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Microbiology

Oil Destructing Extremophilic Actinomycetes, Isolated from Various Types of Soil of Georgia

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ABSTRACT. Oil hydrocarbons are principal pollutants of the environment. We used extremophilic microorganisms, among them mono- and mixed cultures of halophiles, alkalifiles and thermophiles. As seen from the results, biological efficiency of a thermophilic actinomycete *Thermoactinomyces dichotomicus* 84Th is extremely high (71.5%). Among oil destroyers of extremophilic actinomycetes most distinguished are thermophiles, both as mono- and mixed cultures. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *extremophilic actinomycetes, crude oil, oil-destroyers.*

One of the most urgent problems of the modern world is environment purification from different toxic residues. Oil hydrocarbons are the principal pollutants of the environment. Today great attention is paid to the working out of ecologically safe biological technologies for rehabilitation of soils contaminated with crude oil. Bioremediation of toxicant contaminated soils is generally based on activation of endemic microflora and introduction of environmentally adapted microorganisms (bioaugmentation) into soil [1, 2]. In this respect the use of extremophilic microorganisms, among them halophiles, alkaliphiles and thermophiles, adapted to local environment, is most effective.

A prospective method for detoxification of soils contaminated with crude oil is the use of preparations in which microorganisms, active destroyers of hydrocarbons, are incorporated. Introduction of pure cultures capable of oxidation of aliphatic, aromatic and other hydrocarbons, into contaminated soils enhances soil detoxification and stabilization of the processes of biological degradation with comparatively lower economical expenses and is of no danger for environment [3].

The objects of the given study are halophilic, alkalophilic and thermophilic actinomycetes, isolated from different types of soil of Georgia. Among 405 strains of extremophilic actinomycetes halophilic, alkaliphilic and thermophilic strains capable of oil hydrocarbons (hexane, benzene, benzpyrene, naphthalene, crude oil) detoxification have been isolated. It has been stated that among hydrocarbon destroying extremophilic actinomycetes thermophiles of *Thermoactinomyces* genera are most distinguished [4 - 6].

Mono- and mixed cultures of extremophilic actinomycetes with capability of oil detoxification have been selected for introduction into contaminated soil. 1 kg soil was placed into 0.02m^2 pots. 7 days later the soil was artificially polluted with oil (0.5%). According to regularities adopted in former USSR countries, 0.1% dose of oil was considered dangerous, but by international rules it must not exceed 0.05%. Wheat previously germinated and infected with actinomycete cultures was planted in oil polluted soil. Initial amount of actinomycetes introduced into soil was: *Streptomyces levoris* 201A – 99.9×10^6 CFU, *Streptomyces pruniviolaceus* 214A - 54×10^5 CFU,

Streptomyces spp. 278H - 34×10^5 CFU, *Streptomyces streptomycini* 295H – 30.9×10^5 CFU, *Thermoactinomyces* spp.82Th – 10.3×10^2 CFU, *Thermoactinomyces dichotomicus* 84Th – 10.1×10^2 CFU. A small dose of the mineral fertilizer NPK – 50g/m^2, was introduced into soil. 14 variants were selected for the test: 2 different monocultures of alkaliphilic, halophilic and thermophilic strains - *Streptomyces levoris* 201A, *Streptomyces pruniviolaceus* 214A, *Streptomyces* spp. 278H, *Streptomyces streptomycini* 295H, *Thermoactinomyces* spp.82Th, *Thermoactinomyces dichotomicus* 84Th. The following variants as mixed cultures: *Streptomyces* spp. 278H + *Thermoactinomyces* spp.82Th, *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th, *Streptomyces levoris* 201A + *Thermoactinomyces dichotomicus* 84Th, *Streptomyces levoris* 214A + *Thermoactinomyces* spp.82Th, *Streptomyces levoris* 214A + *Streptomyces* spp. 278H + *Thermoactinomyces* spp.82Th, *Streptomyces levoris* 201A + *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th, *Streptomyces levoris* 214A + *Streptomyces levoris* 201A + *Streptomyces* spp. 278H + *Streptomyces streptomycini* 295H have been used.

The process of wheat seedling growth was observed. A difference was found in the development of 8-day old seedlings. Better growth, compared to the control, was observed in the pot with halophilic monoculture - *Streptomyces streptomycini* 295H. An analogous situation was observed with thermophilic actinomycete monoculture - *Thermoactinomyces dichotomicus* 84Th. In the case of mixed cultures the following variants: *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th, *Streptomyces levoris* 214A + *Thermoactinomyces* spp.82Th, *Streptomyces levoris* 201A + *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th are most distinguished. In these variants of mixed cultures there was always one thermophilic actinomycete.

While observing 16-day old seedlings it was found that as a result of the effect of mono- and mixed cultures on seedlings the development was better compared to the control.

The phenotype observation stopped with 2-month old seedlings. As a result, a stimulatory effect of some mono- and mixed cultures was found, which increases leaf dry mass and length. 50% increase in leaf dry mass was observed as the result of the effect of halophilic actinomycetes *Streptomyces* spp. 278H and that of 34.4% of *Streptomyces streptomycini* 295H, compared to the control. According to leaf dry mass increase most interesting are mixed cultures of halophile and thermophile

Table 1

Effect of mono- and mixed cultures of extremophilic actinomycetes on the growth and development and content of dry matter in wheat seedlings

#	Variant	Leaf dry mass		Leaf average length	
		gram	% difference	cm	% difference
1	Control (soil without oil)	3.2	-	32	-
2	*Streptomyces pruniviolaceus* 214A	3.8	18.7	37	15.6
3	*Streptomyces levoris* 201A	4.1	28.1	37	15.6
4	*Streptomyces* spp. 278H	4.8	50	32.5	1.6
5	*Streptomyces streptomycini* 295H	4.3	34.4	40	25
6	*Thermoactinomyces* spp.82Th	3.2	0	41	28.1
7	*Thermoactinomyces dichotomicus* 84Th	3.6	12.5	37	15.6
8	*Streptomyces pruniviolaceus* 214A+ *Thermoactinomyces* spp.82Th	2.9	-9.4	35	9.4
9	*Streptomyces levoris* 201A+ *Thermoactinomyces dichotomicus* 84Th	3.1	-3.2	36.5	14.1
10	*Streptomyces* spp. 278H+ *Thermoactinomyces* spp.82Th	3.7	15.6	31.5	-1.6
11	*Streptomyces streptomycini* 295H+ *Thermoactinomyces dichotomicus* 84Th	4.1	28.1	31.5	-1.6
12	*Streptomyces levoris* 201A+ *Streptomyces streptomycini* 295H+ *Thermoactinomyces dichotomicus* 84Th	3.6	12.5	35	9.4
13	*Streptomyces pruniviolaceus* 214A+ *Streptomyces* spp. 278H+ *Thermoactinomyces* spp.82Th	3.6	12.5	36	12.5
14	*Streptomyces pruniviolaceus* 214A+ *Streptomyces levoris* 201A+ *Streptomyces* spp. 278H+*Streptomyces streptomycini* 295H	2.7	-15.6	32	0

- *Streptomyces* spp. 278H + *Thermoactinomyces* spp.82Th, *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th. 15.6% increase in leaf length was obtained from the effect of alkaliphiles *Streptomyces levoris* 201A, *Streptomyces levoris* 214A and thermophile *Thermoactinomyces dichotomicus* 84Th mono-cultures. In this respect the monoculture of halophilic actinomycete *Streptomyces streptomycini* 295H is most visible (Table 1).

After completion of the test, some soil samples were collected and their microflora studied. Compared to the control, bacterial CFU in all variants increased, and the amount of actinomycetes was higher in the variants: *Streptomyces* spp. 278H + *Thermoactinomyces* spp.82Th, *Streptomyces levoris* 201A + *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th, 214A+ *Streptomyces levoris* 201A + *Streptomyces* spp. 278H + *Streptomyces streptomycini* 295H.

Soil was prepared for the analysis of the effect of actinomycetes in order to determine residual oil. The amount of residual oil was established using weighing method. The analyzed soil sample was dried for 24h, at room temperature. 5 g sample was placed into Soxhlet apparatus and extraction was performed with chloroform. 20ml extract was placed on a porcelain dish to dry.

As a result of the analyses carried out it was established that a great amount of oil was taken up by thermophilic actinomycete monoculture *Thermoactinomyces dichotomicus* 84Th – 71.5% and mixed cultures of alkaliphilic and thermophilic actinomycetes - *Streptomyces levoris* 214A+*Thermoactinomyces* spp.82Th – 68.7%. Over 50% uptake was observed in 2, 3, 7, 10 and 11 variants. Only in one association - *Streptomyces levoris* 201A + *Streptomyces streptomycini* 295H + *Thermoactinomyces dichotomicus* 84Th it was found to be 1.4% (Table 2).

The monocultures of thermophiles are most distinguished among extremophilic actinomycetes. Mixed cultures are efficient in cases when one of the constitutive strains is a thermophile.

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Table 2

Oil uptake by mono- and mixed culture of extremophilic actinomycetes

#	Variant	20 ml extract residue mass, g	Amount of oil residue	Amount of uptaken oil
1	Control (soil with oil)	0.0144	100	0
2	*Streptomyces pruniviolaceus* 214A	0.0069	47.91	52.08
3	*Streptomyces levoris* 201A	0.0096	66.6	33.4
4	*Streptomyces* spp. 278H	0.0067	46.53	53.47
5	*Streptomyces streptomycini* 295H	0.0080	55.5	44.5
6	*Thermoactinomyces* spp.82Th	0.0065	45.1	54.9
7	*Thermoactinomyces dichotomicus* 84Th	0.0041	28.5	71.5
8	*Streptomyces pruniviolaceus* 214A+ *Thermoactinomyces* spp.82Th	0.0045	31.3	68.7
9	*Streptomyces levoris* 201A+ *Thermoactinomyces dichotomicus* 84Th	0.0078	54.2	45.9
10	*Streptomyces* spp. 278H+ *Thermoactinomyces* spp.82Th	0.0071	49.3	50.7
11	*Streptomyces streptomycini* 295H+ *Thermoactinomyces dichotomicus* 84Th	0.0068	47.2	52.8
12	*Streptomyces levoris* 201A+ *Streptomyces streptomycini* 295H+ *Thermoactinomyces dichotomicus* 84Th	0.0142	98.6	1.4
13	*Streptomyces pruniviolaceus* 214A+ *Streptomyces* spp. 278H+ *Thermoactinomyces* spp.82Th	0.0085	59.1	40.9
14	*Streptomyces pruniviolaceus* 214A+ *Streptomyces levoris* 201A+ *Streptomyces* spp. 278H+*Streptomyces streptomycini* 295H	0.0123	87.8	12.2

მიკრობიოლოგია

საქართველოს სხვადასხვა ნიადაგიდან გამოყოფილი ნავთობის დესტრუქტორი ექსტრემოფილური აქტინომიცეტები

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