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Microbiology

Microorganisms of Alluvial-Carbonated and Grey-Brown Soils in East Georgia

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ABSTRACT: The purpose of our investigation was to study the microflora of alluvial-carbonated and grey-brown soils of east Georgia and isolation of inhabiting actynomycetes, as well as investigation of their antagonistic activity. Four samples of soil from the saline alluvial-carbonated and grey-brown soils of East Georgia (environs of the city Rustavi and Bolnisi) were taken. Different physiological groups of microorganisms: saprophytic and amylolytic bacteria, fungi, cellulose-destructors, actinomycetes, nitrificators of the I and II phase and azotobacter. As for the percentage of particular group of microorganisms, in alluvial-carbonated soils saprophytes prevailed. Their amount made 57.2% of the total amount. This proves that plant and animal residues in these soils are enough for transformation processes. Amylolytic bacteria are much as well 91, 19%. Their actinomycetes were isolated from these soils, and their quantitative composition was studied. Among the four isolated strains of actinomycetes most revealed selective antagonistic activity towards the gram-positive and gram-negative microorganisms: *Elythrosporangium brasiliense, Actinosporangium violaceum, Staphylococcus aureus, Escherichia coli, Mycobacterium rubrum 874, Agrobacterium tumefaciens* (causes vine cancer), *Xanthomonas campestris (infects cabbage), Pectobacterium aroideae* From the experimental results it may be concluded that almost all actinomycetes isolated from soils of east Georgia revealed different level of antagonism. Obtained results clear that the pure cultures of actinomycetes, isolated from Rustavi and Bolnisi environs demonstrated selective activity against both, gram-positive and gram-negative microorganisms, as well as against each other. Active processes of transformation of the plant residues and nitrogen fixation takes place in saline alluvial-carbonated (environs of Rustavi), and grey-brown from the environs of Bolnisi. The saline alluvial-carbonated (environs of Rustavi) and grey-brown soils are rich of actinomycetes with selective antagonistic activity. © 2018 Bull. Georg. Natl. Acad. Sci.

Key words: actinomycetes, nitrificators, cellulose-destructors, azotobacter, saprophytes, amylolytic bacteria

Actinomycetes are members of a large group of pleomorphic Gramm-positive bacteria, many of which have some tendency of mycelial growth. The metabolic multilaterality of actinomycetes, accompanied by the production of primary and secondary metabolites of economic importance, made possible their biotechnological applications. They are a promising source of products like antibiotics, enzyme inhibitors, antiparasitic and anticancer agents [1,2]. They are one of the most

Table 1

Number of microorganisms per one gram of the dry soil								
Soil type	Grey-brown	Alluvial-carbonated						
Site of sampling	Environs of Bolnisi	Environs of Rustavi						
	Bl1	**Bl2**	**Rs1**	**Rs2**				
Groups of micro-organisms	number	%	number	%	number	%	number	%
Saprophytes	$6.9\cdot10^{11}$	15.22	$4.6\cdot10^{11}$	2.648	$1{,}3\cdot10^{8}$	57.2	$5.8\cdot10^{10}$	8.81
Cellulose-destructors	61.59	2.710^{-6}	109.07	1.610^{-8}	326.8	$1{,}1\cdot10^{-7}$	54.8	$2\cdot10^{-6}$
Amylolytic bacteria	$9{,}8\cdot10^{10}$	42.8	$6.1\cdot10^{11}$	91.19	$2.5\cdot10^{11}$	44.775	$1.9\cdot10^{12}$	73.52
Anaerobic bacteria	598	$2.6\cdot10^{-7}$	621	$0.9\cdot10^{-7}$	643	$1.1\cdot10^{-7}$	682	0,026
Nitrificators (Iphase)	41122	$1.8\cdot10^{-5}$	$6.7\cdot10^{5}$	10^{-4}	806271	$2.7\cdot10^{-4}$	499426	$1.9\cdot10^{-5}$
Nitrificators (IIphase)	$3.2\cdot10^{5}$	$1.4\cdot10^{-4}$	$1.1\cdot10^{5}$	$1.6\cdot10^{-5}$	229563.3	$7.6\cdot10^{-5}$	$3.6\cdot10^{5}$	$1.4\cdot10^{-5}$
Actinomycetes	$1.2\cdot10^{4}$	$0.5\cdot10^{-5}$	9868.4	$1.5\cdot10^{-5}$	13437.9	$4.5\cdot10^{-6}$	$1.3\cdot10^{4}$	$0.5\cdot10^{-5}$
Microscopic fungi	$3.6\cdot10^{4}$	$1.6\cdot10^{-5}$	$2.9\cdot10^{4}$	$4.4\cdot10^{-6}$	$6.0\cdot10^{4}$	$0.2\cdot10^{-4}$	11940.3	$4.5\cdot10^{-6}$
Total number of microorganisms	$2.3\cdot10^{11}$	$6.6\cdot10^{11}$	$2.6\cdot10^{12}$	$3.0\cdot10^{11}$				

important sources for the discovery of new antibiotics. An important number of drugs and analogs obtained from actinomycetes are successfully introduced in the market and used today in clinical practice. Actinomycetes are still one of the most important sources of chemical diversity and a source to search for novel structures that require the integration of diverse disciplines. These can range from novel strategies to isolate species previously not cultivated, to in silico biosynthetic predictions from whole gene sequences and novel engineered heterologous expression. Thus, studying of the conditions of their evolution and distribution peculiarities is significant [3-5]. Actinomycetes are significant groups of soil-inhabiting associations of microorganisms. They may account for 10 to 30% of the total soil rhizosphere microorganisms. Many of actinomycetes are antagonistic forms. The main source for obtaining different forms of actynomycetes is soil. From this point of view Georgian soils are less studied. The purpose of our investigation was to study the microflora of alluvial-carbonated and grey-brown soils of east Georgia and isolation of inhabiting actynomycetes, as well as investigation of their antagonistic activity.

Materials and Methods

Both the gramm-positive and gramm-negative microorganisms were taken as test objects: *Elythrosporangium brasiliense, Actinosporangium violaceum, Staphylococcus aureus, Escherichia coli, Mycobacterium rubrum 874, Agrobacterium tumefaciens* (causes vine cancer), *Xanthomonas campestris* (infects cabbage), *Pectobacterium aroideae,* as well as actinomycetes isolated from saline soils.

Cultivation of actinomycetes was performed on Krasilnikov's synthesized medium (CP-I). Antagonistic properties were studied by Block's method [6]. Modern methods of soil microflora testing have been used in study [7].

Results and discussion

Four samples of soil, picked from alluvial-carbonated and grey-brown soils of east Georgia were tested.

Following physiological groups of microorganisms were isolated from the tested soils: saprophytic and amylolytic bacteria, fungi, cellulose-destructors, actinomycetes, nitrificators of the I and II phase and azotobacter. Results are presented in Table 1.

Table 2. Antagonistic properties of actinomycetes

Test-object	Antagonistic culture			
	Strain Bl 1	Strain Bl 2	Strain Rs1	Strain Rs2
	Size of the inhibition zone, mm	Size of the inhibition zone, mm	Size of the inhibition zone, mm	Size of the inhibition zone, mm
Elythrosporangium brasiliense	2.7	3.1	1.9	13
Actinosporangium violaceum	2.7	3.0	1.6	1.25
Staphylococcus aureus	0.9	1.3	1.1	0.1
Escherichia coli	1.1	1.6	0.5	0.2
Mycobacterium rubrum 874	1.25	1.5	0.5	0.0
Agrobacterium tumefaciens	0.9	1.3	1.1	0.0
Xanthomonas campestris	0.5	0.5	0.3	0.25
Pectobacterium aroideae	0.8	1.0	1.7	0.0

According to our experimental results, it is clear that the quantitative composition of microorganisms of the tested soils is different.

As for the percentage of particular group of microorganisms, in sample Rs 1 saprophytes prevailed. Their amount made 57.2% of the total amount. This proves that plant and animal residues in these soils are enough for transformation processes. Amylolytic bacteria are mush as well 44.775%.

As for percentage of the particular groups of microorganisms, in Rs 2 sample amylolytic 73.52% and saprophytic 8.81% bacteria prevailed. In Bl 2 sample content of amylolytic bacteria was higher 91.19% and amount of fungi and anaerobic bacteria was low. In Bl 1 sample the amount of amylolytic bacteria was high 42.8% and saprophytes made 15.22%. In all four samples the amount of amylolytic bacteria was high and amount of actinomycetes, azotobacter and cellulose-destructors prevailed. Peculiarities of the distribution of antagonistic actynomycetes in experimental samples were studied on the next step of investigation. Pure cultures of actinomycetes were isolated from the soil samples, and analyzed (four strains: Bl 1, Bl 2, Rs 1, Rs 2) to reveal the antagonistic properties. Experimental results are presented in Table 2.

From the Table it is clear that the physiological activity of actinomycetes towards the experimental test-objects was revealed with different intensity. The most effective appeared to be the culture Bl 2, which inhibited growth and development of *Pectobacterium aroideae* (size of the inhibition zone was 1.0 mm), and *Esherichia coli* (size of the inhibition zone was 1.6mm), *Agrobacterium tumefaciens (*size of the inhibition zone was 1.3 mm), *Elythrosporangium brasiliense* and *Actinosporangium violaceum* (size of the inhibition zone was 3.1-3.0 mm). The strain Bl1revealed antagonism against *Mycobacterium rubrum* 874 (size of the inhibition zone was 1.25 mm), *Elytrosporangium brasiliense* and *Actinosporangium violaceum* (size of the inhibition zone was 2.7 mm).

The strain Rs 1 was antagonistic against *Mycobacterium rubrum* 874 (size of the inhibition zone was 0.5 mm), *Staphylococcus aureus* (size of the inhibition zone was 1.1 mm). and *Pectobacterium aroideae* (size of the inhibition zone was 1.7 mm). *Elytrosporangium brasiliense* (size of the inhibition zone was 1.9 mm). and *Actinos.orangium violaceum* (size of the inhibition zone was 1.6 mm).

Strain of actinomycetes Rs 2 revealed antagonism against, *Actinosporangium violaceum* (size of the inhibition zone was 1.25 mm) *Elytrosporangium brasiliense* (size of the inhibition zone was 1.3 mm) and *Staphylococcus aureus* (size of the inhibition zone was 0.1 mm).

From the experimental results it may be concluded that almost all actinomycetes isolated from saline soils of east Georgia revealed different level of antagonism.

Obtained results show that the pure cultures of actinomycetes, isolated from Rustavi environs demonstrated selective activity against both, gram-positive and gram-negative microorganisms, as well as against each other.

Different authors demonstrated results of isolation of different strains of actinomycetes with antagonistic activity against different number of pathogenic microorganismsi India. Among them there Bacillus cereus, Enterococcus faecalis, Shigella dysenteriae, Klebsiella pneumoniae, Staphylococcus saprophyticus, Streptococcus pyogenes, Staphylococcus epidermidis, Bacillus cereus, Staphylococcus xylosus, Staphylococcus aureus, Enterococcus faecalis, and Staphylococcus aureus.[8,9].

Conclusion

•Active processes of transformation of the plant residues and nitrogen fixation takes place in saline alluvial-carbonated (environs of Rustavi), and grey-brown from the environs of Bolnisi.

•The saline alluvial-carbonated (environs of Rustavi) and grey-brown soils are rich of actinomycetes with selective antagonistic activity.

მიკრობიოლოგია

აღმოსავლეთ საქართველოს ალუვიურ-კარბონატული და რუხ-ყავისფერი ნიადაგის მიკროორგანიზმები

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კვლევების მიზანი იყო აღმოსავლეთ საქართველოს ალუვიურ-კარბონატული და რუხ-ყავისფერი ნიადაგის მიკროფლორის შესწავლა და ამ ნიადაგებში მობინადრე აქტინომიცეტების გამოყოფა, აგრეთვე, მათი ანტაგონისტური აქტივობის შესწავლა. აღმოსავლეთ საქართველოს ალუვიურ-კარბონატული და რუხ-ყავისფერი ნიადაგიდან (რუსთავის და ბოლნისის შემოგარენიდან) აღებულია ნიადაგის 4 ნიმუში. აღნიშნული ნიადაგის ნიმუშებიდან გამოყოფილია მიკროორგანიზმების შემდეგი ფიზიოლოგიური ჯგუფები: საპროფიტული და ამილოლიტური ბაქტერიები, სოკოები, ცელულოზადამშლელები, აქტინომიცეტები, I და II ფაზის ნიტრიფიკატორები და აზოტობაქტერი. შესწავლილ ნიადაგებში განსხვავებულია მიკროორგანიზმების რაოდენობრივი შემადგენლობა. ალუვიურ-კარბონატული ტიპის ნიადაგში ჭარბობს საპროფიტების რაოდენობა 57,2%, ამილოლიტური ბაქტერის რაოდენობა მეტია რუხ-ყავისფერ ნიადაგში 91,19%. ერთ-ერთი მნიშვნელოვანი ჯგუფია აქტინომიცეტები.მრავალი მათგანი ანტაგონისტური ფორმისაა. აქტინომიცეტების გამოყოფილი 4 შტამიდან უმრავლესობა ავლენს შერჩევით ანტაგონისტურ თვისებებს გრამდადებითი და გრამუარყოფითი მიკროორგანიზმების მიმართ. ტესტ-ობიექტებად გამოვიყენეთ შემდეგი მიკროორგანიზმები: ***Elythrosporangium brasiliense,***

Actinosporangium violaceum, Staphylococcus aureus, Escherichia coli, Mycobacterium rubrum 874, Agrobacterium tumefaciens (ვაზის კიბოს გამომწვევი), ***Xanthomonas campestris*** (კომბოსტოს დაავადების გამომწვევი), ***Pectobacterium aroideae.***

კვლევაში გამოყენებულ იქნა ნიადაგის მიკროფლორის შესწავლის თანამედროვე მეთოდები. აქტინომიცეტების კულტივირებას ვახდენდით კრასილნიკოვის სინთეზურ არეზე (CP-I), ხოლო ანტაგონისტურ თვისებებს ვსაზღვრავდით ბლოკის მეთოდით. კვლევის შედეგი გვიჩვენებს რომ ალუვიურ-კარბონატულ და რუხ-ყავისფერ ნიადაგში (რუსთავის და ბოლნისის შემოგარენი) მიმდინარეობს მცენარეული ნაშთების ტრანსფორმაციის და აზოტფიქსაციის პროცესები.

ალუვიურ-კარბონატული და რუხ-ყავისფერი ნიადაგი (რუსთავის და ბოლნისის შემოგარენი) მდიდარია შერჩევითი ანტაგონისტური მოქმედების უნარის მქონე აქტინომიცეტებით. ცდის შედეგი ნათლად ადასტურებს, რომ მიკროორგანიზმთა რაოდენობრივი და თვისობრივი შემადგენლობა დამოკიდებულია ნიადაგის ტიპზე.

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