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SHORT COMMUNICATIONS

A Study of Native Cell Morphology and Cell Surface Relief of Some Gram-Negative Bacteria

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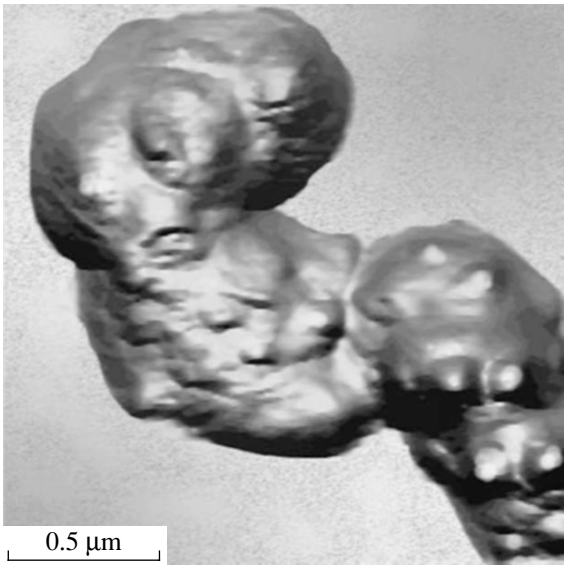
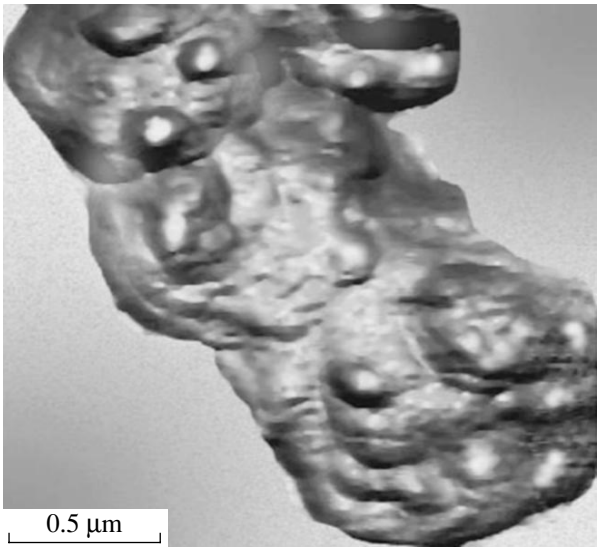
The current techniques for ultrahigh resolution of native biological structures have inherent restrictions. For example, transmission electron microscopy (TEM) imposes high energy loads and high vacuum on the specimen [1]. Along with the variety of light microscopes, a new approach to microscopic examinations has been provided by the development of scanning tunneling microscope (STM) [2] and atomic force microscope (AFM) [3]. The latter belongs to the group of probe microscopes that operate by scanning a specimen with microsensors (probes) through the movement of piezoelectric micromanipulators. A probe (silicon needle) is positioned at the tip of a tiny springy bar (cantilever). When the probe is scanned across the specimen surface, a repulsive force arises that bends the cantilever. This cantilever bending and thus the *Z* coordinate of the needle tip are registered in the course of scanning in each point through a feedback system. The possible range of specimen displacement is 100–200 μm in the *XY* plane and 5–12 μm along the *Z* axis, with an accuracy of one angstrom. As a result, a three-dimensional image of the object surface is formed. Information from the scanner is digitally processed on a computer [5]. The major advantage of AFM is the visualization of intact live microobjects with a high resolution (comparable with that of an electron microscope). The detailed





Figs. 1–3. Cells of *T. mutans* at the late logarithmic growth phase.

Fig. 2.

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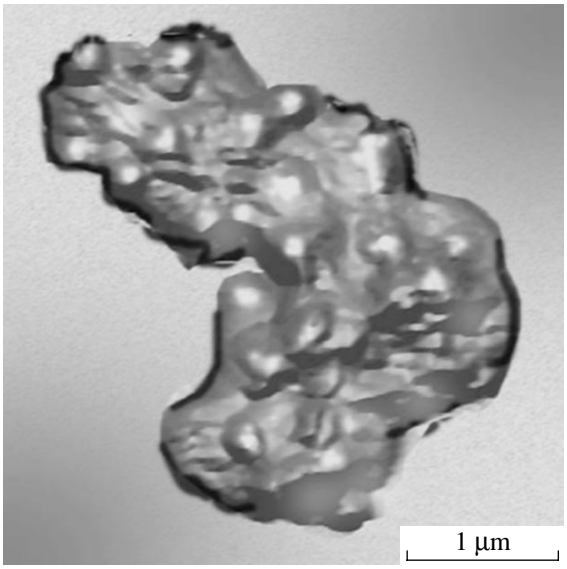


Fig. 3.

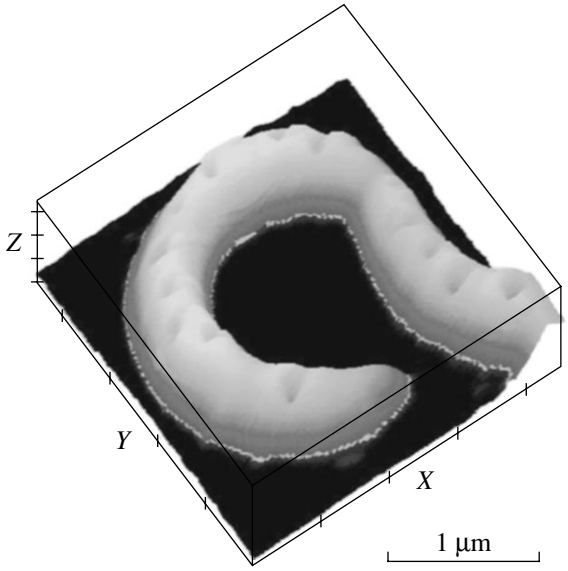


Fig. 4. A part of *S. platensis* trichome.

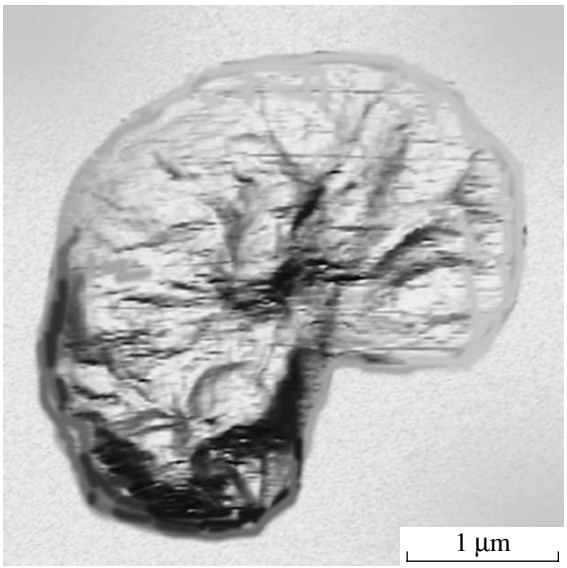
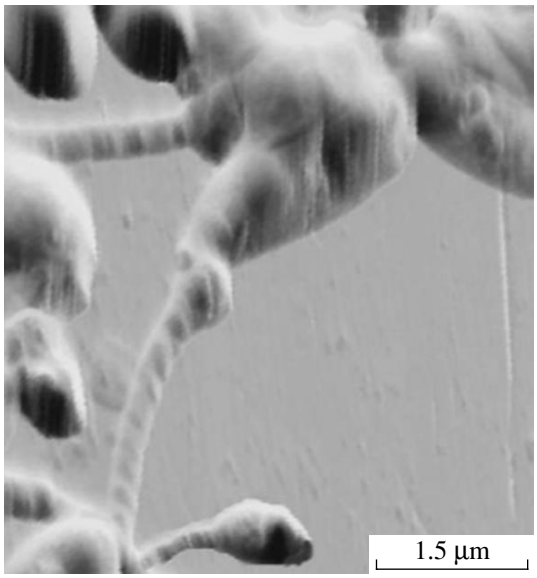


Fig. 5. A toroidal cell of *R. vacuolatum.*



Figs. 6, 7. Cells of *H. vulgare* with hyphae and buds.

description of AFM has been given in several publications [4–6].

The aim of the present work was to examine the features of the shape and surface of living cells under a probe (atomic force) microscope at a high resolution.

Cell surface imaging was performed on a P4-SPM-MDT scanning probe microscope (Nanotekhnologiya, Russia) in the AFM regime with a constant contact. The cantilevers used (Microlevers MSCT-AUHW, Thermo Microscopes, United States) were triangular and 180 μm in length; the bar width and thickness were 18 and 0.6 μm, respectively; the needle was 3 μm in length; the elasticity coefficient was 0.05 N/m; and the resonant frequency was 22 kHz. In all images, the dot number was 256×256.

This study was carried out with bacterial cultures obtained from the collection of the Institute of Microbiology, Russian Academy of Sciences: the prosthecate bacterium *Tuberoidobacter mutans* U2, characterized by large cellular bulges [7]; the methylotrophic bud-

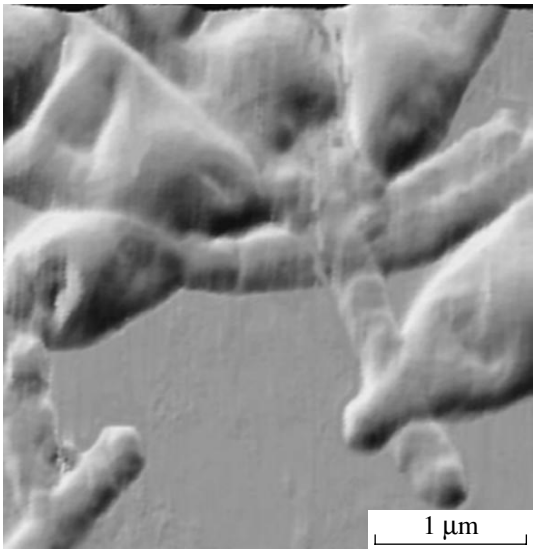


Fig. 7.

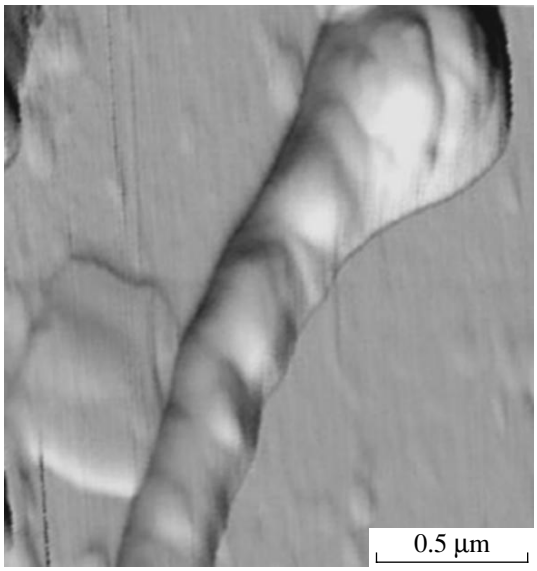


Fig. 8. A part of the *H. vulgare* hyphae.

ding bacterium *Hyphomicrobium vulgare* NP-160, forming hyphae and buds; the budding soil bacterium *Renobacter vacuolatum* [7]; and the cyanobacterium *Spirulina platensis*, obtained from the collection of the Faculty of Geography, Moscow State University. *T. mutans* and *R. vacuolatum* were maintained and grown on PYG medium containing peptone, yeast extract, and glucose (0.1 % each); the budding bacterium *H. vulgare* was grown on a synthetic methanol-containing medium [8]; and the cyanobacterium *S. platensis* was cultivated on Zarrouk medium [9].

Specimens were prepared using a K8 glass support ($35 \times 10 \times 0.8$ mm). Cell suspensions were cleaned of contaminating materials by repeated centrifugation (3–5 times). Cells were adsorbed on a glass surface either by dipping a support into the cell suspension or by pouring a drop of diluted cell suspension onto a glass support and allowing its gradual distribution over the support surface under a cold air flow to obtain a cell density of 1–2 cells/100 μm^2. Preliminary estimation of the cell density was performed under a light microscope. To abate the noise at each point of the image during AFM examination, the signals were averaged over ten measurements.

During AFM imaging, living cells of the bacteria under study were visualized without any preliminary treatment that could have changed the size and morphological peculiarities of the cells. Figures 1–3 demonstrate characteristic features of the bacterium *T. mutans* U2: curved cells with surface outgrowths in the form of bulges. The cell dimensions ($3–5 \times 0.8–1.0$ μm) are consistent with those measured under an electron microscope. Figure 4 shows a three-dimensional image of a part of the *S. platensis* trichome; the observed cell surface is wrinkled, apparently due to differing rigidity of different cell wall layers. The cell shape and dimensions of the budding bacterium *R. vacuolatum* (2×3.65 μm) (Fig. 5) conform to the data obtained using an electron microscope. Slight cell flattening was observed as a result of the specimen drying; this was not observed in the case of spirulina cells, which possess a thick murein layer. Microphotographs of the budding bacterium *H. vulgare* NP-160 (Figs. 6–8) demonstrate morphological peculiarities of cells, hyphae, and buds and thus provide an amount of information comparable to that obtained during electron microscopic examination of intact cells [7]. The application of AFM made it possible to reveal specific spiral convolutions of *H. vulgare* hyphae with a pitch of 0.1 μm at an angle of 35–40° with respect to the long axis, which has not been observed earlier. This emphasizes the definite advantages of atomic force microscopy as compared to transmission electron microscopy.

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