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Population Density and Size of Bacteria in the Course of Cultivation of Their Small Forms

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Abstract—The population density, size, and biomass of the soil bacteria from a mountain meadow-steppe soil of Tajikistan and a light sierozem of the Negev Desert have been analyzed using the method of "cascade" filtration. It was shown that, when cultivating small fractions of soil bacteria, the total number of bacteria increased by 1.5 times and the bacterial size became greater. The number of coarse cells with a size of 1.85 and 0.43 μm essentially increased in both soils. If the contribution of these fractions was about 10–20% in the initial soils, it increased up to 50–60% in the incubated filtrates. The cells with a size of 0.38 and 0.23 μm accounted for about 70% of the total bacteria in the initial soils, while, in the incubated filtrates, the share of 0.23 μm cells composed about 30% in the filtrate and that of 0.38 μm cells reached 45–50% in the filtrate. The average diameter of the bacteria increased from 0.4 to 0.8–0.9 μm; the biomass of bacteria in these filtrates increased by 7–8 times in comparison with the initial soils at the expense of an increasing number of large cells after cultivation.

Keywords: Mollic Leptosols, Haplic Calcisols (Endosalic, Yermic), bacteria, cell size, cultivation, biomass

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INTRODUCTION

It is supposed that the small size of the bacteria in a soil is determined by the natural conditions, which limit the bacterial development by a deficit of nutrients or by stress situations. Small bacteria cells with a minimal size of 0.2 μm, which is considered to be the smallest [14], were found with the help of an epifluorescent microscope in many samples from lakes, rivers, soils, snow, and rainwater as a response of the cells to unfavorable environment and stress factors, which were studied in laboratory experiments [10]. It was found that the cultivation of the obtained nanoforms on rich nutrient media resulted in the return of the initial forms [1, 6, 11, 20].

An earlier study with rearing cells (0.23 μm in diameter) in the filtrates of a soil suspension in a thermostat at a temperature of 28°C demonstrated that the bacterial cells reproduced intensely during incubation, and this was recorded with the classic method by the increase of the population density on glasses [9]. It should be noted that the cell rearing was carried out in a soil suspension without introducing additional nutrient substrates.

The aim of this work was to study the number and size of the bacterial cells before and after cultivation of their small forms (0.23 and 0.38 μm) under favorable moisture and temperature conditions.

OBJECTS AND METHODS

Two samples of soils developing under xerophytic communities were studied. A sample from the surface layer (0–5 cm) of a mountain meadow-steppe shallow subalpine soil (Mollic Leptosol) was taken 20 km northwest of Dushanbe (Tajikistan) at the altitude of 1000 m a.s.l. in November 2010. It was placed into a sterile bag, stored for 8 h at –4°C, and then taken to the laboratory (within 8 h), where it was stored at –18°C.

A sample from a loess-like residual-solonchakous subtropical light sierozem (Haplic Calcisol (Endosalic, Yermic)) was taken in the Negev Desert (Israel) at the altitude of 600–800 m a.s.l. This is an area with an arid subtropical climate with mean monthly temperatures ranging from +12°C in January to +27°C in July and annual precipitation from 50 to 300 mm; no rains take place from June to October. The soil was formed under conditions of a nonpercolative water regime and was characterized by considerable accumulations of carbonates, sulfates, and chlorides in the subsoil.

The samples were subjected to ultrasonic pretreatment using a low-frequency UZDN-1 (22 kHz, 0.44 A, 2 min) disperser [4].

The method of cascade filtration was applied to determine the numbers of bacteria in separate size fractions: 1 mL of soil suspension (1 : 100) was filtered through nuclear filters (Dubna Scientific Center Production) with pore diameters of 1.85, 0.43, 0.38, and

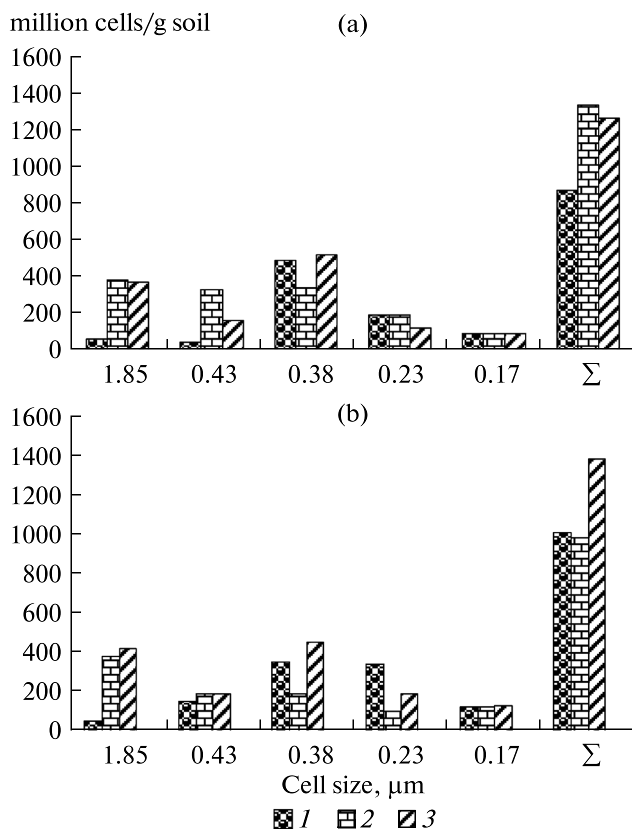

Fig. 1. Distribution by size and total number of bacterial cells (Σ) in the (a) mountain meadow-steppe soil and (b) light sierozem in the (*1*) initial state and after growing in thermostat at 28°C of small bacterial forms of (*2*) 0.23 and (*3*) 0.38 μm in size.

0.23 μm and a membrane filter (Synpore) with a pore diameter of 0.17 μm. The filtration was performed with a vacuum pump and a Bunsen flask. The luminescence of the filters was extinguished by staining with a saturated alcohol solution of Sudan black (Germany). The nuclear filters were placed into this solution for several days and the membrane filters for several hours; then, they were washed out in sterile water, dried, and used for filtering [13].

Four layers of filtering paper were placed on the surface of a metallic screen in a Bunsen flask, a filter (nuclear or membrane) was placed atop and forced against the surface of the device with a metallic ring, and the suspension was added. The suspension was filtered step-by step from the filter with greater pore size to that with a smaller pore size. The number of bacteria was counted on every filter (three replicate filters were used), and the population density was calculated taking conventionally that the cell size was equal to or slightly greater than the pore diameter of the filter on which the bacteria were retained. The calculations were performed on the basis of the assumption that the cells had a spherical shape, and this was confirmed by the data of electron and scanning microscopy [8].

Bacterial suspensions from both soils were filtered through filters of 0.23 and 0.38 μm, and the obtained filtrates were cultivated in a thermostat at 28°C for 136 h; then, "cascade" filtration of these suspensions was carried out, and the obtained results were compared with the data of "cascade" filtration in the initial soils.

The number of cells per 1 g of soil was calculated by the following formula:

$$N = S_1 an / v S_2 c,$$

where N is the cell number per 1 g of soil; S_1 is the area of the specimen, μm^2; a is the number of cells per one field of vision (data averaging was performed for all the filters); n is the dilution of the soil suspension, mL; v is volume of the filtered suspension, mL; S_2 is the area of the visual field of the microscope, μm^2; and c is the weighed portion of soil, g.

When calculating the bacterial number by the commonly adopted method, the dry weight of a bacterial cell of volume 0.1 μm^3 was taken to be equal 2×10^{-14} g [5]. The mass of a cell of real size was proportional to the mass of the cell of volume 0.1 μm^3. The mean square deviation (δ_{n-1}) for the values of the population density of the bacteria in the sample did not exceed 5–10%.

RESULTS AND DISCUSSION

The initial total population of bacterial cells obtained on the filters with "cascade" filtration in the initial soils comprised about 1 billion cells/g of soil in the mountain meadow-steppe soil and light sierozem.

There was a significant difference between the populations of bacterial cells of different fractions in the initial mountain meadow-steppe soil and in the incubated filtrates (Fig. 1a) The number of cells 1.85 μm in size increased after cultivation approximately by sixfold in both fractions (0.23 and 0.38 μm) of the mountain meadow-steppe soil. The number of cells of size 0.43 μm increased 8 times in the fraction of 0.23 μm and by 4 times in the fraction of 0.38 μm. The number of cells of size 0.38, 0.23, and 0.17 μm did not change or was slightly lower than in the initial soil.

As follows from Fig. 1b, the difference between the populations of bacterial cells of different fractions in the initial soil and in the incubated filtrates was not significant in the light sierozem. The number of large cells 1.85 μm in size increased after cultivation eightfold in both fractions (0.23 and 0.38 μm) in the light sierozem. The number of bacterial cells of size 0.43 μm increased insignificantly in both cultivated filtrates, and the number of bacteria of size 0.38 and 0.17 μm practically did not change in the process of cultivation of the filtrate of the fraction of 0.38 μm. The number of cells of size 0.23 μm in both filtrates and of cells of size 0.38 μm in the fraction of 0.23 μm grew twice less in comparison with the initial soil.

It is obvious that the number of large cells 1.85 and 0.43 μm in size increased significantly in both soils. If the contribution of these fractions in the initial soils

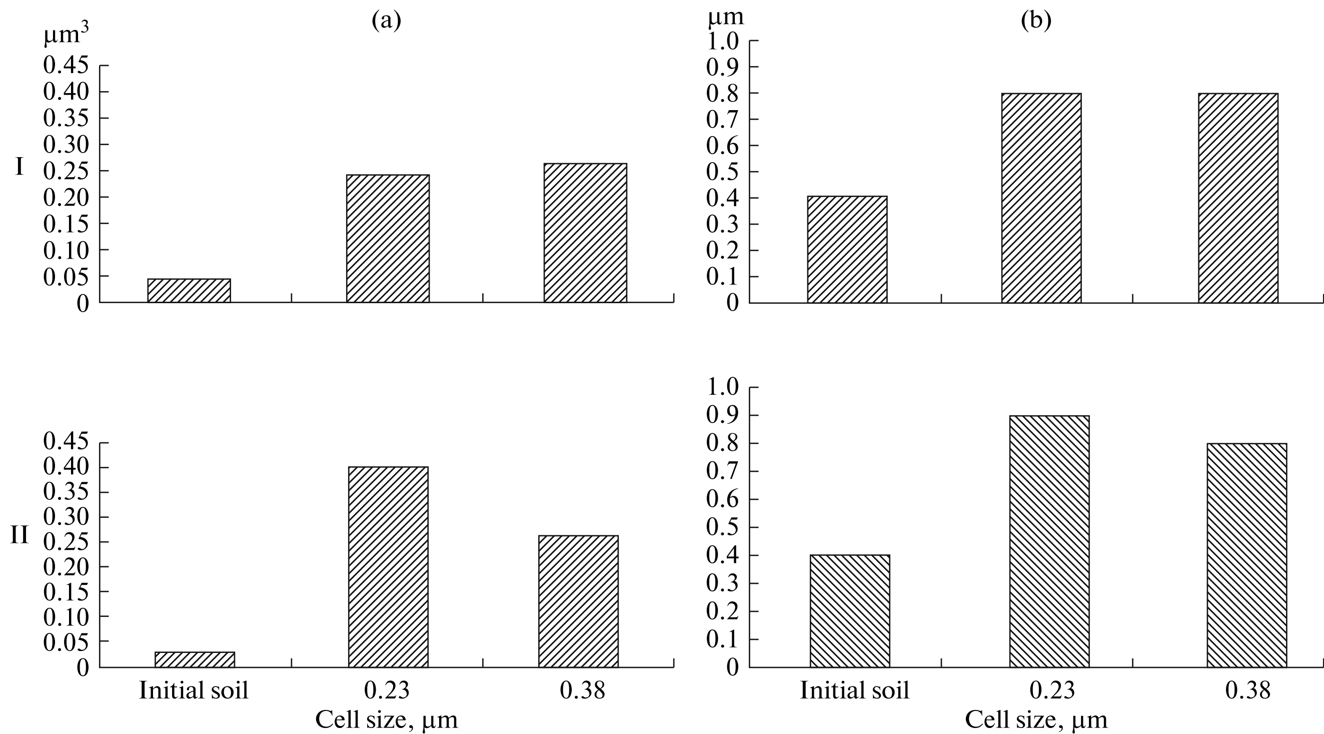

Fig. 2. Volume (a) and diameter (b) of average bacterial cell in initial mountain meadow-steppe soil (I) and light sierozem (II) after growing in thermostat under the temperature 28°C of filtrates 0.23 and 0.38 μm.

was about 10–20%, it increased up to 50–60% in the incubated filtrates. The cells of 0.38 and 0.23 μm comprised about 70% of the total number of bacteria in the initial soils, and, in the incubated filtrates, it was about 30% in the filtrate of cells of 0.23 μm and 45–50% in the filtrate of cells of 0.38 μm. Additionally, a trend was observed in both soils and the cultivated filtrate of 0.23 μm towards the more uniform distribution of the number of large cells (1.85, 0.43, and 0.38 μm), whereas the maximal contribution in the filtrate of 0.38 μm was made by the cells of 1.85 and 0.38 μm, but the number of cells 0.43 μm in size was relatively low. This redistribution resulted in the increase of the average volume and diameter of the bacteria (Fig. 2).

The biomass comprised about 40 μg/g of soil in the initial mountain meadow-steppe soil, and it increased to 250 μg/g in the incubated filtrates of 0.23 and 0.38 μm.

The biomass amounted to approximately 35 μg/g in the initial light sierozem, and it increased in the incubated filtrates 0.23 and 0.38 μm to a value similar to that in the mountain meadow-steppe soil (250–280 μg/g).

As the table shows, the main contribution to the biomass (90–98%) in all the samples was made by the cells 1.85 μm in diameter, about 2–3% was made by the cells 0.38 μm, and all the other size groups amounted to 1%. An approximately fivefold increase of the total biomass occurred at the expense of the biomass of the bacteria 1.85 μm in size.

The weighted mean diameter and average volume of the bacterial cells in the mountain meadow-steppe soil and the light sierozem were calculated on the basis of the data on the population and size of each bacterial fraction.

The cell volume amounted to 0.04 μm^3 in the initial mountain meadow-steppe soil and 0.24 and 0.26 μm^3, respectively, in the incubated filtrates of 0.23 and 0.38 μm. The cell volume amounted to 0.03 μm^3 in the initial light sierozem, and it increased to 0.4 and 0.26 μm^3, respectively, in the incubated filtrates (Fig. 2a).

The average diameter of a bacterial cell in the mountain meadow-steppe soil equaled 0.4 μm in the initial sample, and this value increased to 0.8 μm in the filtrates of 0.23 and 0.38 μm. The average diameter of a bacterial cell in the light sierozem equaled in the initial sample that in the previous soil, and it doubled in the incubated filtrates (Fig. 2b).

According to the available publications, the volume of the bacterial cells in the soils varies in a wide range: from 0.0042 [14] to 1.2 [3] μm^3 in most cases when using the luminescent-microscopic method. Our data for pure soils were approximately similar to the sizes obtained by D.G. Zvyagintsev in 1973, and the size of the cells grown from cell filtrates was in the range of values obtained by another group of scientists [12, 16, 17].

Bacterial biomass in the initial soils and after growing in a thermostat at 28°C of filtrates of 0.23 and 0.38 μm: above the line, μg/g; under the line, %

Cell size, μm	Initial soil	Filtrate, μm	
		0.23	0.38
Mountain meadow-steppe soil			
1.85	38/92	249/98	242/98
0.43	0.4/0.8	3/1	1/0.6
0.38	3/6.5	2/0.84	3/1.3
0.23	0.2/0.57	0.2/0.14	0.2/0.05
0.17	0.05/0.13	0.05/0.02	0.05/0.05
Total biomass	41/100	254/100	246/100
Light sierozem			
1.85	31/89	250/99	276/98
0.43	1/4	2/0.57	2/0.74
0.38	2/6	1/0.35	3/1.09
0.23	0.4/1	0.1/0.06	0.3/0.15
0.17	0.06/0	0.06/0.02	0.07/0.02
Total biomass	35/100	253/100	281/100

CONCLUSIONS

The presence of a "not cultivating" block of bacteria and of a great number of cells with size significantly smaller than 1 μm in the soils intrigued the researchers long ago. Studies of microorganisms responses to stress demonstrated that the effects of unfavorable factors induced the significant physiological rearrangement of bacterial cells "in situ," and this could promote the transition into the noncultivating state and the changing of the cytomorphological characteristics, including the pronounced reduction of the size [2, 7, 15, 18, 19, 21]. It was demonstrated in the experiment that a significant part of the soil bacteria, which had a submicron size and could be considered as nanoforms, increased under favorable conditions not only in terms of the population density but also in terms of the cell size and, thus, additionally contributed to the total biomass. It is fair to say on the basis of these data that the most part of the bacterial pool in the soil passes into the state of hypometabolism or anabiosis under the effects of stress and, first of all, a deficiency of nutrition, and this is attended with the decrease of the cell size to submicron values up to the appearance of a significant number of "nanoforms," which can return to the initial normal cell size under favorable conditions. There is apparently a good reason to account for the presence of some nonvolatile store of nanocells and their deep anabiosis, though we cannot rule out the possible presence of a number of specific "nanoforms."

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