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SHORT COMMUNICATIONS

Growth-Phase Associated Changes in the Reproductive Capacity, Intracellular Polyamines, and Antilysozyme Activity in *Escherichia coli* Batch Culture

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Abstract—Growth-phase associated changes in and relationships between the specific growth rate (μ) characterizing the reproductive capacity of the cells, the contents of intracellular biogenic polyamines (BPA), such as putrescine (P), cadaverine (C), and spermidine (S), and antilysozyme activity (ALA) were studied in 37 strains of *Escherichia coli* grown in batch culture on solid medium. A decrease in μ upon the transition of the culture to the stationary growth phase was accompanied by a decrease in the pool of free BPA, mainly P and C, and by the appearance of ALA. The interrelations between the parameters studied were described as a complex of direct and negative correlations; the combination of low initial P and C contents, reduced P/S and C/S ratios, and a high level of ALA was designated as a *factor of slight inhibition* of *E. coli* reproduction. It is argued that BPA and ALA are integrated in a system controlling both the metabolism and stability of peptidoglycan in *E. coli*.

Key words: Escherichia coli, batch cultivation, reproductive capacity, putrescine, cadaverine, spermidine, antilysozyme activity.

Biogenic polyamines (BPA), such as putrescine (P), cadaverine (C), and spermidine (S), are known to play an important role in the regulation of the cell cycle and growth of *Escherichia coli* in batch culture [1]. Experiments with some gram-negative bacteria showed that P and C are required for cell growth and stabilize the structure of peptidoglycan (PG) [2]. The reproduction of *E. coli* cells was also found to depend on the level of antilysozyme activity (ALA); the antilysozyic factor (anionic peptide) was suggested to play a role in the PG metabolism as an inhibitor of endogenous bacterial muramidases [3, 4]. This has aroused interest in the relationship between BPA, ALA, and the reproductive capacity of *E. coli* cells.

The aim of this work was to study correlations between the growth kinetics, the dynamics of BPA content, and the level of ALA in *E. coli* grown in batch culture on solid medium.

Experiments were carried out with 37 strains of *E. coli* obtained from the Culture Collection of the Institute of Cellular and Intracellular Symbiosis, Ural Division, Russian Academy of Sciences. Inocula grown on nutrient agar for one day to a cell density of 5×10^8 CFU/ml were plated with a loop of 5 mm in diameter onto 1.5% nutrient agar (5 ml per petri dish) and incubated at 37°C. The growth kinetics was estimated by measuring the optical density (OD) of microcolonies with a DM1 densitometer at 560–600 nm every two hours for 24 h with subsequent determination of the biomass (B2–B24, arbitrary units) and calculation of the specific growth rate (μ4–μ24, h^{-1}) [3]. The amounts of free P, C, and S expressed in nmol/mg ADB (ADB stands for absolutely dry biomass) were determined after 0, 4, 8, and 24 h of cell incubation according to the methods described earlier [5], after which the P/S, C/S, and (P + C)/S ratios were calculated. The level of ALA (μg/ml) was determined every two hours of cell incubation by the method described earlier [6]. The results were processed using routine statistical methods, as well as correlation and factor analyses [7].

The growth phase-associated changes in the contents of the biomass and BPA and the level of ALA were revealed in batch cultures of *E. coli* grown on nutrient agar (Fig. 1a). By the 4th hour of cultivation, the amount of BPA in bacterial cells increased by 2.3 times ($p < 0.01$) from its initial value, mainly due to an increase in P and C contents; this was associated with the highest specific growth rate ($\mu = 0.52\ h^{-1}$) of the culture in this period. The subsequent decrease in the BPA pool, which was apparently due to the redistribution of polyamines and changes in their synthesis [8], was accompanied by the retardation of cell growth; from the 8th hour of cultivation, both BPA content and μ declined steadily. Similarly, a 1.3- to 1.9-fold increase in the P/S, C/S, and (P + C)/S ratios (as compared to

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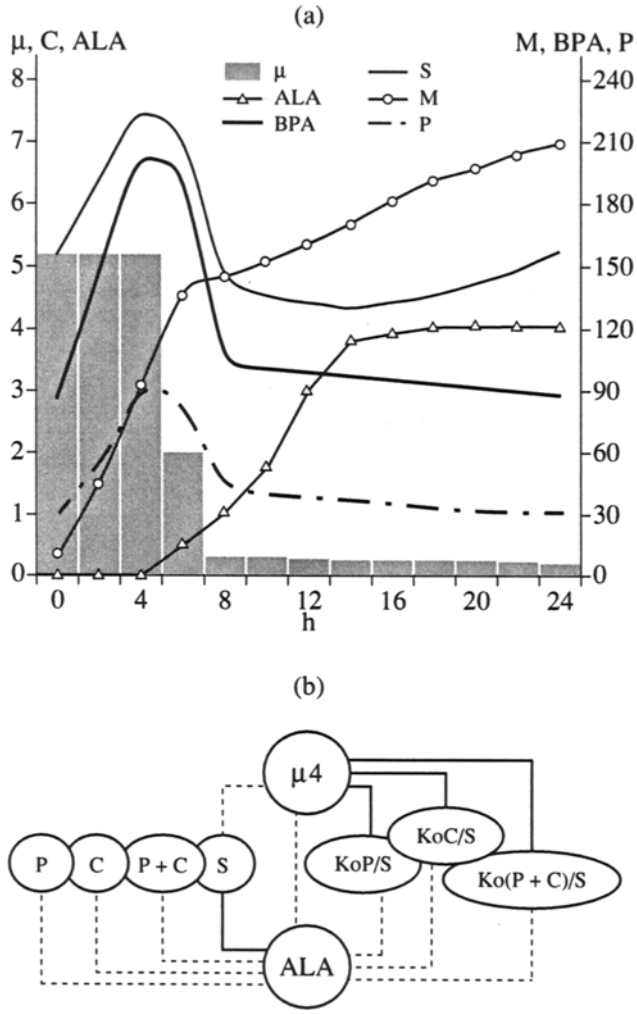

Fig. 1. (a) Chronologic relationship and (b) correlations between growth kinetics, dynamics of BPA content, and ALA level in an *E. coli* batch culture grown on nutrient agar. Left ordinate: specific growth rate (μ), $10 \times h^{-1}$; intracellular S content, nmol/mg ADB; ALA, μg/ml. Right ordinate: biomass (B), arbitrary units; intracellular BPA content, nmol/mg ADB; intracellular P content, nmol/mg ADB. Solid and dotted lines in (b) indicate direct and negative correlations, respectively ($p < 0.05$).

their initial levels) observed in a 4-h culture was followed by their continuous decline by the 8th and 24th hours of cell growth. However, ALA was first revealed after 6–8 h of *E. coli* cultivation; then, its level gradually increased and reached the maximum by 14 to 16 h of cell growth; therefore, the appearance of ALA coincided with the lowering of the intracellular pool of free BPA, a decrease in the P/S, C/S, and (P + C)/S ratios, and the transition of the culture to the stationary growth phase. The observed changes in the characteristics studied indicate a tight coupling of cell growth with the dynamics of both BPA content and the ALA level; on the other hand, a decrease in the amount of free BPA along with an increase in the ALA level in the period of lowering of the reproductive capacity of *E. coli* cells may be considered as a synchronous adaptive reaction of bacteria to stress conditions induced by the changes in the physicochemical parameters of cell cultivation [1, 3, 5].

The relationship between the characteristics of *E. coli* growth was evaluated with the application of correlation analysis, taking a Spirmen reliability coefficient (p) below 0.05. The results schematically depicted in Fig. 1b indicate the existence of a broad spectrum of interrelations between the parameters studied. The relationship between BPA and the specific growth rate at the 4th hour of cell cultivation (μ4) was mainly described by direct correlations (r ranged from 0.60 to 0.80), whereas the relationships between BPA and ALA and between ALA and μ4 were characterized generally by a complex of negative correlations (r ranged from –0.60 to –0.75); however, the correlations of ALA and μ4 with the S content appeared inverted. The relationship between BPA and the reproductive capacity of *E. coli* cells was demonstrated generally by correlations between μ4 and the P/S, C/S, and (P + C)/S ratios rather than between μ4 and the absolute amount of BPA in the cells; this is consistent with the suggestion that it is just the ratios of P and C to S that are important for the reproduction of bacteria [5, 9]. Moreover, the observed interrelations between BPA and ALA on the one hand and μ4 on the other, which have been described earlier for each of these parameters separately in broth cultures of *E. coli* [1, 3], also indicate that BPA and ALA are involved together in the control of *E. coli* growth on solid media. To specify their role in the variations of growth parameters in *E. coli* batch cultures grown on nutrient agar, we applied factor analysis. The key factor determining 35% dispersion was named *the factor of slight inhibition* of *E. coli* reproduction, since it was associated with slow biomass accumulation in the period of 2–6 h of cell growth (B2–B6) and low values of specific growth rate at the 4th hour of cultivation (μ4) (r ranged from –0.84 to –0.97); it was also associated with low initial contents of P, C, and P + C (r ranged from –0.68 to –0.72), reduced P/S, C/S, and (P + C)/S ratios (r ranged from –0.67 to –0.71), and a high level of ALA ($r = 0.89$). These data are indicative of the intimate functional relationships between the studied parameters characterizing the growth of *E. coli* batch cultures.

Taking into account the coupling of both binary cell division and morphogenesis of *E. coli* to the synthesis of the cell wall components [10], it cannot be ruled out that the observed growth-phase associated changes in BPA, ALA, and the reproductive capacity of *E. coli* cells are accompanied by changes in PG metabolism. The redistribution of BPA in bacterial cells may be favorable not only for the stabilization of PG due to its covalent binding of P and C during their release into the periplasmic space [2, 8]; it may also activate the synthesis of the bacterial antilysozymic factor, anionic peptide, which is an inhibitor of lytic hydrolases involved in the balanced autolysis–synthesis of PG in

E. coli [2, 4, 10]. This activation may be due to BPA binding to DNA resulting in the induction of DNA supercoiling and the expression of adaptive genes [1, 5]. From this point of view, both BPA and the antilysozymic factor are involved in the system controlling the stability of bacterial cell walls under stress conditions.

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