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EXPERIMENTAL ARTICLES

Thermotolerant and Thermophilic Actinomycetes from Soils of Mongolia Desert Steppe Zone

A. I. Kurapova[a], G. M. Zenova[a, 1], I. I. Sudnitsyn[a], A. K. Kizilova[b], N. A. Manucharova[a], Zh. Norovsuren[c], and D. G. Zvyagintsev[a]

[a] *Moscow State University, Moscow, Russia*

[b] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[c] *Institute of Biology, Mongolian Academy of Sciences, Ulan Bator, Mongolia*

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Abstract—In the actinomycete complexes of Mongolian desert soils, thermotolerant and thermophilic actinomycetes were found in high abundance, exceeding that of the mesophilic forms. Among the thermotolerant members of the order *Actinomycetales, Streptomyces, Micromonospora, Actinomadura,* and *Streptosporangium* species were most widespread in desert soils. Experiments with soil microcosms demonstrated that thermophilic actinomycetes in desert soils grew, developed, and formed mycelia of the length comparable to that of the mesophilic forms of actinomycetes. Molecular biological investigation of the samples of desert steppe soils by denaturing gradient gel electrophoresis (DGGE) and fluorescent in situ hybridization (FISH) revealed members of the phylum *Actinobacteria*. FISH analysis revealed that the biomass of the metabolically active mycelial actinobacteria in the prokaryotic community of Mongolian desert soils exceeded that of the unicellular *Actinobacteria*.

Keywords: thermotolerant and thermophilic actinomycetes, metabolically active mycelial actinobacteria, actinomycete complexes, desert soils.

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Investigation of effects of the temperature factor on activity of microbial populations is of high theoretical and applied importance. The mechanisms of temperature adaptation are of theoretical interest. Understanding the effect of adaptations to temperature on the rate of microbial respiration (resulting in CO_2 emission) is among the issues of applied interest. Carbon dioxide is a greenhouse gas and variations in its concentration can result in large-scale climatic changes. Soil respiration is considered a more significant factor than technogenic industrial emissions. Effect of temperature on the functional activity of the soil microbial population, including actinomycetes (gram-positive mycelial bacteria) is therefore of importance.

The soils of Mongolian desert steppes are periodically heated to high temperatures (50–60°C) and are characterized by intermittent modes of moistening and nutrient supply.

Existence of extremophilic and extremotolerant soil actinomycetes (acid-tolerant and alkalitolerant, psychrotolerant and thermotolerant, halotolerant and haloalkalitolerant, or xerophilic) does not cause any doubt [1–4].

Mesophilic forms predominate among spore-forming actinomycetes producing developed aerial mycelium and exospores. Thermophilic forms have been described within the genus *Thermoactinomyces* [5], which is presently excluded from the order *Actinomycetales* in accordance with its phenotypic and molecular genetic characteristics [6], as well as among some *Thermomonospora, Microbispora, Saccharopolyspora, Saccharomonospora*, and *Streptomyces* species [5, 7]. The patterns of occurrence of thermotolerant and thermophilic actinomycetes in soils are insufficiently studied.

The optimal growth temperature for mesophilic actinomycetes is from 20 to 42°C. Among these organisms, thermotolerant species exist which survive at 50°C [8]. Moderately thermophilic actinomycetes grow within the 28–60°C temperature range with a growth optimum at 45–55°C [2]. Strictly thermophilic actinomycetes grow at 37 to 65°C with the optimum temperature at 55–60°C [2]. Incubation temperatures of 28, 37, and 45°C are considered optimal for the isolation of soil mesophilic, thermotolerant, and moderately thermophilic actinomycetes, respectively.

The goal of the present work was to isolate the thermophilic and thermotolerant actinomycetes of Mon-

[1] Corresponding author; e-mail: zenova38@mail.ru

Table 1. Characteristics of investigated soils

Sample no.	Soil type	Sampling horizon, cm	C, %	pH	Sampling site
1, 2, 3	Brown, desert steppe sand	A_d, 0–10	0.3	8–9	Mongolia, Southern Gobi, Bulgan sum
4	Gray-brown desert	A1, 6–18	0.29	8–9	Southern Gobi, Khan-bogd-Galdyn Gobi
5	Desert steppe light brown saline meadow	A_d, 0–5	0.67	8.4	Southern Gobi, Nomgon sum
6	Desert steppe light brown medium loamy	A_d, 0–2	0.2	7.9	Southern Gobi, Nomgon sum
7	Desert steppe light brown saline	A_d, 0–3	0.2	7.9	Southern Gobi, Nomgon sum
8	Takyr-like	C, 0–5	2.64	7.3	Southern Gobi, Nomgon sum
9	Desert steppe meadow brown solonchak	A_d, 0–1.5	0.1	7.8	Eastern Gobi aimag, Ulaanbadrakh sum

golian desert steppe soils and to determine their structural, functional, and taxonomic characteristics.

MATERIALS AND METHODS

Subjects of investigation were soils from the desert steppe zone of Mongolia, which are located within the internal Central Asian Basin and belong to the Central Asian landscapes [9] (Table 1).

Isolation and differentiated enumeration of actinomycetes were carried out by plating the dilutions of soil suspensions on solid nutrient media (humus–vitamin agar and the medium with sodium propionate) [10]. For inoculations, air-dry weighted soil samples were used.

The media were supplemented with nystatin in order to suppress fungal growth and with the vitamin B complex (4 μg/L) to improve actinomycete recovery. The plates were incubated for 2–3 weeks at 28 and 37°C and for 4–6 days at 45°C.

Preliminary identification of actinomycetes was carried out by microscopy of the colonies on agar plates with subsequent isolation of the dominant morphotypes on oat agar [10]. For preliminary identification of actinomycete cultures isolated from soil, the following phenotypic characteristics were used: morphology (mycelium fragmentation, spore formation on the substrate and/or aerial mycelium, number of spores in chains, and presence of single spores or sporangia) and chemotaxonomy (presence of L- or *meso*-isomers of diaminopimelic acid and differentiating sugars in whole cell hydrolysates) [11]. Identification manuals [12, 13] and publications [3, 5–7, 14, 15] were used. The results of sequencing (see below) were considered during identification.

Soil microcosms. Development of actinomycetes in soils at different temperatures was investigated using soil microcosms. The biomass and length of mycelium were monitored during the humidification-induced (60% of water saturation) microbial succession in brown desert steppe soil. Soil preparation and initiation of the succession were carried out using the conventional technique [16]. Soil was incubated at 28 and 45°C. Mycelial length in soil was determined by epifluorescence microscopy 0, 4, 10, and 17 days after the onset of succession. The mycelium was stained with water solutions of acridine orange (1 : 10000, 2–4 min).

Mycelial length per 1 g of soil was calculated using the equation:

$$N = S_1\, a\, n / v\, S_2\, c, \quad (1)$$

where N is the length of mycelium (μm) (cell number) per 1 g soil, a is the average length of the mycelium in the microscope field (averaged for all the preparations), S_1 is the area of the preparation (μm^2), n is the dilution of the soil suspension (mL), v is the volume of the drop applied to the microscope slide (mL), S_2 is the area of the microscope field (μm^2), and c is the sample weight (g).

The biomass was calculated from the value 3.9×10^{-8} g, the biomass of 1 m of dry actinomycete mycelium 0.5 μm in diameter [16].

The optimal and limiting temperatures for actinomycete growth were determined from the radial rate of colony growth on Gauze 1 solid medium [10] at 5, 8, 10, 15, 20, 28, 37, 45, 50, and 55°C. The radial rate was calculated from the equation:

$$K_r = (d_2 - d_1)/(t_2 - t_1),$$

where d_1 and d_2 are colony diameters at the initial and final moment of measurement, respectively; t_1 and t_2 are times (days) of the initial and final measurements. The measurements were carried out in 20 repeats.

Xerotolerance of the moderately thermophilic streptomycete *Streptomyces* sp. 315 FR716528 was determined in desiccators with saturated solutions of various salts. Three levels of air humidity were created, corresponding to different levels of water pressure in soil (P) (a_w): extremely low, −96.4 MPa (a_w 0.50); corresponding to the maximal adsorption water capacity (MAW) of the soil, −22.6 MPa (a_w 0.86); and corresponding to the maximal hygroscopic humidity of the soil, −2.8 MPa (a_w 0.98). A drop of the monosporic actinomycete suspension (10^6 spores/mL) was applied to a microscope slide in a desiccator at 28 or 45°C. Air humidity in the desiccators was monitored using a VIring AB digital thermohygrometer with the measurement error not exceeding 1%. After exposure for 6, 24, 48, and 72 h, the slides were examined under a Zeiss Axioskop 2 plus epifluorescence microscope (Germany). The number of germinated (with growth tubes) and ungerminated spores was determined, as well as the length of mycelial germs. After examination, the slides were not returned to the desiccators.

FISH (fluorescent in situ hybridization) analysis was used to assess the biomass of the metabolically active bacterial cells [17–19]. The spectrum of probes used in the present work was specific for the *Bacteria* domain and for the phylum *Actinobacteria* [18]. The following rRNA-specific fluorochrome-labeled oligonucleotide probes were used: 5'-GCT GCC TCC CGT AGG AGT-3' for the *Bacteria* and 5'-TAT AGT TAC CAC CGC CGT-3' combined with the unlabeled oligonucleotide 5'-TAT AGT TAC GGC CGG CCGT-3' for the *Actinobacteria*. The number of metabolically active microbial cells per 1 g of soil was calculated using equation (1).

The samples were analyzed under an Axioskop 2 plus epifluorescence microscope (Zeiss, Germany) with the Filter set 15 for the probes and Filter set 09 for acridine orange staining. The numbers of target microorganisms were determined from the number of hybridized cells in 50 microscope fields for every cell with subsequent recalculation per 1 g of soil.

PCR analysis. Analysis of the amplified 16S rRNA gene from the total DNA isolated from takyr desert soil and of the DNA from pure cultures was based on denaturing gradient gel electrophoresis (DGGE) [20, 21]. DNA from pure microbial cultures was obtained using the Wizard Genomic DNA Purification Kit (Promega, United States). Total DNA from soil was extracted using the PowerSoil DNA Isolation Kit (MO BIO, United States) according to the manufacturer's recommendations. The 626-bp 16S rRNA fragment was amplified using the 341F+GC and 907R universal primers [22].

341F	5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCCCCG CCC CCT CCT ACG GGAGGC AGC AG-3'
907R	5'-CCG TCA ATT CMT TTR AGT TT-3'

M = C : A, R = G : A, always 1 : 1.

The polymerase chain reaction (PCR) was carried out in 50 μl of the reaction mixture containing the following: Master-Mix (Fermentas), 12.5 pmol of both primers, and ~25 ng of the template DNA. The temperature profile used was as follows: first cycle for 5 min at 94°C and 15 cycles (1 min at 94°C, 1 min at 55°C, and 3 min at 72°C). After the last cycle, the mixture was incubated for 7 min at 72°C for elongation.

The PCR fragments were separated by denaturing gradient gel electrophoresis on a DCode Universal Mutation Detection System (BioRad, United States) in the Institute of Microbiology, Russian Academy of Sciences, according to the protocol [23]. PCR fragments were separated in 6% polyacrylamide gel (acrylamide : *bis*-acrylamide ratio of 37.5 : 1) in the 40–65% denaturing gradient (100% denaturant contained 7 M urea and 40% formamide). Electrophoresis was carried out in 1× TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 7.4) at 60°C and 100 V for 16 h. The gel was stained with ethidium bromide (0.5 μg/mL) for 30 min and washed for 30 min. The results were recorded using the GelDoc image system (BioRad, United States). The major visible bands were excised and DNA was eluted with sterile deionized water. The product was then reamplified and used for sequencing

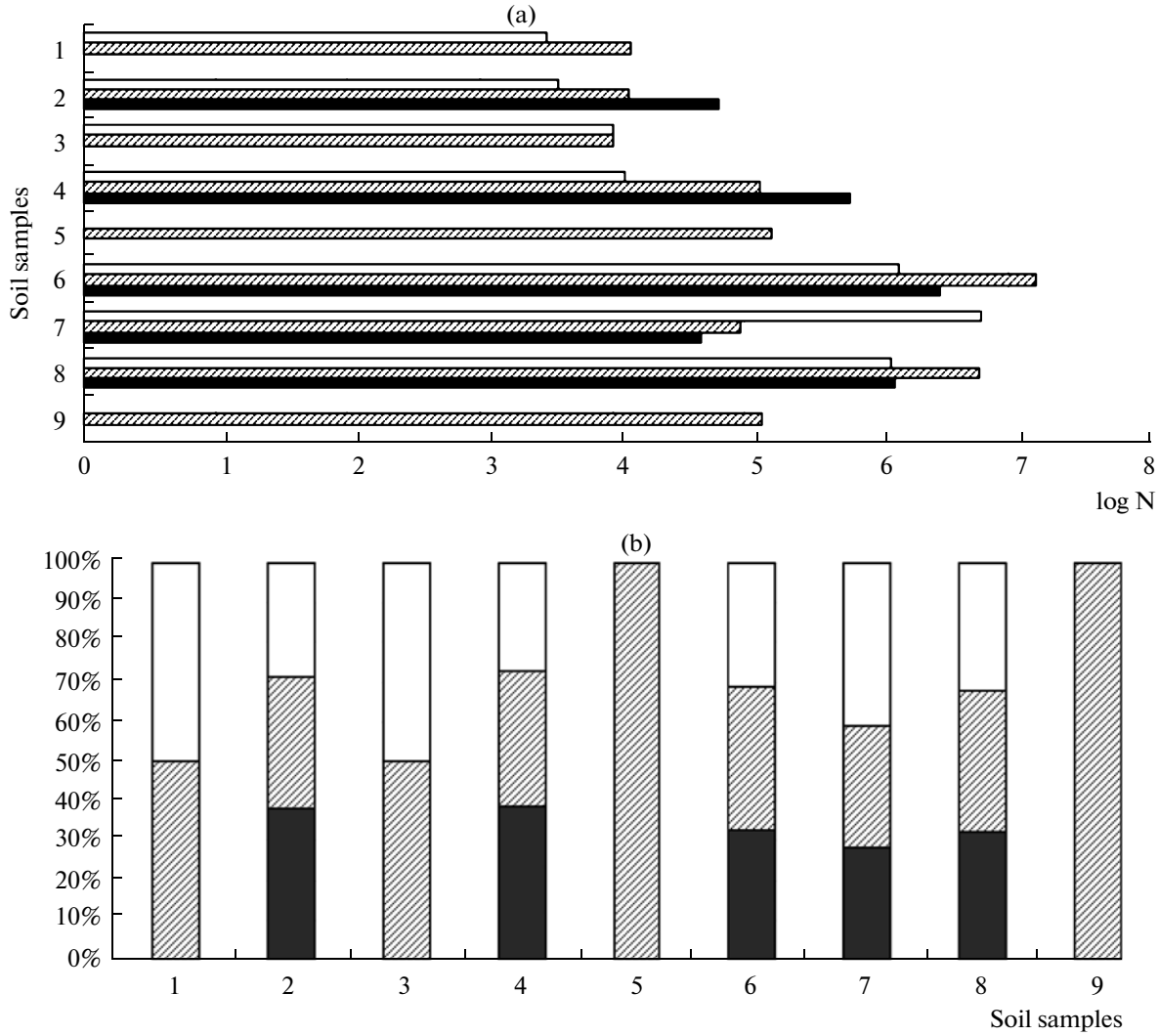

Fig. 1. Abundance (log N) (a) and ratio within the actinomycete complex (b) of mesophilic (clear columns), thermotolerant (hatched columns), and moderately thermophilic actinomycetes (shaded columns) in Mongolian desert soils. N is CFU/g soil.

in the service laboratory. Sequencing of the 16S rRNA genes was carried out in the Bioengineering Center Russian Academy of Sciences using an automated capillary sequencer (Silver Sequence d/ddNTP Mixes, Promega, United States).

The nucleotide sequences were analyzed using the BLAST software package (http://www.ncbi.nlm.nih.gov/blast). The sequences were edited using BioEdit (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.htmL) and aligned using CLUSTAL W 1.75. Phylogenetic trees were constructed using the neighbor-joining (NJ) algorithm implemented in MEGA 4. Statistical reliability of the phylogenetic reconstruction was determined by bootstrap analysis of 1000 alternative trees. The 16S rDNA sequences of the strains were deposited in GenBank under unique accession numbers.

RESULTS AND DISCUSSION

The numbers of thermophilic and thermotolerant actinomycetes in Mongolian desert soils varied from thousands to millions CFU/g, depending on the soil type, and was comparable to or even higher than the number of mesophilic forms (Fig. 1a).

The highest numbers of actinomycetes (up to several millions GFU/g) were found in desert steppe light brown and takyr-like soils. This abundance of actinomycetes, exceeding that of all the other soils investigated, is probably caused by the high content of organic matter (e.g., in takyrlike soil) or the heavier granulometric composition resulting in more favorable conditions of aeration, water exchange, and nutrient supply (in desert steppe light brown medium loamy soil) (Table 1). In all soil samples, thermotolerant actinomycetes were found; moderately thermo-

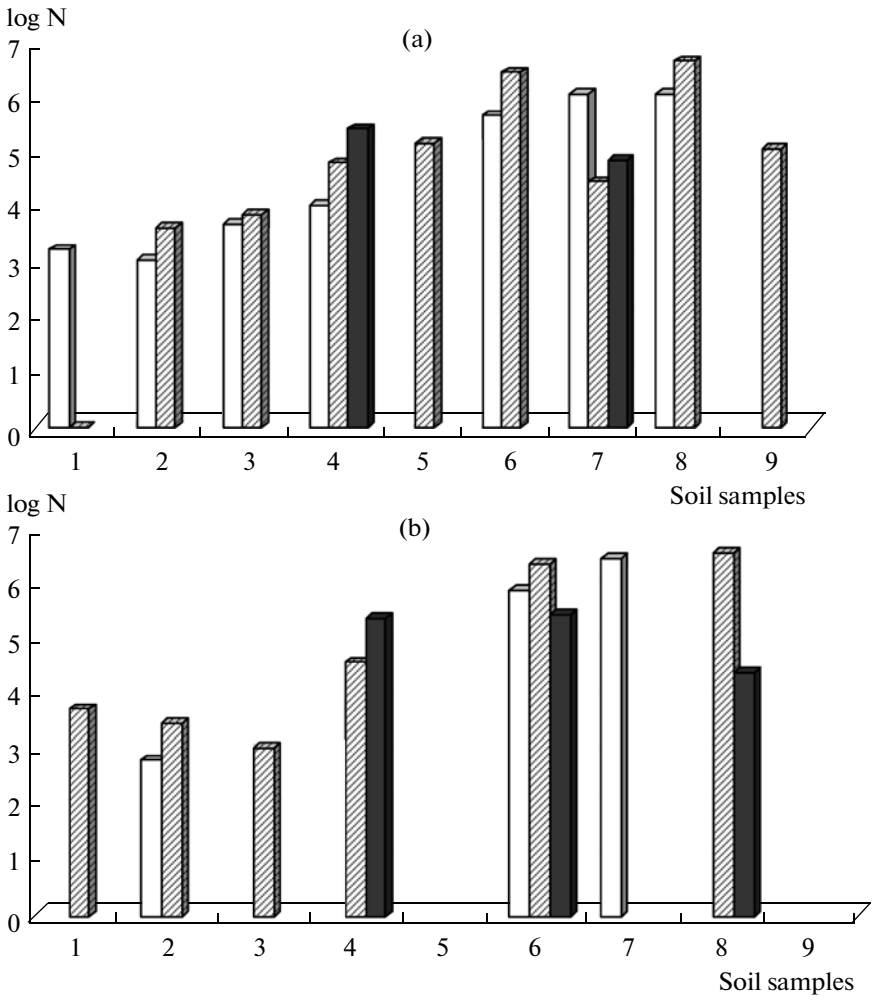

Fig. 2. Abundance (log N) of mesophilic (clear columns), thermotolerant (hatched columns), and moderately thermophilic (shaded columns) species of the genera *Streptomyces* (a) and *Micromonospora* (b) in Mongolian desert soils.

philic forms were present in almost all the samples in numbers comparable to or exceeding the number of mesophiles. Desert steppe light brown medium loamy soil had the highest content of thermotolerant and moderately thermophilic actinomycetes, probably due to more significant heating of loamy soils. Thermotolerant and moderately thermophilic actinomycetes usually constituted a significant part of the actinomycete complexes of desert soils, exceeding the share of mesophilic actinomycetes (Fig. 1b).

The genera *Streptomyces* and *Micromonospora* were the most widespread among *Actinomycetales* of Mongolian desert soils. In the actinomycete complexes of almost all desert soils studied, *Micromonospora* were represented by thermotolerant and thermophilic forms (Fig. 2).

A moderately thermophilic actinomycete was isolated from gray-brown desert soil and identified as *Streptomyces* sp. based on its phenotypic characteristics and 16S rRNA gene sequence. The 16S rDNA sequence of this strain was deposited in NCIB GenBank under accession number *Streptomyces* sp. 315 FR716528.

The nucleotide sequence of the 16S rRNA gene from the thermotolerant strain 175 isolated from brown desert soil and identified as *Streptomyces tendae* according to its phenotypic characteristics and 16S rDNA sequences was deposited in NCIB GenBank under accession number *Streptomyces tendae* 175 FR846234.

In Mongolian takyr-like desert soil, mesophilic actinomycetes were represented by the genus *Streptomyces*, and thermotolerant ones by the genera *Micromonospora, Actinomadura*, and *Streptosporangium*.

The thermotolerant strain 392-1 isolated from the desert takyr-like soil and identified as *Actinomadura* sp. according to its phenotypic characteristics and 16S rDNA sequences was deposited in NCIB GenBank

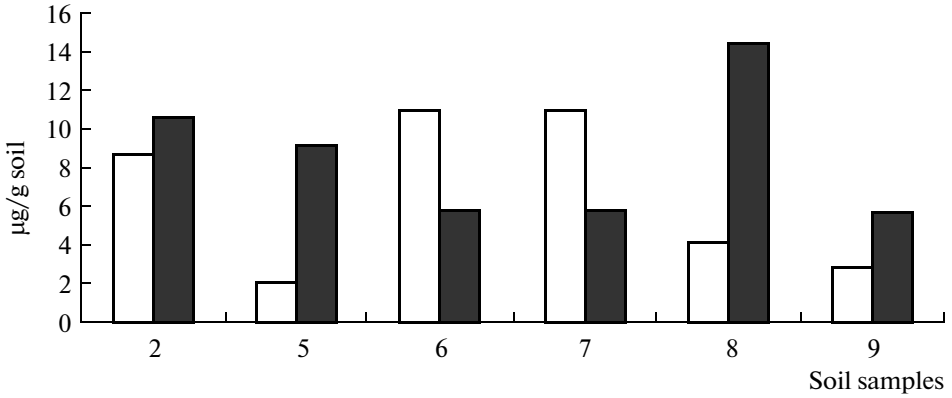

Fig. 3. Actinomycete biomass (μg/g soil) in Mongolian desert soils incubated at 28°C (clear columns) and 45°C (shaded columns).

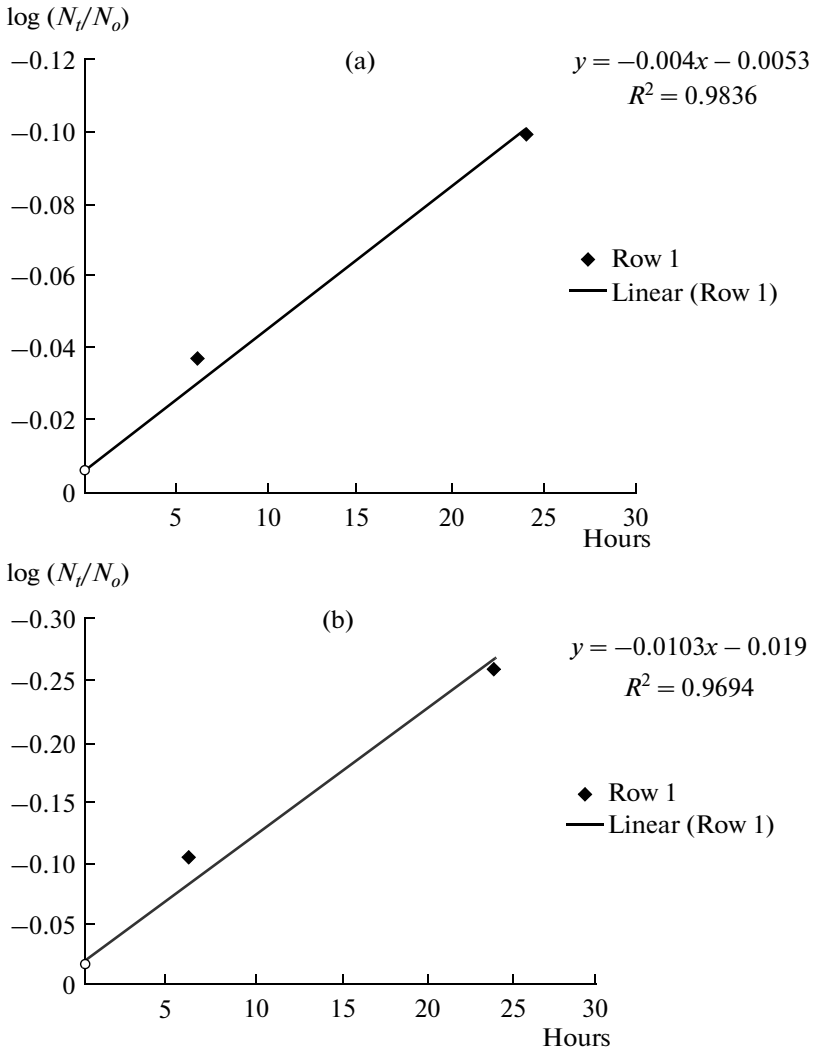

Fig. 4. Germination of the spores of the moderately thermophilic strain *Streptomyces* sp. 315 under low humidity at 28 (a) and 45°C (b): N_o is the number of viable ungerminated spores at the onset of the experiment, N_t is the number of viable ungerminated spores at time t. Graphs averaged for all a_w levels (diamonds) and linear approximation (line).

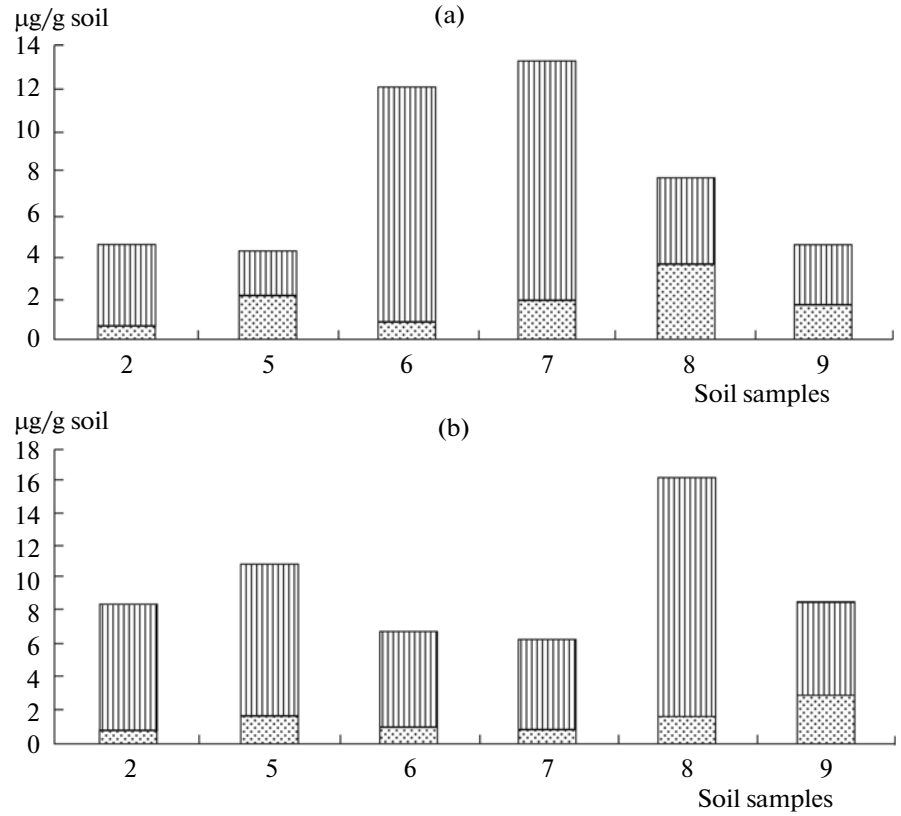

Fig. 5. Ratios of the biomass of active mycelial (hatched columns) and unicellular (dotted columns) *Actinobacteria* in prokaryotic microbial communities of Mongolian desert soils incubated at 28 (a) and 45°C (b) according to FISH analysis and measurement of mycelial length in the samples.

under accession number *Actinomadura* sp. 392-1 FR853173.

The thermotolerant strain S341f isolated from takyr-like desert soil and identified as *Streptosporangium* sp. according to its phenotypic characteristics and 16S rDNA sequences was deposited in NCIB GenBank under accession number *Streptosporangium* sp. S341f FR863175.

Comparison of our results with the literature data on actinomycete content in desert soils shows that the number of actinomycetes revealed in the soils of the Atacama desert (northern Chile) by plating of diluted soil suspensions did not exceed thousands CFU/g soil [24]. Higher abundance of mycelial actinobacteria in the desert soils of Mongolia may result from the relative scarcity of organic matter in Atacama soils (not exceeding 0.03%, compared to 0.2 to 2.64% in different Mongolian desert soils) and from its arid climate of the "driest place on Earth" [24], "the most barren region possible", and "the dryness limit for microbial life". According to their phenotypic and phylogenetic characteristics, most Atacama isolates belonged to the genera *Amycolatopsis, Lechevalieria*, and *Streptomyces* [24].

Actinomycetes of the genus *Geodermatophilus* were isolated from the Negev desert soils (Israel) by plating soil dilutions on solid media and identified based on their phenotypic characteristics [25]. Actinomycetes morphologically resembling the cultured forms of *Geodermatophilus, Actinoplana*, and *Streptomyces* were detected in the Mojave desert soil along the California–Nevada border by selective chemoattraction [26].

Observation of the dynamics of mycelial development in microcosms with the brown desert steppe soil suggested that moderate thermophiles actively grew and reproduced under these conditions, undergoing the entire life cycle. In the course of succession initiated by humidification of the soils, the dynamics of mycelial length and biomass were similar for the mesophilic and thermotolerant actinomycetes. Mycelial length and biomass were of the same order of magnitude for the thermophilic and mesophilic actinomycetes, although in the case of thermophilic actinomycetes, the decrease in mycelial length and biomass by the end of the experiment (17th day) was more drastic. The highest biomass of thermophilic actinomycetes (14.2 μg/g) was observed in takyr-like soil on the fourth day of the experiment. The highest biomass

Table 2. Phylogenetic characterization of actinobacteria from Mongolian desert steppe soil incubated at 28°C (DGGE)

Unicellular

Accession no.	Name	Similarity, %
NC_013441.1	*Gordonia bronchialis* DSM 43247	78
AEUD01000036.1	*Gordonia neofelifaecis* NRRL B-59395 Scaffold36	78
NC_010397.1	*Mycobacterium abscessus* ATCC 19977 chromosome chromosome 1	78
AEAU01000076.1	*Corynebacterium variabile* DSM 44702 CVARI_00080	77
AAMN01000002.1	*Janibacter* sp. HTCC2649 1099316001545	77
NC_014246.1	*Mobiluncus curtisii* ATCC 43063	77
ACKW01000035.1	*Mobiluncus mulieris* ATCC 35243 contig00060	77
NC_014158.1	*Tsukamurella paurometabola* DSM 20162	77
NC_003450.3	*Corynebacterium glutamicum* ATCC 13032	77
NC_013172.1	*Brachybacterium faecium* DSM 4810	76
NC_013174.1	*Jonesia denitrificans* DSM 20603	76
NC_013235.1	*Nakamurella multipartita* DSM 44233	76
ACNO01000030.1	*Rhodococcus erythropolis* SK121 contig00145	76

Mycelial

Accession no.	Name	Similarity, %
ADFC02000001.1	*Streptomyces cf. griseus* XylebKG-1 scaffold_1_Cont1	78
ADFC02000002.1	*Streptomyces cf. griseus* XylebKG-1 scaffold_1_Cont2	78
ADFD01000039.1	*Streptomyces* sp. ACTE ctg00036	78
ABYX01000136.1	*Streptomyces roseosporus* NRRL 11379 cont3.136	78
NZ_CM000913.1	*Streptomyces clavuligerus* ATCC 27064	78
NC_010572.1	*Streptomyces griseus* subsp. *griseus* NBRC 13350	78
NC_013929.1	*Streptomyces scabiei* 87.22	78
NC_003155.4	*Streptomyces avermitilis* MA-4680	78
ACUY02000007.1	*Actinomyces* sp. oral taxon 848 str. F0332	77
NC_013093.1	*Actinosynnema mirum* DSM 43827	77
NC_003888.3	*Streptomyces coelicolor* A3(2)	77
AEDI01000202.1	*Streptomyces violaceusniger* Tu 4113 ctg00307	77
AEYX01000010.1	*Streptomyces griseoaurantiacus* M045 Contig010	77

Table 3. Phylogenetic characterization of actinobacteria from Mongolian desert steppe soil incubated at 45°C (DGGE)

Unicellular		
Accession no.	Name	Similarity, %
NC_013739.1	*Conexibacter woesei* DSM 14684	81
NC_013124.1	*Acidimicrobium ferrooxidans* DSM 10331	80
NC_013235.1	*Nakamurella multipartita* DSM 44233	78
NC_014158.1	*Tsukamurella paurometabola* DSM 20162	78
NC_008578.1	*Acidothermus cellulolyticus* 11B	78
NC_004572.3	*Tropheryma whipplei* str. Twist	79
AEUD01000036.1	*Gordonia neofelifaecis* NRRL B-59395 Scaffold36	82
NC_010397.1	*Mycobacterium abscessus* ATCC 19977 chromosome chromosome 1	82
ACLH01000041.1	*Corynebacterium aurimucosum* ATCC 700975 contig00103	78
AEEE01000021.1	*Mobiluncus curtisii* subsp. *curtisii* ATCC 35241 contig00039	81
ACNO01000030.1	*Rhodococcus erythropolis* SK121 contig00145	81
AEGE01001139.1	*Pseudonocardia* sp. P2 PP201241	81
Mycelial		
Accession no.	Name	Similarity, %
NC_013131.1	*Catenulispora acidiphila* DSM 44928	82
AEYC01000092.1	*Saccharopolyspora spinosa* NRRL 18395 contig_92	81
ADFC02000001.1	*Streptomyces cf. griseus* XylebKG-1 scaffold_1_Cont1	80
ADFD01000039.1	*Streptomyces* sp. ACTE ctg00036	80
ADFC02000002.1	*Streptomyces cf. griseus* XylebKG-1 scaffold_1_Cont2	80
NC_014165.1	*Thermobispora bispora* DSM 43833	80
ABYX01000136.1	*Streptomyces roseosporus* NRRL 11379 cont3.136	80
NC_010572.1	*Streptomyces griseus* subsp. *griseus* NBRC 13350	80
NZ_CM000913.1	*Streptomyces clavuligerus* ATCC 27064	79
NC_013757.1	*Geodermatophilus obscurus* DSM 43160	79
AEDI01000202.1	*Streptomyces violaceusniger* Tu 4113 ctg00307	79
NC_003155.4	*Streptomyces avermitilis* MA-4680	79
NC_013929.1	*Streptomyces scabiei* 87.22	79
ACUY02000007.1	*Actinomyces* sp. oral taxon 848 str. F0332	78
AEYX01000010.1	*Streptomyces griseoaurantiacus* M045 Contig010	78
NC_007333.1	*Thermobifida fusca* YX chromosome	78
NC_003888.3	*Streptomyces coelicolor* A3(2)	78
NC_013093.1	*Actinosynnema mirum* DSM 43827	78
NC_008278.1	*Frankia alni* ACN14a	78
ACFH01000038.1	*Actinomyces urogenitalis* DSM 15434 contig00038	78

of mesophilic forms (11.2 μg/g) was observed in desert steppe light brown saline soil (Fig. 3).

Actinomycete isolates were classified according to their temperature preferences according to the radial growth rates of their colonies. Mesophilic actinomycetes with the highest colony growth rate at 28°C and the growth range from 8 to 37°C, not growing at 45°C, were revealed, as well as thermotolerant ones with the optimal radial rate of colony growth at 37°C and the growth range from 20 to 60°C. *Streptomyces* sp. 315 isolated from Mongolian gray-brown desert soil, which had the highest colony growth rate at 45°C, grew well at 55 and 37°C, and grew poorly at 28°C, was assigned to moderate thermophiles. *Streptomyces aureofaciens* strain K-6, which had the growth optimum at 50°C, grew well at 55 and 37°C, and grew poorly at 28°C, was assigned to moderate thermophiles. *Streptomyces aureofaciens* strains K-6 and K-5, which had the growth optima at 50 and 45°C, respectively, grew well at 55 and 37°C, and grew poorly at 28°C, belonged to moderate thermophiles.

Investigation of the germination of the spores of the moderately thermophilic *Streptomyces* sp. 315 on the slides in desiccators with different humidity values at 28 and 45°C for 72 h showed that the spores germinated at all water activity values investigated (a_w 0.50, a_w 0.86, and a_w 0.98).The value of the coefficient on the graph of spore germination averaged for all a_w levels shows that spore germination at 45°C was twice as rapid than at 28°C (Fig. 4). Thus, *Streptomyces* sp. 315 is moderately thermophilic and xerotolerant.

Investigation of the taxonomic structure of the prokaryotic microbial community of desert steppe soils by in situ hybridization with rRNA-specific fluorochrome-labeled oligonucleotide probes (FISH) showed that metabolically active eubacteria constituted a significant portion of the total bacterial biomass (18 to 59%) in the complex of most soils incubated at 28°C. The share of metabolically active bacteria in the prokaryotic complex of these soils after incubation at 45°C varied from 25 to 82%. Only in exceptional cases (in desert steppe brown saline soils) metabolically active eubacteria constituted less than 10% of the total prokaryotic community. The share of the metabolically active *Actinobacteria* among the metabolically active *Bacteria* members was one-third to a quarter.

FISH analysis combined with the measurement of mycelial length revealed that mycelial forms prevailed among the metabolically active *Actinobacteria*. Unicellular bacteria constituted a smaller portion of the metabolically active actinobacteria. This pattern was observed in the prokaryotic complex of all the soils under study (Fig. 5).

DGGE analysis of the samples revealed the *Actinobacteria* members (Tables 2 and 3). The analyzed sequences from total DNA from soil incubated at 28 and 45°C belonged to mycelial and unicellular actinobacteria. The translated sequences exhibited over 80% similarity to the known sequences from the clone library.

Thus, molecular biological techniques (FISH and DGGE) confirmed the presence of members of the *Actinobacteria* in desert soils.

Actinobacteria have been detected in desert soils by other researchers. For example, the clones related to *Frankia* have been retrieved form the Atacama soils [27].

Investigation of the desert soils demonstrated high abundance of mycelial actinobacteria, with actinobacterial isolates often adapted to high temperature, high salt concentration, and radiation [4, 28]. The moderately thermophilic xerotolerant actinomycete *Streptomyces* sp. 315 was isolated form Mongolian gray-brown desert soil.

Thus, investigation of the actinomycete complexes in the soils of the desert steppe zone of Mongolia, which are periodically heated to high temperatures, suggests that these soils are favorable for growth of thermotolerant and moderately thermophilic actinomycetes, which constitute a share comparable to or greater than that of the mesophilic forms. The ability of actinomycete spores to germinate at very low moisture pressure in the environment (–96.4 MPa, a_w 0.50) [29] enables their adaptation to draught conditions. Xerotolerant actinomycetes actively grow and form mycelia in the soils of the arid zone. The share of the metabolically active mycelial forms is higher than that of unicellular actinobacteria.

A broader spectrum of selective techniques used for the isolation of actinobacteria from desert soils and of specific primers for molecular biological investigation will improve our understanding of the diversity of actinobacteria.

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