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Short Communication

Chromosomal deletions and rearrangements cause coordinate loss of haemolysis, fimbriation and serum resistance in a uropathogenic strain of *Escherichia coli*

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Introduction

The coincidence of virulence factors such as haemolysin, adhesins and O and K antigens among strains of *Escherichia coli* causing urinary tract infections (UTI) is well-documented.[1–4] Indeed it seems that prominent uropathogenic strains belong to a restricted number of clonal types possessing specific constellations of these chromosomally-encoded characters[5] and that such associations frequently reflect a close genetic linkage of virulence genes.[6–8] By assessing the serum sensitivity of spontaneous non-haemolytic mutants arising from chromosomal deletions in a haemolytic serum resistant uropathogenic strain, we are able to suggest that the strong correlation[9] between haemolysis and high levels of serum resistance among urinary isolates may have a similar genetic basis.

Results and discussion

E. coli 536 O6:K15 is a plasmidless UTI isolate of high serum resistance. It carries two independent haemolysin (*hly*) determinants and in addition the determinants of both type 1 and S-type (*sfa*) fimbrial adhesins. Like other UTI strains, 536 gives rise to spontaneous Hly^- mutants at a rate of 10^{-3}–10^{-4} and these mutants may or may not show coordinate loss of the phenotypes associated with fimbriae production.[6] Such mutants are the result of chromosomal deletions and rearrangements which appear to be provoked by repeat sequences associated with the *hly*-region.[10]

A representative number of $O6^+K15^+Hly^-$ mutants (phenotypes of which are listed in Table 1) were taken for serum bactericidal assay. Mutants 536-21, 536-111 and 536-112 were independently isolated but in each case have large (75–100 kb) deletions spanning both *hly* determinants and also rearrangements which result in loss of Type 1 adhesin and Sfa production.[10] These Hly^-, Type 1 $adhesin^-$, Sfa^- mutants showed a drastic reduction in resistance to normal human serum; survival after 3 hours in undiluted serum was only 1–2%, compared to over 200% survival in the case of the wild-type 536 (Fig. 1). In contrast, the mutants 536-14 and 536-18 have

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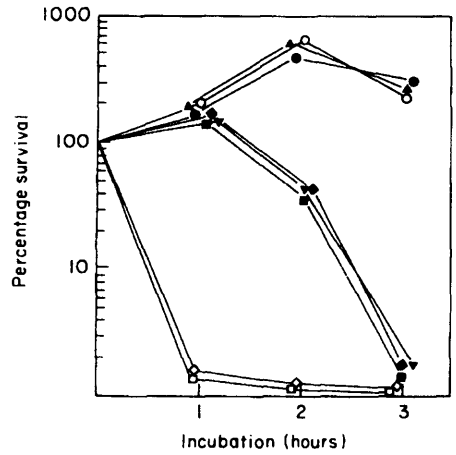

Fig. 1. Resistance to normal human serum of *E. coli* 536 and its mutants. ○, *E. coli* 536 wild-type; ▲, 536-14; ●, 536-18; ■, 536-21; □, 536-32; ◇, 536-33; ▼, 536-111; ◆, 536-112. Mutants 21, 111, 112 have deletions and rearrangements which together eliminate production of haemolysin and both S-type and type 1 fimbrial adhesins. Mutants 14 and 18 have shorter deletions eliminating only one of the two haemolysin determinants.[6,10] Spontaneous mutants 32 and 33 are derivatives of mutant 21 lacking in addition O-side chains.

only a shorter deletion removing one of the *hly* determinants (*hly* I) and these mutants retain the high resistance of the parent.

Despite the ca. 200-fold reductions in 3-hour survival, mutants 21, 111 and 112 were essentially unaltered in the ability to survive the initial hour of bacterial activity. Because this type of delay in killing is reported to be determined by the possession of LPS O-side chains,[3,11] spontaneous O6-negative mutants of the Hly⁻ 536-21 derivative were isolated. The O6⁻Hly⁻Sfa⁻ Type 1⁻ variants 536-32 and 536-33 had lost O6 antigenicity and had lost all resistance to undiluted serum; their sensitivity was equivalent to that shown by rough *E. coli* K-12 laboratory strains.[3]

Loss of haemolysin or adhesin determinants could possibly influence envelope topology directly as they both encode secreted products.[12,13] To test this possibility the mutant 536-21, Hly⁻, Type 1⁻, Sfa⁻ and no longer serum resistant, was transformed with *hly* determinants cloned as previously described[12,14,15] from the Hly-plasmid pHly152 or the chromosomes of 2 common UTI O-types O6 (strain 536) and O18 (strain 764). Serum bacterial assays with these transformants (Fig. 2) showed that

Table 1 Virulence factors of *E. coli* 536 and mutants

Strain	O6	K15	Hly I[a]	Hly II[a]	Sfa[b]	Type 1[c]
536 w.t.	+	+	+	+	+	+
536-14	+	+	−	+	+	+
536-18	+	+	−	+	+	+
536-111	+	+	−	−	−	−
536-112	+	+	−	−	−	−
536-21	+	+	−	−	−	−
536-32	−	+	−	−	−	−
536-33	−	+	−	−	−	−

Elimination of Hly and adhesin production was caused by spontaneous chromosomal deletion and rearrangement.[6,10]

[a] Strain 536 has 2 haemolysin determinants (*hly*I and *hly*II) on its chromosome.[10]

[b] S-fimbrial adhesin associated with mannose-resistant, neuraminidase-sensitive agglutination of bovine erythrocytes.

[c] Type 1 adhesin, mannose-sensitive agglutination of guinea pig erythrocytes and yeast cells.

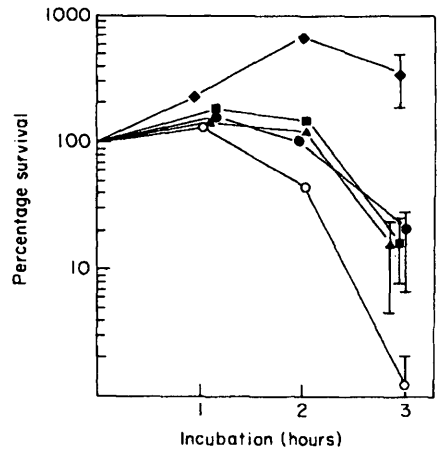

Fig. 2. Resistance to normal human serium of Hly mutant 536-21 transformed with recombinant plasmids and cosmids bearing *hly, sfa* and type 1 adhesin determinants. ◆, 536 wild-type; ○, 536-21 mutant, 536-21 (pGB30), 536-21 (pANN801) i.e. 3 strains; ▲, 536-21 (pANN202-312); ■, 536-21 (pANN5211); ●, 536-21 (pANN5311). pGB30 and pANN801 are cosmids carrying genes for type 1 and S-type adhesins, respectively; pANN202-312, pANN5211 and pANN5311 are pACYC184 plasmids bearing haemolysin genes from 3 separate isolates.

the synthesis and transport of haemolysin did not restore full serum resistance. Nevertheless, the presence of multicopy plasmids carrying the fully functional *hly* determinants did invoke reproducible lower level increases in 3-h survival (20% instead of the 1–2% of 536-21). Introduction of the same recombinant DNAs into the O6 mutant 536-32 had no effect on serum resistance; this seems reminiscent of the O-antigen dependent increase in resistance invoked by FII plasmids.[3]

Introduction into 536-21 of the 2 recombinant cosmids bearing separately the entire type 1 and S-type fimbrial adhesin determinants made no difference to serum sensitivity. This was also the case when double transformants carrying *hly* plasmids and either of the adhesin cosmids were tested; sensitivity was the same as seen when *hly* alone was present (data not shown).

These data suggest that the loss of parental high level serum resistance in the mutants is not due directly to the loss or inactivation of either *hly* or adhesin genes, although the former can in the presence of normal O-side chains contribute to lower level serum resistance. Rather, the effect of the large chromosomal deletions and rearrangements seems to reflect the presence in the parent of an additional serum resistance determinant in this chromosomal region.

Materials and methods

Bacterial strains. The characters of *E. coli* 536 and its derivatives are shown in Table 1. Haemolysis was confirmed with washed human erythrocytes.[1] S-fimbrial adhesin was detected by haemaggutination of washed human and bovine erythrocytes with and without 1% mannose and preincubation with neuraminidase (Merck AG, FRG), electron microscopy and agglutination with specific antiserum.[15] Type 1 fimbrial adhesin was identified by agglutination of yeast cells and guinea pig erythrocytes. The presence and titre of O6 and K15 antigens was controlled by microtitre-agglutination with specific antisera prepared against standard strains (obtained from F. Orskov, Copenhagen). Hly-negative mutants occurred spontaneously at a rate of 10^{-3}–10^{-4} and were isolated on blood agar plates.[6] The O6-negative mutants of 536-21 were spontaneous and commonly seen as rough colonies following subculture. The O6 mutants 536-32 and 536-33 were representative and had identical chromosomal alterations to those seen in 536-21.

Serum bactericidal assay.[3] Cells from an early logarithmic phase broth culture were washed and resuspended in 10 mM Tris-Cl (pH 7.5) to a density of ca. 5×10^6/ml. This suspension was

then diluted 1:10 in fresh normal human serum obtained on the same day from healthy volunteers and incubated with gentle shaking at 37°C. Viable counts were obtained at the beginning of incubation and after 1, 2 and 3 hours. Each strain was tested on 3 separate occasions.

Recombinant DNAs. Recombinant *hly*-plasmids were derived from the Hly-plasmid pHly152 (pANN202-312) and the chromosomes of the urinary tract isolates 536 O6:K15 (pANN5211) and 764 O18ac:K5 (pANN5311). Cloning was achieved via cosmid pJC74 into pACYC184 and the *sfa* and type 1 adhesin determinants from *E. coli* 536 were cloned into cosmid pHC79 to produce, respectively, pANN801 and pGB30.[12,14,15]

Transformation. E. coli 536 derivatives were transformed by a modified $CaCl_2$ procedure[16] and stable inheritance of recombinant DNA confirmed by the presence of phenotypic markers (antibiotic resistance, haemolysis, fimbriation and haem-agglutination) and plasmid DNA.[16]

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