
EXPERIMENTAL
ARTICLES

Actinomycete Complexes in the Rhizosphere of Winter Rye on Soddy Podzolic Soil

I. G. Shirokikh¹ and O. V. Merzaeva

Rudnitskii Zonal Research Institute of Agriculture in the Northeast of Russia, Kirov, Russia

Abstract—The structure of actinomycete complexes in the rhizosphere of winter rye (*Secale cereale* L.) varieties originating from different ecosystems and geographical zones was studied in field experiments on soddy podzolic soil. In addition to streptomycetes, the complexes studied contained actinomycetes of the genera *Micromonospora* and *Streptosporangium*, represented at high occurrence and comparable abundance rates. The rhizosphere of most of the studied rye varieties was dominated by micromonosporas. The antifungal potential of mycelial prokaryotes associated with winter rye was assessed. The taxonomic and functional structure of actinomycete complexes was shown to be similar in rye varieties originating from the nonchernozem zone of Russia. However, the actinomycete complex proved to be much different in the rye variety that developed in the steppe zone of the lower Volga region.

Key words: actinomycete complex, winter rye varieties, rhizosphere, antifungal activity.

Although mycelial prokaryotes are known to produce a wide range of biologically active substances that can strongly affect plants, there is not much evidence in the literature, except for several plant diseases, as to exactly which actinomycetes most commonly form associations with plant roots [1, 2].

A major factor affecting soil microorganisms is root exudates of plants. The dependence of root colonization by bacteria as well as their beneficial action on plants, including antifungal effects, on a genetically predetermined composition of different components of root exudates (e.g., sugars and organic acids) is currently the subject of active research [3–5]. It is not yet known whether the composition of root exudates, determined by the plant's genotype, has a selective role to play with regard to immobile mycelial prokaryotes.

By studying the structure of actinomycete complexes in the rhizosphere of genotypically dissimilar plants, we cannot only uncover the microlocus distribution pattern of mycelial prokaryotes in soil but also understand the peculiar features in the makeup of rhizospheric complexes in different species and varieties of plants. Given that the distinctions among varieties within one species are genetically controlled, taking them into account could be helpful in developing new varieties that would be able to fully exploit the potential of positive interactions with bacteria.

The goal of this work was to study and compare actinomycete complexes formed in the rhizosphere of winter rye varieties originating from different environmental and geographical zones.

MATERIALS AND METHODS

The subject of this study was the structure of actinomycete complexes associated with roots of the following winter rye varieties: Vyatka 2, Falenskaya 4, Kirovskaya 89 (selection from the Research Institute of Agriculture in Northeastern Russia, (RIANR)), and two varieties coming from other regions: Krona (Research Institute of Agriculture in the Central Nonchernozem Region) and Saratovskaya 2 (Research Institute of Agriculture in Southeastern Russia). Samples of roots were obtained by removing plants, at the stage of milk-wax ripeness, together with pieces of soil sticking to the roots. Five samples of each rye variety were collected. The soddy podzolic soil in the test field had the following agrochemical parameters: pH 6.5; P₂O₅, 655 mg/kg; K₂O, 264 mg/kg soil; and humus 3.0%.

Prior to inoculation, predried soil together with roots was exposed to heat at 100°C for 1 h and then used for preparing soil suspensions inoculated on dishes with solid medium in three replicates. The generic composition of actinomycete complexes was studied with the use of a medium containing sodium propionate; the species composition of the genus *Streptomyces* was determined on casein glycerol agar (CGA) [6]. In order to selectively inhibit the growth of non-mycelial bacteria and fungi, nalidixic acid (1 µg/ml) and nystatin (50 µg/ml) were added to the medium. The plated material was incubated at 28°C for 2–3 weeks.

The obtained colonies were estimated according to their morphological types described in our previous paper [7], with an additional group assigned to actinomycetes forming sporangia on air mycelium (preliminarily identified as representatives of the genus

¹ Corresponding author. E-mail: shirokikh@ptlan.com

Streptosporangium). The colonies of the morphological type prevalent in each dish were isolated in pure cultures. The species affiliations of the isolates were identified by their cultural and morphological characteristics using the manual by Gause *et al.* [8]. Each strain was indexed and its belonging to a certain rye variety was recorded for further data processing. The obtained collection comprised a total of 60 strains.

The antibiotic activity of isolated strains was assayed by the method of agar blocks [9]. Test cultures were represented by phytopathogenic fungi *Fusarium avenaceum* 7/2 and 10/2 and *F. sporotrichiella* K-8999-k, isolated from winter rye plants by T.K. Sheshegova (head of the immunity group, RIANR); *Penicillium* sp. and *Trichoderma viride*, isolated from soil; and yeasts *Saccharomyces cerevisiae*, an industrially used strain from the collection of the All-Russia Research Institute of the Beer and Nonalcoholic Beverage Industry, Moscow. The fungi were grown on the Czapek medium and actinomycetes on nutrient agar [10]. The antibiotic activity of strains was evaluated in terms of the size of the zone where growth of the test culture was suppressed. Each experiment was replicated three times.

The structure of rhizospheric actinomycete complexes was characterized in terms of such ecological parameters as the occurrence rate, the dominance rate, and the relative abundance of species and genera in the entire complex [2]. The diversity of actinomycetes was characterized by the Shannon index H [11]. The antibiotic activity of actinomycete complexes was characterized by the occurrence rate of species antagonistic to individual test cultures, the occurrence rate of wide-spectrum antagonistic species (the number of sensitive test cultures being greater than three), and the population share of antagonists with high antibiotic activity (the lysis zone exceeding 20 mm).

The statistical analysis of the data was conducted by standard methods of variance and cluster analysis using STATGRAPHICS software.

RESULTS AND DISCUSSION

The total number of actinomycetes in the rhizosphere of winter rye was different for different rye varieties and changed in the range $(1.2 \pm 0.26) \times 10^6$ to $(2.3 \pm 0.25) \times 10^6$ colony forming units (CFU) in 1 g of substrate, indicating their high density in the plant rhizosphere, which is a fairly peculiar habitat in terms of quality and availability of nutrient substrates, its humidity and microaerophilic conditions, and the content of mineral compounds. The complexes obtained on the medium with sodium propionate most often contained species of the genera *Streptomyces*, *Micromonospora*, and *Streptosporangium*. The occurrence rate of other uncommon actinomycete genera was also quite high (60–100%).

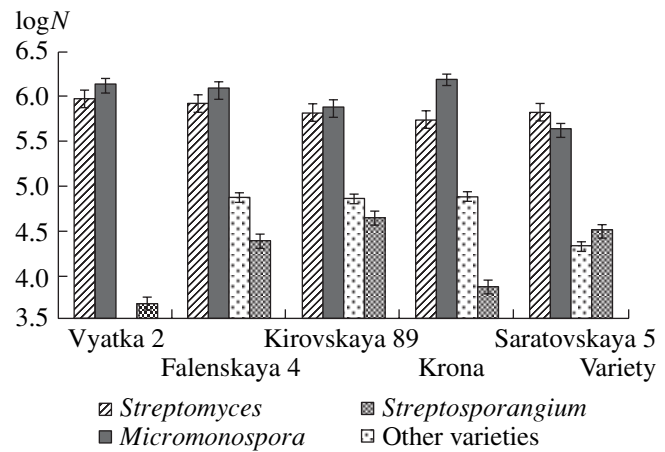


Fig. 1. Abundance (CFU/g, logN) of representatives of different actinomycete genera in winter rye rhizosphere on soddy podzolic soil. The confidence limits for the mean are given for $P = 0.99$.

The number of micromonosporas in the rhizosphere of winter rye turned out to be comparable with that of streptomycetes, ranging from hundreds of thousands to millions of CFU/g (Fig. 1). The population density of *Micromonospora* representatives in winter rye roots varied widely ($P > 0.99$) depending on the rye variety. The largest difference in the degree of root colonization by micromonosporas was observed with two rye varieties—Saratovskaya 5 ($(0.44 \pm 0.22) \times 10^6$ CFU/g) and Krona ($(1.55 \pm 0.21) \times 10^6$ CFU/g)—that originate from substantially different ecological and geographical regions. The average relative abundance of micromonosporas in the winter rye rhizosphere complex estimated over all varieties exceeded that of streptomycetes (Fig. 2) and varied from 38% in the rhizosphere of Saratovskaya 5 to 70% in the rhizosphere of Krona. By contrast, the relative content of streptomycetes was highest in the rhizosphere of Saratovskaya 2 and lowest in complexes of winter rye Krona.

Micromonosporas differ in their ecological characteristics from ubiquitous streptomycetes. Micromonosporas belong to hydrophilic microaerophiles and have more requirements than streptomycetes for vitamins and additional nutrients in the medium. The noted inter-varietal distinctions in root populations of micromonosporas are obviously associated with dissimilar root exudation patterns in genotypically different plants. It is well known that species and varieties of cereal crops can significantly differ in the content of vitamins [12] and organic acids and sugars [3, 5] in their exudates.

The affinity of micromonosporas to vegetal substrates (moss) and residues (e.g., leaf litter and peat) noted in the literature [2, 13] is explained by the fact that certain species of micromonosporas that show good growth on xylose and arabinose are able to degrade pentosans of plant origin [14]. Significant amounts of pentosans present in cell walls of winter rye

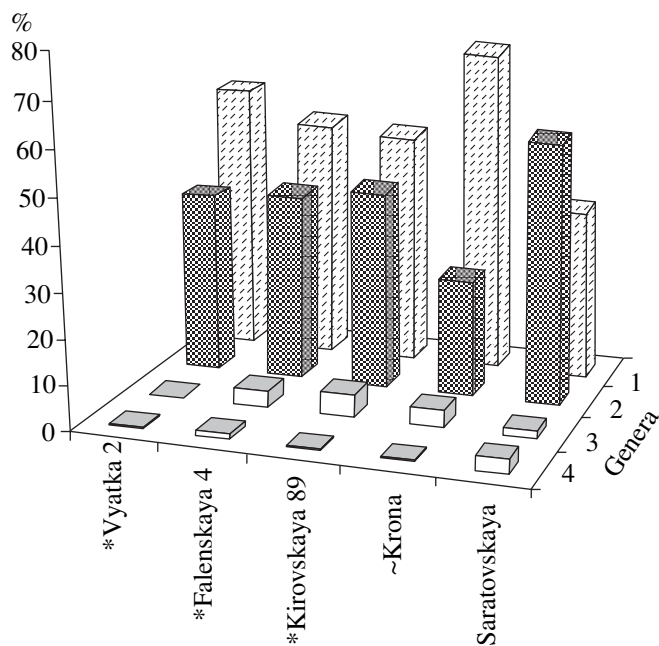


Fig. 2. Relative abundance (%) of representatives of actinomycete genera (1) *Micromonospora*, (2) *Streptomyces*, (3) *Streptosporangium*, and (4) other genera in the rhizosphere complex of winter rye grown on soddy podzolic soil. Names of variants with abundance difference not significant at the confidence level of $P = 0.95$ are prefixed with the same symbol.

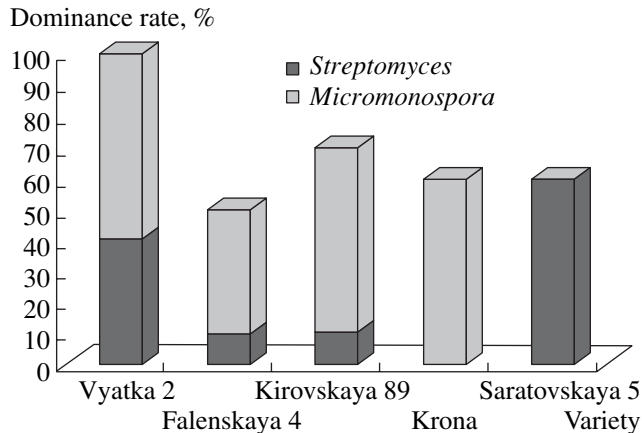


Fig. 3. Dominance rate (%) of representatives of the genera *Micromonospora* and *Streptomyces* in actinomycete complexes of different winter rye varieties.

and characterized by high water-absorbing capacity [15] can be one of the reasons for abundant development, in the rhizosphere complexes of winter rye, of hydrophilic micromonosporous forms which, unlike streptomycetes, produce hydrophilic spores.

Having estimated in this study the prevalence frequency of different organisms, we were able to document the dominant role of micromonosporas in actino-

mycete complexes of winter rye varieties selected under similar bioclimatic conditions of the nonchernozem belt of Russia (Fig. 3). Representatives of *Micromonospora* failed to dominate in the rhizosphere complex of only one rye variety—Saratovskaya 5—selected under qualitatively different environmental conditions of the lower Volga region. The predominant position (60%) in this case belonged to streptomycetes.

The varieties of winter rye differed significantly ($P > 0.99$) in terms of abundance of streptomycetes in their rhizosphere and, in order of decreasing abundance, estimated on CGA medium, is arranged as follows: Kirovskaya 89 > Krona > Vyatka 2 > Saratovskaya 5 > Falenskaya 4.

Considerable distinctions between varieties were documented as regards their occurrence rate and the diversity in their rhizosphere of individual species of the genus *Streptomyces* (Fig. 4). The common dominants in the rhizosphere of all varieties studied were species of the sections and series Cinereus Achromogenes and Albus Albus. However, the dominants in the rhizosphere of the rye variety Falenskaya 4 also included species of the section Imperfectus and the dominants in the rhizosphere of Vyatka 2 included *C. Chromogenes* and Imperfectus. The spectrum of dominants in the rhizosphere of the rye varieties Kirovskaya 89 and Krona also comprised representatives of the series A. Albocoloratus, Helvolo-flavus Helvolus, and Roseus Lavendulae-roseus. The variety Saratovskaya 5 had the most diverse spectrum of dominants, which included species of the sections and series C. Achromogenes, A. Albus, C. Chromogenes, A. Albocoloratus, Roseus Lavendulae-roseus, and Imperfectus. We see, therefore, that the diversity of streptomycetes was highest ($H = 1.94$) in the rhizosphere of Saratovskaya 5 despite their relatively low total abundance. The varieties Vyatka 2 ($H = 1.30$) and Falenskaya 4 ($H = 1.31$) were far behind in this respect, whereas Krona ($H = 1.71$) and Kirovskaya 89 ($H = 1.83$) occupied midrange positions on this scale.

In addition to species of the genera *Streptomyces* and *Micromonospora*, high occurrence in the complex of mycelial prokaryotes in winter rye rhizosphere was shown by representatives of the genus *Streptosporangium*. Favorable conditions for the development of *Streptosporangium* actinomycetes are low pH and high humidity in the environment and low humus content in soil [13]. Streptosporangia were previously found to constitute an integral part of actinomycete complexes in forest and steppe belt soils, occurring more frequently and in greater numbers in northern than in southern soils [2]. Streptosporangia were almost always present in the rhizosphere of winter rye grown on soddy podzolic soil at a density of tens of thousands of CFU per gram substrate, but their relative abundance in the complex did not exceed 2–5% (Fig. 2). The only exception was the rhizosphere of winter rye Vyatka 2, where actinomycetes of the genus *Streptosporangium*

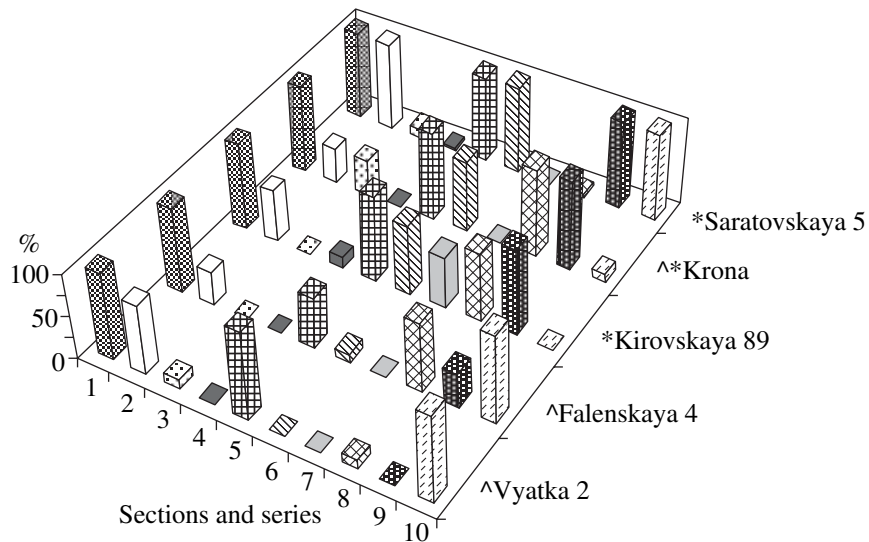


Fig. 4. Occurrence rate of different representatives of streptomycetes in the rhizosphere of winter rye varieties studied: (1) *Cinereus Achromogenes*; (2) *C. Chromogenes*; (3) *C. Aureus*; (4) *C. Violaceus*; (5) *Albus Albus*; (6) *A. Albocoloratus*; (7) *Helvolo-flavus Flavus*; (8) *Helvolo-flavus Helvolus*; (9) *Roseus Lavendula-roseus*; and (10) *Imperfectus*. Names of variants with insignificant difference at the confidence level of $P = 0.95$ are prefixed with the same symbol.

were not found. Like representatives of other rare genera, streptosporangia were never among prevalent organisms in actinomycete complexes of any winter rye variety studied and for this reason were assigned to the group of typical and frequently occurring forms.

Actinomycetes can affect plants in many different ways and one of these is by synthesizing various biologically active compounds and, most importantly, antibiotics. Many actinomycetes and, above all, representatives of the genera *Streptomyces* and *Micromonospora* are known to have strong antagonistic properties towards widely different microorganisms [9]. It is clear that the abundance and wide distribution of these actinomycetes in such a competitive habitat as the rhizosphere arise from both their ability to produce antibiotics and their antibiotic resistance.

The occurrence rate of actinomycetes isolated from the rhizosphere complex of winter rye with antagonistic properties in relation to microscopic fungi was found to be 66%. Thirty-six percent of antibiotically active isolates exhibited high antifungal activity (the diameter of the growth suppression zone exceeding 20 mm). The typical trait of the winter rye actinomycete complex is a high occurrence rate (up to 88%) of narrow-spectrum antagonists.

The highest susceptibility to actinomycetes isolated from the rhizosphere was shown by *Fusarium sporotrichiella* K-8999-k, a fungus that is part of a pathogenic complex known to affect winter rye crops in the Kirov region [16]. Antifungal activity towards this organism was found in 48.5% of isolated actinomycetes. The results of identification of some antibiotically active strains of streptomycetes are given in Table 1. The highest tolerance to antifungal metabolites produced by act-

inomycetes was shown by *Saccharomyces cerevisiae*, a yeast culture that does not have a common habitat with actinomycetes.

The obtained data point to a fairly high antifungal potential of the actinomycete complex in the rhizosphere, and this factor can be important not only for self-defense of winter rye plants against fungal phytopathogens but also in having a healing effect on the entire microbial complex of soil. The phytosanitary effect exerted by the actinomycete complex under crop rotation is well known in plant growing and can be explained by its ability to readily form associations with fluorescent bacteria from the genus *Pseudomonas* and fungi from the genera *Trichoderma* and *Gliocladium* [17], which are active antagonists to phytopathogens. *Micromonosporas* and streptomycetes, which occur widely in the rhizosphere of winter rye, can also play a significant role in controlling the growth of pathogenic fungi.

The actinomycete complexes of different winter rye varieties studied varied in their spectrum of antifungal action and the occurrence frequency of species-antagonists in the rhizosphere (Fig. 5). The widest spectrum of antifungal action was found in the complex of the Vyatka 2 variety. The antifungal activities of actinomycetes associated with roots of the Kirovskaya 89, Krona, and Saratovskaya 5 varieties exhibited antifungal activity against a smaller number of test cultures, and, in the complex of the Falenskaya 4 variety, antagonists to only two fungal cultures were detected and these occurred at a fairly low rate.

The actinomycete complexes of the winter rye varieties studied also differed in other indices of antibiotic activity. For example, the relative abundance of all iso-

Table 1. Active antagonistic strains of actinomycetes identified in the rhizosphere of winter rye on soddy podzolic soil

Work designation	Isolated from rhizosphere of the variety	Section and series	Species	Antibiotics produced [8]
2-V-4	Vyatka 2	Helvolo-Flavus Helvolus	<i>S. globisporus</i>	Actinoxanthine and several unidentified antibiotics
3-K-4	Kirovskaya 89	Roseus Lavendula-Roseus	<i>S. cirratus</i>	Cirramycin (macrolide)
4-S-1	Saratovskaya 5	C. Achromogenes	<i>S. wedmorensis</i>	Phosphonomycin, antibiotic 280
1-S-1	Saratovskaya 5	C. Achromogenes	<i>S. omiyaensis</i>	Chloramphenicol
1-Kr-4	Krona	Helvolo-Flavus Helvolus	<i>S. globisporus</i>	Actinoxanthine and several unidentified antibiotics

lates with the antibiosis capacity increased from 30% in the rhizosphere of the Falenskaya 4 variety to 90% in the rhizosphere complex of Vyatka 2. By contrast, the relative abundance of severely antifungal antagonists fell from 67% in the complex of Falenskaya 4 winter rye to 11% in the actinomycete complex of the Vyatka 2 variety (Table 2). The actinomycete complexes of Kirovskaya 89 and Krona proved to be similar in this respect (33 and 38%, respectively) and, unlike complexes of other varieties, included strains with a wide spectrum of antimicrobial activities, i.e., to as many as four or five test cultures. The actinomycete antagonists from the rhizosphere of Saratovskaya 5 winter rye in more than half of the cases were antagonistic to just one fungal test culture, but most of them (57%) showed high antifungal activity.

The intervarietal dissimilarity of actinomycete complexes in the rhizosphere of winter rye was qualitatively gauged using the obtained numerical characteristics. The distance D in the multidimensional space of taxo-

nomical and functional (antibiosis) features computed by means of cluster analysis between complexes of the Kirovskaya 89 and Krona varieties was smaller ($D = 33.3$) than the distance between complexes of Vyatka 2 and Falenskaya 4 ($D = 71.2$). The largest distance ($D = 172.0$) was observed between the Saratovskaya 5 variety, selected in the chernozem part of Russia, and other winter rye varieties that originate from relatively close geographic regions and are adapted to the conditions prevailing in zonal soddy podzolic soils (Fig. 6).

The results of this study demonstrate that the winter rye rhizosphere is predominantly inhabited by micromonosporas occurring in numbers comparable to those of streptomycetes. Another interesting feature of the population structure of the rhizosphere complex of winter rye is the high occurrence rate of streptosporangia (up to 100%). A higher abundance and occurrence rate of streptosporangial actinomycetes in the rhizosphere of winter rye as compared to the rhizosphere of

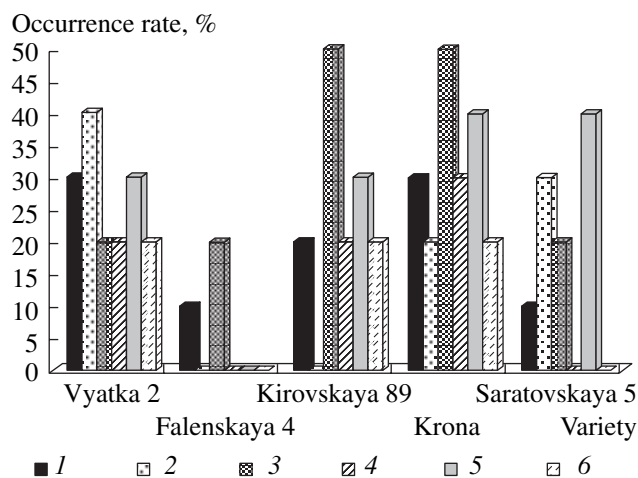


Fig. 5. Antagonistic effect of actinomycetes from rhizosphere of different winter rye varieties on (1) *Fusarium avenaceum* 7/2, (2) *Fusarium avenaceum* 10/2, (3) *Fusarium sporotrichiella* K-8999-k, (4) *Penicillium* sp., (5) *Trichoderma viride*, and (6) *Saccharomyces cerevisiae*. The occurrence rate relates to actinomycetes suppressing fungal growth in a zone larger than 12 mm.

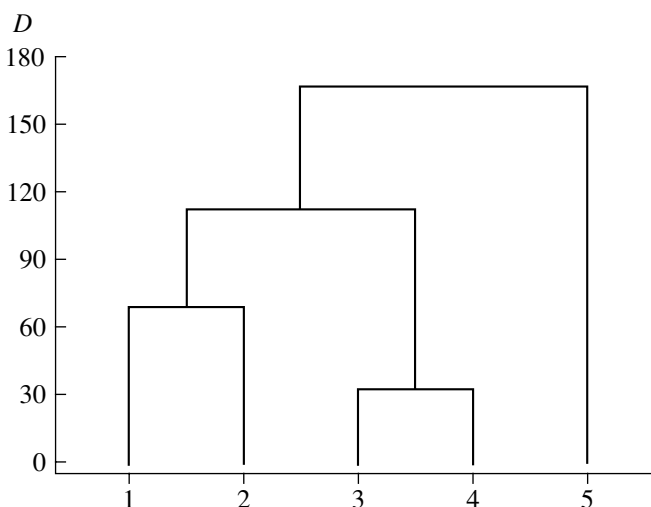


Fig. 6. Similarity dendrogram for actinomycete complexes in the rhizosphere of different winter rye varieties: (1) Vyatka 2; (2) Falenskaya 4; (3) Kirovskaya 89; (4) Krona; and (5) Saratovskaya 5.

Table 2. Indices of antagonistic activities of actinomycetes from the rhizosphere of different winter rye varieties

Variety	Rate of occurrence in the complex		Relative abundance in the complex of species with high antagonistic activity (the lysis zone larger than 20 mm), %
	Antagonist species, %	Those with wide-spectrum action	
Vyatka 2	90	0	11
Falenskaya 4	30	0	67
Kirovskaya 89	60	20	33
Krona	80	20	38
Saratovskaya 5	70	0	57

spring barley [7] can be due to the efficient hydrolytic degradation of larger quantities of plant residues formed by winter crops as compared to spring crops.

By employing quantitative ecological parameters in our study, we were able to identify intervarietal distinctions in the structure of actinomycete complexes of winter rye. Individual varieties were found to differ in terms of abundance, composition of dominants, and the number of species of the genus *Streptomyces* represented in their rhizosphere. The actinomycete complex of Vyatka 2 stood out among other varieties by virtue of having a wider spectrum of antifungal activity and a higher occurrence rate of species-antagonists. In the field, this variety is characterized by high lability and good resistance to most harmful fungal diseases [16].

The obtained results provide sufficient grounds to believe that the genotype of winter rye, which determines the specific composition of its root exudates and the root deposit as a whole, exerts selective influence on the formation of actinomycete complexes associated with plant roots.

REFERENCES

- Kalakutskii, L.V. and Sharaya, L.S., Actinomycetes and Higher Plants, *Usp. Mikrobiol.*, 1990, vol. 24, pp. 26–65.
- Zvyagintsev, D.G. and Zenova, G.M., *Ekologiya aktinomitsetov* (The Ecology of Actinomycetes), Moscow: GEOS, 2001.
- Kravchenko, L.V., Azarova, T.S., and Dostanko, O.Yu., The Effect of the Root Exometabolites of Plants with Different Polyploidy of Their Genomes on the Growth of *Azospirillum brasilense*, *Mikrobiologiya*, 1993, vol. 62, no. 3, pp. 524–529.
- Granssee, A. and Wittenmayer, L., Qualitative and Quantitative Analysis of Water-Soluble Root Exudates in Relation to Plant Species and Development, *Plant Nutr. Soil Sci.*, 2000, vol. 163, no. 4, pp. 381–385.
- Shtark, O.Yu., The Effect of the Wheat Root Exometabolites on the Antagonism of Rhizobacteria with Respect to Phytopathogenic Fungi, *Cand. Sci. (Biol.) Dissertation*, St. Petersburg: VNIISKhM, 2001.
- Zenova, G.M., *Pochvennye aktinomitsety redkikh rodov* (Soil Actinomycetes of Rare Genera), Moscow: Mosk. Gos. Univ., 2000.
- Shirokikh, I.G., Zenova, G.M., and Zvyagintsev, D.G., Actinomycetes in the Rhizosphere of Barley Grown on Acid Soddy Podzolic Soil, *Mikrobiologiya*, 2002, vol. 71, no. 4, pp. 533–537.
- Gauze, G.F., Preobrazhenskaya, T.P., Sveshnikova, M.A., et al., *Opredelitel' Aktinomitsetov* (A Handbook of Actinomycetes), Moscow: Nauka, 1983.
- Egorov, N.S., *Osnovy ucheniya ob antibiotikakh* (Fundamentals of Antibiotic Science), Moscow: Vysshaya shkola, 1979.
- Zenova, G.M., *Pochvennye aktinomitsety* (Soil Actinomycetes), Moscow: Mosk. Gos. Univ., 1992.
- Magurran, A.E., Ecological Diversity and Its Measurement, 1988. Translated under the title *Ekologicheskoe raznoobrazie i ego izmerenie*, Moscow: Mir, 1992.
- Puzik, V.K., The Species and Variety Specificity of the Root Exudates of Cereals with Respect to the Content of Group V Vitamins and Inositol, *Fiziol. Biokhim. Kult. Rast.*, 2002, vol. 34, no. 4, pp. 332–336.
- Zenova, G.M. and Zvyagintsev, D.G., *Raznoobrazie aktinomitsetov v nazemnykh ekosistemakh* (Diversity of Actinomycetes in Terrestrial Ecosystems), Moscow: Mosk. Gos. Univ., 2002.
- The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd ed., Balows, A. et al., Eds., New York: Springer, 1991, vol. 1, p. 1175.
- Ismagilov, R.R., Ayupov, D.S., Vanyushina, T.N., and Ismagilov, R.R., Pentosanes in Winter Rye Grain, in *Ozimaya Rozh': Seleksiya, Semenovodstvo, Tekhnologii, i Pererabotka*, Proc. Int. Conf., Kirov: NIISKh, 2003, pp. 137–139.
- Sheshegova, T.K., The Species Composition and Pathogenicity of Fungi from the Genus *Fusarium* on Winter Rye Crops in the Kirov Region, *Mikol. Fitopatol.*, 2003, vol. 37, no. 2, pp. 85–89.
- Pieta, D., Pastucha, A., and Patkowska, E., The Role of the Organic Substance in the Formation of Communities of Microorganisms, *Ann. Agric. Sci., Ser. Plant Protect.*, 1999, vol. 28, nos. 1/2, pp. 81–92.