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Anti-Carcinogenicity of Probiotics and Prebiotics

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Abstract

Yoghurt, and the lactic acid producing bacteria (LAB; probiotics) that it contains, have received much attention as potential cancer-preventing agents in the diet. It is usually considered that the mechanism of the action is by increasing the numbers of LAB in the colon, which modifies the ability of the microflora to produce carcinogens. Prebiotics such as non-digestible oligosaccharides (NDO) appear to have similar effects on the microflora by selectively stimulating the growth of LAB in the colon.

Evidence for cancer-preventing properties of pro- and prebiotics is derived from studies on faecal enzyme activities in animals and humans, inhibition of genotoxicity of known carcinogens *in vitro* and *in vivo*, suppression of carcinogen-induced preneoplastic lesions and tumours in laboratory animals. Some of these studies indicate that combinations of pro and prebiotics ('synbiotics') are more effective. Epidemiological and intervention studies provide some, albeit limited, evidence for protective effects of products containing probiotics in humans.

Probiotics Prebiotics and Synbiotics

The original definition of a probiotic was 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (Fuller, 1989). Recent definitions are more general, omitting the aspect of intestinal balance. For example Salminen *et al* (1998) define a probiotic as 'a live microbial food ingredient that is beneficial to health'. A probiotic organism should be non-

pathogenic and non-toxic, and also resistant to low pH and to bile salts to improve its chances of survival in the gastrointestinal tract (Fuller, 1991). Most probiotics are members of two genera of lactic acid producing bacteria (LAB), *Lactobacillus* and *Bifidobacterium*, but *Saccharomyces* and *Enterococcus* are also used. Many of the bacteria used for probiotic preparations have been isolated from human faecal samples to maximise the likelihood of compatibility with the human gut microflora and hence enhance their chances of survival.

The concept of probiotics evolved from a theory first proposed by Metchnikoff who suggested that the long, healthy life of Bulgarian peasants could be attributed to their consumption of fermented milk products. A variety of health benefits have been associated with LAB such as improvement of lactose intolerance, regulation of gastrointestinal stasis, resistance to infectious digestive diseases, especially rotavirus-associated diarrhoea in infants, and immunomodulation (Sanders, 1993).

A prebiotic is 'a nondigestible 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' (Gibson and Roberfroid, 1995). A number of poorly digested carbohydrates fall into the category of prebiotics including certain fibres and resistant starches (Silvi *et al* 1998), but the most widely described prebiotics are non-digestible oligosaccharides (NDOs). These are low molecular weight carbohydrates with 2-10 degrees of polymerisation, which are poorly digested in the small intestine thus reaching the colon largely unaltered and can act as a substrate for the colonic microflora. They appear to stimulate specifically the numbers of bifidobacteria and lactobacilli, often at the expense of other microflora components such as bacteroides, clostridia and *Escherichia coli* (Gibson *et al* 1995, Rowland and Tanaka 1993). Combinations of probiotics and prebiotics can result in additive or synergistic effects on gastrointestinal function. The term synbiotic has been proposed for such combinations. A synbiotic has been defined as 'a mixture of probiotics 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 activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare' (Gibson and Roberfroid, 1995)

Role of the Gut Microflora in Cancer

The enormous numbers and diversity of the human gut microflora is reflected in a large and varied metabolic capacity, particularly in relation to xenobiotic biotransformation, carcinogen synthesis and activation. The metabolic activities of the gut microflora can have wide-ranging implications for the health of the host, resulting in both beneficial and detrimental effects (Rowland *et al*, 1999; Rowland and Gangolli 1999)

Evidence from a wide range of sources supports the view that colonic microflora is involved in the aetiology of cancer. The main pieces of evidence are:

Abbreviations

ACF: aberrant crypt foci
AOM: azoxymethane
DMH: 1,2-dimethylhydrazine
FOS: fructo-oligosaccharide
Glu-P-1: 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole
Glu-P-2: 2-aminodipyrido[1,2-a:3',2'-d]imidazole
HFA: human flora associated
IQ: 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline
LAB: lactic acid producing bacteria
MeIQ: 2-amino-3,4-dimethylimidazo[4,5-f]quinoline
MeIQx: 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
MNNG: N-methyl-N'-nitro-N-nitrosoguanidine
NDO: non-digestible oligosaccharide
PhIP: 2-amino-1-methyl-6-phenylimidazo-[4,5b]pyridine
TOS: trans-galactosylated oligosaccharide
Trp-P-1: 3-amino-1,4-dimethyl-5H-pyrido-[4,3-b]indole
Trp-P-2: 3-amino-1-methyl-5H-pyrido[4,3b]indole

- 1) Human faeces have been shown to be mutagenic, and genotoxic substances of bacterial origin have been isolated (Venturi *et al*, 1997)
- 2) Intestinal bacteria can produce, from dietary components, substances with genotoxic, carcinogenic and tumour-promoting activity (Rowland *et al*, 1999)
- 3) Gut bacteria can activate procarcinogens to DNA reactive agents
- 4) Germ-free rats treated with the carcinogen 1,2-dimethylhydrazine have a lower incidence of colon tumours than similarly treated rats having a normal microflora (Reddy *et al* 1975)
- 5) Germ-free rats fed human diets exhibit lower levels of DNA adducts in tissues than conventional rats (Rumney *et al*, 1993).

It follows from the above, that modification of the gut microflora may interfere with the process of carcinogenesis and this opens up the possibility for dietary modification of colon cancer risk. Probiotics and prebiotics, which modify the microflora by increasing numbers of lactobacilli and/or bifidobacteria in the colon, have been a particular focus of attention in this regard. Evidence that probiotics and prebiotics can influence carcinogenesis is derived from a variety of sources:

- Effects on bacterial enzyme activities.
- Antigenotoxic effects *in vitro* and *in vivo*.
- Effects on pre-cancerous lesions in laboratory animals.
- Effects on tumour incidence in laboratory animals
- Epidemiological and experimental studies in humans.

Effects of Probiotics and Prebiotics on Bacterial Enzyme Activities

The ability of the colonic microflora to generate a wide variety of mutagens, carcinogens and tumour promoters from dietary and endogenously-produced precursors is well documented (Rowland, 1995). For example, the enzyme β -glucuronidase is involved in the release in the colon, from their conjugated form, of a number of dietary carcinogens,

including polycyclic aromatic hydrocarbons. Similarly, bacterial β -glucosidase hydrolyzes the plant glycoside cypasin to a carcinogen in the gut. It should be noted however that glycoside hydrolysis by intestinal microflora can result in the generation of potential anti-carcinogenic and anti-mutagenic substances in the form of flavonoids such as quercetin (Rowland, 1995). A major role for the intestinal microflora has been identified in the metabolism of the bile acids cholic and chenodeoxycholic acids to deoxycholic and lithocholic acids, which are thought to possess tumour-promoting activity. Other potential tumour-promoters, namely ammonia, phenols and cresols, are also generated by deamination of amino acids such as tyrosine by intestinal bacteria.

The reaction of nitrite with secondary amines and amides can lead to the formation of *N*-nitroso compounds, many of which possess mutagenic and carcinogenic activity. There is evidence from germ-free rat studies that nitrosation can occur under neutral pH conditions by an enzymic process catalysed by intestinal bacteria (Massey *et al* 1988). Another bacterially-catalysed reaction yielding a reactive substance capable of causing DNA damage and mutation, is the conversion of the cooked food carcinogen 2-amino-3-methyl-3H-imidazo(4,5-*f*)quinoline (IQ) to its 7-hydroxy derivative. The latter, unlike its parent compound is a direct-acting mutagen (Carman *et al*, 1988).

In general, species of *Bifidobacterium* and *Lactobacillus*, have low activities of these enzymes involved in carcinogen formation and metabolism by comparison to other major anaerobes in the gut such as bacteroides, eubacteria and clostridia (Saito *et al* 1992). This suggests that increasing the proportion of LAB in the gut could modify, beneficially, the levels of xenobiotic metabolising enzymes. Studies have been carried out in laboratory animals and humans in order to acquire a greater understanding of the way in which administration of specific probiotics and prebiotics affect gut microflora metabolism.

Table 1. Effects of Probiotics and Prebiotics on Bacterial Enzyme Activity and Metabolic End Products in Laboratory Animals

Species	Endpoint	Pro/ prebiotic	Result	Author
F344 rat	Faecal β -glucuronidase	<i>L. acidophilus</i> (10^9 - 10^{10} cells/day)	Decreased the activity of β -glucuronidase by 40-50%	Goldin and Gorbach (1976)
Lister hooded rat (HFA)	β -glucuronidase and β -glucosidase activity	<i>L. acidophilus</i> or <i>B. adolescentis</i> (10^9 cells/day for three days)	A significant decrease in enzyme activity for <i>L. acidophilus</i> only	Cole <i>et al</i> (1989)
F344 rat	Faecal levels of enzyme reaction products after administration of test substances	<i>L. acidophilus</i>	Animals given <i>L. acidophilus</i> had significantly lower free amines in faeces and 50% less of conjugates	Goldin and Gorbach (1984)
Rat	Faecal SCFA levels	Neosugar (10-20% in diet)	Significantly increased SCFA concentration in faeces	Tokunga <i>et al</i> (1986)
Germ free Lister hooded rat	Various caecal enzymes	TOS (5%w/w in diet for 4 weeks) or TOS + <i>B. breve</i>	β -glucuronidase and nitrate reductase activities, pH and the conversion of IQ to 7-OHIQ significantly reduced in caecal contents of the TOS-fed rats. Bacterial β -glucosidase activity was increased in TOS fed rats	Rowland and Tanaka (1993)
Male Sprague-Dawley rats	Faecal enzymes and ammonia	<i>B. longum</i> (freeze dried) and inulin (5%)	Significant decrease in β -glucuronidase and ammonia. Probiotic plus prebiotic was more effective	Rowland <i>et al</i> (1998)

Table 2. Effects of Probiotics and Prebiotics on Bacterial Enzyme Activities and End Products in Humans

Subjects	Endpoint	Probiotic/ prebiotic	Result	Author
21 healthy subjects	Faecal enzyme activities	Milk supplemented with <i>L. acidophilus</i> (1×10^9 viable bacteria per day)	Faecal β -glucuronidase activity was reduced from 1.7-2.1 units to 1.1 units in all subjects	Goldin and Gorbach 1984(a)
14 colon cancer patients	Faecal β -glucuronidase activity	<i>L. acidophilus</i> (given as a fermented product, between 1.5×10^{11} and 6×10^{11} CFU/ day)	A 14% decrease in mean β -glucuronidase activity after two weeks	Lidbeck <i>et al</i> (1991)
20 healthy male subjects (40-65 years old)	Faecal β -glucuronidase and β -glucosidase activity	<i>L. casei</i> (strain Shirota) 3×10^{11} CFU/ day	Significant decrease in β -glucuronidase and β -glucosidase activity ($P < 0.05$)	Spanhaak <i>et al</i> (1998)
9 healthy adults	Faecal β -galactosidase β -glucosidase and β -glucuronidase	Olifus™ (a commercial fermented milk containing <i>L. acidophilus</i> (3×10^9 bacteria/day) strain A1, <i>B. bifidum</i> B1 (3×10^{10} bacteria/ day), <i>Streptococcus lactis</i> and <i>S. cremoris</i> (3×10^{10} bacteria/day)	No change in faecal β -galactosidase and β -glucuronidase. Significant increase β -glucosidase activity	Marteau <i>et al</i> (1990)
3 male and 9 female healthy subjects	Faecal β -glucosidase and β -glucuronidase activity	Digest™ containing viable <i>L. acidophilus</i> (strain DDS1) (3 eight oz cups of milk containing 2×10^6 CFU/ml per day)	A decrease in β -glucuronidase and β -glucosidase activity	Ayebo <i>et al</i> (1980)
21 young women aged 21-35 years with severe premenstrual syndrome	Nineteen faecal enzyme activities	<i>L. acidophilus</i> and <i>B. bifidum</i> (1×10^9 of each type of bacteria / capsule – three capsules per day	Reduction in β -glucosidase activity. A decrease in β -glucuronidase activity	Bertazzoni -Minelli <i>et al</i> (1996)
Human volunteers	Viable bacterial count	Soy bean oligosaccharides (SOE) 10g/ day	No significant difference in levels of <i>p</i> -cresol, indole or phenol between various dietary periods	Hayakawa <i>et al</i> (1990)

Studies in Laboratory Animals

The effects of probiotics and prebiotics on gut bacterial enzymes have been studied in conventional microflora animals and also germ free rats associated with a human faecal microflora, so called 'Human Flora Associated (HFA) rats. These studies are summarised in Table 1 and some examples are discussed in more detail below.

In a conventional rat study, supplementation of a high meat diet (72% beef) with *Lactobacillus acidophilus* (10^9 – 10^{10} organisms/day) significantly decreased by 40 – 50% the activity of faecal β -glucuronidase and nitroreductase (Goldin and Gorbach 1976). Interestingly the modulating effect of the lactobacillus strain was dependent on the type of basal diet fed – no significant effects were seen when the rats were fed a grain based diet. In an analogous study in HFA rats, Cole *et al* (1989) demonstrated a significant reduction in β -glucuronidase and β -glucosidase activities when *L. acidophilus* was fed for 3 days, with the effect persisting for 7 days after dosing ceased.

These changes in enzyme activities seen after consumption of LAB would in theory be expected to result in changes in rates of metabolism of their substrates *in vivo*, although only if the enzymes catalysed the rate limiting step in their metabolism. Goldin and Gorbach (1984) have confirmed this by showing that a reduction in activity of the bacterial enzymes nitroreductase, azoreductase and β -glucuronidase in rats given oral lactobacilli, was matched by a decrease (of about 50% in comparison to controls) in the excretion in urine of the reaction products of the enzymes.

Studies on the influence of NDOs on gut bacterial enzyme activities in laboratory animals have concentrated on fructo-oligosaccharides and galacto-oligosaccharides. In conventional microflora rats fed a purified diet containing tyrosine and tryptophan, incorporation of a fructo-oligosaccharide ('Neosugar') into the diet at 0.4 – 10%

reduced the faecal concentration of the potential tumour promoter *p*-cresol (Hidaka *et al* 1986). The effect was related to dose of the NDO. Higher dietary concentrations of Neosugar (up to 20%) were found to increase short chain fatty acids and total daily excretion of neutral and acid sterols (Tokunaga *et al* 1986).

The effect of ingestion of trans-galactosylated oligosaccharide (TOS; 5% w/w in diet for 4 weeks) with or without *Bifidobacterium breve*, was studied in HFA rats (Rowland and Tanaka 1993). In the TOS-fed animals, an increase in bifidobacteria and lactobacilli numbers in the caecum was seen, associated with significant decreases in β -glucuronidase and nitrate reductase activities, pH and the conversion of IQ to its directly genotoxic derivative 7-OHIQ. Bacterial β -glucosidase activity was increased presumably as a consequence of elevated numbers of LAB which have a high activity of this enzyme. No evidence for additive or synergistic effects of *B. breve* consumption on enzyme activities was detected. In contrast, in a recent study by Rowland *et al* (1998) in rats given *B. longum*, inulin or both, an increased effect of the synbiotic combination on enzyme activities and faecal bacterial metabolites was reported. For example, feeding of *B. longum* alone resulted in a 30% decrease in activity of β -glucuronidase whereas a 55% decrease was seen in rats given diet supplemented with the probiotic/prebiotic combination.

Studies in Human Subjects

A number of studies have been carried out on the effects of pro-, pre-, and syn-biotics on human subjects which have included measurement of bacterial enzyme activities (Table 2). Goldin and Gorbach (1984) studied volunteers consuming milk supplemented with 10^9 viable lactobacilli per day. Prior to lactobacillus feeding, faecal β -glucuronidase activity ranged between 1.7-2.1 units. This

declined in all 21 subjects after consumption of lactobacilli to a mean value of 1.1 units. The activity returned to baseline values 10 days after consumption of LAB ceased. Lidbeck *et al* (1991) supplemented the diets of 14 colon cancer patients with *L. acidophilus* as a fermented milk product (approximately 3×10^{11} lactobacilli per day) for a period of six weeks and faecal microflora, faecal bile acids and β -glucuronidase activity were measured. Coincident with changes in microflora (an increase of lactobacilli in faeces and a decrease in the numbers of *E. coli*) was a 14% decrease in β -glucuronidase activity. A decrease in total bile acids and deoxycholic acid of 15% and 18%, respectively was also observed. Similar results were obtained by Spanhaak *et al* (1998) who reported a significant decrease in the activity of faecal β -glucuronidase and β -glucosidase activity in a group of twenty healthy male subjects given *L. casei* (approximately 10^{11} CFU/day for a four week test period).

Marteau *et al* (1990) studied nine healthy volunteers before (period 1), during (period 2), and after (period 3) ingesting 100g/day of a fermented milk product ('Olifus') containing *L. acidophilus* (10^7 CFU/g), *Bifidobacterium bifidum* (10^8 CFU/g) and *Streptococcus (Lactococcus) lactis* (10^8 CFU/g) and *S. cremoris (Lactococcus lactis* subsp. *cremoris*) (10^8 CFU/g) for three weeks. Faecal azoreductase and β -glucuronidase activities did not change throughout the three periods. Nitroreductase activities dropped significantly in period 2 and remained at a low level during period 3. There was no change in β -galactosidase activity but β -glucosidase activity significantly increased in period 2 and returned to baseline levels in period 3. *In vitro* the dairy product showed a high β -glucosidase activity that was related to the presence of *B. bifidum*. The decrease in nitroreductase activity still persisted 3 weeks after cessation of ingestion of the fermented dairy product suggesting more prolonged modifications of the colonic microflora.

Ayebo *et al* (1990) assessed the effect of consumption of non-fermented milk containing *Lactobacillus acidophilus* (2×10^6 CFU/ml) on faecal β -glucuronidase and β -glucosidase in a cross-over study in elderly human subjects. Low fat milk was given as a control and diets were consumed for a period of four weeks. Faecal counts of lactobacilli rose during the period of probiotic consumption by approximately one order of magnitude. β -glucuronidase activity decreased slightly after four weeks of lactobacillus feeding. Inconsistencies in β -glucosidase activity were evident as the activity decreased from 0.9 units to 0.45 units during one period of exposure whereas during another period there was no change in activity.

In a study on young women with premenstrual syndrome, Bertazzoni-Minelli *et al.*, (1996) found that consumption of lyophilised *L. acidophilus* and *B. bifidum* (1×10^9 of each type of bacterium / capsule) was associated with only minor changes in β -glucosidase and no significant effects on β -glucuronidase.

Effects in human studies of prebiotics and synbiotics on toxic bacterial metabolites in faeces are few and generally have yielded inconsistent or negative results. Tanaka *et al* (1983) reported no effect of TOS (3 or 10g/day) on faecal ammonia, but did show that simultaneous ingestion of TOS and *B. breve* reduced ammonia concentration in 4 out of 5 subjects.

A study in human volunteers given soy bean oligosaccharides (SOE); (10g/day) with or without simultaneous consumption of *B. breve* demonstrated no significant effects on faecal pH or amino acid breakdown products (*p*-cresol, phenol and indole), despite changes in faecal bifidobacteria numbers (Hayakawa *et al* 1990).

Table 3. Antigenotoxicity of Probiotics and Prebiotics *in vitro* and *in vivo*

Target	Endpoint	Mutagen	Probiotic/ prebiotic	Result	Author
<i>Salmonella typhimurium</i> TA 98	<i>In vitro</i> mutagenicity (Ames)	Trp-P-1, Trp-P-2 and Glu-P-1	Lactic acid bacteria isolated from a traditional Chinese cheese	Lyophilized cells of all strains inhibited Trp-P-1 and Trp-P-2 mutagenicity. Some strains inhibited Glu-P-1	Zhang <i>et al</i> (1990)
<i>Salmonella typhimurium</i> . TA 1538	<i>In vitro</i> mutagenicity (Ames)	Nitrosated beef extract	Ten isolated <i>Lactobacillus</i> strains	<i>L. casei</i> and <i>L. lactis</i> inhibited mutation by > 85% and <i>L. sake</i> and <i>L. confusus</i> had no effect	Pool-Zobel <i>et al</i> (1993)
<i>Salmonella typhimurium</i>	<i>In vitro</i> mutagenicity (Ames)	Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, Trp-P-1, Trp-P-2	22 strains of intestinal bacteria	The majority of strains inhibited mutagenicity	Morotomi <i>et al</i> (1986)
<i>Salmonella typhimurium</i> TA100 and TA97	<i>In vitro</i> mutagenicity (Ames)	Nitrovin and 2-aminofluorene	Nine strains of LAB	Significant anti-genotoxic activity exerted by six of the nine strains tested	Ebringer <i>et al</i> (1994)
Female, 4 week old BALB/c mice	<i>In vitro</i> binding and <i>in vivo</i> mutagenicity in liver	B(a)P, AFB1, IQ, MeIQ, MeIQx, PhIP and Trp-P-2	<i>L. acidophilus</i> and <i>B. longum</i>	Bacterial strains tested were able to bind carcinogens <i>in vitro</i> . No effect on <i>in vivo</i> mutagenicity or absorption	Bolognani <i>et al</i> (1997)
Male Sprague-Dawley rats	<i>In vivo</i> DNA damage in colon (Comet assay)	MNNG and DMH	<i>L. acidophilus</i> , <i>L. gasseri</i> , <i>L. confusus</i> , <i>B. longum</i> , <i>B. breve</i> , <i>S. thermophilus</i> , <i>L. acidophilus</i>	Most LAB tested strongly inhibited genotoxicity in the colon. <i>S. thermophilus</i> had no effect. Heat treatment abolished probiotic effect	Pool-Zobel <i>et al</i> (1996)
F344 rats (human flora associated)	<i>In vivo</i> DNA damage in colon (Comet assay)	DMH	Lactulose (3% in diet)	Lactulose significantly decreased extent of DNA damage ($P < 0.05$)	Rowland <i>et al</i> (1996)

Table 4. Effect of Probiotics/Prebiotics and Synbiotics on Colonic Aberrant Crypt Foci in Laboratory Animals

Species	Carcinogen	Probiotic/Prebiotic	Stage of exposure to Pro/Prebiotic*	Result	Author
Male F344 rats	AOM (s.c.)	Lyophilized <i>B. longum</i> (1.5% and 3% dietary)	Initiation and promotion	Significant inhibition of total ACF ($P < 0.01$). Significant reduction in total AC per colon ($P < 0.001$)	Kulkarni and Reddy (1994)
Male F344 rats	AOM (s.c.)	<i>B. longum</i> (1×10^8 cells/ g of feed, rats fed <i>ad libitum</i>) lactulose (2.5%) or both	Initiation and promotion	Significant reduction in ACF in rats consuming BI, L, BI + L ($P, 0.05$). Rats fed BI+L had significantly fewer ACF than rats consuming BI or L alone	Challa <i>et al</i> (1997)
Male F344 rats	DMH (i.p.)	<i>Bifidobacterium</i> sp. (6×10^9 cells/animal/day) in cell suspension, or fermented milk. Skim milk powder.	Initiation and promotion	Significant ($P < 0.05$) inhibition of AC: 61% (<i>Bifidobacterium</i>) 51% (skim milk) 49% (fermented milk)	Abdelali <i>et al</i> (1995)
Male Sprague-Dawley rats.	AOM (s.c.)	<i>B. longum</i> (4×10^8 viable cells/g of diet) or inulin (5%w/w diet).	Promotion	Total ACF decreased by 74% in rats treated with probiotic + prebiotic (synbiotic effect). Numbers of the large ACF (> 4 AC per focus) were significantly decreased ($P < 0.05$) by 59% in rats fed probiotic + prebiotic	Rowland <i>et al</i> (1998)
Male wistar rats	DMH (gavage)	Skim milk, skim milk + bifidobacteria (10^9 /day) skim milk + fructooligosaccharide and skim milk + bifidobacteria + fructooligosaccharide; <i>L. acidophilus</i> (10^8)	Promotion	Inconsistent results	Gallaher <i>et al</i> (1996)
Weanling male F344 rats	AOM (s.c.)	Inulin (10%) in diet	Initiation and promotion	No significant effect on ACF but reduced the number of AC/cm ²	Rao <i>et al</i> (1998)
F344 rats	AOM (s.c.)	<i>L. acidophilus</i> NCFMTM (lyophilized) in diet	Initiation and promotion	Significant suppression of colonic ACF	Rao <i>et al</i> (1999)
Male F344 rats	AOM (s.c.)	Oligofructose (10%) or inulin (10%) in diet	Initiation and promotion	Significant inhibition of ACF/ colon – more pronounced for inulin ($P < 0.0006$) than for oligofructose ($P < 0.02$). Crypt multiplicity also inhibited in animals fed inulin ($P < 0.02$) or oligofructose ($P < 0.04$)	Reddy <i>et al</i> (1997)

*The 'initiation and promotion' protocol involves feeding the probiotic for about 1 week, followed by dosing with the carcinogen and then continued probiotic administration until animals are sacrificed prior to ACF assessment. In the 'promotion' protocol, the rats are dosed with carcinogen prior to probiotic treatment.

s.c. = subcutaneous; i.p. = intraperitoneal

Anti-Genotoxicity of Probiotics and Prebiotics *in vitro* and *in vivo*

More direct evidence for protective properties of probiotics and prebiotics against cancer has been obtained by assessing the ability of cultures to prevent DNA damage and mutations (which is considered to be an early event in the process of carcinogenesis) in cell cultures or in animals (Table 3).

The effect of LAB on the induction of mutations by a wide variety of model carcinogens *in vitro* has been studied using the Ames Salmonella assay. The carcinogens used include N-nitrosocompounds N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and N-methylnitrosourea (MNU) heterocyclic amines (*e.g.* IQ and related compounds) and aflatoxin B1. Overall the results indicate that the various LAB can inhibit genotoxicity of dietary carcinogens *in vitro*. The degree of inhibition was strongly species dependent. For example Pool-Zobel *et al* (1993) demonstrated that *Lactobacillus casei* and *L. lactis* inhibited the mutagenic activity of nitrosated beef by over 85% whereas *Lactobacillus confusus* (*Weissella confusa*) and *Lactobacillus sake* had no effect.

It seems likely that these results together with similar results by other workers, are a consequence of binding of

the mutagens by the LAB (Zhang *et al*, 1991 Bolognani *et al* 1997). Whether such a mechanism operates *in vivo* is questionable, since binding appears to be highly pH dependent and easily reversed and does not appear to affect uptake of carcinogens from the gut, neither does it have any apparent *in vivo* effect on mutagenicity in the liver (Bolognani *et al*, 1997).

Using the technique of single cell microgel electrophoresis (Comet assay), Pool-Zobel *et al* (1996) investigated the ability of 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 - *L. acidophilus* (isolated from a yoghurt), *Lactobacillus gasseri*, *L. confusus*, *B. breve* and *Bifidobacterium longum*, prevented MNNG-induced DNA damage when given at a dose of 10^{10} cells/kg body weight, 8 hours before the carcinogen. In most cases the DNA damage was reduced to a level similar to that in untreated rats. *Streptococcus thermophilus* was not as effective as the other LAB strains.

The protective effect was dose dependent: doses of *L. acidophilus* representing 50 and 10% of the original dose were less effective in reducing MNNG-induced DNA damage. Importantly, heat-treatment of *L. acidophilus* abolished its antigenotoxic potential indicating the

importance of viable cells. Similar results were obtained when the LAB strains were tested in rats given DMH as the DNA damaging agent. Again, all the lactobacilli and bifidobacteria strongly inhibited DNA damage in the colon mucosa, whereas *S. thermophilus* was much less effective. There was evidence of strain differences in antigenotoxic effects: Of three strains of *S. thermophilus*, two were ineffective and one exhibited protection against DNA damage.

The Comet assay has also been used to evaluate the effect of a prebiotic, lactulose, on DNA damage in the colonic mucosa. 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 (Rowland *et al.*, 1996).

The above results provide evidence that both LAB and prebiotics may have protective effects against the early stages of colon cancer.

Effect of Probiotics and Prebiotics on Pre-Cancerous Lesions in Laboratory Animals (Table 4)

Aberrant crypts (AC) are putative pre-neoplastic lesions seen in the colon of carcinogen-treated rodents. In many cases a focus of two or more crypts is seen and is termed an aberrant crypt focus (ACF). Aberrant crypts are induced in colonic mucosa of rats and mice by treatment with various colon carcinogens such as azoxymethane (AOM), DMH and IQ. The findings of significantly more ACF with four or more crypts in rats with tumours compared with those without tumours suggests that large ACF may be a predictor of eventual tumour incidence (Pretlow *et al.*, 1992). Studies have employed various treatment regimes with differences in the sequence of exposure to carcinogen and probiotic/prebiotic to allow conclusions to be drawn about the stage of carcinogenesis affected. In the majority of studies the protocol involved feeding the probiotic for about 1 week, followed by dosing with carcinogen and then continued probiotic administration until animals were killed prior to ACF assessment. Thus the probiotic treatment covered both initiation and early promotion stages. In the 'promotion' protocol, the rats were dosed with carcinogen prior to probiotic treatment.

Effect of Probiotic Treatment Alone

A variety of studies have been carried out using AOM or DMH to determine the effects of specific probiotics on ACF formation. Kulkarni and Reddy (1994) reported inhibition in ACF formation of about 50% when male F344 rats were fed *B. longum* in the diet (1.5% and 3% of a lyophilised culture containing 2×10^{10} CFU/g) for 5 weeks and injected subcutaneously with AOM once weekly for 2 weeks. Since dietary treatments were started 5 weeks prior to administration of the carcinogen dose results do not allow deductions to be made about the stage of carcinogenesis affected. There were no differences between the animals fed the 1.5% and 3% *B. longum* diets.

A similar study was carried out by Challa *et al.* (1997) who observed a 23% reduction in total colonic ACF and a 28% reduction in total AC in rats given a diet containing 0.5% *B. longum* (1×10^8 viable cells/g of feed). Animals were fed the experimental diet before treatment with AOM and throughout the experiment.

Abdelali *et al.* (1995) compared the effects of *Bifidobacterium* species administered in diet and also fed as a fermented milk product. The amounts of organisms consumed were similar (6×10^9 cells/day). DMH was given 4 weeks after the LAB and the latter treatments continued for a further 4 weeks before ACF assessment. The dietary bifidobacteria appeared to be slightly more effective in reducing ACF than the bifidobacteria - fermented milk (61% and 49% reduction respectively). Interestingly however, skim milk alone reduced ACF numbers by 51%.

Rowland *et al.* (1998) in a study of *B. longum* (6×10^9 CFU/day) in AOM-treated Sprague Dawley rats, demonstrated a significant reduction of 26% in total ACF by comparison to control animals. The changes were seen only in small ACF (1-3 AC per focus). Since the probiotic treatment began 1 week after the carcinogen exposure, the results indicate an effect on the early promotional phase of carcinogenesis.

Not all ACF studies with probiotics have yielded positive effects. Gallagher *et al.* (1996), who used a 'promotion' protocol with *B. longum* and *L. acidophilus*, obtained inconsistent results, which they attributed to differences in ages of rats when DMH was administered.

Table 5. Effect of Probiotics and Prebiotics on Tumour Incidence in Laboratory Animals

Species	Endpoint	Carcinogen	Probiotic/ prebiotic	Result	Author
F344 rats	Incidence of colon tumours	DMH	<i>L. acidophilus</i>	Colon tumour incidence lower in probiotic fed animals (40% vs 77% in controls)	Goldin and Gorbach (1980)
F344 rats	Colon, liver, mammary tumours (incidence & multiplicity)	IQ in diet	<i>B. longum</i> (1×10^{10} live bacterial cells in diet)	Suppression of colon ($P < 0.05$), liver ($P < 0.05$) and mammary (NS) tumour incidence	Reddy and Rivenson (1993)
C57BL/CJ-Min/+mice	Colon tumour incidence	N/A	Short chain fructo-oligosaccharides (5.8%)	Significant reduction in colon tumours ($P < 0.01$)	Pierre <i>et al.</i> (1997)
Male F344 rats	Incidence and multiplicity of colon tumours	DMH (s.c.)	<i>Lactobacillus</i> GG ($2-4 \times 10^{10}$ organisms/ day)	Lower incidence of tumours ($P < 0.012$) and tumour multiplicity ($P < 0.001$) when rats given LGG throughout experiment. No effect when LGG given after DMH	Goldin <i>et al.</i> (1996)

s.c = subcutaneous; i.p. = intraperitoneal

Prebiotic and Synbiotic Treatments on Colonic Aberrant Crypt Foci (ACF)

Prebiotics alone appear to give inconsistent results on carcinogen-induced ACF induction which may be partly a consequence of differences in carcinogen and treatment regimes used. For example Rao *et al* (1998) reported that inulin (10% in diet) had no significant effect on total ACF in the colon, or their multiplicity, in F344 rats, although curiously a significant decrease in ACF/cm² of colon was reported. The study by Gallaher *et al* (1996) on *Bifidobacterium* species and FOS (2% in diet) gave inconsistent results with only 1 out of 3 experiments showing a decrease in DMH-induced ACF. Differences in FOS dose and carcinogen treatment regimes may be responsible for the discrepancy between this study and those of Challa *et al* (1997) and Rowland *et al* (1998).

Reddy *et al* (1997) compared short- (FOS) and long-chain (inulin) oligosaccharides incorporated at a level of 10% in the diet on AOM-induced ACF in rats. The NDOs were fed before carcinogen treatment and throughout the experiment and significant decreases of approximately 25% and 35% respectively in total ACF were reported. The decreases seen were almost entirely in the smaller ACF (< 3 AC per focus) and inhibition by inulin appeared to be more pronounced than that of FOS.

Similar results were obtained by Rowland *et al* (1998) who reported a decrease of 41% in small ACF when inulin (5% in diet) was given 1 week after AOM dose. No effect of inulin on large ACF was observed.

Challa *et al* (1997) demonstrated a small reduction (22%) in total ACF in AOM treated F344 rats when the synthetic, non-digestible disaccharide lactulose was incorporated in the diet at 2%. Both Challa *et al* (1997) and Rowland *et al* (1998) studied the effect of combined treatment of probiotic and prebiotic on ACF numbers. The combination of *B. longum* and lactulose resulted in a 48% inhibition of colonic ACF, which was significantly greater

than that achieved by either *B. longum* or lactulose alone (Challa *et al*, 1997). Similarly Rowland *et al* reported a decrease in total ACF of 74% in rats given *B. longum* plus inulin (by comparison to a 29% and 21% reduction achieved by *B. longum* or inulin alone). Importantly, the combined administration of probiotic and prebiotic reduced large ACF by 59%, whereas the individual treatments had no effect (Rowland *et al*, 1998).

Effect of Probiotics and Prebiotics on Tumour Incidence in Laboratory Animals (Table 5)

Goldin and Gorbach (1980) investigated the effect of *L. acidophilus* on colon tumour incidence in rats treated with DMH. A reduction in colon cancer incidence (40% vs 77% in controls) was evident in animals receiving *L. acidophilus* after 20 weeks but no difference was discernible at 36 weeks suggesting that the lactobacilli had increased the latency period, or induction time, for tumours.

Administration of dietary *B. longum* (0.5% lyophilised *B. longum* in diet, 1x10¹⁰ live bacterial cells/ day) significantly inhibited the formation of IQ-induced colon and liver tumours and multiplicity (tumours/animal) of tumours in colon, liver and small intestine in male rats (Reddy and Rivenson 1993). The percentage decrease in tumour incidence was 80% in liver and 100% in colon. In female rats, dietary supplementation with *Bifidobacterium* cultures also decreased the IQ induced mammary carcinogenesis to 50% and liver carcinogenesis to 27% of that on the control diet, but the differences were not significant. There were however significant changes in tumour multiplicity in the mammary gland.

A mouse model has recently been developed (*Min* mice) in which the animals are heterozygous for a non-sense mutation of the *Apc* gene, the murine homologue of APC. These mice, which develop spontaneous adenomas throughout the small intestine and colon within a few weeks

Table 6. Effect of Probiotics and Prebiotics on Cancer in Humans - Epidemiological Studies

Subjects	Type of cancer	Probiotic/prebiotic	Result	Author
289 population controls and 133 breast cancer cases.	Breast cancer	Fermented milk products (yoghurt, buttermilk and kefir).	Fermented milk consumption (> 225g/ day) reduced odds ratio (OR) to 0.5	van't Veer <i>et al</i> (1989)
Control group 182 men and 245 women. Cancer cases 109 men and 62 women.	Small and large colon adenoma, and colon cancer	Yoghurt	Inverse relationship yoghurt consumption (0.5-1/ day) with risk of large adenomas in men and women	Boutron <i>et al</i> (1996)
152 proximal colon cancer patients, 201 distal colon cancer patients and 618 general population controls.	Colon cancer	Fermented milk	Inverse association of colon cancer with the consumption of fermented milk	Young and Wolf (1988)
746 colon cancer patients and 746 controls.	Colon cancer	Yoghurt	Protective effect against colon cancer	Peters <i>et al</i> (1992)
331 men and 350 women with adenomatous polyps of colon/rectum and controls (9,159 men and 8,585 women).	Colorectal adenomas	Fermented dairy products	Inverse association (nonsignificant) between yoghurt consumption and adenomas in men and women	Kampmann <i>et al</i> (1994a)
232 colon cancer patients and 259 population controls.	Adenocarcinoma of colon	Fermented dairy products	Positive, significant association (OR 1.52) in men; negative, nonsignificant association in women	Kampmann <i>et al</i> (1994b)

have been used for testing of chemopreventive agents targeted against cancerous lesions. In one such study *Min* mice were fed various diets containing wheat bran, resistant starch or FOS (5.8% in diet) for 6 weeks. Tumour numbers remained unchanged from the control (fibre free diet) in the mice fed either wheat bran or resistant starch, but a significant reduction in colon tumours was observed in rats receiving the diet supplemented with FOS. Furthermore 4 out of the 10 FOS fed animals were totally free of colon tumours (Pierre *et al*, 1997).

Goldin *et al* (1996) investigated the effect of "Lactobacillus GG" (*Lactobacillus rhamnosus* GG) in DMH treated rats given either before, during and after DMH exposure (initiation + promotion protocol) or after (promotion protocol) the carcinogen treatment. Using the former protocol, a significant decrease was seen in the incidence of colon tumours (71% vs 100% in control rats), and the number of tumours per tumour-bearing animal (1.7 vs 3.7 in controls). However when Lactobacillus GG was administered after DMH, no decrease in tumour incidence was seen indicating that the effect of the LAB was on initiation stage rather than on promotion stage of tumorigenesis. In this study, the rats were fed basal diets either high or low in fat content. Although the decrease in colon tumour incidence induced by the probiotic was similar on the two diets, the effects on tumour multiplicity were more pronounced in the animals fed a high fat diet.

Probiotics and Cancer in Human Epidemiological Studies (Table 6)

A case-control study in the Netherlands showed that certain fermented dairy products may confer a protective effect against breast cancer. The results indicated that consumption of > 225 g per day of fermented dairy products (yoghurt, buttermilk, curds and kefir) reduced the odds ratio to 0.50 (van't Veer *et al*, 1989).

Results from a case-control study by Boutron *et al* (1996) showed a significant ($P=0.03$) inverse relationship between risk of large colonic adenomas in both men and women and consumption of moderate amounts (0.5 – 1 pot per day) of yoghurt. The odds ratios (OR) were 0.6 and 0.5 respectively for the two levels of yoghurt consumption. There was no relationship between colorectal cancer risk and yoghurt consumption. Other population-based case

control studies have provided evidence of inverse associations of colorectal cancer risk and consumption of fermented dairy products (Young and Wolf, 1988) and yoghurt (Peters *et al*, 1992) and Kampmann *et al* (1994a) reported a non-significant inverse relationship between yoghurt consumption and colonic adenomas. This finding, however, was not confirmed in a further case control study in the Netherlands of colorectal cancer risk and fermented dairy products which revealed a small significant positive association in men (OR 1.52) and a small, non-significant inverse association in women (OR 0.77) (Kampmann *et al* 1994b).

Probiotics and Cancer in Human Intervention Studies (Table 7)

Intervention studies to evaluate the ability of probiotics to prevent cancer in humans have been based largely on the use of biomarkers for assessing cancer risk. Due their non-invasive nature, markers in faeces and urine have been mostly used. For example, the aqueous phase of human faeces (faecal water) is considered to be an important source of inducers and modulators of carcinogenesis in the colon and methods exist for assessing biological activities related to cancer risk in such samples.

In order to determine whether the cytotoxicity and genotoxicity of faecal water were affected by a change in dairy product intake, 18 healthy males and females were shifted from their normal dairy product-rich diet to a dairy product-free diet in a cross-over design study (Glinghammar *et al*, 1997). Faecal water cytotoxicity, analysed by the HT29 cytotoxicity assay, indicated a decrease in cell survival from 34% to 20% when dairy products were excluded from the subjects diet. This assay is considered to reflect potential tumour-promoting activity and suggests that dairy products may have beneficial effects. The comet assay (single cell gel electrophoresis) was used to analyse the genotoxicity of faecal water which indicated no differences due to dietary intervention.

Hayatsu and Hayatsu (1993) examined the effect of 3 week oral administration of *L. casei* in 6 healthy non-smokers on the urinary mutagenicity of dietary fried ground beef using the Ames assay (*Salmonella typhimurium* TA 98, with S9 mix). The 6 people were divided into two groups, one group for administration of the *L. casei* 3×10^{10} cells/

Table 7. Effect of Probiotics and Prebiotics on Cancer/Cancer Biomarkers in Human Intervention Studies

Subjects	Endpoint	Probiotic/ prebiotic	Result	Author
18 healthy male and female subjects	Faecal water cytotoxicity in HT29 cells and genotoxicity (Comet assay)	Dairy products vs low dairy product diet	Decreased cytotoxicity of faecal water during high dairy product intake effect. No effect on genotoxicity	Glinghammar <i>et al</i> (1997)
6 healthy subjects	Urinary mutagenicity after fried beef consumption	<i>L. casei</i>	Decrease in urinary mutagenicity ($P < 0.001$)	Hayatsu and Hatatsu (1993)
11 healthy subjects	Urinary and faecal mutagenicity after fried beef consumption	<i>L. acidophilus</i>	Probiotic consumption decreased urinary mutagen excretion by 50% to 70% and faecal mutagen excretion by 30%	Lidbeck <i>et al</i> (1992)
20 patients with colonic adenomas	Cell proliferation in rectal mucosa biopsies	<i>L. acidophilus</i> and <i>B. bifidum</i>	LAB administration reduced rectal proliferation only in patients with high basal proliferation rates	Biasco <i>et al</i> (1991)

day and another group for supplementation with *L. casei* 1.5×10^{11} cells/day for three weeks. Urine was collected before meat meals and 0-12 and 12-24 hour urines were collected after the meat meal. A suppressive effect of *L. casei* administration was observed (6-67% of the control group mutagenicity) when control urinary mutagenicity was compared to test sample results. The average decrease in activity (12 hour urine collection stage) was 47.5% and the decrease was statistically significant.

Lidbeck *et al* (1992) carried out a study involving 11 subjects fed fried hamburgers as part of their diet (days 0-2) which are a source of pyrolysate mutagens detectable in urine. "Lactococcus milk" was given as a control (10^{10} - 10^{11} /day) two days prior to dietary supplementation with fried hamburgers until 6 days after. The probiotic *Lactobacillus acidophilus* was given to the second group at a dose of 1.5×10^{11} cells per day starting again two days before the hamburger addition and lasting for a further 6 days afterwards. Consumption of LAB decreased urinary excretion of mutagens by 50% to 70% and excretion of faecal mutagens was decreased by 30%.

Increased cell proliferation in the mucosal crypts is considered to be a marker of elevated cancer risk. In a study of the effect of LAB on cell proliferation in the rectal mucosa, Biasco *et al*, (1991) administered six capsules containing 10^9 *L. acidophilus* and 10^9 *B. bifidum* daily for a period of 3 months to 20 patients with colonic adenomas. Four rectal biopsies were taken at baseline and after treatment, and cell proliferation in the upper part of the rectal mucosal crypts was assessed by tritiated thymidine incorporation. Overall, no significant differences were detected in crypt cell proliferation before and after treatment. Eight patients having elevated cell proliferation rates, however, showed a significant decrease in proliferation after LAB (0.21 ± 0.03 vs 0.10 ± 0.03 , $P < 0.03$).

Aso *et al* (1995) investigated the administration of *L. casei* (Biolactis powder, 3g/day) on the recurrence of superficial transitional cell carcinoma of the bladder after trans-urethral resection in 125 patients. In patients with either primary multiple tumours or single recurrent tumours, the recurrence free rate increased from 54.9% in placebo group to 79.2% in the *L. casei* group. There was no significant effect however in patients with recurrent multiple tumours, who had very poor prognosis. The authors suggest that a stimulation of the immune system by the lactobacilli may be an important factor in its effect on the patients.

Mechanisms of Anticarcinogenicity and Antigenotoxicity

Binding of Carcinogens

There are a large number of reports describing the adsorption or binding *in vitro* by LAB and other intestinal bacteria, of a variety of food-borne carcinogens including the heterocyclic amines formed during cooking of meat, the fungal toxin Aflatoxin B1, benzo(a)pyrene and the food contaminant AF2 (Morotomi and Mutai, 1986; Orrhage *et al* 1994; Zhang *et al* 1990; Zhang and Ohta 1991; Bolognani *et al* 1997). 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.

However, although binding represents a plausible mechanism for the inhibition of genotoxicity and mutagenicity by LAB *in vitro*, it does not appear to have any influence *in vivo*. Bolognani *et al* (1997) demonstrated that simultaneous administration to mice of LAB with various carcinogens had no effect on absorption of the compounds from the gastrointestinal tract, nor did it affect the *in vivo* mutagenicity of the carcinogens in the liver. It should be noted that these results conflict with those of Zhang and Ohta (1993), who found that absorption from the rat small intestine of Trp-P-1 was significantly reduced by co-administration of freeze-dried LAB. However, the latter study was confounded by the use of rats that had been starved for 4 days, which would induce severe nutritional and physiological stresses on the animals.

Effects on Bacterial Enzymes, Metabolite Production

The studies listed in Tables 1 and 2 demonstrate that the increase in concentration of LAB as a consequence of consumption of LAB and/or prebiotics leads to decreases in certain bacterial enzymes purported to be involved in synthesis or activation of carcinogens, genotoxins and tumour promoters. This would appear to be due to the low specific activity of these enzymes in LAB (Saito *et al* 1992). Such changes in enzyme activity or metabolite concentration have been suggested to be responsible for the decreased level of preneoplastic lesions or tumours seen in carcinogen-treated rats given pro and pre biotics (Reddy and Rivenson 1993; Rowland *et al* 1998). Although a causal link has not been demonstrated, this remains a plausible hypothesis.

Stimulation of Protective Enzymes

Many of the food-borne carcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons, are known to be conjugated to glutathione, which appears to result in inactivation. The enzyme involved, glutathione transferase (GSH), is found in the liver and in other tissues including the gut. Challa *et al* (1997) in a study of the effect of *B. longum* and lactulose on AOM-induced ACF in the colon, showed that the activity of GSH in the colonic mucosa was inversely related to the ACF numbers. Such a mechanism of protection would be effective against a wide range of dietary carcinogens

Increase in Immune Response

Another mechanism suggested by Perdigon *et al* (1998) by which probiotics exert anti-tumour activity is by reducing the inflammatory immune response. In a study of tumour growth in DMH treated mice, yoghurt was found to suppress the inflammatory immune response with an increase in IgA secreting cells and in CD4+ T lymphocytes. In those animals, a marked reduction in tumours was seen. An immune mechanism was also proposed to explain the increase in time before tumour recurrence in bladder cancer patients given *L. casei* (Aso *et al* 1995) although no supporting evidence for the mechanism was presented. Such studies are consistent with the work of Schiffrin *et al* (1996) and Marteau *et al* (1997) who have provided evidence of modulation of the immune system in human subjects consuming probiotics. The changes seen were increased phagocytic activity of monocytes and

granulocytes and increases in levels of antibody secreting cells. The significance of these changes in relation to tumour development has not been established.

Conclusions

Overall, studies in *in vitro* systems and in a wide range of animal models provide considerable evidence that probiotics, and to a lesser extent prebiotics, have the potential to reduce colon cancer risk. The evidence from humans is less compelling, but nevertheless is suggestive of a cancer-preventing effect of fermented foods. Clearly what is now needed, are carefully controlled intervention studies in human subjects using biomarkers of cancer risk. The data from animal studies would suggest that using a combination of pro- and prebiotics may be the most effective strategy to maximize any anticarcinogenic effects.

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