Genetics of Escherichia coli Uropathogenicity: Analysis of the O6:K15:H31 Isolate 536*

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Summary

E. coli strain 536 (O6:K15:H31) isolated from a case of acute pyelonephritis, expresses S-fimbrial adhesins, P-related fimbriae, common type I fimbriae, and hemolysins. The respective chromosomally encoded determinants were cloned by constructing a genomic library of this strain. Furthermore, the strain produces the iron uptake substance, enterochelin, damages HeLa cells, and behaves in a serum-resistant mode. Genetic analysis of spontaneously arising non-hemolytic variants revealed that some of the virulence genes were physically linked to large unstable DNA regions, termed "pathogenicity islands", which were mapped in the respective positions on the E. coli K-12 linkage map. By comparing the wild type strain and mutants in vitro and in vivo assays, virulence features have been evaluated. In addition, a regulatory cross talk between adhesin determinants was found for the wild-type isolate. This particular mode of virulence regulation is missing in the mutant strain.

Zusammenfassung

Der E. coli Stamm 536 (O6:K15:H31), isoliert aus einem Patienten mit akuter Nierenbeckenentzündung, zeichnet sich durch die Ausprägung von S-Fimbrien Adhäsinen, P-verwandten Fimbrien, "common type I" Fimbrien und Hämolysinen aus. Ausgehend von einer Genbank des Stammes 536 konnten die entsprechenden chromosomal kodierten Gene geklont werden. Weiterhin produziert der Stamm die Eisenaufnahmesubstanz Enterochelin, schädigt HeLa-Zellen und verhält sich Serum-resistent. Genetische Untersuchungen an

* Dedicated to Dr. I. Ørskov and to Dr. F. Ørskov on the occasion of their 70th birthday.

Introduction

The majority of urinary tract infections (UTI) can be attributed to Escherichia coli pathogens causing cystitis or pyelonephritis. E. coli UTI isolates exhibit specific virulence properties, enabling colonization of and survival in the urinary tract and leading to severe tissue damage in the case of acute pyelonephritis (9, 21, 28). Fimbrial adhesins mediating binding to host cell surfaces, serum resistance properties, iron uptake substances, and toxin production have been shown to be involved in the infectious process (8, 13, 19). Furthermore, the type of lipopolysaccharide composition (O antigen) and capsule formation (K antigen) are important (1, 13, 17). In the last decade, numerous attempts have been made to study uropathogenicity on a molecular level. Cloning and characterization of virulence determinants from various strains (e.g. J 96, 2980, 20025, IH 3034; 12, 16, 18, 20, 23) belonging to different serotypes provided an insight into the molecular mechanisms of the uropathogenicity of E. coli. The present overview of the genetics is dealing with aspects of the virulence of the uropathogenic E. coli strain 536.

Isolation and Serotyping of the Uropathogenic Strain E. coli 536

E. coli strain 536 had been isolated from a patient suffering from acute pyelonephritis at the Institut für Hygiene und Mikrobiologie, University of Würzburg in 1980 (2). Serotyping of strain 536 revealed carriage of the O6 lipopolysaccharide antigen, which is frequently found among UTI isolates (3, 31). Furthermore, capsule type K15 and H31 flagellin antigen have been determined (2, 10).

Virulence Features of E. coli Strain 536

As shown by phenotypic characterization, strain 536 exhibits hemolytic properties and mannose-resistant agglutination of human and bovine erythrocytes, as well as mannose-sensitive agglutination of yeast cells (2, 11). Detailed genetic analysis (see below) revealed the existence of two hemolysin (hly) determinants, gene clusters encoding the S-fimbrial adhesin (sfa), P-related fimbriae (prf), and common type I fimbriae (pil) (3, 11, 14). Furthermore, the ability to survive in non-immune serum could be observed (7). The strain produces the iron uptake substance enterochelone (Rabsch and Reissbroth, personal communication) but does not produce aerobactin. In addition, strain 536 produces cytotoxic effects in HeLa cells (Hessemann, unpublished) which might be due to the expression of the cytotoxic necrotizing factor (cnf, 4, 5). A schematic diagram of the virulence features of E. coli strain 536 is given in Fig.1.
Genetics of the UTI Isolate E. coli 536

Serum resistance

Fig. 1. Schematic representation of E. coli strain 536.

Isolation of Non-pathogenic Mutants

Non-pathogenic variants of the wild type strain were isolated by screening single colonies on blood agar plates for loss of the hemolytic property. Such mutants occurred at a frequency of approximately $10^{-3}$ (14). Besides mutants, generated in vitro, non-hemolytic variants could also be isolated following infection of the rat urinary tract (mutant 536-22; 6). Further phenotypic analysis revealed that the loss of the hemolytic phenotype is accompanied by a reduced mannose-resistant and mannose-sensitive agglutination activity, the loss of fimbria formation and of toxicity for HeLa cells. The mutants were also serum-sensitive (7, 11, 14, Blum et al., in preparation). The isolated mutants had been very helpful for evaluating the virulence properties of the wild type strain in vivo and in vitro models, and led to the identification of particularly unstable DNA regions, termed "pathogenicity islands" (14), which comprise physically linked virulence genes (6) and also influence the expression of pathogenicity determinants still present on the chromosome (see below).

In vivo and in vitro Virulence Assays

To test the influence of the pathogenicity factors mentioned above on the virulence of the strains, the wild type isolate 536 and the mutant 536-21 were analysed in various in vitro and in vivo assays (see Table 1). It can be seen that adhesion to uroepithelial cells and serum resistance were markedly reduced in the mutant strain as compared to the wild type isolate. Furthermore, the generation of leukotrienes and histamine produced by human granulocytes and rat mast cells following incubation with strain 536 dropped considerably when using mutants 536-21 (15, 29). In addition, the mutants had also lost their in vivo virulence demonstrated by the use of different animal assays. As shown in different studies (7, 8, 19), the introduction of cloned determinants coding for hemolysin and/or adhesins into mutant 536-21 increased the virulence capacity of the transformants. Thus, it could be demonstrated that the expression of cloned adhesins contributed to colonization of the strains while
hemolysin production revealed an effect on mouse lethality and inflammatory response. Following transformation with different plasmids encoding virulence factors and subsequent chromosomal integration of the plasmid DNA, strains were isolated which exhibited an in vivo virulence comparable to that of the wild-type strain (19).

**Cloning and Mapping of Virulence-associated Genes**

The *E. coli* strain 536 does not harbour plasmids, consequently genetic attempts to analyse its virulence properties started with the construction of a genomic library of this strain, and of one of the non-hemolytic mutant 536-21 generated in vitro (2, 14). The cosmid vector pHC 79 was used for random cloning of 35-40 kB (kilobases) stretches of chromosomal DNA in *E. coli* K-12 HB 101. Various recombinant cosmids were identified according to the phenotypes of hemolysis and agglutination in the mannose-sensitive and mannose-resistant manners (Table 2). The S-fimbrial adhesin and Type 1 fimbriae were cloned on cosmids pANN 801 and pGB 30, respectively.

### Table 1. In vivo and in vitro assays for determination of virulence properties of strain 536 (wild type) and mutant 536-21

<table>
<thead>
<tr>
<th>virulence assay</th>
<th>Wild-type 536</th>
<th>Mutant 536-21</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion (bacteria/cell)</td>
<td>6.2</td>
<td>1.9</td>
<td>19</td>
</tr>
<tr>
<td>Serum resistance (% survival/3 h)</td>
<td>250</td>
<td>0.1</td>
<td>7</td>
</tr>
<tr>
<td>Lethality (mouse)(LD&lt;sub&gt;100&lt;/sub&gt;)</td>
<td>7 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>1 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>7, 8</td>
</tr>
<tr>
<td>Inflammation (leukotriene C&lt;sub&gt;4&lt;/sub&gt;/pg)</td>
<td>25</td>
<td>0</td>
<td>15, 29</td>
</tr>
<tr>
<td>Nephrovirulence (rat) (renal counts/log)</td>
<td>4.8</td>
<td>2.7</td>
<td>19</td>
</tr>
<tr>
<td>Lethality (rat) (% lethality/48 h)</td>
<td>31</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table 2. Characterization of cosmids derived from a genomic library of *E. coli* strain 536

<table>
<thead>
<tr>
<th>Strain/cosmid</th>
<th>Sfa</th>
<th>Pil</th>
<th>Prf</th>
<th>Hly I</th>
<th>Hly II</th>
</tr>
</thead>
<tbody>
<tr>
<td>536 WT (wild-type)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HB101 (pANN 801)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HB101 (pGB 30)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HB101 (pANN 10-21)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HB101 (pCos 473)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HB101 (pCos 10)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HB101 (pHC 79)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Sfa, S-fimbrial adhesin; Pil, type 1 fimbriae; Prf, P-related fimbriae; Hly I, II, hemolysin I, II.*
Furthermore cosmids conferring hemolytic properties to the E. coli K-12 host strain were identified (pCos 10; pCos 473). Using a DNA probe specific for P fimbriae derived from the \(\text{pap}\) determinant of strain J96 (18), it could be shown that cosmids pANN 10–21 and pCos 473 carried sequences highly related to P fimbriae (6). Subcloning of this particular region confirmed the expression of fimbriae, which were identified electron-microscopically. Agglutination with human erythrocytes or Gal-Gal coated latex beads specific for P fimbriae, however, has not been observed (6, Vetter et al., in preparation). Thus, the corresponding determinant was termed \(prf\) (P-related fimbriae; 6).

The data elaborated by cosmid analysis revealed a physical linkage of the determinants coding for one hemolysin (hlyII) and P-related fimbriae (prf). The hlyI gene cluster was not linked to hlyII and prf. By analysing cosmids from a genomic library of the mutant strain 536-21, it has become evident, that there were large chromosomal deletions in the genome of this strain which comprises hlyI, hlyII and prf, which could be mapped by chromosome walking (14). Two distinct unstable DNA regions carrying the above mentioned determinants were identified. Preliminary data speak in favour of the location of the genes for cytotoxic necrotizing factor (cnf) in one of these regions.

The gene clusters for \(sfa\) and \(pil\) were still present on the mutant genome, but they were poorly expressed (Table 3). By constructing Hfr strains of the E. coli wild type isolate 536, the location of the unstable regions could be mapped according to the E. coli K-12 linkage map at 55 min (region I) and 97 min (region II). Additionally, the \(sfa\) and \(pil\) determinants were identified at positions 91 min and 98 min, respectively. The data have been summarized in Fig. 2.

Table 3. Genetic and phenotypic characterization of wild-type strain 536 and mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>(sfa)</th>
<th>(pil)</th>
<th>(prf)</th>
<th>(hlyI)</th>
<th>(hlyII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>536</td>
<td>uropathogenic</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>wild-type</td>
<td>expressed</td>
<td>expressed</td>
<td>expressed</td>
<td>expressed</td>
<td>expressed</td>
</tr>
<tr>
<td>536–21</td>
<td>laboratory</td>
<td>present</td>
<td>present</td>
<td>deleted</td>
<td>deleted</td>
<td>deleted</td>
</tr>
<tr>
<td></td>
<td>mutant</td>
<td>repressed</td>
<td>repressed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>536–22</td>
<td>generated</td>
<td>present</td>
<td>present</td>
<td>deleted</td>
<td>deleted</td>
<td>deleted</td>
</tr>
<tr>
<td></td>
<td>in vivo</td>
<td>repressed</td>
<td>repressed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Mutant Strains

To yield further insight into the physical structure of the “pathogenicity islands” of strain 536, the genomes of the wild type isolate and of mutant 536–21 were analysed by pulsed field gel electrophoresis. In Fig. 3 (A), it can be seen that the genomic \(XbaI\) profiles of the strains differ, as a result of the loss of the unstable DNA regions. Using DNA probes derived from the cloned virulence genes in Southern hybridizations (Fig. 3 B–F), it could be demonstrated that two fragments of 370 kb and 50 kb in size reacted.
with the \textit{hly} probe in the wild type strain, while the \textit{prf} probe hybridized only in the 370 kb fragment. No hybridization occurred in the mutant genome. Using an \textit{sfa}-specific probe, a 470 kb \textit{XbaI} fragment could be detected in both strains, whereas the wild type reacted with a \textit{pil} probe in a 320 kb fragment and the mutant, in a 500 kb fragment. To prove that the fragments of 370 kb and 320 kb were adjacent in the wild type, and that the loss of the \textit{hly} region II resulted in a 500 kb fragment, hybridization was carried out by using a DNA probe comprising the fusion point of this region which had been derived from the genomic library of mutant 536-21. It can be seen that the DNA probe termed HD1 (14) hybridized in the respective fragments. In this way, the size of the deleted region II could be calculated to be 190 kb (Fig. 4), while the size of the unstable region I had been determined before by Knapp et al. by chromosome walking, which was 70 kb (14).

The deletion events comprise the \textit{hly}, and \textit{prf} determinants, while the \textit{sfa} and \textit{pil} gene clusters are still present on the chromosome of the mutant. This was confirmed by using DNA probes spanning the entire coding regions of these determinants (11). Phenotypic analysis, however, revealed a reduced expression of type 1 fimbriae and S-fimbrial adhesins (7, 11, 14; cf. Table 3). To evaluate the differences in expression of \textit{sfa} more precisely, \textit{sfa–lacZ} translational fusions were generated in wild type and mutant variants which had a Tn5 insertion in the original \textit{lac} gene (27). Determination of LacZ expression in these strains, reflecting the degree of Sfa production, revealed a more than 10-fold decrease of the LacZ values for the mutant as compared to the wild type (Table 4).

These data strongly suggest a \textit{trans} regulatory effect of a gene product encoded by the unstable DNA regions on the expression of the \textit{sfa} gene. Analysis of mutants which had lost either of the two regions revealed that the pathogenicity island II was responsible for this effect (14). Since in this region, the \textit{prf} determinant is located (6), and \textit{sfa} and \textit{P} fimbriae determinants share a high degree of homology according to their \textit{trans} acting regulatory cistrons (25, 26), it might be concluded, that a positive cross-talk between \textit{prf} and \textit{sfa} exists.
Fig. 3. Genomic XbaI patterns of E. coli strain 536 wild type (lane 1) and mutant 536-21 (lane 2) obtained by pulsed field gel electrophoresis (A) and Southern hybridization using hly (B), pap (C), pil (D), and sfa (E) specific fragments and the cloned junction fragment HD 1 of hly region II (F, see Fig. 4) as DNA probes. Yeast chromosomes (Y), lambda concatemers (L), and HindIII cleaved lambda DNA (M) were used as DNA size markers.

Unstable DNA regions as observed for strain 536 seem to be a general phenomenon, as in O18:K1 strains and in the O4:K6 isolate J96, DNA regions similar to the pathogenicity island II have been detected which also carry physically linked virulence determinants (6).
Fig. 4. Map of the hlyII region in strains 536 and 536-21 obtained by XbaI cleavage. The locations of gene clusters are given for pil, prf, hlyII, and sfa. In the upper part, the E. coli K-12 linkage map is given.

Table 4. Determination of Sfa expression in E. coli 536 wild-type (WT) and mutant 536-21 via sfa-lacZ translational gene fusions

<table>
<thead>
<tr>
<th>Strain</th>
<th>LacZ units</th>
</tr>
</thead>
<tbody>
<tr>
<td>536 WT</td>
<td>5321 (^1)</td>
</tr>
<tr>
<td>536-21</td>
<td>5516 (^1)</td>
</tr>
<tr>
<td>536 WT (lacZ::Tn5)</td>
<td>14 (^1)</td>
</tr>
<tr>
<td>536-21 (lacZ::Tn5)</td>
<td>12 (^1)</td>
</tr>
<tr>
<td>536 WT (sfa-lacZ)</td>
<td>602</td>
</tr>
<tr>
<td>536-21 (sfa-lacZ)</td>
<td>52</td>
</tr>
</tbody>
</table>

\(^1\) Induced with IPTG.

Conclusion

Cloning of virulence genes from the chromosome of uropathogenic E. coli isolates enabled molecular studies of the properties determining pathogenicity (13). Furthermore, using cloned fragments as DNA probes in Southern hybridization, the determinants could be mapped in the genome, as well as the occurrence of virulence genes among various isolates could be analysed for epidemiological purposes (3, 22, 23). There is a dynamic situation in the chromosome of extraintestinal E. coli isolates, as some virulence factors are located on unstable DNA regions (6, 14) which also influence the expression of other virulence genes on the chromosome (11, 14). One might speculate about the biological advantages of such deletions. A resulting smaller genome favours a decrease of the generation time of the bacteria which can be useful at certain stages during the infectious process, and stable negative phenotypes due to the loss of the genes may be helpful for circumvention of the immune response. Furthermore, the origin of the virulence-associated chromosomal islands might be of particular interest. Recently, Zagaglia et al. (30) reported on the integration of virulence plasmids into the
chromosome of enteroinvasive E. coli and *Shigella flexneri*. Maybe these UTI E. coli "pathogenicity islands" originate from the integration of hemolysin plasmids frequently found among isolates from animal sources (13). A lot of questions arise with regard to the regulatory events in the expression of virulence factors *in vitro* and *in vivo*. It is known that virulence factors are not expressed constitutively (27). Rather, they are controlled by environmental parameters, leading to different levels of expression (24). Additionally, there might be a cross-talk between different gene clusters. Future studies with strains harbouring gene fusions of virulence determinants with reporter genes will be useful for evaluating coordinate virulence gene expression which is a crucial feature of uropathogenic *E. coli*.

To study these various aspects of uropathogenicity, the genetically well characterized *E. coli* strain 536 represents a useful model.

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References


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