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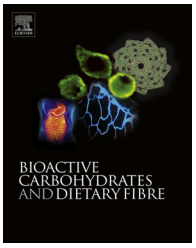

Inhibition of the adhesion of *Escherichia coli* to human epithelial cells by carbohydrates

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ABSTRACT

Mannose-containing carbohydrates provide an alternative therapeutic approach for controlling bacterial infections. The aim of this work was to compare the effects of depolymerised konjac glucomannan and other carbohydrates (including simple sugars) on the inhibition of the adhesion of *Escherichia coli* to human cheek epithelial cells. Sucrose, glucose, commercial galacto-oligosaccharides (GOS), maltodextrin, inulin, glucomannan hydrolysates (GMH) and mannose were assessed for this purpose. The average cell counts of *E. coli* adhering to the epithelial cells were found to be significantly higher ($P<0.01$) in sucrose, glucose, commercial galacto-oligosaccharides (GOS), maltodextrin and inulin compared to mannose or GMH. The use of mannose-rich carbohydrates as an approach to reduce bacterial infections could prove a successful approach although large scale studies in humans are required to characterise further the specific role of such carbohydrates with different pathogens.

1. Introduction

Many Gram negative bacteria, particularly species of the family Enterobacteriaceae, have been shown to possess pili or fimbrial structures (Antão, Wieler, & Ewers, 2009; Bidhendi et al., 2007; Duguid & Old 1980). The pili are composite structures comprising fimbrial protein subunits where some major subunits form the bulk of the pili structures. These organelles facilitate epithelial colonisation by cell adherence in both human and animal hosts; including by *Escherichia coli* (Antão et al., 2009; Clegg & Geriach, 1987). Bacterial adherence to epithelial cells has been reported to be a significant step toward creating infections (Ofek, Mirelman, & Sharon, 1977; Wolska, Zabielska, & Jakubczak, 2006). In general, the fimbriae of *E. coli* mediate the attachment of such microorganisms to human urinary tract epithelial cells (Bidhendi et al., 2007; Eden & Hansson, 1978; Wullt, 2003) and agglutinate guinea pig erythrocytes (Eden & Hansson, 1978; Firon, Ashkenazi, Mirelman, Ofek, & Sharon, 1987) or monkey kidney cells (Salit & Gotschlich, 1977).

Antimicrobial strategies involving anti-adhesive components from natural products are valuable alternatives to traditional antibiotic therapy (Signoretta, Canepari, Stauder, Vezzulli, & Pruzzo, 2012). Examples of 'natural' compounds with activity against bacterial adhesion are cranberry juice (Gupta et al., 2007) and oligosaccharides extracted from human milk (Hickey, 2012). The key therapeutic factors within such products are the carbohydrates. These (sugars and oligosaccharides) are recognised by the bacteria fimbrial lectins and block the adhesion of the bacteria to epithelial cells (Sharon, 2006).

The objective of this work was to compare the effects of depolymerised konjac glucomannan with other carbohydrates including simple sugars on the inhibition of adhesion of *E. coli* cells to human epithelial cells.

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2. Materials and methods

2.1. Materials

The carbohydrates used in this study included maltodextrin (STAR-DRY 5, Tate & Lyle), commercial galacto-oligosaccharides (GOS, Local Chemist), glucose (G/0500/53, Fischer Scientific, UK), inulin (13754, Sigma) mannose (M4625, Sigma) and sucrose (18219, Fluka). In addition, (konjac) glucomannan hydrolysate (GMH, Glycologic Limited, Glasgow) was also used. The GMH was prepared by enzymatic hydrolysis of konjac flour using cellulase (Al-Ghazzewi, Khanna, Tester, & Piggott, 2007). Nutrient broth/agar (CM0001, CM003), tryptone soya broth (TSB, CM0129) and phosphate buffered saline tablets (PBS, BR0014G, pH 7.2±0.2) were obtained from Oxoid Ltd., Basingstoke, UK.

2.2. Bacteria and growth conditions

A strain of *E. coli* NCTC 8623 (HPA culture collections, Salisbury, UK) was grown in nutrient broth/agar supplemented with 0.1% glucose (w/v) for 18 h at 37 °C, then subcultured several times in TSB under the same conditions to enhance the production of fimbriae. The bacterial cells were pelleted by centrifugation (1500*g*, 10 min), washed three times in PBS (pH 7.2±0.2) then re-suspended to a cell concentration of about 10^8 CFU ml^{-1}.

2.3. Isolation of buccal and pharynx epithelial cells

Buccal and pharynx epithelial cells were collected from the human oral cavity using a sterile spatula. Cells were removed from the spatula by agitation in PBS (pH 7.2±0.2). The epithelial cells were washed three times with centrifugation (100*g*, 10 min) in PBS to remove the unattached bacteria. The cells were harvested at about 10^5 cells ml^{-1}. A pipette was then used to suck the cell suspension in and out several times in order to separate the aggregated cells and produce a uniform cell suspension.

2.4. Carbohydrates preparation

The carbohydrates (40 mg ml^{-1}) were dissolved separately in sterile PBS. The *E. coli* suspension (0.5 ml) was pre-incubated statically in Bijou bottles with the chosen carbohydrate (0.5 ml) for 1 h at 37 °C before use in the adhesion investigation.

2.5. Adhesion protocol

The adherence of the *E. coli* to the epithelial cells was assessed by mixing the washed cell suspension (0.5 ml) and washed bacterial suspension (0.5 ml) with the desired carbohydrate (0.5 ml). All mixtures were incubated at 37 °C for 2 h in a shaking water bath. Following incubation, the non-adherent bacteria were separated from the epithelial cells by washing the bacterial – epithelial cells – carbohydrate mixtures in PBS three times with differential centrifugation. The harvested pellets were then re-suspended in 0.05 ml PBS and placed on slides, let to dry, and then stained with Gentian violet for 10 min. Any excess stain was washed off and the slides well dried prior to examining using light-field microscopy (×100). The numbers of bacteria adhering to 30 well-defined epithelial cells were counted in each experiment. Three experiments were conducted to obtain the mean number of bacteria adhering to the cells. The data were analysed for statistical variance. Differences were considered as statistically significant at $P<0.05$.

2.6. Statistical analysis

Data were evaluated statistically by ANOVA test using the Statistical Analysis Tool of Microsoft Excel 2010, Microsoft Office Professional Edition 2010. The results were considered significant when *P*-values were $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

3. Results and discussion

The average number of *E. coli* cells adhering to the epithelial cells are shown in Fig. 1. These are also shown pictorially in Fig. 2. The order of inhibition of adherence (in descending order) of carbohydrates was sucrose, glucose, GOS, maltodextrin, inulin, GMH then mannose. One-way ANOVA showed the mannose-containing carbohydrates were significantly different ($P<0.01$) from the other carbohydrates towards inhibiting the adherence of bacteria. However, no difference was found between GMH and mannose (Table 1). The GOS showed a significant difference ($P<0.05$) with sucrose but no difference with glucose, maltodextrin or inulin.

Inhibition of bacterial adhesion using mannose-rich carbohydrates could lead to a novel therapy approach against bacterial infections (Hartmann et al., 2012; Sharon, 2006). For large intestine applications, mannose would not be appropriate as it is absorbed in the small intestine – unlike the GMH. A number of studies have discussed the role of some oligosaccharides with respect to the prevention of cellular adhesion by pathogens such as *Neisseria meningitides* (Hakkarainen et al., 2005), *L. monocytogens* (Ebersbach, Anderson, Bergstrom, Hutkins, & Licht, 2012), *Streptococcus zooepidemicus*, *Pseudomonas aeruginosa* (King, Young, Nequin, & Carnevale, 2000; Lecuyer, et al., 2011), *E. coli* and *Salmonella* spp. (Antão et al., 2009; Becker & Galletti, 2008; Pereira

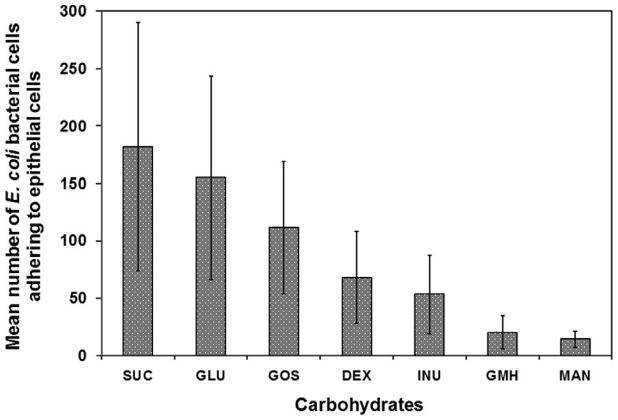

Fig. 1 – Number of *E. coli* (Mean±SD) adhering to epithelial cells in the presence of sucrose (SUC), glucose (GLU), galacto-oligosaccharides (GOS), maltodextrin (DEX), inulin (INU), glucomannan hydrolysates (GMH) and mannose (MAN).