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EXPERIMENTAL ARTICLES

Isolation of Actinomycetes from Soil Using Extremely High Frequency Radiation

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Abstract—A new method employing extremely high frequencies (EHFs) is proposed for the selective isolation of actinomycetes from soil. The pretreatment of soil suspensions with EHF wavelengths of 5.6 and 7.1 mm led to a nonselective isolation of actinomycetes. At the same time, the irradiation of soil suspensions within wavelength bands of 3.8–5.8 and 8–11.5 mm considerably augmented the total number of isolated actinomycetes and increased the fraction of the isolated rare genera by 2 and 7 times, respectively. The rare actinomycete genera were represented by *Actinomadura*, *Microtetraspora*, *Nonomuraea*, *Micromonospora*, *Amycolatopsis*, *Pseudonocardia*, *Saccharotrix*, and *Streptosporangium*.

Key words: actinomycetes, rare genera of actinomycetes, EHF radiation.

The pretreatment of substrates from natural habitats is one of the methods used for the selective isolation of actinomycetes. For this purpose, researchers employ various chemical compounds, such as phenol [1] and chloramine T [2], and physical agents, such as ultraviolet light [3], ultrasound [4], superhigh frequency (SHF) radiation [5], electric pulses [6], and thermal treatment [7, 8]. To the best of our knowledge, extremely high frequency (EHF) radiation of millimeter wavelengths has not yet been used to enhance the recovery of actinomycetes from soil.

On the other hand, since the mid-1960s, the effect of millimeter waves has been extensively studied with respect to their action on various living organisms, from bacteria to mammals. At present, EHF radiation is employed in many microbiological applications. It is known to exert a growth-promoting effect on heterotrophic [9] and photosynthesizing [10] microorganisms or a growth-inhibiting effect on microbial cells [11], protozoans, and algae [12].

These data imply that EHF radiation inhibiting the growth of one microorganism may promote the selective isolation of other microorganisms which are tolerant to such radiation or even stimulated by it. The present work was aimed at studying the possibility of employing EHF radiation for the isolation of particular actinomycete genera.

MATERIALS AND METHODS

Experiment design. Soil suspensions placed in test tubes or petri dishes were irradiated with EHFs from the bottom using G4-141, G4-142, P2-65, and P2-69 generators (Russia). Electromagnetic energy was transmitted with the aid of a waveguide duct containing an attenuator, a phase-shifting circuit, a control power meter, and plug-in horn radiators with particular radiation patterns. Emitted radiation had a nonthermal intensity and was amplitude-modulated at a frequency of 1 kHz.

Soil suspensions were pretreated by irradiating them either with two monochromatic wavelengths of 5.6 and 7.1 mm or within two wavelength bands of 3.8–5.8 and 8–11.5 mm. To this end, soil samples (chernozem from the Voronezh region) were ground in a mortar, sieved, and thoroughly mixed. A 100-mg portion of thus prepared soil mixture was suspended in 10 ml of water. The suspension was diluted 10- and 100-fold, and aliquots of each dilution were either irradiated (experimental soil suspension dilution) or not (control soil suspension dilution) with EHFs.

Experimental and control soil suspension dilutions were plated onto Gauze agar 2 [13] supplemented with 10 μg/ml nalidixic acid to inhibit bacteria showing creep growth and 50 μg/ml nystatin to inhibit the growth of fungi [14]. It is known that nalidixic acid at the concentration mentioned does not inhibit the growth of actinomycetes [15]. The plates were incubated at 28°C for two weeks. In some experiments, the incubation was continued for 6 weeks in order to detect slow-growing species.

Data statistics. The number of actinomycetes detected in soil was expressed as CFU per gram of soil. The results were statistically processed using the methods described by Ashmarin and Vorob'ev [16] and Plokhinskii [17].

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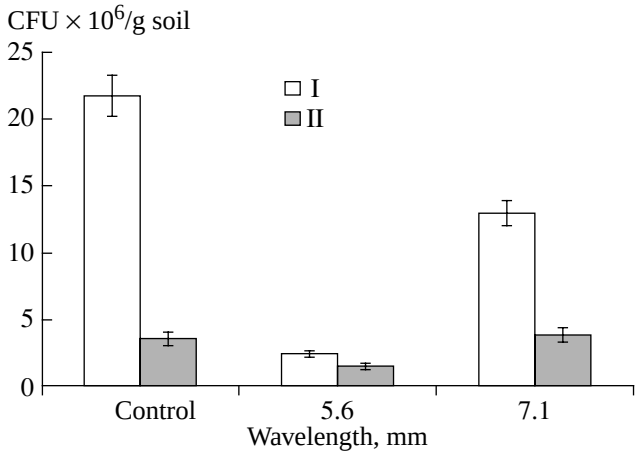

Fig. 1. The effect of monochromatic EHF radiation (5.6 and 7.1 mm) on the number of viable (I) unicellular bacteria and (II) actinomycetes detected in soil.

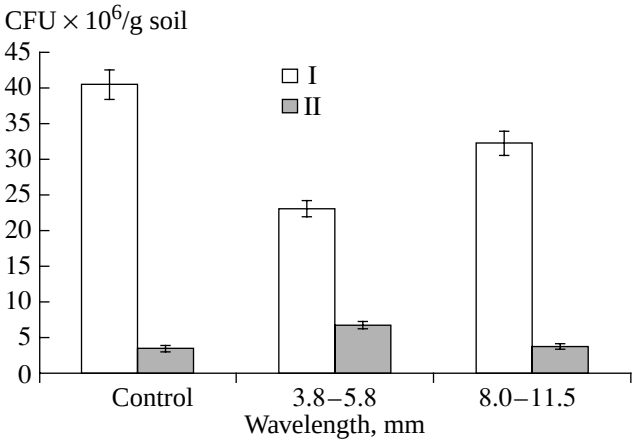

Fig. 2. The effect of EHF irradiation within two wavelength bands (3.8–5.8 and 8–11.5 mm) on the number of viable (I) unicellular bacteria and (II) actinomycetes detected in soil.

Taxonomic identification. The generic status of isolates was determined from their morphological properties and the chemical composition of their cell walls. To determine the structure of sporangia, the isolates were grown on Gauze agar 1 and oat agar [13] and examined under a Jenaval light microscope. The cell-wall diaminopimelic acid and sugars were analyzed in whole-cell hydrolysate by thin-layer chromatography on cellulose plates (Merck, Germany) as described elsewhere [18–20].

Culture antagonism. The antagonistic properties of the isolates were studied by cultivating them on Gauze agar 2 with the following test cultures: *Escherichia coli* K-13, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* 209P, *S. aureus* 209P/UF-2, *Micrococcus luteus* ATCC 9341, *Bacillus mycoides* R-537, *B. subtilis* ATCC 6533, and *Saccharomyces cerevisiae* INA S-1.

RESULTS AND DISCUSSION

The irradiation of soil suspensions with monochromatic EHF radiation of wavelength λ = 5.6 mm was found to diminish the population of unicellular bacteria and actinomycetes by factors of 10 and 2.5, respectively (Fig. 1). In the case of irradiation with the EHF wavelength λ = 7.1 mm, the decrease in the population of unicellular bacteria was 2-fold, whereas the population of actinomycetes changed insignificantly. Therefore, the irradiation of soil suspensions with 5.6- and 7.1-mm wavelengths proves to be efficient for the nonselective isolation of actinomycetes, without a noticeable prevalence of rare actinomycete genera (all *Actinomycetales* genera except for the genus *Streptomyces*). Meanwhile, rare actinomycete genera are of the most interest from the viewpoint of obtaining valuable biologically active substances.

Table 1. The effect of EHF radiation on the number of isolates of rare actinomycete genera obtained from soil

EHF wavelengths	Percentage of isolates of rare genera		
	Micromonospora	other rare genera	total
Control	37.4	4.1	41.5
3.8–5.8 mm	37	8.4	45.4
8–11.5 mm	29.7	30.6	60.3

Note: The total number of isolates in each experimental variant is taken to be 100%.

Assuming that other millimeter waves could be efficient for the selective isolation of rare actinomycete genera, we irradiated soil suspensions with EHFs within wavelength bands of 3.8–5.8 and 8–11.5 mm. In the case of 3.8- to 5.8-mm wavelengths, the population of unicellular bacteria decreased by a factor of 2 and the population of actinomycetes increased by the same factor (Fig. 2). In the case of 8- to 11.5-mm wavelengths, the population of unicellular bacteria decreased about 1.5-fold and the population of actinomycetes changed insignificantly.

The taxonomic analysis of isolated actinomycetes showed a prevalence of rare actinomycete genera among the isolates that were obtained from the irradiated soil suspensions (Table 1). Taking into account that *Micromonospora* is the most widespread and well-studied rare actinomycete genus, the genetic composition of isolates was analyzed with respect to this genus. In the control, the fraction of *Micromonospora* isolates was 37.4% and that of other rare genera was only 4.1%. Among the isolates that were obtained from the soil suspensions irradiated with 3.8- to 5.8-mm wavelengths, the fraction of *Micromonospora* species was almost the same, whereas the percentage of representatives of other rare genera (including *Actinomadura*,

Table 2. The effect of EHF radiation on the number of isolates of various actinomycete genera obtained from soil

Actinomycetes	Control		3.8–5.8 mm		8–11.5 mm	
	number	%	number	%	number	%
Streptomyces	72	58.5	114	54.5	40	39.7
Micromonospora	46	37.4	77	37	30	29.7
Actinomadura, *Microtetraspora* and *Nonomurae*	3	2.5	9	4.3	22	21.7
Amycolatopsis and *Pseudonocardia*	0	0	5	2.4	6	5.9
Saccharotrix	0	0	2	0.9	0	0
Streptosporangium	1	0.8	0	0	1	1
Unidentified genera	1	0.8	2	0.9	2	2
Total number of isolated actinomycetes	123	100	209	100	101	100
Number of isolates of rare genera	51	41.5	95	45.4	61	60.3

Microtetraspora, *Nonomuraea*, *Amycolatopsis*, *Pseudonocardia*, and *Saccharotrix* (Table 2)) increased more than twofold. Among the isolates that were obtained from the soil suspensions exposed to 8- to 11.5-mm wavelengths, the fraction of *Micromonospora* species slightly decreased, whereas the percentage of representatives of other rare genera increased from 4.1 to 30.6, i.e., by a factor of about 7.5 (Table 1). The fraction of representatives of the genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* turned out to be as high as 21.7% (Table 2). Irradiation with 8- to 11.5-m wavelengths also augmented the relative number of isolates of the rare genera *Amycolatopsis*, *Pseudonocardia*, and *Streptosporangium*.

The investigation of the antibiotic activity of actinomycete isolates (except those of the genus *Micromonospora*) showed that the exposure of soil suspensions to radiation within the wavelength bands 3.8–5.8 and 8–11.5 mm increased the fraction of isolates with antibiotic activity by 13 and 20%, respectively.

Thus, the pretreatment of soil suspensions with EHF radiation of certain wavelengths and wavelength ranges affects soil unicellular bacteria and actinomycetes in different ways and facilitates the isolation of rare actinomycete genera. Presumably, this is due to the fact that EHF radiation inhibits the growth of unicellular bacteria and promotes the resuscitation of dormant actinomycetes in soil.

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