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Enterohaemorrhagic *Escherichia coli* O157 and non-O157 serovars differ in their mechanisms for iron supply

Andreas U. Kresse[a], Ilse Rienäcker[a], Ana Maria Valle[b,1], Hartmut Steinrück[c], Hermann Claus[a], Shelley M. Payne[b], Helmut Tschäpe[a], Peter H. Williams[d,*], Rolf Reissbrodt[a]

[a]*Robert Koch Institute, Wernigerode Branch, Burgstraße 37, D-38855 Wernigerode, Germany*
[b]*Section of Molecular Genetics & Microbiology, University of Texas at Austin, Austin, TX 78712, USA*
[c]*Federal Institute for Risk Assessment, Diedersdorfer Weg 1, D-12277 Berlin, Germany*
[d]*Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK*

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Abstract

Clinical isolates of enterohaemorrhagic *Escherichia coli*, both O157 and non-O157 serotypes, were investigated for siderophore production, for growth promotion by haem and esculetin in iron-restricted conditions, for production of enterohaemolysin and esculin hydrolase, and for the presence of the *chuA* and *ehx* genes by PCR. As expected, all the strains produced enterobactin, but the prevalence of other factors varied among the serovars tested. None of the O157 and O26 strains produced aerobactin or "colibactin", whereas among other enterohaemorrhagic *E. coli* non-O157 serovars the frequencies of aerobactin and "colibactin" production were similar to those of commensal *E. coli* strains. The ability to use ferric esculetin for growth in iron-limited media was markedly more prevalent among non-O157 serovars and less prevalent among O157 strains compared with commensal *E. coli* strains. Almost all O157, O26 and O103 strains expressed enterohaemolysin, compared with only 50% of other non-O157 strains. Similarly, almost all O157 and O26 strains utilised haem as a host iron source; the frequency of haem use by other non-O157 strains was generally lower and variable among serovars, such that none of the O103:H2 isolates tested used haem as an iron source. The gene *chuA*, which encodes the haem transport protein ChuA and which is prevalent in O157:H7 strains, was only rarely noted among non-O157 serovars of enterohaemorrhagic *E. coli*, even among isolates that could use haem as an iron source. Overall our data demonstrate that O157:H7 and non-O157 serovars, in particular O26:H$^-$/H11 and O103:H2, use distinctly different strategies for obtaining iron, and suggest two evolutionary distinct lines of enterhaemorrhagic *E. coli*.

Keywords: Enterohaemorrhagic *E. coli*; Iron supply; Siderophores; Haem; Esculetin; Enterohaemolysin

Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) are important food-borne pathogens that represent a significant public health issue in industrialised countries.

*Corresponding author. Tel.: +44 116 252 3317; fax: +44 116 252 3378.
E-mail address: phw2@le.ac.uk (P.H. Williams).
[1]Present address: Texas Department of State Health Services, Austin, TX 78756, USA.

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EHEC infections in humans are characterised by diarrhoea with a range of manifestations from watery to bloody, but the most feared sequelae are haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. The key virulence marker of EHEC, and the main cause of haemolytic uraemic syndrome, is the production of Shiga toxins (Stx). Other virulence factors include a plasmid-encoded enterohaemolysin (Ehx) and the adhesin intimin (Eae) which is encoded by the chromosomal pathogenicity island known as the locus of enterocyte effacement (Nataro and Kaper, 1998; Reissbrodt, 1998).

One of the main considerations in bacterial infections in multicellular hosts is the availability of iron for microbial growth. To overcome iron stress, EHEC strains appear to use several strategies to obtain iron from host sources. The principal siderophore of *E. coli* is enterobactin (synonymous with enterochelin); essentially all *E. coli* strains, whether or not they are pathogenic, produce this siderophore in conditions of iron stress. Among extraintestinal *E. coli* strains that cause septicaemia and urinary tract infections, the citrate-hydroxamate siderophore aerobactin appears to be an important contributor to pathogenesis (Carbonetti et al., 1986; Selander et al., 1986; Warner et al., 1981; Williams, 1979). Moreover, some uropathogenic *E. coli* secrete catecholate siderophores of the salmochelin group (Hantke et al., 2003), and a number of *E. coli* isolates (including 30–40% of commensal strains, our unpublished results) produce an additional siderophore that is detectable in cross-feeding tests with the indicator strain *Microbacterium flavescens* JG-9 (Payne, 1994). Investigations of the chemical structure of this novel siderophore, provisionally designated ''colibactin'', are in progress. Furthermore, approximately 35% of *E. coli* isolates, as well as other bacteria such as *Listeria monocytogenes*, enterococci and *Yersinia* spp., produce an enzyme that can hydrolyse the plant glycoside esculin; the hydrolysis product, esculetin (6,7-dihydroxycoumarin), reacts with ferric iron to form a black compound which apparently functions as a siderophore (Coulanges et al., 1996; Farmer and Kelly, 1991). In addition to iron uptake via siderophores, some intestinal pathovars of *E. coli*, like many other enteric pathogens, utilise host iron-binding compounds such as haem and haemoglobin as iron sources (Mills and Payne, 1995; Otto et al., 1992; Stoebner and Payne, 1988; Stojiljkovic and Hantke, 1992; Wyckoff et al., 1998). The haem iron transport system of EHEC O157:H7, which is TonB dependent, comprises a 69-kDa transport protein encoded by *chuA* that is expressed in response to iron limitation (Torres and Payne, 1997).

In this study, we have investigated the distribution of these various iron-supplying systems among the most frequently isolated EHEC serovars in Germany. Our data demonstrate that O157:H7 and non-O157 serovars, in particular O26:H$^-$/H11 and O103:H2, use distinctly different strategies for obtaining iron.

Material and methods

Collection and characterisation of bacterial strains

EHEC isolates of different serovars, 155 of O157:H7/H$^-$ (all sorbitol non-fermenters), 31 of O26:H$^-$/H11, 28 of O103:H2 and 82 of other non-O157 types (see footnote of Table 1), were isolated from individual cases or outbreaks in Germany between 1996 and 2000 and deposited in the culture collection of the Robert Koch Institute, Wernigerode. Determination of virulence markers (Shiga toxin by ELISA and PCR; enterohaemolysin phenotypically and by PCR; intimin by PCR), as well as biochemical and serological characterisation of strains were performed as previously described (Prager et al., 1998).

Siderophore cross-feeding tests

The EHEC serovars were examined in siderophore cross-feeding tests as previously described (Reissbrodt and Rabsch, 1988) using the following siderophore indicator strains: *Salmonella enterica* serovar Typhimurium enb-7 for detection of enterobactin, *E. coli* LG1522 for detection of aerobactin, and *M. flavescens* JG-9 for detection of ''colibactin''.

Growth promotion assays

Routine screening of the ability of EHEC strains to utilise haem as an iron source was performed on Luria agar containing 1 mg/ml ethylenediamine di-ortho-hydroxyphenylacetic acid (EDDHA). Filter paper discs (5 mm diameter) were loaded with 10 μg of haem and placed onto the agar surface surrounded by spot-inoculated iron-starved EHEC isolates at a distance of approximately 1 cm from the discs. Plates were incubated at 37 °C for 24 h. Growth of culture spots close to the haem discs was regarded as evidence of growth promotion by haem. Additionally, growth promotion was also checked on Luria agar containing EDDHA on which the bacteria had previously been seeded and on which filter paper discs loaded with various concentrations of haem and esculetin were placed. Growth of the bacteria around the discs was regarded as evidence of growth promotion by these compounds. Growth promotion by haem and esculetin was also checked in a Bioscreen C apparatus (Thermolabsystems, Helsinki, Finland) by inoculating EHEC strains into nutrient broth (SIFIN, Berlin, Germany) containing bovine