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Role of Plasmids in the Formation of *E. Coli* Polycellular Forms

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The formation of polycellular forms by *E. coli* strain K-12 cells containing F-like plasmids pAP22-2 and pAP42 was studied by the method of small-angle laser scattering. The efficiency and patterns of the resultant cell associations depend on genetic characteristics of the studied plasmids.

Key Words: *E. coli; polycellular forms (cell associations); plasmids; conjugative piles*

Bacteria of many species form supracellular (polycellular) forms [1,3,5,6]. This phenomenon attracts special interest as a high risk factor for the appearance and unfavorable course of an infectious process in humans and animals [9]. The resultant colonies, biofilms, and other integral populations are characterized by functional specialization of cells constituting these forms and provide certain advantages for these cells (high resistance to antibacterial drugs and immune defense factors of human organism, more effective use of nutrient components of the medium, *etc.*). The mechanism underlying the formation of polycellular association is determined by activity of specific surface formations of bacterial cell, such as rods, adhesion fimbria, conjugative pili, *etc.* [10]. However, the role of bacterial plasmids determining the synthesis of individual variants of sex pili remains virtually unknown. We studied the impact of genetically different F-like plasmids for the efficiency of polycellular forms generation by *E. coli* cells.

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MATERIALS AND METHODS

The study was carried out on the cells of C600 reference auxotrophic strain (thr leu Str), K-12 strain derivative. Plasmid-free bacteria and variants containing pAP22-2 and pAP42 F-like plasmids were studied. These plasmids were previously identified at our Department and labeled by inserting Tn1 and Tn9 transposons containing ampicillin- and chloramphenicol-resistance genes, respectively, in their structures [4,8]. Polycellular forms of the studied bacteria cultured under standard conditions in liquid nutrient medium (meat/peptone broth), were detected by Malvern 3600 Ec particle size laser diffraction analyzer. This device based on the laser low-angle dissemination provides data on cell distribution by size and shape in a bacterial population [6].

Conjugation transfer of plasmids was realized in plasmid-containing donor cell cross-over with appropriate recipient bacteria by the standard method [2].

RESULTS

Quantitative and volume distribution of C600F$^-$, C600pAP22-2::Tn1, and C600pAP42::Tn9 cells was

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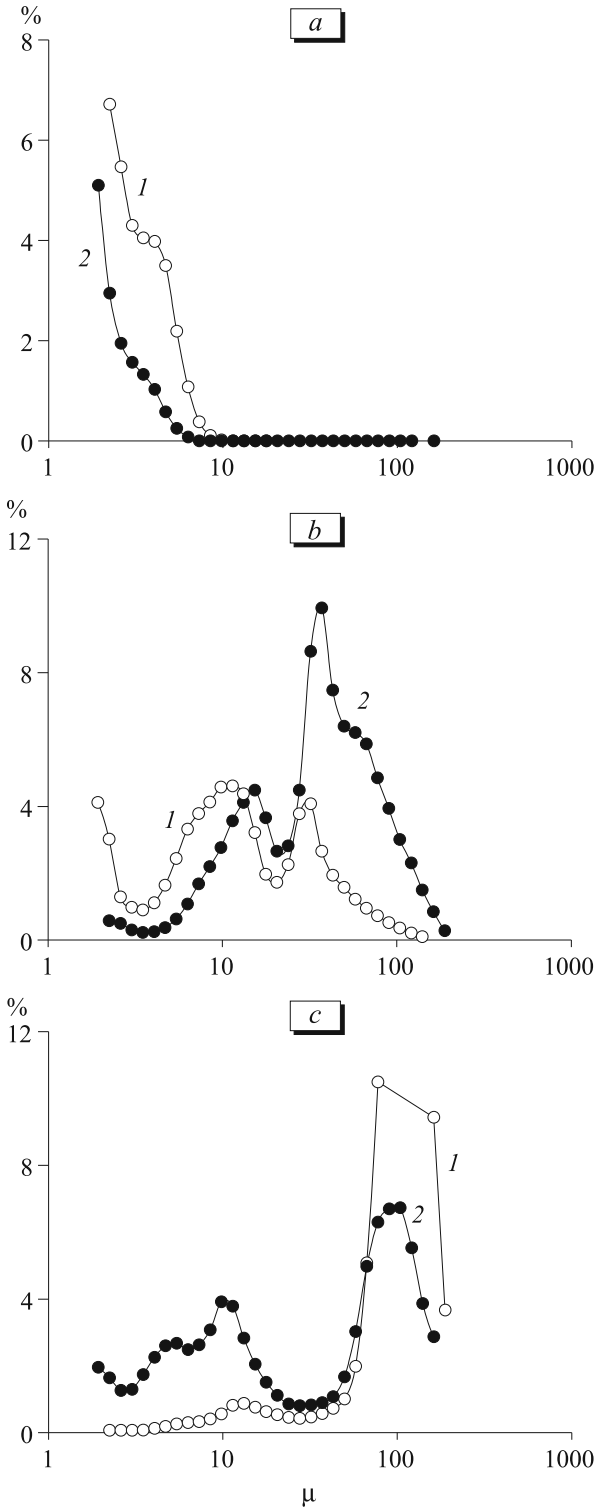

Fig. 1. Numerical (*2*) and volume (*1*) distribution of cells and associations in *E. coli* culture in the logarithmic growth phase. *a*) C600; *b*) C600pAP42::Tn9; *c*) C600pAP22-2::Tn1. Cell concentration in suspension 10^8/ml.

evaluated in broth cultures of these bacteria in the poststationary growth phase. Groups varying in size from 1 to 100 μ were obtained (Fig. 1, *a*). At maximum numerical distribution of cells of about 2 μ, the volume distribution was <1 μ, which corresponded to the presence of just solitary cells. Their volume percentage is low (about 12%). Hence, no cell associations were present in these cases. On the other hand, the size spectra of cells belonging to isogenic variants of C600 cells containing F-like pAP22-2::Tn1 and pAP42::Tn9 plasmids indicate the presence of polycellular forms of different size.

Numerical and volume spectra of C600pAP22-2::Tn1 cells were also analyzed (Fig. 1, *c*). Two groups of different size with maxima at 15 and 100 μ were distinguished at volume distribution of cell forms from 1 to 100 μ. In the volume distribution peak of 15 μ the number of cells was high, but their volume was small. On the other hand, it the peak of 100 μ few very

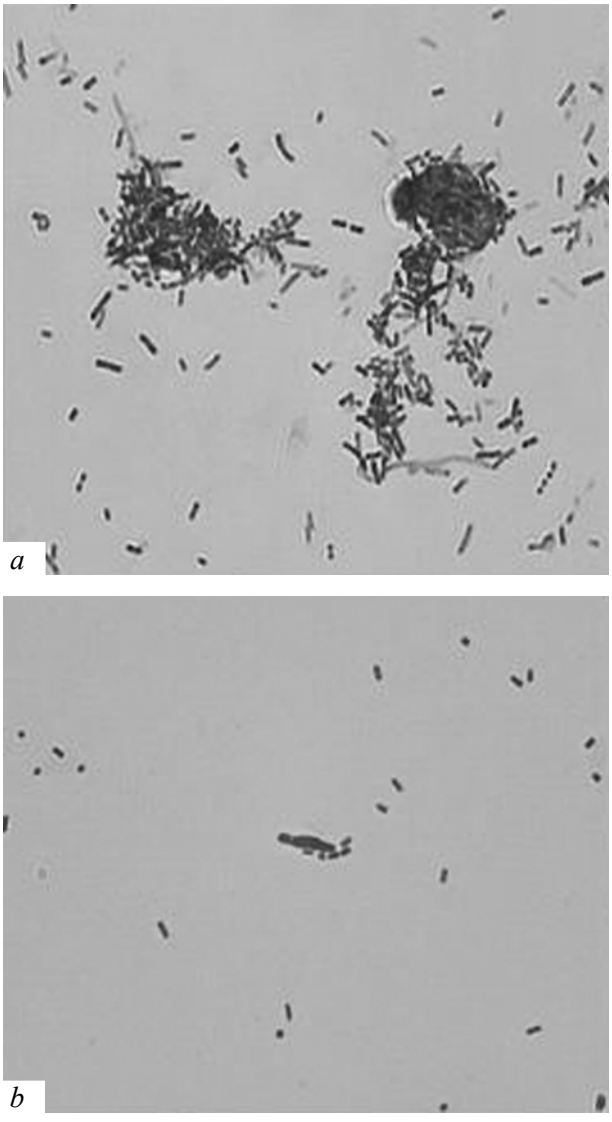

Fig. 2. Suspensions of *E. coli* cell culture. Gram staining, ×100. *a*) C600pAP22-2::Tn1; *b*) C600pAP42::Tn9.

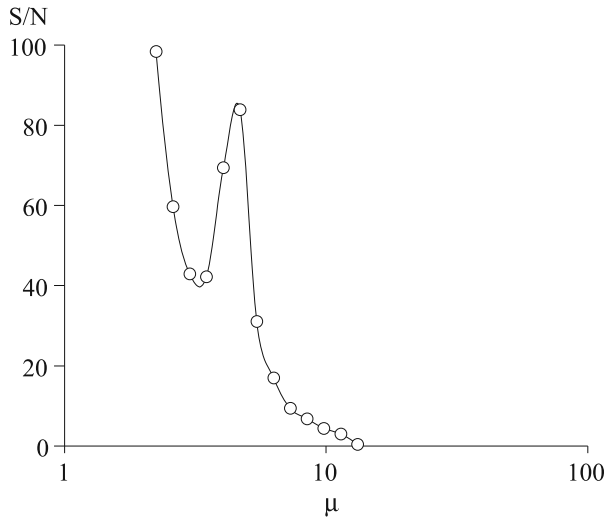

Fig. 3. Incidence of volume distribution of *E. coli* C600 cells. The "signal/noise" ratio for curve *1* in Fig. 1, *a* is shown.

large particles were detected. Analysis of numerical distribution of C600pAP42::Tn9 cells 3 to 100 μ in size (Fig. 1, *b*) showed two groups of cells of similar size with peaks of 15 and 40 μ. Their numerical and volume percentages increased.

Hence, volume distribution of particles in plasmid-containing cell populations indicates the presence of larger particles, presumably cell associations. These data were additionally confirmed by microscopic examination of Gram stainedf smears of these bacteria. The studied preparations contained solitary and polycellular *E. coli* forms (Fig. 2). Analysis of 100 visual fields showed 63% fields with polycellular forms and 37% with solitary formations, that is, the volume percent of polycellular forms is greater, while their number is low (mainly 1-3 associations per visual field).

Hence, the presence of two studied F-like plasmids in *E. coli* cells is an important factor contributing to the formation of polycellular forms of these bacteria. Presumably, these plasmids differently modify the patterns of the forming cell associations. Comparison of the curves (Fig. 1, *b*, *c*) shows different patterns of one-size groups of cell associations for the bacteria containing pAP22-2::Tn1 and pAP42::Tn9 plasmids. Cells containing C600pAP22-2::Tn1 plasmid formed larger, but less numerous associations, while in case of C600pAP42::Tn9 plasmid numerous small associations were formed. In order to verify these data, an experiment with 5 repetitions of volume distribution of cells was carried out as exemplified by *E. coli* C600 (Fig. 3).

Hence, it seems that the effects of the studied plasmids on the formation of *E. coli* polycellular forms are determined by genetic characteristics of these plasmids and specific surface formations (conjugative pili) synthesized under their control. Our previous results indicating atypical phenomenon of the C600pAP22-2::Tn1 and C600pAP42::Tn9 plasmid incompatibility and specific features of genetic regulation of their conjugative transfer [4,5] support this conclusion.

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