Escherichia coli Strain M17: Analysis of Adhesive Phenotype as a Factor of Host Colonization and/or Pathogenicity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 4, pp. 424-428, April, 1999
Original article submitted June 2, 1998

FimH adhesins M_1L and M_1H are differently adapted to colonization of primary normal and secondary pathological niches of human *E. coli.* The M^L phenotype is typical and optimal for intestinal *E. coli,* while M^H phenotype predominates among pathogenic strains and is undesirable for the eubiotic intestinal *E. coli* strain M17.

Key Words: *colibacterin; adhesion; colonization; pathogenicity*

Preparations of normal microflora (eubiotics) are widely used for prevention and treatment of dysbacteriosis and other gastrointestinal disorders [1]. They are based on live cultures of specially selected symbiont strains of normal human microflora and are characterized by numerous useful properties. Colibacterin is a representative of this group of drugs; it is based on *Escherichia coli* producer strain M17 obtained by modifications of producer strain Mutaflor [13].

Adhesion of bacterial cells to surface receptors plays an important role in the microorganism-host interactions [7]. Type 1 pili ensuring mannose-sensitive binding represent the most prevalent adhesive organelles. Adhesin is presented by FimH protein coded by *fimH* gene in the *fim* cluster of the bacterial chromosome. Structural mutations yielded variants of FimH adhesin and hence, various adhesive phenotypes [11]. The most prevalent phenotypes are M^H and M^L ensuring high and low, respectively, levels of adhesion to the model substrate yeast mannan.

Previously we compared adhesive activity of 3 producer strains and detected mannose-sensitive adhesion via type 1 pili [2]. Related strains M17 and Mutaflor have similar M^H phenotype, while Simbioflor producer strain has M^L phenotype.

Human and animal large intestine is the primary niche for *E. coli* as a member of normal microflora and a reservoir for *E. coli* which can cause enteric and non-enteric diseases. The majority (about 80%) of human intestinal *E. coli* have M^L phenotype. *E. coli* strains causing urogenital infections mostly (at least 70%) have M^H phenotype [11].

We investigated the functional differences between these phenotypes and their role in colonization of various niches in the host and analyzed whether the M^H phenotype is desirable for the intestinal *E. coli* strain M17.

MATERIALS AND METHODS

All strains were stored in small aliquots in 15% glycerol at -80° and grown in LB or BHI (Difco) media at 37°C for 18 h. Commercial *E. coli* producer strains M17, Mutaflor, and Simbioflor for probiotic agents were used (maintained at Laboratory of Biomedical Technologies, I. M. Setchenov Moscow Medical Academy). Recombinant isogenic strains (from the collection of E. V. Sokurenko, University of Tennessee, Memphis) were obtained by transduction of KB18 strain with plasmids containing *fimH* genes of different origin and phenotype: KB21 (chimeric consensus *fimH,* M^L), KB23 (from PC31, M^H), KB54

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0007-4888/99/0004-0386$22.00 ©1999 Kluwer Academic/Plenum Publishers

(CI#12, M^H), KB59 (CI#3, M^L), KB91 (F-18, M^L), KB92 (KS-54, M^H), and KB96 (MJ#2-4, M^H).

Adhesive activity of bacterial cells was studied by the yeast aggregation and erythrocyte agglutination tests [2]. Adhesive phenotype was determined by the growth test [2]. Bacterial adhesion to epithelial cells was evaluated by the number of bound bacteria in a Giemza-stained specimen under a light microscope [14]. Inhibition of bacterium binding to buccal epithelial cells (BE) was studied by the inverted adhesion test [12]. Human salivary specimens were collected and treated as described previously [5]. The inhibition was assessed by IC_{50}, the concentration of inhibitor reducing adhesion by 50% [10].

In studies of adhesive activity we used mannan from *Saccharomyces cerevisiae* (MN), α-methyl-D-mannopyranoside, bovine pancreatic RNase B, BSA, bovine lactoferrin, bovine intestinal mucin (Sigma), mannosylated BSA (M_1BSA, EY Laboratories), 3,6-Di-O-(-D-mannopyranosyl)-D-mannopyranose-BSA (α_{1-3}, α_{1-6}-D-mannotriose-BSA, M_3BSA, V-Labs), epithelial cells J82 (human urinary bladder, strain HTB44), A498 (human kidney, strain HTB1), MDCK (Madin—Darby canine kidney cells), MPM (murine peritoneal macrophages)(American Type Culture Collection), BE, erythrocytes of different origin, and baker's yeasts.

The results were statistically processed using Cricket Graph software and Fischer's method.

RESULTS

Adhesion of 7 isogenic recombinant strains with M^L and M^H adhesive phenotypes was studied: KB91, KB21, KB59, KB54, KB23, KB92, and KB96 to MBCA and M_3BSA. Then binding with substrate pairs MN+M_1BSA and RNase+M_3BSA was analyzed (Fig. 1). A strict correlation was found between strain binding to MN and M_1BSA, on the one hand (Fig. 1, *a*, r=0.98, p<0.005), and their binding to RNase and M_3BSA, on the other (Fig. 1, *b*, r=0.77, p<0.05). There was no correlation between adhesion to MN and M_3BSA (r=0) or between adhesion to RNase and M_1BSA (r=-0.3). Binding of KB54 strain to MBSA was 12-16 times higher than of strain KB91, and both strains equally bound to M_3BSA. Inhibition of adhesion to M_1BSA and M_3BSA with monomannosides was similar to inhibition of binding to MN and RNase, respectively: reactions of bacterial cells with MN and M_1BSA were much more sensitive to inhibition with α-methyl-D-mannopyranoside than binding to RNase and M_3BSA (for strains KB54 and KB91, the RNase IC_{50}:MN IC_{50} was 45 and 22, respectively, while M_3BSA IC_{50}:M_1BSA IC_{50} was 50 and 2, respectively). It means that M^H (but not M^L) adhesins ensure intense binding to solitary terminal mannoside residues (M_1) in mannose-enriched or hybrid oligosaccharide structures. On the other hand, both FimH adhesins react with unsubstituted trimannoside groups. Therefore, we shall further call M^L and M^H phenotypes M_1L (intense binding to M_1) and M_1H (low binding to M_1), respectively.

Then we compared the binding of M_1L and M_1H strains to different cell types (Table 1). The adhesive activity of M_1L strains was considerably lower than that of M_1H strains in all experiments, except adhesion to BC and yeast cell agglutination, where their binding activity was approximately the same. Recently we demonstrated that *E. coli* M_1H possess a wider spectrum of binding receptors than M_1L [10]. Such differences in adhesion to different cell types indicate that the

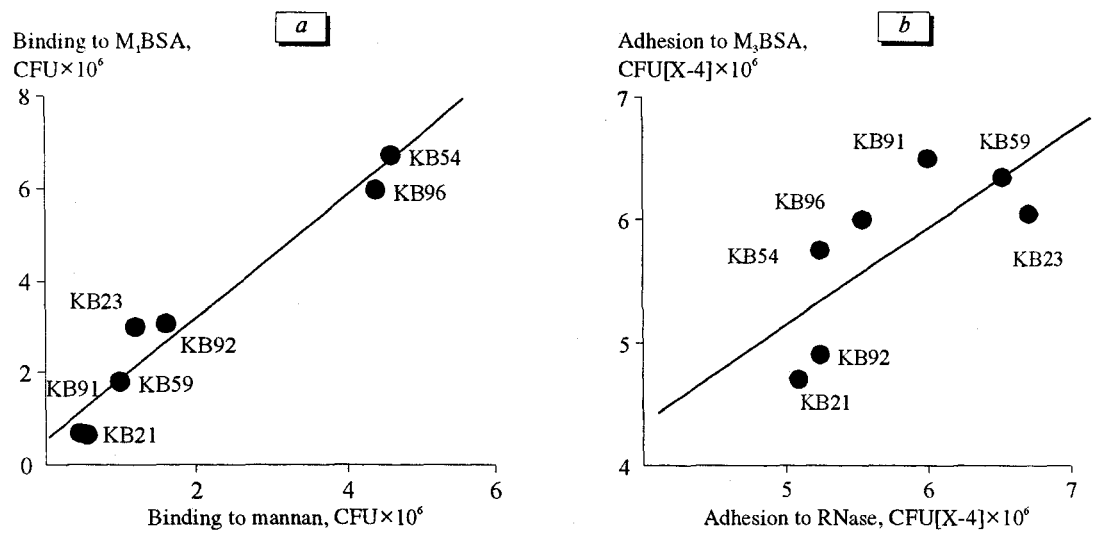


Fig. 1. Analysis of correlations of adhesion of 7 M_1L and M_1H recombinant *E. coli* strains to mannosylated BSA (M_1BSA) and mannan (*a*); α_{1-3}, α_{1-6}-D-mannotriose-BSA (M_3BSA), and RNase B (*b*).

TABLE 1. Adhesive Activity of *E. coli* with Different FimH towards Different Cells (*M*±*m*)

FimH type	Yeasts, dilution	MPM cells	A498 cells	J82 cells	MDCK cells	BC/well	Erythrocytes (highest dilution causing agglutination)			
		number of bacteria per epithelial cell					guinea pig	sheep	horse	man
M_1L	16	2±2	4±2	5±2	5±2	185±15	2	0	0	0
M_1H	16	11±2	41±5	42±4	24±4	194±12	16	1	4	1

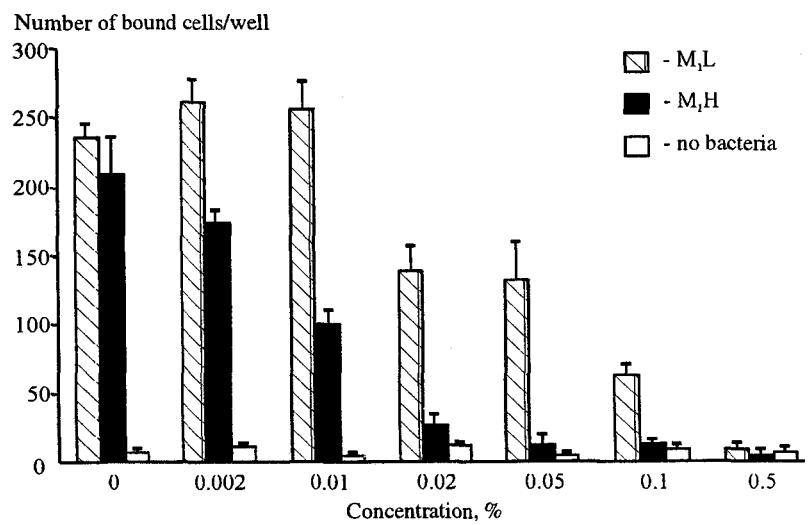
variability of receptor specificity of different FimH adhesin determines strong differences in the microorganism tropism to tissues, which is important for the microorganism-host interactions.

Comparative analysis of adhesive activity of 7 recombinant M_1L and M_1H strains showed a strict correlation between bacterial adhesion to human bladder epithelial cells J82 and binding to monomannoside receptors MN and M_1BSA (r=0.98, p<0.005). Similar data were obtained with human kidney epitheliocytes A498 (r=1, p<0.005). This correlation explains the predominance of M_1H adhesive phenotype in *E. coli* strains causing urinary infections. Bacterial adhesion to epithelial cells and binding to trimannoside receptor structures did not correlate (r=0.025-0.08).

The M_1L phenotype predominates in intestinal *E. coli*. Type 1 pili are not expressed in the intestine [8], and therefore it is obvious that the M_1L phenotype offers selective advantages for transitory colonization of the oropharyngeal area, facilitating the spread of bacteria from one individual to another [4]. The number of mucosa-bound bacteria depends on adhesin affinity to epithelial cell receptor and on the effect of inhibitors in the fluid washing the mucosa surface [9]. Both types of *E. coli* (M_1L and M_1H) similarly adhere to BC. Therefore, the above-mentioned advantage of the M_1L phenotype should consist in higher resistance to inhibition by mannose-containing agents. Though high concentrations of α-methyl-D-mannopyranoside completely inhibit adhesion of strains of both types, at intermediate concentrations adhesion is much more resistant to inhibition (IC_{50} M_1L/IC_{50} M_1H=15, Fig. 2).

Though free mannose never occurs in nature, the mucous layer is washed by glycoproteins carrying exposed terminal mannose residues [3]. Some immobilized glycoproteins effectively bind both phenotypes (RNase B, bovine lactoferrin, *etc.*), while others react only with *E. coli* M_1H (MN, bovine intestinal mucin, *etc.*) [10]. Adhesion of the M_1L strain was much more resistant to inhibition (Table 2).

The majority of potential inhibitors of bacterial adhesion in the oropharynx are salivary components. Therefore, we investigated the effect of whole saliva on the interaction of *E. coli* with BC. M_1L adhesion was 5 times less sensitive to inhibition than M_1H adhesion (Fig. 3). The absolute level of M_1L strain adhesion in the presence of whole saliva was 4 times higher than of M_1H (the difference decreased with saliva dilution). Under natural conditions, this offers selective advantages for the M_1L phenotype by faci-



olved α-methyl-D-mannopyranoside on interactions of isogenic M_1L and M_1H *E. coli* strains with buccal epithelial cells.

litating bacterial adhesion to oropharyngeal cells at the initial stages of colonization in the presence of salivary components and/or by decreasing the probability of further existence in the Fim$^+$ phase, which is unfavorable for intestinal colonization.

These data mean that FimH variants are differently adapted to colonization of the primary normal and secondary pathological niches of human *E. coli*. We believe that M_1L phenotype is functionally optimal for the physiological role of type 1 pili, namely, colonization of the oropharyngeal area, ensuring effective colonization of a new host. M_1H phenotype poorly adapted for this function was lost for the commensal *E. coli* population: it rarely occurs among intestinal *E. coli*, results from structural mutations of the gene, and is poorly adapted to bacterial adhesion in the presence of natural inhibitors.

High virulence of *E. coli* strains towards the urinary system due to the presence of M_1H adhesin FimH can be a factor maintaining the FimH polymorphism. Dissemination of *E. coli* through infected urine is typical of urinary infections. This additional source of dissemination promotes colonization of a carrier organism and can compensate, to a certain extent, the impaired capacity for primary colonization of a new host. The FimH M_1H phenotype in intestinal strains of *E. coli* can be compensated by simultaneous expression of other bacterial factors, for example, uncommon adhesive structures, such as S and P fimbriae, *etc.* This compensatory pathway is in line with the theory of fixation of unfavorable mutations [6]. Therefore, functional abnormality of the allele may serve as a factor potentiating bacterial virulence.

These findings indicate that M_1H (M^H) phenotype of commercial *E. coli* strain M17 is undesirable for the intestinal strain and should be removed or replaced. The decrease in the adhesive activity should not essentially modify the main function of the eubiotic, colonization of the intestine, because the stage of colonization of the oropharynx is important only in administration of low doses of bacteria, which is not the case in probiotic therapy [10].

TABLE 2. Effects of Soluble Glycoproteins on Interactions of BC and *E. coli* Strains with Different FimH ($M \pm m$)

Inhibitor, mg/ml	IC_{50}	
	M_1L	M_1H
MN	5.0±0.3	0.5±0.2
Bovine intestinal mucin	1.7±0.1	0.6±0.1
Bovine RNase	0.9±0.2	0.08±0.02
Bovine lactoferrin	2.1±0.2	0.08±0.03

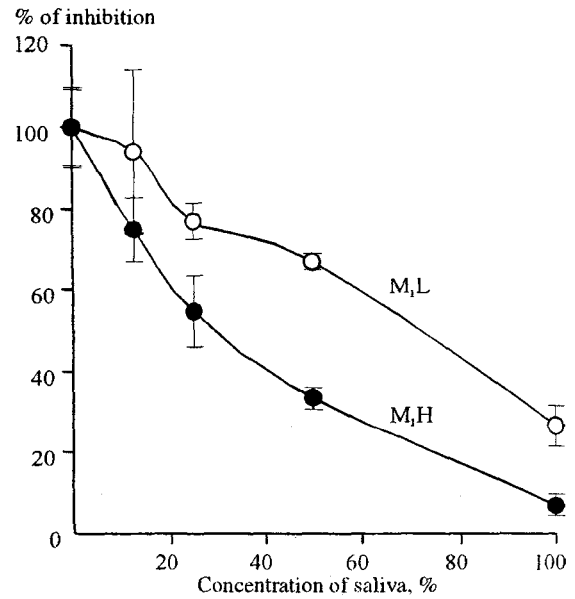


Fig. 3. Effect of whole human saliva on binding of isogenic M_1L and M_1H *E. coli* strains with buccal epithelial cells.

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