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SHORT COMMUNICATIONS

The Morphological and Physiological Differences between Fast- and Slow-Growing *Escherichia coli* Cells

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The adaptive response of microbial cells to a deficiency of nutrients involves two different strategies, the strategy of fast growth [1] and the strategy of slow growth [2]. The physiological mechanisms of switching to these strategies are still poorly understood, largely because of the difficulty associated with the methodology of analysis of individual bacterial cells belonging to different clones. In this work, some morphological and physiological parameters of *Escherichia coli* strains characterized by different specific growth rates μ in a minimal synthetic medium were comparatively studied by the method of flow cytofluorimetry.

The *Escherichia coli* strains B and 392 used in this work were obtained from the collection of microbial cultures at the Department of Microbiology, Kazan State University, and from the Kazan Research Institute of Epidemiology and Microbiology, respectively. Strain B was characterized by a more intense respiration and a higher maximum specific growth rate (μ_{max} = 0.375 h^{-1}) than strain 392 (μ_{max} = 0.258 h^{-1}). Based on the difference in their specific growth rates, strains B and 392 were arbitrarily called the fast-growing strain and the slow-growing strain, respectively. The strains were grown at 37°C in shaken (120 rpm) 250-ml flasks in a synthetic medium containing (g/l) Na_2HPO_4, 6; KH_2PO_4, 3; NaCl, 0.5; and NH_4Cl, 1. After autoclaving, the medium was supplemented with 10 ml of 0.01 M $CaCl_2$, 1 ml of 1 M $MgSO_4 \cdot 7H_2O$, and 10 ml of 20% glucose. The medium was inoculated (to a density of about $(3–5) \times 10^7$ cells/ml) with a 16-h-old *E. coli* culture grown at 37°C in nutrient broth in shaken (120 rpm) flasks. The culture turbidity was measured at 670 nm using a KFK-3 photocolorimeter.

The membrane potential $\Delta\Psi$ of cells was measured by the method of Novo *et al.* [3], with the exception that the red-fluorescent membrane probe chloromethyl-X-rosamine (CMXR) was used instead of diethyloxacarbocyanine. The fluorescence of the probe was excited by 488-nm-wavelength laser light at a rate of no more than 1000 cells/s and recorded using a FacsCalibur flow cytofluorimeter (Becton Dickinson, United States). The red fluorescence of CMXR was proportional to $\Delta\Psi$. In addition to this parameter, we also measured the forward and sideward light scattering of cells (FSC and SSC, respectively), FSC being an index of the size of cells, and SSC being an index of the degree of cytoplasm granularity. The results of these measurements were processed with the aid of the Kolmogorov–Smirnov statistics.

The flow cytofluorimetric analysis of fast- and slow-growing cells revealed statistically significant differences between them, the slow-growing cells being characterized by greater sizes, a higher degree of cytoplasm granularity, and a higher value of the membrane potential $\Delta\Psi$ (Fig. 1, I). Similar data on the size of *E. coli* cells cultivated over a long period of time in a medium with a low content of glucose were reported by Mongold and Lenski [4], who considered their results as an indication of the existence of a relation between the adaptive potential of cells and their size.

The high degree of cytoplasm granularity in slow-growing cells suggests that they accumulate some metabolic products and/or that the intracytoplasmic membrane structures of such cells are morphologically more developed than those of fast-growing cells.

Unexpectedly, the membrane potential $\Delta\Psi$ of slow-growing cells was higher than that of fast-growing cells. To the best of our knowledge, there are no data in the literature concerning the dependence of the membrane potential of bacterial cells on their growth rate and other physiological parameters. At the same time, Bobyleva *et al.* [5] reported on a decrease in the mitochondrial membrane potential $\Delta\Psi_m$ of rat hepatocytes in the order hypothyroid state > euthyroid state > hyperthyroid state and on an increase in the respiration rate of hepatocytes in the same order. These observations allowed Babyleva to conclude that the degree of coupling between phosphorylation and electron transfer in hepatocyte mitochondria is at a maximum under hypothyroid conditions and is at a minimum under hyperthyroid conditions. Taking into account the

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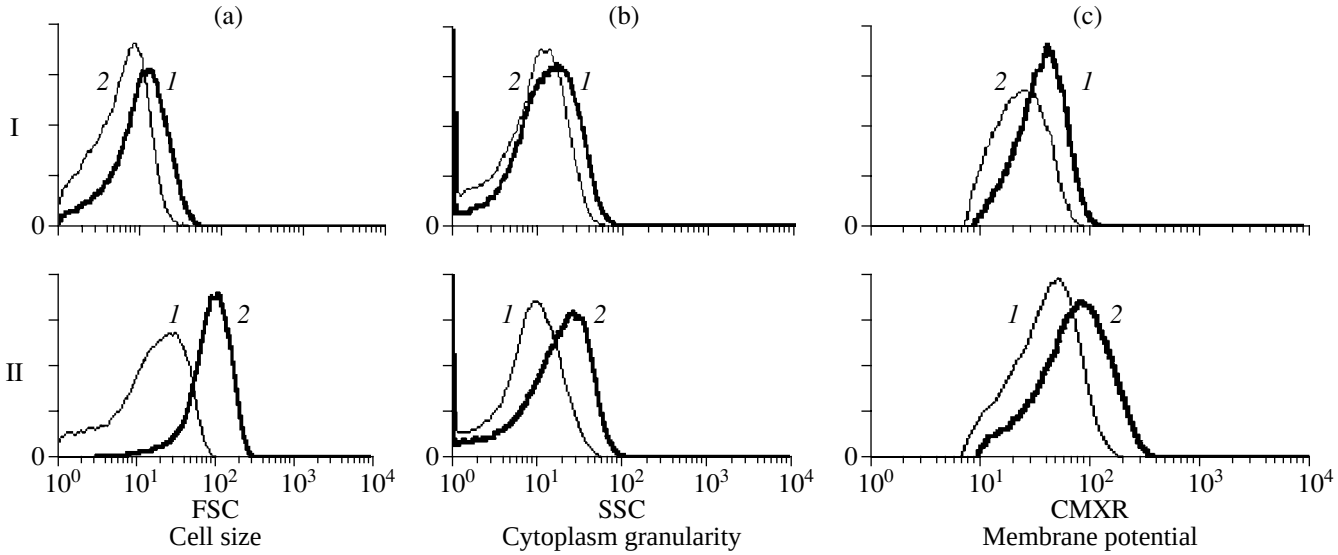

Fig. 1. The size (a), the cytoplasm granularity (b), and the membrane potential (c) of *E. coli* cells as evaluated from the results of three independent measurements. The results are expressed numerically in arbitrary units as $M \pm m$. (*I*) Slow-growing (*1*) and fast-growing (*2*) cells from the retardation growth phase: (a) 13.55 ± 0.11 (*1*) and 8.27 ± 0.17 (*2*) ($P < 0.001$); (b) 16.25 ± 0.22 (*1*) and 11.29 ± 0.24 (*P*) ($P < 0.001$); (c) 44.22 ± 0.23 (*1*) and 28.26 ± 0.08 (*2*) ($P < 0.001$). (*II*) Slow-growing cells from the logarithmic growth phase (*1*) and after 120 h of cultivation (*2*): (a) 22.35 ± 0.07 (*1*) and 103.70 ± 0.22 (*2*) ($P < 0.001$); (b) 9.58 ± 0.03 (*1*) and 21.74 ± 0.13 (*2*) ($P < 0.001$); (c) 47.66 ± 0.12 (*1*) and 92.78 ± 0.29 (*2*) ($P < 0.001$).

hypothesis that the mitochondria of eukaryotic organisms have evolved from bacterial cells, the high value of the $\Delta\Psi$ of slow-growing *E. coli* cells may also be considered as an indication of the adaptive increase in the degree of oxidative phosphorylation coupling in bacterial membranes.

If the increase in the size, the cytoplasm granularity, and the membrane potential of slow-growing cells is part of their adaptive response to cultivation in minimal media, these parameters must presumably increase during the long-term cultivation of such cells in such media. To verify this supposition, slow-growing *E. coli* cells were incubated for 5 days in a culture medium without changing it (Fig. 1, II). This experiment showed that, indeed, all of the parameters mentioned increased in the course of cultivation, indicating that the morphophysiological alterations in the slow-growing *E. coli* cells have an adaptive nature.

Thus, in response to a deficiency of nutrients, slow-growing *E. coli* cells increase their size, degree of cytoplasm granularity, and value of membrane potential. These morphophysiological changes likely give these cells an advantage over fast-growing *E. coli* cells.

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