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Primary Screeningof Substanceswith Potential Antitumor Activity

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ABSTRAC
A primary screening of antitumor substances was carried out among strains of actinomycetes isolated from the samples of natural substrates of arid zones in the Ile-Balkhash region. Antitumor properties of actinomycetes against *Staphylococcus aureus*209P(*S. aureus* 209P) and its mutants UF-2 and UF-3 were studied using the agar block technique. The diameter of growth inhibition zone was measured after incubation of the test microorganisms at a temperature of 37 °C for 24 hours.
16 strains of actinomycetes (10,2%) from sandy soils and from the plant rhizosphere of the Kapshagai area virtually having no effect or only slightly affecting the growth of wild-type culture.22 strains of actinomycetes (14,0%) from takyrs and takyr-like soils of the Balkhash area had an activity against staphylococcal mutants two or more times higher than against the stock staphylococcal strain.This strains may be the potential producers of antitumor antibiotics.It was established that 24.2% of the strains of actinomycetes may be potential producers of antitumor antibiotics.
***Keywords*:**antitumor antibiotics, actinomycetes, arid zones, bacterial test systems, UF-2 and UF-3 mutants of S. aureus 209P.

I. INTRODUCTION

The fight against malignant tumors ranked in one of the first places among the causes of mortality in almost all countries of the world is one of the major challenges facing the present-day medicine [1, 2]. Currently in our country and abroad, the studies are being carried out targeted at finding active antitumor antibiotics [3, 4].The screening technique involving physicochemical and bacterial test systems is important to solve the problem as the most cost effective approach to testing a large number of substances. If bacteria and fungi can be directly used in the experiments as test organisms when being cultured in common laboratory media, the search for antitumor antibiotics requires living cells and tissues. Currently, there is no unified standard screening procedure for antitumor agents [5].

A number of indirect research methods are based on the *in vitro* use of various biological objects – cultures, microorganisms and other biological systems that are capable of detecting growth inhibitors of various origins. As a result of the action of ultraviolet radiation and urethane able to obtain mutants*Staphylococcus aureus* 209R (*S. aureus* 209P), *Bacillus subtilis*(*B. subtilis*), *Escherichia paracoli*, *Escherichia coli* with oxidation disorder resembling oxidation in the cancer cell. Comparing the sensitivity of the method with Ehrlich ascites cells and a method of biochemical mutants of microorganisms, it must be concluded that the biochemical mutants - more sensitive tests to determine the anti-tumor action of microorganisms [6].

Using bacterial test systems allows primary screening of a large number of substances in a short time with their further study in the classical test systems is very important for oncology and of great theoretical significance and applied relevance. Using a biochemical staphylococcal mutant UV 3, 4 new antibiotics have been successfully isolated from the most active producers: 1832 and 2169 (*Bac. cereus* as a producer), antibiotic 17 (*Bac. mycoides* as a producer), and antibiotic 4957 [7].

The main objective was to identify the producers of biological inhibitors among soil actinomycetes,

which could be considered as potential antitumor antibiotics. In this connection, the normal staphylococcal culture and its mutants with defective respiration were used. Used *in vitro* assays were meant for the primary detection of new biological growth inhibitors in order to subsequently concentrate and partly purify these antibiotics and thereby prepare them for testing in the *in vivo* experiments on animals.

Therefore, the most intensive search for antitumor antibiotics is carried out among this group of ray fungi. Soil samples from around the world are constantly analyzed in search of the new superpotent antibiotics. One of the most productive sources of antibiotics is a genus *Streptomyces* [8].

II. MATERIALS AND METHODS

The strains of actinomycetes isolated from the samples of natural substrates of arid zones in the Ile-Balkhash region and showed activity against the gram-positive test microorganism *Bacillus subtilis* ATCC 6633 were chosen as the objects of the study.

Antitumor properties of actinomycetes against *S. aureus* 209P and its mutants UF-2 and UF-3 were studied using the agar block technique [7]. The diameter of growth inhibition zone was measured after incubation of the test microorganisms at a temperature of 37 °C for 24 hours.

Staphylococcal mutants have an altered metabolism, approximate to the tumor cell, with oxidative defect resembling oxidation in a cancer cell and are of interest as the bacterial models for primary screening of antitumor drugs. The ability of the drug under study to inhibit the growth of the mutant culture is considered to be a desired effect, virtually having no effect or only slightly affecting the growth of wild-type culture

In the mathematical data processing, the standard methods for finding the means and their mean errors were used [9]. Statistical significance of the results obtained was determined by Student t-test. Differences were considered statistically significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

In the first stage of the study, 157 strains of actinomycetes were selected, having activity against gram-positive test microorganism *B.subtilis* ATCC 6633. These strains were examined for activity against *S. aureus* 209P and its mutants UF-2 and UF-3. Antibacterial activity of actinomycetes against the investigated staphylococci is presented in Tables 1 and 2.

36 strains of actinomycetes from sandy soils, and 6 strains from the plant rhizosphere showed activity against *S. aureus* 209P. High activity was observed in 12 strains (28,6%) - the diameter of the test culture inhibition zone was more than 50 mm. The most active were the strains isolated from sandy soils of the Kapshagai area - K8/5 (60 mm), K11/3 and K6/9 (58 mm), and a strain B10/3 (58 mm) from the Balkhash area. 10 strains of actinomycetes revealed bacteriostatic action, 7 strains had a diameter of growth inhibition zone of 0-10 mm.

31 of 47 actinomycete strains isolated from the sandy soil and rhizosphere of sand plants, showed an effect on the stock and mutant cultures at the same time; 11 strains (K1/4, R2/10, R1/3, R2/7 K7/14, K10/7, K11/2, B1/4, B10/2, B 6/4, B1/9) exhibited high activity against mutants and low against staphylococcus; 5 strains (K2/7, K11/2 B 6/4 K1P2, K4P3) exhibited selective antagonistic effects on biochemical mutants of bacteria without affecting the stock staphylococcal strain (Table 1).All actinomycetes from takyrs and takyr-like soils of the Balkhash area showed antimicrobial activity against *S. aureus* 209P (Table 2).

Table 1 - Antimicrobial properties of actinomycetes isolated from the sandy soils and rhizosphere of sand plants in the Ile-Balkhash region against *S. aureus* 209P and UF-2 and UF-3 mutants of *S. aureus*209P

Strain number	Diameter of the test microorganism growth inhibition zone, mm		
	*S. aureus*209P	Mutants of *S. aureus*209P	
		UF-2	UF-3
Sandy soils			
K1/4	17±0,3	44±0,1	45±0,2
K11/6	54±0,2	43±0,2	42±0,1
K2/2	17±0,1	28±0,1	30±0,3
K1/3	18±0,2	48±0,3	46±0,3
K2/7	0	40±0,2	42±0,1
K11/3	58±0,4	40±0,1	40±0,2
K1/10	54±0,1	43±0,1	42±0,1
K11/1	55±0,2	48±0,2	49±0,2
K6/9	58±0,2	58±0,3	60±0,2
K7/3	50±0,1	48±0,1	48±0,3
K9/4	40±0,1	68±0,2	67±0,1

K6/5	50±0,3	58±0,2	57±0,4
K2/10	20±0,4	38±0,1	40±0,2
K11/5	50±0,1	58±0,3	60±0,1
K2/17	43±0,2	50±0,1	50±0,2
K2/11	40±0,1	20±0,3	22±0,1
K7/4	40±0,2	55±0,3	57±0,2
K8/5	60±0,1	58±0,1	59±0,1
K11/9	48±0,2	50±0,1	48±0,1
K6/5	50±0,3	58±0,3	58±0,3
K11/16	44±0,1	48±0,3	46±0,2
K11/7	50±0,1	50±0,2	52±0,2
K9/2	25±0,4	40±0,1	44±0,1
K9/5	32±0,1	48±0,2	48±0,1
K7/14	14±0,2	54±0,1	56±0,2
K10/7	17±0,1	44±0,1	45±0,1
K11/2	0	20±0,2	23±0,2
B10	45±0,2	56±0,2	58±0,1
B6/2	46±0,3	40±0,1	42±0,3
B1/4	20±0,1	45±0,3	48±0,2
B10/3	58±0,1	65±0,3	67±0,2
B1/2	21±0,3	20±0,1	23±0,1
B10/2	10±0,1	20±0,2	22±0,1
B2/4	44±0,2	40±0,1	42±0,2
B1/6	25±0,1	32±0,1	35±0,3
B6/12	20±0,3	24±0,2	25±0,1
B6/4	0	20±0,3	21±0,1
B1/9	10±0,1	40±0,1	42±0,3
B2/1	34±0,2	32±0,1	32±0,2
Sandplantrhizosphere			
K5R5	15±0,2	42±0,1	40±0,1
K1R2	0	35±0,3	37±0,2
K6R3	20±0,2	50±0,2	50±0,2
K4R3	0	28±0,2	30±0,1
B1R12	30±0,1	34±0,1	36±0,3
B3R5	40±0,1	50±0,3	52±0,1
B2R2	30±0,3	40±0,1	43±0,3
B1R11	25±0,1	20±0,1	18±0,1

Table 2 - Antimicrobial properties of actinomycetes isolated from the soils and plant rhizosphere of the clay deserts in the Balkhash area against *S. aureus* 209P and UF-2 and UF-3 mutants of *S. aureus*209P

Strain number	Diameter of the test microorganism growth inhibition zone, mm		
		Mutants of *S. aureus*209P	
	*S. aureus*209P	UF-2	UF-3
Takyrs			
T3/1	23±0,2	25±0,1	26±0,1
T5/2	30±0,1	60±0,2	61±0,3
T1/9	28±0,3	68±0,1	65±0,1
T6/10	43±0,3	40±0,2	43±0,2
T1/7	36±0,1	70±0,3	70±0,1
T2/8	24±0,2	28±0,1	30±0,2
T6/7	43±0,1	30±0,1	33±0,2
T3/3	20±0,2	28±0,3	30±0,1
T1/11	18±0,1	40±0,1	38±0,1
T2/7	28±0,1	38±0,1	38±0,1
T2/2	35±0,3	40±0,2	45±0,2
T6/11	10±0,1	44±0,3	42±0,1
T6/1	46±0,2	48±0,2	50±0,3

T1/10	27±0,2	35±0,2	38±0,2
T2/3	25±0,1	20±0,1	22±0,1
T6/8	10±0,3	28±0,1	30±0,2
T3/4	34±0,3	60±0,3	62±0,3
Takyr-like soils			
Tv1/3	10±0,2	24±0,3	25±0,1
Tv2/7	37±0,1	38±0,1	40±0,1
Tv7/1	30±0,3	45±0,1	48±0,1
Tv5/3	17±0,1	48±0,1	50±0,1
Tv7/10	30±0,2	32±0,2	35±0,4
Tv5/1	20±0,2	44±0,1	46±0,1
Tv5/7	17±0,1	48±0,2	46±0,1
Tv3/1	36±0,3	50±0,1	51±0,2
Tv7/2	32±0,1	45±0,3	48±0,1
Tv5/2	20±0,1	44±0,3	45±0,3
Tv7/7	10±0,2	25±0,1	28±0,2
Tv2/6	37±0,2	38±0,1	40±0,2
Tv7/8	30±0,2	45±0,2	48±0,1
Tv5/9	17±0,3	48±0,1	50±0,2
Tv7/6	30±0,1	32±0,2	35±0,1
Tv5/8	20±0,2	44±0,1	46±0,2
Tv5/6	17±0,1	48±0,3	46±0,1
Tv2/3	38±0,1	38±0,1	40±0,3
Tv7/12	30±0,3	45±0,2	48±0,2
Tv5/4	17±0,1	48±0,3	50±0,1
Tv7/6	30±0,2	32±0,1	35±0,2
Tv2/2	36±0,1	70±0,1	68±0,2
Tv5/11	17±0,1	48±0,2	46±0,1
Plant rhizosphere			
Tv2R1	35±0,2	38±0,1	40±0,2
Tv3R3	18±0,1	25±0,3	27±0,3
Tv1R5	22±0,2	25±0,2	28±0,1
Tv2R7	12±0,3	20±0,1	22±0,1
Tv5R2	22±0,1	50±0,3	52±0,1
Tv4R5	0	20±0,1	23±0,3
Tv6R9	20±0,2	38±0,2	39±0,3
Tv1R3	42±0,1	62±0,1	63±0,1

72.2% (13 strains) and 61.5% (8 strains), respectively, had the diameter of the test microorganism growth inhibition zone of more than 20 mm. Strains T6/1 (46 mm), T6/10 (43 mm) and T6/7 (43 mm) isolated from takyrs, and strains and Tv2/7 (37 mm), Tv2/6 (37 mm), Tv2/3 (38 mm), isolated from takyr-like soils, revealed the highest activity (Fig.1).

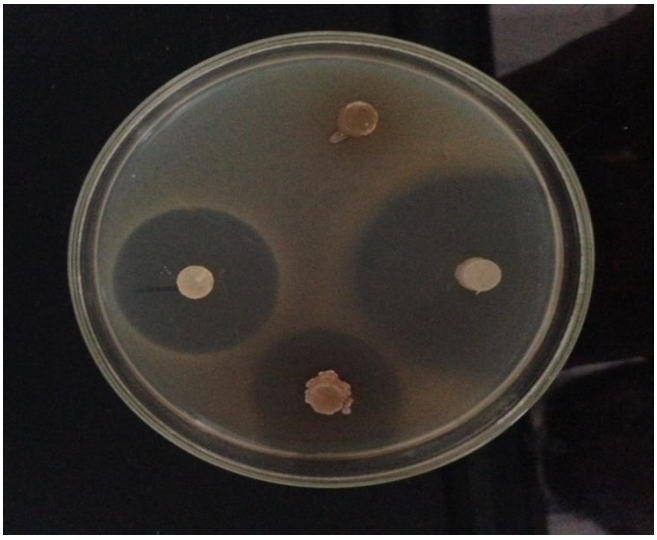

Figure 1 -Activity strains of actinomycetes against mutant UF-3 of *S. aureus*

9 strains of actinomycetes from the plant rhizosphere of takyr-like soils were active against *S. aureus*209R, 2 strains possessed bacteriostatic activity. The highest activity against *S. aureus* 209R was recorded in the strain Tv2R1 (35 mm) isolated from the rhizosphere of the plant *Ferula tataricum*.

All the studied strains were active against UF-2 and UF-3 mutants of *S. aureus* 209P. Strains T1/7 (70 mm), T1/9 (68 mm), Tv2/2 (70 mm) Tv1R3 (62 mm), and Tv5R2 (50 mm)showed a high level of activity.

26 strains exerted an effect on the stock and mutant cultures simultaneously. 21 actinomycete strains - T5/2, T1/9, T1/7, T6/8, T1/11, T6/11, T3/4, T1/3 Tb5/3, Tb5/7, Tb5/1, Tb5/8, T5/2, T7/7, T5/9, T5/6, T5/11, Td2P7, Tb5P2, Tb4P5, Tb6P9 had an activity against staphylococcal mutants two or more times higher than against the stock staphylococcal strain, and one strain - Tb4P5 had selective antagonistic effect on biochemical mutants of bacteria, showing no activity against the stock staphylococcal strain.

Thereby, 10,2% of the strains of actinomycetes from sandy soils and from the plant rhizosphere, and 14,0% of the strains of actinomycetes from takyrs and takyr-like soils of the arid zones in the Ile-Balkhash region had an activity against staphylococcal mutants. This strains may be the potential producers of antitumor antibiotics. The next step in the search for new antibiotics is to test the activity of selected cultures on the stage of culture fluids.

After obtaining the initial antibiotic preparations, the antitumor activity will be tested on a model of antitumor growth - secondary growth of the fungus *Fusariumbulbigenum var. blasticola*, as well as in the cell culture - H9 (*Human lymphoma*) – the human lymphoma. Antibiotics which exhibited activity against gram-positive test microorganisms will be studied with regard to drug-resistant clinical staphylococcal strains.

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