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SOIL BIOLOGY

# Actinomycetal Complex of Light Sierozem on the Kopet-Dag Piedmont Plain

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**Abstract**—The population density of actinomycetes in the samples of light sierozem from the Kopet Dag piedmont plain (75 km from Ashkhabad, Turkmenistan) reaches hundreds of thousand CFU/g soil. The actinomycetal complex is represented by two genera: *Streptomyces* and *Micromonospora*. Representatives of the *Streptomyces* genus predominate and comprise 73 to 87% of the actinomycetal complex. In one sample, representatives of the *Micromonospora* genus predominated in the complex (75%). The *Streptomyces* genus in the studied soil samples is represented by the species from several sections and series: the species of section *Helvolo-Flavus* series *Helvolus* represent the dominant component of the streptomycetal complex; their portion is up to 77% of all isolated actinomycetes. The species of other sections and series are much less abundant. Thus, the percentage of the *Cinereus Achromogenes* section in the actinomycetal complex does not exceed 28%; representatives of the *Albus* section *Albus* series, *Roseus* section *Lavendulae-Roseus* series, and *Imperfectus* section belong to rare species; they have been isolated not from all the studied samples of light sierozem, and their portion does not exceed 10% of the actinomycetal complex.

*Keywords:* light sierozem, mycelial actinobacteria (actinomycetes)

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## INTRODUCTION

Altitudinal sequence of soils in the soil cover is observed in the Kopet-Dag Mountains. This is caused by the change of climatic conditions with altitude: the increase of precipitation amount and decrease of air temperature.

Kopet-Dag piedmont plain is one of the main regions of irrigated farming in Turkmenistan. Sierozems (Haplic Calcisols) are typical soils of mountains and occur up to the altitude of 1500 m. They are formed on loesses. The belt of mountain brown (cinnamonic) soils is situated above 1500 m. Sierozems are the most fertile soils of Turkmenistan. However, rugged topography and water deficiency limit wide using of these soils in agriculture. Soils lose their plant cover owing to cattle overgrazing, and this causes desertification on the plains and increase of probability of mudslides in mountains. Soil erosion, which is especially intense in the piedmont belt of the Kopet-Dag Range, is also dangerous.

Sierozems of Kopet-Dag piedmont plain with humus content from 1 to 3–4% and high carbonate content occupy the piedmont plains and lower mountain slopes. Light sierozems are common in the piedmont, typical sierozems occupy lower mountain belt, and dark sierozems confined to higher altitudes. Cinnamonic mountain soils are developed on the highest parts of mountain plateaus and ranges of Kopet-Dag and Kugitangtau Ranges [2].

Desert foothills and piedmont plains of Kopet Dag are dominated by low-shrub plant communities: white and silky wormwood species and ephemeral herbs. Herbaceous plants of ephemeroid type (bulbous bluegrass, thick-stem sedge, and ferula) and ephemeras are spread in Kopet-Dag piedmont plains and foothills. One can see feather-grass and wheat grass steppes on the plateaus and gentle slopes in the middle and upper mountain belts (beginning from 1000 m and higher); juniper groves start at the altitude of 1500 m. The gorges in the Western Kopet-Dag Range are abound in wild fruit trees and shrubs (grapes, apple, hawthorn, cherry plum, almond, pomegranate, walnut, fig, and wild nard).

## OBJECTS AND METHODS

The samples studied were taken from the upper horizon of light sierozem (pH of water extract 8, humus content 1.5%), in the Kopet-Dag piedmont plain at a distance of 75 km from Ashkhabad, Turkmenistan, (sample numbers 1, 5, 7, and 10), as well as soil material from the rhizosphere of plants (sample numbers 3, 4, and 9) [7].

† Deceased.

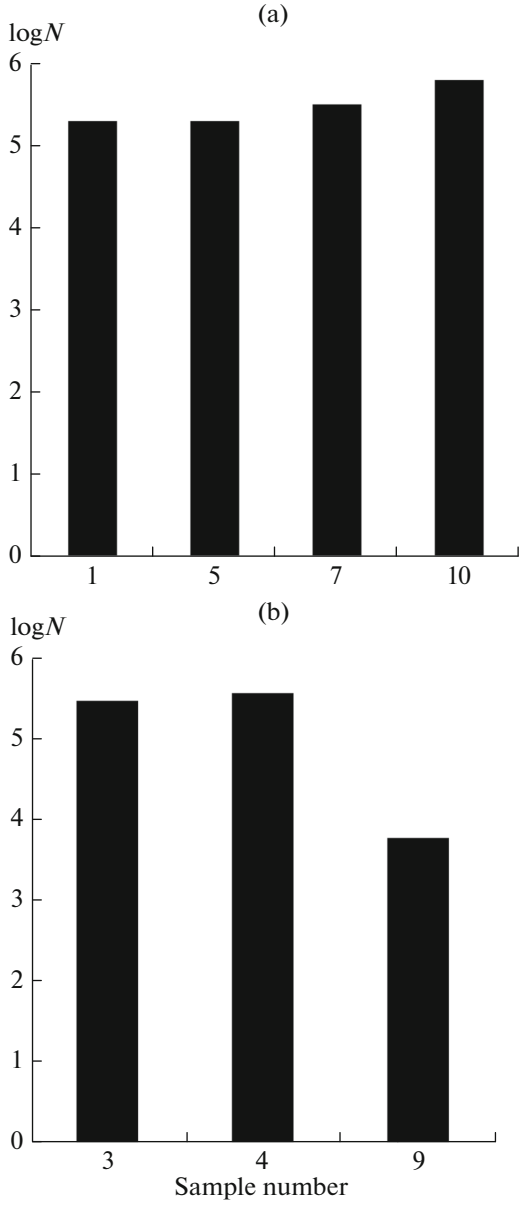


**Fig. 1.** Population density (*N,* CFU/g) of actinomycetes isolated from the samples of (a) light sierozem and (b) rhizosphere of light sierozem.

The traditional method of inoculation on the agar mineral medium Gauze–1 from the dilutions of soil suspension was used for differentiated accounting and isolation of actinomycetes from the studied soils [1]. The air-dry soil samples were used for inoculation.

The Nystatin antibiotic (50 μg/mL of medium), inhibiting the growth of microscopic fungi), and complex of B vitamins group (5 μg/mL of medium) were added. The cultures were incubated in the thermostat under temperature 28°C during 4–6 days on the Gauze—1 media and humus-vitamin agar, and during 2–3 weeks on the medium with sodium propionate [2–5].

Molecular method of hybridization in situ (FISH method, fluorescent in situ hybridization) was used to evaluate the biomass of metabolically active cells of *Bacteria* Domain and separate the phylogenetic group of *Actinobacteria*. Preparations with fluorescence-labelled probes were processed according to the method [8]. The rRNA-specific fluorescence-labelled oligonucleotide probes were used with nucleotide sequence for *the Bacteria* Domain: 5' GCT GCC TCC CGT AGG AGT 3' and for the *Actinobacteria* group: 5' TAT AGT TAC CAC CGC CGT 3' in combination with not labelled oligonucleotide 5' TAT AGT TAC GGC CGC CCGT-3'.

Actinomycetes were preliminarily identified during microscopic examination of colonies in the dishes with nutrient medium with subsequent isolation of representatives of dominant morphotypes into pure cultures on oat agar [4]. The following morphological characteristics were taken into account for the preliminary identification of actinomycetes cultures isolated by inoculation on the nutrition media from the soil: presence/absence of mycelium fragmentation, spore formation on substrate and/or aerial mycelium, number of spores in chains, and presence of single spores and sporangia [9]. Isolated strains of actinomycetes were identified by phenotypic (cultural, morphological, chemotaxonomic, and physiological) characteristics [9].

## RESULTS AND DISCUSSION

Population densities of actinomycetes isolated from the studied samples of light sierozem onto the mineral medium Gauze–1 comprised hundreds of thousand CFU/g of soil (Fig. 1). The portion of actinomycetes from the total number of bacteria grown on mineral medium Gauze–1 inoculated from soil samples comprised 14 to 55% depending on the sample. Actinomycetic complex in soil samples was presented by two genera: *Streptomyces* and *Micromonospora*. Representatives of *Streptomyces* genus dominated the actinomycetic complexes in three studied samples; they composed 73 to 87% of actinomycetic complex (Fig. 2a). Only in one sample of light sierozem, the actinomycetic complex was numerically dominated by the representatives of *Micromonospora* genus, which comprised 75% of the complex.

The *Streptomyces* genus was presented in the samples by species of following sections and series: section *Helvolo-Flavus* series *Helvolus*, section *Albus* series *Albus*, and section *Cinereus* series *Achromogenes*. Representatives of species of section *Helvolo-Flavus* series *Helvolus* were found in all samples (their number varied from 4 to 57% of the total number of actinobacteria in the studied samples). The species of other sections were presented not in all samples. For example, the portion of species – representatives of *Cinereus Achromogenes* section in actinomycetic complex of the studied samples did not exceed 28%; representatives of

section *Albus* series *Albus*, section *Roseus* series *Lavendulae-roseus* and *Imperfectus* belonged to rare species in the studied soils; they were found not in all samples, and their portion did not exceed 10% of actinomycetic complex.

The number of mycelial actinobacteria amounted to hundreds of thousands CFU/g in the rhizosphere. The sample of the soil rhizosphere 9, taken in the rhizosphere of *Halocharis hispida*, where the number of actinomycetes grown on mineral medium Gauze–1 amounted to thousands CFU/g of soil, was an exception. The number of actinomycetes comprised almost half of bacterial community in the samples of the rhizosphere of Kashgar tamarisk (*Tamarex hispida*) and *Aeluropus repens* (39 and 46% respectively). In the sample 9 of the rhizosphere of *Halocharis hispida*, actinomycetes comprised only 1% of bacterial community in light sierozem. Actinomycetic complex in rhizosphere soil taxonomically was characterized by domination of the representatives of *Micromonospora* genus (Fig. 2b). Representatives of *Streptomyces* genus were found only in one sample of the rhizosphere soil under *Aeloropus repens*, and they comprised only 15% of bacterial community population, and were presented by the species of one section *Albus* series *Albus*. The *Streptomyces albolongus* species was the most abundant streptomycete species in this sample of the rhizosphere soil.

Salinization in sierozems of the studied region is usually of the sulfate type. Population density of actinomycetes (hundreds of thousand/g of soil), isolated by inoculation on Gauze–1 medium in the light sierozem of Kopet-Dag piedmont plain with sulfate character of salinization was similar to that in steppified desert light brown salinized soil of Mongolia with the same type of salinization [6]. The study of prokaryotic microbial communities in two samples of light sierozem (soil sample 5 and rhizosphere soil 9) with the hybridization in situ method with the help of 16S rRNA specific oligonucleotide probes, determining representatives of phylogenic group of *Actinobacteria*, demonstrated that the biomass of metabolically active representatives of this group comprised 34% in the rhizosphere soil (sample 9), and 29% (sample 5) at the depth of the 0–5 cm in the common soil sample (sample 5) of biomass of all metabolically active bacteria in prokaryotic microbial communities in sierozems. Metabolically active mycelial actinobacteria were more abundant in comparison with one-cellular bacteria, and domination of mycelial actinobacteria over one-cellular ones was less pronounced in the rhizosphere soil.

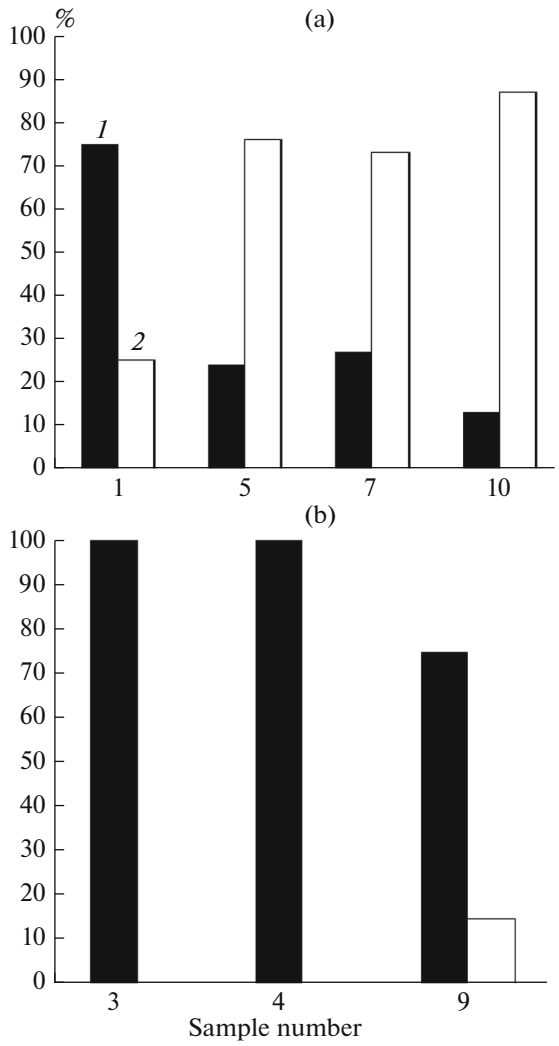


**Fig. 2.** The ratio between population densities of separate genera of actinomycetes isolated from the (a) light sierozem and (b) rhizosphere of light sierozem: *1—Micromonospora* and *2—Streptomyces*.

## CONCLUSIONS

It was found that mycelial actinobacteria (actinomycetes) comprised 14 to 55% of prokaryotic microbial complex in the samples of light sierozem and were presented by *Streptomyces* and *Micromonospora* genera. The *Streptomyces* genus was presented in actinomycetic complex of the studied samples of light sierozem by species of sections and series *Helvolo-Flavus Helvolus* and *Albus Albus*, while the species of sections and series *Cinereus Achromogenes*, *Cinereus Chromogenes*, and *Roseus Lavendulae-roseus* were less typical. Representatives of *Micromonospora* genus were presented in every studied sample of light sierozem, but they dominated only in sample 1 (up to 75% of all isolated actinomycetes). Most isolated cultures of streptomycetes were halotolerant; their growth was recorded on the medium with 7% NaCl.

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