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EXPERIMENTAL ARTICLES

Peculiarities of Growth and Morphological Differentiation of Acidophilic and Neutrophilic Soil Streptomycetes

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Abstract—Acidophilic actinomycetes are shown to possess a special mechanism of adaptation to low pH conditions, which shows up in their ability to grow faster on acidified than on neutral media and to adjust the pH of the medium to a level favorable for the formation of aerial mycelium and spores. On nutrient media with pH below 5, neutrophilic actinomycetes either fail to grow or grow much slower than on neutral media; they do not alkalize the medium and do not form aerial mycelium.

Key words: streptomycetes, acidophilic soil actinomycetes, radial growth rate.

The notion of acidophilic and acidotolerant actinomycetes appeared in the literature fairly recently, in the 1970s [1, 2]. Until that, all actinomycetes were thought to be neutrophilic. Even today, biological properties and ecological functions of acidophilic actinomycetes and the role of these organisms in the community of soil microorganisms are insufficiently studied.

In our previous studies of actinomycete complexes isolated from different soils on mildly acidic (pH 4.5–5.3) and neutral (pH 7.0) nutrient media, we accumulated a culture collection of acidophilic and neutrophilic streptomycetes and found that the number of acidophilic actinomycetes in acidic and mildly acidic soils was higher than that of neutrophilic ones [3, 4].

The goal of this work was to compare the radial growth rates and patterns of morphological differentiation in soil streptomycetes isolated on acidic and neutral media.

MATERIALS AND METHODS

The subjects of investigation were 20 strains of streptomycetes isolated from different-type soils using neutral (pH 7.0) and acidified (pH 4.5 and 5.3) Gause 1 nutrient medium (Table 1). The isolation media were acidified with a phosphate buffer prepared by combining the solutions of 1/15 M Na_2HPO_4 and 1/15 M KH_2PO_4 taken in ratios as required [5]. In the preparation of nutrient media with pH 4 to 5, the agar and the liquid buffer with the ingredients of the Gause 1 medium were sterilized separately. The species were identified using the *Actinomycete manual* [7].

Given the linear growth dynamics of streptomycete colonies [8], the colony radial growth rate was chosen as a measure of culture growth. The rate was determined by gauging the size of a visible colony twice: 3 to 5 days after inoculation, when the colony first appeared on the nutrient agar in the petri dish, and 6 to 8 days after inoculation, when the colony size increase became evident with the naked eye. The colony diameter was gauged with a pair of compasses in two orthogonal directions to within one-tenth of a millimeter. The colonies were grown on Gause 1 agarized media with pH values ranging from 3.0 to 8.0. The nutrient media were acidified using a phosphate–citrate buffer composed of 0.2 M Na_2HPO_4 and 0.1 M citrate solutions taken in the required proportions [5]. Cultures of streptomycetes were inoculated by stabbing agarized media. Each test was replicated ten times. The samples were incubated at 28°C. The radial growth rate was estimated as

$$Kr = \frac{d_2 - d_1}{t_2 - t_1},$$

where d_1 and d_2 are the results of the first and the second colony diameter measurements (mm) at times (days) t_1 and t_2, respectively. The cultural features of streptomycetes isolated from soils of different types using media with different pH values (4.5–5.3, and 7.0) were recorded.

The dynamics of cytodifferentiation of streptomycete mycelium was studied by the following method [9]. Sterile slides were placed in petri dishes at an angle to the surface of agarized Gause 1 medium inoculated with a streptomycete culture. The dishes were incubated at 28°C. The slides were withdrawn on the third, sixth, ninth, and 15th day of culture growth, fixed with Carnoi liquid [10], stained with methylene blue (1%), and examined under a light microscope (at 40× and 900×). Changes in the nutrient medium pH in the course of culture growth were monitored using a creo-

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Table 1. Streptomycete strains studied

Streptomycete strain	pH of the isolation medium	Soil the strain was isolated from
Streptomyces narbonensis (C_2)*	4.5	Alluvial meadow soil
S. pallidoviolaceus (C_3)	4.5	Alluvial meadow soil
S. xanthocidicus (C_7)	4.5	Alluvial meadow soil
S. viridobrunneus (A_5)	4.5	Alluvial meadow soil
S. achromogenes (A_6)	4.5	Alluvial meadow soil
S. iverini (A_7)	4.5	Alluvial meadow soil
S. flavogriseus (A_{10})	4.5	Alluvial meadow soil
S. thermoviolaceus (C_5)	5.3	Alluvial meadow soil
S. roseofulvus (C_6)	5.3	Alluvial meadow soil
S. xanthocidicus (A_2)	5.3	Alluvial meadow soil
S. viridobrunneus (1_1)	5.3	Lowland peat soil
S. viridogenes (E)	5.3	Soddy-podzolic soil
S. fumosus (G)	5.3	Soddy-podzolic soil
S. argenteolus (B_7)	7.0	Chestnut solonetz soil
S. karnatakensis (9_1)	7.0	Lowland peat soil
S. viridobrunneus (12_1)	7.0	Lowland peat soil
S. candidus (13_1)	7.0	Lowland peat soil
S. gelaticus (VI_1)	7.0	Ordinary chernozem
S. violaceoruber (I_1)	7.0	Gray forest soil
S. roseus (D_2)	7.0	Mountain permafrost taiga soil

*Parenthesized are designations of the strains in the collection of the Department of Soil Biology, Moscow State University.

sol bromide purple indicator [5] and a portable pH-meter with a glass electrode.

RESULTS AND DISCUSSION

Our studies of the radial growth rate of streptomycetes on agarized nutrient medium at pH ranging from 4.0 to 8.0 showed that all cultures isolated from different soils on a nutrient medium with pH 5.3 shared a common feature: the K_r maximum occurred at about pH 5.0, and culture growth was possible in the pH interval of 4.0–8.0 (Fig. 1a). In streptomycetes isolated from soils at neutral pH of the medium, the maximum K_r was attained in the pH range of 6.0–7.0, and the pH interval where growth was possible was 5–9 (Fig. 1b). The first group of streptomycetes was regarded as acidophilic and the second one as neutrophilic.

The key distinguishing feature between acidotolerant and acidophilic actinomycetes is claimed to be the capacity of the former and incapacity of the latter to grow at neutral pH values [11]. In our view, this is not quite correct because the capacity to grow under neutral conditions could be due to the actinomycete's adaptation to its soil habitat, characterized by microzonality. For this reason, we apply the term acidophilic to streptomycetes that show maximum radial growth rates on acid media.

The medium pH at which the radial growth rate attains its maximum was found to correlate with the pH of the nutrient medium on which the culture was isolated from soil.

The cultural properties of acidophilic and neutrophilic streptomycetes grown on acidified and neutral media were found to differ in several respects.

Neutrophilic streptomycetes either totally failed to grow on low-pH media, or their growth was much weaker than on neutral media. When grown on acidified media, neutrophilic streptomycetes did not form aerial mycelium, their substrate mycelium remained colorless, and no formation of soluble pigment was observed.

Acidophilic streptomycetes produced larger colonies on acidified media than on neutral media; on acidified media, their aerial mycelium was formed much later than on neutral media, and the coloration of the substrate mycelium and medium was less intense.

The mycelium differentiation dynamics was studied in an acidophilic strain of *Streptomyces fumosus* (Fig. 2a) and a neutrophilic strain of *Streptomyces roseus* (Fig. 2b).

By the third day of growth on Gause 1 medium (pH 7.2), the acidophilic streptomycete produced a colony with developed substrate and aerial mycelia and long tightly coiled spiral sporophores (1–3 convolu-

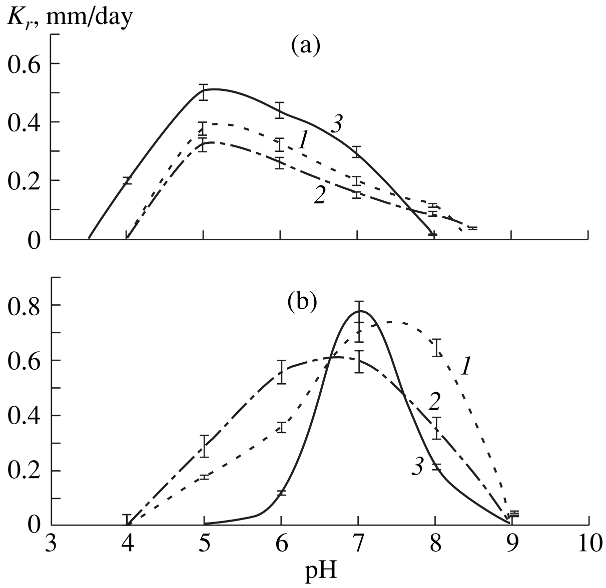

Fig. 1. Ranges of medium pH enabling growth of streptomycetes (a) isolated from soils on acidified media (*1*, *Streptomyces fumosus*, *2*, *S. xanthochromogenes*, and *3*, *S. viridogenes*) and (b) isolated from soils on neutral media (*1*, *S. viridobrunneus*, *2*, *S.argenteolus*, and *3*, *S. roseus*).

tions). The spores were released by the ninth day of culture growth. On a medium with pH 6.2, the substrate mycelium and sterile aerial mycelia were formed by the third day of growth; the sporophores shaped as hooks and spirals were generated by the sixth to ninth day of growth, and the spores were released on the 15th day. The substrate mycelium formed by the third day of growth at pH 5.3 had thicker hyphae than that developing at higher pH values; the aerial mycelium was sterile. The sporophores shaped as hooks and loops were not formed until the ninth day of culture growth, and spirals were formed only by the 15th day. On a medium with pH 4.5, naked colonies with no aerial mycelium appeared by the third day of growth. An aerial mycelium was formed only by the 15th day, and it was sterile and poorly developed.

On medium with neutral pH, the neutrophilic strain formed colonies with developed substrate mycelium and aerial mycelium having long spiral-shaped sporophores of one to three coils already by the third day of growth. At pH 6.2, colonies with sterile aerial mycelium were observed. On medium with pH 5.3, the growth was very poor and the colonies had no aerial mycelium. At pH 4.5, no growth was observed.

It follows that the distinctive features of morphological differentiation of the acidophilic streptomycete on acidified media compared to that on neutral media are elongated stages of the life cycle and retarded generation of aerial mycelium and spores. Despite the relatively high radial growth rates (i.e., the high pace of substrate colonization) exhibited by acidophilic streptomycetes on acidified media, their generative activities were relatively repressed. On the other hand, the life cycle stages of the neutrophilic streptomycete grown on such media are reduced, and sterile aerial mycelia are formed. At even greater medium acidities, no aerial mycelia are formed or growth fails to proceed at all.

Our study of the change in the nutrient medium pH during the course of growth of streptomycetes showed (Table 2) that the pH value of the Gause 1 medium increased from 5.3 to 6.0 on the ninth day of growth. The dynamics of mycelium differentiation in the acidophilic *S. fumosus* grown on a medium with initial pH 5.3 indicates that this rise in the medium pH coincided with the period of the spore-forming activity of the culture (Fig. 2).

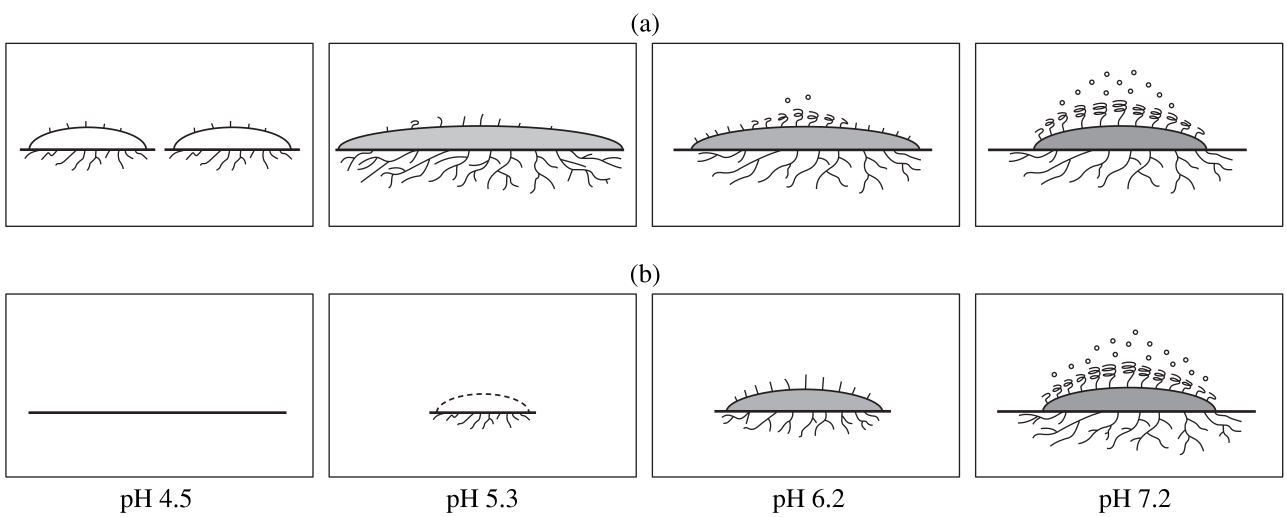

Fig. 2. Morphological differentiation of streptomycetes on media with different pH values: (a) the acidophilic streptomycete *Streptomyces fumosus* and (b) the neutrophilic streptomycete *S. roseus.*

Table 2. Variation of medium acidity in the course of growth of acidophilic and neutrophilic actinomycetes

Strain designation	Streptomycete species	Color of indicator-containing medium* on the 9th day of growth	pH of the medium on the 9th day of culture growth
		Acidophilic actinomycetes	
l_1	*Streptomyces viridobrunneus*	Pinkish brown	6.2
A_5	*S. viridobrunneus*	Pinkish brown	6.1
G	*S. fumosus*	Brownish pink	6.2
E	*S. viridogenes*	Pinkish brown	6.2
A_2	*S. xanthocidicus*	Pink	6.1
A_6	*S. achromogenes*	Pink	6.1
A_7	*S. iverini*	Pink	6.0
C_5	*S. thermoviolaceus*	Pinkish brown	6.2
C_6	*S. roseofulvus*	Pinkish	5.9
C_7	*S. xanthocidicus*	Pink	5.9
		Neutrophilic actinomycetes	
D_2	*S. roseus*	No color	5.3
9_1	*S. karnatakensis*	No color	5.3
12_1	*S. viridobrunneus*	No color	5.3
13_1	*S. candidus*	No color	5.3
1_1	*S. violaceoruber*	No growth	–
VI_1	*S. gelaticus*	No color	5.3
B_7	*S. argenteolus*	No growth	–
10_2	*S. albus*	No color	5.3
14_2	*S. hydroscopicus*	No color	5.3
14_3	*S. albidoflavus*	No color	5.3

* Mineral Gause 1 medium (pH 5.3)with the creosol bromide purple indicator was used, which is colorless in acid media and turns purple in alkaline media.

No change in the pH of the medium was detected during growth of the neutrophilic strain on acidified Gause 1 medium.

The high rate of colonization of acidified medium by the acidophilic streptomycete and the formation of a developed substrate mycelium, apparently, help this organism increase the pH of the medium from 5.3 to 6.0, i.e, to a level making possible the formation of spores and the completion of the life cycle. Such an increase in the pH value of the medium could be accomplished through utilization of ingredients of the phosphate–citrate buffer by the organism or by release of certain products in the medium. A similar change in the cultivation medium acidity was previously observed with acidophilic bacteria [12].

It can be concluded that acidophilic actinomycetes have a special mechanism of adaptation to low pH values, consisting in their ability to grow faster on acidified media than on neutral ones and to elevate the medium pH to the level conducive to the development of aerial mycelium and spore formation. The existence of this mechanism allows acidophilic actinomycetes to develop in acidic soils without facing competition with neutrophilic actinomycetes.

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