend on the biochemical composition of root exudates, which is different for different plant species and varieties [17].

The actinomycete complex of the barley var. Novichok showed the widest range of antagonistic activity, both antibacterial and antifungal (Fig. 3). The range of the antagonistic activity of the actinomycete complex of the barley var. Kumir was also wide. The actinomycete complex of the barley var. 889-93 showed a relatively narrow range of antagonistic activity but was characterized by the high occurrence rates (given in parentheses) of actinomycete species antagonistic to the bacteria *A. lipoferum* 257 (40%) and *P. aeruginosa* 381 (60%) and to the fungi *F. sporotrichiella* K-8999-z (40%) and KK-794-27 (40%). The range of the antagonistic activity of the actinomycete complex of the barley var. 999-93 was the most narrow.

The antagonistic streptomycetes isolated in this work were found to belong to different series of the section *Cinereus* (Table 2). The species *S. clavuligerus* and

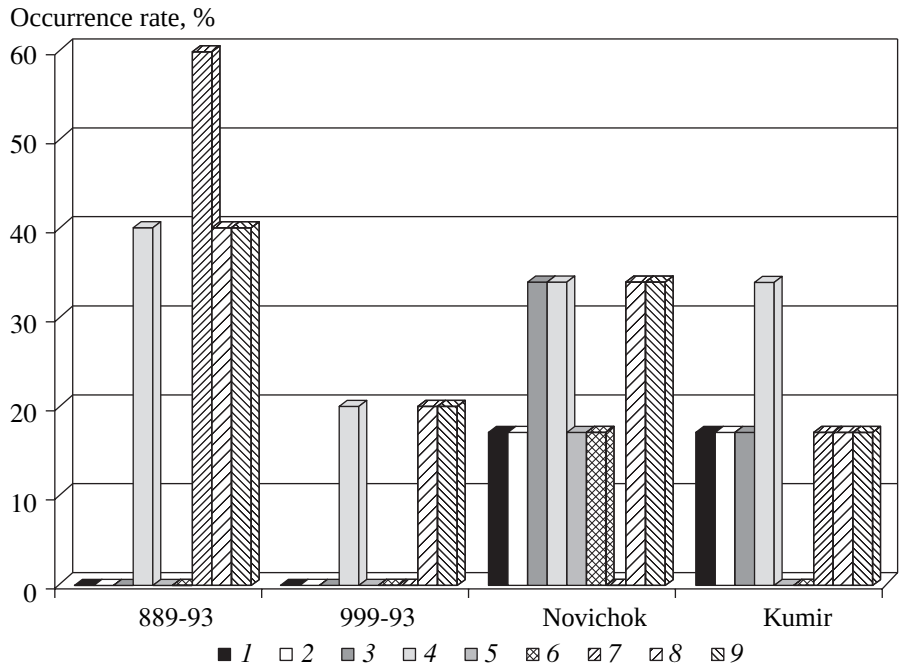


**Fig. 3.** The occurrence rates of the barley root–colonizing actinomycetes antagonistic to (*1*) *Flavobacterium* sp. L30, (*2*) *A. simplex* Dr. 12, (*3*) *A. mysorens* 7, (*4*) *A. lipoferum* 257, (5) *B. subtilis* KC-1, (*6*) *P. aeruginosa* 381, (*7*) *P. fluorescens* 540, (*8*) *F. sporotrichiella* K-8999-z, and (*9*) *F. sporotrichiella* KK-794-27. Presented are the actinomycetes that produced the zones of inhibition of bacterial growth with radii greater than 3 mm and the zones of inhibition of fungal growth with radii greater than 5 mm.

*S. thermoflaves* showed high antifungal activity. The species *S. griseoluteus*, which was isolated from the barley var. Kumir roots, suppressed the growth of a wide range of bacteria.

A comparison of the diversity indices and the antagonistic activity ranges of actinomycetes colonizing different barley varieties showed that there is a direct correlation between the species diversity of actinomycetes in a particular complex and their ability to produce antibiotics. This is in agreement with the conception that the ability of actinomycete to produce antibiotics stems from the competition of microorganisms in natural habitats.

In the next set of experiments, we estimated the functional and taxonomic similarity of different actinomycete complexes (Table 3). The Sorensen similarity coefficients *Ks* calculated from the occurrence rates of different streptomycete sections and series were found to be higher than those calculated from the occurrence rates of actinomycete species with different trophic requirements and antagonistic properties. As a whole, the degree of similarity of the actinomycete complexes of the barley varieties 999-93 and 889-93 ($K_S$ = 0.50–

**Table 2.** Antagonistically active actinomycetes isolated from the rhizoplane of barley plants grown in soddy podzolic soil

| Isolate | Barley variety | Streptomycete section and series | Species affiliation | Antibiotics produced [13] |
|---|---|---|---|---|
| 126b | 889-93 | *Cinereus Chromogenes* | *S. mirabilis* | Miramycin |
| 128a | Novichok | *Cinereus Chromogenes* | *S. plicatus* | Amicetin, bamicetin, plicacetin |
| 127a | 999-93 | *Cinereus Aureus* | *S. flavovirens* | Actinomycin, pilaromicin |
| 130c | 889-93 | *Cinereus Aureus* | *S. thermoflavus* | SF-733 [Jap. Pd 17150, 1967] |
| 132c | Novichok | *Cinereus Achromogenes* | *S. clavuligerus* | Cephalosporin, penicillin, cefamycin C |
| 130b | 889-93 | *Cinereus Achromogenes* | *S. pseudogriseolus* | Xanthomycin |
| 133b | Kumir | *Cinereus Aureus* | *S. griseoluteus* | Griseoluteins A and B |

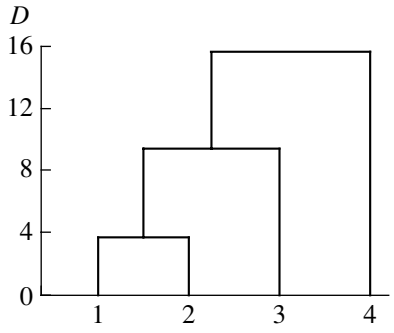


**Fig. 4.** The similarity dendrogram of actinomycetes colonizing the roots of the barley varieties (1) 889-93, (2) 999-93, (3) Novichok, and (4) Kumir.

0.77) was lower than the degree of similarity of the actinomycete complexes of the barley varieties Novichok and Kumir ($K_S$ = 0.70–0.84). The actinomycete complexes colonizing the barley varieties 999-93 and Kumir exhibited the least similarity ($K_S$ = 0.43–0.67). The cluster analysis of the actinomycete complexes showed that all of them belong to one cluster. Within the cluster, the actinomycete complexes somewhat differ, the complex of the barley variety 999-93 differing the most (Fig. 4).

Thus, the mycelial prokaryotic complexes associated with the roots of genotypically different barley plants are similar in the taxonomic structure but differ in functional characteristics. The actinomycete complex of the barley variety 999-93 is distinguished by the minimal values of the species abundance and diversity, the widest range of utilizable carbon sources, and the most narrow range of antagonistic activity. In contrast, the actinomycete complexes of the barley varieties Novichok, 889-93, and Kumir show relatively high values of the species abundance and diversity, the narrow range of utilizable ingredients of root exudates, and the wide range of antagonistic activity.

These variety-specific differences in the colonization of the barley roots by mycelial prokaryotes may be of interest to plant breeders, especially in relation to the problem of soil infections. The selection of plants with an enhanced ability to accommodate soil actinomycetes with particular antagonistic properties may be one of the possible ecologically sound ways of phytopathogen control in the plant rhizosphere.

**Table 3.** S'orensen similarity coefficients $K_S$ of the actinomycete complexes colonizing the rhizoplane of barley plants grown in soddy podzolic soil

| Barley variety | 999-93 | Novichok | Kumir |
|---|---|---|---|
| Taxonomic similarity | | | |
| 889-93 | 0.77 | 0.94 | 0.84 |
| 999-93 | | 0.70 | 0.67 |
| Novichok | | | 0.84 |
| Similarity in trophic requirements | | | |
| 889-93 | 0.56 | 0.71 | 0.64 |
| 999-93 | | 0.66 | 0.47 |
| Novichok | | | 0.76 |
| Similarity in antagonistic activity | | | |
| 889-93 | 0.50 | 0.48 | 0.49 |
| 999-93 | | 0.41 | 0.43 |
| Novichok | | | 0.70 |

## REFERENCES

1. Orazova, M.Kh., Polyanskaya, L.M., and Zvyagintsev, D.G., The Structure of the Microbial Community in the Barley Root Zone, *Mikrobiologiya*, 1999, vol. 68, no. 1, pp. 127–133.
2. Kalakutskii, L.V. and Sharaya, L.S., Actinomycetes and Higher Plants, *Usp. Mikrobiol.*, 1990, no. 2, pp. 25–26.
3. Krol, M.J. and Kobus, J., The Occurrence of Microorganisms in the Rhizosphere of Barley Cultivated on Gray Brown Podsolic Soil Developed from Loess, *Fob. Univ. Agric. Stetin. 201 Agricult.*, 1999, vol. 78, pp. 129–138.
4. Shirokikh, A.A., Shirokikh, I.G., and Rodina, N.A., Microbial Population Density on the Roots of Various Barley Varieties Grown in Acid and Limes Soddy Podzolic Soil, *Pochvovedenie*, 2001, no. 9, pp. 1097–1102.
5. Zenova, G.M., *Pochvennye aktinomitsety redkikh rodov* (Soil Actinomycetes of Rare Genera), Moscow: Mosk. Gos. Univ., 2000.
6. Gauze, G.F., Preobrazhenskaya, T.P., Sveshnikova, M.A., *et al.*, *Opredelitel' aktinomitsetov* (Manual of Actinomycetes), Moscow: Nauka, 1983.
7. Egorov, N.S., *Osnovy ucheniya ob antibiotikakh* (Fundamentals of the Science of Antibiotics), Moscow: Vysshaya Shkola, 1979.
8. *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Zvyagintsev, D.G., Ed., Moscow: Mosk. Gos. Univ., 1991.
9. Magurran, A.E., Ecological Diversity and Its Measurement, 1988. Translated under the title *Ekologicheskoe raznoobrazie i ego izmerenie*, Moscow: Mir, 1992.
10. Katsy, E.I., The Genetic, Biological, and Ecological Aspects of Motility and Chemotaxis in Phytopathogenic, Symbiotic, and Plant-associated Bacteria, *Usp. Sovrem. Biol.*, 1996, vol. 116, no. 5, pp. 579–592.
11. Sealy, L.J., Gochnauer, M., and McCully, M.E., Do Plant Roots Select Their Microflora?, *Am. J. Bot.*, 1989, vol. 76, no. 6, pp. 57–58.
12. Bolton, H., Frederickson, J.K., and Elliot, L.F., Microbial Ecology of the Rhizosphere, *Soil Microbial Ecol-*

*ogy*, Metting, F.B., Ed., New York: Marcel Dekker, 1992, pp. 27–36.

13. Grayston, S.J., Wang, S., Campbell, C.D., and Edwards, A.C., Selective Influence of Plant Species on Microbial Diversity in the Rhizosphere, *Soil Biol. Biochem.*, 1998, vol. 30, no. 3, pp. 369–378.

14. Kim, T.K., Gilk, W.K., and Ghur, A.Y., A Mathematical Model for pH Patterns in the Rhizospheres of Growth Zones, *Plant Cell Environ.*, 1999, vol. 22, pp. 1527–1538.

15. Shirokikh, I.G., The Structural Organization of Actinomycete Complexes in the Rhizosphere of Barley Plants Grown in Acid Soddy Podzolic Soil, *Tez. dokl. Vseros. konf. "Sel'skokhozyaistvennaya mikrobiologiya v XIX–XXI vekakh* (Proc. All-Russia Conf. "Agricultural Microbiology in the 19–21 Centuries"), St. Petersburg: VNII Sel'sk. Mashinostr., 2001, p. 82.

16. Golovlev, E.L., Ecological Strategy of Bacteria: Specific Nature of the Problem, *Mikrobiologiya*, 2001, vol. 70, no. 4, pp. 437–443.

17. Kravchenko, L.V., Rhizosphere As a Region of Interaction of Microorganisms and Plants, *Tez. dokl. Vseros. konf. "Sel'skokhozyaistvennaya mikrobiologiya v XIX–XXI vekakh* (Proc. All-Russia Conf. "Agricultural Microbiology in the 19–21 Centuries"), St. Petersburg: VNII Sel'sk. Mashinostr., 2001, p. 59.