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SHORT COMMUNICATIONS

Electron Microscope Investigation of the Biofilms of a Binary Bacterial Community

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The presence of locomotory organs (flagella or pili) and synthesis of adhesins and glycocalix are the main conditions for the formation of structured bacterial communities [1, 2]. The majority of bacteria possess these characteristics. At the present time, numerous transgenic microorganisms (TM) have been created in which certain characteristics are manifested to a lesser degree than in the wild type strains. Accidental leakage to open ecosystems is possible for TM that are used in agriculture or veterinary medicine. They bring new genetic information to the community and can form stable associations (biofilms) with native species; these structures will contribute to their prolonged survival in the environment [3].

One of the stress factors in aquatic environments is the concentration of dissolved inorganic ions. Osmotic stress can cause the death of bacteria or delay their growth and reproduction. Heavy metals accumulating in aquatic ecosystems can affect both macro- and microbiological communities. Bacteria have various mechanisms to counteract the stresses caused by heavy metals; these include binding the metal into complex compounds, their transformation into less toxic compounds, and their excretion from the cell [4, 5]. It is necessary to take into account that under the conditions of cumulative effects of abiotic factors of natural (variable medium salinity) and anthropogenic origin (ions of heavy metals), interpopulation relations can change and the balance between microbial species in the ecosystem can be disrupted. The survival rate of TM under stress conditions can possibly increase as a result of their interaction with native microorganisms of the ecosystem. Experimental confirmation of this assumption was obtained by the combined cultivation of TM with environmental halotolerant bacteria [6].

The present work was devoted to the investigation of the structures of biofilms formed during the combined growth of halotolerant bacteria *Alcaligenes* sp. 1–1 isolated from the bradlish Lake Shira, and TM *Bacillus subtilis* 2335 under stress conditions (variability of NaCl content and the presence of copper ions in the culture medium). *B. subtilis* 2335 contained the plasmid pBMB105 with genes of human α2-interferon and kanamycin resistance. The cells were cultivated in test tubes without shaking at 22°C for three weeks for biofilm formation at the air–liquid interface.

Monocultures of the strains investigated formed biofilms on the liquid surface in media with increasing salinity (0.05–5% NaCl). Under the double stress, in the presence of Cu^{2+} and NaCl in medium, *Alcaligenes* sp. 1–1 formed biofilms under the low concentration of Cu^{2+} (50 mg/l). Increase of the copper content in the nutrient medium up to 250 mg/l inhibited the formation of structured communities. Under the maximum concentrations of Cu^{2+} (250 mg/l) and NaCl (5%), bacilli aggregated in flakes in the medium column 1–2 days after inoculation. Later, cell aggregates dissociated, and culture growth was not observed.

During the combined growth of *B. subtilis* 2335/pBMB105 and *Alcaligenes* sp. 1–1 in media with increased salinity (0.05–5%) and maximum content of copper ions (250 mg/l), the cells of the binary community formed thin unstable films at the air–liquid interface (Fig. 1). The structure of the biofilms of the binary community in the medium with 2.5% NaCl and 250 mg/l Cu^{2+} was investigated using transmission electron microscopy. Analysis of the biofilm profiles revealed their heterogeneity in thickness. Analysis of ultrathin sections revealed the heterogeneity of cell composition of the biofilms. Environmental bacteria *Alcaligenes* sp. 1–1 were the main component. They were rod-shaped cells gram-negative with a typical dense packing of DNA in the nucleoplasm [7]. Cells of *B. subtilis* 2335/pBMB105 were smaller and had an electron-dense cytoplasm. These two species occupied different niches in the biofilm. The dominant strain *Alcaligenes* sp. 1–1 formed a biofilm proper, which bordered both with the liquid nutrient medium and with

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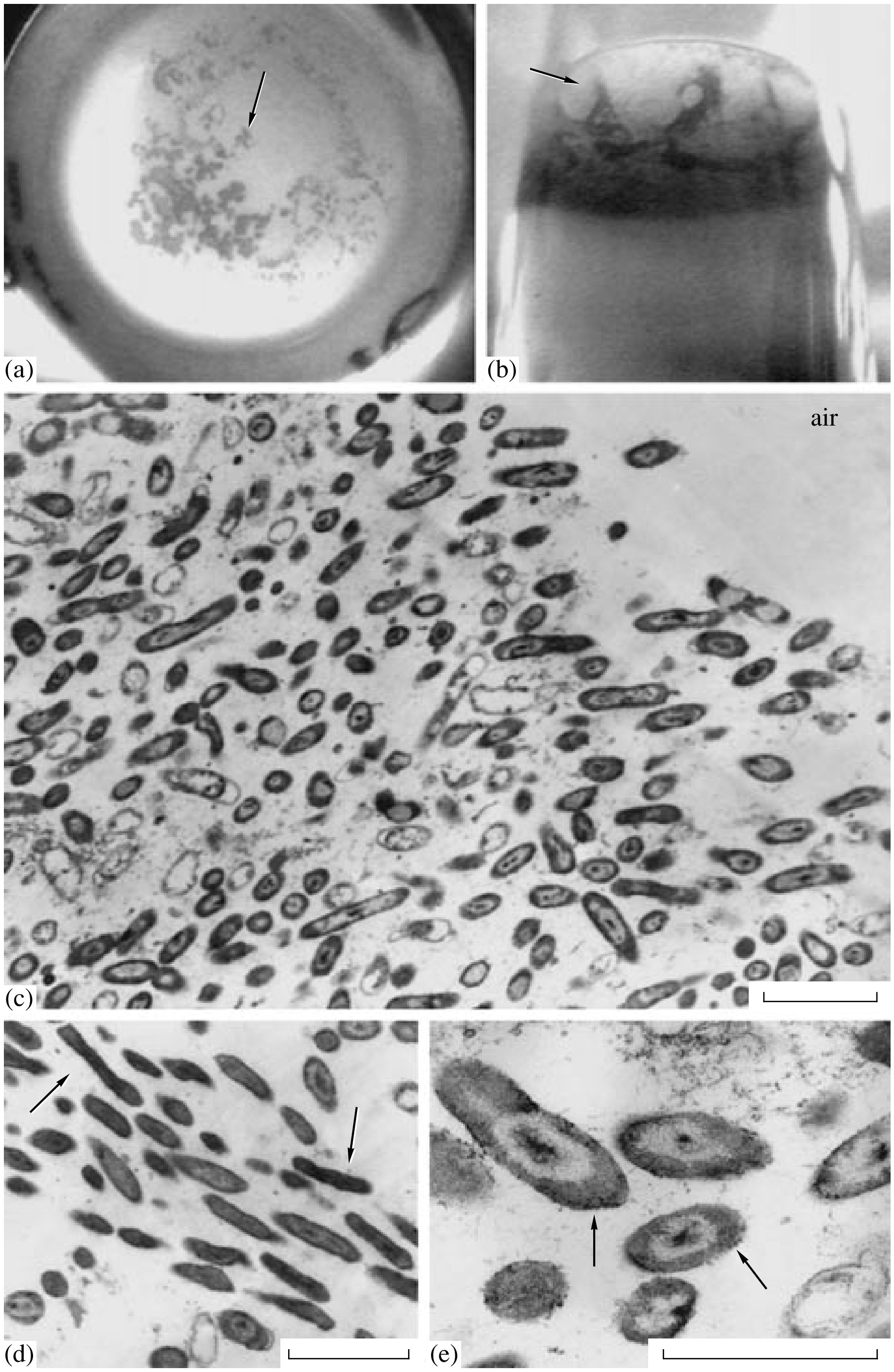

Fig. 1. The formation of biofilm on the surface of liquid medium under the combined growth of environmental halotolerant bacteria *Alcaligenes* sp. 1–1 and TM *B. subtilis* 2335/pBMB105: (a) formation of the first microcolonies on the surface; (b) 7-day biofilm of heterogeneous thickness; (c) ultrathin cross section of a part of the biofilm adjucent the air; (d) cells of *B. subtilis*; (e) electron-dense metal accumulations in cytoplasm and cell envelope of *Alcaligenes* sp. 1–1 (indicated by the arrows). Scale bar, 1 μm.

air. Bacilli were localized, as small groups, near the air boundary or above the upper boundary of the biofilm. Probably, halotolerant environmental bacteria started to grow on the surface of the liquid, while bacilli later formed their colonies at the boundary with air. We have previously shown that interpopulation relations between these strains change under stress conditions [6]. *B. subtilis* 2335/pBMB105 exhibited a bacteriostatic effect on *Alcaligenes* sp. in the absence of stress factors, whereas halotolerant bacteria suppressed the growth of *B. subtilis* under 2.5% NaCl and 250 mg/l Cu^{2+}. It is known that the formation of microcolonies in the multispecies biofilms is an adaptive bacterial strategy contributing to their survival in the course of their interactions with other species [8]. Small electron-dense particles of metal were localized in the bacterial cell envelope and cytoplasm. Bacteria *Alcaligenes* sp. 1–1 accumulated copper particles more actively, and bigger metal accumulations were revealed in their cytoplasm (Fig. 1).

Thus, halotolerant bacteria were more resistant to the maximum used concentration of copper ions and formed biofilms; however, *B. subtilis* 2335/pBMB105 were able to survive in the community with natural bacteria in spite of their negative effect. The possibility of TM survival in natural aquatic ecosystems as biofilms on different biotic and abiotic surfaces can therefore be assumed.

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