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ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM DIFFERENT SOIL TYPES IN THE KARABAKH REGION OF THE REPUBLIC OF AZERBAIJAN

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Abstract

The distribution and antimicrobial properties of microorganisms, especially actinomycetes, in the various soil types in the Karabakh region was studied. The richest soil sample in actinomycetes was the soil of Shusha, which contained 46 strains. Samples taken from the soils of Gubadli and Fizuli contained 22 and 20 strains of actinomycetes, respectively. Among them, both white and gray aerial spore-forming mycelia were observed. The studied actinomycetes exhibited varying levels of antimicrobial activity, which was most pronounced against the Gram-positive bacterium *Bacillus subtilis*. All nine examined strains, based on their morphological and cultural traits, belongув to the genus *Streptomyces*.

Keywords: *Karabakh soi lmicroorganism;, actinomycetes; antimicrobial activity; test organisms*

1. Introduction

As is well known, microorganisms, especially actinomycetes, and the biologically active substances and antibiotics they synthesize are widely used in various fields. Recent studies have shown that pathogenic microorganisms do not possess sufficient antagonistic resistance to various diseases. Soil is a major reservoir for many microorganisms. Various factors can influence soil microorganisms, or directly affect the internal environment of the cell. It is rare to distinguish between these two forms of influence. The question of the distribution and role of actinomycetes in the soil is highly relevant. Soil studies should include an examination of the characteristic representatives of the microbial communities in these habitats. On the other hand, the study of soil actinomycetes is undoubtedly of significant importance for biotechnologists. The search for producers of new antibiotics and enzymes with specific pH activity optima, the use of actinomycete populations for biocontrol and bioremediation, and the fight against phytopathogenic fungi are practical tasks for which knowledge of actinomycetes can be highly valuable [2].

Actinomycetes are producers of a variety of biologically active compounds with diverse chemical structures, exhibiting antibacterial, antifungal, and antitumor activities. Most antibiotics have been isolated from actinomycetes, particularly from the widely distributed genus *Streptomyces*. *Streptomyces* are fast-growing microorganisms, easily isolated from natural sources, and simple to cultivate. Currently, researchers are focusing on the isolation and study of representatives from rare genera of actinomycetes, which may serve as potential producers of new, yet-to-be-explored antibiotics [3, 4]. The aim of this study is to

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investigate the antimicrobial properties of actinomycetes isolated from various soil areas in the Karabakh region.

2. Materials and Methods

The objects of the study were actinomycetes isolated from various soils of the Karabakh region (Shusha city, and the districts of Gubadly and Fuzuli).Microbiological analysis of the soil samples was conducted using the dilution method. To isolate and maintain the actinomycetes, we used the mineral agar medium of Gauze 1 [5]. Identification was carried out based on morphological and cultural characteristics. The selected actinomycetes were studied for the shape of the spore-bearing structures, the surface characteristics of spore coats, the coloration of aerial mycelium, the coloration of substrate mycelium, and the presence of soluble pigments [1, 6].

The antibiotic activity of the isolated actinomycetes was evaluated using the agar block method [7], with *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella sp.*, and *Acinetobacter baumannii* as test cultures. The nutrient medium used for inoculating these test cultures was meat-peptone agar (MPA). The antibiotic activity was assessed based on the zone of lysis around the agar block.

3. Results and discussion

The study showed that actinomycetes are widespread to varying degrees in the soil samples taken from different areas of Karabakh (Table 1).

Table 1. Distribution of Actinomycetes in Various Soils of the Karabakh Region of the Republic of Azerbaijan

Soil Sample (Collection Zones)	Soil Type	Total Number of Microorganisms (CFU/g)	Number of Actinomycetes	
			(CFU/g)	(CFU/%)
Shusha	Gray Meadow Soil	56	46	82
Gubadly	Mountain ForestSoil	38	22	57
Fuzuli	Chestnut Soil	37	20	54

As seen from the data in Table 1, the highest number of microorganisms and actinomycetes was observed in the soil samples taken from the city of Shusha (gray meadow soils), with a total of 56 CFU/g of microorganisms, of which 46 CFU/g were actinomycetes. In the soil samples taken from the Gubadly district (mountain forest soils), the total number of microorganisms was 38 CFU/g, of which 22 CFU/g were actinomycetes. In the soil samples from the Fuzuli district (chestnut soils), the total number of microorganisms was 37 CFU/g, with 20 CFU/g being actinomycetes. Thus, the total number of microorganisms and actinomycetes depends on the soil type.

Soil samples from different areas of Karabakh exhibit distinct actinomycete landscapes. In the soil taken from the city of Shusha, actinomycetes with white aerial mycelium predominated (Fig. 1).

Fig. 1. Actinomycete cultures isolated from the soils of Shusha city

In the soils taken from the Fuzuli and Gubadly districts, actinomycetes with gray aerial mycelium predominated. It was found that the soil type affects not only the number of actinomycetes but also the pigment-producing properties of their aerial mycelium, which is taken into account during their classification (Fig. 2, 3).

Fig. 2. Actinomycete cultures isolated from the soils of the Fuzuli district.

Fig. 3. Actinomycete cultures isolated from the soils of the Gubadly district.

The actinomycete colonies were subcultured onto test tubes with slanted mineral agar of the Gauze 1 medium. Thus, the isolated strains were obtained in pure culture. During the study, 88 actinomycete strains were isolated from the examined soil samples, of which 73 strains were cultured for further investigation. The antimicrobial properties of the selected strains were tested against pathogenic test cultures. Antibiotic activity was assessed based on the zone of lysis around the placed agar block.Among the isolated strains, antimicrobial activity was investigated in 9 strains: Sh1, Sh2, Sh3, G1, G2, and G4, F1, F3, and F4.

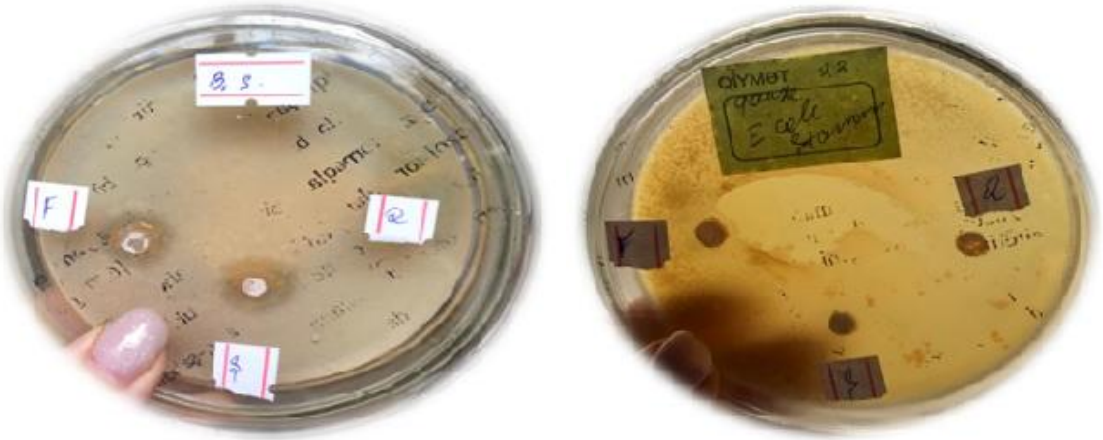
*Escherichia coli***,** *Bacillus subtilis***,** *Klebsiella sp*.**,** *Acinetobacter baumannii***,** *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were used as test cultures. The antimicrobial activity of the studied strains exhibited varying levels of activity against different test cultures. The results of the study are presented in Table 2. The actinomycete strains differed in their antimicrobial activity. The studied actinomycetes exhibited varying levels of antagonistic activity, which was more pronounced against Gram-positive bacteria. The highest activity was observed against the Gram-positive bacterium *Bacillus subtilis*.

All tested strains were active against this bacterium, but the most active were the strains Sh2, F4, and G4 (the diameter of the lysis zone was 16 mm). Against *Staphylococcus aureus*, 8 strains demonstrated activity, with the lysis zone ranging from 8 to 14 mm. It should be noted that the selected strains did not show high antibiotic activity against the Gram-negative *Escherichia coli*. Only one strain, Sh1, exhibited antimicrobial activity, but it was very weak (the diameter of the lysis zone was 2 mm). All strains demonstrated antimicrobial activity against *Pseudomonas aeruginosa,* with the lysis zone ranging from 6 to 14 mm.

Table 2. Antimicrobial Properties of Actinomycetes Isolated from Different Soil Types in Karabakh

Strains	Test Cultures (lysis zone, mm)					
	E.coli	*Staph. aureus*	*Bac. Subtilis*	*Pseud. aeruginosa*	*Klebsella sp.*	*Acinetob. baumanii*
Sh1	2	14	14	11	9	12
Sh2	-	6	16	14	16	10
Sh3	-	8	12	9	14	8
F1	-	8	12	10	-	8

F3	-	-	15	12	10	9
F4	-	12	16	12	-	10
G1	-	14	8	8	-	8
G2	-	12	12	10	-	9
G4	-	14	16	6	12	10



1-Bacillus subtilis *2-Escherichia coli*

Fig. 4. Antimicrobial activity of actinomycete strains against

Against *Klebsiella sp.* 5 out of 9 strains demonstrated antimicrobial activity, with the lysis zone ranging from 9 to 16 mm. Among the selected strains, only one (Sh1) exhibited antagonistic activity against all test cultures. Thus, based on this study, it can be concluded that the 9 investigated actinomycete strains, isolated from various soil types in Karabakh, exhibit antibiotic activity to varying degrees, with the highest activity observed against Gram-positive bacteria (*Bacillus subtilis*). All 9 studied strains, based on their morphological and cultural characteristics, belong to the genus *Streptomyces*.

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