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SOIL BIOLOGY

Psychrotolerant Actinomycetes of Plants and Organic Horizons in Tundra and Taiga Soils

M. S. Dubrova, G. M. Zenova, A. V. Yakushev, N. A. Manucharova, E. P. Makarova, D. G. Zvyagintsev, and I. Yu. Chernov

Faculty of Soil Science, Moscow State University, Moscow, 119991 Russia

E-mail: zenova38@mail.ru

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Abstract—It has been revealed that in organic horizons and plants of the tundra and taiga ecosystems under low temperatures, actinomycetal complexes form. The population density of psychrotolerant actinomycetes in organic horizons and plants reaches tens and hundreds of thousands CFU/g of substrate or soil, and decreases in the sequence litters > plants > soils > undecomposed plant remains > moss growths. The mycelium length of psychrotolerant actinomycetes reaches 220 m/g of substrate. Application of the FISH method has demonstrated that metabolically active psychrotolerant bacteria of the phylum Actinobacteria constitute 30% of all metabolically active psychrotolerant representatives of the Bacterià domain of the prokaryotic microbial community of soils and plants. Psychrotolerant actinomycetes in tundra and taiga ecosystems possess antimicrobial properties.

Keywords: tundra, mycelium, FISH method, metabolically active actinomycetes, diversity, ecosystems

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INTRODUCTION

It was traditionally considered that actinomycetes adapt with difficulty to low temperatures and that there are no representatives among them capable of surviving at temperatures close to 0°C. However, reports have appeared on the isolation of psychrotolerant actinobacteria from terrestrial and aquatic ecosystems. Psychrotolerant representatives of the genera *Streptomyces, Nocardia* and *Geodermatophilus* have been isolated from soils of Iceland and Antarctica [12]. A new genus of *Subtercola boreus* gen. nov., sp. nov. actinobacteria has been isolated from cold groundwater in Finland. Its growth was observed at a temperature of 2°C with an optimum at 15–17°C [14]. Psychrotolerant actinomycetes have been isolated from Antarctic lichens [17]. A wide search for producers of antimicrobial substances is being conducted among psychrophilous actinomycetes [11, 15]. In Antarctica, psychrophilous actinomycete *Streptomyces* sp. strain no. 8, which is a producer of such antibiotics as azalomycin B, nigericin, and a nonpolyene macrolide antibiotic, which consists of two components that suppress the growth of gram-positive bacteria, yeast, and phytopathogenic fungi, has been isolated from the Edmonson glacier [10]. A search for antagonists to the pathogenic bacteria *Streptococcus mutans* and *S. oralis* among psychrophilous soil actinomycetes have shown a high degree of *Streptococcus mutans* sensitivity to *Dactilosporangium* sp., while *Streptococcus oralis* proved sensitive to streptomycetes [15]. In cold environments, psychrotolerant actinomycetes possess significant potential for the production of biologically active substances. In addition, in northern conditions, mycelial actinobacteria, which constitute a significant part of the hydrolytic microbial complex of soil and plant substrates, form a microbial complex of ecosystems. At the same time, actinomycetes, the vital activity of which is associated with organic soil horizons and plants of tundra and taiga ecosystems, have been studied insufficiently.

The aim of this work is a comparative study of the population density and biomass of metabolically active mycelial representatives of the phylum Actinobacteria, their taxonomic position, temperature preferences, and antimicrobial properties in organic soil horizons and plants of northern regions.

EXPERIMENTAL

The objects of the study were plants and organic horizons of tundra and taiga soils, samples of which were taken in growths of green moss and undecomposed moss remains on the surface of typical peat cryozem in the Yamal Peninsula; the moss horizon of raw-humus gleyic cryozem in the moss–lichen tundra near the city of Vorkuta; in sphagnum residues of peat oligotrophic soil in the Tver oblast; in litters of Siberian pine plantations on iron-illuvial podzols on the Solovetsky Islands, and in plants of the Taimyr tundra (table). The actinomycetal complexes of the organic

Characterization of objects of study

Region	Subzone	Soil	Substrate for isolation of actinomycetes
Taimyr Peninsula, Tareya village	Typical tundra	Gley tundra	Plants
Central Yamal Peninsula	Typical tundra	Peat cryozem	Green moss and undecomposed moss remains
Vorkuta city region	Moss–lichen tundra	Raw-humus cryozem	Green moss
Large Solovetsky Island	North taiga	Iron-illuvial podzol	Litters of forest biomes
Tver oblast, Zapadnodvinskii district	South taiga	Peat oligotrophic	Sphagnum remains

soil horizons and plants of northern regions were compared with the soil actinomycetal complexes of the studied ecosystems.

To isolate and quantify the actinomycetes in plant substrates, the method of surface inoculation of plant substrates on the dense Gauze medium was used [1]. Inoculations were incubated at 5, 20, and 28°C.

The temperature limits of the growth of isolated psychrotolerant actinomycetes were determined using the indicator of the radial growth rate of colonies [3].

The development of mycelium of psychrotolerant actinomycetes in the sphagnum residues of the peat oligotrophic soil was observed in microcosms incubated at 5 and 20°C. The microbial succession in a microcosm was initiated by moistening of the sphagnum remains to 60% of the total moisture capacity (**CM**). To calculate the length of the actinomycete mycelium, an Axioskop2+ luminescent microscope was used. In microcosms, the length of the mycelium of actinomycetes was calculated on days 1, 3, 7, 14, and 28 after the initiation of succession. To stain the mycelium, a water solution of orange acridine (1 × 10000; 2–4 min) was used. The length of the mycelium in 1 g of soil was calculated using the formula

$$N = S_1 an / vS_2 c,$$

where N is the length of the mycelium, m/g of soil; a is the mean length of the mycelium in the field of vision (averaging was performed over all preparations); S_1 is the area of preparation, μm^2; n is the index of dilution of the soil suspension, mL; v is the volume of the drop applied to the glass, mL; S_2 is the area of the field of vision of the microscope, μm^2; and c is the soil sample, g [7].

The antimicrobial properties of psychrotolerant actinomycetes were determined using the block method [4].

The molecular hybridization method in situ (FISH method: fluorescent in situ hybridization) was used to estimate the biomass of metabolically active cells of the Bacteria domain, as well as of the separate phylogenetic group Actinobacteria. The hybridization of preparations with fluorescent-labeled probes was performed according to the method [5, 16]. rRNA-specific fluorescent-labeled oligonucleotide probes with the following sequences of nucleotides were used. For the Bacteria domain: 5' GCT GCC TCC CGT AGG AGT 3'; for the phylum Actinobacteria: 5'TAT AGT TAC CAC CGC CGT 3' in combination with the unlabeled oligonucleotide 5-TAT AGT TAC GGC CGC CCGT-3.

The isolated strains of psychrotolerant actinomycetes were identified by their phenotypical (cultural, morphological, chemotaxonomical, and physiological) [4] and molecular–genetic characteristics (sequence of the *16S rRNA* gene). The phylogenetic position of isolated psychrotolerant actinomycetes was determined based on the sequencing of the *16S rRNA* gene. DNA was isolated from the bacteria biomass using the set of reagents in the Wizard Genomic DNA Purification Kit (Promega technologies, United States) according to the manufacturer's recommendations with insignificant modifications [6]. To carry out a polymerase chain reaction and further sequencing of PCR fragments of the *16S rRNA* gene, a universal primer system was used [9, 13]. A full-size copy of the gene was obtained on a Mastercycler personal (Eppendorf 11F, Germany) device using the following primers: 11F 5'-AGAGTTTGATCMTGGCTCAG-3' 1492R5'-TACGGYTACCTTGTTACGACTT-3', where M = C or A, Y = C or T.

The volume of the amplification mixture was 50 μL and had the following composition: 1 × DNA buffer of the BioTaq polymerase (17 mM $(NH_4)_2SO_4$, 67 mM *tris*-HCl, pH 8.8, 2 mM $MgCl_2$); 12.5 nmol of each of dNTP, 50 ng of the DNA-matrix; 5 pmol of the respective primers (11F and 1492R), 3 units of the BioTaq DNA polymerase (Dialat LTD, Russia).

The temperature–temporal profile of the polymerase chain reaction was as follows: the first cycle at a temperature of 94°C, 9 min; 1 min at a temperature of 55°C, 2 min at 72°C, and the following 30 cycles: 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and the final cycle: 7 min at 72°C.

A primary analysis of the similarity of nucleotide sequences of the *16S rRNA* gene of the studied strain was performed using the BLAST program (http://

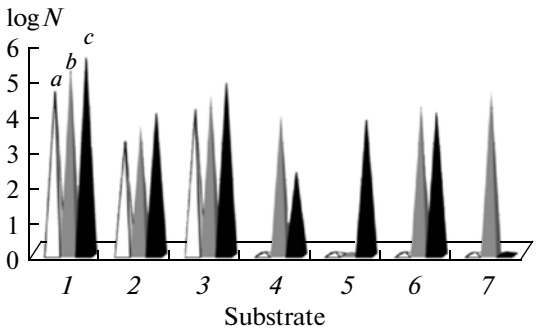

Fig. 1. Number (log N, N – CFU/g of substrate) of actinomycetes isolated from various organic horizons and plants of tundra and taiga ecosystems at different temperatures of incubation of inoculations: (*a*) 5°C, (*b*) 20°C, (*c*) 28°C; (*1*, *2*, *3*) litters of forest plantations on iron-illuvial podzols; (*4*, *5*) green moss on the surface of the typical peat cryozem; (*6*) green moss on the surface of the raw–humus gleyic cryozem; and (*7*) undecomposed moss remains on the surface of the typical peat cryozem.

www.ncbi.nlm.nih.gov/blast). The sequences were edited using the BioEdit editor (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html), while their multiple leveling was accomplished using the CLUSTAL W 1.75 program. Dendrograms were built using the "neighbor-joining" (NJ) algorithm in the MEGA 4 program. The statistical reliability of the phylogenetic reconstructions was assessed using the Bootstrap method by building 1000 alternative trees. Nucleotide sequences of the *16S rRNA* gene of the isolated strains were deposited in Gen Bank NCIB and the cultures were given individual access numbers.

RESULTS AND DISCUSSION

In the organic horizons of tundra ecosystems, such as moss growths and undecomposed remains on the surface of cryozems, no psychrotolerant actinomycetes were found during incubation of the inoculations at 5°C (Fig. 1). Psychrotolerant actinomycetes were isolated from the organic horizons at a temperature of 20°C in thousands to tens of thousands CFU/g of substrate. The fraction of psychrotolerant actinomycetes (isolated during incubation of inoculations at a temperature of 20°C) is comparable to or exceeds the fraction of mesophilic forms (isolated during incubation of inoculations at 28°C) in complexes of actinomycetes of mosses and undecomposed remains in tundra ecosystems. The number of psychrotolerant actinomycetes isolated from the organic horizons of cryozem (mosses and their remains) proved comparable to the number of psychrotolerant forms in tundra cryozems [4]. From tundra cryozems, the psychrotolerant actinomycetes were isolated mainly during the incubation of inoculations at 20°C in an amount that did not exceed thousands of CFU/g of soil. Only in a single case were psychrotolerant forms observed in inoculations incubated at 5°C in the raw-humus gleyic cryozem at a depth of 9–12 cm. The fraction of psychrotolerant actinomycetes in the soil actinomycetal complex exceeded the fraction of mesophilous forms [4].

Observation of the phenotypic parameters [1, 8] of the isolated actinomycetes allowed us to ascribe all cultures to the genus *Streptomyces*. The isolated streptomycetes are divided into species sections and series according to the actinomycetes guide [1].

The variety of psychrotolerant actinomycetal complexes of moss growths, remains, and mineral horizons of the tundra cryozem is insignificant. Actinomycetes are represented mainly by *Streptomyces*, while the number of species sections and series is limited: only *Cinereus achrhmogenes* and *Albus albus* occur.

A significant amount of psychrotolerant forms, which often exceeds the number of mesophilic forms and reaches tens and hundreds of thousands CFU/g of substrate, was revealed in the substrates of forest plantations on iron-illuvial podzols of Large Solovetsky Island during incubation of inoculations at 5 and 20°C. As in moss growths and remains in cryozems, the psychrotolerant actinomycetes constitute a more significant fraction (about 70% of the total content of all actinomycetes) in the actinomycetal complex compared with the mesophilic actinomycetes. The number of psychrotolerant and mesophilic forms of actinomycetes in the upper humus horizon of iron-illuvial podzols proved smaller compared with the number of actinomycetes in the litters of forest biomes and did not exceed thousands of CFU/g of soil. No actinomycetes were isolated from the podzol at 5°C. The diversity of streptomycetes in forest litters and podzols, as well as in tundra cryozems, was small and manifested itself in the dominance of streptomycetes of the species section and the series *Cinereus Achrhmogenes* and *Albus albus* in the actinomycetes complex of podzol and forest litters.

The number of psychrotolerant and mesophilic actinomycetes isolated from plants of Taimyr tundra biotopes was from tens to hundreds of thousands CFU/g of substrate; however, in most cases, psychrotolerant actinomycetes were isolated during incubation of inoculations at 20°C (Fig. 2). Only from some plants were psychrotolerant actinomycetes isolated during incubation of inoculations at 5°C. We were unable to isolate psychrotolerant actinomycetes from bog plants and plants of the pattern-ground tundra.

In the tundra gley soil of the Taimyr tundra, the content of psychrotolerant actinomycetes was smaller compared with the number of the mycelial prokaryotes on plants and constituted thousands and tens of thousands CFU/g of soil.

Thus, the population density of psychrotolerant actinomycetes isolated from plant substrates of tundra and taiga land ecosystems varies from thousands to tens and hundreds of thousands CFU/g of substrate depending on the temperature of incubation of inoculations. A dependence of psychrotolerant actino-

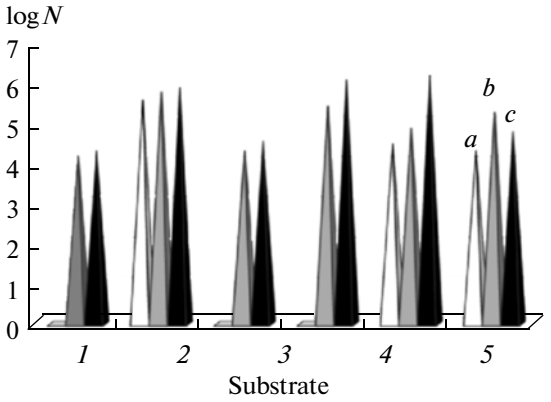

Fig. 2. Number of actinomycetes isolated from various plants of Taimyr tundra at different temperatures of incubation of inoculations: (*a*) 5°C, (*b*) 20°C, (*c*) 28°C; (*1*) viviparous sheep's-fescue (*Festuca vivipara*), (*2*) *Papaver pulvinatum*, (*3*) *Oxytropis adamsiana*, (*4*) mountain sorrel (*Oxyria digyna*), and (*5*) Arctic sandwort (*Minuartia arctica*); (*1*, *4*, *5*) plants of the Dryadetum biotope; and (*2*, *3*) plants of the meadow biotope.

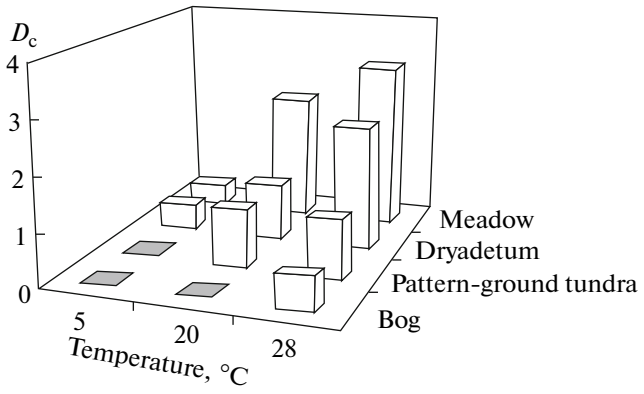

Fig. 3. Diversity coefficient of streptomycetes (D_c) isolated from the plants of the Taimyr biotopes at various temperatures of incubation of inoculations calculated as a ratio of the mean number of species sections and series of actinomycetes in a certain biotope to the number of samples of plants in the biotope.

mycetes on the type of the substrate can also be distinctly traced: it decreases in the sequence litters > plants > soils > undecomposed plant residues > moss growths.

A tendency toward a decrease in species diversity of streptomycetes with a decrease in the incubation temperature of inoculations in the following temperature set is observed: 28 > 20 > 5°C. The number of specific sections of streptomycetes significantly decreases in the indicated temperature series. It manifests itself most distinctly for plants and the primitive skeletal soil in Taimyr tundra ecosystems (Fig. 3).

The application of dispersion analysis showed that the incubation temperature reliably influences the species diversity of streptomycetes isolated from plants. The diversity value reliably depends on the temperature of incubation of cultures. The contribution of temperature to the total dispersion is 30%. The dependence of the streptomycetes diversity on the occurrence of tundra plants in a certain biotope proves to be unreliable.

In the Dryadetum biotope, the most frequent psychrotolerant streptomycetes were of the species sections and series *Cinereus Achromogenes* and *Helvolo-Flavus Helvolus*, while in the meadow biotope, *Helvolo-Flavus Helvolus* and *Albus Albus* predominated. In the primitive skeletal soil, dominance of actinomycete species of the *Helvolo-Flavus Helvolus* section and series was found. In inoculations from meadow plants *Papaver pulvinatum* and *Oxytropis adamsiana* and the soil under them, psychrotolerant violet actinomycetes *Streptomyces violaceoruber* (section *Cinereus*, series *Violaceus*) were revealed, which are rare forms in the terrestrial ecosystems of northern regions [2].

The development of the mycelium of psychrotolerant actinomycetes was observed in microcosms with sphagnum remains collected from the surface of peat oligotrophic soil. The microcosms were incubated at a temperature of 5 or 20°C. The microbial succession was initiated by moistening (60% of total moisture capacity). The investigations showed that the mycelium of psychrotolerant actinomycetes grows and develops in microcosms incubated at 5 and 20°C. In moss residues, the length of the mycelium by day 4 of the experiment reached 140 m/g and remained at this level to day 21 during incubation of the microcosm at 5°C, while during incubation of the microcosm at 20°C, the length of the mycelium increased to 220 m/g in the undecomposed remains to day 21 of the growth (Fig. 4).

Study of the prokaryotic microbial community of substrates using hybridization in situ and *16S rRNA*-specific oligonucleotide probes, which determine the representatives of the Actinobacteria phylogenetic group, showed that the biomass of metabolically active representatives of this group is from 10 to 23% of the biomass of all metabolically active representatives of the Bacteria domain of the prokaryotic microbial communities in the studied substrates (undecomposed moss remains, litters of the forest biomes and plants) during incubation of the latter at 5°C and from 13 to 32% during incubation of the studied plant substrates at a temperature of 20°C. It was noted that with the increase in the temperature of incubation of plants and moss remains, the fraction of representatives of the Actinobacteria phylum from all bacteria of the prokaryotic community increases, while with the increase in the temperature of incubation of the litter this fraction somewhat decreases. In the phylum Actinobacteria of the microbial prokaryotic community of the studied plant substrates in the tundra and taiga ecosystems, the psychrotolerant metabolically active

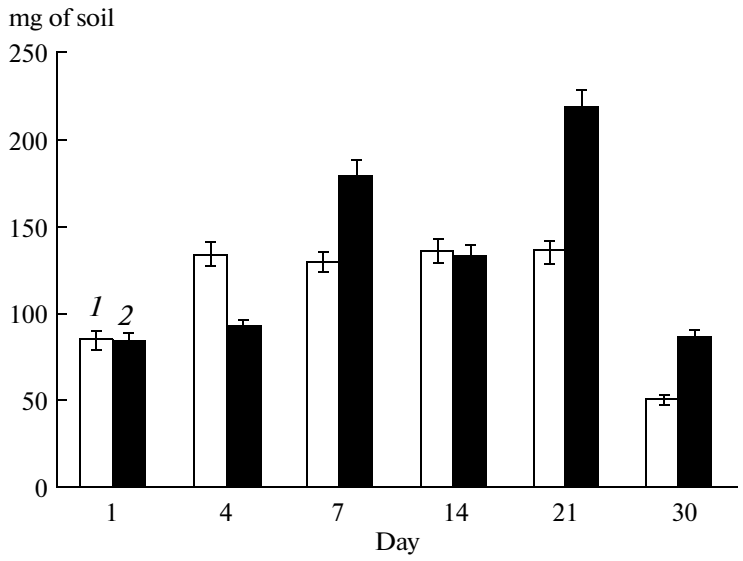

Fig. 4. Dynamics of the length of actinomycetes mycelium in the course of succession initiated by the moistening of the sphagnum remains on the oligotrophic peat soil at various temperatures of incubation of these remains: (*1*) 5; (*2*) 20°C.

mycelial actinobacteria constitute a larger fraction compared with the single-celled ones. With the increase in the incubation temperature from 5 to 20°C, the fraction of metabolically active single-cell actinobacteria in prokaryotic microbial communities of the sphagnum remains and forest litter increases. An exception is tundra plants, during incubation of which at 5°C the metabolically active psychrotolerant single-cell representatives dominate in the phylum Actinobacteria of the prokaryotic microbial community, and with the increase in the temperature of incubation of the substrate to 20°C, their population density decreases and metabolically active psychrotolerant mycelial forms become dominant in the phylum Actinobacteria (Fig. 5). In tundra soils, as a rule, in the phylum Actinobacteria, mycelial psychrotolerant actinobacteria predominate.

A collection of cultures of psychrotolerant actinomycetes isolated from the tundra and taiga ecosystems was compiled. The isolated cultures were identified by their phenotypical characteristics as representatives of *Streptomyces*. Specific identification was performed using phenotypic and/or molecular-genetic characteristics (nucleotide sequences were determined in the *16S rRNA* molecule). Nucleotide sequences of the *16SrRNA* gene from isolated strains were deposited in GenBank NCBI and the cultures were given individual access numbers.

Using calculation of the radial rate of growth of streptomycete colonies, the temperature range of their growth has been established. A group of strains of psychrotolerant streptomycetes (*S. parvus* 18FR846234 isolated from moss growths on the surface of the typical peat cryozem in tundra at the territory of the Central Yamal Peninsula, *S. helvaticus* strains 8.5.2 and 8.5.3 isolated from the forest litter on the surface of the iron-illuvial podzol (Large Solovetsky Island), and *S. lavendulae* strain DN5.1 isolated from the Taimyr tundra plants) had a growth range of 2 to 37°C; the maximum value of the radial growth rate of colonies was recorded at 20°C. The moderate psychrophile streptomycetes *Streptomyces beijiangensis* 5–4 FR837628 isolated from the typical peat cryozem (Central Yamal) was characterized by a temperature range of growth from 2 to 30°C and maximum radial growth rate of colonies at 5°C [3]. Thus, it was shown experimentally that streptomycetes isolated from the studied plant substrates and soils of tundra and taiga ecosystems are psychrotolerant in their temperature preferences.

A study of the antimicrobial activity of psychrotolerant streptomycetes isolated from plant substrates of northern regions revealed cultures possessing antibacterial, antiyeast, and antistreptomycete properties. *Streptomyces lavendulae* strain DN.5.1 isolated from the Taimyr tundra plants manifested antagonism to the following test cultures: *Pseudomonas fluorescens, Candida* sp., *Saccharomyces cerevisiae, Streptomyces chrysomallus; Streptomyces parvus* moss 18FR846235 isolated from moss growths on the surface of the typical peat cryozem at the territory of Central Yamal, manifested antimicrobial properties against *Saccharomyces cerevisiae*, *Streptomyces chrysomallus*, and other streptomycetes isolated from the Taimyr tundra plants. The psychrotolerant actinomycetes isolated from the typical peat cryozem possess antagonistic activity to gram-negative bacteria of the genera *Aquaspirillum* and *Bacteroides*, fungi of the genera *Fusarium* and *Penicillium*, and fungi *Mucor riemalis* and *Cladosporium herbarum*.

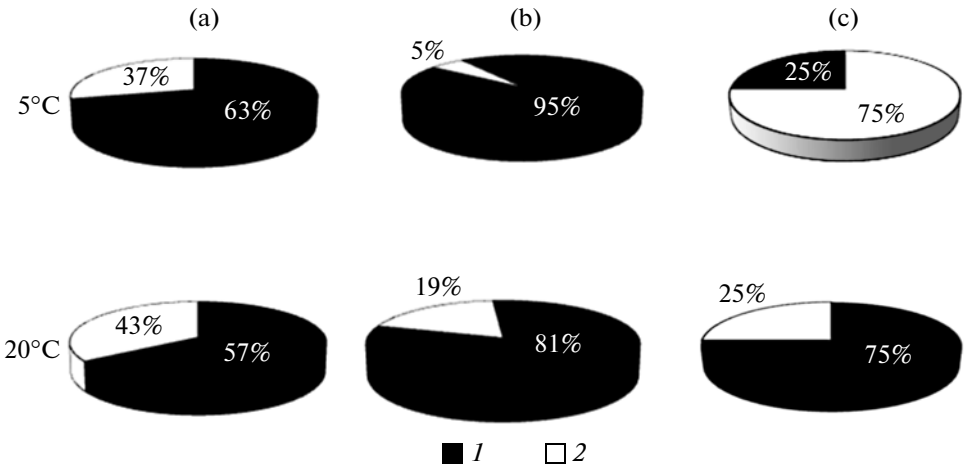

Fig. 5. Ratio of (1) mycelial and (2) single-cell representatives of the Actinobacteria phylogenetic group in the prokaryotic microbial communities of the sphagnum combing of (a) the oligotrophic peat soil, (b) litters of forest plantations on iron-illuvial podzol, and (c) plants of the Taimyr tundra.

CONCLUSIONS

It was shown experimentally that in the organic horizons (moss growths, undecomposed moss remains, and litters) of soils and tundra and taiga plants, psychrotolerant actinomycetes are found in tens and hundreds of thousands CFU/g of plant substrate. The number of psychrotolerant actinomycetes differs in various plant substrates and decreases in the sequence litters of forest plantations > tundra plants > soil > undecomposed moss remains > moss growths.

In the actinomycete complexes of plant substrates, psychrotolerant actinomycetes are comparable in number with mesophilous forms and often constitute a larger fraction in the actinomycete complex. The study showed the development of the actinomycete mycelium, an increase in its length in moss remains on peat oligotrophic soil in the succession initiated by moistening (up to 60% of the total moisture capacity), and incubation of the undecomposed moss remains at 5 and 20°C. Using the FISH molecular–biological method, it was established that the metabolically active psychrotolerant representatives of the phylum Actinobacteria constitute from 10 to 32% in the biomass of all metabolically active psychrotolerant representatives of the Bacteria domain of the prokaryotic microbial communities of organic and mineral soil horizons and plants of tundra and taiga ecosystems. In the Actinobacteria phylum of the Bacteria domain of prokaryotic microbial communities of organic and mineral soil horizons, mycelial psychrotolerant metabolically active representatives, as a rule, prevail over single-cell psychrotolerant metabolically active actinobacteria. In the microbial communities of tundra plants during incubation at 5°C in the phylum Actinobacteria of the Bacteria domain, single-cell psychrotolerant metabolically active actinobacteria dominate.

It was noted that the psychrotolerant actinomycetes isolated from soils and the tundra and taiga plants possess some antimicrobial properties to the gram-negative bacteria, yeast, and streptomycetes. It was established that in the northern regions, a significant fraction of the Bacteria domain of prokaryotic microbial communities of ecosystems is made up of metabolically active psychrotolerant mycelial actinobacteria, which form a hydrolytic complex and participate in the creation of cenotic bonds and redox processes in soils.

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