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EXPERIMENTAL ARTICLES

Actinomycetes in the Rhizosphere of Barley Grown on Acid Soddy Podzolic Soil

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Abstract—The study of various factors (soil acidity, the variety of barley, and their developmental phases) on the rhizosphere actinomycete complex showed that it is soil acidity that substantially influences the population of rhizosphere actinomycetes. The effect of soil acidity was most likely due to the different tolerance of rhizosphere actinomycetes to high concentrations of the aluminum and hydrogen ions. The developmental phases of barley correlated with the population indices of only one genus of actinomycetes, *Micromonospora*.

Key words: plant rhizosphere, actinomycete complex, soil acidity, plant variety.

Actinomycetes comprise about 25% of the soil microbial complex when they are enumerated by the plate method on standard nutrient media [1, 2]. The content of the actinomycete mycelium in the rhizosphere, estimated by luminescence microscopy, amounts to 20% of the total bacterial biomass [3].

Little is known about the taxonomic composition of actinomycetes in the plant rhizosphere. According to some data available in the literature, the plant rhizosphere is inhabited by members of the genera *Streptomyces*, *Micromonospora*, and *Nocardia* [4].

Actinomycetes are able to form symbiotic associations with plants [4] and algae [1]. The biologically active compounds produced by actinomycetes may considerably influence the development of plants. This stimulates the interest of researchers in the relationship between mycelial prokaryotes and plants under particular soil conditions.

The aim of the present work was to study factors that promote the colonization of the plant rhizosphere by actinomycetes and to investigate the composition of the actinomycete complex of the barley rhizosphere in acid soddy podzolic soil.

MATERIALS AND METHODS

Investigations were carried out within the scope of the vegetation experiment performed by researchers from the Laboratory of Selection and Primary Seed Production, Rudnitskii Research Institute of Agriculture in Northeastern Russia. The cultivated soddy podzolic soil in the experimental farm Fedyakovo situated in the Kirov region had a pH_{H_2O} 3.9 and a content of mobile aluminum equal to 10.8 mg/100 g soil. The acidity of the control soil was corrected by adding lime.

Barley plants were grown in the summer season using 1.5-m^3 seedling pans. The moisture and temperature conditions were natural. Two barley varieties, 999-93 and 889-93, were tolerant to mobile aluminum and two varieties, Dina and Kumir, were susceptible to it.

For microbiological analysis, plants were withdrawn together with the soil on their roots. This soil was then removed from the roots, dried, weighed, heated at 100°C for 1 h, and used for the preparation of aqueous suspensions. The soil suspension dilutions were plated onto an agar medium with sodium propionate [5]. The plates were incubated at 28°C for 2–3 weeks. The actinomycete colonies were enumerated by distinguishing the following three morphotypes: (1) actinomycetes producing an aerial nonseptate mycelium with spore chains (they were preliminarily classified into the genus *Streptomyces*); (2) actinomycetes producing a substrate mycelium with single spores and a scanty, if at all, sterile nonseptate aerial mycelium (they were preliminarily classified into the genus *Micromonospora*); and (3) actinomycetes producing sporangia, single spores, or short chains of spores, which were larger than those of streptomycetes, on an aerial mycelium (they were combined into a group of rare genera of actinomycetes). The representatives of these three groups of actinomycetes were isolated in pure cultures for their further identification.

The results obtained were analyzed for the occurrence and abundance rates of actinomycete genera. The occurrence rate was defined as the ratio of the number of the soil samples in which a given genus was detected to the total number of the soil samples analyzed. The

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Correlation between some soil and plant parameters (soil acidity and the variety and developmental phase of barley plants) and the population of various actinomycete genera in the barley rhizosphere

Factor	Number of degrees of freedom	Sum of squares	Fisher statistic	Significance level
Streptomycetes				
Soil acidity	1	1752.6	11.23	0.99*
Barley phase	1	234.7	1.50	0.78
Barley variety	3	4379.5	9.35	0.99*
Micromonosporas				
Soil acidity	1	176688	22.28	0.99*
Barley phase	1	106257	13.40	0.99*
Barley variety	3	385359	16.20	0.99*
Actinomycetes of rare genera				
Soil acidity	1	51	5.53	0.95
Barley phase	1	2.5	0.57	0.39
Barley variety	3	165.7	5.98	0.99*
Total actinomycetes				
Soil acidity	1	219041	29.86	0.99*
Barley phase	1	107949	14.72	0.99*
Barley variety	3	474941	21.58	0.99*

* The asterisks mark statistically significant values.

abundance rate was defined as the ratio of the soil samples in which a given genus comprised more than 50% of all the genera detected to the total number of the soil samples analyzed.

The effect of various factors (soil acidity, the developmental phase of plants (ear formation and maturity), and plant variety) on the population indices of rhizosphere actinomycetes was evaluated in terms of variance analysis.

RESULTS AND DISCUSSION

The number of actinomycetes in the rhizosphere of the barley plants grown in the acid aluminum-containing soddy podzolic soil varied from $(0.3 \pm 0.2) \times 10^4$ to $(3.2 \pm 0.8) \times 10^4$ CFU/g soil, depending on the variety and the developmental phase of barley plants.

The actinomycete complex of the barley rhizosphere was dominated by the genera *Streptomyces* and *Micromonospora*. The actinomycetes of other genera were two to three orders less frequent and abundant.

According to some data available in the literature, the species composition of actinomycetes in the plant rhizosphere is mainly determined by soil parameters, whereas the abundances of particular species depend largely on the plant [4]. The variance analysis of the results obtained in this study showed that soil acidity and plant variety exert a statistically significant effect on the population density of streptomycetes and micromonosporas in the rhizosphere and that variations in the population density of the actinomycetes are determined by soil acidity and, to a lesser degree, by the barley variety (see table).

The average number of actinomycetes in the rhizosphere of the barley plants grown in the experimental acid soil was $(12.6 \pm 2.55) \times 10^4$ CFU/g soil, i.e., six times less than in the control (limed) soil ($(1.87 \pm 0.87) \times 10^4$ CFU/g) (Fig. 1a). The number of the rhizosphere actinomycetes was dependent on the plant variety, changing from $0.5 \pm 0.47) \times 10^4$ CFU/g soil for the barley var. Kumir to $(7.5 \pm 2.5) \times 10^5$ CFU/g soil for the barley var. 889-93 (Fig. 1b). In the limed soil, variations in the number of actinomycetes inhabiting the rhizosphere of different barley varieties also reached two orders of magnitude.

The number of rhizosphere actinomycetes of rare genera widely varied depending on the barley variety, whereas changes in the population density of these actinomycetes with respect to soil acidity and the developmental phase of plants were less pronounced (Figs. 1a–1c).

There was a correlation between the number of the rhizosphere micromonosporas and the developmental phase of barley plants: the micromonosporas were an order of magnitude more abundant in the phase of barley maturity than in the phase of ear formation (Fig. 1c). At the same time, the population of the rhizosphere streptomycetes and rare genera did not exhibit statistically significant dependence on the developmental phase of plants (Fig. 1c).

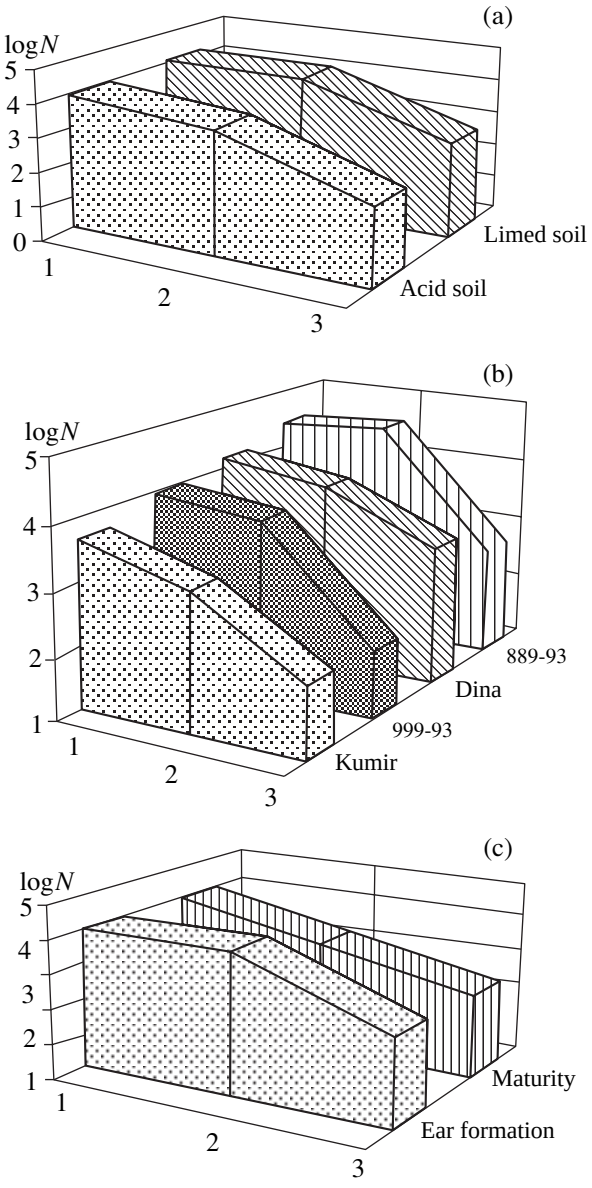

Fig. 1. The population of various groups of rhizosphere actinomycetes as a function of (a) soil acidity, (b) barley variety, and (c) the developmental phase of barley plants: (1) *Streptomyces*, (2) *Micromonospora*, and (3) rare genera. The number of actinomycetes (*N*) is expressed in CFU/g rhizosphere soil.

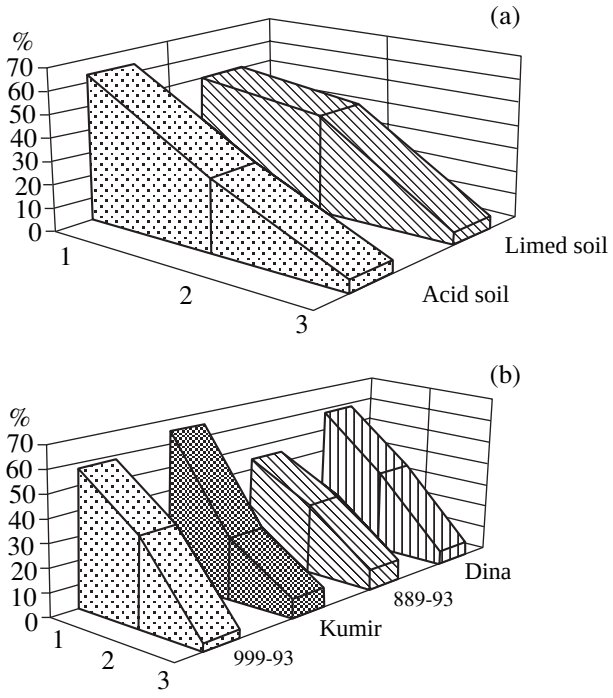

Fig. 2. The fraction of various genera in the rhizosphere actinomycete complex as a function of (a) soil acidity and (b) barley variety: (1) *Streptomyces*, (2) *Micromonospora*, and (3) rare genera.

Thus, the population of the rhizosphere actinomycetes was a function of soil acidity to a greater extent than of the other factors studied. The developmental phases of barley plants exhibited a correlation with only the population indices of the rhizosphere micromonosporas.

The fraction of the rhizosphere streptomycetes in the acid soil was larger, while that of the rhizosphere micromonosporas was smaller, than in the limed soil (Fig. 2a). The fraction of the rhizosphere actinomycetes of rare genera was small (5.2–5.5% of the total actinomycetes) in both acid and limed soils (Fig. 2a).

The fraction of streptomycetes varied from 44.6% in the rhizosphere of the barley var. 889-93 to 65.5% in the rhizosphere of the barley var. Kumir (Fig. 2b). The fraction of micromonosporas varied from 26.4% in the rhizosphere of the acid-susceptible barley var. Kumir to 38.4% in the rhizosphere of the barley var. 999-93, which is more tolerant to soil acidity. The fraction of rare genera in the rhizosphere actinomycete complex was small and varied from 3.3 to 8.6%, depending on the barley variety.

Irrespective of the all studied factors (soil acidity, barley variety, and the developmental phase of plants), the occurrence rate of the rhizosphere streptomycetes and micromonosporas was 100%. At the same time, the occurrence rate of the rare genera of actinomycetes (Figs. 3a, 3b) was higher in the limed soil (87%) than in the acid soil (75%). Similarly, it was higher in the rhizosphere of the barley var. Dina (100%) than in the rhizosphere of the other barley varieties (75%).

The rhizosphere actinomycete complex was dominated by streptomycetes in both acid and limed soils, with the abundance rates of 75 and 62%, respectively (Fig. 4a). The abundance rate of the streptomycetes varied from 75% in the rhizosphere of the acid-susceptible barley varieties Dina and Kumir to 50% in the rhizosphere of the acid-tolerant barley varieties 999-93 and 889-93.

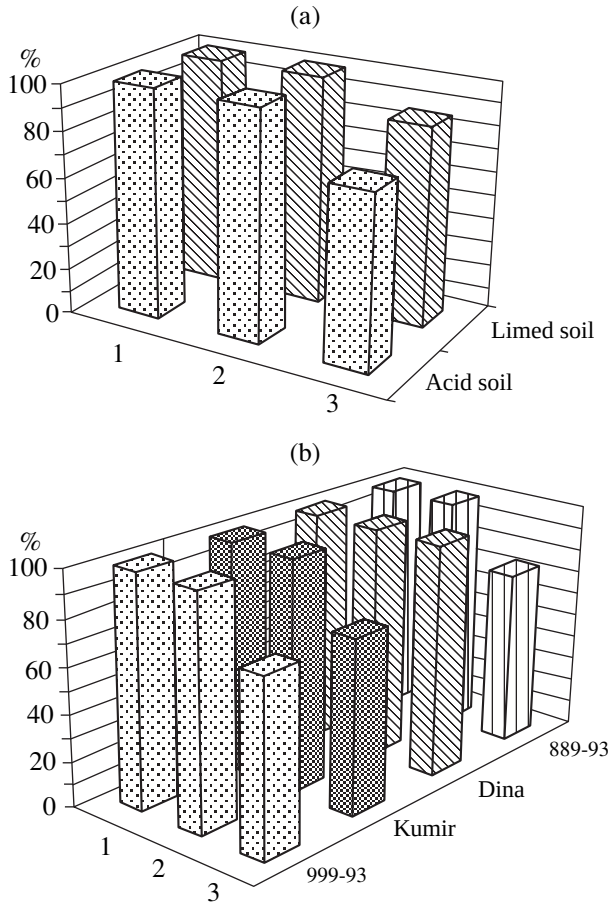

Fig. 3. The occurrence rate of various actinomycete genera in the barley rhizosphere as a function of (a) soil acidity and (b) barley variety: (1) *Streptomyces*, (2) *Micromonospora*, and (3) rare genera.

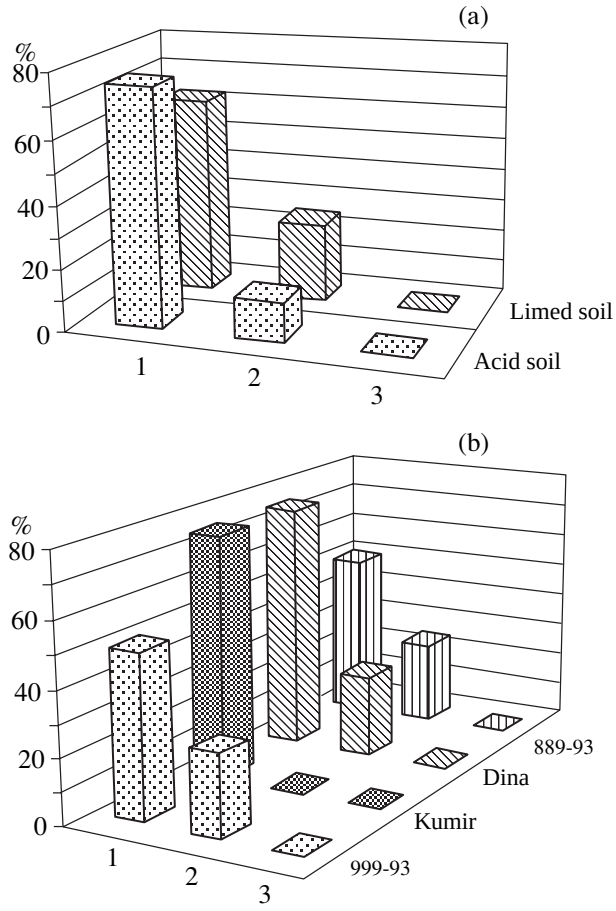

Fig. 4. The abundance rate of various actinomycete genera in the barley rhizosphere as a function of (a) soil acidity and (b) barley variety: (1) *Streptomyces*, (2) *Micromonospora*, and (3) rare genera.

The abundance rate of the rhizosphere micromonosporas in the acid and limed soils amounted to 12.5 and 25%, respectively, and was almost the same (25%) in the rhizosphere of all the barley varieties studied except for the variety Kumir. The rare actinomycete genera were present in the rhizospheres of all the barley varieties in minor amounts.

Thus, our investigations showed that soil acidity, which is due to the presence of hydrogen and aluminum ions in the soil, exerted a statistically significant effect on the abundance and the composition of the actinomycete complex in the barley rhizosphere. In the acid soil, the total number of actinomycetes was smaller, whereas the fraction and the occurrence rate of streptomycetes were higher, than in the limed soil. Unlike the population indices of the streptomycetes, the population indices of the rhizosphere micromonosporas were higher in the limed soil. The occurrence rate of the rare genera of actinomycetes was lower in the acid than in the limed soil, being low in both types of soil when compared with the occurrence rates of the rhizosphere streptomycetes and micromonosporas. Therefore, an inference can be made that the rhizosphere streptomycetes are more tolerant to the toxic effect of high concentrations of the hydrogen and aluminum ions than the rhizosphere micromonosporas. There is a high positive correlation (r = 0.98) between the fraction of micromonosporas in the rhizosphere actinomycete complex and the pH of the rhizosphere soil.

The population density of actinomycetes was higher in the rhizosphere of the acid- and aluminum-tolerant barley variety 889-93 than in the rhizosphere of the less tolerant barley varieties. The rhizosphere actinomycete complex of this barley variety was found to contain less streptomycetes and more micromonosporas (in the phase of ear formation) and rare actinomycete genera than the rhizosphere of the other barley varieties. Conversely, the rhizosphere actinomycete complex of the aluminum-susceptible variety Kumir contained more streptomycetes and less micromonosporas than the rhizosphere of the aluminum-tolerant barley varieties. Streptomycetes were more abundant in the rhizospheres of the acid-susceptible barley varieties Kumir

and Dina than in the rhizospheres of the acid-tolerant barley varieties 889-93 and 999-93.

The specific effect of the barley variety on the composition of the rhizosphere actinomycete complex can be accounted for by the different abilities of the barley varieties to neutralize acids present in the rhizosphere soil [6], as well as by the different composition of the pentosan-containing root exudates, which may promote the development of particular actinomycete genera [7].

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