Colon Cancer Prevention by Lactic Acid Bacteria

Joseph RAFTER,^{1*} Kazuhiro HIRAYAMA^{1,2} and Hisanaga HORIE^{1,3}

¹Department of Medical Nutrition, Karolinska Institutet, NOVUM, S-141 86 Huddinge, Sweden

²Present address: Laboratory of Veterinary Public Health, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–8657, Japan

³Present address: Department of Surgery, Jichi Medical School, 3311–1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329–0498, Japan

Received for publication, December 13, 2000

While a myriad of healthful effects have been attributed to the probiotic lactic acid bacteria, perhaps the most controversial remains that of anticancer activity. There is no direct experimental evidence for cancer suppression in humans as a result of consumption of lactic cultures in fermented or unfermented dairy products. However, there is a wealth of indirect evidence, based largely on laboratory studies, in the literature and this will be summarised in the present paper. Reports in the literature, regarding the anticancer effects of lactic acid bacteria, fall into the following categories: *in vitro* studies, *in vivo* studies in laboratory animals, epidemiological studies correlating cancer and certain dietary regimes and dietary intervention studies in human volunteers.

Key words: colon cancer; chemoprevention; probiotics; intestinal microflora

LABORATORY STUDIES

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. (46) reported, using the comet assay, that *Lactobacillus acidophilus*, *L. gasseri*, *L. confusus*, *Streptococcus thermophilus*, *Bifidobacterium breve* and *B. longum* were antigenotoxic toward *N*-nitro-*N*-nitrosoguanidine (MNNG). 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 MNNG-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 preneoplastic lesions or tumours induced by carcinogens. Goldin et al. (17) 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 aberrant crypt foci (ACF), which are thought to be precursor

lesions of colon cancer, induced by azoxymethane (AOM) (3). Although *B. adolescentis* culture and its supernatant did not show an inhibitory effect in this study (3), feeding of bifidobacteria suppressed the ACF formation induced by AOM (9, 32) or DMH (2, 18). Consumption of *B. longum* or inulin was associated with a decrease in AOM-induced colonic small ACF in rats and combined administration significantly decreased the incidence of large ACF (53). In addition, it has been reported that colonisation of bacteria with an ability to produce genotoxic compounds and high β -glucuronidase activity enhanced progression of ACF induced by DMH in rats, and that the additional colonisation of *B. breve* reduced the number of ACF with four or more crypts/focus and crypt multiplicity which are reliable predictors of malignancy (43).

Reddy and Rivenson (52) reported that lyophilised cultures of *B. longum* administered in the diet to rats inhibited liver, colon and mammary tumours, induced by the food mutagen IQ. In another study, Kohwi et al. (30) demonstrated the potential of two *Bifidobacterium* species, *B. infantis* and *B. adolescentis*, injected either subcutaneously or intraperitoneally into BALB/c mice to inhibit 3-methylcholanthrene-induced tumours. Goldin and Gorbach (14) 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. Feeding of fermented milk increased the survival rate of rats with chemically induced colon cancer (59). Dietary administration of a lyophilised culture of *B. longum* resulted in a significant suppression of colon tumour incidence

*Corresponding author. Mailing address: Department of Medical Nutrition, Karolinska Institutet, NOVUM, S-141 86 Huddinge, Sweden. Phone: +46–8–58583717. Fax: +46–8–7116659.

and tumour multiplicity and also reduced tumour volume induced by AOM in rats (61). Ingestion of *B. longum* also significantly inhibited AOM-induced cell proliferation, ornithine decarboxylase activity and expression of the ras-p21 oncoprotein.

There is additional direct evidence for anti-tumour activities of lactic acid bacteria obtained in studies using pre-implanted tumour cells in animal models. It has been demonstrated that feeding of fermented milk or cultures containing lactic acid bacteria inhibited the growth of tumour cells injected into mice (12, 31). Repeated intralesional injection of live or dead *Bifidobacterium* cells inhibited the growth of Meth-A tumour cells transplanted s.c. into syngenic BALB/c mice (30). Sekine et al. (63) 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 humans (24), we exploited human flora associated (HFA) mice to test the effects of a probiotic mixture on a parameter of relevance for colon carcinogenesis i.e. DNA adduct formation. Indeed, the results from a previous report, from our laboratory, demonstrated that human intestinal microflora had different effects than mouse microflora concerning DNA adduct formation after exposure to mutagens (20). The probiotic mixture, Biothree[®], used in this study contained *Streptococcus 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 (62). It has also been reported that *B. mesentericus* TO-A stimulated the growth of *Bifidobacterium* by producing 3,3-dihydroxyazetidine (26, 56). 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, the results of this study demonstrated that the above probiotic mixture had an effect to significantly decrease the DNA adduct formation in the colonic epithelium induced by the food mutagen 2-amino-9H-pyrido[2,3-b]indole (2-

amino- α -carboline; AAC), given by gavage. Two possible mechanisms may be involved: reduction of direct exposure to AAC and/or induction of DNA repair of the DNA adducts in the colonic epithelium (see discussion on mechanisms below).

HUMAN STUDIES

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 *L. acidophilus* was given to volunteers on a fried meat diet known to increase faecal mutagenicity, a lower faecal mutagenic activity was noted on day 3 compared to day 3 when fried meat and ordinary fermented milk were given (37). 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 to 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 and Hayatsu (23) also demonstrated a marked suppressing effect of orally administered *L. casei* on the urinary mutagenicity arising from ingestion of fried ground beef in the human. It is possible that the *L. acidophilus* supplements are influencing excretion of mutagens by simply binding them in the intestine (see discussion on mechanisms below).

As yet, there are few epidemiological studies addressing the association between fermented dairy products and colorectal cancer. 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 (55). 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 (25, 39). In two population-based case-control studies of colon cancer, an inverse association was observed for yoghurt (47) and cultured milk consumption (67), adjusted for potential confounding variables. 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 (36, 65). On the other hand, two companion American prospective studies, the 1980–1988 follow up of the NHS and the 1986–1990 follow up of the HPFS, did not provide evidence that

Fig. 1. Potential mechanisms by which probiotic bacteria may exert anticancer effects.

intake of dairy products is associated with a decreased risk of colon cancer (28). In a cohort study in the Netherlands, it was shown that the intake of fermented dairy products was not significantly associated with colorectal cancer risk in an elderly population with a relatively wide variation in dairy product consumption, although a weak nonsignificant inverse association with colon cancer was observed (29).

MECHANISMS BY WHICH LACTIC ACID BACTERIA INHIBIT COLON CANCER

The precise mechanisms by which lactic acid bacteria may inhibit colon cancer are presently unknown. However, such mechanisms might include: enhancing the host's immune response; binding and degrading potential carcinogens; quantitative and/or qualitative alterations in the intestinal microflora incriminated in producing putative carcinogen(s) and promoters (e.g. bile acid-degrading bacteria); producing antitumorigenic or antimutagenic compounds in the colon; alteration of the metabolic activities of intestinal microflora; alteration of physicochemical conditions in the colon; effects on physiology of the host (Fig. 1).

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. (63) 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 (11, 33, 60). *Lactobacillus casei* strain Shirota (LcS) 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, intrapleural administration of LcS into tumour-bearing mice has been shown to induce the production of several cytokines, such as IFN- γ , IL-1 β and TNF- α , in the thoracic cavity of mice, resulting in the inhibition of tumour growth and increased survival (40). These findings suggest that treatment with LcS has the potential to ameliorate or prevent tumourigenesis through modulation of the host's immune system, specifically cellular immune responses. An additional study has indicated that oral administration of BLP, a preparation of viable *Lactobacillus casei* YIT 9018, potentiated systemic immune responses that modified T-cell functions in tumour bearing mice (27). 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 (58).

Binding and Degrading Potential Carcinogens

Bacterial cells in addition to certain plant cell walls may be an important factor in determining the ratio of bound to free (bioavailable) toxins in the colon. Mutagenic compounds, commonly found in the Western meat rich diet, can be bound to the intestinal and lactic acid bacteria *in vitro* and binding correlated well with the reduction in mutagenicity observed after exposure to the bacterial strains (38, 44, 45, 69). The binding appears to be a physical phenomenon, mostly due to a cation-exchange mechanism and it has been suggested that cell wall peptidoglycans and polysaccharides are the two most important elements responsible for the binding (68). Although little is known about the fate of bound mutagens in the human gastrointestinal system, Zhang and Ohta (70) 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 (Trp-P-1) from the small intestine in rats and that this was accompanied by decreased levels of this food mutagen in blood. However, there is a danger in extrapolating these results to health claims for humans as the reversibility of mutagen binding to cultures *in vivo* is unknown. Furthermore, the biologically significant levels of mutagens and of lactic acid bacteria in the human system are unknown. Lactobacilli have also been shown to degrade nitrosamines (51).

Quantitative and/or Qualitative Alterations in the Intestinal Microflora

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 (1, 55) suggesting 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 precarcinogens.

Production of Antitumorigenic or Antimutagenic Compounds in the Colon

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 (5). Arimochi et al. (3) showed an inhibitory effect of *L. acidophilus* on ACF formation in the colon of rats, induced by azoxymethane, and enhanced removal of *O*⁶-methylguanine from the colon mucosal DNA and that these effects came from culture supernatants, not from bacterial cells. In addition, it has been demonstrated that dietary administration of lyophilized cultures of *Bifidobacterium longum* strongly suppressed azoxymethane-induced colonic tumour development and that this effect was associated with a decrease in colonic mucosal cell proliferation and colonic mucosal and tumour ornithine decarboxylase and ras-p21 activities (49).

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 car-

cinogenic aglycones in 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 the above faecal enzyme activities which focused attention on these bacteria as possible anticancer agents (2, 15, 32, 53). Lactic acid bacteria also reduced the specific activities of faecal enzymes in human volunteer studies (1, 19, 35, 57). Goldin and Gorbach (16) 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 21 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 days of feeding. A reversal of the effect was observed within 10–30 days of stopping *Lactobacillus* feeding, indicating that continuous consumption of these bacteria was necessary to maintain the effect. The authors suggested that the observed reduction of these enzymes may explain the reduced colon cancer incidence in rats fed viable *L. acidophilus* (14). 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 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 humans. Indeed, the origin of the carcinogens causing this disease in humans is still to a large extent unknown.

Alteration of Physicochemical Conditions in the Colon

Modler et al. (41) 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 (53), though some other studies did not detect a significant change in intestinal pH (2, 8).

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 7 α -dehydroxylase on primary bile acids)

in the colon (66). 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 (4). It has been demonstrated that a 6-week administration of *L. acidophilus* to colon cancer patients resulted in lower concentrations of soluble bile acids in faeces (34). 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 a definite trend towards decreased levels of soluble bile acids was 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* (7). 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 in the above mentioned study (34) 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.

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 (64) as well as the colonic mucosa (13) has 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 (46) and glutathione S-transferase levels (9) and to reduce hepatic uridine diphosphoglucuronyl transferase activity (2), enzymes which are involved in the metabolism of carcinogens in rats.

Butyrate, a metabolite of some probiotic strains, promotes differentiation and apoptosis in a variety of colon tumour cell lines (10, 22). Apoptosis is a central feature in the regulation of cell number and the elimination of nonfunctional, harmful, or abnormal cells in the colon (21, 48). Therefore, apoptosis induced by butyrate could affect the removal of the DNA adducts from the colonic epithelium. Arimochi et al. investigated the effect of several strains of intestinal bacteria on the for-

mation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and DNA adducts in the rat colon (3). They showed that culture supernatants of *Lactobacillus acidophilus* inhibited the ACF formation and that the inhibitory effect was due to the enhanced removal of *O*⁶-methylguanine from the colonic mucosal DNA. They suggested that a metabolite of *L. acidophilus* in the culture supernatants might induce *O*⁶-methylguanine-repair enzyme, methylguanine-methyltransferase. Therefore, metabolites of probiotic bacteria might be inducing DNA-adduct-repair enzymes after exposure to mutagens found in the diet.

Lactic acid bacteria or a soluble compound produced by the bacteria may interact directly with tumour cells in culture and inhibit their growth (50, 54). 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 (6).

CONCLUDING REMARKS

Thus, a number of studies indicate that administration of bifidobacteria or lactobacilli alone or with fermentable carbohydrate (defined as a prebiotic) can alter colonic microflora populations and decrease the development of early preneoplastic lesions and tumours. However, several studies failed to demonstrate that bifidobacteria or lactobacilli administration alters colonic microflora or has effects on the host (8, 42). At present, the results from the epidemiological studies do not appear to support the results from experimental studies examining lactobacilli and colon cancer prevention. The reason for this is unclear but might be explained by differences in bacterial strains, with the strains being used in the experimental studies surviving better in the gastrointestinal tract than the strains present in fermented dairy products. It should also be emphasised that great care must be exercised in extrapolating the results of the above *in vitro* and animal studies to the human system. Many of the animal studies exploit specifically bred strains of mice and whether one can extrapolate antitumour activity in these animals to humans is somewhat unclear. It also must be kept in mind that the composition and metabolic activities of intestinal flora of experimental animals are significantly different from those of humans. Exploitation of human-flora-associated animals (germ-free animals inoculated with whole human intestinal flora) can be one of the solutions to this problem (20). Results of administering lactic cultures intravenously, intraperi-

toneally and intralesionally (often used in animal studies) may not be compatible with oral consumption in humans. Many of the antitumour activities attributed to lactic cultures have been suggested to involve an enhanced function of the immune response. More work needs to be done to identify the specific strains and strain characteristics responsible for antitumour effects and the mechanisms by which these effects are mediated. However, even with these reservations in mind and mindful of the limited number of human studies described above, the use of lactic cultures for human cancer suppression is interesting, holds promise and certainly deserves more scrutiny. The latter should involve carefully designed clinical studies to corroborate the wealth of experimental studies.

Acknowledgements. This work was supported by a grant from the Swedish Cancer Society.

REFERENCES

- (1) Ayebo AD, Angelo IA, Shahani KM. 1980. Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. *Milch Wissenschaft* **35**: 730–733.
- (2) Abdelali H, Cassand P, Soussotte V, Daubeze M, Bouley C, Narbonne JF. 1995. Effect of dairy products on initiation of precursor lesions of colon cancer in rats. *Nutr Cancer* **24**: 121–132.
- (3) Arimochi H, Kinouchi T, Kataoka K, Kuwahara T, Ohnishi Y. 1997. Effect of intestinal bacteria on formation of azoxymethane-induced aberrant crypt foci in the rat colon. *Biochem Biophys Res Commun* **238**: 753–757.
- (4) Bruce WR. 1987. Recent hypotheses for the origin of colon cancer. *Cancer Res* **47**: 4237–4242.
- (5) Biffi A, Coradini D, Larsen R, Riva L, Di Fronzo G. 1997. Antiproliferative effect of fermented milk on the growth of a human breast cancer cell line. *Nutr Cancer* **28**: 93–99.
- (6) Baricault L, Denariac G, Houri J-J, Bouley C, Sapin C, Trugnan G. 1995. Use of HT-29, a cultured human colon cancer cell line, to study the effect of fermented milks on colon cancer cell growth and differentiation. *Carcinogenesis* **16**: 245–252.
- (7) Biasco G, Paganelli GM, Brandi G, Brillanti S, Lami F, Callegari C, Gizzi G. 1991. Effect of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on rectal cell kinetics and fecal pH. *Ital J Gastroenterol* **23**: 142.
- (8) Bartram HP, Scheppach W, Gerlach S, Ruckdeschel G, Kelber E, Kasper H. 1994. Does yogurt enriched with *Bifidobacterium longum* affect colonic microbiology and fecal metabolites in healthy subjects. *Am J Clin Nutr* **59**: 428–432.
- (9) Challa A, Rao DR, Chawan CB, Shackelford L. 1997. *Bifidobacterium longum* and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. *Carcinogenesis* **18**: 517–521.
- (10) Calabresse C, Venturini L, Ronco G, Villa P, Chomienne C, Belpomme D. 1993. Butyric acid and its monosaccharide ester induce apoptosis in the HL-60 cell line. *Biochem Biophys Res Commun* **195**: 31–38.
- (11) De Simone C, Vesely R, Bianchi Salvadori B, Jirillo E. 1993. The role of probiotics in modulation of the immune system in man and in animals. *Int J Immunother* **9**: 23–28.
- (12) Friend BA, Farmer RE, Shahani KM. 1982. Effect of feeding and intraperitoneal implantation of yogurt culture cells on Ehrlich ascites tumor. *Milch Wissenschaft* **37**: 708–710.
- (13) Fang W-F, Strobel HW. 1978. Activation of carcinogens and mutagens by rat colon mucosa. *Cancer Res* **38**: 2939–2944.
- (14) Goldin BR, Gorbach SL. 1980. Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *J Natl Cancer Inst* **64**: 263–265.
- (15) Goldin BR, Gorbach SL. 1984. Alterations of the intestinal microflora by diet, oral antibiotics and *Lactobacillus*: decreased production of free amines from aromatic nitrocompounds, azo dyes and glucuronides. *J Natl Cancer Inst* **73**: 689–695.
- (16) Goldin BR, Gorbach SL. 1984. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. *Am J Clin Nutr* **39**: 756–761.
- (17) Goldin BR, Gualtieri LJ, Moore RP. 1996. The effect of *Lactobacillus GG* on the initiation and promotion of DMH-induced intestinal tumors in the rat. *Nutr Cancer* **25**: 197–204.
- (18) Gallaher DD, Stallings WH, Blessing LL, Busta FF, Brady LJ. 1996. Probiotics, cecal microflora, and aberrant crypts in the rat colon. *J Nutr* **126**: 1362–1371.
- (19) Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL. 1980. Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. *J Natl Cancer Inst* **64**: 255–261.
- (20) Hirayama K, Baranczewski P, Åkerland J-E, Midtvedt T, Möller L, Rafter J. 2000. Effects of human intestinal flora on mutagenicity of and DNA adduct formation from food and environmental mutagens. *Carcinogenesis* **21**: 2105–2111.
- (21) Hall PA, Coates PJ, Ansari B, Hopwood D. 1994. Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. *J Cell Sci* **107**: 3569–3577.
- (22) Hague A, Elder DJ, Hicks DJ, Paraskeva C. 1995. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *Int J Cancer* **60**: 400–406.
- (23) Hayatsu H, Hayatsu T. 1993. Suppressing effect of *Lactobacillus casei* administration on the urinary mutagenicity arising from ingestion of fried ground beef in the human. *Cancer Lett* **73**: 173–179.
- (24) Hirayama K, Itoh K, Takahashi E, Mitsuoka T. 1995. Comparison of composition of faecal microbiota and metabolism of faecal bacteria among 'human-flora-associated' mice inoculated with faeces from six different human donors. *Microbial Ecol Health Dis* **8**: 199–211.
- (25) Intestinal Microecology Group, International Agency for

- Research on Cancer. 1977. Dietary fibre, transit time, fecal bacteria, steroids, and colon cancer in two Scandinavian populations. *Lancet* **2**: 207–211.
- (26) Iino H, Fukaya K, Hirasawa Y, Shimizu K, Seo G. 1993. Stimulation of bacterial growth of some strains of *Bifidobacterium* by a crude preparation of metabolites from *Bacillus mesentericus* TO-A. *Biomed Lett* **48**: 73–78.
- (27) Kato I, Endo K, Yokokura T. 1994. Effects of oral administration of *Lactobacillus casei* on antitumor responses induced by tumor resection in mice. *Int J Immunopharmacol* **16**: 29–36.
- (28) Kampman E, Giovannucci E, van't Veer P, Rimm E, Stampfer MJ, Colditz GA, Kok FJ, Willett WC. 1994. Calcium, vitamin D, dairy foods, and the occurrence of colorectal adenomas among men and women in two prospective studies. *Am J Epidemiol* **139**: 16–29.
- (29) Kampman E, Goldbohm RA, van den Brandt PA, van't Veer P. 1994. Fermented dairy products, calcium, and colorectal cancer in the Netherlands cohort study. *Cancer Res* **54**: 3186–3190.
- (30) Kohwi T, Imai K, Tamura A, Hashimoto Y. 1978. Antitumor effect of *Bifidobacterium infantis* in mice. *Gann* **69**: 613–618.
- (31) Kato I, Kobayashi S, Yokokura T, Mutai M. 1981. Antitumor activity of *Lactobacillus casei* in mice. *Gann* **72**: 517–523.
- (32) Kulkarni N, Reddy BS. 1994. Inhibitory effect of *Bifidobacterium longum* cultures on the azoxymethane-induced aberrant crypt foci formation and fecal bacterial β -glucuronidase. *Proc Soc Exp Biol Med* **207**: 278–283.
- (33) Kato I, Yokokura T, Mutai M. 1983. Macrophage activation by *Lactobacillus casei* in mice. *Microbiol Immunol* **27**: 611–618.
- (34) Lidbeck A, Geltner-Allinger U, Orrhage KM, Ottava L, Brismar B, Gustafsson J-A, Rafter JJ, Nord CE. 1991. Impact of *Lactobacillus acidophilus* supplements on the faecal microflora and soluble faecal bile acids in colon cancer patients. *Microbial Ecol Health Dis* **4**: 81–88.
- (35) Ling WH, Korpela R, Mykkanen H, Salminen S, Hanninen O. 1994. *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. *J Nutr* **124**: 18–23.
- (36) Le MG, Moulton LH, Hill C, Kramer A. 1986. Consumption of dairy product and alcohol in a case-control study of breast cancer. *J Natl Cancer Inst* **77**: 633–636.
- (37) Lidbeck A, Overvik E, Rafter J, Nord CE, Gustafsson J-A. 1992. Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in feces and urine in humans. *Microbial Ecol Health Dis* **5**: 59–67.
- (38) Lankaputhra WEV, Shah NP. 1998. Antimutagenic properties of probiotic bacteria and of organic acids. *Mutat Res* **397**: 169–182.
- (39) Malhotra SL. 1977. Dietary factors in a study of colon cancer from cancer registry, with special reference to the role of saliva, milk, and fermented milk products and vegetable fibre. *Med Hypotheses* **3**: 122–134.
- (40) Matsuzaki T. 1998. Immunomodulation by treatment with *Lactobacillus casei* strain Shirota. *Int J Food Microbiol* **41**: 133–140.
- (41) Modler GW, McKellar RC, Yaguchi M. 1990. Bifidobacteria and bifidogenic factors. *Can Inst Food Sci Technol J* **23**: 29–41.
- (42) Nielsen OH, Jorgensen S, Pedersen K, Justesen T. 1994. Microbiological evaluation of jejunal aspirates and fecal samples after oral administration of bifidobacteria and lactic acid bacteria. *J Appl Bacteriol* **76**: 469–474.
- (43) Onoue M, Kado S, Sakaitani Y, Uchida K, Morotomi M. 1997. Specific species of intestinal bacteria influence the induction of aberrant crypt foci by 1,2-dimethylhydrazine in rats. *Cancer Lett* **113**: 179–186.
- (44) Orrhage K, Sillerstrom E, Gustafsson J-A, Nord CE, Rafter J. 1994. Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. *Mutat Res* **311**: 239–248.
- (45) Pool-Zobel BL, Munzner R, Holzapfel WH. 1993. Antigenotoxic properties of lactic acid bacteria in the *S. typhimurium* mutagenicity assay. *Nutr Cancer* **20**: 261–270.
- (46) Pool-Zobel BL, Neudecker C, Domizlaff I, Ji S, Schillinger U, Rumney C, Moretti M, Vilarini I, Scarsellati-Sforzolini R, Rowland I. 1996. *Lactobacillus*- and *Bifidobacterium*-mediated antigenotoxicity in the colon of rats. *Nutr Cancer* **26**: 365–380.
- (47) Peters RK, Pike MC, Garabrant D, Mack TM. 1992. Diet and colon cancer in Los Angeles County, California. *Cancer Causes Control* **3**: 457–473.
- (48) Potten CS, Wilson JW, Booth C. 1997. Regulation and significance of apoptosis in the stem cells of the gastrointestinal epithelium. *Stem Cells* **15**: 82–93.
- (49) Reddy BS. 1998. Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. *Br J Nutr* **80**: S219–S223.
- (50) Reddy GV, Friend BA, Shahani KM, Farmer RE. 1983. Antitumor activity of yogurt components. *J Food Protect* **46**: 8–11.
- (51) Rowland IR, Grasso P. 1975. Degradation of *N*-nitrosamines by intestinal bacteria. *Appl Microbiol* **29**: 7–12.
- (52) Reddy BS, Rivenson A. 1993. Inhibitory effect of *Bifidobacterium longum* on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline, a food mutagen. *Cancer Res* **53**: 3914–3918.
- (53) Rowland IR, Rumney CJ, Coutts JT, Lievense LC. 1998. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis* **19**: 281–285.
- (54) Reddy GV, Shahani KM, Benerjee MR. 1973. Inhibitory effect of yogurt on Ehrlich ascites tumor cell proliferation. *J Natl Cancer Inst* **50**: 815–817.
- (55) Shahani KM, Ayebo AD. 1980. Role of dietary lactobacilli in gastrointestinal microecology. *Am J Clin Nutr* **33**: 2448–2457.
- (56) Seo G, Akimoto Y, Hamashima H, Masuda K, Shiojima K, Sakuma C, Sasatsu M, Arai T. 2000. A new factor from *Bacillus mesentericus* which promotes the growth of *Bifidobacterium*. *Microbios* **101**: 105–114.
- (57) Spanhaak S, Havenaar R, Schaafsma G. 1998. The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune pa-

- rameters in humans. *Eur J Clin Nutr* **52**: 899–907.
- (58) Sekine K, Kawashima T, Hashimoto Y. 1994. Comparison of the TNF- α levels induced by human-derived *Bifidobacterium longum* and rat-derived *Bifidobacterium animalis* in mouse peritoneal cells. *Bifidobacteria Microflora* **13**: 79–89.
- (59) Shackelford LA, Rao DR, Chawan CB, Pulusani SR. 1983. Effect of feeding fermented milk on the incidence of chemically-induced colon tumors in rats. *Nutr Cancer* **5**: 159–164.
- (60) Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A. 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J Dairy Sci* **78**: 491–496.
- (61) Singh J, Rivenson A, Tomita M, Shimamura S, Ishibashi N, Reddy BS. 1997. *Bifidobacterium longum*, a lactic acid-producing intestinal bacterium inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis. *Carcinogenesis* **18**: 833–841.
- (62) Seo G, Shimizu K, Sasatsu M, Kono M. 1989. Inhibition of growth of some enteropathogenic strains in mixed cultures of *Streptococcus faecalis* and *Clostridium butyricum*. *Microbios Lett* **40**: 151–160.
- (63) Sekine K, Toida T, Saito M, Kuboyama M, Kawashima T, Hashimoto Y. 1985. A new morphologically characterized cell wall preparation (whole peptidoglycan) from *Bifidobacterium infantis* with a higher efficacy on the regression of an established tumor in mice. *Cancer Res* **45**: 1300–1307.
- (64) Venitt S. 1988. Mutagens in human faeces and cancer of the large bowel. *In* Role of the Gut Flora in Toxicity and Cancer, Rowland IR (ed), Academic Press, London, p. 399–460.
- (65) van't Veer P, Dekker JM, Lamers JWJ, Kok FJ, Schouten EG, Brants HAM, Sturmans F, Hermus RJJ. 1989. Consumption of fermented milk products and breast cancer: a case-control study in the Netherlands. *Cancer Res* **49**: 4020–4023.
- (66) Weisburger JH, Wynder EL. 1987. Etiology of colorectal cancer with emphasis on mechanism of action and prevention. *In* Important Advances in Oncology, De Vita VT, Hellman S, Rosenberg SA (eds), JB Lippincott, Philadelphia, p. 197–220.
- (67) Young TB, Wolf DA. 1988. Case-control study of proximal and distal colon cancer and diet in Wisconsin. *Int J Cancer* **42**: 167–175.
- (68) Zhang XB, Ohta Y. 1991. Binding of mutagens by fractions of the cell wall skeleton of lactic acid bacteria. *J Dairy Sci* **74**: 1477–1481.
- (69) Zhang XB, Ohta Y. 1991. *In vitro* binding of mutagenic pyrolyzates to lactic acid bacterial cells in human gastric juice. *J Dairy Sci* **74**: 752–757.
- (70) Zhang XB, Ohta Y. 1993. Microorganisms in the gastrointestinal tract of the rat prevent absorption of the mutagen-carcinogen 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole. *Can J Microbiol* **39**: 841–845.