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The effects of probiotics on colon cancer development

Joseph Rafter

Department of Medical Nutrition, Karolinska Institutet, NOVUM, S-141 86 Huddinge, Sweden

While several effects beneficial to health have been attributed to the probiotic lactic acid bacteria, perhaps the most interesting and controversial remains that of anti-cancer activity. The vast majority of studies in this area deal with protective effects against colon cancer. There is no direct experimental evidence for cancer suppression in human subjects as a result of the consumption of probiotic cultures in fermented or unfermented dairy products. However, there is a wealth of indirect evidence, based largely on laboratory studies. Reports in the literature, regarding the anti-cancer effects of lactic acid bacteria, fall into the following categories: *in vitro* studies, animal studies, epidemiological studies and human dietary intervention studies. Examples of these reports will be given in the present review. The mechanisms by which probiotic bacteria may inhibit colon cancer are still poorly understood. However, several potential mechanisms are being discussed in the literature and these will also be addressed in the present review.

Colon cancer: Probiotics: Cancer prevention

Introduction

Probiotics, an example of a functional food, have been the focus of intense research activity in recent years and have been defined as 'living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition' (Guarner & Schaafsma, 1998; Ouwehand *et al*. 2002). The term 'probiotics' usually refers to highly selected lactic acid bacteria; for example, *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus* spp. They have defined gut-survival properties and associated biological activities and can be ingested in fermented milk products or as a supplement. The list of beneficial effects attributed to probiotic bacteria is extensive (Salminen *et al*. 1998) and includes: alleviation of lactose-intolerance symptoms; serum cholesterol reduction; anti-cancer effects; alleviating constipation; relieving vaginitis, to name but a few. The vast majority of studies on the anti-cancer effects deal with colorectal cancer (CRC), although some have investigated breast (van't Veer *et al*. 1989) and bladder cancer (Ohashi *et al*. 2002).

Mortality from CRC is second only to that of lung cancer in men and breast cancer in women and has shown little sign of decreasing in the last 20–30 years. Diet makes an important contribution to CRC risk (World Cancer Research Fund/American Institute for Cancer Research, 1997) implying that the risks of CRC are potentially reducible. Evidence from a wide range of sources supports the view that the colonic microflora are involved in the aetiology of CRC. This has led to an intense interest in factors that can modulate the gut microflora and their metabolism. These are: probiotics, prebiotics ('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'; Salminen *et al*. 1998), and synbiotics (combinations of pro- and prebiotics).

Evidence for the protective effects of pro- and prebiotics against cancer is derived from *in vitro* studies, animal models, epidemiology and human intervention studies.

Overall, the supportive evidence is stronger for probiotics than prebiotics (possibly because the latter have only recently come to prominence) and is recently suggestive that synbiotics are more effective than either pro- or prebiotics alone. The evidence from animal studies provides the strongest support for anti-cancer effects and data from human studies (epidemiology and experimental) are limited.

Evidence from human studies

The consumption of lactobacilli by healthy volunteers has been shown to reduce the mutagenicity of urine and faeces associated with the ingestion of carcinogens in cooked meat. When *Lactobacillus acidophilus* was given to eleven volunteers on a fried-meat diet known to increase faecal mutagenicity, a lower faecal mutagenic activity (not significant) was noted on day 3 compared with day 3 when fried meat

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido(4,3-*b*)indole.

Corresponding author: Dr Joseph Rafter, fax +46 8 711 66 59, email joseph.rafter@mednut.ki.se

and ordinary fermented milk were given (Lidbeck *et al*. 1992). High levels of mutagenicity also appeared in urine on days 2 and 3 of the fried-meat and ordinary fermented milk dietary regimen. During *L. acidophilus* administration, the urinary mutagenic activity on days 2 and 3 was significantly lower compared with the ordinary fermented milk period. In most cases, an increase in the number of faecal lactobacilli corresponded to a lower mutagen excretion, particularly in urine. Hayatsu & Hayatsu (1993) also demonstrated a marked suppressing effect of orally administered *L. casei* on the urinary mutagenicity arising from the ingestion of fried ground beef in human volunteers.

As yet, there are few epidemiological studies addressing the association between fermented dairy products and CRC. The consumption of large quantities of dairy products such as yoghurt and fermented milk containing *Lactobacillus* or *Bifidobacterium* may be related to a lower incidence of colon cancer (Shahani & Ayebo, 1980). An epidemiological study performed in Finland demonstrated that, despite the high fat intake, colon cancer incidence was lower than in other countries because of the high consumption of milk, yoghurt, and other dairy products (Intestinal Microecology Group, International Agency for Research on Cancer, 1977; Malhotra, 1977). In two population-based case–control studies of colon cancer, an inverse association was observed for yoghurt (Peters *et al*. 1992) and cultured milk consumption (Young & Wolf, 1988), adjusted for potential confounding variables. In another case–control study, an inverse relationship for yoghurt consumption with the risk of large colon adenomas in men and women was reported (Boutron *et al*. 1996). It can also be mentioned that an inverse relationship has been demonstrated between the frequency of consumption of yoghurt and other fermented milk products and breast cancer in women (Le *et al*. 1986; van't Veer *et al*. 1989). On the other hand, two companion American prospective studies, the 1980–1988 follow-up of the Nurses' Health Study and the 1986–1990 Health Professionals follow-up study, did not provide evidence that the intake of dairy products is associated with a decreased risk of colon cancer (Kampman *et al*. 1994*a*). In a cohort study in The Netherlands, it was shown that the intake of fermented dairy products was not significantly associated with CRC risk in an elderly population with a relatively wide variation in dairy product consumption, although a weak non-significant inverse association with colon cancer was observed (Kampman *et al*. 1994*b*). In summary, it would appear that the case–control studies indicate protective effects while the prospective studies do not.

In conclusion, data from human intervention studies are of paramount importance in providing evidence that probiotics, prebiotics or fermented milk consumption are causally related to a reduction in cancer risk. Thus, this is an area of high priority for future studies. Presently, however, the lack of well-validated biomarkers for colon cancer limits the relevance of such studies although a wide range of potential biomarkers of risk are under development. Once such markers are available, it will become possible to perform studies in healthy volunteers, at-risk groups and patients. It will be important to define dose and time relationships and it would appear at present, from animal studies, the most profitable approach would be to use combinations of pro- and prebiotics. There will also be, in the near future, the opportunity to exploit genomics and proteomics in investigations of the effects of pro- and prebiotics on gene expression and post-transcription events in colonic biopsies and to identify human groups responsive to pro- and prebiotic intervention. It will also be particularly important to use data on mechanisms of action to develop hypothesis-based intervention studies in human subjects.

Of relevance here is a clinical trial which is presently ongoing, i.e. to examine the effect of a synbiotic preparation on colon cancer risk biomarkers in human participants. The SYNCAN project is funded by the European Union, and involves eight research centres in Europe (SYNCAN, 2004). It involves a 12-week randomised, double-blind, placebo-controlled trial of a food supplement containing *Lactobacillus* GG, *Bifidobacterium* Bb-12 and Raftilose Synergyl in adenoma patients. In this study, all of the 'state of the art' colon cancer risk biomarkers, including colonic mucosal markers, faecal water markers and immunological markers, are being measured. It is hoped that the results of the SYNCAN study will provide much-needed information on the cancer-protective effects of synbiotics in man and supply us with additional valuable information on the underlying mechanisms.

Evidence from laboratory animal studies

There are several good animal models for colon cancer that have proved useful for identifying dietary factors which may protect us against the development of this tumour. The end points used are the tumours themselves or early lesions, such as aberrant crypt foci (ACF). ACF are putative pre-neoplastic lesions from which adenomas and carcinomas may develop. In recent years, there have been many studies, using these models, which clearly demonstrate a protective effect of dietary supplements of lactic acid bacteria against colon tumour development.

The oral administration of lactic acid bacteria has been shown to effectively reduce DNA damage, induced by chemical carcinogens, in gastric and colonic mucosa in rats. Pool-Zobel *et al*. (1996) reported, using the comet assay, that *L. acidophilus*, *L. gasseri*, *L. confusus*, *Streptococcus thermophilus*, *Bifidobacterium breve* and *B. longum* were antigenotoxic toward *N*'-nitro-*N*-nitrosoguanidine. These bacteria were also protective toward 1,2-dimethylhydrazine (DMH)-induced genotoxicity. Metabolically active *L. acidophilus* cells, as well as an acetone extract of the culture, prevented *N*'-nitro-*N*-nitrosoguanidine-induced DNA damage, while heat-treated *L. acidophilus* was not antigenotoxic. Among different cell fractions from *L. acidophilus*, the peptidoglycan fraction and whole freeze-dried cells were antigenotoxic.

Certain strains of lactic acid bacteria have also been found to prevent putative pre-neoplastic lesions or tumours induced by carcinogens. Goldin *et al*. (1996) showed that a specific strain of *L. casei* subsp. *rhamnosus* designated GG can interfere with the initiation or early promotional stages of DMH-induced intestinal tumorigenesis and that this effect is most pronounced for animals fed a high-fat diet. Overnight cultures of *L. acidophilus* also inhibited the formation of ACF, induced by azoxymethane (AOM)

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(Arimochi *et al*. 1997). Although *B*. *adolescentis* culture and its supernatant fraction did not show an inhibitory effect in the study of Arimochi *et al*. (1997), the feeding of bifidobacteria suppressed the ACF formation induced by AOM (Kulkarni & Reddy, 1994; Challa *et al*. 1997) or DMH (Abdelali *et al*. 1995; Gallaher *et al*. 1996). The consumption of *B*. *longum* or inulin was associated with a decrease in AOM-induced colonic small ACF in rats and the combined administration significantly decreased the incidence of large ACF (Rowland *et al*. 1998). In addition, it has been reported that colonisation by bacteria with an ability to produce genotoxic compounds and high β-glucuronidase activity enhanced the progression of ACF induced by DMH in rats. However, the additional colonisation by *B*. *breve* reduced the number of ACF with four or more crypts/focus and crypt multiplicity, which are reliable predictors of malignancy (Onoue *et al*. 1997).

Reddy & Rivenson (1993) reported that lyophilised cultures of *B*. *longum* administered in the diet to rats inhibited liver, colon and mammary tumours, induced by the food mutagen 2-amino-3-methyl-3*H*-imidazo(4,5-*f*)quinoline. Goldin & Gorbach (1980) showed that dietary supplements of *L*. *acidophilus* not only suppressed the incidence of DMH-induced colon carcinogenesis but also increased the latency period in rats. The feeding of fermented milk increased the survival rate of rats with chemically induced colon cancer (Shackelford *et al*. 1983). The dietary administration of a lyophilised culture of *B*. *longum* resulted in a significant suppression of colon tumour incidence and tumour multiplicity and also reduced tumour volume induced by AOM in rats (Singh *et al*. 1997). The ingestion of *B*. *longum* also significantly inhibited AOM-induced cell proliferation, ornithine decarboxylase activity and expression of the ras-p21 oncoprotein. Recently, there was a report on the anti-tumorigenic activity of the prebiotic inulin, enriched with oligofructose, in combination with the probiotics *L*. *rhamnosus* and *B*. *lactis* in the AOM-induced colon carcinogenesis rat model (Femia *et al*. 2002). The authors concluded that, while a possible protective effect of probiotics was observed, the results indicated that the prebiotic decreased AOM-induced carcinogenesis. The mechanisms by which the pre- and probiotics act are less clear, but the data presented suggested that they may act through a combination of mechanisms involving an increase in SCFA production, lower proliferative activity and a variation in the expression of some enzymes involved in the pathogenesis of colon cancer (for a discussion on mechanisms, see p. 279).

There is additional direct evidence for anti-tumour activities of lactic acid bacteria obtained in studies using preimplanted tumour cells in animal models. It has been demonstrated that the feeding of fermented milk or cultures containing lactic acid bacteria inhibited the growth of tumour cells injected into mice (Kato *et al*. 1981; Friend *et al*. 1982). Sekine *et al*. (1985), using whole peptidoglycan isolated from *B*. *infantis* strain ATCC15697, reported that a single subcutaneous injection significantly suppressed tumour growth. In addition, five intralesional injections resulted in 70 % tumour regression in the mice.

More recently, mindful of the fact that the composition and metabolic activities of the intestinal flora of experimental animals are significantly different from those of human hosts (Hirayama *et al*. 1995), we exploited human flora-associated mice to test the effects of a probiotic mixture on a parameter of relevance for colon carcinogenesis, i.e. DNA adduct formation (Horie *et al*. 2003). Indeed, the results from a previous report, from our laboratory, demonstrated that human intestinal microflora had different effects from mouse microflora concerning DNA adduct formation after exposure to mutagens (Hirayama *et al*. 2000). The probiotic mixture, Biothree®, used in the Horie *et al*. (2003) study contained *S*. *faecalis* T-110, *Clostridium butyricum* TO-A and *Bacillus mesentericus* TO-A, which are acid resistant in contrast to most bacteria, which do not survive contact with gastric acid. It has been reported that *S*. *faecalis* T-110 and *C*. *butyricum* TO-A showed strong symbiosis with each other and the growth of enteropathogens (enterotoxigenic *Escherichia coli*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *C*. *difficile* and *C*. *botulinum*) was inhibited in mixed cultures of *S*. *faecalis* T-110 and *C*. *butyricum* TO-A (Seo *et al*. 1989). It has also been reported that *Bacillus mesentericus* TO-A stimulated the growth of *Bifidobacterium* by producing 3,3-dihydroxyazetidine (Iino *et al*. 1993; Seo *et al*. 2000). Biothree® is used as a clinical therapy in Japan. It is effective for the improvement of symptoms caused by abnormal intestinal flora, i.e. diarrhoea and constipation. Interestingly, Horie *et al*. (2003) demonstrated that the probiotic mixture had an effect in that it significantly decreased the DNA adduct formation in the colonic epithelium induced by the food mutagen 2-amino-9*H*-pyrido(2,3-*b*)indole (2-amino-alpha-carboline), given by oral administration. Two possible mechanisms may be involved: a reduction of direct exposure to 2-amino-alpha-carboline and/or an induction of DNA repair of the DNA adducts in the colonic epithelium (for a discussion on mechanisms, see p. 281).

Mechanisms by which probiotic bacteria may be inhibiting colon cancer

The precise mechanisms by which lactic acid bacteria may inhibit colon cancer are presently unknown. However, such mechanisms might include: an alteration of the metabolic activities of intestinal microflora; an alteration of physicochemical conditions in the colon; the binding and degrading of potential carcinogens; quantitative and/or qualitative alterations in the intestinal microflora incriminated in producing putative carcinogen(s) and promoters (for example, bile acid-metabolising bacteria); the production of anti-tumorigenic or anti-mutagenic compounds; enhancement of the host's immune response; effects on the physiology of the host. These potential mechanisms will now be addressed individually.

Alteration of the metabolic activities of intestinal microflora

Many foreign compounds are detoxified by glucuronide formation in the liver before entering the intestine via the bile. The bacterial enzyme β-glucuronidase has the ability to hydrolyse many glucuronides due to its wide substrate specificity, and thus may liberate carcinogenic aglycones in

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the intestinal lumen. Several other bacterial enzymes have also been suggested to be implicated in the carcinogenic process, releasing carcinogens in the intestinal tract. Interestingly, it was these earlier observations that feeding lactic acid bacteria supplements in the diets of rodents significantly decreased the activities of some of these faecal enzyme activities which focused attention on these bacteria as possible anti-cancer agents (Goldin & Gorbach, 1984*a*; Kulkarni & Reddy, 1994; Abdelali *et al*. 1995; Rowland *et al*. 1998). Lactic acid bacteria also reduced the specific activities of faecal enzymes in human volunteer studies (Ayebo *et al*. 1980; Goldin *et al*. 1980; Ling *et al*. 1994; Spanhaak *et al*. 1998). Goldin & Gorbach (1984*b*) studied the effect of feeding *L. acidophilus* strains NCFM and N-2 on the activity of three bacterial enzymes (β-glucuronidase, nitroreductase and azoreductase) in twenty-one healthy volunteers. Both strains had similar effects and caused a significant decline in the specific activity of the three enzymes in all subjects after 10 d of feeding. A reversal of the effect was observed within 10–30 d of stopping *Lactobacillus* feeding, suggesting that continuous consumption of these bacteria was necessary to maintain the effect. Thus, in summary, the animal and human studies do indicate that feeding certain lactic cultures can result in a decrease of faecal enzymes that may be involved in the formation of carcinogens. It is important to mention here that the reports published to date do not always find reductions in the same enzymes, although findings with β-glucuronidase and nitroreductase are most consistently positive. However, we still do not know how or whether a reduction in these enzyme activities affects cancer rates in man. Indeed, the origin of the carcinogens causing this disease in man is still to a large extent unknown.

Alteration of physicochemical conditions in the colon

Modler *et al*. (1990) have suggested that large-bowel cancer could be influenced directly by reducing intestinal pH, thereby preventing the growth of putrefactive bacteria. In rats given inulin-containing diets with or without *B. longum*, an increase in caecal weight and β-glucosidase and a decrease in caecal pH were observed (Rowland *et al*. 1998), though some other studies did not detect a significant change in intestinal pH (Bartram *et al*. 1994; Abdelali *et al*. 1995).

Dietary fat has been considered a risk factor for colon cancer, and it has been suggested that this phenomenon may be mediated by increased levels of bile acids (mainly secondary bile acids, produced by the action of bacterial 7α-dehydroxylase on primary bile acids) in the colon (Weisburger & Wynder, 1987). One hypothesis regarding colon carcinogenesis involves a cytotoxic effect on the colonic epithelium exerted by bile acids in the aqueous phase of faeces (soluble bile acids), followed by an increased proliferation of cells in the intestine (Bruce, 1987). It has been demonstrated that a 6-week administration of *L. acidophilus* fermented milk supplements to colon cancer patients resulted in lower concentrations of soluble bile acids in faeces (Lidbeck *et al*. 1991). Although the decrease in the concentration of bile acids in this fraction of faeces was not significant (perhaps due to a low number of patients or a limited supplementation period), it was of interest that decreased levels of soluble bile acids were observed in the colon cancer patients receiving *L. acidophilus* fermented milk supplements. In another study, patients with colonic adenomas participated in a 3-month study, where *L. acidophilus* was administered together with *B. bifidum* (Biasco *et al*. 1991). During this period, the faecal pH was reduced significantly, and patients having a higher proliferative activity in the upper colonic crypts than that calculated for subjects at low risk for colon cancer showed a significant decrease after therapy with the lactic acid bacteria. In view of the results from the study of Lidbeck *et al*. (1991), it is interesting to speculate that this latter effect was in part due to decreased levels of bile acids in the aqueous phase of faeces.

Binding and degrading potential carcinogens

Bacterial cells in addition to certain plant cell walls may be an important factor in determining the bound:free (bioavailable) toxins ratio in the intestine. Mutagenic compounds, commonly found in the Western meat-rich diet, can be bound to the intestinal and lactic acid bacteria *in vitro* and binding has been found to be correlated well with the reduction in mutagenicity observed after exposure to the bacterial strains (Orrhage *et al*. 1994). Morotomi & Mutai (1986) investigated the ability of twenty-two strains of intestinal bacteria to bind the mutagenic pyrolyzates and compared their ability to that of some dietary fibres. 3-Amino-1,4-dimethyl-5*H*-pyrido(4,3-*b*)indole and 3-amino-1-methyl-5*H*-pyrido(4,3-*b*)indole (Trp-P-2) were effectively bound to all gram-positive and some gram-negative bacterial cells, maize bran, apple pulp and soyabean fibre. When the mechanism of binding of Trp-P-2 to *L. casei* YIT 9018 and maize bran was investigated, it was shown to be pH dependent, occurred instantaneously and was inhibited by the addition of metal salts, indicating a cation-exchange mechanism. The mutagenicity of Trp-P-2 for *Salmonella typhimurium* TA98 in the presence of S9 mix was inhibited by the addition of *L. casei* YIT 9018 to the reaction mixture, indicating that bound Trp-P-2 did not cause mutation under the assay conditions.

Although little is known about the fate of bound mutagens in the human gastrointestinal system, Zhang & Ohta (1993) showed that freeze-dried cells of lactic acid bacteria, intestinal bacteria and yeast significantly reduced the absorption of 3-amino-1,4-dimethyl-5*H*-pyrido(4,3-*b*)indole from the small intestine in rats and that this was accompanied by decreased levels of this food mutagen in blood. A more recent study demonstrated a reduced uptake of the food mutagen, Trp-P-2, and its metabolites in various tissues of mice supplemented with dietary lactic acid bacteria (Orrhage *et al*. 2002). In addition, the consumption of lactobacilli by human volunteers has been shown to reduce the mutagenicity of urine and faeces associated with the ingestion of carcinogens in cooked meat (Lidbeck *et al*. 1992; Hayatsu & Hayatsu, 1993). In view of the *in vitro* results referred to here, it is possible that the lactic acid bacteria supplements are influencing the uptake and/or excretion of mutagens by simply binding them in the intestine. Lactobacilli have also been shown to degrade

nitrosamines (Rowland & Grasso, 1975). This is of some interest as nitrosamines have been shown to be carcinogenic in animal models and these compounds have been detected in human faeces.

Quantitative and/or qualitative alterations in the intestinal microflora

The consumption of fermented milk containing *L. acidophilus* has been shown to reduce significantly the counts of faecal putrefactive bacteria such as coliforms and increase the levels of lactobacilli in the intestine (Ayebo *et al*. 1980; Shahani & Ayebo, 1980). This suggests that supplemental *L. acidophilus* has a beneficial effect on the intestinal microecology by suppressing the putrefactive organisms that are possibly involved in the production of tumour promoters and putative pre-carcinogens. However, the mechanisms underlying these effects are still poorly understood.

Production of anti-tumorigenic or anti-mutagenic compounds

Lactic acid bacteria or a soluble compound produced by the bacteria may interact directly with tumour cells in culture and inhibit their growth (Reddy *et al*. 1973, 1983). Lactic acid bacteria significantly reduced the growth and viability of the human colon cancer cell line HT-29 in culture and dipeptidyl peptidase IV and brush-border enzymes were significantly increased, suggesting that these cells may have entered a differentiation process (Baricault *et al*. 1995). Milk fermented by *B. infantis*, *B. bifidum*, *B. animalis*, *L. acidophilus* and *L. paracasei* inhibited the growth of the MCF7 breast cancer cell line and the anti-proliferative effect was not related to the presence of bacteria (Biffi *et al*. 1997). These findings suggest the presence of a soluble compound produced by lactic acid bacteria during milk fermentation or the microbial transformation of some milk components in a biologically active form.

Enhancing the host's immune response

One explanation for tumour suppression by lactic acid bacteria may be mediated through an immune response of the host. Sekine *et al*. (1985) suggested that *B. infantis* stimulates the host-mediated response, leading to tumour suppression or regression. In addition, there are studies to suggest that lactic acid bacteria play an important role and function in the host's immunoprotective system by increasing specific and non-specific mechanisms to have an anti-tumour effect (Kato *et al*. 1983; De Simone *et al*. 1993; Schiffrin *et al*. 1995). *L. casei* strain Shirota has been shown to have potent anti-tumour and anti-metastatic effects on transplantable tumour cells and to suppress chemically induced carcinogenesis in rodents. Also, the intrapleural administration of *L. casei* strain Shirota into tumour-bearing mice has been shown to induce the production of several cytokines, such as interferon-γ, IL-1β and TNF-α, in the thoracic cavity of mice, resulting in the inhibition of tumour growth and increased survival (Matsuzaki, 1998). These findings suggest that treatment with *L. casei* strain Shirota has the potential to ameliorate or prevent tumorigenesis through modulation of the host's immune system, specifically cellular immune responses. An additional study has indicated that the oral administration of BLP, a preparation of viable *L. casei* YIT 9018, potentiates systemic immune responses that modify T-cell functions in tumour-bearing mice (Kato *et al*. 1994). It has also been demonstrated that *B. longum* and *B. animalis* promote the induction of inflammatory cytokines (IL-6, TNF-α) in mouse peritoneal cells (Sekine *et al*. 1994).

Effects on physiology of the host

Lactobacilli are one of the dominant species in the small intestine, and these micro-organisms presumably affect metabolic reactions occurring in this part of the gastrointestinal tract. The ileal mucosa (Venitt, 1988) as well as the colonic mucosa (Fang & Strobel, 1978) have the capacity to absorb mutagenic compounds from the intestinal lumen whereafter the compounds are passed into the bloodstream, either unchanged or as metabolites. In addition, lactic acid bacteria have been shown to increase colonic NADPH-cytochrome P-450 reductase activity (Pool-Zobel *et al*. 1996) and glutathione S-transferase levels (Challa *et al*. 1997) and to reduce hepatic uridine diphosphoglucuronyl transferase activity (Abdelali *et al*. 1995), enzymes which are involved in the metabolism of carcinogens in rats. Arimochi *et al*. (1997) showed an inhibitory effect of *L. acidophilus* on ACF formation in the colon of rats, induced by AOM, and enhanced removal of O^6-methylguanine from the colon mucosal DNA and that these effects came from culture supernatant fractions, not from bacterial cells. In addition, it has been demonstrated that the dietary administration of lyophilised cultures of *B. longum* strongly suppresses AOM-induced colonic tumour development and that this effect is associated with a decrease in colonic mucosal cell proliferation, and colonic mucosal and tumour ornithine decarboxylase and ras-p21 activities (Reddy, 1998).

Conclusion

Many health-promoting effects are attributed to the probiotic bacteria and some of these effects have more scientific support than the anti-cancer effect. The strongest evidence for the anti-cancer effects of probiotics comes from animal studies; evidence from human studies (epidemiology and experimental) is still limited. An important goal for the future should be carefully designed human clinical trials to corroborate the wealth of experimental studies.

Also, as discussed earlier (p. 279), there are several possible mechanisms that could explain how lactic acid bacteria might protect against tumour development in the colon. It is possible that different strains target different mechanisms. All of the mechanisms have various degrees of support, mainly originating from *in vitro* and animal experiments and some of them even have some support from human clinical studies. Thus, more work needs to be done to identify the specific strains and strain characteristics responsible for specific anti-tumour effects and the mechanisms by which these effects are mediated. However,

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even with these reservations in mind and with awareness of the limited number of human studies available, the use of lactic cultures for human cancer suppression is interesting, holds promise and certainly deserves more scrutiny.

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