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## Analysis of the variability of S-fimbriae expression in an *Escherichia coli* pathogen

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### 1. SUMMARY

The uropathogenic *Escherichia coli* wild-type strain 536 produces S-fimbriae, P-related fimbriae and type I fimbriae. Using immuno-colony dot and ELISA techniques, variants were detected showing an increased degree of S-fimbrial production. It was demonstrated by immunofluorescence microscopy that in normal (wild-type) and hyper-S-fimbriated *E. coli* populations non-fimbriated cells also exist, and that the percentage of S-fimbriated and non-fimbriated bacteria was roughly identical in either population. Hyper-S-fimbriated variants could be stably maintained. The transition from wild-type to hyper-S-fimbriation, which occurs spontaneously, is markedly higher than vice versa. Southern blot analysis of the S fimbrial adhesin (*sfa*) determinants of normal and hyper-fimbriated strains revealed no marked difference in the gene structure.

### 2. INTRODUCTION

Fimbrial adhesins enable pathogenic bacteria to colonize eukaryotic tissue surfaces [1]. Extraintestinal *Escherichia coli* isolates causing urinary tract infections (UTI), new born meningitis (NBM) or cases of sepsis are able to produce several types of fimbrial adhesins which can be distinguished by their different receptor specificities. S fimbrial adhesins (Sfa) produced by NBM and, to a minor extent by UTI strains are able to bind to terminal sialyl- $\beta$  [2,3] lactose sequences of glycoproteins [2]. The S fimbrial adhesin complex consists of four proteins. One of these, SfaA, represents the major subunit of the fimbrial structure (16.5 kDa). SfaS, a minor subunit protein of 14 kDa represents the sialic acid binding molecule [3]. The S-fimbrial determinants are not expressed constitutively, but rather are influenced by several factors including growth rate, temperature and composition of cultivation media [4]. In addition a spontaneous ON and OFF switching of S-fimbriation has been described [5]. Here we report on the occurrence of three different phases of S fimbrial expression in an *E. coli* wild-type isolate: 'Nor-

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mal' fimbriated, non-fimbriated and hyper-fimbriated bacteria.

### 3. MATERIALS AND METHODS

#### 3.1. Bacterial strains and plasmids

*E. coli* strain 536 (O6:K15:H31), isolated from a patient suffering from a urinary tract infection, was obtained from the Institute für Hygiene und Mikrobiologie, Würzburg [6]. The variant strain designated 536-53 exhibiting a hyper-S-fimbriated phenotype was isolated from a single colony strongly reacting with anti-SfaA antibodies in an immuno-colony-dot assay. *E. coli* strain 536-17B1 is a Tn*phoA* mutant (*sfa*::Tn*phoA*) displaying a Sfa<sup>-</sup> phenotype (ref. 7; Hacker et al. in preparation). The *E. coli* K-12 strain HB101 was used in control experiments. For generation of the *sfa*-specific-DNA probe, plasmid pANN801-13 (Ap<sup>R</sup>) was used [8]. The recombinant DNA was maintained in *E. coli* HB101 under antibiotic pressure (ampicillin conc. 50 µg/ml).

#### 3.2. Media and reagents

Bacteria were grown in Luria Bertani (LB) broth. For detection of fimbriae, bacteria were cultivated on solid media. FITC (fluorescein-isothiocyanate)-conjugated goat-anti-rabbit IgG antibodies were obtained from Sigma, F.R.G. Peroxidase-conjugated swine-anti rabbit IgG antibodies were purchased from Dako, Denmark. All other chemicals were a gift from Sigma, F.R.G.

#### 3.3. Antibody preparation

S-fimbriae specific antiserum was prepared from rabbits immunized with purified fimbrial protein (SfaA) eluted from a sodium dodecyl sulphate-polyacrylamide electrophoresis gel [9]. The preimmune serum did not react with any fimbriae isolated. The specificity of anti-SfaA serum was tested in Western blot analysis using fimbriae preparations of *E. coli* strain 536. There was no cross-reaction detectable to the other fimbrial subunits of this strain (data not shown).

#### 3.4. Immuno-colony-dot assay

Bacterial colonies were replica plated on nitrocellulose filters and grown overnight on LB medium. Immuno-colony-dot procedure was then carried out as described by van Die et al. [10].

#### 3.5. Whole-cell ELISA

Whole cell ELISA was performed as described previously [11]. Bacteria were harvested after growth on solid media and OD<sub>600</sub> was adjusted to 0.8. 150 µl of this suspension were used. Values were determined at 490 nm using a microtiter plate reader (Flow, F.R.G.).

#### 3.6. Immunofluorescence microscopy

Bacteria were harvested from LB plates and washed in TBS (50 mM Tris-HCl, pH 7.5/0.9% NaCl). An aliquot of the bacterial suspension was dropped onto a glass slide and incubated for 10 min at room temperature (RT). Then 25 µl of a 0.5% glutaraldehyde solution (v/v in H<sub>2</sub>O) were added. After a further incubation of 10 min at RT, the glass slide was washed three times in TBS. Then 50 µl of a TBS/3%BSA (bovine serum albumin) solution was dropped onto the fixed bacteria. After 30 min incubation at RT the BSA solution was removed and antibody I (anti-SfaA) solution was added. Incubation was for 30 min at 37°C in a humidified glass chamber. Thereafter the slide was washed three times with TBS and further incubation with the FITC-labelled antibodies followed. After final washing the preparations were examined with a fluorescence microscope (Leitz, Dialux 20).

#### 3.7. DNA techniques and Southern hybridization

Isolation of chromosomal and plasmid DNA, restriction enzyme cleavage and subsequent agarose gel electrophoresis were performed according to protocols of Maniatis et al. [12]. Southern hybridization was performed as previously described [13]. DNA fragments used as probes were eluted from agarose gels via electroelution and <sup>32</sup>P-labelled by nick translation [12].

## 4. RESULTS

### 4.1. Isolation of a hyper-S-fimbriated variant of the *E. coli* wild type strain 536

The *E. coli* wild-type strain 536 expresses three types of fimbriae, S-fimbriae, P-related fimbriae and Type 1 fimbriae [14]. Antibodies were raised against the major S fimbrial subunit protein SfaA (16.5 kDa) of the wild type strain 536. Using immuno-colony-dot analysis, approximately one out of 100 colonies of the wild-type strain exhibited a stronger reaction with anti-SfaA antibodies (Fig. 1), indicating an overproduction of S-fimbriae. One of these variants, designated 536-53 was further characterized. Immuno-colony-dot analysis of the 536-53 strain revealed that all colonies reacted very strongly, and no colony similar to the normal fimbriated wild-type could be observed (data not shown).

### 4.2. Quantification of the expression of S-fimbriae

To compare the S-fimbriae production of the wild type strain 536 with the variant 536-53 more precisely, a whole cell ELISA using anti-SfaA antibodies was performed. Fig. 2 clearly demonstrates a significant increase of adsorption in variant 536-53 compared with that of the wild type strain, confirming the overproduction of S-fimbriae in the variant strain. In control experiments an S fimbrial negative mutant 536-17B1 (*sfa::Tn<sub>phoA</sub>*) still expressing P-related fimbriae and Type 1 fimbriae (data not shown) and the *E. coli* K12 strain HB101 were used, which did not show a significant reaction with the anti-SfaA specific antibodies.

### 4.3. Analysis of fimbriation of single bacterial cells by immunofluorescence microscopy

For further analysis, the wild-type strain 536 and the variant 536-53 were examined via immunofluorescence microscopy using the anti-SfaA antibodies. In Fig. 3 it can be seen that in either population, bacterial cells reacting with the antibodies and non-reacting cells co-exist, indicating the presence of S-fimbriated and non-S-fimbriated cells. In both strains, 536 wild-type and 536-53, approximately 60–70% of the cells displayed S-fimbriation. Therefore we concluded that hyper-

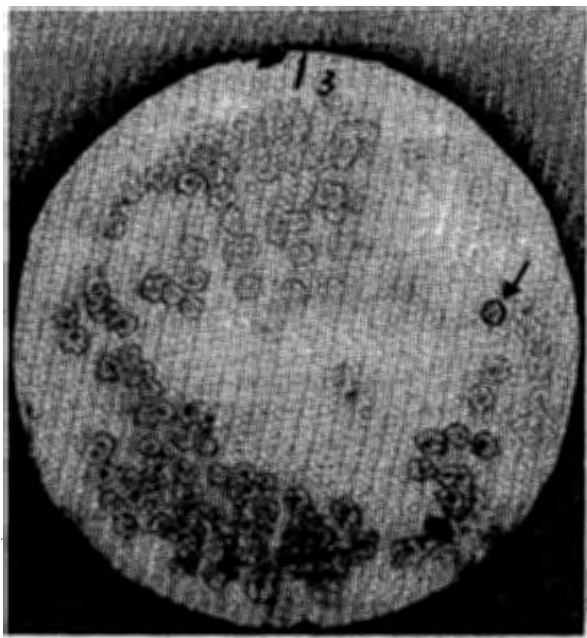


Fig. 1. Immuno-colony-dot analysis of strain *E. coli* 536, using anti-SfaA antibodies. One of the stronger reacting colonies (arrow) of the wild-type population was isolated and designated 536-53, exhibiting a hyper-S-fimbriated phenotype.

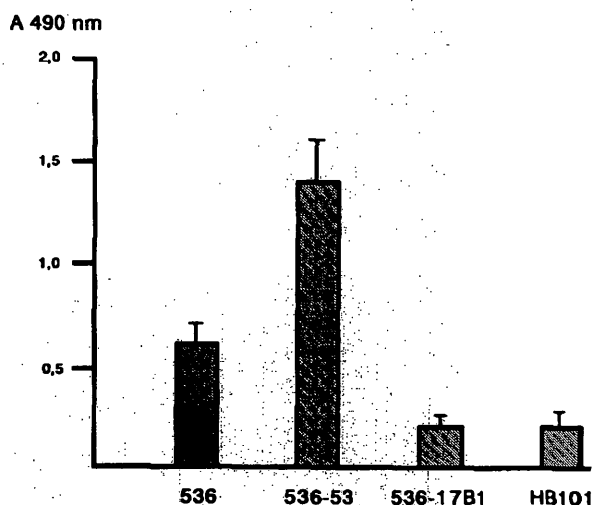


Fig. 2. Whole-cell ELISA of *E. coli* strains 536, 536-53, 536-17B1 and HB101 using anti-SfaA antibodies. The values represent absorbance at 490 nm ( $A_{490}$ ). Standard deviation is given.

reaction observed with the anti-SfaA antiserum in the ELISA is not due to an increased percentage of S-fimbriated cells within the 536-53 population, but rather is due to a higher degree of S-fimbriation per bacterial cell. Indeed this was confirmed by electronmicroscopic examination (data not shown).

#### 4.4. Genetic analysis of the *sfa* determinants from the genomes of the wild-type and hyper-S-fimbriated variant

In order to analyse the gene structure of the *sfa* determinants of strains 536 (wild-type) and 536-53, chromosomal DNA was isolated and hybridized against the 9.0-kb *sfa*-specific DNA fragment, derived from plasmid pANN 801-13, comprising the whole *sfa* determinant [15]. The chromosomal DNAs were double digested with *Pst*I and *Sph*I to increase the number of fragments, which might have been helpful for detection of minor genomic variations. But as can be seen in Fig. 4, there is no detectable difference in the hybridization pattern, indicating that genomic alterations do not occur within the *sfa*-gene structure, when normal S-fimbriated cells become hyper-S-fimbriated.

## 5. DISCUSSION

In this report we describe the variation of S-fimbriae expression in the *E. coli* wild-type strain 536. Using anti-SfaA antibodies, recognizing the major S-fimbrial subunit in different immunological assays we could demonstrate that three distinguishable levels of S-fimbriae production exist. It is presumed that single cells can be normally S-fimbriated (ON status), as well as hyper-S-fimbriated (HYP) or non-S-fimbriated (OFF). Hyper-fimbriated variants carry more fimbriae per bacterial cell than the normal fimbriated bacteria. Spontaneous transitions of one state to another occur. We describe here for the first time the stable isolation and characterization of a hyper-S-fimbriated variant of an *E. coli* wild-type strain. Transition to the HYP level occurs at a rate of  $10^{-2}$ . Precise calculation of a reversion to the ON level has not been carried out, but the frequency seems to be much lower than transition in the opposite direction. Genetic analysis revealed that there are no marked differences in the gene structure of *sfa* determinants of the wild-type and the hyper-S-fimbriated strain. Other mechanisms be-

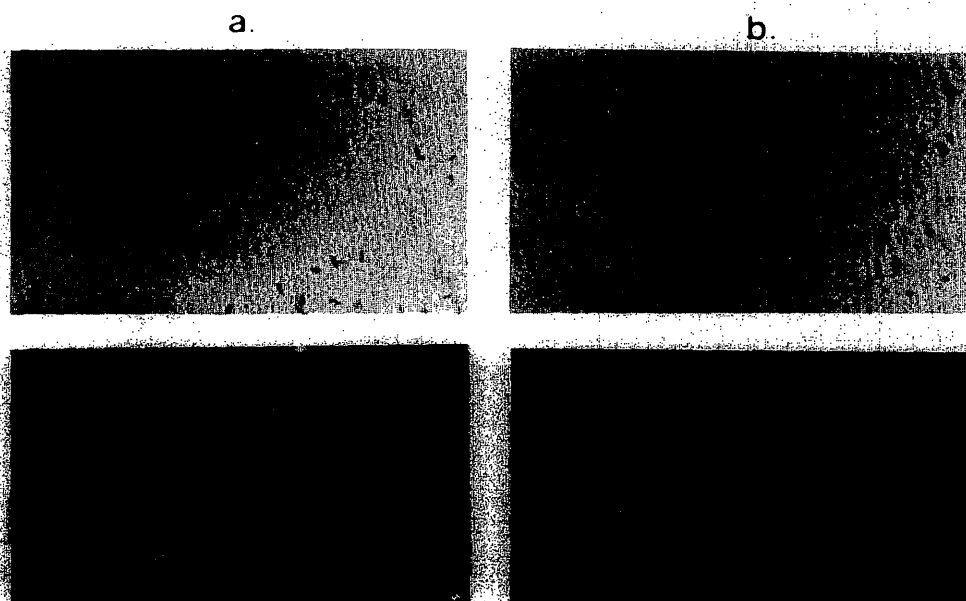


Fig. 3. Immunofluorescence microscopy of strains *E. coli* 536 (a) and 536-53 (b) using anti-SfaA antibodies. The same sections are shown without (above) and with fluorescence (below). S-fimbriated bacteria (appearing bright after fluorescence stimulation) (+) and non-S-fimbriated bacteria (-) are indicated by arrows.

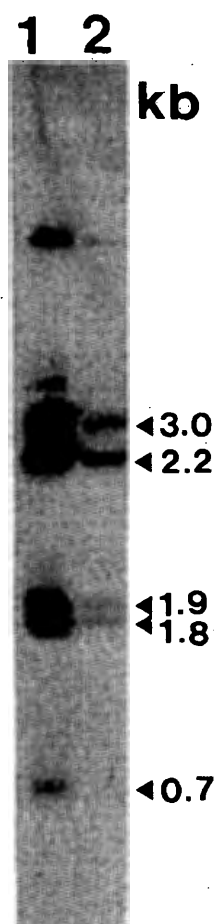


Fig. 4. Southern blot analysis of *Pst*I-*Sph*I cleaved genomic DNA of the wild-type strain 536 (lane 1) and the hyper-S-fimbriated variant 536-53 (lane 2), using the 9.0-kb *Eco*RV fragment from pANN801-13 as DNA-probe. DNA sizes of internal *sfa*-specific fragments are indicated.

yond major rearrangements might be responsible for the altered phenotype.

Genetic mechanisms for the ON-OFF switching of the expression of a certain phenotype have been described by several authors. Ou and co-workers report on the variation of the Vi capsular antigen of *Citrobacter freundii*, which is regulated by an insertion-excision of an IS 1-like DNA element [16]. In an analogous way the exopolysaccharide (EPS) production of the marine bacterium *Pseudomonas atlantica* is switched ON-OFF by an insertion element [17]. Another mode of control is responsible for the phase variation of Type 1

fimbriae in *E. coli*. The inversion of a DNA element leads to either the ON or OFF state [18,19]. Phase variation has been further reported for P-fimbriated as well as for S-fimbriated pathogenic *E. coli* strains, where in contrast to the above mentioned examples, the underlying control mechanisms have not been elucidated [5,20]. Also hyper-fimbriation has been described for Type 1 fimbriae in *E. coli* K-12 [21]. Van der Woude and co-workers reported on a mode of controlling 987P-fimbriae expression in enterotoxigenic *E. coli* where the number of fimbriae per bacterial cell is regulated [22]. As one can see, different levels of expression of a certain phenotype are commonly found in pathogenic as well as on non-pathogenic bacteria. In the case of pathogenic bacteria, the advantages of altering the level of expression of certain virulence traits are obvious. Adaptation to changing environmental conditions in the course of an infection might be mediated by OFF-ON-HYP switching a certain phenotype.

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