ACTA ABP BIOCHIMICA POLONICA

Vol. 61, No 2/2014
389–392
on-line at: www.actabp.pl

Communication

Type 1 fimbriae in commensal *Escherichia coli* derived from healthy humans*

Paweł Pusz✉, Ewa Bok, Justyna Mazurek, Michał Stosik and Katarzyna Baldy-Chudzik

Department of Biological Sciences, University of Zielona Góra, Poland

Type 1 fimbriae are one of the most important factors of *Escherichia coli* adaptation to different niches in the host. Our study indicated that the genetic marker — *fimH* gene occurred commonly in commensal *E. coli* derived from healthy humans but expression of the type 1 fimbriae was not observed. Identification of fim structural subunit genes (*fimA–fimH*) and recombinase *fimE* and *fimB* genes showed that many of the strains were carrying an incomplete set of genes and the genes expression study revealed that in strains with complete set of fim genes, the *fimC* gene, encoding the chaperone protein, was not expressed.

Key words: commensal *E. coli,* type 1 fimbriae, gene expression

Received: 31 October, 2013; revised: 17 March, 2014; accepted: 01 April, 2014; available on-line: 20 May, 2014

INTRODUCTION

Type 1 fimbriae, the fimbrial adhesins, are important for *Escherichia coli* adherence to both biotic and abiotic surfaces and are responsible for specific interactions with mannose-containing receptors found in many host tissues. This surface structure plays a role in the infection and colonization process of human body by two mechanisms: adherence and invasion. It is also believed to be an important virulence factor in many extraintestinal infections in humans, especially infections of urinary tract caused by *E. coli* (Mitra *et al*., 2013).

The type 1 fimbrial adhesin is encoded by a *fim* gene cluster (Fig. 1), including nine genes required for its biosynthesis (Iida *et al.,* 2001), the *fimA* gene encoding the major subunit, *fimF* and *fimG* genes encoding two minor subunits and *fimH* gene coding the mannose-sensitive tip of fimbriae. There are two genes involved in transport and assembly of type 1 fimbriae within the *fim* gene cluster: *fimC* and *fimD*. The *fimC* gene encodes a chaperone protein that helps to translocate fimbrial proteins through the periplasm and *fimD* gene encodes an integral outer membrane protein that serves as an usher. Furthermore, *fimI* gene encodes a structural component (Schwan, 2011). The expression of the *fim* structural subunit genes (*fimA–fimH* genes) is controlled by an invertible promoter region (known as the '*fim* switch', *fimS*). Inversion of the 314 bp switch element is the basis of phase-variable expression of type 1 fimbriae (Blumer *et al*., 2005) and is catalyzed by two site-specific recombinases encoded by *fimB* and *fimE* genes located upstream of the *fimS* region. Phase variation yields a heterogenous set of piliated and unpiliated bacteria and may account for differential expression of type 1 fimbriae in different body sites.

The role of the type 1 fimbriae in *E. coli* in colonization of gastrointestinal tract is still uncertain, therefore here we investigated the presence and expression of fimbrial genes responsible for the active form of type 1 fimbriae in commensal *E. coli* isolated from healthy humans.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strains were isolated from fecal samples derived from 54 healthy adults living in Zielona Góra. Fecal samples were inoculated into agar m-FC. After 24h of incubation at 44.5°C, blue cultures were passaged onto the MacConkey's agar. Lactopositive strains were subjected to standard biochemical IMVC (indol, methyl-red-voges-proskauer, citrate) tests for *E. coli* identification. *E. coli* strains derived from the same person/sample were screened for confirmation of their genomic diversity by the BOX-PCR fingerprinting method (Baldy-Chudzik & Stosik, 2003). All strains were stored in glycerol broth at –80°C and for subsequent analysis were cultured in Luria-Bertani (LB) media. In total, 127 unique *E. coli* strains were identified.

Agglutination tests. Mannose-sensitive haemagglutination was determined in phosphate-buffered saline (PBS) with a 5% suspension of sheep red blood cells sensitized by Tannic Acid, in the presence or absence of 3% D-mannose (Sigma). Bacterial cultures were grown in LB broth for 16 h under shaking conditions, then washed in PBS, and suspended to an $OD_{600} \sim 0.5$. The test was conducted in 96-microwell plates. Twenty-five microliters of the erythrocyte suspension with or without D-mannose was mixed with an equal volume of a bacterial suspension. The haemagglutination reaction was checked after 60 min and 24h under microscope and it was considered to be mannose sensitive when it was inhibited by D-mannose.

Identification of *fim* genes by PCR. Genes constituting the *fim* operon were identified by PCR with primers described in Table 1. Representative probes of PCR amplicons for particular genes were sequenced (Genomed) and compared with target sequences in genetic sequence database — GenBank (NCBI).

Restriction analysis of PCR products for identification of the switch position (ON/OFF) in promoter region of the *fim* operon. The switch position of

✉e-mail: p.pusz@wnb.uz.zgora.pl
*Presented at the 3-rd Workshop on Microbiology „MIKROBIOT 2013" in Łódź, Poland.

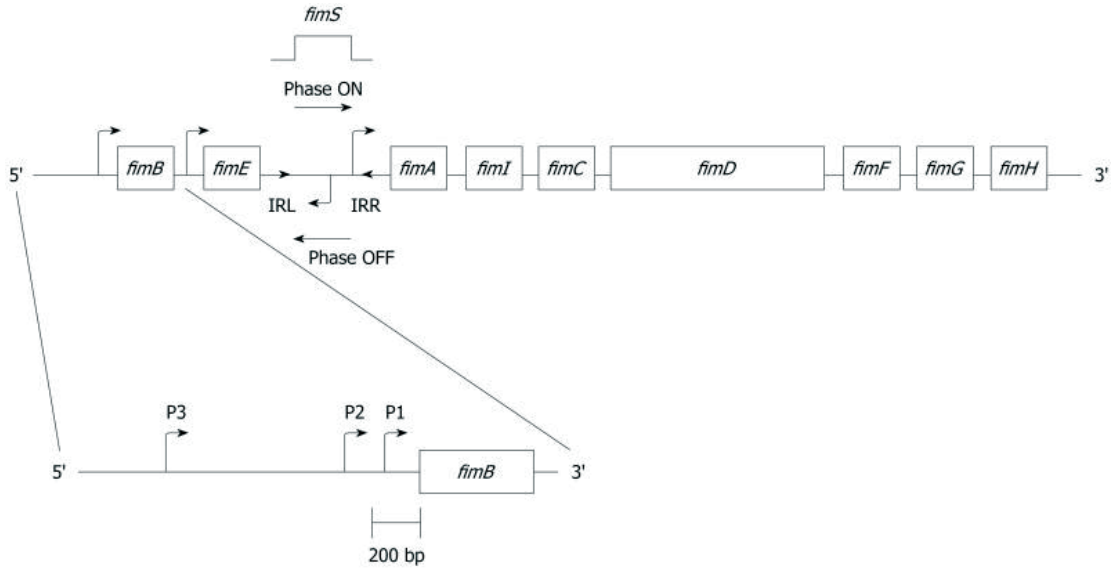

Figure 1. Scheme of the type 1 fimbriae operon (Schwan, 2011).

Table 1. Primers used in this study.

Gene	Function	Primer names and DNA sequences (5′-3′)		References	Product size (bp)	GenBank Accession no.
fimA	major subunit	FimA-F FimA-R	AGTTAGGACAGGTTCGTACCGCAT AAATAACGCGCCTGGAACGGAATG	Hernandes *et al*., 2011	315	KC405511.1
		FimA-F* FimA-R*	GGACAGGTTCGTACCGCATC ACGTTGGTATGACCCGCATC	Blumer et al. 2005	151	KC405511.1
fimB	regulator	FimB-F FimB-R	CGAATCACTCCTTAAAGCAG GGCGTAACATGTGCGGATGAA	Schwan *et al*., 2002	379	EU890414.1
		FimB-F* FimB-R*	ACTGGAGATTCATCCGCACA GTCGTCCTCTGGCTCTATCC	This study	179	EU890414.1
fimC	chaperone	FimC-F FimC-R	GGGTAGAAAATGCCGATGGTG CGTCATTTTGGGGGTAAGTGC	Ewers *et al*., 2007	477	FJ866110.1
		FimC-F* FimC-R*	CTCGCAATTATCAGCCGCAT GCATTTTCAAGAACCCGGGT	This study	182	FJ866110.1
fimE	regulator	FimE-F FimE-R	AAACGTCGTTATCTTACCGG TTTCTTTCCCATAATCCGGC	Hernandes *et al*., 2011	550	EU890408.1
		FimE-F* FimE-R*	TATGAATTGGCGGAGCGTGGTG AAACGAGCAGCATTACTGGCGGTAT	Blumer et al. 2005	153	EU890408.1
fimH	adhesin	FimH-F FimH-R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	Chapman *et al*., 2006	508	FJ865819.1
		FimH-F* FimH-R*	GCTGTGATGTTTCTGCTCGT AAAACGAGGCGGTATTGGTG	This study	168	FJ865819.1
fimI	structural protein	FimI-F FimI-R	GACGGTCAATATGGGGCAAA TTTTTACCATCCGCGACACC	Blumer *et al*., 2005	153	AF286465.1
		FimI-F* FimI-R*	GACGGTCAATATGGGGCAAA TTTTTACCATCCGCGACACC	Blumer et al. 2005	153	AF286465.1
gapA	reference gene for real-time PCR	GapA-F* GapA-R*	GTTGTCGCTGAAGCAACTGG AGCGTTGGAAACGATGTCCT	Blumer et al. 2005	170	AF424784.1

F*and R* — sequences of primers used in real-time PCR

Table 2. Identification of the *fim* structural subunit genes and recombinase genes in commensal *E. coli* derived from healthy humans.

Number of *E. coli* n (%)	Genes essential for expression of type 1 fimbriae in *E. coli.*					
	fimB	fimE	fimA	fimI	fimC	fimH
72 (59.0)	+	+	+	+	+	+
39 (32.0)	+	+	-	+	+	+
4 (3.3)	+	+	-	+	-	+
2 (1.6)	-	+	-	+	+	+
2 (1.6)	-	+	+	+	+	+
1 (0.8)	+	-	-	-	-	+
1 (0.8)	-	-	+	-	-	+
1 (0.8)	-	-	-	-	-	+
Total 122	116 (95.1%)	119 (97.5%)	75 (61.5%)	119 (97.5%)	115 (94.3%)	122 (100%)

the *fimS* (fragment size of 559-bp containing the *fimA* promoter) was identified by PCR and subsequently restriction analyzed with HinfI, according to the Nowrouzian and coworkers (2007).

Analysis of gene expression by Real-Time PCR. Total RNA was extracted from cultures with an optical density of 0.6 (600 nm) using the SV Total RNA Isolation System (Promega), according to the manufacturer's protocol. The RNA sample was quantitated based on absorption at 260 nm, and purity was determined by the ratio of absorption values at 260 and 280 nm. 2 μg of RNA were treated with DNase I (Roche) at a concentration of 1U/1μg of RNA for 60 min. at room temperature. DNase I was inactivated by adding EDTA to a final concentration of 8 mM and heating the samples at 75°C for 10 min. cDNA synthesis and real-time PCR reactions were performed using DyNAmo™ SYBR® Green 2-Step qRT-PCR Kit (Finnzymes). Reverse transcription was performed in a 20-μl reaction mixture containing 0.5 μg of RNA, 300 ng of random hexamer primer set, 2x RT buffer (containing dNTP mix and 10 mM $MgCl_2$), M-MuLV RNase H+ reverse transcriptase (containing RNase inhibitor), RNase free water, as described by the manufacturer. The no-reverse transcriptase controls (-RT) were prepared for each DNase-treated RNA sample. cDNA synthesis was performed under the following conditions: primer extension at 25°C for 10 min, cDNA synthesis at 37°C for 30 min, reaction termination at 85°C for 5 min (inactivation of M-MuLV), cooling the sample at 4°C. Real-time PCR was performed in a 20 μl volume containing: 1 μl cDNA, 2x master mix (containing modified hot-start Tbr DNA polymerase, SYBR® Green I, optimized PCR buffer, 5 mM MgCl2, dNTP mix), 400 nM of each primer, and sterilized molecular-grade water, according to the manufacturer's protocol. The sequences of specific primers for each target gene are described in Table 1.

Real-time PCR was performed under the following cycle conditions: denaturation at 95°C for 5 min and 45 cycles of 94°C for 10 s, 60°C (*fimA*), 60°C (*fimB*), 61°C (*fimC*), 61°C (*fimE*), 60°C (*fimH),* 58°C (*fimI*) and 60°C (*gapA*) for 20 s, and 72°C for 30 s, followed by a 10-min extension at 72°C (Rotor Gene-3000). In each analysis, the negative control probes (no- template control (NTC) and no-reverse transcriptase control (-RT)) were included and no expression profiles (the fluorescence curves) were observed before the onset of 40 cycles of PCR. Positive controls of expression were performed by using primers specific for the gene encoding D-glyceraldehyde-3-phosphate dehydrogenase A (gapA). To ensure specificity of the PCR products, melting curve analysis was performed. Each PCR was performed in duplicate.

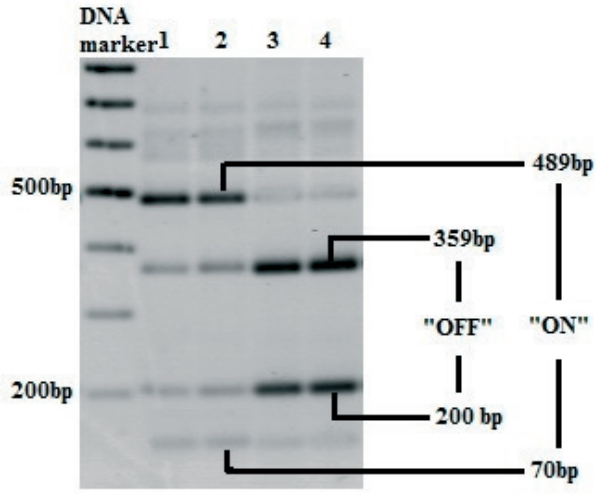

Figure 2. Detection of the switch phase orientation of the *fimS* promoter of the type 1 fimbriae operon in *E. coli* using PCR-RFLP by HinfI enzyme cleavage.
Cleaved PCR product of strains 1 and 2 indicate the switch phase in the "ON" position while strains 3 and 4 display the "OFF" position.

RESULTS AND DISCUSSION.

In fecal samples from healthy people, 127 unique *E. coli* strains were identified. The whole set of strains was tested for the presence of the *fimH* gene which is believed to be a genetic marker for type 1 fimbriae. The *fimH* gene commonly occurred in the analyzed strains (122/ 96,1%). Adhesion phenotype of *E. coli* was tested by haemagglutination assays. The results revealed that all of 122 *fimH*$^+$ strains were negative for type 1 fimbriae expression.

Detection of the *fim* structural subunit genes (*fimA-fimH*) and recombinase *fimE* and *fimB* genes was performed by PCR and revealed the presence of all of the tested genes in 72 strains, but in 50 strains the gene set was incomplete. The absence/deletion of *fimA* gene (32%) was most commonly observed and in other cases (9%), the deletion of more than one gene was observed. The detailed results are shown in Table 2.

For 72 strains carrying a complete set of genes, the PCR-RFLP Hinf1 analysis was performed to character-

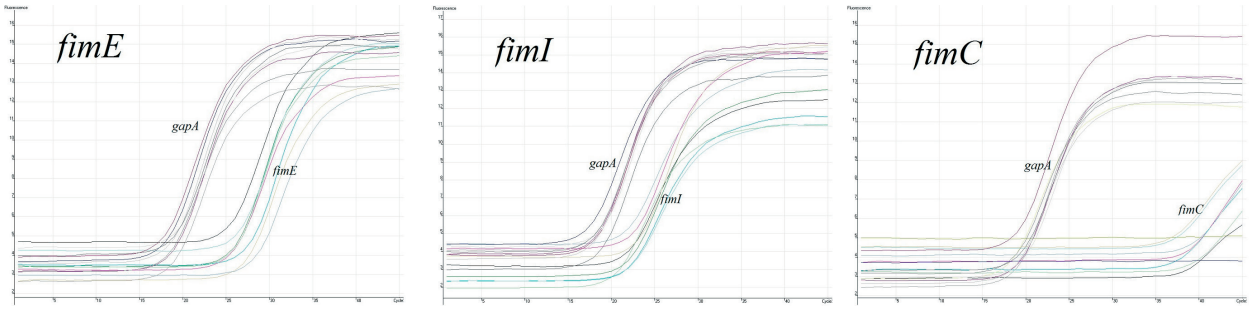

Figure 3. The *fim* genes expression profile — fluorescence curves of the tested genes and the reference gene — *gapA*. Profiles of *fimE* and *fimI* show expression of genes while the profile of *fimC* shows lack of expression.

ize the switch orientation of the *fimS* promoter region (Fig. 2). In 53 strains the promoter was found to be in the "ON" position (active promoter).

For 53 strains with the active promoter (ON position) the expression analyses of *fimB*, *fimE, fimA, fimI, fimC* and *fimH* genes were performed by real-time PCR. Expression of the *fimC* gene encoding a chaperone protein and *fimH* gene encoding adhesin were not observed in any of the tested strains (Fig. 3).

Among 127 commensal *E. coli* of human origin, 43.3% of strains had a deletion of at least one of the *fim* genes responsible for the type 1 fimbriae expression, and 41.7% revealed lack of expression of the *fimC* gene. Preliminary research indicated that 85% of the tested strains did not have ability to expressi type 1 fimbriae, which may suggest that the lack of these fimbriae is an adaptive feature of *E. coli* to the habitation of the human intestine environment. Further research will explain the lack of *fimC* gene expression.

Acknowledgement

This work was supported by the MNiSW grant – N N304 176 340.

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