Regulation of Type 1 Pili Expression as Exemplified by *Escherichia Coli* Strain M17

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Studies of *E. coli* strain M17 colibacterin and its derivatives showed that *fimH* gene regulates morphological characteristics of type 1 fimbria and cell piliation on the whole. Gene *fimH* has a negative effect and decreased the percentage of piliated cells in the population and piliation of individual cells, which does not agree with its role of a positive regulator.

Key Words: *adhesion; type 1 pili; electron microscopy; colibacterin*

Type 1 pili are expressed on the surface of many pathogenic, commensal, and parasitic bacteria. They play an important role in microbial colonization of human organism and in the development of pathological processes ensuring mannose-sensitive binding to different substrates [1,5]. Genes responsible for the synthesis of type 1 pili are located in the *fim* cluster of bacterial chromosome. The majority of these genes are trans-complementary, *i. e.* gene copies are present in other structures, for example, in plasmids. Gene *fimH* is responsible for the synthesis of fimbrial protein FimH adhesin of a certain type, while gene *fimB* positively regulates type 1 pili expression in cells [5]. An extra copy of gene *fimB* irreversibly activates the synthesis and expression of type 1 pili in bacterial cell [5].

We previously obtained derivatives of *E. coli* strain M17 (commercial producer of probiotic colibacterin) characterized by different combinations of *fimH* and *fimB* genes [1]. Here we investigated the mechanisms underlying regulation of adhesive activity at the cell and molecular levels and the resultant changes in morphological and quantitative characteristics of *E. coli* type 1 pili.

MATERIALS AND METHODS

All strains were stored in small aliquots in 15% glycerol at -80°C and cultured in LB or BHI (Difco) media at 37°C (18-24 h). *E. coli* strain M17 (M^H) (commercial producer of probiotic colibacterin) and its derivatives M17dF (*fimH::npt,* $FimH^-$), M17/pPKL91 (*fimB*), M17dF/pPKL91 ($FimH^-$, *fimB*), M17dF/pGB17-9 (F18 *fimH, fimB*) were used. The strains are maintained at the Department of Microbiology, Russian University of Peoples' Friendship.

Adhesive activity of bacterial suspensions was evaluated by the yeast aggregation test [1,9].

Preparations for electron microscopy were made using original modification of the known methods described below. Electron microscopy was carried out using a Zeiss microscope. The size of pili was evaluated visually and expressed in arbitrary units (ocular grid divisions, 1 arb. unit corresponds to 700 nm at ×4000 and 370 nm at ×8000).

The data were processed statistically using standard tests for normal distribution; the mean values, standard deviations, and confidence intervals were calculated, and the distribution curves were plotted on the basis of incidence in a sampling.

RESULTS

Electron microscopy of type I pili requires special processing of samples. First of all, the specific nature of the examined object (cell with pili) does not allow the use of common ultrathin sections, because pili are not seen on such sections. Second, type 1 pili are very unstable structures and should be handled with care. Methods for making such preparations are usually

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TABLE 1. Piliation of *E. coli* Isogenic Strains (*M*±*m*)

Parameter	M17	M17/pPKL91	M17dF	M17dF/pPKL91	M17dF/pGB17-9
Percentage of cell without pili	4.0	2.0	3.5	14.8	4.0
Number of pili per unit of cell perimeter	4.0±0.3	1.70±0.18	1.30±0.14	0.40±0.04	1.90±0.26
Size of pili, arb. units	1.30±0.05	1.00±0.02	2.5±0.1	2.60±0.16	1.20±0.04
Yeast aggregation, titer (maximum dilution inducing aggregation)	1:32	1:32	0	0	1:32

based on application of cell suspension on film-coated lattices. Different authors used different contrasting agents (alcoholic and aqueous ammonium molybdate, uranyl acetate, phosphotungstic acid, *etc.*) and different order of procedures and conditions of contrasting (temperature, pH, *etc.*) [2-4,6,8]. In our comparative studies we used these methods, but failed to attain the desired results. Many preparations contained numerous disordered fimbriae or were contaminated with crystals of contrasting agents and water. Under electron microscope the pili rapidly lost their contrast, which makes impossible long examinations. These factors impeded correct and accurate estimation of the number and size of pili on bacterial cells. The parameters required in this study were attained only with the following modified method.

Fresh bacterial culture was twice washed with phosphate buffered saline (PBS, gentle centrifugation at no more than 2000 rpm) and the concentration of bacterial cells was adjusted to 10^{10}/ml. Bacterial suspension was mixed (1:1) with 2% aqueous solution of phosphotungstic acid (pH 6.0). After 2-h incubation, 10 μl of the mixture was rapidly applied onto a nickel lattice (300 holes) floating on the surface of pure water and coated with a Parlodium film with 4-6 fine holes along the perimeter. The lattice taken from the water is almost dry and ready to microscopy in several minutes. For deeper contrasting the preparations were incubated in 1% OsO_4 vapors in PBS for 10 min.

This method ensured the desired purity of the preparation, degree and stability of pilus contrasting, and maximum stability of the pili.

The parameters of *E. coli* type 1 pili were examined using a set of 5 isogenic *E. coli* strains, the first of which was *E. coli* M17. Previous experiments showed that this strain was characterized by adhesive activity, determined by the presence of type 1 pili [1] and possessed highly adhesive M^H phenotype determined by chromosome copy of *fimH* gene. The following derivatives of strain M17 were obtained: M17: M17 *fimH::npt* (M17dF), in which adhesin chromosome gene was inactivated by insertion of neomycinphospha-

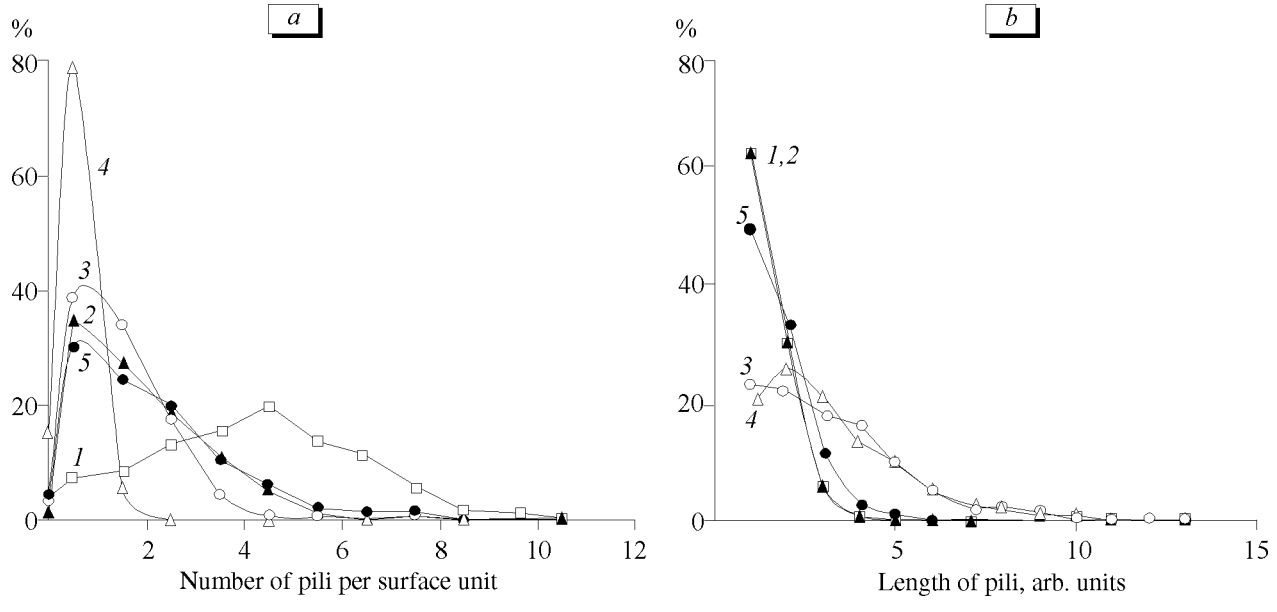

Fig. 1. Distribution of piliated cells (*a*) and pili of different length (*b*) in the population of *E. coli* strain M17 (*1*) and its derivatives M17/pPKL91 (*2*), M17dF (*3*), M17dF/pPKL91 (*4*), and M17dF/pGB17-9 (*5*).

tase gene (zero adhesive activity); M17/pPKL91, into which an extra plasmid copy of *fimB* gene was inserted (positive regulation of type 1 pili expression by cells); M17dF/pPKL91, inactive adhesin gene and additional copy of *fimB*; M17dF/pGB17-9, in which inactive adhesin chromosome gene was compensated for by an extra plasmid copy of *fimH* gene from normal intestinal *E. coli* F18 strain (low adhesive M^L phenotype), pGB17-9 plasmid containing an extra copy of regulatory *fimB* gene.

The following parameters were estimated (Table 1): percentage of piliated bacteria in the population (200-cell sample), pilation of individual bacteria (200-cell sample), and size of pili (100-cell sample). All operations were carried out with simultaneous control of adhesive activity in the yeast aggregation test.

The number of pili on cells (piliation) was evaluated as follows: perimeter of each piliated cell and number of pili on it were estimated at ×4000, the mean number of type 1 pili per perimeter unit (PU) was estimated (Table 1). The sample consisted of 200 randomly cells selected in 3 preparations for each strain. The incidence of cells with a certain number of pili per PU in the sample and the percentage of these cells in the population were estimated. The data were used for plotting curves representing distribution of piliated cell in the population (Fig. 1, *a*).

The initial M17 strain with highly adhesive phenotype under standard conditions was characterized by

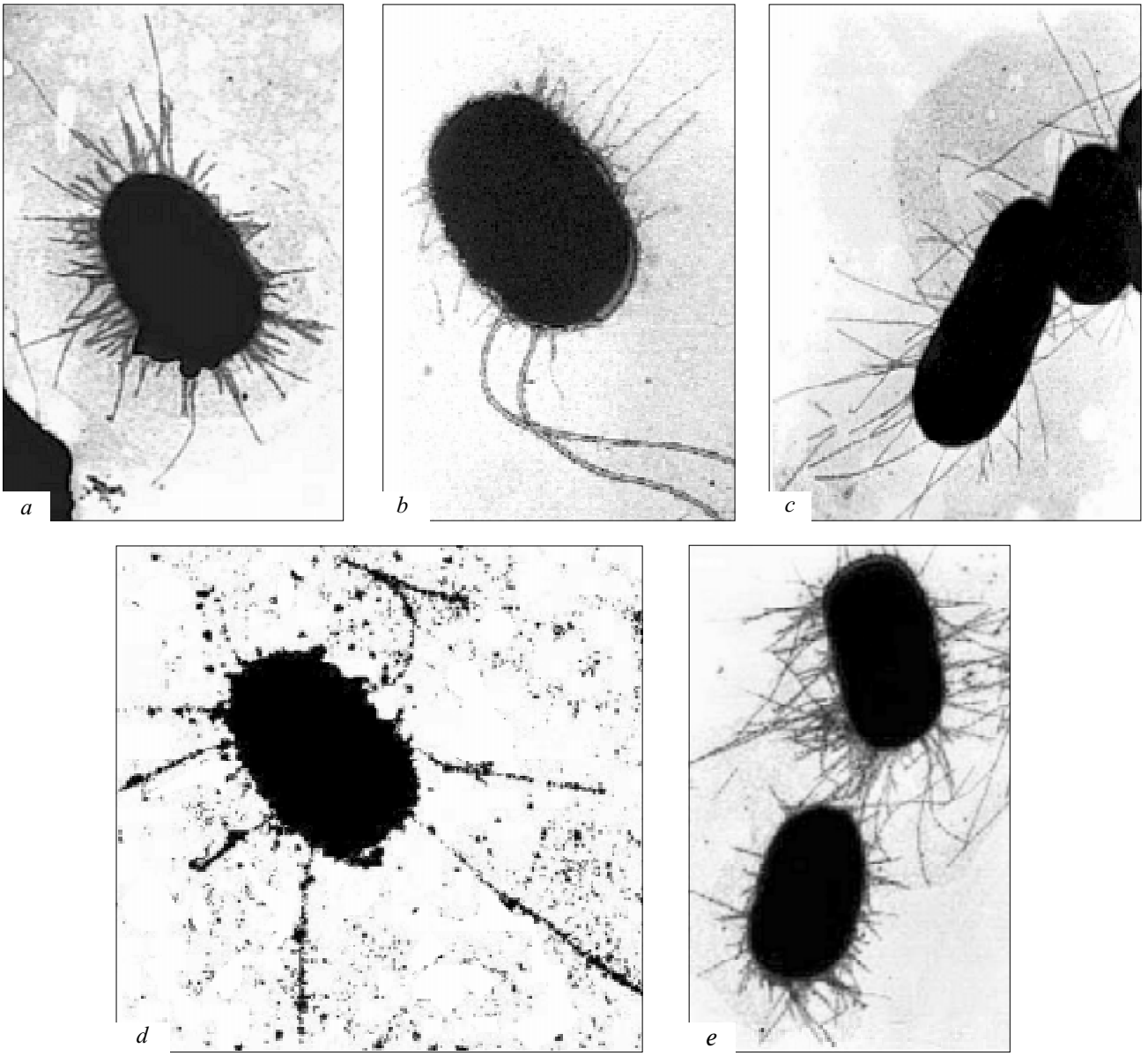

Fig. 2. Electron microphotography of type 1 pili on *E. coli* cells: strains M17 (*a*), M17/pPKL91 (*b*), M17dF (*c*), M17dF/pPKL91 (*d*), and M17dF/pGB17-9 (*e*).

near-normal distribution of piliated cells in the population (Table 1). Frequency distribution of the number of pili was observed in the range of 0 (4% cells) to 10 piles/PU (1% cells) with a maximum at 4-5 pili/PU (19.5% cells) (Fig. 1, Fig. 2, *a*).

The mean number of pili in other recombinant isogenic strains was much lower than in the initial M17 strain (Table 1). In these strains the frequency distribution of the number of pili in piliated cells was shifted towards the zero (Fig. 1, *a*). This shift of the piliation profile for strain M17dF was not surprising. According to published data, product of *fimH* gene is involved in the initiation of pilus synthesis and determined their number. Hence, inactivation of *fimH* gene leads to disorders in pilus synthesis (Fig. 2, *c*).

The incidence of piliated cells in strains M17/pPKL91 and M17dF/pPKL91 was quite contrary to the expected. These strains contain just an extra copy of *fimB* gene [1,5,8]. Therefore we expected a higher percentage of piliated cells and a higher degree of piliation compared to the initial strains M17 and M17dF, respectively. However, the piliation profiles of these strains were shifted towards zero (Table 1, Fig. 1, *a*) and their maxima corresponded to 1-2 (27.5%) and 0-1 pili/PU (79.6%), respectively. This regularity was most expressed in strain M17dF/pPKL91 and could not be explained only by inactivation of the adhesin gene, because the percentage of cells without fimbriae in the population increased more than 4-fold in comparison with strain M17dG, while other cells were just negligibly piliated (Fig. 2, *b, d*).

Piliation profile of strain M17dF/pGB17-9 was similar to that of strain M17/pPKL91 (Table 1, Fig. 1, Fig. 2, *e*), which confirmed the regularity demonstrated above, as both strains had different but intact copies of adhesin gene and the same extra copies of *fimB* regulator.

The size of pili was evaluated as follows: sizes of all pili in each selected bacterial cell were evaluated at ×8000 and expressed in arbitrary units, the mean size of pili for each strain was calculated (Table 1). To this end, 50-100 cells in three preparations were analyzed. Pili corresponding to certain length intervals were counted and their percentage in the total pool of cells of this strain was evaluated. The data were used for plotting the profiles of pilus size distribution on bacterial cells in a population (Fig. 1, *b*).

The results suggest that adhesin gene *fimH* plays the key role in the regulation of the length of fimbriae. Inactivation of *fimH* gene in strain M17dF led to the appearance of longer pili in comparison with the initial strain M17 (Fig. 1, *b*; Fig. 2, *a, c*). Insertion of an intact copy of *fimH* gene with low-adhesive phenotype to strain M17dF/pGB17-9 returns the profile of pilus length distribution to the variant close to the initial (Fig. 1, *b*, curve *5*). It seems that *fimH* gene product is essential not only for initiation, but also for termination of pilus synthesis as well and affects both the number of pili and their shape.

Gene *fimB* had no effect on the length of pili: the curves for strain pairs M17 — M17/pPKL91 and M17dF — M17dF/pPKL91 were virtually identical (Fig. 1, *b*). Presumably the role of *fimB* gene for pilus expression is not confined to positive regulation and is to be further investigated with other cells.

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