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ORIGINAL CONTRIBUTION

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Effect of lactobacilli, bifidobacteria and inulin on the formation of aberrant crypt foci in rats

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Summary *Background* Our studies were aimed at investigating the effect of lactic acid producing bacteria (LAB) or inulin, a natural source of non-digestible oligosaccharides derived from chicory, on the induction by carcinogens of aberrant crypt foci (ACF) in the colon, which are considered to be early precursor lesions of neoplasia. *Methods* Strains of *Bifidobacterium longum, Lactobacillus casei* and *Lactobacillus acidophilus* were administered to rats fed a purified high starch diet, under a variety of treatment protocols including daily gavage, via the drinking water and in the diet. The rats were treated with methyl-N-nitrosourea, 1,2-dimethylhydrazine, or azoxymethane (AOM) to induce ACF. *Results* In general, no consistent significant changes in ACF numbers were detected in these experiments. In one study, the basal diet of the rats was changed to one containing a higher level of fat (corn oil). Under these conditions, a significant decrease in AOM-induced colonic ACF was seen in rats given *L. acidophilus* or inulin. In a concurrent group of animals fed a low fat diet, no significant decrease in ACF was observed. *Conclusions* The results indicate that the type of diet fed can influence the detection of protective effects of LAB and oligosaccharides and that against the background of a diet with a level of fat typical of a Western diet, evidence for a protective effect of *L. acidophilus* and inulin towards colon cancer was obtained

Key words probiotics – lactic acid bacteria – inulin – prebiotics – colon cancer

Introduction

A wide variety of dietary components have been ascribed potential modulatory roles in cancer, both beneficial, such as starch [1], antioxidants [2], fruit and vegetables [3] and calcium [4], and detrimental, such as fat [5] and red meat [6]. Amongst those components of the diet thought to have a protective role, attention has focused in recent years on fermented milk products and the bacteria, particularly lactobacilli and bifidobacteria, associated with them [7]. These lactic acid producing bacteria (LAB) are usually termed probiotics, i. e. live microbial food ingredients that are beneficial to health [8]. Probiotics have been shown to prevent DNA damage and mutations *in vitro* [9] and *in vivo* [10, 11] and to inhibit tumour cell growth *in vitro* [12]. Consumption of LAB has also been shown to suppress carcinogen-induced tumorigenesis in rodents [13–16] and to reduce, in human volunteers, urinary and faecal excretion of mutagens associated with the consumption of carcinogens in fried meat [17].

Various mechanisms have been suggested by which LAB such as lactobacilli and bifidobacteria may have protective effects against colon cancer [7, 18], including binding and inactivation of the carcinogens by the LAB

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[19, 20], and modification of gut bacterial metabolism of endogenous and xenobiotic compounds associated with the generation of genotoxic and carcinogenic products [21]. In general, species of *Bifidobacterium* and *Lactobacillus* have low activities of such xenobiotic metabolizing enzymes [22], suggesting that increasing the proportion of LAB in the gut microflora could result in an overall decrease in enzyme activity in the colon.

In many studies it is considered that the protective effects of probiotics are a consequence of increased numbers of LAB in the large intestine. Such increases in colonic LAB can be also be achieved by ingestion of non-digestible oligosaccharides. Oligosaccharides are a diverse group of short chain-length sugars (many occurring naturally in numerous edible plants), which on reaching the colon are fermented and appear to stimulate preferentially, indigenous populations of lactobacilli and bifidobacteria [22–26]. The term 'prebiotic' has been coined to describe such compounds [27]. There is some evidence that prebiotics can inhibit some stages of the carcinogenic process; for example, we have demonstrated that Lactulose, a synthetic non-digestible disaccharide, decreases the degree of carcinogen-induced DNA damage in the colon of rats [28]. In the present study we have investigated inulin, a natural source of oligosaccharides derived from chicory [29].

Our studies were aimed at investigating the effect of LAB or inulin administration on the induction in the colon of aberrant crypt foci (ACF), which are considered to be early precursor lesions of neoplasia [30–32]. ACF lesions similar to those found in carcinogen-treated rodents have also been observed in colorectal cancer patients [31] and several groups have used the formation of ACF as a predictive measure of the modulatory role of dietary (and endogenous) components [31, 32], including pro- and pre-biotics [33–36] in colon carcinogenesis.

Material and methods

Animals and diets

Male Sprague Dawley rats (OLAC 1976 Ltd) age 3–5 weeks were fed a purified diet (SSA) containing 5 % w/w corn oil [37]. In some experiments, a high fat diet (CO25) containing 25 % (w/w) corn oil was given. The corn oil replaced starch on an energy basis such that the protein, vitamin and mineral content per kJ in the SSA and CO25 diets were identical (Table 1).

Table 1 Composition of high fat (CO25) and low fat (SSA) diets

	CO25		SSA	
Ingredient	g/kg	kJ	g/kg	kJ
Maize starch	450.0	7200	704.0	11264
Casein	245.0	4165	200.0	3400
Corn oil	249.0	9213	50.0	1840
Mineral mix	43.0	_	35.4	–
Vitamin mix	12.3	–	10.0	–
Total energy (kJ/kg)		20578		16504

Bacterial strains and culture media

Bifidobacterium longum was provided by Professor C Romond (Université de Lille, France), *Lactobacillus acidophilus* and *Lactobacillus casei* by Professor Holzhapfel (Bundesforschungsanstalt für Ernährung [BfE], Karlsruhe, Germany) and the other *Lactobacillus casei* strain (designated BSN) was obtained from Group Danone, Paris.

These three *Lactobacillus* strains were chosen on the basis of their demonstrated ability to inhibit MNNG and DMH-induced genetic damage to the colon of rats [8, 9].

Lactobacilli were cultured in MRS broth (Oxoid Ltd, Basingstoke, UK); *Bifidobacterium longum* was cultured in Brain Heart Infusion supplemented (BHIs) broth containing, in 500 ml deionized water, Brain Heart Infusion broth (Oxoid) 18.5 g, Yeast extract (Oxoid) 2.5 g, resazurin solution (0.25 mg/ml) 2 ml, cysteine HCl 0.25 g, hemin solution (0.5 mg/ml) 5 ml, vitamin K (Sigma Chemicals, Poole Dorset, UK) 0.1 ml.

Viable numbers of lactobacilli and bifidobacteria in suspensions and freeze-dried preparations used in the experimental studies were determined by plating serial dilutions (prepared in sterile Ringers for lactobacilli and in sterile Brucella broth for bifidobacteria) on MRS or BHIs agars respectively and incubating aerobically for lactobacilli, or in an anaerobic cabinet (Don Whitley Scientific, Shipley, West Yorkshire, UK) for bifidobacteria, for up to five days at 37 °C.

Chemicals

1,2-Dimethylhydrazine dihydrochloride (DMH), azoxymethane (AOM), methyl-N-nitrosourea (MNU) and inulin were purchased from Sigma Chemicals (Poole, Dorset UK). Inulin was derived from chicory root and consisted mainly of longer chain oligosaccharides with a degree of polymerization of 12 to approximately 60 [29].

Aberrant crypt foci (ACF) assays

Experiment 1

Preparation of lactobacillus suspensions. *L. casei* BSN was inoculated from frozen (liquid nitrogen) cultures

into 500 ml MRS broth and incubated for 16 h aerobically at 37 °C in flasks, without shaking. The cultures were centrifuged at 2,900 x g for 1 h at 4 °C and the pellets subsequently washed with phosphate buffered saline (PBS) and centrifuged at 2,900 x g for 30 min. The resultant pellets were combined and diluted in sterile deionized water to the original volume and stored at 4 °C. The suspension was used without further dilution for administration by gavage, or was diluted by 1/10 and 1/1000 with sterile water each day to provide drinking water for the rats. Fresh lactobacillus cultures were prepared approximately once per week.

Animal treatments. Nine groups of 10 male Sprague Dawley rats (7 weeks old), fed SSA diet were given by gavage either distilled water (1 group, negative control), 60 mg 1,2-dimethylhydrazine (DMH)/kg (6 groups) or 150 mg methyl-N-nitrosourea (MNU)/kg (2 groups) and killed, by carbon dioxide asphyxia, either 4 or 12 weeks after carcinogen dosing. Two of the DMH-treated groups (positive controls) were given deionized water to drink and killed at 4 weeks or 12 weeks, two groups were given *L. casei* in the drinking water at a concentration of approximately $3x10^8$ viable cells/ml (killed at 4 and 12 weeks), one was given *L. casei* in the drinking water at a concentration of approximately $2.5x10^6$ viable cells/ml (killed at 12 weeks) and one was given a single oral dose by gavage of $3x10^9$ viable *L. casei* cells immediately before the DMH dose and then deionized water to drink until being killed at 4 weeks. Of the two MNU-treated groups, one was designated as a control and the other was given *L. casei* ($3x10^8$ viable cells/ml) in the drinking water; both groups were killed at 4 weeks. Where *L. casei* was put in the drinking water, this was given for one week prior to carcinogen dosing and then continuously until the rats were killed. Half the negative control group was killed at each timepoint. Colons were removed from all animals and examined for ACF as described below.

Experiment 2

Preparation of lactobacillus and bifidobacterium suspensions. *L. casei* BfE was cultured in 500 ml MRS broth as described in experiment 1. *B. longum* was inoculated from a liquid nitrogen culture into 50 ml BHIs broth. After 48 h incubation at 37 °C in an anaerobic cabinet (Don Whitley Scientific Ltd, Shipley, West Yorkshire, UK), the resulting culture was used to inoculate 500 ml BHIs broth, which was incubated for a further 48 h under the same conditions. The bacterial cultures were pelleted by centrifuging at 2,900 x g for 1 h. The supernatants were subsequently decanted in air (*L. casei*) or in an anaerobic cabinet (*B. longum*) and the pellet washed once in sterile deionized water (*L. casei*) or in sterile anaerobic saline (*B. longum*). The bacterial cells were stored as a pellet at 4 °C for up to 4 d and then resuspended in sterile saline (or anaerobic saline for *B. longum*) before use to give a suspension 5x the concentration of the original culture.

Animal treatments. At approximately 7 weeks of age, 6 groups of 8 male Sprague Dawley rats, fed SSA diet, were administered by gavage DMH as 2 doses of 25 mg/kg given 2 days apart (3 groups) or as 4 daily doses of 12.5 mg/kg (3 groups). Two groups of rats on each dose regimen were also given a saline suspension (2 ml) of either *L. casei* BfE (approximately $3x10^9$ viable cells/ml) or *B. longum* (approximately $4x10^7$ viable cells/ml) daily by gavage. Controls were given 2 ml saline. Treatment with LAB continued throughout the experimental period, from 2 days before the first DMH dose until the day of necropsy, 4 weeks after the last DMH dose, when the colons were removed and treated as below.

Experiment 3

Preparation of freeze-dried *L. acidophilus* cultures. An overnight culture (500 ml) of *L. acidophilus* in MRS was used to inoculate 9 l MRS, which was then incubated aerobically at 37 °C for 21 h. The resulting culture was centrifuged at 2,900 x g for 30 min, the pellet washed once in sterile deionized water and the final pellet resuspended in 50 ml of a 12 % skimmed milk, 12 % malt extract solution. The resulting cell suspension was snap-frozen in liquid nitrogen and dried under vacuum. Three batches were prepared, each yielding approximately 19 g freeze-dried cell preparation containing on average approximately 10^9 viable cells/g. This was then mixed into the SSA diet at a level of 0.5 %.

Animal treatments. Forty-eight male Sprague Dawley rats were fed SSA from 4 weeks old and at approximately 6 and 7 weeks of age, given two subcutaneous doses (one week apart) of AOM (12.5 mg/kg). One week after the last AOM dose, the rats were divided into 6 groups and fed one of six experimental diets. These comprised either SSA or CO25 alone, or these diets supplemented with either freeze-dried *L. acidophilus* preparation (0.5 % w/w, $5x10^9$ viable cells/kg diet), or inulin (5 % w/w in diet). Since the freeze-dried lactobacillus preparation contained skimmed milk and malt extract as cryopreservatives, the control and inulin-supplemented diets were also supplemented with corresponding amounts of skimmed milk and malt extract.

After 4 weeks on the experimental diets, the rats were killed and the colons removed and examined for ACF as described below.

Assessment of aberrant crypt foci (ACF)

This was performed as described by Bird *et al* [30] with minor modifications. Briefly, each colon, slit longitudinally, was placed on a piece of white card, mucosal surface uppermost and the surface cleaned gently with 0.9 % saline. The back of the card was sprayed with absolute ethanol and the card and its attached colon placed upside down in 10 % buffered formalin for 24 h for fixation of the tissue. The colons were then stained with 0.2 % methylene blue in saline, to identify the ACF under a low power stereo microscope (Carl Zeiss Ltd., Welwyn Garden City, Herts, UK). The number of ACF was determined, together with their distribution according to number of aberrant crypts per focus.

Statistical analysis

Results were subjected to analysis of variance using the Minitab statistical package. Individual means were compared using the Least Significant Difference criterion [38].

Results

Preliminary studies

Experiments were conducted to assess the ability of a number of carcinogens to induce ACF. A comparison of the abilities of N-methyl-N-nitroso-guanidine (MNNG) and another direct-acting nitrosamide, MNU, to induce ACF in the colons of Sprague Dawley rats revealed marked differences in potencies, particularly when compared to the effects of DMH. No ACF were induced by oral doses of 15, 30 and 75 mg MNNG/kg body weight or MNU, at an oral dose of 30 mg/kg whereas an oral dose of 100 mg MNU/kg, induced an average of 18 aberrant crypts per colon. A further study investigated the ability of a low dose (18.75 mg/kg) of DMH, known to cause DNA damage in the Comet assay [11], to induce ACF. Six weeks after exposure to the carcinogen, the number of ACF were in the range of 1–3 per colon, an insufficient number to study the potentially protective properties of LAB. In the following studies, the ability of LAB to modulate ACF formation was assessed using a variety of experimental protocols.

Experiment 1. Effect of *L. casei* on DMH- and MNU-induced ACF

As in all preliminary studies, no ACF were detected in rats that were not treated with carcinogens; consequently in the subsequent studies, untreated controls were not included.

In experiment 1, two concentrations of *L. casei* BSN in drinking water were used. Rats given the higher concentration consumed in the range of approximately 16–19 ml/d, giving a viable *L. casei* consumption of 4.8–5.9 x 10^9/d, whilst rats given the lower concentration consumed in the range of approximately 17–18 ml/d, giving a viable *L. casei* consumption of 4.2–4.5 x 10^7/day. In addition, groups of animals were killed at 4 and 12 weeks after exposure to DMH (60 mg/kg) to study the development of the ACF lesion. In DMH-treated control rats, the total number of ACF was more than three fold higher at 12 weeks (74.6 ± 36.7) than at 4 weeks (17.5 ± 12.6) and the percentage of ACF containing more than four aberrant crypts was also higher in the 12 week animals (9.5 %) than in the 4 week animals (1.1 %; Table 2). Treatment of the rats with lactobacilli did not reduce the number of DMH-induced ACF per colon in the animals killed at 4 or 12 weeks (Table 2). In fact, a significant increase in total ACF per colon was seen in the animals given 2.5x10^6 *L. casei* BSN /ml drinking water (Table 2). The percentage of ACF with more than 4 crypts was not markedly affected by any of the lactobacillus treatments. In rats treated with MNU, a slight, but not statistically significant, decrease in ACF per colon was seen when lactobacilli were administered in the drinking water. The percentage of foci with more than 4 aberrant crypts was also decreased by *L. casei* in MNU-treated rats (Table 2).

Table 2 Experiment 1: effect of *L. casei* BSN on DMH- and MNU-induced ACF in rats

Experimental Period[a]	Carcinogen	*L. casei*	ACF/colon (% > 4 crypts)
4/12 wk control	– (water)	–	0 (0.0)
4wk	DMH	–	17.5 ± 12.6 (1.1)
4wk	DMH	3x10^8/ml wk –1 to 4	31.4 ± 25.3 (0.3)
4wk	DMH	3x10^9/rat one dose	21.7 ± 14.5 (0.9)
12wk	DMH	–	74.6 ± 36.7 (9.5)
12wk	DMH	3x10^8/ml wk –1 to 12	86.9 ± 26.2 (11.0)
12wk	DMH	2.5x10^6/ml wk –1 to 12	131.1 ± 52.2* (8.2)
4wk	MNU	–	37.0 ± 26.2 (5.4)
4wk	MNU	3x10^8/ml wk –1 to 4	23.9 ± 16.4 (0.0)

Values for ACF per colon are means ± SD for 10 rats. The value marked with an asterisk is significantly different from the DMH control ($P < 0.01$; analysis of variance). Numbers in parentheses represent the percentage of ACF containing more than 4 aberrant crypts.

[a] Number of weeks between carcinogen treatment and killing of rats

Experiment 2. Effect of *L. casei* BfE and *B. longum* on ACF induced after multiple doses of DMH

In this experiment, a dose of DMH (50 mg/kg) known to induce between 50 and 100 ACF was divided into 2 or 4 smaller doses. This was in an attempt to circumvent the perceived problem that previously used doses of the carcinogen may be too high to permit detection of protective effects of the administration of LAB. Because of concerns about the viability of the LAB (particularly the bifidobacteria) in drinking water, fresh cultures were prepared approximately twice per week, the cells harvested by centrifugation and stored as pellets at 4 °C. Pellets were then resuspended in sterile saline each day and administered by gavage. The administration of DMH as 2 x 25 mg/kg doses consistently induced more ACF than the same total amount of DMH given as 4 doses (Fig. 1), with the most marked effects being seen in the control ($P < 0.01$) and *B. longum*-dosed groups ($P < 0.001$). *L. casei* BfE treatment appeared to decrease, *B. longum* to increase total ACF in rats given 2 x 25 mg DMH/kg (these two groups differing significantly from each other, $P < 0.01$). However despite the large differences between control and LAB-treated groups (66 ACF vs 36 ACF for *L. casei* BfE) the results did not achieve statistical significance due to the high variability of the data.

Experiment 3. Effect of *L. acidophilus* and inulin on ACF induction by AOM – influence of high and low fat diets

It was considered that the purified SSA diet (high starch/low fat) previously used for the ACF experiments may have been too 'protective' and that a diet high in fat (previously shown to promote the induction of ACF; 34) might provide a better control diet for studies of LAB effects. Thus the aim of this experiment was to determine whether feeding a high-fat diet to rats could modify the induction of ACF by AOM and furthermore could modulate the influence of lactobacilli or oligosaccharides on ACF induction. AOM was used instead of DMH since it was felt that subcutaneous injections of AOM rather than oral doses of DMH might reduce variations in carcinogen exposure and thus possibly in number of ACF induced in the colon.

Food intake of rats on the SSA diet was approximately 20 g/d resulting in an intake of *L. acidophilus* of approximately $1x10^8$ viable cells/rat/day. The rats on the CO25 diet consumed, on average, 16.5 g diet/day, resulting in an approximate *L. acidophilus* intake of $8.25x10^7$ viable cells/rat/day. Energy intake (and hence intake of protein, vitamins and minerals) of the rats on the basal diets was similar (mean 333 ± 32 kJ/rat/day for SSA and 338 ± 19 kJ/rat/day for CO25).

Consumption of the high-fat diet alone did not significantly alter the incidence of total ACF (Fig. 2), nor the incidence of larger foci (mean number of ACF with > 4 aberrant crypts/focus was 13.5 ± 12.19 for SSA and 14.0 ± 11.07 for CO25-fed rats). The incorporation of either lactobacilli or inulin into the SSA diet was associated with a small, but non-significant decrease in ACF (Fig. 2). In contrast, when incorporated into the CO25 diet, both *L. acidophilus* and inulin significantly decreased, by 38 and 48 % respectively, the number of AOM-induced ACF (Fig. 2). The decrease was most apparent in foci with 1–3 aberrant crypts per focus (Fig. 3).

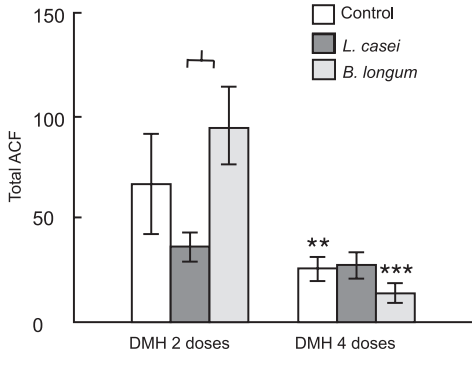

Fig. 1 Effect of *L. casei* BfE or *B. longum* on ACF induced by 50 mg DMH /kg administered in 2 or 4 doses. The rats (8 per group) were given a daily gavage of 2 ml saline (control), 2 ml *L. casei* suspension ($3 x 10^9$ cells/ml) or 2 ml *B. longum* suspension ($4 x 10^7$ cells/ml). Two days after the first dose of bacteria, the rats were given oral doses of DMH 2 x 25 mg/kg, 2 days apart (dose 1), or 4 daily doses of 12.5 mg/kg (dose 2). Bacteria treatments continued until day of necropsy, 4 weeks after the last DMH dose. Asterisks indicate statistically significant effects between dose 1 and dose 2 for the same dietary treatment ** $P < 0.01$; *** $P < 0.001$ (ANOVA). *B. longum* and *L. casei*-treated groups given 2 DMH doses differed significantly (++$P < 0.01$).

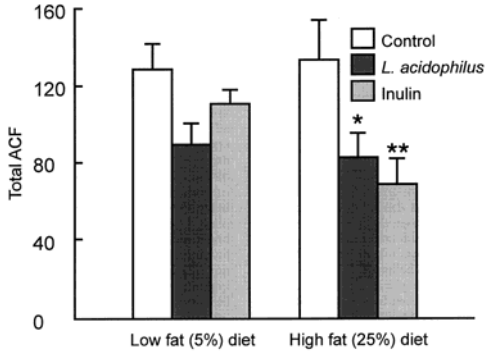

Fig. 2 Effect of *L. acidophilus* (0.17 % w/w) or inulin (5 % w/w) incorporated into a low fat or high fat diet on the induction of ACF in colons of rats treated with AOM (2 x 25 mg/kg).The rats were transferred to the experimental diets 1 week after the last AOM dose and were killed 4 weeks later. Values shown are means ± SEM for 8 rats. Asterisks indicate statistically significant effects of either L. acidophilus or inulin treatment by comparison to the corresponding control. * $P < 0.05$; ** $P < 0.01$ (ANOVA).

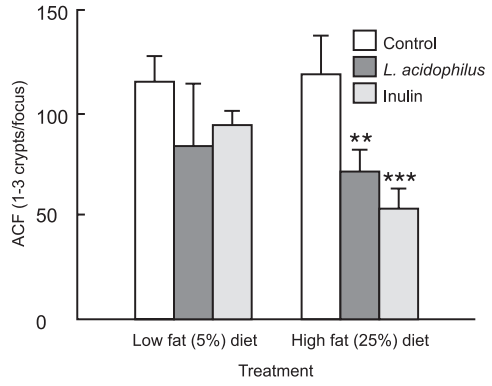

Fig. 3 Effect of *L. acidophilus* (0.17 % w/w) or inulin (5 % w/w) incorporated into a low fat or high fat diet on the induction of ACF with 1–3 crypts per focus in colons of rats treated with AOM (2 x 25 mg/kg). For further details see legend to Fig. 1.

Discussion

In the first two experiments, administration of lactobacilli or bifidobacteria, either in the drinking water or by gavage, did not appear to inhibit carcinogen-induced ACF formation. A more pronounced inhibition of ACF formation was achieved when a diet containing a higher level of fat than the standard purified diet was used. The level of fat in the CO25 diet (about 45 % of total energy) is comparable to that in the normal Western European diet, which is associated with a high risk of colon cancer. Interestingly, consumption of the CO25 diet did not increase the incidence of carcinogen-induced ACF, but had a marked effect on the ability of lactobacilli and inulin to inhibit ACF formation. The total number of ACF and the total number of ACF with one to three crypts formed were significantly lowered by dietary supplementation with the lyophilized *L. acidophilus* and an even stronger inhibition was apparent with the diet supplemented with inulin. Inulin has previously been shown to stimulate the levels of indigenous LAB in the gut [26] and, like other indigestible oligosaccharides, may provide a more effective method of raising the concentration of these bacteria in humans than feeding live organisms. The reason why an inhibitory effect was observed in the third experiment and not in earlier experiments may be related to the naturally protective nature of the high starch / low fat diet that had previously been used. It could also be due to the fact that AOM rather than DMH was used as the inducing carcinogen and that in the last experiment, whilst the total number of ACF induced was greater (providing greater scope for a protective effect), the extent of variability between counts within a group was reduced, allowing statistically significant differences to be detected. The provision of lactobacilli in the diet may also have been a contributory factor, since although the numbers of viable organisms consumed were not necessarily higher than in previous experiments, the presence of the skimmed milk and malt extract, together with consumption with food, may have afforded greater protection to the organisms when passing through the acid environment of the stomach. It is also possible that *L. acidophilus* has greater inhibitory potential than the *L. casei* strains or *B. longum*. In a study of effects of various probiotics on DMH-induced tumours in rats, McIntosh *et al.* [16] noted that *L. acidophilus* was more inhibitory to large intestinal tumours than *L. rhamnosus, B. animalis* or *Streptococcus thermophilus*. It is noteworthy that although *B. longum* did not exhibit inhibitory effects in the present study, suppression of ACF by this organism was observed by Rowland et al. [36]. Interestingly in the latter study, the basal diet fed to the animals was a high fat diet.

The inhibitory effect observed with inulin may have been due to its prebiotic activity of stimulating of indigenous LAB with naturally high inhibitory properties. Alternatively, the protective properties of inulin may be via another mechanism, such as an increase in short chain fatty acids generated by fermentation of the non-digestible oligosaccharides by the resident gut microflora.

Previous experiments had shown that LAB given 8 h prior to DMH exposure prevents DNA damage in the colon of rats, indicating that the protective activity is probably due to a direct interaction of DMH-metabolites and LAB in the colon [11]. In contrast, in the experimental protocol employed here, LAB were given several days after the DMH application. Thus the protective effect occurs in cells which have already been genetically damaged. One possible mechanism is by inducing apoptosis in the carcinogen-damaged cells as has been recently demonstrated for inulin-derived fructo-oligosaccharides in DMH-treated rats [39]. However, before concluding that *L. acidophilus* or inulin consumption can reduce the risk of colon carcinogenesis, some caution is necessary. Whilst it is generally accepted that ACF may indeed be pre-neoplastic lesions, several studies have failed to find a significant correlation between total ACF and eventual tumour incidence [40–43], although the number of large ACF was a more consistent predictor [43, 44]. It has also been suggested that crypt multiplicity (number of aberrant crypts per focus; [40]), or number of very large ACF with > 14 crypts per focus [42] are better indicators of tumour formation. In our final experiment, statistically significant differences between groups were seen only in the total number of ACF and number of small ACF.

In conclusion, results have been obtained which tentatively suggest that consumption of probiotics and/or prebiotics in the form of non-digestible oligosaccharides (particularly in conjunction with what might generally be considered as a 'high risk' diet) may modulate the development of ACF, of which a proportion might otherwise develop into tumours.

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