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**EXPERIMENTAL ARTICLES**

# Actinomycetes of the Genus *Micromonospora* in Meadow Ecosystems

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**Abstract**—Investigations showed that micromonosporas, along with streptomycetes, are the major inhabitants of floodplain meadow ecosystems, where their population varies from tens of thousands to hundreds of thousands of CFU per g substrate. In spring, the population of micromonosporas in soil and on the plant roots was found to be denser than that of streptomycetes.

*Key words*: micromonosporas, streptomycetes, actinomycete complex, biogeocenosis, ecosystem.

Actinomycetes of the genus *Micromonospora* produce many antibiotics, including aminoglycosides, macrolides, maquarimicides, and anticancer antibiotics, as well as enzymes and other physiologically active substances. Like streptomycetes, micromonosporas synthesize hydrolases, due to which they are able to degrade cellulose, chitin, pentosans, and other recalcitrant organic substances [1].

The ecology of micromonosporas is still poorly studied. Although they were first isolated from soil in 1932 [2], micromonosporas have long been believed to be predominantly aquatic organisms.

This work was aimed at studying the distribution of micromonosporas in the floodplain meadow biogeocenoses of the southern taiga subzone of Russia.

## MATERIALS AND METHODS

Samples of plant substrates, soil, and river mud were taken from the meadow ecosystems of the Protva River floodplain in the Borovsk region, Kaluga oblast. Some characteristics of these ecosystems are summarized in the table. The ecosystems under study are situated on the right bank of the Protva River and represent either low floodplain meadows, which are flooded every year, or high meadows on the river bank slope, which are flooded on the average every six years. The vegetative period in the Borovsk region lasts 120–140 days. The hydrologic and temperature conditions in this region, with distinct seasonal changes in the soil moisture content, are favorable for biochemical processes in the soils [3].

The alluvial meadow soils under study had the following profiles: Ad (0–3 cm), A (3–29 cm), B1 (29–52 cm), BC (52–101 cm) in the low floodplain and AKd (0–3 cm), A1 (3–25 cm), B1 (25–87 cm), BC (87–105 cm) in the high floodplain.

To isolate actinomycetes, suspensions prepared from the samples of soil and plant substrates were plated onto selective agar media with sodium propionate [4]. The media were supplemented with 1 μg/ml

Some characteristics of the biocenoses under study

| Biocenosis | Soil or substrate | Substrates and horizons |
|---|---|---|
| Grass–forb–legume floodplain meadow | Low alluvial floodplain meadow | Plants (leaves and stalks of grasses; flowers of forbs; leaves, stalks, and roots of legumes) and soil horizons Ad, A1, B1, and BC |
| Grass–forb floodplain meadow | High alluvial floodplain meadow | Plants (leaves, stalks, and roots of grasses and flowers, leaves, stalks, and roots of forbs) and soil horizons Ad, A1, B1, and BC |
| Aquatic ecosystem | River mud | Leaves, stalks, and roots of aquatic plants and river mud |

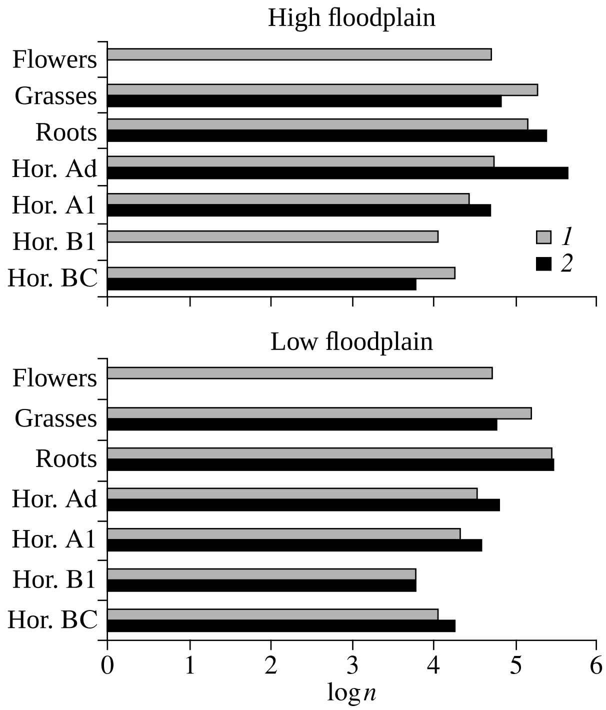


**Fig. 1.** Vertical distribution of the actinomycete genera (*1*) *Streptomyces* and (*2*) *Micromonospora* in the floodplain meadow biogeocenoses in spring.

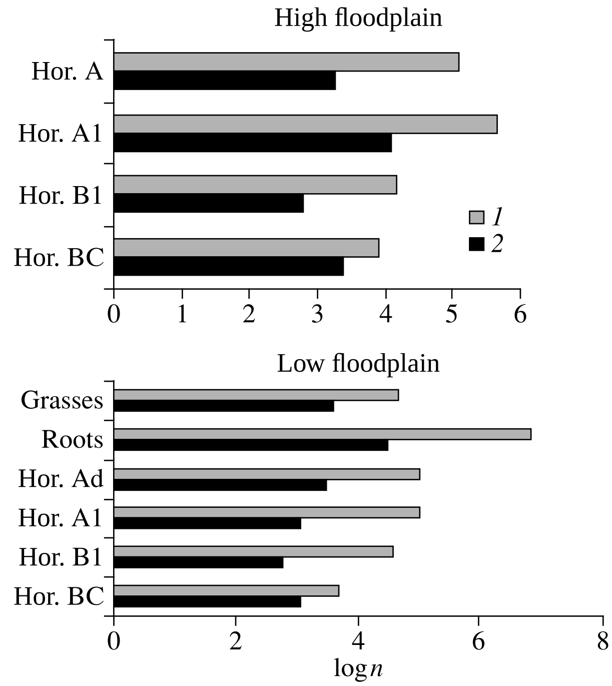


**Fig. 2.** Vertical distribution of the actinomycete genera (*1*) *Streptomyces* and (*2*) *Micromonospora* in the floodplain meadow biogeocenoses in autumn.

nalidixic acid to inhibit the growth of creeping bacteria and 50 μg/ml nystatin to inhibit the growth of fungi [4]. The suspensions were heated at 70°C for 10 min. The inoculated plates were incubated at 28–30°C for 3 weeks.

To detect representatives of different actinomycete genera, the incubated plates were examined under an optical microscope at a magnification of ×400 for the presence of aerial and substrate mycelia and sporangia, as well as for the type of sporophores and the arrangement of spores (single, paired, or chains) on the aerial and/or substrate mycelium. Colonies belonging to each morphotype were enumerated, and each morphotype was isolated in a pure culture. Actinomycetes were isolated and cultivated using oat agar or Gauze 1 medium [5]. The isolates were identified on the basis of their morphological and chemotaxonomic characteristics. Morphological characteristics were determined by growing actinomycetes in the form of a groove [6] or on microscope slides placed in a humid chamber [7]. Chemotaxonomic characteristics (the type of the cell wall and the type of differentiating sugars) were determined chromatographically [8].

The isolated actinomycetes were preliminarily identified to a generic level using the identification criteria of Bergey's Manual [7, 9]. Representatives of the genus *Micromonospora* were identified based on the definitive classification schemes presented in Bergey's Manual and that described by Bibikova and Ivanitskaya [7, 9, 10].

Numerical ecological indices (the relative occurrence rate and the relative frequency of domination) were determined as the ratio of the number of samples in which a given genus was encountered to the total number of the samples analyzed (the relative occurrence rate) and as the ratio of the number of samples in which the representatives of a given genus comprised more than 50% of the representatives of all the detected genera to the total number of the samples analyzed (the relative frequency of domination).

The ecological indices of micromonosporas were compared with those of the best studied soil streptomycetes.

## RESULTS AND DISCUSSION

The population of micromonosporas in the alluvial floodplain meadow biogeocenoses varied from tens of thousands to hundreds of thousands of CFU per g substrate and was comparable with that of soil streptomycetes. In spring, the population of micromonosporas in the upper soil horizons and on the plant roots was denser than that of streptomycetes. In the overground horizon, micromonosporas were detected only on grasses, where their population was lower than that of streptomycetes (Fig. 1). The number of micromonosporas in the low and high floodplain biogeocenoses virtu-

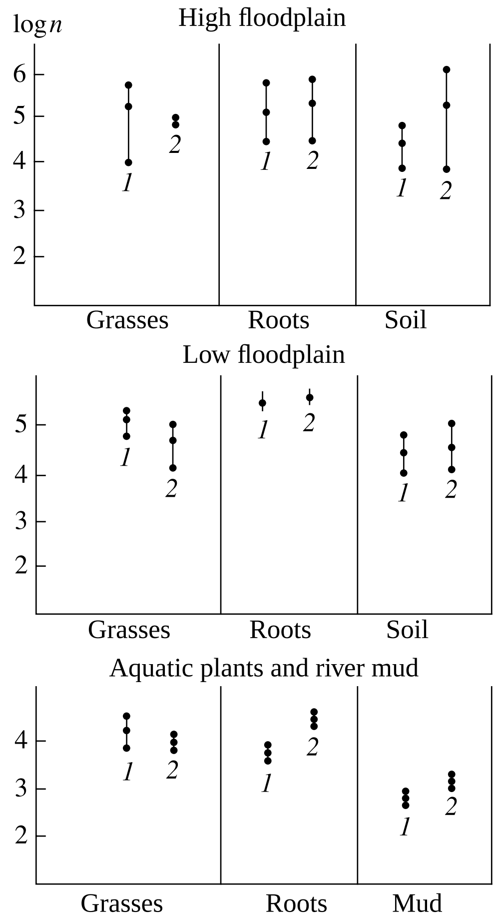


**Fig. 3.** The mean values and the variation limits of the population density of the actinomycete genera (*1*) *Streptomyces* and (*2*) *Micromonospora* in the Protva River floodplain meadow biogeocenoses and in the river ecosystem in spring.

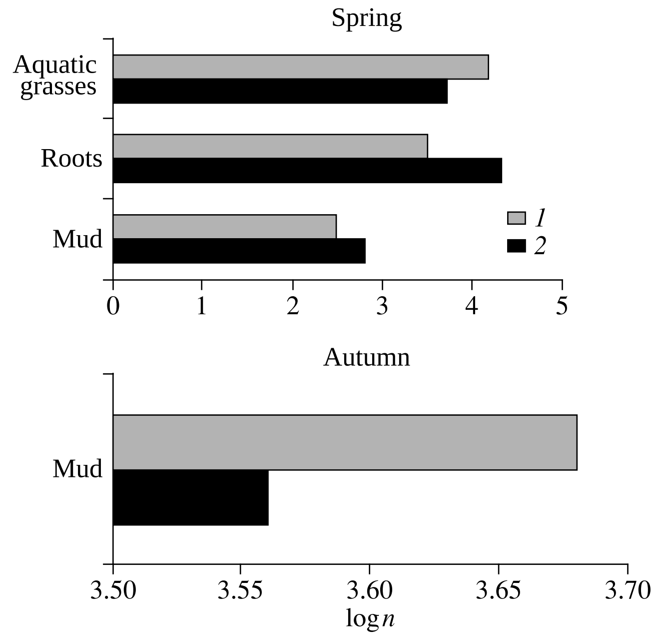


**Fig. 4.** Distribution of the actinomycete genera (*1*) *Streptomyces* and (*2*) *Micromonospora* in the Protva River ecosystem in spring and autumn.

ally did not differ, except that the number of micromonosporas in the soil horizon Ad in the high floodplain turned out to be an order of magnitude greater than in the same horizon of the low floodplain.

By autumn, the number of micromonosporas in the floodplain meadow biogeocenoses decreased to make up tens of thousands of CFU/g substrate (Fig. 2), whereas the number of soil streptomycetes did not decrease, so that the number of micromonosporas in all horizons of the biogeocenoses turned out to be 1 to 1.5 order of magnitude smaller than the number of streptomycetes.

The variation in the micromonospora population was maximal in the soil horizons of meadow biogeocenoses (Fig. 3). The maximum, average, and minimum values of the micromonospora population of soils and plant roots were greater than those of soil streptomycetes in both the low and high floodplain biogeocenoses. It is obvious that the alluvial floodplain meadow soils, which are subject to regular flooding and hence are enriched in the river mud sediments, favor the growth of micromonosporas, since they (and their spores) are hydrophilic [11] and are more nutritionally demanding than streptomycetes [1].

In the river mud and on the leaves, stalks, and roots of aquatic plants, the number of micromonosporas varied from thousands to tens of thousands of CFU/g substrate. In spring, the population of micromonosporas in these habitats was denser than that of streptomycetes (Fig. 4). By autumn, the populations of micromonosporas and streptomycetes in the mud increased concurrently.

An analysis of the ecological indices of actinomycetes showed that micromonosporas frequently occur in all horizons of the floodplain meadow biogeocenoses and in the river ecosystem (Fig. 5). The relative occurrence rate of micromonosporas in the soil horizons and on the plant roots was as high as 100%. On the grasses of the floodplain meadows, the occurrence rate of streptomycetes was higher than that of micromonosporas.

Micromonosporas dominated actinomycete complexes in the soils and on the plant roots in the floodplain meadow biogeocenoses and in the river mud and on the aquatic plant roots (Fig. 6).

A comparative ecological analysis of micromonospora populations in the floodplain meadow, forest, and steppe biogeocenoses [11] showed that the content of micromonosporas in the soils of floodplain meadows is relatively high, which may be due to the favorable

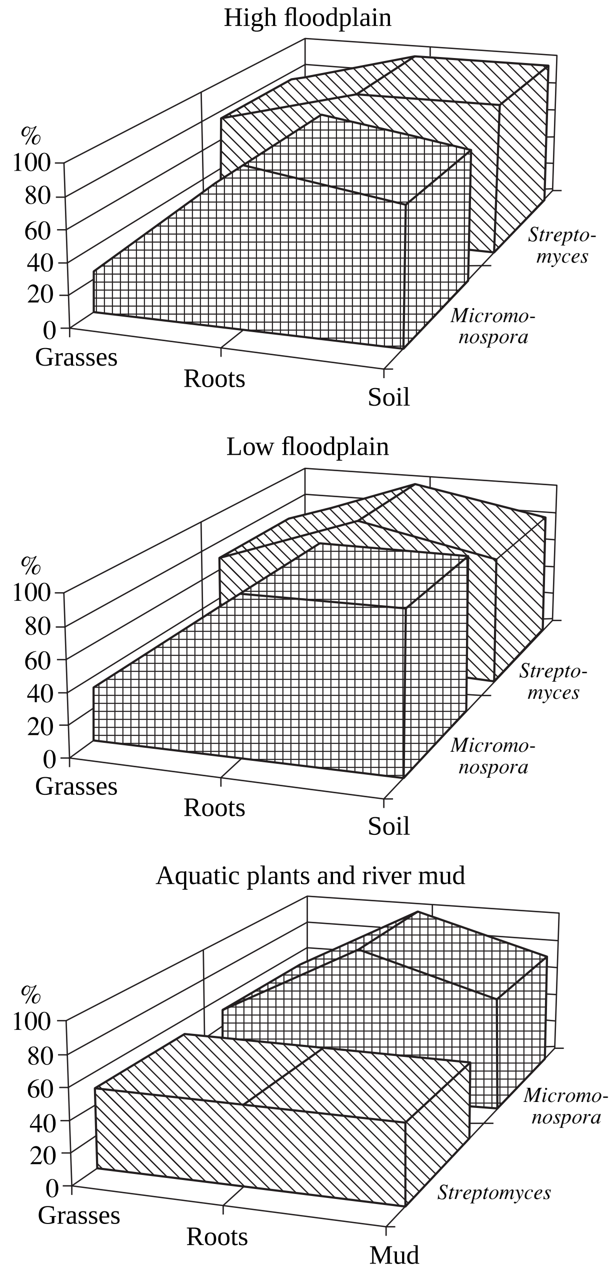


**Fig. 5.** The relative occurrence rates of the actinomycete genera *Streptomyces* and *Micromonospora* in the floodplain meadow biogeocenoses and in the river ecosystem.

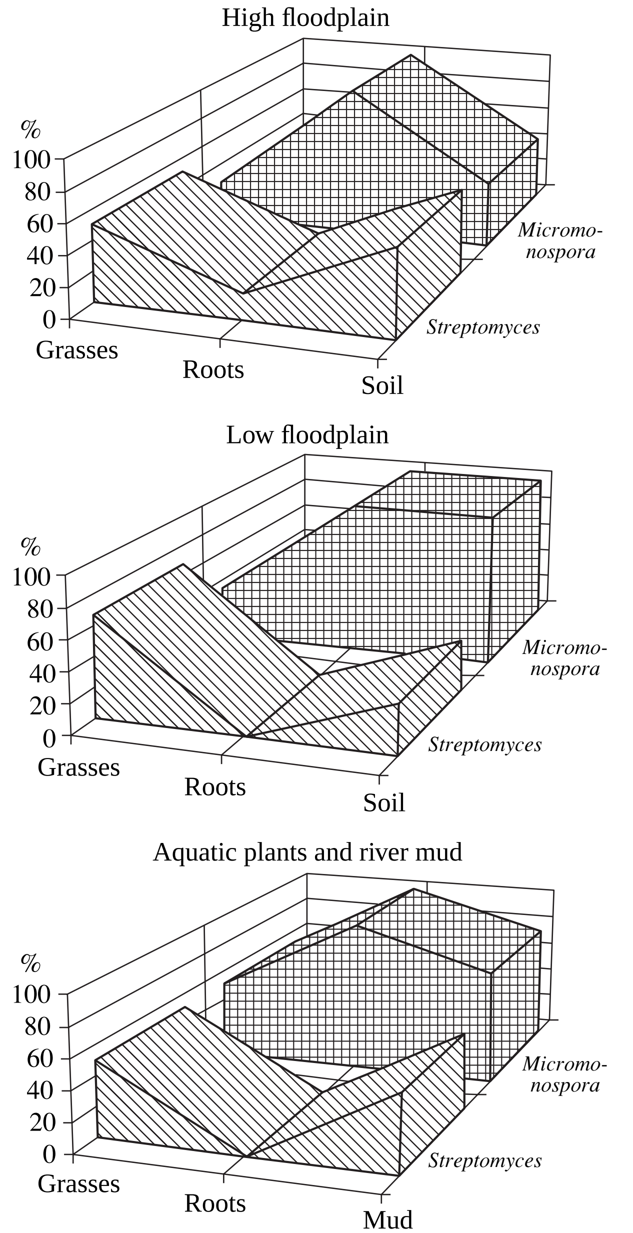


**Fig. 6.** The relative domination frequency of the actinomycete genera *Streptomyces* and *Micromonospora* in the floodplain meadow biogeocenoses and in the river ecosystem.

hydrological and nutritional conditions of floodplains, characterized by regular flooding and high waters, the deposition of river sediments and the formation of the floodplain warp, which, when mixed with rotting plant debris, is very fertile [12]. In the floodplain ecosystems, soil-forming processes occur at the boundary between oxic and anoxic environments. As a result, the floodplain meadow biogeocenoses and river ecosystems are dominated by micromonosporas, which are hydrophilic microaerophiles, nutritionally more demanding than streptomycetes.

The floodplain meadow biogeocenoses are dominated by the micromonospora subgroups *Aurantiaca* and *Cinnamomea*, which seem to be promising as potential producers of antibiotics.

## ACKNOWLEDGMENTS

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