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EXPERIMENTAL ARTICLES

Alkaliphilic Soil Actinomycetes

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Abstract—The actinomycete complex of alkaline soils was found to be dominated by alkaliphilic streptomycetes, which showed maximal radial rates of colony growth at pH 8. At pH values of 7 and 10, the growth of these streptomycetes was poor. Alkaliphilic streptomycetes can be morphologically differentiated from other actinomycetes based on their high radial rates of colony growth and increased spore formation in alkaline media as compared to neutral media.

Key words: alkaliphilic soil actinomycetes, alkaline soils, radial rate of colony growth.

It is known that actinomycetes grow well in neutral and slightly alkaline media. The optimal growth of actinomycetes in alkaline media was first described by Baldacci [1]. Alkaliphilic actinomycetes were first isolated from various soils by Taber [2]. Study of such actinomycetes has led to the description of new taxa [3–5] and the discovery of alkaline proteases and new antibiotics [6, 7]. Taxonomically, the recently described alkaliphilic mycelial prokaryotes belong to the genera *Streptomyces* and *Nocardiopsis* [8–10].

Study of actinomycetes isolated from alkaline soils is of interest from the standpoint of their position in the microbial complex of alkaline soils and may considerably contribute to our knowledge of microbial biodiversity. The aim of this work was to investigate the streptomycete complex isolated from alkaline soils.

MATERIALS AND METHODS

The objects under investigation were soils with high values of actual acidity (see table): alluvial saline meadow soil on carbonate deposits, chestnut saline soil, brown semidesert soil, crusted saline soil, and meadow saline soil. Actinomycetes were counted by plating soil suspension dilutions onto Gauze agar medium 1 [11]. In order to suppress the growth of fungi and nonmycelial prokaryotes, the medium was supplemented with, respectively, nystatin (50 μg/ml) and nalidixic acid (10 μg/ml). The actinomycetes were isolated using media with pH values of 7.0 and 9.0. The pH of the

Characteristics of the soil samples under study

Ecosystem	Soil, horizon, sampling depth	pH_{H_2O}	Location
Saline	Saline chestnut soil (sample 1); saline crust (sample 2); 0–6 cm	9.0 9.0	Ulan-Ude, terrace of Lake Orengoiskoe
	Crusted saline soil (sample 3); saline crust, crusted saline soil (samples 1–4); 0–6 cm	9.4 9.0	Bank of Lake Sul'fatnoe Caspian Depression, Buzachi Peninsula
	Meadow saline soil (horizon 1, 0–8 cm; horizon 2, 8–14 cm)	7.1 7.2	Astrakhan
	Brown semidesert soil (Ad, 0–7 cm; B1, 7–22 cm)	7.3 7.3	The same
Alluvial	Saline alluvial meadow soil on carbonate deposits		Volgograd oblast, Ilovlinskii region, the environs of station Kachalino
	Ad	7.4	
	A	7.4	
	ABsa	7.5	
	BCcasa	7.9	

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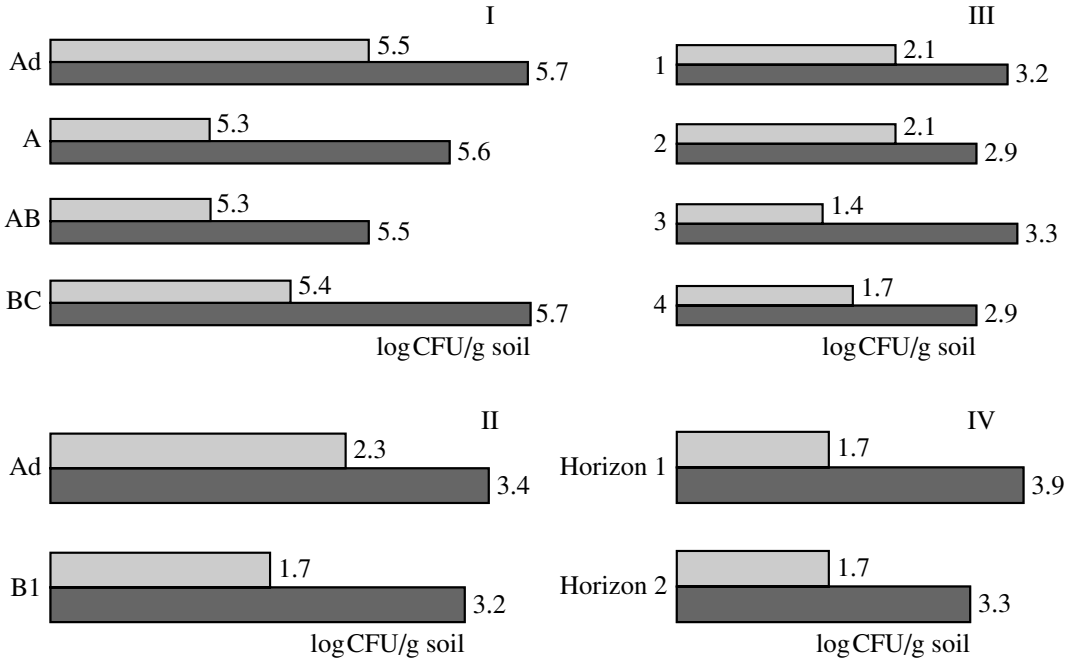

Fig. 1. The number of streptomycetes (in log CFU/g soil) isolated on neutral (gray bars) and alkaline (black bars) Gauze media from (I) saline alluvial meadow soil (horizons Ad, A, AB, and BC); (II) brown semidesert soil (horizons Ad and B1); (III) crusted saline soil (depth 0–6 cm, samples 1–4); and (IV) meadow saline soil (horizon 1, 0–8 cm; horizon 2, 8–14 cm).

media was adjusted by adding a buffer solution containing 1/15 M $Na_2HPO_4 \cdot 2H_2O$ and 1/15 M KH_2PO_4. The inoculated media were incubated at 28–30°C for 10 days.

The actinomycetes were preliminarily assigned to the genus *Streptomyces* according to the identification criteria given in *Bergey's Manual* [12]: the presence of aerial or substrate mycelium, the absence of mycelial fragmentation, the presence of spore chains on the aerial mycelium, the absence of spores on the substrate mycelium, the presence of L,L-diaminopimelic acid, and the absence of differentiating sugars in whole-cell hydrolyzates. Then, the actinomycetes were isolated in pure cultures using oat agar [11] and identified to a species level according to the *Handbook of Actinomycetes* [11].

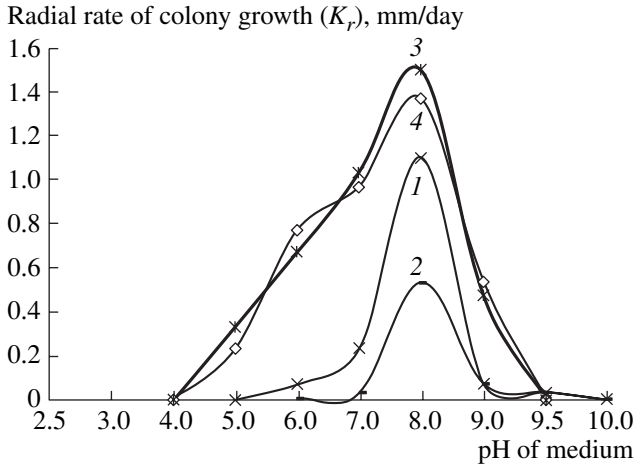

Fig. 2. Ranges of pH suitable for growth of alkaliphilic streptomycetes as indicated by the radial rate of colony growth (K_r): (*1*) *S. xanthochromogenes* 8410, (*2*) *S. resistomycificus* 8402, (*3*) *S. varsoviensis* 69, and (*4*) *S. chromofuscus* strain C.

Optimal and extreme values of pH for growth of the actinomycetes were determined from the radial rates of colony growth on Gauze agar 1 with pH values equal to 3, 4, 5, 6, 7, 8, 9, and 10. The pH of the medium was adjusted and maintained by adding a buffer solution containing 0.2 M $Na_2HPO_4 \cdot 2H_2O$ and 0.1 M citric acid.

The radial rate of colony growth was calculated according to the formula $K_r = (d_2 - d_1)/(t_2 - t_1)$, where d_1 and d_2 are the mean diameters of the colonies (a total of 20 colonies were measured) on the 3rd (t_1) and 8th (t_2) days of incubation, respectively.

A morphological description of the isolated streptomycetes was attained using agar media with pH values of 6.0, 7.0, and 8.4. The pH of the media was adjusted with a phosphate buffer solution. The shape of the sporophores and spores was determined with a scanning electron microscope. The streptomycetes were morphologically differentiated according to the following method [12]: Sterile cover glasses were placed onto the inoculated Gauze agar 1 plates at an angle to the agar surface. The plates were incubated for 5, 10, and 15 days. Then, the glasses were placed onto specimen slides and examined under an Axiostar microscope (400× and 1000×). Changes in the pH of the media dur-

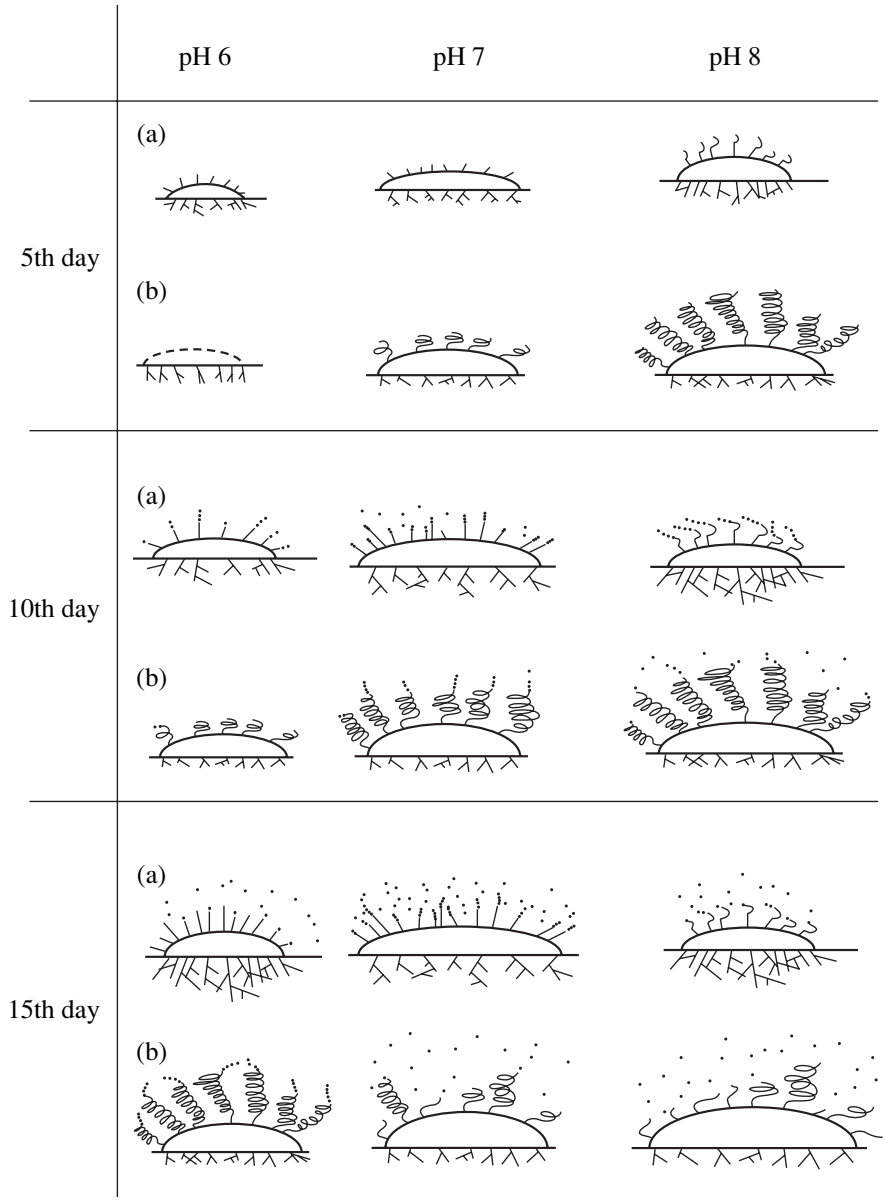

Fig. 3. Schematic representation of colony formation for (a) neutrophilic and (b) alkaliphilic streptomycetes on agar media with different pH values.

ing cultivation were recorded with the aid of a portable pH meter equipped with a glass electrode.

RESULTS AND DISCUSSION

Alkaline soils with pH values higher than 7 were found to contain more streptomycetes capable of growing at pH 9 than those capable of growing at neutral pH. The difference between the number of such streptomycetes reached one order of magnitude in the case of saline soils (solonchaks) (Fig. 1).

The coefficient of alkaliphilicity (K_{alk}) of the actinomycete complex, calculated as the ratio of the number of actinomycetes isolated at pH 9 to the number of actinomycetes isolated at pH 7, was found to be more than unity for all of the soils studied. This result implies that the actinomycete complex of alkaline soils is mainly represented by alkaliphilic actinomycetes.

The streptomycetes isolated at pH 9 from all of the soils were dominated by gray forms (Category IV and Gray Series) [13]. The streptomycete complexes isolated from the soils at pH 7 were characterized by specific dominant species. Since streptomycetes implement the so-called searching strategy, which combines oligotrophy and rapid colonization of new territories, their growth is almost linear and their growth rate is almost

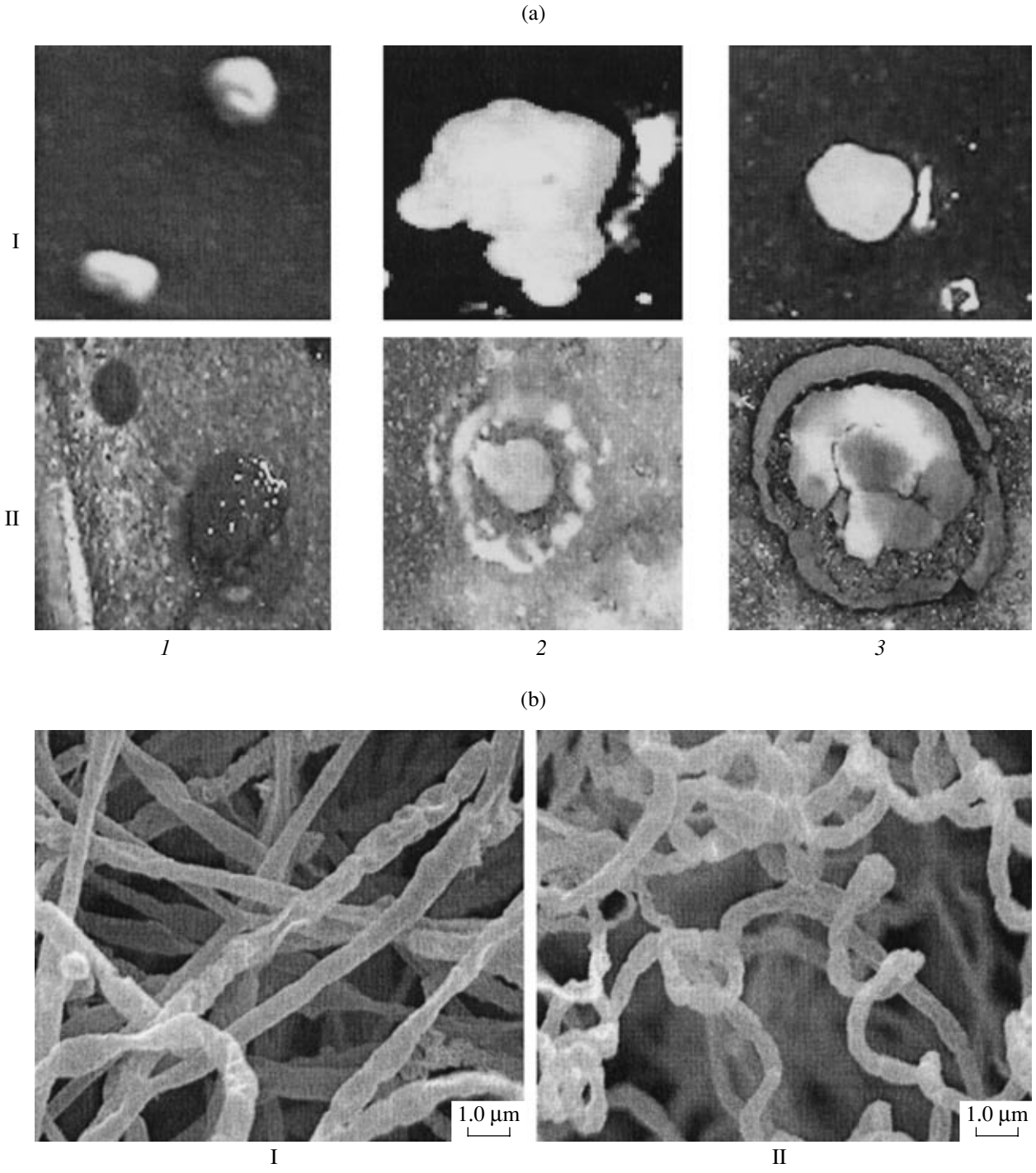

Fig. 4. Microphotographs of (a) colonies and (b) sporophores and spores produced on (*1*) acidic, (*2*) neutral, and (*3*) alkaline agar media by (I) neutrophilic and (II) alkaliphilic streptomycetes.

constant [14]. This circumstance allows the optimal and extreme values of pH for growth of streptomycetes to be determined from the radial rates of increase in the diameter of a colony at different pH values of the agar media. In fact, such measurements give a curve describing the tolerance of mycelial prokaryotes to the medium acidity.

The alkaliphilic streptomycetes isolated from the soils under study showed optimal growth at pH 8.0 but can grow within the pH range of 5.0 to 9.5–10.0 (Fig. 2). That there is such a wide range of pH values suitable for the growth of alkaliphilic streptomycetes is probably due to the significant heterogeneity of the soils, which contain microzones with different actual acidity.

It should be noted that we do not share the viewpoint of some authors [15] that actinomycetes can be divided into alkalitolerant and alkaliphilic types according to their ability (and, respectively, inability) to grow at pH 7.0. The ability of alkaliphilic soil actinomycetes to grow under neutral and even acidic conditions may be a consequence of their adaptation to life in soils, where pH can considerably vary in different microzones. It would be reasonable to suggest that actinomycetes are alkaliphilic if they show high radial rates of colony growth under alkaline conditions but not at pH 7.0 or lower.

Due to the specific features of their habitats, many alkaliphilic microorganisms are also halophilic. We checked the halophilicity of the alkaliphilic actinomycetes isolated from the crusted saline soil by measuring the radial rate of colony growth on the agar media supplemented with different salt concentrations. The pH of these media was optimal for actinomycete growth. It was found that the alkaliphilic strain *S. varsoviensis* 69 is moderately halophilic and grows best at a salt concentration of 6% in the medium. The possibility of the morphological differentiation of soil streptomycetes was studied using the neutrophilic strain *S. canescens* 7402 and the alkaliphilic strain *S. resistomycificus* 8402.

The neutrophilic streptomycete, incubated on either acidic or alkaline agar medium for 5 days, produced colonies with developed substrate and aerial mycelia containing short and straight sporophores (Fig. 3). By the 10th day of growth, smooth spores had formed (Fig. 4). These spores were released from chains in the next 5 days (Fig. 3). The 5-day-old streptomycete colonies grown on neutral media were larger in size than when grown on acidic and alkaline media (Fig. 4). Furthermore, the spores were released sooner (by the 10th day of growth) than in the case of the acidic and alkaline media (Fig. 3).

The alkaliphilic strain incubated on acidic agar medium for 5 days produced colonies lacking aerial mycelium (Figs. 3, 4). After 10 days of growth, we could observe aerial mycelium and spores arranged in short spiral chains containing 1–3 turns. At that time, the pH of the medium changed from 5.3 to 6.0. The spores had been released from the chains by the 15th day of growth. The 5- day-old colonies grown on neutral agar medium were characterized by the presence of substrate and aerial mycelia. The latter contained short spiral sporophores (1–3 turns). By the 10th day, spores had been formed and had begun to be released. The 5-day-old colonies produced on agar medium with pH 8.4 were larger than colonies of the same size grown on neutral or acidic agar (Fig. 4). At that time, the aerial mycelium contained spiral sporophores with 6–8 turns (Figs. 3, 4). By the 10th day of growth, when the pH of the medium had decreased from 8.4 to 7.0, smooth spores were released.

Thus, the morphology of the alkaliphilic streptomycetes cultivated on acidic media is characterized by prolonged life-cycle stages and a delayed formation of aerial mycelium and spores as compared to their morphology when cultivated on neutral and alkaline media. The colonization rate of substrates (i.e., the radial rate of colony growth) of the alkaliphilic streptomycetes is higher on alkaline media than on neutral and acidic media. The neutrophilic streptomycete that was cultivated on alkaline media has lower rates of colonial growth and spore formation than when it was cultivated on neutral media. Such growth characteristics suggest that the alkaliphilic streptomycetes have evolved specific adaptive mechanisms for the conditions of life under high pH values. These mechanisms allow them to colonize alkaline media better than neutral media.

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