Inhibitory Effect of *Bifidobacterium longum* Cultures on the Azoxymethane-Induced Aberrant Crypt Foci Formation and Fecal Bacterial β-Glucuronidase (43817)

NALINI KULKARNI AND BANDARU S. REDDY[1]

Division of Nutritional Carcinogenesis, American Health Foundation, Valhalla, New York 10595

Abstract. **Epidemiologic and experimental studies suggest that consumption of fermented milk products and lactic bacterial cultures that are used to ferment the dairy products, decrease the incidence of certain types of cancer. The present study was designed to determine the effect of lyophilized cultures of *Bifidobacterium longum* (*B. longum*), a lactic bacteria, on the azoxymethane (AOM)-induced preneoplastic lesions such as aberrant crypt foci (ACF) formation in the colon and on fecal bacterial β-glucuronidase activity in male F344 rats. At 5 weeks of age, groups of animals were fed the AIN-76A (control) and the experimental diets containing 1.5% and 3% lyophilized cultures of *B. longum*. At 10 weeks of age, all animals received sc injection of AOM dissolved in normal saline at a dose rate of 20 mg/kg body wt, once weekly for 2 weeks. The animals were necropsied 6 weeks after the last AOM injection, and the ACF were visualized under light microscopy in the formalin-fixed, unsectioned methylene blue-stained colons where they were distinguished by their increased size, more prominent epithelial cells, and pericryptal space. The cecal contents were analyzed for bacterial β-glucuronidase activity. The feeding of lyophilized cultures of *B. longum* significantly inhibited the ACF formation (53%) and the crypt multiplicity in the colon. A significant decrease in the fecal bacterial β-glucuronidase was also observed in the animals fed the diets containing *Bifidobacterium* supplements as compared with control diet. These results demonstrate that *B. longum* in diet influences the metabolic activity of certain types of intestinal microflora that are involved in the production of β-glucuronidase. Furthermore, the findings also suggest that *B. longum* supplements inhibit ACF formation, an early preneoplastic marker of malignant potential in the process of colon carcinogenesis.** [P.S.E.B.M. 1994, Vol 207]

Colorectal cancer is the second leading cause of cancer deaths in the United States (1). Epidemiological and experimental studies provide evidence that nutritional factors play a role in the etiology of colon cancer (2–4). In recent years, there has been a growing awareness of fermented milk, yogurt, and certain bacterial cultures that ferment the dairy products in human and animal health (5–8). Several *in vitro* and animal model studies show that these products may possess antimutagenic and anticarcinogenic properties (9–12). Bodana and Rao (12) demonstrated the antimutagenic activity of fermented milk with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Goldin and Gorbach (9) showed that dietary supplements of *Lactobacillus acidophilus* not only reduced the incidence of 1,2-dimethylhydrazine (DMH)-induced colon cancer, but also increased the latency period. These studies indicate that fermentation of milk may result in the formation of certain inhibitors of carcinogenesis. In addition to *Streptococcus* and *Lac-*

[1] To whom requests for reprints should be addressed at Division of Nutritional Carcinogenesis, American Health Foundation, One Dana Road, Valhalla, NY 10595.

Received April 21, 1994. [P.S.E.B.M. 1994, Vol 207]
Accepted July 13, 1994.

0037-9727/94/2073–0278$10.50/0

tobacillus, Bifidobacteria also ferment milk and other dairy products, and oral administration of live cultures or the products containing *Bifidobacteria* colonize in the colon (13). Several ecological studies also indicate the existence of *Bifidobacteria* in the colons of adult humans (13). The beneficial role of *Bifidobacteria* pertaining to the improvement of intestinal flora and its antitumorigenic property has been reviewed by Ishibashi and Shimamura (13). The major physiological effects of Bifidobacteria relate to improvement of intestinal flora by preventing colonization of pathogens including *Escherichia coli* and *Clostridium*. The cell wall preparations from heat killed *B. infantis* have been shown to possess antitumorigenic effect in BALB/c mice inoculated with syngeneic Meth A fibrosarcoma tumor cells (14). Pool-Zobel *et al.* (15) demonstrated antimutagenic properties of fermented dairy products containing viable *Bifidobacteria*. In addition, *B. longum* has been shown to have protective effect against the lethal activity of endotoxin from *E. coli* in germfree mice (16). Furthermore, there are studies to indicate that cultures of *B. longum* increase the host's immune response (17). In our laboratory, studies with both male and female F344 rats have shown that feeding cultures of *B. longum* inhibited 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced colon, liver and mammary carcinogenesis (7).

Dietary intake of fermented milk containing lactic bacteria and pure cultures of *L. acidophilus* have been shown to reduce the counts of colonic putrefactive bacteria, increase the levels of Lactobacilli and decrease the metabolic activity of certain classes of intestinal microflora as indicated by fecal bacterial β-glucuronidase (6, 18). Although there is no evidence that AOM is metabolized by the bacterial β-glucuronidase, this enzyme is believed to be largely responsible for the hydrolysis of glucuronide conjugates in the colon and thus important in the generation of toxic and carcinogenic substances (19, 20).

Recently, several investigators have identified and characterized microscopic lesions, namely, *aberrant crypt foci* (ACF), in whole mount preparations of colon from animals treated with carcinogen reflecting the earliest identifiable precursor lesions of colon to indicate the initiation of carcinogenic process (21–23). ACF appear in the rat colon within 5 days of colon carcinogen administration (24). Studies in humans have suggested that colonic ACF are putative precursor lesions from which adenomas and andenocarcinomas will develop (25). Although many reports indicate these precursor lesions as predictors of colon tumors, there are few studies which did not show a significant correlation between the number of ACF and alteration in the incidence of colon cancer (26, 27). However, it has been shown that four or more aberrant crypts/focus was a consistent predictor and can be used as a biomarker with malignant potential to predict the tumor incidence in male F344 rats (28). Furthermore, animal model studies have shown the inhibition of ACF by certain candidate chemopreventive agents suggesting that ACF assay could be used to evaluate several agents for their potential chemopreventive properties (24, 29). The purpose of this study was to determine the effect of cultures of *B. longum,* a lactic bacteria indigenous to human intestine, on the colon carcinogen-induced ACF formation and fecal bacterial β-glucuronidase activity in the rat.

Materials and Methods

Animals, Diets, and Carcinogen. Male F344 rats were obtained from Charles River Breeding Laboratories (Kingston, NY). Lyophilized *B. longum* (BB-536) cultures were kindly provided by Morinaga Milk Industry Co., Ltd. (Zama City, Japan). The methods of culturing, harvesting, and lyophilizing *B. longum* as provided by Morinaga Milk Industry were described (Ishibashi N, Shimamura S. Personal communication). *B. longum* was cultured in a medium containing glucose (2.0%), peptone (1.0%), yeast extract (1.0%), and salts (0.5%). The cells were harvested by centrifugation and washed using a saline solution. After mixing with a cryoprotectant solution containing sodium glutamate (1.0%) and sucrose (3.5%), the cells were lyophilized. The lyophilized *B. longum* culture material contained about 80% carbohydrate, protein, and amino acids, and some vitamins (Ishibashi N, Shimamura S. Personal communication). Each gram of lyophilized material contained about 2×10^{10} live bacterial cells. Although fermented dairy products containing *Bifidobacteria* are not common in the United States, several bifidus-fermented dairy products and beverages used in Europe and Japan contain about 10^7 bacterial cultures/ml (13). Azoxymethane (AOM), a colon carcinogen, was purchased from Ash-Stevens (Detroit, MI). All ingredients of semipurified diets were obtained from Dyets, Inc. (Bethlehem, PA) and stored at 4°C prior to preparation of the diets. The composition of semipurified diet is as follows (30): casein, 20%; D,L-methionine, 0.3%; corn starch, 52%; dextrose, 13%; corn oil, 5%; Alphacel, 5%; mineral mix (AIN-76A), 3.5%; vitamin mix (AIN-76A), 1%; and choline bitartrate, 0.2%. Lyophilized cultures of *B. longum* were added to the control diet at the expense of dextrose. Because of relatively small amounts of lyophilized bifidus cultures added to the control diet, no adjustments were made to the fat, protein, and vitamin content of the basal diet. The diets were prepared weekly and stored at 4°C in air-tight plastic containers filled with N_2.

Experimental Procedure. Four-week-old male F344 rats were quarantined for 1 week. At 5 weeks of age, groups of animals were fed the AIN-76A (control)

and experimental diets containing 1.5% and 3% lyophilized *B. longum* cultures. At 10 weeks of age, groups of animals received sc injection of AOM dissolved in normal saline at a dose rate of 20 mg/kg body wt, once weekly for 2 weeks or normal saline alone. The experiment was terminated 6 weeks after the last AOM injection. All animals were sacrificed by CO_2 euthanasia and the cecum was removed, tied and stored anaerobically under a stream of oxygen-free gas (5% Hydrogen, 10% CO_2, 85% Nitrogen) at −20°C for subsequent analysis of fecal β-glucuronidase activity.

Analysis of Aberrant Crypt Foci. For ACF assay, the colons were removed, flushed with Kreb's Ringer salt solution, slit open along the longitudinal median axis and fixed flat between filter papers in 10% buffered formalin for 24 hr. Each colon was then cut into 2-cm segments and stained with 0.2% methylene blue dissolved in Kreb's Ringer solution for 5–10 min. They were then placed on microscope slides with the mucosal side up and aberrant crypts were scored under light microscope at a magnification of ×40 or 100 according to the standard procedures (21–23). Briefly, the aberrant crypt foci were identified from the normal crypts by their increased pericryptal zone, elliptic or circular luminal opening, and greater thickness of the epithelial lining containing one or more crypts, and were recorded for each colon. Crypt multiplicity was determined as the number of crypts in each focus and categorized as those containing one, two, three, and four or more crypts/focus. Finally, the sum of aberrant crypts from all the foci represented the total number of aberrant crypts for each animal.

β-Glucuronidase Assay. The cecum was weighed and its contents were transferred to a tube containing prereduced phosphate-buffered saline (PBS) at pH 7.0. The composition of prereduced PBS is as follows: 1 g of dibasic potassium phosphate, 1 g monbasic potassium phosphate, 10 g sodium bicarbonate, and 2 g sodium chloride, all dissolved in 1100 ml distilled water and autoclaved before use. The samples were vortexed for 1 min and centrifuged at 500 rpm for 5 min to remove the undigested food materials. The slurry was transferred to a fresh tube and subsequently used for enzyme assay. Fecal β-glucuronidase activity was assayed by the method previously used in our laboratory (31). Briefly, an aliquot of fecal suspension was sonicated at 0°C for 30 sec and centrifuged at 20,000*g*. The reaction mixture consisting of 0.1 ml of supernatant (enzyme source) preparation, 0.1 ml of 0.1 *M* phenolphthalein glucuronide (pH 7.0) as substrate, and 0.8 ml of 0.1 *M* phosphate buffer (pH 7.0) was incubated at 38°C for 30 min in a water bath. The reaction was terminated by adding 2.5 ml of 0.01 *M* alkaline glycine buffer and 2.5 ml distilled water. The phenolphthalein liberated was measured at 540 nm using a spectrophotometer, and the enzyme activity was expressed as milligrams of phenolphthalein liberated per hour per gram feces.

Figure 1. Mucosal surface view of AOM-induced unsectioned rat colon stained with methylene blue showing crypt foci with five aberrant crypts; magnification ×100.

Statistical Analysis. The data were analyzed statistically by analysis of variance and Duncan's multiple range test.

Results

The body weights of animals fed the control and *Bifidobacterium* diets were comparable and no significant differences among the groups were observed throughout the study (data not shown). Figures 1 and 2 show the photomicrograph of colonic ACF of animals treated with carcinogen or normal saline (vehicle). As shown in Figure 1, the ACF were distinguished by their increased size, thicker epithelial lining, and increased pericryptal zone, and were observed in the AOM-treated animals fed the control and Bifidobacterium diets. It should also be noted that Figure 1 shows a focus containing five crypts. There were no ACF formed in animals treated with vehicle (normal saline) as shown in Figure 2.

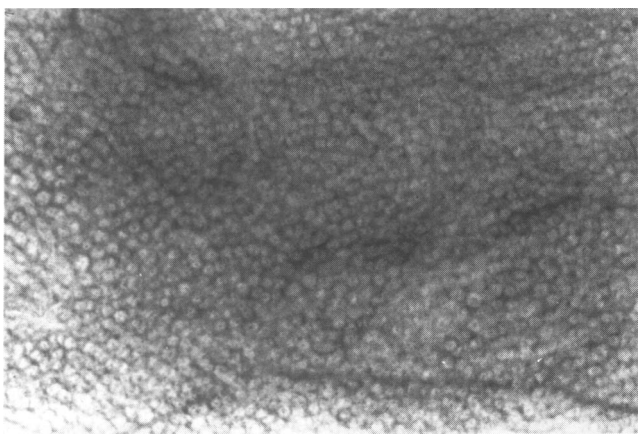

Figure 2. Mucosal surface of normal rat colon treated with saline with no aberrant crypts; magnification ×40.

Table I summarizes the effect of *B. longum* on ACF formation and its characteristics. A significant inhibition in the development of total number of ACF was observed in animals fed 1.5% and 3% Bifido diets as compared with those fed the control diet ($P < 0.01$–0.001). In addition, total number of aberrant crypts per colon were also significantly reduced in animals fed the cultures of *B. longum* as compared with the animals fed control diet ($P < 0.001$). The percentage inhibition of ACF ranged from 43 to 53 in animals fed the Bifidus cultures. There were, however, no differences between the animals fed 1.5% and 3% *B. longum* diets. The crypt multiplicity was analyzed by categorizing the number of crypts/focus. Among crypt multiplicity (number of crypts/focus), two crypts/focus were higher in all three groups as compared with one, three, and four or more crypts/focus. But the crypt multiplicity (one, two, three, or four or more crypts/focus) was significantly reduced ($P < 0.001$) in the animals fed the cultures of *B. longum* as compared to those fed the control diet.

The effect of *B. longum* on fecal bacterial β-glucuronidase activity is shown in Table II. A significant decrease in the activity of β-glucuronidase was observed in the animals fed the *B. longum* diets as compared with those fed control diet ($P < 0.001$). There was, however, no difference among the *B. longum* diets.

Discussion

The present study was undertaken to determine (i) the modifying effect of cultures of *B. longum* on early colonic preneoplastic lesions using ACF formation in a widely used animal model and (ii) the metabolic activity of gut microflora using β-glucuronidase as an indicator. The findings of this study demonstrate the protective effect of *B. longum* towards the AOM-induced ACF formation as compared with control diet, suggesting that this lactic bacteria might have an inhibitory effect in the formation of AOM-induced colon tumors. This was based on the evidence that four or more aberrant crypts/focus was a consistent predictor of tumor incidence (35). The results of the present study, however, could not differentiate whether the inhibitory effect *B. longum* on ACF is at the initiation or postinitiation stage of carcinogenesis because the cultures were administered before, during, and after the AOM treatment until termination of the study. With regard to the modulating effect of tumor promoters and inhibitors on ACF development, McLellan and Bird (32) have demonstrated that feeding of diets containing 1% disulfiram (DSF) 9 days prior to and 14 days after single injection of AOM or DMH resulted in complete inhibition of ACF in CF1 mice. DSF has been shown to inhibit colon cancer in mice given DMH or its metabolite, AOM (33, 34). Previous studies demonstrated that crypt multiplicity was higher in the animals fed cholic acid, a known colon tumor promoter suggesting the importance of this parameter of ACF as an early marker (35). Therefore, this characteristic appears to be responding to modulating effect of tumor promoters. Among the aberrant crypt foci characteristics, crypt multiplicity (number of crypts per focus) has been considered as one of the consistent predictors of tumor outcome (35). Our results show that the occurrence of two crypts/focus was higher as compared with one, three or four crypts/focus in all dietary groups. However, feeding of *B. longum* cultures not only reduced the formation of one, two, and three crypts/focus but also significantly inhibited the formation of four or more crypts/focus. A number of animal model studies have demonstrated that dietary *L. acidophilus* or *B. longum* inhibit DMH- or IQ-induced colon carcinogenesis in laboratory animal models (7, 9). The results of the present study, which indicate that lyophilized cultures of *B. longum* a lactic acid-producing bacteria indigenous to human colon, inhibited the formation and multiplicity of preneoplastic ACF in the colon, provide some evidence for potential colon tumor-inhibitory properties of lactic cultures and fermented dairy products.

Though the mode of action of *B. longum* is not completely understood, it is possible that it can alter the physiological condition in the colon by affecting

Table I. Effect of *Bifidobacterium longum* on AOM-Induced ACF Formation in Male F344 Rats

Dietary regimen	Total no. of ACF/colon	No. of foci containing				Total no. of aberrant crypts/colon
		1 Crypt	2 Crypts	3 Crypts	4 Or more crypts	
Control diet	101 ± 16.0[a]	17.6 ± 4.9	39.4 ± 9.9	25.9 ± 5.7	19.0 ± 5.3	249 ± 54
1.5% Bifido	52.9 ± 14.6[b]** (48)[c]	6.6 ± 4.5[b]*** (63)	21.1 ± 5.9[b]*** (46)	12.7 ± 3.7[b]*** (51)	12.5 ± 5.6[b]* (34)	142 ± 37[b]*** (43)
3% Bifido	47.1 ± 19.3[b]*** (53)	5.5 ± 5.3[b]*** (69)	19.1 ± 3.3[b]*** (52)	11.8 ± 1.3[b]*** (54)	10.6 ± 4.7[b]** (44)	130 ± 46[b]*** (48)

[a] Values are mean ± SD; $n = 11$.
[b] Significantly different from control diet; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
[c] Values in parenthesis represent percentage of inhibition when compared with control diet.

Table II. Effect of *Bifidobacterium longum* on the Fecal Bacterial β-Glucuronidase in Male F344 Rats

Dietary regimen	β-Glucuronidase[a]
Control diet	3.3 ± 1.2[b]
1.5% Bifido	2.2 ± 0.65[c]
3% Bifido	1.9 ± 0.68[c]

[a] Values are expressed as mg of phenolphthalein liberated/hr/g of feces.
[b] Values are mean ± SD; $n = 11$.
[c] Significantly different from control diet, $P < 0.001$.

the metabolic activity of intestinal microflora. The species of *Lactobacilli* and *Bifidobacterium* as dietary supplements have been shown to have a suppressive effect towards several enteropathogenic organisms such as *E. coli* and *C. perfringens* (16, 36, 37). In this study, we have shown that *B. longum* significantly reduced the colonic β-glucuronidase activity. From the reduced activity of β-glucuronidase, it is clear that there could be a decreased production of active carcinogenic metabolites being transported to the colon. In this connection, Tohyama *et al.* have shown that administration of *B. breve* reduced the activity of bacterial enzymes such as β-glucuronidase, tryptophanase, and lysine decarboxylase in feces (38). Furthermore, Goldin and Gorbach have observed that Lactobacillus supplements significantly decreased the levels of bacterial β-glucuronidase (19, 39). The significance of this bacterial enzyme should be considered in the light of its importance in the etiology of colon cancer (40).

In conclusion, the results of this study demonstrate that lyophilized cultures of *B. longum,* a lactic acid bacillus in the colon, inhibit the formation of AOM-induced preneoplastic lesions in the colon and fecal bacterial β-glucuronidase. Further studies are required to corroborate these findings on the inhibition of AOM-induced colon tumorigenesis in this model system.

We thank Donna Virgil for the preparation of manuscript and the staff of Research Animal Facility for technical assistance. The study was supported by USPHS Grant CA 17613 from the National Cancer Institute.

1. Boring CC, Squires TS, Tong T. Cancer Statistics. CA-A Cancer J Clin **43**:7–26, 1993.
2. Veer PV, Dekker JM, Lamers JWJ, Kok FJ, Schouten EG, Brants HAM, Sturmans F, Hermus RJJ. Consumption of fermented milk products and breast cancer: A case-control study in The Netherlands. Cancer Res **49**:4020–4023, 1989.
3. Reddy BS. Dietary fiber and colon cancer: Animal model studies. Prev Med **6**:559–565, 1987.
4. Potter JD, McMichael AJ. Diet and cancer of the colon and rectum: A case-control study. J Natl Cancer Inst **76**:557–569, 1986.
5. Rao DR, Pulusani SR, Chawan CB. Natural inhibitors of carcinogenesis: Fermented milk products. In: Reddy BS, Cohen LA, Eds. Diet, Nutrition, and Cancer: A Critical Evaluation. Boca Raton, FL: CRC Press, Vol **2**:pp63–75, 1986.
6. Shahani KM, Ayebo AD. Role of dietary *lactobacilli* in gastrointestinal microecology. Am J Clin Nutr **33**:2448–2457, 1980.
7. Reddy BS, Rivenson A. 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, 1993.
8. Mitsuoka T. *Bifidobacteria* and their role in human health. J Indus Microbiol **6**:263–268, 1990.
9. Goldin BR, Gorbach SL. Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. J Natl Cancer Inst **64**:263–265, 1980.
10. Shackelford LA, Rao DR, Chawan CB, Pulusani SR. Effect of feeding fermented milk on the incidence of chemically induced colon tumors in rats. Nutr Cancer **5**:159–164, 1983.
11. Bourlioux P, Pochart P, Karger Basel. Nutritional and health properties of yogurt. Wld Rev Nutr Diet **56**:217–258, 1988.
12. Bodana AR, Rao DR. Antimutagenic activity of milk fermented by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. J Dairy Sci **73**:3379–3384, 1990.
13. Ishibashi N, Shimamura S. *Bifidobacteria:* Research and development in Japan. Food Technol **47**:126–135, 1993.
14. Sekine K, Toida T, Saito M, Kuboyama M, Kawashima T, Hashimoto Y. 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, 1985.
15. Pool-Zobel BL, Munzner R, Holzapfel WH. Antigenotoxic properties of lactic acid bacteria in the *S. typhimurium* mutagenicity assay. Nutr Cancer **20**(3):261–270, 1993.
16. Yamazaki S, Kamimura H, Momose H, Kawashima T, Ueda K. Protective effect of *Bifidobacterium* monoassociation against lethal activity of *Escherichia coli*. Bifidobacteria Microflora **1**:55–59, 1982.
17. Ueda K. Immunity provided by colonized enteric bacteria. Bifodobacteria Microflora **5**:67–72, 1986.
18. Ayebo AD, Angelo IA, Shahani KM. Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. Milchwissenschaft **35**:730–733, 1980.
19. Goldin BR, Gorbach SL. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. J Natl Cancer Inst **57**:371–375, 1976.
20. Weisburger JH, Grantham PH, Horton RE, Weisburger EK. Metabolism of the carcinogen N-hydroxy-N-flourenylacetamide in germ-free rats. Biochem Pharmacol **19**:151–162, 1970.
21. McLellan EA, Bird RP. Specificity study to evaluate induction of aberrant crypts in murine colons. Cancer Res **48**:6183–6186, 1988.
22. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. Cancer Lett **37**:147–151, 1987.
23. Rao CV, Desai D, Simi B, Kulkarni N, Amin S, Reddy BS. Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. Cancer Res **53**:4182–4188, 1993.
24. Wargovich MJ, Harris C, Chen CD, Palmer C, Steele VE, Kelloff GJ. Growth kinetics and chemoprevention of aberrant crypts in the rat colon. J Cell Biochem **16**(Suppl G):51–54, 1992.
25. Pretlow TP, Barrow BJ, Ashton WS, O'Riordon MA, Pretlow TG, Jurcisek JA, Stellato TA. Aberrant crypts: Putative preneoplastic foci in human colonic mucosa. Cancer Res **51**:1564–1567, 1991.
26. Hardman WE, Cameron IL, Heitman DW, Contreras E. Demonstration of the need for end point validation of putative bio-

markers: Failure of aberrant crypt foci to predict colon cancer incidence. Cancer Res **51**:6388–6392, 1991.
27. Bird RP. Effect of cholic acid on the number and growth of aberrant crypt foci: Putative preneoplastic lesions. Proc Am Assoc Cancer Res **32**:147, 1991.
28. Pretlow TP, O'Riordan MA, Somich GA, Amini SB, Pretlow TG. Aberrant crypts correlate with tumor incidence in F344 rats treated with azoxymethane and phytate. Carcinogenesis **13**:1509–1512, 1992.
29. Pereira MA, Barnes LH, Rassman VL, Kelloff GV, Steele VE. Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. Carcinogenesis **15**:1049–1054, 1994.
30. Reddy BS, Sugie S. Effect of different levels of omega-3 and omega-6 fatty acids on azoxymethane-induced colon carcinogenesis in F344 rats. Cancer Res **48**:6642–6647, 1988.
31. Reddy BS, Hanson D, Mangat S, Mathews L, Sbaschnig M, Sharma C, Simi B. Effect of high-fat high-beef diet on fecal bacterial enzymes and fecal bile acids and neutral sterols. J Nutr **110**:1880–1887, 1980.
32. McLellan E, Bird RP. Effect of disulfiram on 1,2-dimethylhydrazine- and azoxymethane-induced aberrant crypt foci. Carcinogenesis **12**:969–972, 1991.
33. Wattenberg LW. Inhibition of dimethylhydrazine-induced neoplasia of the large intestine by disulfiram. J Natl Cancer Inst **54**:1005–1006, 1975.
34. Wattenberg LW, Lam LKT, Fladmoe AV, Borchert P. Inhibitors of colon carcinogenesis. Cancer **40**:2432–2435, 1977.
35. Magnuson BA, Carr I, Bird RP. Ability of aberrant crypt foci characteristics to predict colon tumor incidence in rats fed cholic acid. Cancer Res **53**:4499–4504, 1993.
36. Vincent JG, Veonett RC, Riley RG. Antibacterial activities associated with *Lactobacillus acidophilus*. J Bacteriol **78**:477–482, 1959.
37. Mutai M, Tanaka R. Ecology of *Bifidobacterium* in the human intestinal flora. Bifidobacteria Microflora **6**:33–41, 1987.
38. Tohyama K, Tanaka R, Kobayashi Y, Mutai M. Relationship between the metabolic regulation of intestinal microflora by feeding *Bifidobacterium* and host hepatic function. Bifidobacteria Microflora **1**:45–50, 1982.
39. Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL. Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. J Natl Cancer Inst **64**:255–261, 1980.
40. Reddy BS, Engle A, Simi B, Goldman M. Effect of dietary fiber on colonic bacterial enzymes and bile acids in relation to colon cancer. Gastroenterology **102**:1475–1482, 1992.