Microbiology, Vol. 70, No. 3, 2001, pp. 365–367. Translated from Mikrobiologiya, Vol. 70, No. 3, 2001, pp. 421–423.

SHORT COMMUNICATIONS

Relationship between the Cell Size and Antilysozyme Activity in *Escherichia coli* Batch Cultures

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Received September 26, 2000

The growth of bacterial batch cultures is known to depend on cultivation conditions. However, the intraspecific variability of bacterial cell sizes was observed even under identical cultivation conditions [1], which was probably due to the species-specific regulation of the synthesis of the cell wall peptidoglycans (PG). Taking into account the relationship between bacterial morphogenesis and the operation of the cellular hydrolytic enzymes that are responsible for the balanced autolysis–synthesis of PG [2], the possibility cannot be excluded that the level of antilysozyme activity (ALA) is involved in the regulation of the cell size. In enterobacteria, the cell size is probably controlled by the antilysozyme (AL) factor [3], which represents an anionic peptide located in the periplasm and serving as an endogenous inhibitor of autolytic muramidases [4]. It has been demonstrated for *Escherichia coli* that bacteria with a high AL activity exhibit a reduced reproductive ability [5]. However, there are no data on the relationship between the AL activity and the size of *E. coli* cells.

The aim of the present work was to study the relationship between the cell size, the AL activity, and the growth characteristics of *E. coli* batch cultures.

The study was carried out with 80 strains of *E. coli* obtained from the collection at the Institute of Cellular and Intracellular Symbiosis and strain *E. coli* K-12 (no. 240367) obtained from the Tarasevich State Institute of Standardization and Control of Medical and Biological Preparations in Moscow, Russia. To prepare material for inoculation, bacteria were grown on nutrient agar (NA) for one day and then were washed off of the agar to give a cell concentration of 5×10^8 CFU/ml. 0.3-ml aliquots of this suspension were inoculated into test tubes with 2.7 ml of nutrient broth (NB) or were plated, using a 5-mm-diameter inoculating loop, onto petri dishes with 5 ml of 1.5% NA. The tubes and agar plates were cultivated at 37°C for 24 h. The growth of bacteria in the NB was followed by measuring the optical density (OD) of the culture on a KFK-2 photoelectrocolorimeter (0.5-cm cuvette; 540 nm); the growth of microcolonies on NA was evaluated with a DM1 densitometer at 560–600 nm. The mass of bacterial cells (M) was expressed in arbitrary units (a.u.); the specific growth rate (μ), expressed in h^{-1}, was calculated as described in the papers [4, 5]. The optical density of broth cultures and the cell suspensions prepared from microcolonies was measured at 440 and 540 nm. The cell size was evaluated as an integral parameter R (also expressed in arbitrary units) calculated by the formula: $R = 5/y$, where y is the wave exponent calculated by the formula: $y = LN(OD_{440}/OD_{540})/LN(540/440)$ [6]. AL activity (μg/ml) was assayed at 2-h intervals as described earlier [7]. Data were statistically processed according to the manual [8].

The biomass, cell size, and the AL activity of *E. coli* K-12 cells cultivated in NB or on NA were found to be closely related (Figs. 1a and 1b). Thus, at the beginning of the exponential phase (1–2 h of growth), when μ was low, the sizes of bacterial cells grown in NB and on NA were at a maximum (R = 4.7 and 5.6 a.u., respectively), exceeding their initial size (3.2 a.u.) by 1.5–1.8 times. During the period of active growth (3 and 5 h of cultivation, respectively), the cell sizes diminished to 4.0 and 3.7 a.u., respectively. In the stationary phase (from 4 and 6 h of growth to 24 h), the sizes of NB- and NA-grown cells decreased further, amounting to 3.7 and 3.2 a.u., respectively. At the same time, the AL activity of NA-grown cells could be detected only after 6 h of growth (i.e., during the transition to the stationary phase) and reached a maximum (3.0 μg/ml) by the 14th h of cultivation (Fig. 1b). These results seem to indicate the synchronous adaptive responses of *E. coli* cells to varying cultivation conditions and suggest a close functional relationship between ALA and the growth-regulating processes in bacterial cells [4].

The comparative study of 80 *E. coli* strains grown in NB and on NA for 24 h showed that bacterial cells grown in liquid medium were 15.2% larger than those grown on solid medium ($R = 3.8 \pm 0.1$ and 3.3 ± 0.1 a.u., respectively; $p < 0.05$). However, the particular strains were polymorphic with respect to cell sizes: the R values of NB- and NA-grown cells varied from 2.9 to 5.3 and from 2.8 to 4.3 a.u., respectively. The observed effect of environmental factors on the morphological characteristics of bacterial cells suggests the existence of endogenous factors responsible for the intraspecific size variability of *E. coli* cells.

The analysis of the AL activity and the cell size of 80 *E. coli* strains grown in NB and on NA for one day

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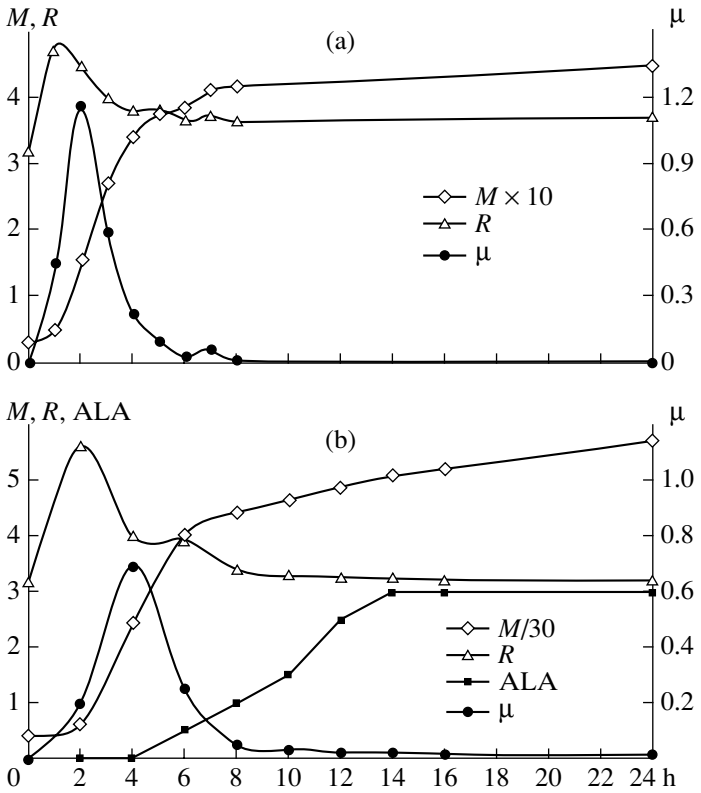

Fig. 1. Relationship between the growth rate (μ, h^{-1}), the biomass (*M*, a. u.), the cell size (*R*, a. u.), and ALA (μg/ml) in *E. coli* batch cultures grown (a) in NB and (b) on NA.

revealed the existence of a positive correlation between these parameters ($r = 0.5$ and 0.3, respectively; $p < 0.05$) at the beginning of the cultivation period (within 2 and 4 h of growth, respectively). During the period of growth retardation (4 and 6 hours of cultivation, respectively), the correlation between AL activity and cell size was absent, while it became negative ($r = –0.4$ and –0.3, respectively; $p < 0.05$) in the late stationary phase (24 h of cultivation). The observed ambiguous relationship between the cell size and the AL activity of *E. coli* cell was probably due to the growth phase–related dynamics of the synthesis of the antilysozyme factor and its role in PG metabolism as an endogenous inhibitor of bacterial muramidases [4, 5]. It should be noted that the regulation of the bacterial cell cycle includes the control of the ribosome activity; the formation of DNA-binding proteins of the PIS and other types; the regulation of the amount and activity of autolysins at the level of their transcription, translation, and cellular location; the functioning of RNases and serine proteinases; and the formation of the complexes of teichoic and lipoteichoic acids. In addition, bacterial cells possess one more mechanism for cellular regulation, which involves the interactions between the antilysozyme factor and autolysins.

On the basis of present-day knowledge, we may imagine the following sequence of events occurring in the stationary-phase *E. coli* cells transferred to the fresh medium and cultivated in a batch mode. In the lag phase, the cells gradually lose their antilysozyme factor; this activates autolytic enzymes and leads to an increase in the cell size because of the incorporation of new blocks into the cellular PG [9]. In the exponential growth phase, when the AL activity of *E. coli* cells is very low, the active growth of bacteria is promoted by the high activities of autolytic enzymes, which are directly involved in cell reproduction and the formation of division septa between daughter cells [10]. The accumulation of metabolites in the culture liquid induces the production of the antilysozyme factor in *E. coli* cells, which results in the inhibition of bacterial muramidases and in the growth retardation. In the stationary phase, the AL activity of cells increases further due to the secondary synthesis of the antilysozyme factor; this resulted in the strong inhibition of bacterial growth.

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