INTERACTION OF TWO STRAINS OF *ESCHERICHIA COLI* O157 WITH HUMAN EPITHELIAL CELLS

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ABSTRACT:

Escherichia coli strains with the O157 LPS serotype are often related with foodborne infections. These bacteria are extracellular pathogens which have developed a specific mode to manipulate host cell cytoskeleton using a Type III secretion system by which they modify host microvilli and form specific attacling-and-efacing lesions. *E. coli* O157 strains of different origin and H serotype have been previously shown to vary in their interaction patterns with cultured cells. Adherent bacteria form compact structures known as localized adherence (LA) loci, or are more diffuse in a localized adherence-like (LAL) pattern. Here we compare the adhesion patterns characteristic for one *E. coli* O157:H- and one *E. coli* O157:H7 strain.

Key words: *E. coli O157:H-, E. coli O157:H7, bacteria-host cell interaction*

Introduction

Escherichia coli strains with the O157 LPS serotype are often the cause of foodborne infections and are related with gastrointestinal or systemic health disorders with variable acuteness [Kaper J. et al., 2004]. While the heaviest cases are due to the release of Shiga-like toxins, strains incapable of Shiga toxin synthesis may interact with host intestinal epithelia and cause heavy diarrhea. These bacteria are extracellular pathogens which use their Type III secretion system to manipulate host cell cytoskeleton and form specific attaching-and-effacing (A/E) lesions [Kaper J. et al., 2004; Vallance B., B. Finlay. 2000] which represent areas of altered epithelial microvilli called pedestals over which the bacteria attach. Furthermore, pedestals together with the adherent bacteria may occur in groups which may be compact and are known as localized adhesion loci (LA) or more diffuse in a localized adherence-like (LAL) pattern [Mora A. Et al., 2009].

E. coli O157 strains of different origin and H serotype may vary in their interaction patterns with cultured cells. Adherent bacteria may group in compact structures known as localized adherence (LA) loci, or be more diffuse in a localized adherence-like (LAL) pattern.

Here we compare the adhesion patterns of two Shiga toxin-negative strains, *E. coli* O157:H- and *E. coli* O157:H7.

Materials and Methods:

Bacterial strains and cultivation

E. coli O157:H-, A2CKSS, NCIPD, a Stx-negative strain, and *E. coli* O157:H7CCUG 44875, a strain with blocked capacity to form Stx [Schmidt et al. 1999] were used in this study. The strains were cultivated overnight at 37^{o}C in TSB. Bacteria were peletted, resuspended in 0.9% NaCl and calibrated to 10^9 CFU by MacFarland standard. They were then added to antibiotic-free DMEM medium at concentrations of 10^6 and 10^4.

Epithelial cells cultivation and innoculation

HeLa cells were grown to 80-90% confluence on microscopic cover glasses in DMEM medium supplemented with 10% FCA and antibiotics. The medium was withdrawn, the cells were washed in three changes of PBS to remove antibiotics, and then the DMEM-suspended bacteria were added. The co-cultivation was performed at 37^{o}C, 5% CO_2 for s up to 6 hours.

Morphological evaluation of the bacterial adhesion patterns

The glasses were washed, fixed with phosphate buffered formalin, stained with Giemsa stain and mounted with Canada balsam. 20 random pictures were taken from each slide and the number of bacteria in 10 adhesion loci per picture was counted. The results were processed by MS Excel.

Results and Discussion

Within the *E. coli* O157 group of strains, most extensively studied are several Shiga toxin producing strains of the *E. coli* O157:H7 serotype. In natural isolates however there is a variety of Shiga toxin presence/absence as well as of H serotypes. Among them *E. coli* O157:H- is considered as an emerging pathogen in Europe [Jenke et al. 2010; Nielsen et al. 2011].

Examination of the Giemsa-stained samples revealed distinct adhesion patterns characteristic for each of the two strains. Notably, in both cases not all HeLa cells were associated with bacteria and there is a kind of selectivity with only some of the eukaryotes interacting with the pathogens. *E. coli* O157:H- adhered to the surface of the HeLa in loose groups visualized as distinct dark dots (Fig. 1A, B).

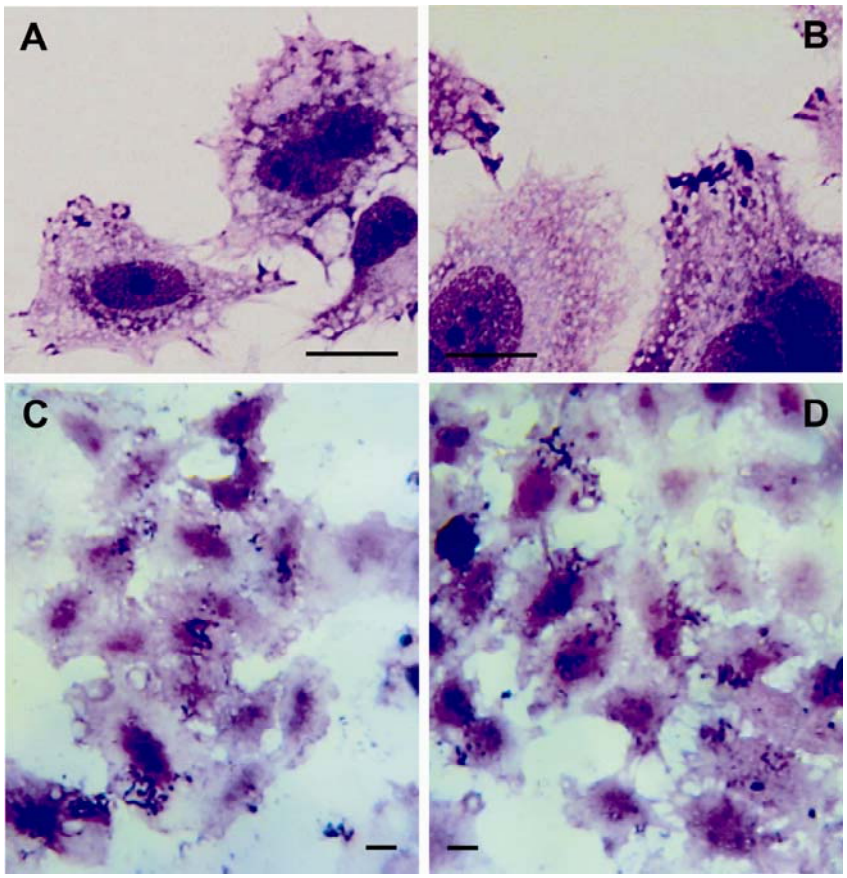

Fig. 1. Localised-like adhesion of E. coli O157:H- A2CK (A, B); Localised adhesion of E. coli O157:H7 CCUG 44875 (C, D). Bar = 5 μm.

Single adherent bacteria could rarely be observed hence this strain can be characterized as having a LAL adhesion pattern. *E. coli* O157:H7 on the other hand formed typical LA loci. They contained numerous bacterial cells (Fig. 1C, D).

Since the two strains apparently differed by the amounts of bacteria involved in a given adhesion locus, we further quantitated the number of pathogen cells per adhesion locus. Fig. 2 illustrates the distinction between the two strains with this regard. While E. *coli* O157:H- adhered

most often in groups of 3 to 10 bacterial cells, *E. coli* O157:H7 attached predominantly in groups of 10 to 30.

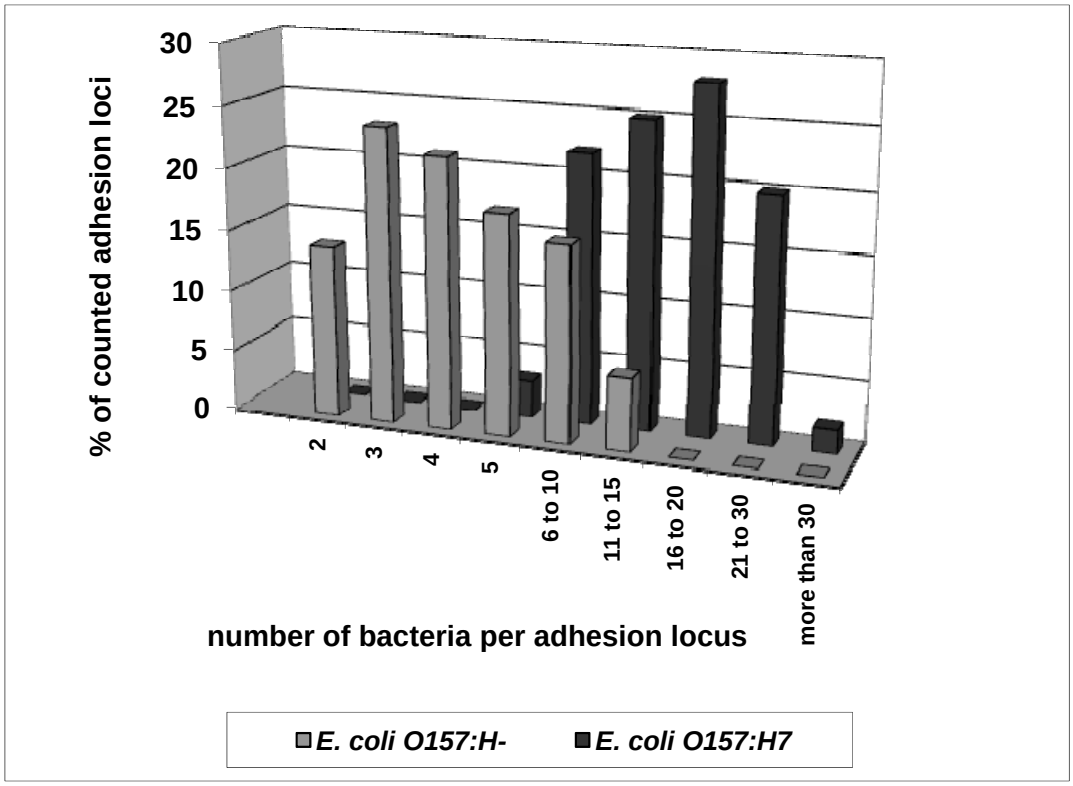

Fig. 2. Distribution of localized adhesion patterns within the two strains.

It is known from literature that the formation of this type of adhesion loci is due to bacterial manipulation of the host cytoskeleton [Kaper et al. 2004]. Hence the present results imply also different degree of alteration of the host cell cytoskeleton by the two strains. Interestingly, using fluorescent phalloidin we were unable to detect actin rearrangements in HeLa cells co-cultivated with *E. coli* O157:H- (unpublished data). This was also the observation on a number of other clinical isolates [Mora et al. 2009]. On the other hand, this strain appears capable to modulate the tubulin cytoskeleton and to change the localization of dynein and VDP, two molecules involved with the intracellular transport of vesicles [Topouzova-Hristova et al., 2011].

These observations imply that the interactions of *E. coli* O157 strains with eukaryotic host cells may involve more and more variable mechanisms than the hitherto well-explored Type III secretion-based host cell actin rearrangements.

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