Antigenotoxicity of probiotics and prebiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells

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Received 15 October 2003; received in revised form 12 March 2004; accepted 16 March 2004
Available online 4 June 2004

Abstract
Six strains of lactic acid producing bacteria (LAB) were incubated (1 × 10^8 cfu/ml) with genotoxic faecal water from a human subject. HT29 human adenocarcinoma cells were then challenged with the resultant samples and DNA damage measured using the single cell gel electrophoresis (comet) assay. The LAB strains investigated were Bifidobacterium sp. 420, Bifidobacterium Bb12, Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus bulgaricus and Enterococcus faecium. DNA damage was significantly decreased by all bacteria used with the exception of Strep. thermophilus. Bif. Bb12 and Lact. plantarum showed the greatest protective effect against DNA damage. Incubation of faecal water with different concentrations of Bif. Bb12 and Lact. plantarum revealed that the decrease in genotoxicity was related to cell density. Non-viable (heat treated) probiotic cells had no effect on faecal water genotoxicity.

In a second study, HT29 cells were cultured in the presence of supernatants of incubations of probiotics with various carbohydrates including known prebiotics; the HT29 cells were then exposed to faecal water. Overall, incubations involving Lact. plantarum with the fructooligosaccharide (FOS)-based prebiotics Inulin, Raftiline, Raftilose and Actilight were the most effective in increasing the cellular resistance to faecal water genotoxicity, whereas fermentations with Elixor (a galactooligosaccharide) and Fibersol (a maltodextrin) were less effective. Substantial reductions in faecal water-induced DNA damage were also seen with supernatants from incubation of prebiotics with Bif. Bb12.

The supernatant of fermentations involving Ent. faecium and Bif. sp. 420 generally had less potent effects on genotoxicity although some reductions with Raftiline and Elixor fermentations were apparent.

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Keywords: Probiotics; Prebiotics; Oligosaccharides bifidobacteria; Lactobacilli; Faecal water; DNA damage; Antigenotoxicity

1. Introduction
There is considerable evidence that the colonic microflora is involved in the aetiology of colorectal cancer. For example, gut bacteria can produce, from dietary components, substances with genotoxic, carcinogenic and tumour promoting activity [1]. During recent years there has been considerable interest in dietary agents that can modify the gut microflora and hence potentially modulate colon cancer risk. In this regard, probiotics and prebiotics have received particular attention. Salminen et al. (1998) define a probiotic as ‘a live microbial food ingredient that is...
beneficial to health'. They usually comprise lactic acid producing bacteria (LAB) of the genera Lactobacillus and Bifidobacterium, which when consumed increased the proportion of LAB in faeces. In general species of Bifidobacterium and Lactobacillus have relatively low activities of the enzymes involved in carcinogen formation and metabolism by comparison to other major anaerobes in the gut such as bacteroides, eubacteria and clostridia [2]. In contrast to probiotics, prebiotics provide a means of indirectly stimulating numbers of LAB in the colon. A prebiotic has been defined as a 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that have the potential to improve host health' [3].

A number of poorly digested carbohydrates fall into the category of prebiotics including certain fibres and resistant starches [4], but the most widely described prebiotics are non-digestible oligosaccharides (NDOs). These are low molecular weight carbohydrates with 2–10 degrees of polymerization, which are poorly digested in the human intestine thus reaching the colon largely unaltered and which can act as a substrate for the colonic microflora. They appear to stimulate specifically numbers of bifidobacteria and lactobacilli, often at the expense of other microflora components such as bacteroides, clostridia and E. coli [4,5].

The anticarcinogenicity of probiotics and prebiotics has been extensively reviewed by Burns and Rowland [6]. In general probiotic and prebiotic supplementa-
tion has been shown to have protective effects against a broad range of events such as induction of DNA damage in the colonic mucosa of rats [7], formation of aberrant crypt foci in rats [8], tumour incidence in animal models [9–12] and alteration of bacterial enzyme activities thereby reducing the levels of en-
zymes involved in carcinogen formation [13,14]. It is noteworthy that in some of these studies, combina-
tions of probiotics and prebiotics resulted in additive or synergistic effects on colonic lesions [8]. The term symbiotic has been proposed for such combinations. A symbiotic has been defined as 'a mixture of probi-
otics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or ac-
tivating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare' [3].

Identifying pro-, pre-, and symbiotics with potential anticancer properties using animal models is time consuming and expensive, with the number and variety of these agents that are available. In vitro methods can provide a more practical alternative and, given the cru-
cial importance of DNA damage in the initiation and progression of colon cancer [15], reduction in geno-
toxicity is considered a highly relevant endpoint.

There are a number of reports describing the adsorp-
tion or binding in vitro by LAB and other intestinal bacteria, of a variety of food-borne carcinogens includ-
ing the heterocyclic amines formed during cooking of meat, the fungal toxin Aflatoxin B1, benz(a)pyrene and the food contaminant AF2 [16–20]. In several of these studies, a concomitant decrease in mutagenicity was reported. The extent of the binding was dependent on the mutagen and bacterial strain used—in general greatest binding was seen with the heterocyclic amines and least with Aflatoxin B1 and AF2. The adsorption appeared to be a physical phenomenon, mostly due to a cation exchange mechanism. In these studies specific food-associated mutagens have been used, although it is not certain whether these compounds play a role in the aetiology of colorectal cancer since the identity of carcinogens in the colon has not been established. A viable alternative to using model chemical carcino-
gens is faecal water.

It is likely that the agents involved in the aeti-
ology of colon cancer are associated with the aqueous phase of the faecal stream in the gut. This fraction (faecal water) prepared by high speed centrifugation of human faecal samples, has been shown to contain biologically active substances that are cytotoxic to mammalian cells. We have shown, using the single cell gel electrophoresis (comet) assay, approximately 30% of all faecal water samples from human volun-
teers to be highly genotoxic towards a human colon cell line [21]. Since mutations and other genetic changes are critical factors in the initiation and de-
velopment of colorectal cancer [15], this observation is of potentially great significance for the aetiology of the disease. The use of faecal water in conjunction with the comet assay and human colon cell lines thus provides a useful and highly relevant in vitro model to investigate dietary components for potential anti-
A healthy male, non-smoking individual with no history of gastrointestinal disease, and identified in preliminary studies as having genotoxic activity in faeces, provided a faecal sample for the study. Faecal water was prepared according to Venturi et al. [21]. Briefly, the complete faecal sample was homogenized in a stomacher for 2 min and centrifuged at 50,000 \( \times g \) for 2 h at 10\( ^\circ \)C. The supernatant was distributed to 1.5 ml Eppendorf tubes and stored at −20\( ^\circ \)C prior to analysis.

2.2. Bacterial cultures

Lactobacillus plantarum was obtained from Rhodia Food, Rue de Clemencierie, BP 32, F-38360 Sassenage, France. Bifidobacterium Bb12 supplied by Chr. Hansens, Laboratoryum, Denmark A/S originated from the German culture collection DSM 20215 designated as Bifidobacterium bifidum of human origin. Enterococcus faecium strain S13 and Bifidobacterium sp. 420 originated from Wisby (Danisco), Niebull, Germany. All cultures were supplied via Uniq plc, Wootton Bassett, UK.

All LAB were cultured for 16 h anaerobically at 37 \( ^\circ \)C in MRS (de Man, Rogosa, Sharpe) broth (Oxoid Ltd., Basingstoke, UK) and re-suspended in Ringers solution. Population density was determined by reference to an optical density curve and adjusted with Ringers solution to provide \( 2 \times 10^8 \) cfu/ml. All LAB cultures were kept on ice prior to use. In some experiments, cell cultures of Bif. Bb12 and Lact. plantarum were diluted further in Ringers solution to provide cultures with a range of cell densities from approximately \( 8 \times 10^7 \) to \( 2 \times 10^5 \) cfu/ml. In studies to evaluate the effect of viability on anti-genotoxic activity, the LAB suspensions were placed in a boiling water bath for 10 min. The viability of the resulting suspensions was determined by plating onto MRS agar and incubating anaerobically at 37\( ^\circ \)C.

2.3. Prebiotics

The prebiotics used in the study were Raftiline HP (long chain fructooligosaccharides (FOS), degree of polymerization (DP) 22–25; ORAFTI Active Food Ingredients, Tienen, Belgium), Raftilose (short chain FOS (DP 3–7); ORAFTI), Inulin (mixture of long and short chain FOS, derived from chicory root; Sigma Chemical Co., UK) Elixor (galactooligosaccharides; Borculo Whey Products, Needesweg, The Netherlands), Actilight 950P (short chain FOS, Beghin Meiji Industries, France) and Fibersol (maltodextrin; Matsutani, Chemical Industry Co. Hyogo, Japan).

2.4. Probiotic/prebiotic fermentations

Broth for the fermentations with pro-and prebiotics was prepared according to the formula for MRS with the exclusion of glucose as follows (ingredients per litre): peptone, 10 g; Lab Lemco powder, 8 g; yeast extract, 4 g; Tween-80, 1 ml; di-potassium hydrogen phosphate, 2 g; sodium acetate 3H\(_2\)O, 5 g; tri-ammonium citrate, 2 g; magnesium sulphate 7H\(_2\)O, 0.2 g; manganese sulphate 7H\(_2\)O, 0.2 g; magnesium sulphate 4H\(_2\)O, 0.05 g. Oligosaccharide or r-glucose was added to provide a final concentration of 2% (w/v).

Control incubations of LAB in MRS without added carbohydrate, and of each prebiotic without added LAB, were also included.

Probiotics were cultured with each prebiotic anaerobically overnight (16 h) at 37\( ^\circ \)C. Optical density was read at 600 nm in order to confirm that maximum population density had been reached. The culture was then centrifuged at 10,000 rpm for 10 min to remove the probiotic cells. The supernatant, containing the end products of fermentation, was removed, aliquoted to tubes, and stored at −20\( ^\circ \)C prior to incubation with HT29 cell line. All samples were neutralised to pH 7.0 with 0.1 M NaOH prior to assays.

2.5. Mammalian cell culture

HT 29 cells were obtained from the European Collection of Animal Cell Cultures (ECACC), Salisbury, UK. Dulbeccos Minimum Essential Medium
(DMEM) was obtained from Gibco Life Technologies Ltd., Paisley, Scotland. HT29 cells were cultured in Roux flasks as monolayers in DMEM containing 10% foetal bovine serum, 20 mM glutamine and 100 units per litre penicillin/streptomycin. Cells were cultured for 6–7 days (>75% confluence) at 37°C with 5% CO2 and 95% filtered air. The medium was changed every 2 days. Cells were washed with Hanks balanced salt solution (HBSS) for 2 min and re-suspended by the addition of trypsin (0.25% trypsin-EDTA) at 37°C for 5 min. Cells were centrifuged at 1250 rpm for 3 min and cells re-suspended in serum and penicillin/streptomycin free DMEM.

3. Experimental design

3.1. Studies on probiotics

Because the presence of bacterial cells interfered with the analysis of the comet images, it was not possible to incubate HT29 cells in the presence of both LAB and faecal water. Consequently, the faecal water was pre-incubated with LAB. Thus these studies examine the ability of the probiotics directly to decrease the DNA damaging potential of the faecal water. The bacterial suspensions were incubated anaerobically in Eppendorf tubes at 37°C for 30 min 1:1 with faecal water (100 ml LAB + 100 ml faecal water). Final population of bacteria in the assay was 1 × 10^9 cfu/ml. Faecal water/phosphate buffered saline (PBS) (1:1) was included for all incubations as a control. All incubations were carried out in triplicate. After incubation, samples were centrifuged at 10,000 × g for 10 min to remove the bacteria present. The supernatant (100 ml) was added to a new Eppendorf tube and 400 ml of HT29 cells (2.5 × 10^6 cells/ml) were added and incubated on ice for 5 min. Positive (75 μM hydrogen peroxide) and negative (PBS) controls were included for all experiments.

3.2. Studies on probiotic/prebiotics combinations (end-products of fermentation)

These studies were designed to examine the effect of fermentation products on the cellular resistance of the HT29 cells to faecal water genotoxins. The protocol used was based on that of Abrahamse et al. [22]. Briefly, HT29 cells (2.5 × 10^6 ml^-1 in 450 μl of serum-free DMEM) were incubated with fermentation supernatants (450 μl) for 30 min at 37°C. The mixture was centrifuged at 285 × g for 5 min and the supernatant was discarded. HT29 cells were resuspended in serum-free DMEM (450 μl) and exposed to genotoxic faecal water for 5 min on ice. The comet assay was carried out to measure single strand breaks in DNA. All samples were run in triplicate and positive and negative controls were run for each experiment. Trypan blue exclusion tests were carried out to assess cytotoxicity: all tests showed <10% cytotoxicity after incubation with test samples.

3.3. Comet assay

The assay was performed essentially as described by Venturi et al. [21]. The incubations of HT29 cells with faecal water with and without pro- and prebiotic treatments, together with the positive (hydrogen peroxide) and negative controls (PBS), were centrifuged for 5 min at 285 × g. The supernatant was discarded and the cell pellet re-suspended in 75 μl of 0.85% low melting point agarose (LMPA) in phosphate buffer saline (PBS) and maintained in a water bath at 40°C. The suspension was added to previously prepared gels (normal agarose; 1%) on frosted slides and coverslips were added. The gels were chilled at 4°C and a further protective layer of LMPA added. The slides were immersed in Lysis buffer (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris) for 1 h at 4°C and then placed in electrophoresis buffer and allowed to unwind for 20 min before running at 26 V, 300 mA for 20 min. After electrophoresis, gels were washed in neutralization buffer (0.4 M Tris, pH 7.5 with concentrated HCl) three times 5 min washes (4°C). Gels were stained with 50 μl of ethidium bromide (2 μg/ml) prior to scoring. Images were analyzed at 400× magnification using a fluorescence microscope. Olive Tail Moment was recorded using Komet 3.0 image analysis software (Kinetic Imaging Ltd. Liverpool). For each slide 100 cells were scored. Positive (hydrogen peroxide; 75 μM) and negative (PBS) controls were included for all experiments.
3.4. Statistical analysis

The 75th percentile was calculated from 100 cells per gel (each sample in triplicate) and the mean of each set of data was used in the statistical analysis (Venturi et al. [21]). Differences between means were evaluated by ANOVA ($P < 0.05$).

4. Results

Incubation of the HT29 colonic adenocarcinoma cell line with faecal water at 37 °C for 5 min caused no detectable cytotoxicity in the trypan dye exclusion assay—all tests showed <10% cytotoxicity after incubation with test samples. Negative controls (HT29 cells incubated with PBS) resulted in tail moment values ranging between 0.38 and 0.69. Treatment of cells with $75\mu$M hydrogen peroxide (positive control) induced mean tail moment of $14.4 \pm 1.02$ ($n = 5$). Incubations with faecal water alone, induced considerable DNA damage, yielding tail moments of $10.5 \pm 1.34$ (mean ± S.E.M.).

4.1. Studies on probiotics alone

Incubation of the LAB strains *Bif. Bb12*, *Bif. sp. 420*, *Lact. plantarum*, *Lactobacillus bulgaricus* and *Ent. faecium* at cell densities of $1 \times 10^8$ cfu/ml (final concentration) with the faecal water prior to exposure to HT29 cells, significantly decreased ($P < 0.05$) the induced DNA damage in the target cells (Fig. 1). The greatest protective effects against genotoxic damage were observed with *Bif. Bb12* and *Lact. plantarum* (65 and 63% reductions in genotoxicity, respectively). Smaller, but nonetheless significant ($P < 0.05$), decreases in faecal water-induced DNA damage were caused by the organisms *Lact. bulgaricus*, *Ent. faecium* and *Bif sp. 420* (37, 23 and 24% reduction, respectively). In contrast *Streptococcus thermophilus* at the same cell density showed no significant lowering of faecal water genotoxicity (Fig. 1).

Heat treatment of bacterial cultures resulted in non-viable cell suspensions (data not shown). When heat-treated probiotics were incubated with faecal water (Fig. 1), none showed any significant effect on induced DNA damage ($P > 0.05$) indicating the need for cultures of probiotics to be viable in order to decrease the DNA damaging potential of faecal water.

To investigate the effect of probiotic cell density on inhibition of genotoxicity, suspensions of the two most effective probiotics (*Bif. Bb12* and *Lact. plantarum*) were diluted prior to incubation with faecal water (Figs. 2 and 3). There was a cell density-related decrease in inhibition of genotoxicity with both bacterial strains. At cell densities at, or below, $1.6 \times$
Fig. 2. Effect of concentration of \textit{Lact. plantarum} on faecal water genotoxicity. Faecal water was incubated with either PBS or \textit{Lact. plantarum} at various cell densities for 30 min at 37°C. The bacteria were removed by centrifugation and the supernatant tested for genotoxic activity toward HT29 cells in the comet assay. Values shown are means and S.E.M. of three incubations and those marked with an asterisk differ significantly from control (FW alone). ANOVA \((P < 0.05)\).

10^6\text{cfu/ml} for \textit{Lact. plantarum} and 4 \times 10^5\text{cfu/ml} for \textit{Bif. Bb12} no significant effect on DNA damage was observed (Figs. 2 and 3).

4.2. Studies on pro and prebiotic combinations

Pre-incubation of HT29 cells for 30 min with MRS broth containing glucose, or the prebiotics Raftiline, Elixor, Raftilose, Inulin, Fibersol, Actilight, or no NDO, had no significant effect on the genotoxic effects of subsequent faecal water exposure (Table 1).

Table 1 Effect of incubation of HT29 cells with carbohydrates on cellular resistance to faecal water genotoxins

<table>
<thead>
<tr>
<th></th>
<th>H_2O_2</th>
<th>PBS</th>
<th>Faecal water only</th>
<th>Glucose</th>
<th>Raftiline</th>
<th>Elixor</th>
<th>Raftilose</th>
<th>Inulin</th>
<th>Fibersol</th>
<th>FOS</th>
<th>No NDO</th>
</tr>
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<tbody>
<tr>
<td>S.E.M.</td>
<td>0.24</td>
<td>0.33</td>
<td>0.54</td>
<td>0.75</td>
<td>0.47</td>
<td>0.75</td>
<td>0.55</td>
<td>0.24</td>
<td>0.17</td>
<td></td>
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<tr>
<td>(P)</td>
<td>0.126</td>
<td>0.390</td>
<td>0.081</td>
<td>0.785</td>
<td>0.639</td>
<td>0.405</td>
<td>0.992</td>
<td>0.659</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Prebiotics, prepared in MRS basal medium (minus carbohydrate source), were incubated at a final concentration of 10 mg/ml for 30 min at 37°C with HT29 cells. The cells were collected by centrifugation and exposed to faecal water and DNA damage assessed by comet assay. Values shown are means and S.E.M. (\(n = 3\); except for positive and negative controls where \(n = 1\)) for tail moment. \(P\) values were calculated (ANOVA and least significant difference) for comparisons with faecal water alone.

HT29 cell incubations with fermentation supernatants of \textit{Lact. plantarum} and the prebiotics Raftiline, Elixor, Raftilose, Inulin, Fibersol and Actilight before exposure to genotoxic faecal water resulted in significant reductions in tail moment of 80, 62, 84, 69 and 79%, respectively (Fig. 4a). In contrast, preincubation of HT29 cells with supernatant derived from incubations of \textit{Lact. plantarum} and Elixor, resulted in a much smaller decrease (34%) in genotoxicity, similar to that achieved with \(d\)-glucose or in the absence of added oligosaccharide (Fig. 4a).

Incubation of HT29 cells with supernatants from fermentations of \textit{Bif. Bb12} and the prebiotics Elixor, Raftilose, Inulin, and Actilight substantially increased cellular resistance to the genotoxic effects of faecal water, with reductions in genotoxicity of 49, 57, 83,
and 79% respectively being observed. In contrast, supernatants from Fibersol and glucose incubations had more modest effects and Raftiline showed no activity (Fig. 4b).

The effects on cellular resistance to faecal water genotoxins of fermentation products of prebiotics with \textit{Ent. faecium} were very variable (Fig. 5a), with only Raftiline and Inulin fermentations having marked effects on genotoxicity (76 and 52% reduction, respectively). In the case of Actilight fermentation, cellular resistance to faecal water genotoxicity was actually decreased.

Highly variable effects on cellular resistance were also seen with fermentation supernatants of \textit{Bif.}
Fig. 5. Effect of supernatants of fermentations of carbohydrates with *Ent. faecium* (a) or *Bif. sp. 420* (b) on cellular resistance of HT29 cells to faecal water genotoxins. For further details see legend to Fig. 4.

sp. 420 (Fig. 5b). Fermentations with Raftiline and Elixor resulted in reductions of 52 and 55% respectively, whereas Insulin and Actilight actually increased genotoxicity and Raftilose and Fibersol had no effect.

5. Discussion

The results of this study show that certain strains of LAB were able to counteract the genotoxic potential of faecal water, which is considered to play a potential role in induction of colorectal cancer.

The effect was species specific, not just a generalised bacterial inhibition, since the different types of bacteria exhibited different inhibitory potencies at the same cell densities. For example *Lact. plantarum* was a more effective inhibitor (63% inhibition) than *Lact. buccaricus* (37% inhibition) at the same cell density. Similarly *Bif. Bb12* was a more potent inhibitor than *Bif. sp. 420*. *Strep. thermophilus*, one of the main organisms normally used in yoghurt production, had no significant inhibitory effect at 10⁸ cfu/ml.

These data are consistent with other in vitro studies using specific carcinogens and the Ames mutagenicity assay as an endpoint [16,19,20], which showed that various LAB can inhibit genotoxicity of dietary carcinogens in vitro. The degree of inhibition was strongly species dependent in some studies. For example Pool-Zobel et al. (1993) demonstrated that *Lact. casei* and *Lact. lactis* inhibited the mutagenic activity of nitrosated beef by over 85% whereas *Lact. confusus* and *Lact. sake* had no effect.

The direct inhibitory effect of probiotics on the genotoxic potential of faecal water was strongly dependent on the cell concentration in the present study. With *Lact. plantarum* cell densities at, or lower than, 1.5 × 10⁶ cfu/ml there was little or no inhibitory effect on faecal water genotoxicity. The corresponding critical value for *Bif. Bb12* was 4 × 10⁵. It is difficult to determine whether sufficient numbers of viable probiotics would be achieved in the human colon to have a significant effect on faecal water genotoxicity in vivo. However, there is considerable evidence that oral administration of probiotics can suppress genotoxic damage to the colonic mucosal cells in rats. For example Pool-Zobel et al. [7] used the comet assay to investigate the ability of a range of species of LAB to inhibit DNA damage in the colon mucosa of rats treated with the carcinogens MNNG or 1,2-dimethylhydrazine (DMH). All the strains of lactobacilli and bifidobacteria tested—*Lact. acidophilus* (isolated from a yoghurt), *Lact. gasseri*, *Lact. confusus*, *Bifidobacterium breve* and *Bifidobacterium longum*, prevented MNNG-induced DNA damage when given at a dose of 10¹⁰ cells/kg body weight, 8h before the carcinogen. In most cases the DNA damage was reduced to a level similar to that in untreated rats. The protective effect was also dose dependent: doses of *Lact. acidophilus* representing 50 and 10% of the original dose were less effective in reducing...
MSNG-induced DNA damage. *Strep. thermophilus* was not as effective as the other LAB strains in this study—a finding consistent with our results. Non-viable probiotics had no significant effect on faecal water genotoxicity in the present study. It would appear that active processes are involved and that the antigenotoxic effect of the probiotics studied here is not due to non-specific binding of the genotoxins to cell wall components such as has been suggested previously [15–18] since the latter does not require viable cells. When the probiotics were incubated with prebiotics and the resulting culture supernatants tested for induction of cellular resistance to faecal water genotoxins in the comet assay, a wide range of effects were seen that were dependent on both the probiotic strain and the prebiotic used. Bacterial incubations in the absence of a carbohydrate source exhibited little or no effect on resistance to faecal water-induced DNA damage as did supernatants of fermentations of probiotics with glucose. In contrast, probiotic incubations with oligosaccharides resulted, in many cases, in substantial reductions in response to subsequent faecal water challenge. Fermentations involving *Lact. plantarum* were the most effective in increasing cellular resistance to faecal water genotoxicity. Significant reductions in genotoxic response were seen with all the prebiotic substrates used as well as with glucose and even in the absence of a carbohydrate source. Culture supernatants of *Lact. plantarum* with the longer chain oligosaccharide mixtures, Inulin and Raftiline, and the short chain FOS were particularly effective with reductions of 79–84% in genotoxicity being seen. Substantial increases in cellular resistance to faecal water-induced DNA damage were also seen with supernatants from incubation of *Bif. Bb12* with all prebiotics except with Raftiline. It is noteworthy that *Lact. plantarum* and *Bif. Bb12* exerted the most potent direct antigenotoxic effect when incubated alone with faecal water. Overall, fermentations of the FOS-based carbohydrates were more effective than the galactooligosaccharide-based Elixor.

The supernatants of fermentations involving Ent. faecium and *Bif. sp.* 420 generally had less potent effects on cellular resistance to genotoxicity although some effects with Raftiline and Elixor fermentations were apparent. Unexpectedly, supernatants of FOS incubation with these two bacteria resulted in an increase in genotoxic damage. There is no obvious explanation for such an effect.

The comet assay has previously been used to evaluate the in vivo effect of a prebiotic, lactulose, on DNA damage in the colon. Rats that were fed a diet containing 3% lactulose and given DMH, exhibited less DNA damage in colon cells than similarly treated animals fed a sucrose diet. In the latter animals, the percentage of cells with severe DNA damage comprised 33% of the total compared with only 12.6% in the lactulose-fed rats [23].

In conclusion we have shown that certain LAB have the ability to reduce the genotoxic effects of faecal water in human colon cells in culture. Viability of the bacterial cells was a prerequisite for the reduction in faecal water genotoxicity and the probiotic effect was shown to be dose dependent. The degree of protection conferred on the host by probiotic supplementation was also species dependent with *Lact. plantarum* and *Bif. Bb12* being particularly effective. It was also apparent that the end products of fermentation of certain probiotics and prebiotics increased cellular resistance to subsequent faecal water challenge. Fermentations involving *Lact. plantarum* and *Bif. Bb12* were particularly effective, especially in combination with Inulin and Actilight. The different degrees of inhibition of genotoxicity observed with the various combinations of pre- and probiotics suggest that this may be a consequence of different end products of metabolism. The main metabolic products of dietary carbohydrate fermentation in the colon are the short chain fatty acids, predominantly acetate, propionate and butyrate [24]. There is evidence from studies using the comet assay that these compounds, particularly acetate and β-butyrate, can reduce the activity of model genotoxins in colon cells [22]. Butyrate has been shown to induce glutathione-S-transferase π1 in cultured human colon carcinoma cells [25], which would potentially enhance the detoxification capacity of the cells towards carcinogens. However, it is unlikely that this mechanism is operating in the present study, since the exposure time (30 min) would be too short for induction of glutathione transferase. In the study by Stein et al. [25], enzyme activity was not detected until 3 days after butyrate exposure. A more likely mechanism may be related to the efficient utilization of acetate and butyrate as energy sources by colon cells [22].
Although there are many studies in laboratory animals indicating that administration of probiotics and/or prebiotics reduces the incidence of tumours and precancerous lesions in the colon [8–11,26,27], evidence in human is lacking. However, a recent intervention study in which human volunteers consumed yoghurt containing strains of \textit{Lactobacillus acidophilus} and \textit{Bifidobacterium longum} has demonstrated a reduction in faecal water genotoxicity, in comparison to a conventional yoghurt [28].

Overall, our results suggest that \textit{Lactobacillus plantarum} and \textit{Bifidobacterium} \textit{Bb12} have intrinsic antigenotoxic potential and may have protective effects against the early stages of colon cancer and certainly are worthy of further evaluation in in vivo assays for anticarcinogenic effects.

Acknowledgements

This study was part of a research programme funded by Uniq plc. We are grateful for the helpful advice of Dr. John Stevens and Dr. Chris Griggs.

References


