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EXPERIMENTAL ARTICLES

Spore Germination and Mycelial Growth of Streptomycetes at Different Humidity Levels

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Abstract—This study is the first to show the ability of streptomycetes to develop at a very low humidity level. All of the streptomycetes studied produced growth at low humidity (a_w 0.86 and 0.67). This capacity was most markedly pronounced in *Streptomyces odorifer*, whose spores were capable of germinating, and mycelial germs increased in length, at the air humidity a_w 0.50. The formation of lateral branches (mycelium branching) at this humidity was noted only in single *S. odorifer* germs and only after 72 h of incubation. Study of streptomycete growth on an agarized medium with different osmotic pressures, created by various glycerol concentrations in the medium, showed that, at a_w 0.67, the spores of all the streptomycetes studied germinate, producing mycelial germs but not microcolonies. The ecological significance of mycelial prokaryotes in soil microbial communities that develop and function under conditions of extremely low humidity is discussed.

Key words: streptomycetes, humidity, spore germination, microcolonies.

Humidity is an important factor influencing the capacity of microorganisms for growth and development [1]. The humidity range in which an organism is able to develop determines its distribution in terrestrial ecosystems.

Certain fungi are considered to be the most xerophilic components of the soil biocenosis [2, 3]. They can develop at a_w 0.60 [4]. One organism capable of growing under such conditions is the fungus *Xeromyces bysporus* [5]. Prokaryotes are much more dependent on moisture than fungi, and most of them develop at an activity of water (a_w) equaling 0.97 or higher [1]. Extreme halophiles are an exception.

Actinomycetes are more resistant to soil desiccation than other bacteria [6]. The exospores of streptomycetes retain viability under conditions of absolute desiccation. The spores in the *Actinoplanes* sporangia retain viability for a long time in dried soil and leaf samples [7]. In the soils of arid regions, actinomycetes occupy an important place in the complex of soil prokaryotic organisms [8]. However, no experimental evidence of the vegetative growth of mycelial prokaryotes at low humidity values is available in the relevant publications. The results of studies performed with pure actinomycete cultures testify to the fact that the tolerance of actinomycetes to dehydration is determined by the resistance of the spores; the vegetative hyphae of mycelial prokaryotes are quickly injured by desiccation [9].

The aim of the present study was to determine the rates of spore germination and mycelium growth exhibited by streptomycetes at different humidity levels.

MATERIALS AND METHODS

Four streptomycete cultures isolated from different habitats and kept in the culture collection of the Department of Soil Biology in the Faculty of Soil Science at Moscow State University were the subjects of this study: *Streptomyces odorifer* 1, isolated from an enrichment culture of the cyanobacterium *Oscillatoria terebriformis* (Ag.) Elenk. emend., which is a component of a natural algobacterial cenosis; *S. rectiviolaceus* 3, isolated from sago palm plants; *S. pallidoviolaceus* C3, isolated from forest podzolic soil; and *S. violaceoruber* 7, isolated from the bottom sediments of Lake Balkhash.

The rates of streptomycete spore germination and mycelium growth were determined at different humidity levels, which were achieved using two methods.

The first method consisted of controlling the air humidity in desiccators with salt solutions (see table) [10]. Three humidity levels were used: (1) a_w 0.50 (–96.4 MPa; relative humidity (RH) of 50%), (2) a_w 0.86 (–22.6 MPa; RH of 86%), and (3) a_w 0.98 (–2.8 MPa; RH of 98%).

Monospore streptomycete suspensions (10^5 cells/ml) were applied to microscope slides (one drop was spread over an area of 1 cm^2), dried to attain an air-dry state, and the average spore number in a microscope field was counted under an Axiostar microscope at 1000×. The slides were placed in desiccators, where certain air humidity levels were created, and incubated in a ther-

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Comparison of the main humidity measurement units

Activity of water, a_w	RH, %	Moisture tension, MPa	Soil moisture potential	Desiccator with saturated solution of
0.98	98	–2.8	–27.6	K_2SO_4
0.86	86	–22.6	–223.0	KCl
0.50	50	–96.4	–961.5	$Ca(NO_3)_2$

mostat at 28 ± 1°C. The air humidity level in the desiccators was monitored using a Viking AB digital moisture meter, in which case the air humidity measurement error did not exceed 1%. The slides were examined under a microscope after 8, 24, and 72 h of incubation in the desiccator. The number of geminated spores (the number of spores with germ tubes) was counted and the length of the mycelium germs determined. An examined slide was not returned to the desiccator, and the following determinations were performed with a slide that remained in the desiccator.

The second method, proposed by the authors of the present paper, used an agarized medium (glycerol–nitrate agar [11]) with different osmotic moisture tensions. The osmotic tension was controlled by varying the glycerol concentration in the medium. The relationship between the osmotic tension and the glycerol concentration was determined in advance (see Fig. 1). Three humidity values were used: (1) a_w 0.67 (–53.6 MPa; RH of 67%), (2) a_w 0.86 (–22.6 MPa; RH of 86%), and (3) a_w 0.98 (–2.8 MPa; RH of 98%). Creation of a humidity below a_w 0.67 using glycerol appeared impossible.

The radial growth rate of the streptomycete colonies was determined on agarized media with different osmotic tensions. For this purpose, the streptomycete spores were placed on the surface of the agarized medium, which was contained in a Petri dish, using a preparation needle (the stab-inoculation technique). The dishes were incubated in a thermostat for 14 days at 28°C in desiccators. The humidity in the desiccators was monitored by means of the same Viking AB moisture meter. The diameter of the streptomycete colonies was measured under a microscope equipped with an ocular micrometer or under a magnifying glass using a sliding caliper. Measurements were made every two days over a period of 14 days. The radial colony growth rate was calculated using the formula

$$K_r = (d_2 - d_1)/(t_2 - t_1),$$

where d_1 and d_2 are the colony diameters (mm) at the initial (48th hour) and final measurements, respectively, and t_1 and t_2 are the times of the initial and the final measurements (days). The measurements were performed in 20 replicates.

If streptomycete colony formation was not observed after 14 days of growth, the number of sites on the Petri dishes in which spore germination (the formation of germ tubes) had occurred was counted under a microscope, and the percentage of stab inoculations that had resulted in spore germination was calculated.

The capacity of a streptomycete to develop at different humidity levels was estimated according to the following parameters: the presence of germinated spores, the intensity of mycelium germ branching, the forma-

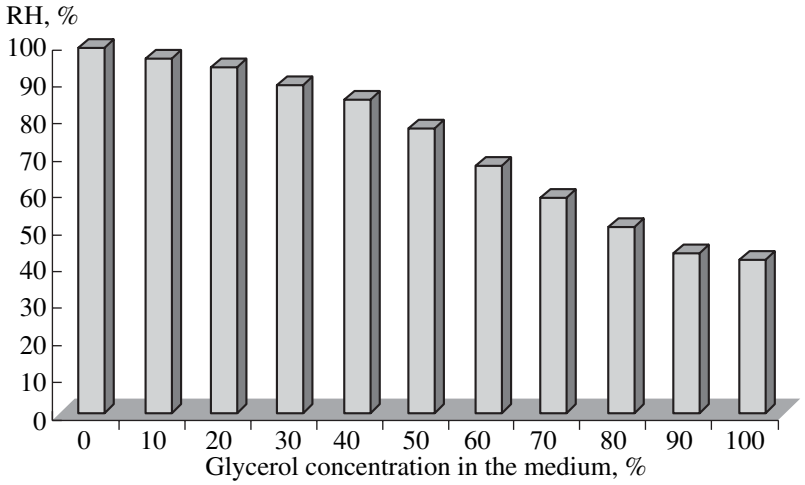

Fig. 1. Dependence of humidity on the glycerol concentration in the medium.

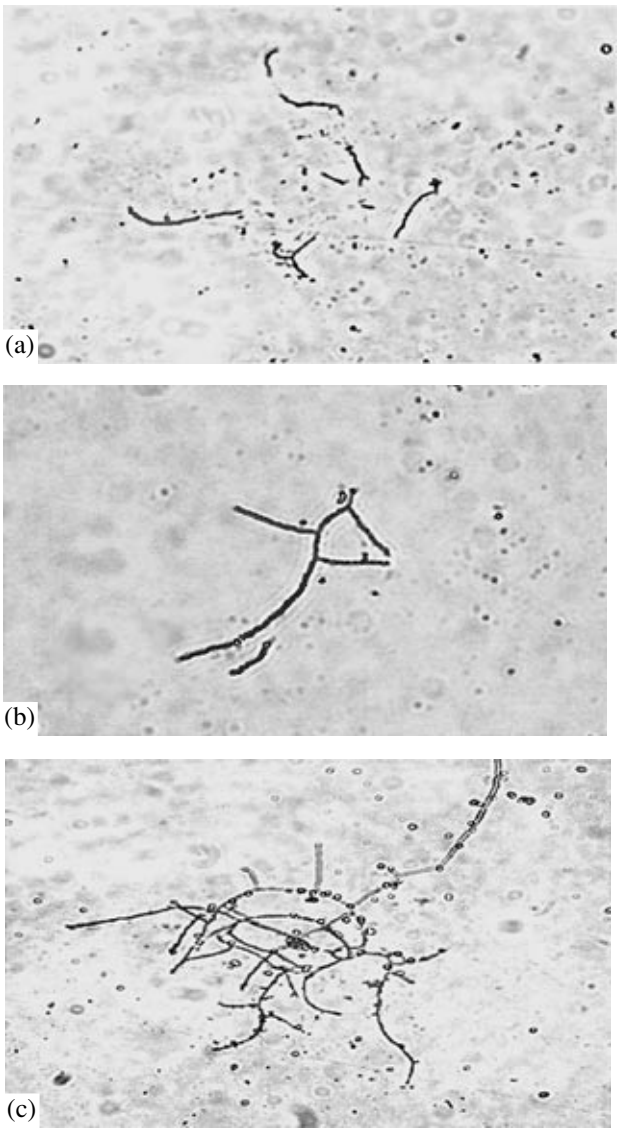

Fig. 2. *S. odorifer* mycelium after 72 h of development under different humidity conditions: (a) a_w 0.50; (b) a_w 0.86; and (c) a_w 0.98.

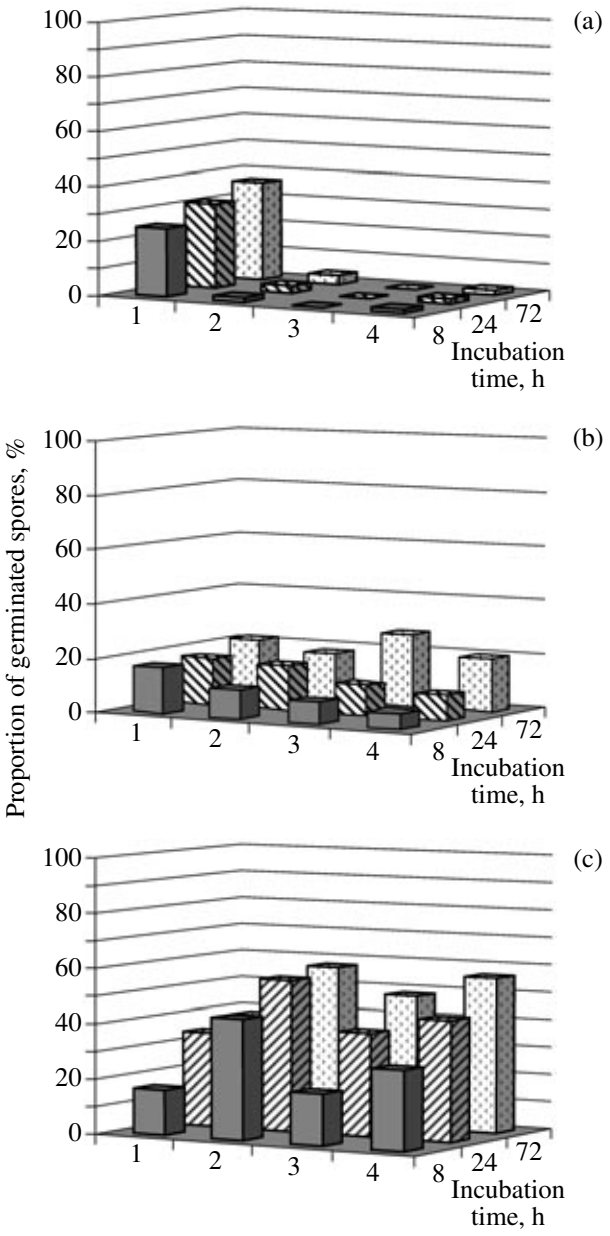

Fig. 3. Rate of spore germination in (1) *Streptomycetes odorifer* 1, (2) *S. rectiviolaceus* 2, (3) *S. pallidoviolaceus* C3, and (4) *S. violaceoruber* 7 at air humidities of (a) a_w 0.50, (b) a_w 0.86, and (c) a_w 0.98.

tion of microcolonies, the formation of aerial mycelium, and spore formation on the aerial mycelium.

RESULTS AND DISCUSSION

Our experiments showed that streptomycete spores were able to geminate at a_w 0.50. This capacity was noted to the highest degree in *Streptomyces odorifer* (see Fig. 2). After only 8 h of incubation in the desiccator, the number of germinated spores (spores with germ tubes) constituted 25% of the total spore number, and, after 72 h, this figure had increased to 36.4% (Fig. 3a). At this humidity level, the spores of *S. rectiviolaceus* and *S. violaceoruber* germinated at a lower rate (3.4 and 1.7% of the total spore number after 72 h of incubation). The spores of *S. pallidoviolaceus* did not germinate at all at this humidity.

At a_w 0.86 and a_w 0.98, the spores of all the streptomycetes studied germinated (Figs. 3b, 3c). At a_w 0.98, the most active germination was observed for the spores of *S. rectiviolaceus* and *S. violaceoruber* (57.3 and 56.2% of the total spore number after 72 h of incubation). The spores of *S. pallidoviolaceus* germinated in smaller numbers (48.2% of the total spore number after 72 h of incubation). The germination rate of *S. odorifer*

spores at a high humidity level (a_w 0.98) was approximately the same as at a low humidity level (a_w 0.50).

Study of streptomycete growth at different humidity levels showed that, at a_w 0.50, not only spore germination but also germ elongation occurred. At a_w 0.50, the most active growth was noted for the germs of *Streptomyces odorifer*: after 8 h, the average length of the germs was 3.1 μm; after 72 h, it was 8.3 μm (Fig. 4a). Mycelial growth in *S. rectiviolaceus* and *S. violaceoruber* occurred at a significantly lower rate: after 72 h, the average length of their germs did not exceed 2.9 and 2.0 μm, respectively.

At a_w 0.86, the length of the germs of all the streptomycetes studied increased with time. Under these conditions, the average length of the germs for *S. odorifer* constituted 5.1 μm after 72 h of incubation; in the other streptomycetes studied, it did not exceed 3.4 μm (Fig. 4b).

The greatest increase in mycelium length was noted in all of the streptomycete cultures studied at a_w 0.98 (Fig. 4c). However, the mycelium growth rate of *S. odorifer* at this humidity level was not reliably greater than it was at a_w 50.0. This result is probably connected with the fact that the strain studied was isolated from an enrichment culture of cyanobacteria that develop on the soil surface.

The formation of lateral mycelium branches at a_w 0.50 was noted only in some *S. odorifer* germs and only after 72 h of incubation; the average length of these lateral branches did not exceed 1.5 μm. At a_w 0.86, the formation of lateral branches after 24 h of incubation was observed in the strains whose spores were able to germinate at a_w 0.50, namely, in *S. odorifer, S. rectiviolaceus*, and *S. violaceoruber*; after 72 h, this was observed in *S. pallidoviolaceus.*

At a_w 0.98, the formation of lateral branches was observed in all of the streptomycetes studied as early as after 24 h of incubation; the formation of microcolonies occurred after 72 h.

When studying the growth of streptomycetes on an agarized medium in which the osmotic tension was varied by changing the glycerol concentration in the medium, we established that, at a_w 0.67, the spores of all of the streptomycetes studied germinated (germ tubes and mycelium germs formed); however, microcolony formation was not observed. The greatest percentage of stab inoculations that resulted in spore germination was noted for *S. violaceoruber* (90%); lesser percentages were recorded for *S. odorifer* (85%), *S. rectiviolaceus* (70%), and *S. pallidoviolaceus* (65%).

At a_w 0.86, the formation of microcolonies was observed in all of the streptomycetes studied. The highest rate of radial colony growth was noted for *S. odorifer* (8.8 μm/h); after 14 days of growth on the agarized medium, visible colonies (but no more than 3 mm in diameter) developed, and the development of sterile aerial mycelium was noted. In *S. rectiviolaceus*, the radial colony growth rate attained 6.7 μm/h; after 14 days, colonies devoid of aerial mycelium developed.

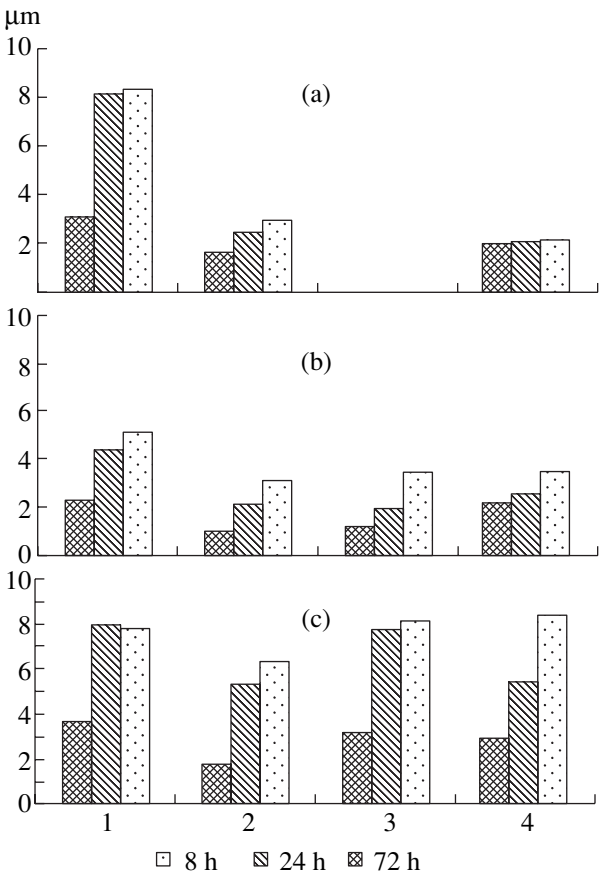

Fig. 4. Length of streptomycete mycelium germs under different air humidity conditions. See Fig. 3 for other designations.

In *S. violaceoruber*, the rate of radial colony growth was 5.9 μm/h; after 14 days, colonies with sterile aerial mycelium developed. The lowest rate of radial colony growth (5.1 μm/h) was noted for *S. pallidoviolaceus*, which developed no visually observable colonies.

At a_w 0.98, all the streptomycetes studied formed visually observable colonies on the agarized medium. Aerial mycelium with spores was formed in the colonies of all of the cultures except *S. pallidoviolaceus.* The colony growth rate in all the strains studied was significantly higher (two- to sevenfold) at this humidity than at a_w 0.86. The highest colony growth rate was noted in *S. violaceoruber.*

Thus, we revealed that the spores of streptomycetes are able to germinate and produce mycelium germs that increase in length at a low level of air humidity (a_w 0.50). This capacity is the most markedly pronounced in *Streptomyces odorifer.* The formation of lateral mycelial branches at a_w 0.50 was noted only in some germs of *S. odorifer* and only after 72 h of incubation. The average length of the lateral branches did not exceed 1.5 μm. When we studied the growth of streptomycetes on an agarized medium in which the osmotic tension was varied by changing the concentration of glycerol, we found that, at a_w 0.67, the spores of

all the streptomycetes studied germinated but no microcolony formation occurred.

These findings allow us to conclude that the activity of mycelial prokaryotes in soil may occur under low humidity conditions that are barely suitable for the activity of nonmycelial bacteria. Apparently, the capacity of actinomycete spores to germinate under low humidity conditions was conducive to their appearance as one of the first terrestrial organisms on dry land and to their participation in the processes of primary soil formation.

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