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SOIL BIOLOGY

Composition of Bacterial Communities of the Main Types of Bog Plants

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Abstract—Direct and inoculation methods indicated high concentrations of bacteria on the following plants: *Carex nigra*, *Eriophorum vaginatum*, *Drosera rotundifolia*, and *Ledum palustre*. The correlation between bacterial abundance, species, and organs of the studied plants was revealed. Proteobacteria were shown to predominate in the phyllosphere, while bacilli and actinomycetes prevailed in rhizoplane and rhizosphere. Members of the genera *Pseudomonas* and *Erwinia* predominated in the phyllosphere. High values of the nitrogen-fixing activity of bacterial populations in the rhizosphere were revealed, which reached a maximum in the rhizosphere under sundew.

Keywords: bog, plants, bacteria, abundance, taxonomic composition, nitrogenase activity

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INTRODUCTION

A large number of both Russian and foreign studies are devoted to microbial communities of sphagnum moss and high-bog peat [1, 3, 11, 17, 20]. However, the microbial composition of herbaceous plants and shrubs growing in high bogs remains insufficiently studied. Such research is essential, since the functions of these plants are very important for the maintenance and functioning of wetland ecosystems. Herbaceous plants and heather shrubs were shown to significantly contribute to the development and maintenance of the microrelief. In particular, they contribute to the formation of hummocks with stable temperature and humidity, which favorable for the development of sphagnum [18]. Moreover, the presence of *Carex rostrata* and *Scheuchzeria palustris* in bog communities intensified emission of methane into the atmosphere. During the daytime, 52–60% of methane emitted from the bog surface is transported via these plants [9]. The taxonomic composition of epiphytic bacterial communities has been studied only on sundew. Molecular biological methods have revealed 63 bacterial morphotypes. Among them, 34 *nifH* genes, which indicate the nitrogen-fixing activity of members of numerous genera isolated from phyllosphere and rhizosphere of sundew have been identified [14].

The goal of the present study was to determine the abundance of epiphytic and saprotrophic bacterial communities of sedge, cottongrass, sundew, and wild rosemary, as well as to study their taxonomic composition and nitrogen-fixing activity.

MATERIALS AND METHODS

Samples of representatives of herbaceous plants were collected from a shrub–cottongrass–sphagnum pine forest (Tver oblast, West Dvina forest-bog station of the Institute of Forest Science, Russian Academy of Sciences) at the end of May 2016. These were black sedge (*Carex nigra* (L.) Reichard), tussock cottongrass (*Eriophorum vaginatum* L.), round-leaved sundew (*Drosera rotundifolia* L.), and wild rosemary (*Ledum palustre* L.). The latter is a representative of heather shrubs. Their leaves, stems, branches (phyllosphere), root surface (rhizoplane), and rhizosphere under the plants were analyzed.

Bacterial abundance was determined by the direct method using luminescent microscopy [8]. Bacterial cells were preliminarily desorbed for 2 min with a Bandelin Sonopuls HD 2070 ultrasonic disperser (Germany) at 50% power. Preparations were obtained according to the standard procedure, stained with an aqueous solution of acridine orange, and examined (120 fields of view per sample) under a LUMAM-IZ

Table 1. Bacterial abundance in microloci of bog plants

Plant	Microlocus	Bacterial abundance	
		direct method, cells/g	inoculation method, CFU/g
Black sedge	Phyllosphere	2.4×10^9	1.1×10^7
	Rhizoplane	4.2×10^9	1.0×10^5
	Rhizosphere	4.4×10^9	1.6×10^5
Tussock cottongrass	Phyllosphere	2.3×10^9	4.3×10^8
	Rhizoplane	2.2×10^9	18.6×10^5
	Rhizosphere	4.4×10^9	1.0×10^5
Round-leaved sundew	Phyllosphere	0.6×10^9	1.4×10^6
	Rhizoplane	0.9×10^9	2.1×10^5
	Rhizosphere	1.2×10^9	1.1×10^5
Wild rosemary	Phyllosphere	$(1.4–2.1) \times 10^9$	9.2×10^5
	Rhizoplane	4.2×10^9	10.3×10^5
	Rhizosphere	$(7.5–7.8) \times 10^9$	7.1×10^5

luminescent microscope (Russia). Bacterial abundance in 1 g of sample (N_B) was determined according to the formula $N_B = S_1an/vS_2c$, where S_1 is the area of the preparation (μm^2), a is the average number of bacteria per field of view, n is the dilution factor of the suspension (mL), v is the volume of the drop applied to the slide (mL), S_2 is the area of the microscope's field of view (μm^2), and c is the weighed sample (g).

The abundance and taxonomic composition of bacteria of the saprotrophic block were determined by plating on agar glucose–peptone–yeast medium. Nystatin (50 mg/0.5 L of medium) was added to inhibit fungi. For inoculation, the suspension remaining after preparations for luminescent microscopy was used. Plating was performed from the experimentally selected tenfold dilutions in five replicates. Incubation was carried out for 2–3 weeks at room temperature. The total number of bacteria was expressed in colony-forming units (CFU) per 1 g of sample. Differentiated counts of bacterial colonies of various taxonomic groups were performed. The main representatives of bacteria were isolated into pure cultures. Isolated strains were identified to the genus level based on morphological, cultural, and chemotaxonomic characteristics [7, 10]. Predominant taxa were determined by sequencing of nucleotide sequences of 16S rDNA gene using BLAST software [16].

To determine the **actual nitrogen fixation**, soil from the rhizosphere (2–3 g) was placed in 15 mL penicillin vials, hermetically covered with rubber stoppers with metal clips, supplemented with acetylene (1 mL), and incubated for 2 h at 25°C. Afterward, 1 mL of sample was collected from the vials with a syringe, and the amount of produced ethylene was measured with a Kristall-2000 chromatograph (Russia) with a flame ionization detector (column length, 1 m; diameter, 3 mm; filler, Porapak N 80/100). The temperature of the column was 60°C, the temperature of the detector was 160°C, and the temperature of the evaporator was 100°C. The flow rate of the carrier gas (N_2) was 50 mL/min, the flow rate of air was 280 mL/min, and the flow rate of hydrogen was 28 mL/min [8]. The number of replicates was from three to five. The nitrogen-fixing activity was expressed in ng C_2H_4/g h.

Glucose solution (1 mL) was preliminarily added to the sample (1% of soil weight) in order to determine the potential nitrogen fixation. Further steps were carried out as described above.

RESULTS AND DISCUSSION

The bacterial abundance determined by direct counts varied from 0.6 to 2.4 billion cells/g in the phyllosphere, from 1.2 to 4.2 billion cells/g in the plant rhizoplane, and from 0.9 to 7.8 billion cells/g in soil from the rhizosphere. Comparison of plant-associated microloci revealed a tendency to an increase in bacterial abundance in the rhizosphere. In all microloci, its maximum values were found for wild rosemary and minimum values, for sundew (Table 1).

Analysis of the abundance of the epiphytic-saprotrophic complex of plant bacteria made it possible to determine the maximum values for the density of bacteria in the phyllosphere: 10^6–10^8 CFU/g. In the rhizoplane and rhizosphere, the abundance of bacteria was one to three orders of magnitude lower. Plants should be placed in the following sequence in order of decreasing density of bacterial populations in the plant phyllosphere: cottongrass – sedge – sundew – wild rosemary (Table 1). In contrast to herbaceous plants,

Table 2. Taxonomic composition of bacterial communities in microloci of bog plants

Plant	Microlocus	Relative abundance of the group, %		
		proteobacteria	bacilli	actinomycetes
Black sedge	Phyllosphere	100	0	0
	Rhizoplane	0	53	47
	Rhizosphere	10	89	1
Tussock cottongrass	Phyllosphere	100	0	0
	Rhizoplane	100	0	0
	Rhizosphere	27	45	28
Round-leaved sundew	Phyllosphere	100	0	0
	Rhizoplane	88	12	0
	Rhizosphere	54	46	0
Wild rosemary	Phyllosphere	25	75	0
	Rhizoplane	12	69	19
	Rhizosphere	10	89	1

the abundance of bacteria was approximately the same ((7–10) × 10^5 CFU/g) in all microloci of wild rosemary. Thus, the number of bacteria of the epiphytic–saprotrophic complex was significantly higher in the phyllosphere of herbaceous plants than on wild rosemary (heather shrub). Their low density on wild rosemary leaves is probably related to the high content of essential oils, which inhibit the growth of microorganisms.

Gram-negative bacteria predominated in the composition of bacterial complexes of the phyllosphere and rhizoplane of herbaceous plants. Bacilli and actinomycetes predominated only on the surface of sedge roots. A different composition of bacterial complexes was revealed on wild rosemary: predominance of bacilli in all microloci (Table 2). Proteobacteria prevailed in sphagnum, while bacilli were subdominants; the share of actinomycetes did not exceed 10% [1].

Analysis of the prevailing cultures of proteobacteria isolated from the phyllosphere of sedge, cottongrass, and sundew carried out with the 16S rDNA method indicated that proteobacteria were represented both by aerobic bacteria of the genus *Pseudomonas* and facultatively anaerobic bacteria of the genus *Erwinia* in all herbaceous plants. Members of these genera are typical of the phyllosphere of both agricultural plants and weeds [4, 15, 19].

Table 3. Nitrogen-fixing (NF) activity of rhizosphere under studied plants

Plant	NF	NF with glucose
	ng C_2H_4/g substrate h	
Black sedge	0.098	0.235
Tussock cottongrass	0.286	0.302
Round-leaved sundew	0.318	0.297
Wild rosemary	0.227	0.423

The actual nitrogen-fixing activity of the bacterial communities in the rhizosphere of herbaceous plants varied in the range 0.098–0.318 ng C_2H_4/g substrate h, increasing in the following sequence: sedge–wild rose–cottongrass–sundew (Table 3). In general, the obtained actual nitrogen-fixation values were 1.5–3 times lower than those determined for the activity of molybdenum-dependent nitrogenase in the main soil types of European Russia [13]. However, nitrogen fixation in soils is known to have a high spatial variability in natural conditions [6, 12].

In the variants with the addition of glucose, the nitrogen-fixation activity in the rhizosphere under all plants increased considerably. A slight decrease in indices of potential nitrogen fixation was observed only under sundew.

CONCLUSIONS

The luminescent microscopy method indicated a high abundance of bacteria on bog plants, which reached billions of cells per gram of substrate. The maximum values were determined in the rhizosphere. The abundance of bacteria in the epiphytic–saprotrophic complex was higher in the phyllosphere. Analysis of the taxonomic composition of bacterial communities of bog plants revealed the prevalence of proteobacteria in the phyllosphere, among which members of the genera *Pseudomonas* and *Enterobacter* predominated, typical representatives of eccrisotrophs assimilating plant exometabolites. The prevalence of bacilli and actinomycetes on some plants (sedge and cottongrass) in the rhizoplane and rhizosphere indicated the presence of hydrolytic bacteria in these microniches, which are necessary for the assimilation

of various polymers that accumulate on suppressed parts of the plants. This type of distribution of ecological–trophic groups of bacteria in the aboveground and underground layers is characteristic of all land plant species [2, 5]. Measurements of nitrogen-fixing activity in the rhizosphere under bog plants made it possible to detect rather high values, which increased with the addition of glucose. Thus, due to nitrogen-fixing bacteria, peat soils are enriched in nitrogen.

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