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GENETICS AND PATHOGENIC ROLE OF ESCHERICHIA COLI HAEMOLYSIN

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ABSTRACT

While clear evidence exists for the direct involvement of cytolysins in the pathogenesis of Gram-positive bacteria, the significance of Gram-negative haemolysins remains unclear. This paper presents briefly data indicating a role for haemolysin production in infections caused by Escherichia coli and also experiments which have allowed an analysis of the molecular basis of the haemolysis among pathogenic and non-pathogenic strains of this species.

KEYWORDS

Toxicity; plasmids; gene-cloning.

INTRODUCTION

E. coli, though normally a friendly commensal member of the intestinal flora is sometimes responsible for intestinal and extraintestinal infections of man and animals. Strains causing diarrhoeal infections possess colonization factors (in humans CFAI or CFAII) and produce one or both of the heat-labile (LT) and h at-stable (ST) toxins. In contrast, those isolated from extraintestinal infections such as those of the urinary tract (UTI) seem to possess a constellation of factors none of which alone appear to define the pathogenicity of the strains (Sack, 1980; Evans et al., 1981). UTI E. coli generally belong to one of the small number of O-types; O1, O2, O4, O6, O9, O18 and O75 causing about one half of infections. Among these O-types strains frequently possess K-antigens or produce colicin V and there is a very high incidence of both fimbriation, associated most frequently with the mannose-resistant haemagglutination (MRHA) types V, VI or VII, and production of haemolysin.

RESULTS AND DISCUSSION

The incidence of haemolytic (Hly^+) strains is substantially higher among E. coli causing infections of the urinary tract and blood than among those isolated from the faecal flora of healthy humans (Table 1). The incidence of Hly^+ strains is particularly high (over 50%) among the four O-types O4, O6, O18 and O75 which account for a third of UTI cases and haemolysis may also , within these O-types, be associated closely with certain types of MRHA, e. g. type VI in O18, which facilitate adherence to urinary tract epithelial cells.

TABLE I Incidence of haemolysin production among E. coli from normal faecal flora and extraintestinal infections

Source	Percentage of hly+	Authors
Normal faecal flora	8.4	So et al., 1975
	5	Minshew et al., 1978
	7.3	DeBoy et al., 1980
	6	Green and Thomas, 1981
	9.9	this study 1)
	4.9	this study 2)
Urine	26	Vahlne, 1945
	35	Tschäpe and Rische, 1974
	56	Cooke and Ewins, 1974
	49	Minshew et al., 1978 3)
	35.8	Nimmich et al., 1980
	35	DeBoy et al., 1980
	38	Green and Thomas, 1981
	44	Hughes et al., 1981
	34.7	this study
Blood	35	Minshew et al., 1978
	50	DeBoy et al., 1980

1) Strains obtained from Institut für Hygiene und Mikrobiologie, Universität Würzburg

2) Strains obtained from a childrens hospital in Peru

3) only E. coli rough forms tested.

In addition, there is a strong correlation between haemolysin production and both high levels of resistance to complement action (serum resistance), which is influenced by both plasmid and chromosomal determinants (Taylor and Hughes, 1979; Taylor and Robinson, 1980), and toxicity for mice after intraperitoneal infection (Fig.1).

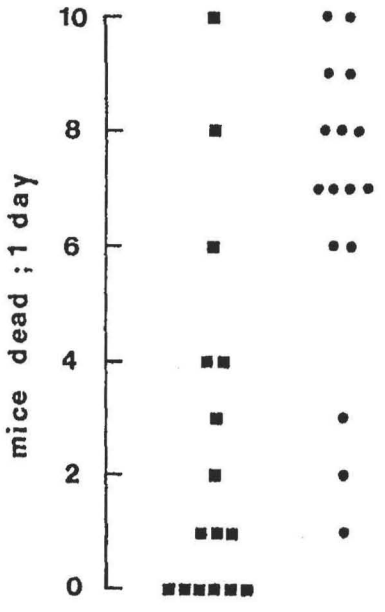


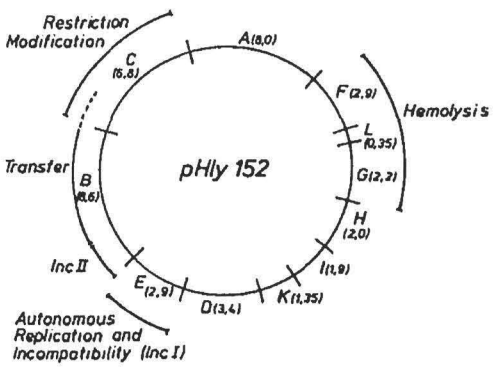
Fig. 1 Toxicity for mice of haemolytic (●) and non-haemolytic (■) E. coli isolated from urinary tract infections. $2x10^8$ organisms per mouse were injected by the intraperitoneal route. No multiplication of bacteria was evident from examination of spleens of mice which survived.

From examination of mutants and transferring hly genes into suitable strains it appears that the hly determinant itself probably does not influence directly serum sensitivity but does contribute to toxicity. The specific action of haemolysin in urinary infection remains to be elucidated as does its association with other factors in what appears to be the multicomponent basis of pathogenicity among extraintestinal strains.

The haemolytic strains originally examined were faecal isolates and as with many other pathogenicity factors the hly determinant was found to be carried on large ($40\text{-}90\times10^6$ Mr) selftransmissable plasmids (Smith and Halls, 1967; Goebel and Schrempf, 1971). One such plasmid, pHly 152 of the incompatibility group I_2 was taken for genetic analysis (Noegel et al., 1981; Goebel et al., 1981). The plasmid, of molecular weight 40×10^6, was first mapped using several restriction endonucleases and then mutagenized by insertional inactivation using the ampicillin resistance transposon Tn3. Two kinds of Hly$^-$ mutants were obtained: those producing no active haemolysin (type I) and those producing active intracellular haemolysin which was not secreted (type II). By observing the increased size and number of restriction fragments which had received the transposon, the mutational insertions were located on EcoRI F, L, G and HindIII E and C fragments (Fig. 2), covering a stretch of DNA of 3.5×10^6 (compared to the 1.2×10^6 coding for the cistrons A and B of heat-labile enterotoxin ; Dallas et al., 1979). Tn3 insertions leading to a complete loss of Hly-activity map within a region of about 3500 base pairs (bp) whereas insertions preventing secretion map immediately to the right in a region of about 1500 bp (Fig.3).

Fig. 2

Circular Map of pHly 152



The relevant fragments were cloned into the vectors pACYC 184 or RSF 2124 and the recombinant DNAs were introduced into Hly-negative mutants by transformations. It was found that recombinant plasmids carrying either EcoRI F or HindIII E were able to complement (i.e. return to activity) mutants of type I having Tn3 insertions in the first 500 bp of the 5000 bp region. Type II mutants could be complemented by plasmids carrying EcoRI G. The other Hly$^-$ mutants with Tn3 insertions in the middle 3000 bp part of the haemolysin determinant are complemented only by recombinant DNA carrying a Bam-Sal-fragment spanning a large of the whole determinant.

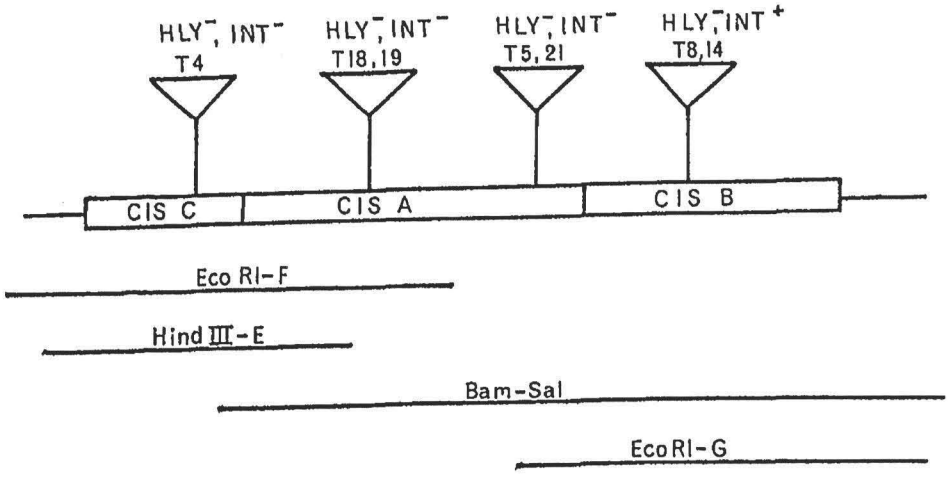


Fig. 3
Haemolysin determinant of pHly152

These and other data suggest that the haemolysin consists of 3 cistrons, *cisA* encoding a haemolysin precursor which seems to be activated by the product of *cisC*, the active haemolysin being then transported across the outer membrane by *cisB* product(s). An additional point of interest is that the cloning of the *cisA* region, or indeed the whole *hly* determinant, proved to be very difficult. This seems to reflect a lethal effect on the cell following higher expression of the gene product as has recently been shown by inserting the *Bam-Sal* fragment downstream of inducible transcription activity (Goebel *et al.*, 1981).

As haemolysis is not a very specific reaction and indeed as characterized Hly-plasmids had been shown to belong to unrelated *inc* types (DelaCruz *et al.*, 1979) it was necessary to investigate the similarity of *hly* determinants carried by different strains. This was done (together with F. DelaCruz presently of Bristol Univ., UK) by the Southern technique (Southern, 1975), i.e. hybridizing ^{32}P-labelled cRNA from the pHly152 restriction fragments mentioned earlier to *EcoRI* or *HindIII* restriction fragments generated from 4 Hly-plasmids (e.g. Fig.4) isolated from different sources and belonging to different *inc* groups.

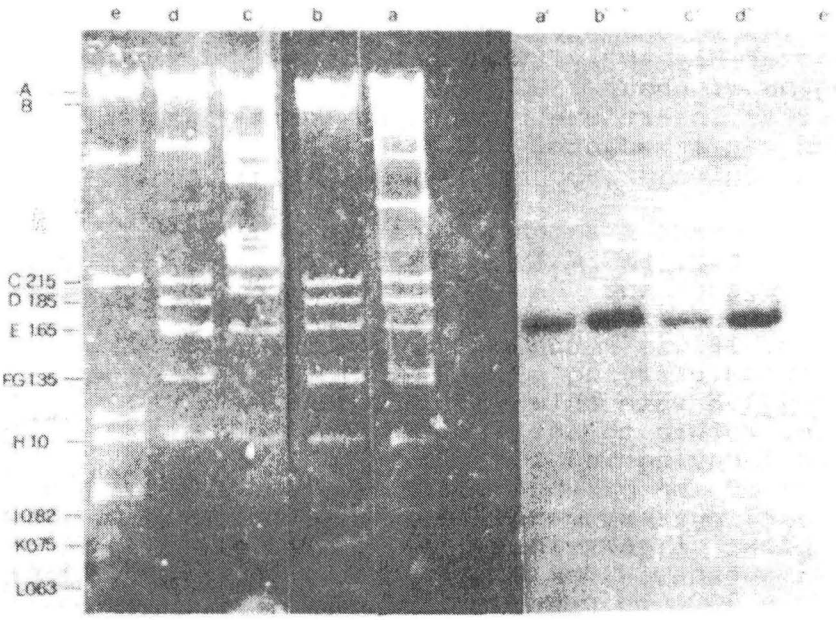


Fig. 4: Example of Southern hybridization performed with cloned *HindIII*-E (containing *cisC*) and *HindIII* cleaved DNA of 4 plasmids of different origin: pSU316 (a), pHly152 (control, b), pSU233 (c), pSU105 (d), pSU5 (e). All show homology in the same fragment, i.e. *HindIII*-E, except pSU316 which demonstrates homology in a smaller fragment (not vivible on this print).

The plasmids tested all hybridized wll with radioactive probes derived from *cisA*, *cisB* and *cisC* of pHly152, indicating that the plasmid-borne *hly* determinants are very similar. There appears to be a rather defined right end (i.e. of *cisB*) whereas the left end (the *cisC* end) varies to a significant extent (DelaCruz *et al.*, 1980), thus allowing the possibility that control regions of these determinants vary on different plasmids.

Surprisingly, examination of UTI haemolytic *E. coli* revealed that generally (i.e. in over 90% of cases) these strains do not carry Hly-plasmids but rather carry their *hly* determinants on the chromosome. A large number of such strains tested could not transfer their *hly* genes to *E. coli* K12 and in many cases the strains carry no plasmids at all. Several lines of evidence suggest nevertheless that the chromosomal genes are similar to those previously examined. Haemolysin proteins isolated from *E. coli* carrying plasmid and chromosomal *hly* genes (M. Härtlein and C. Hughes, unpublished results) have very similar biochemical properties. Mutants of type I and Type II can be isolated from chromosomal strains and chromosomal *hly* determinants can complement mutated cistrons of pHly152. To confirm these indications, hybridization studies have been carried out between radioactive RNA or DNA derived from pHly152 and DNA from strains with chromosomal *hly* determinants.

The results confirm that chromosomally located hly determinants show extensive homology with their plasmid-borne counterparts. The mechanism by which hly determinants have spread thoughout chromosomal and extrachromosomal replicons of pathogenic and non-pathogenic E. coli is as yet unclear.

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