ANTAGONISTIC PROPERTIES OF STREPTOMYCES STRAINS ISOLATED FROM THE TECHNOGENIC SOILS OF KYRGYZSTAN

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The antagonistic properties of Streptomyces bacterium strains against to phytopathogens of agricultural crops were studied. Among the studied strains were selected N3.3/OR; N4KG1; N1KG1; IVOR-K; IOR-K(1), III-OR-K; N2KG2; N3.1/OR as a very strong, wild spectral antibiotic effective antagonists, and the Streptomyces long-ispororuber N6KG2; Streptomyces londisporoflavus N3-OR; Streptomyces albiianatus N2KG1 strains had good antagonistic activities against to Penicillum test-culture. In future, the isolated Streptomyces strains as biotechnologically strong potentiated ones for agricultural production of ecologically clean biological products against to phytopathogens should be recommended.

Keywords: Streptomyces bacterium, strains, test-culture, antagonistic properties, technogenic soils, ISP4 nutrient medium

The antagonistic soil microorganisms by emitting of antibiotics will destroy the cells of phytopathogenic bacterium and hyphae of micromycetic fungus then they will stop their existence in the conditions of nutrient substrate competition. Due to necessity of complex protection of agricultural crops and woods from the phytopathogenic microorganisms, it is important to study microbiological facilities that include antibiotic forming antagonistic bacterium.

Antibiotic forming capacity of Actinomycets is an intensive investigation object of many countries of the world [1, 3, 6]. The antibiotics obtained from Actinomycetes comparatively to chemical preparations are well absorbed, non-toxic, and easy to dissolve in nature, it does not pollute the environment [9, 8]. Several characteristics of microbiological products: selectivity to phytopathogens, high activity allows using them in low concentration; and prevents the accumulation in agricultural products and the environment. Using of antagonistic micro cultures against to phytopathogens will protect the plant from bacterium and fungus development not only during of growing seasons also during of storage of agricultural products and seeds [5].

The keen demand of time – obtaining of high activity and stable cultures of microorganisms became one of the main directions of modern microbiology and biotechnology. Microbiological production need to antagonistic-bacterium obtained by highly effective genetic-engineering method. The antagonistic-bacteria are tolerant to different fungicides at least during of yield conservation, and adapted to hydrothermal conditions of soil.

The aim of investigation is a study of antagonistic features of *Streptomyces* cultures and selection of high effective strains.

Materials and methods of research

17 strains of bacterium belongs to *Streptomyces* family from laboratory collection were used as object of investigation. The used strains were isolated from different types of soils of Kyrgyzstan's mining plant's radioactive residues storage areas – Orlovka, Orlovka-Boordu, Kadzy-Say (table 1). Described Streptomyces bacteria were selected as tolerant indicators (8) to high concentrated heavy metals.

The names of *Streptomyces* culture species, the soil's type and place of isolation, and others are demonstrated in the table 1.

Streptomyces bacteria isolated from the Kyrgystan's technogenic soils were identified by Gauze's and Bergey's manuals (Gauze, 1983; Bergey, 1994).

The antagonistic properties of *Streptomyces* strains were checked by methods of 3th times repetition of on two different nutrient medium (ISP 4 and Chapek), perpendicular lines (Rudakov, 1971), agar blocks (Egorov, 1986).

Spore forming Gram (+) bacterium – *Bacillus subtilis*, micromycetic fungi – *Penicillum sp.* cultures were used as Test-cultures. Antagonistic activity was identified by mm measuring of test-object's destroyed zones.

Results of research and their discussion

To effective show of antagonistic activities the nutrient mediums for creations of optimum conditions for growing of Actinomycets strains and test-cultures were selected.

1. IVOR-K strain of *Streptomyces fumosus* since 6-day of investigation made worse the *Bacillus subtilis* test-culture's growing. During all days of investigation (6; 10; 15) the lysis zones of other test-cultures (*Penicillum sp.*) did not exceed 2 mm (tab. 2, fig. 1, a). On the results of investigation was identified that this strain of *Streptomyces* is a good antagonist

for bacillary bacteria (*Bacillis subtilis),* but not for micromycets fungus. At 6-day of investigation IVOR-K strain made worse the lysis zone of test-culture to 25 mm. This strain of *Streptomyces* bacteria emits the antibiotic extracts in big number that stopped the growing of test-culture.

2. IIOR-K strain of *Streptomyces luridus* at the Chapek nutrient medium after 6 days of investigation made worse the growing of Bacillus subtilis making the lysis zones 7 mm; after 8-15 days size of lysis zone was 10 mm, at ISP 4 nutrient medium during 8-15 days size of lysis zone did not exceed 5 mm. So, Streptomyces IIOR-K strain showed the medium antagonistic influence against to spore-forming bacteria (fig. 1, b). And during of all days (6, 8, 10, 15) of investigation other test-culture Penicillum's lysis zone did not exceed 2 mm (tab. 2). The growing zones of microorganisms were more actively at ISP 4 medium then Chapek ones.

Table 1

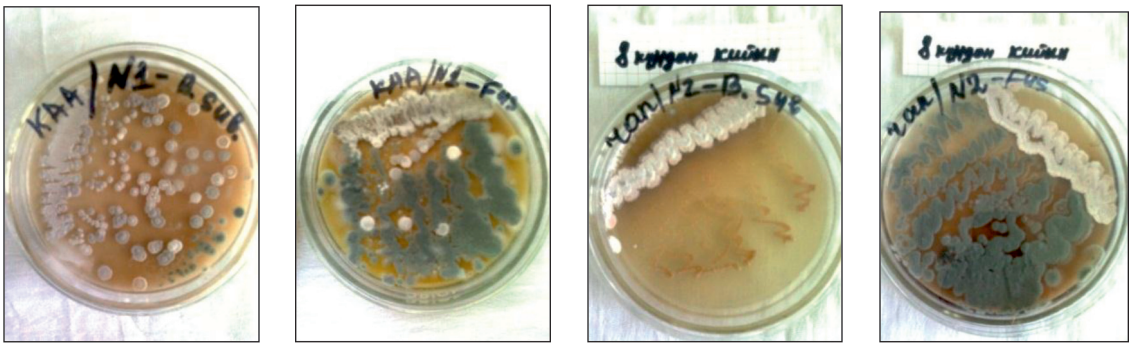
The species content of bacteria belongs to Streptomyces family

N	Collection numbers of Streptomyces cultures	Species names of strains	Sections	Series	Place of strain's isolation
1	IV OR-K	Streptomyces fumosus	Cinereus	Chromogenes	5 km from Boordu radioactive uranus residues storage (Orlovka town, Kemin region), type of soil is light-brown
2	II OR-K	Streptomyces luridus	Roseus	Fradiae	1 km from Orlovka-Kashka residues storage, type of soil is gray
3	N3.2/OR	Streptomyces griseoruber	Cinereus	Violaceus	200 m from Orlovka mining plant
4	N3.1/OR	Streptomyces chromofuscus	Cinereus	Chromogenes	200 m from Orlovka mining plant
5	OR-K(1)	Streptomyces roseochromogenes	Roseus	Fuscus	Orlovka-Bordu restudies storage
6	OR-K(2)	Streptomyces lincolnensis	Roseus	Lavendulae-Roseus	Orlovka-Bordu residues storage
7	N3.3/OR	Streptomyces rubrogriseus	Cinereus	Violaseus	200 m from Orlovka residues storage
8	III-OR-K	Streptomyces mellinus	Roseus	Fradiae	3 km from Orlovka-Bordu residues storage, gray and black soils
9	N3-OR	Streptomyces londisporoflavus	Helvolo-Flavus	Helvolus	Orlovka residues storage
10	ml-3.5	Streptomyces viridogenes	Cinereus	Chrysomallus	Orlovka town, soils around "Jansyz" pond
11	ml-3.6	Streptomyces heliomycini	Cinereus	Aureus	"Jansyz" pond lees
12	N2KG1	Streptomyces albiianatus	Roseus	Fradiae	200 m from Kadzy-Say mining plant
13	N2KG2	Streptomyces tauricus	Roseus	Roseoviola-seus	200 m from Kadzy-Say mining plant
14	N6KG2	Streptomyces longispororuber	Roseus	Ruber	50 km from Kadzy-Say radioactive uran residues storage area (Barskoon v.)
15	N6KG4	Streptomyces steffisburgensis	Azureus	Glaucescens	50 km from Kadzy-Say radioactive uran residues storage area (Barskoon v.)
16	N1KG1	Streptomyces griseomycini	Cinereus	Achromogenes	200 m from Kadzy-Say mining plant
17	N4KG1	Streptomyces chromofuscus	Cinereus	Chromogenes	3-5 km from Kadzy-Say mining plant

Table 2

Antagonistic activity of Streptomyces strains

N	Collection number of Streptomyces culture	ISP4 medium								Chapek nutrient medium							
		Days of investigation (6; 8; 10; 15)															
		Bacillus subtilis				Penicillum sp.				Bacillus subtilis				Penicillum sp.			
		6	8	10	15	6	8	10	15	6	8	10	15	6	8	10	15
1	IV OR-K	–	–	–	–	2	2	2	1	25	25	25	25	2	2	2	2
2	II OR-K	5	5	5	5	2	2	2	2	7	10	10	10	1	1	2	2
3	N3.2/OR	3	5	5	17	1	1	1	1	3	3	3	3	1	1	1	1
4	N3.1/OR	2	2	2	2	1	1	1	1	15	15	15	15	1	1	1	1
5	OR-K(1)	10	11	11	11	2	2	2	2	10	11	11	24	1	1	1	0.5
6	OR-K(2)	9	9	9	9	1	1	1	1	8	8	8	8	2	2	2	2
7	N3.3/OR	12	12	12	12	1	1	1	1	15	40	55	57	1	1	1	1
8	III-OR-K	3	3	17	17	1	2	2	2	5	10	10	11	1	2	2	5
9	N3-OR	4	4	4	4	1	1	1	1	10	12	12	12	1	2	2	5
10	ml-3.5	–	–	–	–	1	1	1	0	15	15	16	16	3	2	2	1
11	ml-3.6	3	9	11	11	1	1	1	1	3	3	3	3	2	2	4	4
12	N2KG1	2	2	2	2	1	1	1	1	3	3	3	3	1	2	2	10
13	N2KG2	–	–	–	–	1	1	1	0	15	15	16	16	0	0	0	0
14	N6KG2	1	1	1	1	35	35	35	35	1	1	0	0	–	–	–	–
15	N6KG4	1	1	1	1	1	1	1	1	–	–	–	–	–	–	–	–
16	N1KG1	24	24	27	27	1	1	0.5	0.5	25	25	25	25	1	1	1	1
17	N4KG1	25	25	50	50	1	1	1	1	18	18	30	30	2	2	2	2



(a) (b) (c) (d)

Fig. 1. Antagonism of IVOR-K strain to 2-test-cultures (a) B. subtilis and (b) Penicillum sp.; lysis zones of IIOR-K strain to (c) B. subtilis and (d) Penicillum sp.

3. N3.2/OR strain of *Streptomyces griseoruber* during 15 days showed antagonistic activity at the ISP 4 nutrient medium more than at Chapek, in other words in 10-15 days was observed thinning of Bacillus subtilis test-culture, lysis zone was 17 mm (fig. 2, a). At the two studied nutrient mediums N3.2/OR, strain did not show antagonistic activity against to Penicillium test-culture, lysis zone did not exceed 1 mm.

4. N3.1/OR strain of *Streptomyces chromofuscus* after 6 days made the lysis zone of Bacillus subtilis 15 mm. Until the last days of investigation, test-culture did not grow into N3.1/OR strain. So, this strain showed the strong antagonistic activity against to spore-forming bacteria (fig. 2, b).

5. OR-K (1) strain of *Streptomyces roseochromogenes* at 15 days made the lysis zone of *Bacillus subtilis* 24 mm. At all days (6; 8; 10; 15) of investigation, the lysis zone of Penicillium was 2 mm. At the both of nutrient mediums the growing zones of actinomycets strain and bacteria were active (fig. 3).

(a) (b) (c)

Fig. 2. The antagonistic activities of N3.2/OR strain against to test-cultures (a) B.subtilis; (b) Penicillium sp. and N3.1/OR strain to (c) test-culture – B. subtilis

(a) (b)

Fig. 3. The antagonistic struggle of OR-K(1) strain against to test-cultures (a) B.subtilis and (b) Penicillium sp.

6. OR-K(2) strain of *Streptomyces lincolnensis* – in 15 days the lysis zone of *Bacillus subtilis* was estimated 9 mm. During of checking days (6; 8; 10; 15) Penicillum test-culture's lysis zone did not exceed 1-2 mm; it means that both of investigated strain and Penicillum have a similar antagonistic capacity at ISP 4 and Chapek nutrient mediums.

7. N3.3/OR strain of *Streptomyces rubrogriseus* – at Chapek nutrient medium the lysis zone of Bacillus subtilis after 6 days was 15 mm, after 8 days was 16-40 mm, in 10 days was estimated 55 mm, after 15 days was 57 mm. In other words, the test-culture is almost completely destroyed. At ISP 4 nutrient medium in 15 days, *Basillus subtilis* retreated to 12 mm. During of checking days (6; 8; 10; 15) Penicillum test-culture's lysis zone did not exceed 1mm. The growing zones of actinomycets were more active at Chapek than ISP 4 medium (tab. 1).

8. IIIOR-K strain of *Streptomyces mellinus* in 15 days showed more antagonistic activity on ISP 4 nutrient medium than Chapek. In other words, in 10-15 days it as N3.2/OR strain destroyed Bacillus subtilis test-culture making the lysis zone 17 mm. At studied two nutrient mediums, antagonistic activity of IIIOR-K against to Penicillum was not strong, because a size of lysis zone did not exceed 2 mm. But, Streptomyces II-IOR-K strain did not inoculated by test-culture, so maybe emitting metabolite's effects of both microorganisms are equally.

9. N3-OR strain of *Streptomyces londisporoflavus* – in 15 days at Chapek nutrient medium the lysis zone of Bacillus subtilis was 15 mm, and during investigation days (6; 8; 10; 15) at ISP 4 medium it's lysis zone estimates 4 mm. In 15 days at Chapek nutrient medium the lysis zone of Penicillum was 5 mm and at ISP 4 at 6; 8; 10 days it estimated 1 mm, at 15 days it was 2 mm and Penicillum was thinning. So N3OR strain showed the antagonistic activity against to Penicillum.

(a) (b) (c) (d)

Fig. 4. The antagonism of ml-3.5 strain against to test-cultures (a) B.subtilis; (b) Penicillium sp. and Ml-3.6 strain to test-culture – B. subtilis (c) and Penicillium sp. (d)

(a) (b) (c)

Fig. 5. The antagonistic struggles of N2KG1 strain against to test-cultures (a) B. subtilis and (b) Penicillium sp. and (c) – N2KG2 strain to B. subtilis

10. ml-3.5 strain of *Streptomyces viridogenes* at ISP 4 and Chapek nutrient mediums dilutes the air mycelium of *Bacillus subtilis* and *Penicillum* test-cultures, and the rise of other colonies of the Streptomyces bacterium among test culture (fig. 4, a, b).

11. Ml-3.6 strain of *Streptomyces heliomycini* at Chapek nutrient medium in 15 days made the lysis zone of Bacillus subtilis test-culture 3 mm. And at ISP 4 nutrient medium in 15 days it estimated 11 mm, therefore this nutrient medium (KAA) makes good condition for isolating antibiotic substances of named strain, so this strain is a good antagonist against to Bacillus subtilis (fig. 4, c). The lysis zone of Penicillum at Chapek nutrient medium was 4 mm, the growing of test-culture at both of nutrient mediums was thinned (fig. 4, d).

12. N2KG1 strain of *Streptomyces albianatus* – during 6-, 8-, 10-, 15 days of investigation lysis zone of Bacillus subtilis did not exceed 3 mm. At Chapek nutrient medium the lysis zone of Penicillum between 6-10 days was 1-2 mm, at 15th day estimated 20 mm. So, N2KG1 strain could be recommended as active antagonist against to Penicillum (fig. 5, a, b).

13. N2KG2 strain of Streptomyces tauricus after 6 days destroyed the Bacillus subtilis test-culture to 15 mm (fig. 5, c), but was not observed the antagonistic influence to Penicillum.

14. *Streptomyces longispororuber* N6KG2 of Streptomyces bacterium had bad growing ability at Chapek nutrient medium. Named strain during antagonism with Bacillus subtilis changed some of cultural-morphological properties (pic.6a) and between 15 days substrate mycelium of N6KG2 strain emitted into substrate a green pigment belonging to culture. Emitting of such pigment in big quantity is explained by that strain has a strong antibiotic ability. At Chapek nutrient medium antagonistic activity of N6KG2 strain against to Penicillum test-culture was a high: in 15 days the lysis zone was estimated to 35 mm (pic. 6, b).

15. N6KG4 strain of *Streptomyces steffisburgensis* in comparison with other cultures

had a bad antagonistic activity, because during investigation days (6; 8; 10; 15) lysis zones of both test-cultures did not exceed 2 mm.

16. N1KG1 strain of *Streptomyces griseomycini* showed the active growing at both KAA and Chapek nutrient mediums. After 6 days of investigation a lysis zone was estimated 24 mm (pic. 6 c), and during 8, 10, 15 days size of lysis zone was 27 mm. N1KG1 strain is a strong antagonist against to bacillus subtilis test-culture was proved on the results of investigation (tab. 1). During investigation it was observed that the lysis zone of Penicillum test-culture did not exceed 1 mm, but its air mycelium had very thinned.

17. N4KG1strain of *Streptomyces chromofuscus* during antagonism struggle after 6 days destroyed Bacillum subtilis that had lysis zone 18 mm, after 8-10-15 days its lysis zone estimated 30 mm. So, was observed that N4KG1 strain is a strong antagonist against to Bacillus subtilus test-culture. The lysis zone of Penicillum did not exceed 1-2 mm, but till last day of investigation did not observe the strain transition into itself.

Conclusions

On the results of investigation was observed that many of *Streptomyces* strains are good antagonists against to Bacillus bacterium than Micromycets fungi. So, on the investigation's results the following strains N3.3OR; N4KG1; N1KG1; IVOR-K; IOR-K(1); III-OR-K; N2KG2; N3.1/OR were selected as strongest antagonists against to *Bacillus subtilis* test-culture and N3OR; IIOR-K, OR-K (2) strains were selected as a strong antagonists.

The *Streptomyces* N6KG2; N3-OR; N2K-G1strains comparatively to above described strains during investigation days (6; 8; 10; 15) made the lysis zones of *Penicillum spp.*1-20 mm and was observed that the substrate mycelium of named fungus growing became low. So, on the results of investigation 3 strains of *Streptomyces* bacterium: N6KG2 strain of Streptomyces longispororuber; N3OR strain of *Streptomyces londisporoflavus;* N2KG1 strain of *Streptomyces albiianatus* were selected as stronger antagonists against to *Penicillum* test-culture.

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