MICROBIOLOGY AND IMMUNOLOGY

Molecular Genetic Analysis of *Escherichia coli* Type I Adhesins

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Protein FimH is an adhesive terminal subunit of *Escherichia coli* type I pili. The content of FimH on the surface of a bacterial cell does not directly depend on FimH expression, but is limited by expression of other *fim* cluster genes encoding structural subunits and proteins responsible for assembly of pili. Piline domain, a component of FimH, cannot incorporate into the fimbria independently, without lectin domain. It seems that fimbriae containing only piline domain are not formed because of inability of the piline domain to initiate the process of assembly.

Key Words: *strains; plasmids; adhesin; molecular genetic analysis*

Mannose-sensitive type I pili with FimH adhesin incorporated in their terminal fragment are expressed on the surface of virtually all *E. coli* and the majority of other representatives of *Enterobacteriaceae* family (*e.g.*, *Salmonella*, *Klebsiella*, *Enterobacter*) [6]. The bacterium usually carries 200-500 peritrichious pili on its surface, each 7 nm in diameter and 0.2-2.0 μ long; such a pilus is a polymer formed by about 1000 copies of the main structural subunit, FimA protein forming an α-helix [4].

All components of type I fimbriae are determined on bacterial chromosome (9200 n. p. cluster consisting of 9 genes carrying structural, regulatory, and transporting functions). In addition to gene encoding the above-mentioned FimA subcomponent, there are three other structural genes encoding subunits FimF, FimG, and FimH. These genes are presented by a lesser number of copies and determine the number, length, and adhesive activity of pili [8-10]. FimH is located at the tip of fimbria and is a subunit directly recognizing the receptor and binding to it. *fimE* and *fimB* genes regulate phase expression of the pili.

Structural components of fimbriae are synthesized as precursors with N-signal sequences cleaved off during transfer through the inner membrane. FimC protein acts as a periplasmic chaperon transporting structural subunits of the pilus from the inner to the outer membrane. The regulatory component FimD located in the inner membrane serves as a platform for pilus assembly; it recognizes the complex consisting of chaperon and structural component and promotes their dissociation with subsequent transfer of the structural protein to the growing fimbria. Fimbrial growth starts with FimH; FimF and FimG then join it for the formation of the fimbrial tip, after which the axial cylinder consisting predominantly of FimA is formed [7,11].

FimH consists of two domains: lectin domain (LD) "recognizing" the structure of mannose receptors on the surface of the target cells and specifically reacting with them and piline domain (PD) binding FimH to the fimbria. Two domains are connected with a linker not belonging to any of them [5,15].

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We studied the role of some factors essential for FimH incorporation into the final structure of the fimbria.

MATERIALS AND METHODS

"Wild" *E. coli* strains (fecal strain F18 and laboratory strain K12) used for cloning *fim* cluster and its elements were taken from the collection of E. V. Sokurenko. Recombinant strains were obtained on the basis of two FimH-zero variants (KB18 and KBC235) (Table 1). Strain AAEC191 (K12*fim*$^-$) was transformed with pPKL114 plasmid carrying the entire *fim* operon with inactivated *fimH* for the construction of strain KB18 [3,8,13]. KBC235 strain carried inactivated *fimH* on the chromosome and possessed plasmid pPKL9 carrying *fimB*-positive regulator of type I pili expression [13]. These strains were transformed with plasmids with different numbers of copies per cell (Table 2). Plasmid pFU was created on the basis of pRS425 [12] and contained 9200 n. p. encoding genes for the expression of type I pili (unpublished data). Vector pACYC184, from which *TcR* (tetracycline resistance) gene was removed and *fimH* was cloned under *bla* promoter control, served the basis for pGB plasmid [13]. A fragment consisting of 6 histidines and a signal peptide were attached to PD N-terminal amino acid in strain BV60. In ELT310 strain the fragment consisting of 6 histidines was located between the whole *fimH* gene and the signal peptide (Fig. 1).

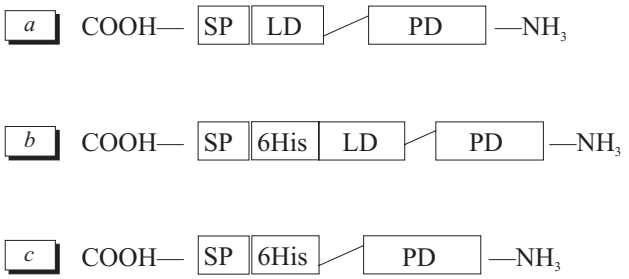

Fig. 1. Scheme of FimH structure in wild strain (*a*) and in constructions described in the paper: ELT310 (*b*) and BV60 (*c*). PD: piline domain; LD: lectin domain; SP: signal peptide.

E. coli binding to monomannose (1 M) substrate was evaluated as described previously [14]. The main stages of the experiment included

TABLE 1. Strains Used in the Study

Strain	Host strain	Plasmids	References
KB18	AAEC191 (*fim*-null)	pPKL114	[3,13]
KBC235	CI10#9 (*fimH*-null)	pPKL9	[13]
BV21 (pGB/*fimH*)	KBC235	pGB/*fimH*, pPKL9	
BV20 (pBeloBAC/*fimH*)	KBC235	pBeloBAC/*fimH*, pPKL9	
ELT246 (pBeloBAC/*fim*)	AAEC191	pBeloBAC/*fim*-operon	
ELT235 (pFu/*fim*)	AAEC191	pFu/*fim*-operon	
BV60 (PD+6His)	KB18	pGB/PD6His, pPKL114	
ELT310 (FimH+6His)	KB18	pGB/*fimH*6His, pPKL9	

TABLE 2. Plasmids Used in the Study

Plasmid	Number of copies per cell	Components	References
pGB	about 15	*cat* (CmR), p15A ori, *fimH*	[13]
pBeloBAC	1	T7 promoter, SP promoter, *lacZa*, *cat* (CmR), ori2 (oriS)	[15]
pFu	about 20	*bla*(ApR), *LEU2*, pMB1 ori	[5]
pPKL9	about 20	*bla*(ApR), *fimB*, pMB1 ori	[13]
pPKL114	about 20	*bla*(ApR), ColE1 ori, *fim*-operon (*fimH*-null)	[3,8,13]

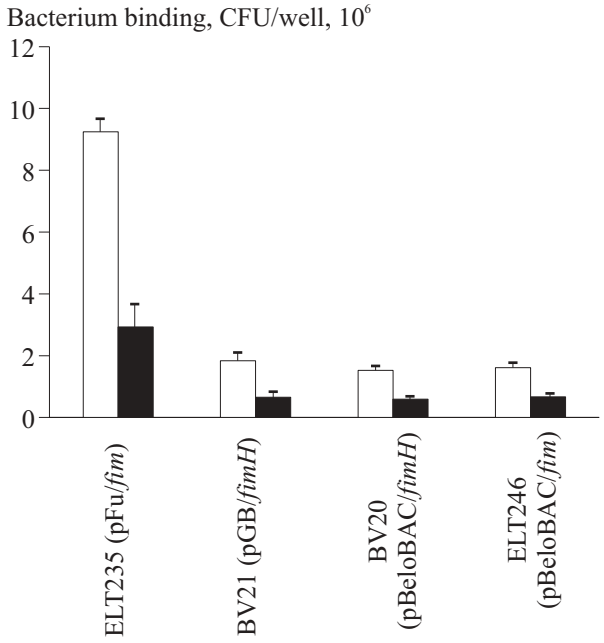

Fig. 2. Mannane binding by the strains in the presence of inhibitor α-methylmannoside (dark bars) and without it (light bars).

radioisotope labeling of *E. coli* by culturing the bacterium in a medium containing ^{3}H-thymidine. The strains were then incubated with yeast mannane (model 1M substrate) prefixed on the plastic surface of wells of a microtitration plate. This was paralleled by incubation with the inhibitor (α-methylmannoside). The absolute number of bacteria bound to the substrate was calculated from the known concentration of bacteria and radioactive signal from one bacterium.

Binding to antifimbrial and anti-fimH antibodies was carried out similarly, with antibodies in 1:100 dilution immobilized in the wells instead of mannane [14].

Binding to horseradish Ni-peroxidase (Ni-HP) was evaluated by the "growth investigation" test [1]. Ni-HP was immobilized on the plastic surface of wells, the plates were incubated with bacteria, washed from free bacteria, and then bound bacteria were cultured in a nutrient medium. Optical density was determined on an automated reader for microplates (Molecular Devices, Inc., Menlo Park).

The results were statistically processed using Student—Fisher *t* test.

RESULTS

We studied the relationship between FimH incorporation in the pili located on the bacterial surface and the level of cytoplasmic expression of FimH alone and together with other structural genes. The degree of ligand binding by strains BV20 (pBeloBAC/*fimH*) and ELT246 (pBeloBAC/*fim*-operon) was the same (Fig. 2), which attests to the same amount of FimH incorporated in the pili. In strain ELT246 the entire *fim*-operon is expressed only from a monocopy plasmid, this imitating its expression from chromosome, while *fimH* is the last gene of the operon of 7000 n. p. in size and is characterized by the lowest level of transcription. In strain BV20 *fimH* is also expressed from a monocopy plasmid, but separately from *fim* cluster located on chromosome (with inactivated *fimH*). Hence, FimH is expressed from a promoter located on the plasmid and ensuring a higher level of expression in comparison with strain ELT246. Therefore, the content of FimH incorporated in the pili does not depend on the level of its expression in the cell. In order to confirm this, we evaluated binding to mannane for strain BV21 with transcription of FimH parallel to chromosomally located *fim* cluster, but from a monocopy plasmid, while the level of FimH expression in the cytoplasm is much higher than in strains ELT246 and BV20. Mannane binding of strain BV21 was slightly higher in comparison with two other strains (Fig. 2), which also confirms our observation, indicating that even a significant increase of FimH expression in the cytoplasm does not lead to a significant increase of its content on cell surface as an adhesive component of the pili.

The study of 1M-binding capacity of strain ELT246 (pFu/*fim*-operon) expressing the whole *fim* operon from a multicopy plasmid (when all genes essential for the synthesis of type I pili, except *fimH* are expressed at the level significantly higher than in other strains), showed that mannane binding by strain ELT235 increased several-fold in comparison with strains BV20, BV21, and ELT246 (that is, in this case the level of FimH incorporation in the pili was higher). Hence, in order to increase the content of FimH incorporated in the pili on the bacterial surface, the cytoplasmic expression of all fimbrial genes should be increased, in addition to FimH expression. It seems that the limiting factor for FimH incorporation is the number of other minor structural subunits of the fimbria (FimF and FimG), essential for the formation of the pilus tip, and a sufficient level of FimC (chaperone) and FimD (regulatory component), essential for pilus assembly.

The next step of our study was to clear out whether the entire FimH was needed for incorporation in the fimbria or PD, considered to be its incorporative subunit, was sufficient. We created BV60 strain, in which only the synthesis of PD with signal sequence was expressed from pGB plasmid; this strain, however, also contained pPKL114 plasmid, from which the rest information of the *fim* cluster was expressed. It was shown that PD expressed

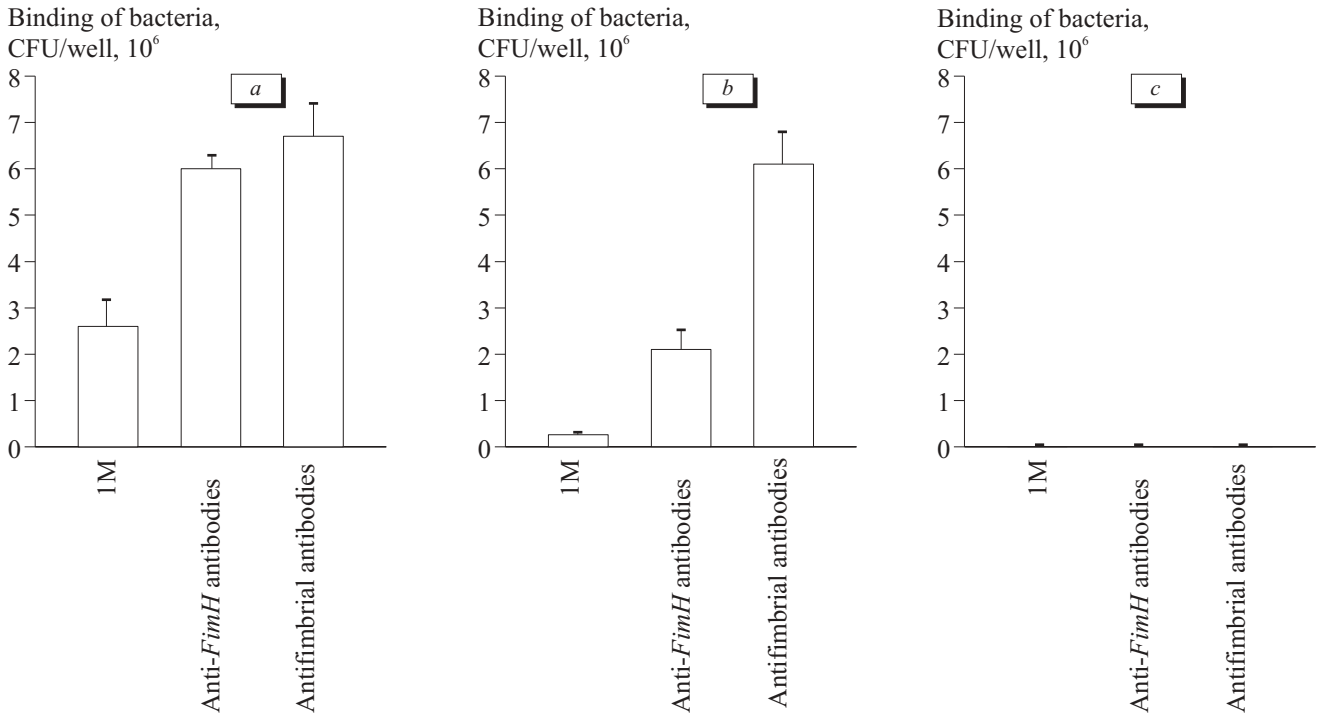

Fig. 3. Binding of mannane and antibodies by the strains. *a*) wild strain; *b*) strain ELT310 (FimH+6His); *c*) strain BV60 (PD+6His).

without LD was effectively transported into the periplasm and existed there in a stable form (unpublished data). In order to detect PD in fimbriae between the signal peptide and PD, a marker containing 6 histidines and characterized by high affinity to nickel, was inserted instead of the linker. As was expected, BV60 did not bind mannane because of the absence of LD (Fig. 3). However, it did not bind anti-FimH-antibodies, antifimbrial antibodies, and Ni-HP, this indicating the absence of PD incorporation in the fimbria; moreover, no type I pili formed. On the other hand, strain ELT310, in which a sequence of 6 histidines was inserted between the LD N-terminal amino acid of whole FimH and the signal peptide, exhibited a high level of binding to antifimbrial antibodies, anti-FimH-antibodies, and Ni-HP. Hence, insertion of 6 histidines did not prevent FimH transport to the cell surface and its incorporation in the fimbria. It seems that inability of PD to incorporate in the fimbria is explained by impossibility of its transportation through the outer membrane and initiating the synthesis of pili without LD.

The receptor-binding function of FimH depends on the conformation of its N-terminal site. This is confirmed by crystallographic findings, indicating that N-terminal phenylalanine is involved in interactions with mannose receptor [5,6]. According to our data, despite the presence of fimbria containing FimH with the histidine marker in strain ELT310, these bacteria do not bind mannane. It seems to be due to masking of the binding package or modification of conformation predetermined by the presence of N-terminal histidines.

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