Probiotics and colon cancer prevention

Graeme H McIntosh, BUSc, PhD (ANU)

CSIRO Division of Human Nutrition

This review examines some of the evidence regarding probiotic bacteria as agents to reduce the risk of colon cancer in humans. While some of the evidence using rodent models of colon cancer is convincing for a reduction in cancer incidence and burden with the introduction orally of such bacteria as *Bifidobacterium longum*, *Lactobacillus acidophilus* and gg, convincing evidence in humans is more difficult to find. It consists of epidemiological studies or marker intervention studies using faecal enzymes, faecal bile acids or urinary/ faecal mutagens from microbial activity as measures of cancer risk, following probiotic introduction. Taken together these sources of data provide limited support for the hypothesis that probiotic bacteria are effective in cancer prevention.

Introduction

Colon cancer is a major health problem in Westernised cultures like Australia, and diet is considered to be a major factor influencing its prevalence. Diets containing high animal proteins and fat and low dietary fibre have been identified as being associated with greatest risk. Recent research has also focused on the influence that gastrointestinal microflorae have on outcome. A large and complex microbiological population inhabiting the colon was seen by some as being established early in life and relatively unchangeable by external factors. However, other research has suggested that it is manipulatable by dietary and microbiological means as well as by antibiotic therapy. The side effects of modern antibiotic therapy may include significant disturbances of the gut microflora and have been part of the motivation to find ways of achieving treatment of disease using 'desirable bacteria' as an alternative therapy. Insofar as the microflora can influence the immune system, nutrient metabolism, detoxification and carcinogen activation mechanisms and thereby the expression of a number of disease processes affecting the bowel, a better knowledge of their contribution to health and disease is warranted.

Probiotics are defined as live microbial food supplements which benefit the host by improving its intestinal microbial balance[1]. Yoghurt is a traditional and common vehicle for such probiotics (Lactobacilli and Bifidobacteria species being most often used in this role). They have a significant target, gastrointestinal disturbances and diseases. Wider claims include their value as a life extender, an elixir of life. Elie Metchnikoff[2], the Russian Nobel Prize biologist, popularised the view that some lactic acid bacteria were capable of increasing length of life, supporting his theory with observations of Bulgarians who ate yoghurt regularly and showed remarkable longevity. At the time, it created a world-wide interest in yoghurt but in the ensuing 70 or so years little attention was given to this claim. However, in the last 20 years, with the upswing in colon cancer and inflammatory bowel diseases in Westernised countries, it has been given increasing attention by researchers.

Useful reviews by Mitsuoka[3], Adachi[4], Marteau *et al*[5], and Ballongue[6], have discussed much of the groundwork research studies, in an area where Japanese and French researchers have made significant contributions.

From North America reviews are provided by Fernandes *et al*[7], Fernandes and Shahani[8], Gorbach and Goldin[9], Sanders[10]; while from Scandinavia Lidbeck *et al*[11], Salminen[12], Rafter[13] useful reviews have also been provided.

Research with regard to use of probiotics in prevention of colon cancer is reviewed in this paper. It must be appreciated that lack of knowledge of the carcinogenic process, the complexity of colonic function, and lack of techniques for adequately identifying specific strains of bacteria, has held back progress significantly. Nevertheless there have been some impressive advances, which I believe are bringing us nearer to predicting a protective diet and/ or probiotic strategy for reducing high rates of colon cancer. The anti-tumour action of probiotics have been proposed as:

(1) direct suppression of the carcinogens and/ or procarcinogens by binding, blocking, removing;
(2) inhibition of bacteria which directly or indirectly convert procarcinogens to carcinogens by enzyme activity etc.
(3) activation of the host's immune system to antitumourigenesis

Correspondence address: CSIRO Division of Human Nutrition, GPO Box 10041 Gouger Street, Adelaide South Australia 5000
Tel: +618 303 8884 Fax: +618 303 8899

(4) reduction of the intestinal pH, thereby altering microbial activity, solubility of bile acids, mucus secretion etc.
(5) alteration of colonic motility and transit time.

Malhotra[14], a medical officer with the Indian railways, reported on the gastrointestinal cancers in India, and proposed that the much lower incidence of colon cancers in northern people was associated with the significant consumption of dairy (including fermented) foods, cereals and vegetable dietary fibres in the regular diet. Southern diets by contrast were low residue highly digestible diets and tended to create a more alkaline colonic milieu. Similar differences have been noted for rural northern versus southern urban populations of Sweden[16], and in Finland versus Denmark[15]. The difference in each case was a reduction to one half or one third the colon cancer incidence and mortality.

Table 1. Chemical colon cancer studies in rats and mice

				% rats with colon carcinoma
Goldin and Gorbach (17)	DMH (S/C) rats n =22	Beef Beef + *L. acidophilus*		77% 40%
				% dead at 36 wks (colon carcinoma)
Shakelford et al (26)	DMH (S/C) n = 25 F344 rats	Skim milk SM + *L. bulgaricus* SM + *S. thermophilus*		28% 7% 10%
			Tumours /tba	**% rats with colon tumours**
Takano et al (27)	DMH (S/C) rats n = 9	Control + *L. helveticus* and *C. utilis*	2.6 1.0*	100% 66%
			Aberrant crypts/colon	
			Wk 20	**Wk 30**
Koo and Rao (28)	DMH (S/C) CF1 mice n = 21	• C +*B. longum* + 5% neosugar	14 7*	20 10*
				Aberrant crypts/colon
Kulkarni and Reddy (29)	AOM (S/C) rats n = 11	• C +*B. longum* 1.5% +*B. longum* 3.0%		249 142* 130*

S/C = subcutaneous; DMH = dimethylhydrazine; C= control; tba = tumour bearing animals * Significance at p< .05

Research up to the mid 1980s was mainly concerned with the direct or indirect anti-tumour action of streptococci, lactobacilli, and bifidobacteria studied in animals and to a lesser extent in man[17-20]. To induce the effect, bacteria were often injected systemically and/ or cancers were transplanted into mice. *Bifidobacterium longum* had a direct inhibitory effect on liver tumours in the mouse[21]. In the BALB/C mouse, *B. infantis and B. adolescentis* injected subcutaneously or intraperitonelly had an antitumour effect[22]. The number of tumours developed by mice with an intestinal flora including *Eschericia coli, Enterococcus faecalis*, and *Clostridium paraputrificum* was considerably reduced if *B. longum* was present[21]. Feeding fermented milks or cultures containing *Lactobacillus acidophilus, L. bulgaricus* and/ or *L. casei* inhibited Ehrlich ascites tumour cell growth or growth of Sarcoma 180 in mice[23,24].

Goldin and Gorbach[17,19,25] used the dimethyl-hydrazine (DMH) rat model to help assess the impact of lactobacilli on intestinal tumours and their studies and others are presented in Table 1. It was shown[17] that the high incidence of DMH induced colon carcinomas in rats fed beef could be lowered from 77 to 40% when *L. acidophilus* was fed simultaneously with the beef diet.

Other studies using the same or similar experimental cancer models have largely confirmed this early observation, although as can be seen there have been differing bacteria tested and endpoints of assessment used. More recently, *Lactobacillus* GG, a known human gut commensurate has been shown also to reduce incidence and tumour numbers of chemically induced colon cancers[9].

These animal studies have been paralleled by human and animal faecal enzyme studies, assessing nitro-reductase, β glucuronidase, azoreductase and/ or urease activity to predict risk of colon cancer. The hypothesis relies on the assumption that modulation of deconjugating and/ or dehydroxylating enzymes found in certain colonic bacteria but not in others will alter risk of carcinogens being generated from procarcinogenic agents or released from bound form into the gut contents, as they traverse the large intestine. Displacement by probiotic bacteria (of undesirable bacteria) will effect significant change. Significant results (Table 2) have been achieved with this approach to assessment of risk.

Table 2. The effects of oral consumption of lactic cultures on faecal enzyme activity

Reference	Bacteria used	Reduction of faecal enzyme activity
Goldin et al (18)	*L. acidophilus* (7 subjects)	+ nitroreductase - azoreductase + β-glucuronidase - steroid 7-α-dehydroxylase
Goldin and Gorbach (20)	*L. acidophilus* (7 subjects)	+ nitroreductase - azoreductase + β-glucuronidase
Goldin and Gorbach (19)	*L. acidophilus* NCFM *L. acidophilus* N-2 (22 subjects)	+ nitroreductase + azoreductase + β-glucuronidase
Marteau et al (30)	Milk fermented with *L. acidophilus B.bifidum*, and mesophilic cultures(9 subjects)	+ nitroreductase + azoreductase - β-glucuronidase β-glucosidase
Goldin et al (31)	*Lactobacillus GG* frozen concentrate (8 subjects)	+ β-glucuronidase
Lidbeck et al (12)	*L. acidophilus* milk (12 subjects)	? β-glucuronidase
Ling et al (32)	*L.gg* (64 subjects)	+β-glucuronidase + nitroreductase + glycocholic acid hydrolase
Kulkarni and Reddy (29)	*B. longum* F344 Rats (33 rats)	+ β-glucuronidase

+ = statistically significant positive, results; - = negative results; ? = results not definitive.

For example, Lidbeck *et al*[11], produced a significant increase in lactobacilli and dietary calcium by feeding *L. acidophilus* fermented milk to colon cancer patients for 6 weeks. Faecal enzyme activity was reduced 14% and soluble faecal bile acids 38%, but both results were not significant. They attributed this result to small number (n=12) of patients and the large variability in enzyme activity between patients. Ling *et al*[32] have shown a greater reduction in faecal enzymes (40%) with the feeding for 4 weeks of lyophylised *lactobacillus* GG and dietary fibre as cereal rye, relative to controls. Urinary paracresol, a mutagenic metabolite of protein, was also significantly reduced (18% $p<0.05$). Bartram *et al*[33] showed they could increase the faecal excretion of *B. longum* with oral supplements of the bacteria via yoghurt($>10^9$ cfu/L) and lactulose, and that breath hydrogen increased and mouth-cecum transit time increased, but no other changes (such as bile acids, SCFA, pH) were observed. They attributed this to significant gut microflora stability.

It is apparent from the above studies that there are differences of opinion as to which bacteria offer most potential for human health and cancer prevention, as well as considerable variation in background diets which could significantly influence outcome of such studies. This could account for some of the large differences in results, and present a possible obstacle to progress. To help sort out the bacteria most likely to be effective against colon cancer cells Baricault and co-workers[34] introduced the use of an *in vitro* cultured colon cancer cell (HT-29) assay. The test relied on inhibition of cells to grow into a confluent layer, or to differentiate under the influence of inhibitory bacteria. In an examination of a number of the probiotic bacteria being used currently they identified *Lactobacillus helveticus* and *Bifidobacterium* (species not named) as being effective, whereas *Lactobacillus acidophilus* was not.

Study of the growth requirements of Lactobacilli and of optimal healthy diets have led to the recognition of some desirable substrates for fermentative bacteria *in vivo*, which when fed alter significantly the proportion of beneficial bacteria present in the colon. without the need to orally supplement bacteria. It could be argued that this aspect of diet may have a bigger impact on health objectives than the provision of probiotic bacteria orally. Ecological studies of faecal microflora support this well[35,36]. These 2 reports point very clearly to the impact of diet on colonic microflora and colon cancer risk but come to opposite conclusions regarding the relevance of Bifidobacteria species to colon cancer risk. In a workshop summary report Roberfroid *et al*[37] referred to the circumstantial evidence of colonic microflora on cancer risk, and proposed the absorption and metabolism of mutagens and carcinogens as the primary role in prevention, while SCFA production from carbohydrate fermentation as seen as secondary in its influence.

Studies in my laboratory have identified the potential of whey proteins to significantly reduce cancer incidence (to one half) relative to red meat and soybean protein in the DMH rat cancer model[38]. This raises the possibility that dairy foods may offer, apart from any probiotic influence, high quality proteins which protect the rat gut from chemical carcinogenesis by an as yet undefined mechanism. It also highlights undesirable characteristics shared by two disparate sources of protein, soybean and red meat. In several of the studies reviewed, grilled or dried beef is used as a background diet to enable a significant improvement to be achieved with probiotics. A recent study by Reddy and Rivenson[39] is of interest in this regard. They have used the now well characterised meat mutagen IQ (2-Amino-3 Methylimidazo) (4,5 -f) quinoline to induce cancers in male and female rats. It is capable of producing breast, liver and colon tumours in rats and mice. When *B. longum* was fed at 0.5% as a lyophilised culture to rats there was 100% suppression of colon tumours, 80% suppression of liver tumours and in females 50% suppression of mammary tumours. Whatever the mechanism for this inhibitory influence, it is an impressive demonstration of a probiotic effect.

A number of studies have reported the use of specific agents to improve the growth of desirable gut microflora such as Bifidus growth factors[46]. They fall into the category of dietary fibre or fibre like components (such as resistant starch, oligosaccharides) which have the attribute of passing undigested through the small intestine to supply a substrate for the colonic bifidobacteria[41-44]. A list of some of the agents reported to be beneficial is shown in Table 3. In general their presence in the diet significantly influences the total counts of bifidobacteria in faeces. For example, with 9g/day gluconic acid, 10 healthy volunteers showed a significant increase ($p<0.001$), while less desirable bacteria like *C. perfringens* fell in number and *Enterobacteriaceae* stayed constant[44].

Table 3. Some oligosaccharides used to promote bifidobacteria *in vivo*[40]

Lactulose, Lactitol, Lactobionic Acid
Neosugar P®
Transgalactosylated oligosaccharides
Galactooligosaccharides, Oligomate®
Gluconic Acid
Xylooligosaccharides
Fructooligosaccharides
Maltooligosaccharides
Stachyose, Raffinose

Provided that increasing bifidobacteria can be identified with reducing risk of cancer, these types of studies support a view that such perturbation of gut flora is in a desirable direction.

Finally there has been considerable research investigating the bacterial and plant cell wall components (peptidoglycans, β-glucans and other polysaccharides) for their influence as an anticancer-strategy in stimulating the immune system via the gut associated lymphoid tissue[45]. This represents a relatively new and challenging area for future research.

Conclusion

There is a promising future for research into probiotic bacteria, to open up a better understanding of the contribution to health of a well constituted balanced microflora in the large intestine. Its potential for

prevention of colon cancer is currently under active investigation, with both animal and human studies contributing. Both approaches appear to be valid and necessary, albeit caution should be exercised in extrapolating animal results directly to humans.

While some of the data supports the view that probiotics as freeze dried powder/ capsules or as yoghurts provide protection from colon cancer, the nature of the diet and/ or components provided by the yoghurt vehicle must also be taken into account.

This means carefully controlled experiments are needed to provide reliable interpretation.

Acknowledgements

I wish to acknowledge the assistance of Leanne Griffiths, Librarian, CSIRO Division of Human Nutrition, Dr Martin Playne of the CSIRO Division of Food Science and Technology, Highett Victoria, for help with this task, and the Dairy Research and Development Corporation for its research grant support.

Probiotics and colon cancer prevention

Graeme H McIntosh

Asia Pacific Journal of Clinical Nutrition (1996) Volume 5, Number 1: 48-52

原生菌（Probiotic）和結腸癌的預防

摘要

這篇綜述檢查了一些有關原生菌（Probiotic Bacteria）做為減低人體結腸癌危險率的因素的証據。雖然嚙齒動物的結腸癌模型在減低癌症的發生率有一些使人信服的証據，但在人體口服這類細菌如勞根式不規則小杆菌（Bifidobacterium Longum）、醋酸性乳酸杆菌（Lactobacillus Acidophilus）仍較難找到使人信服的証據。這包括原生菌（Probiotic）介入後的流行病學調查或標記介入研究，應用糞便酶、糞便膽酸或尿／糞微生物活性誘變劑來測定癌症的危險率。綜合地說，這些資料對原生菌（Probiotic）防癌假說的支持有限。

References

1. Fuller R. Probiotics-an overview. In Human Health: the contribution of microorganisms. 1994. Springer-Verlag, London. 63-73.
2. Metchnikoff E. The prolongation of life. CP Putnams Sons, New York, 1908: 161-183.
3. Mitsuoka T. Intestinal bacteria and health. Harcourt Brace Jovanovich Japan Inc. Tokyo 1978.
4. Adachi S. Chapter 10. Lactic acid bacteria and the control of tumors in The Lactic Acid Bacteria Vol. 1. The lactic acid bacteria in Health and Disease. BJ. Wood ed. Elsevier Applied Science, London. 1992; 233-261.
5. Marteau P, Pochart P, Bouhnick Y, Rambaud J-C. The fate and effects of transiting non pathogenic organisms in the human intestine. World Rev. Nutr Dietet.1993; 71: 1-17.
6. Ballongue J. Chapter 13. Bifidobacteria and probiotic action. In Lactic Acid Bacteria. S Salminen and A von Wright eds. Marcel Dekker Inc, New York. 1993; 357-428.
7. Fernandes, CF, Shahani KM and Guner MA. Therapeutic role of dietary lactobacilli and lactobacilli fermented dairy products. FEMS Microbiology Reviews. 1987; 46: 343-356.
8. Fernandes CF and Shahani KM. Anticarcinogenic and immunological properties of dietary lactobacilli. J Food Protect. 1990; 53: 704.
9. Gorbach SL and Goldin BB. Nutrition and gastrointestinal microflora. Nutrition Reviews. 1991; 50(12): 378-381.
10. Sanders, ME. Chapter 14. Lactic Acid Bacteria as Promotors of Human Health. In Functional Foods ed. Israel Goldberg. Chapman & Hall, New York. 1994; 294-322.
11. Lidbeck A, Nord CE, Gustaffson JA, Rafter JJ. Lactobacilli anticarcinogenic activities and human intestinal microflora. Eur J Cancer Prevention. 1991; 1: 341-353.
12. Salminen S and von Wright A. Lactic Acid Bacteria. Marcel Dekker Inc, Basel, New York. 1993
13. Malhotra SL. 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. Medical Hypothesis. 1977; 3: 122-126.
14. Rafter JJ. The role of lactic acid bacteria in colon cancer prevention. Scand J Gastroenterol. 1995; 30: 497-502.
15. IARC Study. Dietary fibre transit time faecal bacteria steroids and colon cancer in two Scandinavian populations. 1977. Lancet II: 207-211.
16. Rosen M, Nystrom L, Wall S. Diet and cancer mortality in the counties of Sweden. Am J Epidemiol. 1988; 127: 42-49.
17. Goldin BR. Effect of *Lactobacillus acidophilus* dietary supplements on 1, 2-dimethylhydrazine dihydrochloride induced intestinal cancer in rats. J Natl Cancer Inst. 1980; 64: 263-265.
18. Goldin BR, Swenson L, Dwyer J, Sexton M and Gorbach SL. Effect of diet and *Lactobacillus acidophilus* supplements on human faecal bacterial enzymes. J Natl Cancer Inst. 1980; 64(2): 255-261.
19. Goldin BR and Gorbach SL. The effect of oral administration of Lactobacillus and antibiotics on intestinal bacterial activity and chemical induction of large bowel tumor. Dev. Indust. Microbiol. 1984; 25: 139-144.
20. Goldin BR and Gorbach SL. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. Amer J Clin Nutr. 1984; 39: 756-761.
21. Mitzutani T and Mitsuoka T. Effect of intestinal bacteria on incidence of liver tumors in grotobiotic C3H/He male mice. J Natl Cancer Inst. 1979; 63: 1365-70.
22. Kowki T, Imai K, Tamura A, Hashimoto Y. Antitumor effect of *Bifidobacterium infantis* in mice. Gann 1978; 69: 613-8.
23. Reddy GV, Friend BA, Shahani KM and Farmer RE. Antitumor activity of yogurt components. J Food Protect. 1983; 46(1): 8-11.
24. Kato I, Kobayashi S, Yokokura T and Mutai M. Antitumor activity of *Lactobacillus casei* in mice. Gann. 1981; 72: 517-523.
25. Goldin BR. Chemical induction of colon tumors in animals: an overview. Prog Clin Biol Res. 1988; 279: 319-333.
26. Shakelford LA, Rao DR, Chawan CB and Pulusain SR. Effect of feeding fermented milk on the incidence of chemically induced colon tumors in rats. Nutrition Cancer 1983; 5: 159-163.
27. Takano T, Arai K, Murota I, Hayakawa K, Mitzutani T, and Mitsuoka T. Effects of feeding sour milk on longevity and tumorigenesis in mice and rats. Bifidobacteria Microflora. 1985; 4: 31-37.
28. Koo M and Rao AO. Long term effect of Bifidobacteria and neosugar on precursor lesions of colonic cancer in CF1 mice. Nutrition Cancer. 1991; 16: 249-257.

29. Kulkarni N and Reddy BS. Inhibitory effect of bifidobacterium longum cultures on the azoxymethane-induced aberrant crypt foci formation and fecal bacterial β-glucuronidase. PSEBM. 1994; 207: 278-283.
30. Marteau P, Pockart P, Flourie B, Pellier P, Santos L, Desjeux JF and Rambaud JC. Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. Amer J Clin Nutr 1990; 52: 658-688.
31. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gaultiere L and Salminen S. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. Digestive Dis and Sci. 1992; 37: 121-128.
32. Ling WH, Korpela R, Mykkanen H, Salminen S, Haminen O *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J Nutr 1994; 124: 1823.
33. Bartram HP, Scheppach W, Gerlach S, Ruckdeschel G, Kelber E and Kasper H. Does yogurt enriched with *Bifidobacterium longum* affect colonic microbiology and fecal metabolites in healthy adults. Amer J Clin Nutr 1994; 59: 428-432.
34. Baricault L, Denariaz G, Houri JJ, Bouley C, Sapin C and Trugnan G. 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. 1995; 16: 245-252.
35. Benno Y and Mitsuoka T. Effect of diet and ageing on human fecal microflora. Bifidobacteria Microflora. 1991; 10(2): 89-96.
36. Moore WEC and Moore LH. Intestinal floras of populations that have a high risk of colon cancer. Appl. Environment Microbiol. 1995; 61: 3202-7.
37. Roberfroid MB, Bornet F, Bouley C and Cummings, JH. Colonic microflora: nutrition and health. Nutr Reviews. 1995; 53: 127-130.
38. McIntosh GH, Regester GO, LeLeu RH, Royle PJ and Smithers GW. Dairy proteins protect against dimethylhydrazine-induced intestinal cancers in rats. J Nutr. 1995; 124(4): 809-816.
39. Reddy BS and Rivenson A. Inhibition effect of *Bifidobacterium longum* on colon mammary and liver carcinogenesis induced by 2-Amino-3methylimidazo (4,5-f) quinoline, a food mutagen. Cancer Res. 1993; 53: 3914-3918.
40. Playne MJ. Probiotic Microorganisms in Recent Advances in Microbiology, GL Gilbert ed. 1995; 3: 215-25.
41. Ito M, Deguch Y, Matsumoto K, Kimura M, Onodera N and Yajima T. Influence of galactooligosaccharides on the human fecal flora. J Nutr. Sc. Vitaminol. 1995; 39: 635-640.
42. Rowland IR and Tanaka R. The effect of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with a human faecal microflora. J Applied Bacteriology. 1993; 74: 667-674.
43. Morishita Y and Konishi Y. Effects of high dietary cellulose on the large intestinal microflora and short chain fatty acids in rats. Lett. Applied Microbiol. 1994; 19: 433-435.
44. Asano T, Yuasa K, Kunugita K, Teraji T and Mitsuoka T. Effects of gluconic acid on human fecal bacteria. Microbial Ecology in Health & Disease. 1994; 7: 247-256.
45. Waldron KW and Selvendran RR. Bioactive cell wall and related components from herbal products and edible plant organs as protective factors. In Food and Cancer Prevention: Chemical and Biological Aspects. eds. KW Waldron, IT Johnson and GR Fenwick. Royal Society of Chemistry, Cambridge. 1993; 307-326.