Effect of *Lactobacillus acidophilus* KFRI342 on the development of chemically induced precancerous growths in the rat colon

Jin-Hee Chang,^{1,2} Youn Young Shim,³ Seong-Kwan Cha,² Martin J. T. Reaney³ and Kew Mahn Chee¹

Correspondence

Youn Young Shim
younyoung.shim@usask.ca
Kew Mahn Chee
cheekm@korea.ac.kr

¹College of Life Sciences and Biotechnology, Korea University, 5-1 Anam-dong, Sungbuk-gu, Seoul 136-701, Republic of Korea

²Traditional Foods Research Center, Korea Food Research Institute, San 46-1, Baekhyun-dong, Bundang-gu, Sungnam-si, Kyunggi-do 463-746, Republic of Korea

³Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

Lactobacillus acidophilus KFRI342, isolated from the Korean traditional food kimchi, was investigated for its suitability as a dietary probiotic. The effects of *L. acidophilus* KFRI342 on the development of chemically induced (1,2-dimethylhydrazine; DMH) precancerous cytological changes of the colon were investigated in rats. Forty-five male F344 rats were randomly divided into three dietary groups. The control group received a high-fat diet (HF), a second group received a high-fat diet containing the carcinogen (HFC), and a final group received a high-fat diet containing the carcinogen and *L. acidophilus* KFRI342 (HFCL). *L. acidophilus* KFRI342 was administered orally three times per week at 2×10^9 c.f.u. ml⁻¹. *L. acidophilus* KFRI342 treatments decreased the number of *Escherichia coli* in faecal samples, the enzyme activities of β -glucuronidase and β -glucosidase, and plasma triglyceride concentration compared to the HF and HFC treatments ($P < 0.05$). *L. acidophilus* KFRI342 consumption also decreased the ratio of aberrant crypts to aberrant crypt foci incidence and the number of aberrant crypts in HFCL rats. Therefore, *L. acidophilus* showed potential probiotic activity as an inhibitor of DMH-induced symptoms in live rats. Our *in vivo* studies indicate that *L. acidophilus* from kimchi may be suitable as a probiotic for human use.

Received 10 June 2011
Accepted 21 October 2011

INTRODUCTION

Lactic acid bacteria (LAB) are considered to be beneficial probiotic organisms that inhibit harmful intestinal bacteria. They improve lactose malabsorption in humans, increase immune function (Maldonado Galdeano *et al.*, 2007) and prevent cancer (de Roos & Katan, 2000). Several of the most prevalent cancers develop as a result of an interaction between endogenous and environmental factors, most notably the diet. An epidemiological study by Garófolo *et al.* (2004) reported that 35 % of all types of cancer are thought to be due to inadequate diet among these causal factors.

Abbreviations: AC, aberrant crypt; ACF, aberrant crypt foci; DMH, 1,2-dimethylhydrazine; EC, *E. coli* plate count; FER, food efficiency ratio; HDL, high-density lipoprotein cholesterol; HF, high-fat diet; HFC, high-fat diet containing the carcinogen; HFCL, high-fat diet containing the carcinogen and *L. acidophilus*; LAB, lactic acid bacteria; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; TPC, total plate count of aerobic bacteria.

Carcinogenesis is a complex process involving events at several levels, from molecular to cellular to morphological. It can be divided into three main phases: initiation, promotion and progression (Fodde, 2002). In colorectal cancer, the initiation phase can be recognized by the formation of lesions in the intestine called aberrant crypt foci (ACF), which can develop into cancerous tissue (Bird & Good, 2000). These lesions have often been used as biomarkers for the initial phase of colorectal cancer in rats induced with 1,2-dimethylhydrazine (DMH) (Demarzo & Garcia, 2004). This genotoxic carcinogen is frequently used to induce experimental colon carcinogenesis in rodents. DMH is an alkylating agent that targets DNA, and induces the formation of methyl adducts with DNA bases, point mutations, micronuclei and sister chromatid exchanges (Choudhary & Hansen, 1998), thus yielding macroscopically visible neoplasms in a dose-dependent manner (Sequeira *et al.*, 2000). Development of colon cancer is a multistep process involving a series of pathological alterations ranging from discrete microscopic mucosal lesions and ACF to malignant

tumours (Takayama *et al.*, 1998). ACF are induced specifically by carcinogens that predominantly elicit colonic tumours, and are considered to be precursors of colon cancer (Wargovich *et al.*, 1995). Multiplicity of ACF increases with time and appears to be a predictor of tumour outcome. Thus, the DMH-induced colon cancer model in rats is a good tool to investigate the relationship between ACF and colon cancer, and also to evaluate agents with potential chemopreventive properties prior to preclinical studies (Wargovich *et al.*, 1995). This efficient animal-tumour model was a useful approach to study *Lactobacillus acidophilus* KFRI342 during the initiation and post-initiation period, and to understand colon carcinogenesis.

Previous *in vivo* studies with LAB showed the suppression of pre-neoplastic lesions and chemically induced colon tumours in rodent models. Literature between 1996 and 2005 showed that probiotics had anti-carcinogenic effects against chemically induced tumours or ACF (Commane *et al.*, 2005). Goldin *et al.* (1996) investigated the effect of diets enriched with *Lactobacillus rhamnosus* strain GG on the development of DMH-induced tumours in rats. There was a lower incidence of colonic tumours in rats fed *L. rhamnosus* GG before, during and after exposure to DMH compared to rats that had only received the probiotic after nine weekly injections of the carcinogen. In addition, the authors concluded that the probiotics inhibited the initiation or early promotion phase of carcinogenesis. In a similar experiment, Rowland *et al.* (1998) studied the impact of prebiotic diets on the promotion phase of carcinogenesis. Oral administration of prebiotics was initiated 1 week after carcinogen exposure. Subsequently, the impact of both probiotic and prebiotic on ACF formation was observed. Dietary *Bifidobacterium longum* inhibited azoxymethane (AOM)-induced ACF formation (Rowland *et al.*, 1998). In addition, the simultaneous administration of these probiotics with the prebiotic inulin increased this effect. Interestingly, there was a decrease of faecal ammonia, which is considered to be a tumour promoter. Rowland *et al.* (1998) proposed that the probiotics act during the promotion phase of carcinogenesis, as administration of the diets was delayed by 1 week after exposure to the carcinogen.

We isolated *L. acidophilus* KFRI342 from the Korean traditional food kimchi (Chang *et al.*, 2010). In this study, *L. acidophilus* KFRI342 was administered orally to F344 rats to determine how it affects the growth of pathogenic bacteria and blood biochemical parameters over a 10-week period. In addition, the effect of this *L. acidophilus* strain on the early development of cancer was examined by studying the formation of ACF in rats injected with DMH. Oral administration of *L. acidophilus* KFRI342 inhibited the development of pre-neoplastic lesions and lowered the populations of both *Escherichia coli* and aerobic bacteria of the microbiota, which have been associated with carcinogenesis.

METHODS

Materials. DMH, *p*-nitrophenyl β -D-glucopyranoside, *p*-nitrophenyl β -D-glucuronide and general laboratory chemicals were purchased from Sigma. Corn oil and lard were obtained from Samyang Co. The biochemical assays for fasting plasma levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglyceride (TG) were supplied by Asan Pharmaceutical Co. and Wako Chemical.

Production of *L. acidophilus* KFRI342. *L. acidophilus* KFRI342 production was performed as previously described (Chang *et al.*, 2010). In brief, *L. acidophilus* KFRI342 grown to late-exponential phase was collected by centrifugation (VS-15000, Vision Scientific) at 5000 g, 4 °C for 10 min, and then washed with 0.9% NaCl. The freeze-dried powder (1.2 g) was resuspended in 0.9% NaCl, with a final *L. acidophilus* KFRI342 concentration of 2×10^9 c.f.u. ml⁻¹.

Animal maintenance. Forty-five male F344 rats, 5 weeks old, with an average body weight of 130 g, were purchased from Central Lab. Animal Inc. (Seoul, Republic of Korea), and acclimatized by feeding *ad libitum* with the American Institute of Nutrition AIN-76A purified rodent diet (Central Lab. Animal Inc.) for 1 week. The rats were then randomly divided into three dietary groups ($n=15$) that were administered 10 weeks of treatment as described in Fig. 1. The control group (HF) received a high-fat diet resembling the diet of some Western human populations at high risk of colon cancer according to Femia *et al.* (2002): AIN-76A supplemented with 15% fat (corn oil/lard mixture; 1:1, w/w). The rats were fed a high-fat diet during this experiment, which represented the promotion phase of carcinogenesis, because ACF are dysplastic regions of the colon that are thought to represent the earliest precursors of colon cancer. The second group (HFC) received the high-fat diet containing the carcinogen DMH, and the third group (HFCL) received the high-fat diet containing the carcinogen and *L. acidophilus* KFRI342. *L. acidophilus* KFRI342 in the form of a freeze-dried powder was administered orally three times per week at 2×10^9 c.f.u. ml⁻¹. All groups were allowed to consume the diets *ad libitum*. Body weight and food consumption were measured weekly. The rats were housed in polycarbonate cages with paper chip bedding in an animal holding room under controlled conditions (23–28 °C, 12 h light/12 h dark cycle, 55% humidity). All animal protocols were approved by the institutional Animal Care and Use Committee of the Korea Food Research Institute (KFRI, Kyunggi-do, Republic of Korea).

Chemical induction of colon cancer. All animals except the control group (HF) were injected intramuscularly weekly for 10 weeks with 20 mg per kg body weight of DMH (a chemical inducer of carcinogenesis in the colon) dissolved in an aqueous solution containing 1 mM EDTA at pH 6.5. The control rats (HF) were injected with saline.

Blood collection and biochemical analysis. At the end of the experiment, the food was removed and experiments were performed between 9 a.m. and 12 noon. Fasting blood samples were first obtained 12 h after overnight fasting. The blood samples were obtained from the tail vein of the rats at the start and the end of the experiment, and were centrifuged at 3000 g for 15 min at 4 °C. The plasma was isolated and stored at -70 °C before analysis. The concentrations of serum TC, HDL, LDL and TG were measured using Bayer Advia Reagent Packs, and assayed on a Bayer ADVIA 1650 chemistry system analyser.

Gross evaluation of visceral organs. All surviving animals were sacrificed under ether anaesthesia after 10 weeks. The following organs were collected and rinsed with 0.9% NaCl to remove adherent blood: liver, left and right kidney, spleen and colon. The gross

Fig. 1. Experimental design for DMH-induced colon carcinogenesis. There were 15 male F344 rats per group. HF, high-fat diet; HFC, high-fat diet + 1,2-dimethylhydrazine (DMH); HFCL, high-fat diet + DMH + *L. acidophilus* KFRI342. The diet was AIN-76A purified rodent diet with 7.5 % (w/w) lard and 7.5 % (w/w) corn oil added. DMH (20 mg per kg⁻¹ body weight) was injected intramuscularly on the thigh at weekly intervals. *L. acidophilus* KFRI342 was orally administered three times per week at 2×10^9 c.f.u. ml⁻¹.

anatomies of the visceral organs of each rat were inspected. The weights of each organ in the test and control groups were compared, and the lengths of the colon segments were measured.

Faecal samples. Faecal samples of the three groups were collected weekly from each rat and analysed for pH, microbial population counts, and bacterial enzyme activity. The samples were frozen at -70°C within 1 h after being placed in anaerobic containers and were stored therein until analysed.

pH and microbial analysis. The pH of the faecal samples was determined in distilled water at room temperature (20°C) with an Orion ROSS pH meter and combined ROSS glass electrode (Thermo Scientific) standardized with pH 4.01 and 7.01 standard buffer solutions (Merck). Each of the faecal samples was analysed to determine the total plate count of aerobic bacteria (TPC) and *E. coli* count (EC). Twenty-five grams of faecal sample was aseptically obtained from each lot and homogenized with the appropriate amount of sterile 0.1 % peptone water (PW, Gelysate; BBL) to make a 10^{-1} dilution. The resulting homogenate was serially 10-fold diluted with PW. The TPC and EC were determined by surface plating 1.0 ml of appropriate dilutions onto Petrifilm aerobic count plates and *E. coli* count plates (3M Microbiology Products) (Soylemez *et al.*, 2001), respectively, followed by incubation for 48 h at 37°C . The numbers of blue colonies, which are associated with gas formation, were recorded. The TPC and the EC analyses are reported as $\log_{10}$ c.f.u. [(g faeces)⁻¹].

Faecal bacterial enzyme assays. Faecal samples of the three groups were homogenized in 0.9 % NaCl and centrifuged at 2000 g, 4°C for 15 min. Each faecal sample (0.1 g) was suspended in 0.9 ml 0.1 M phosphate buffer (pH 7.0, containing 0.5 % cysteine) and this suspension was used as an enzyme source. The faecal bacterial activities were assayed as follows. The 1.0 ml reaction mixture, consisting of 0.8 ml 2.0 mM *p*-nitrophenyl β -D-glucuronide (for β -glucuronidase) or *p*-nitrophenyl β -D-glucopyranoside (for β -glucosidase) in 0.1 M sodium phosphate buffer (pH 7.0) and 0.2 ml of the enzyme solution (suspended faecal sample), was incubated at 37°C for 30 min in a shaking water bath, and then 1.0 ml 0.5 M NaOH was added. The stopped reaction mixture was centrifuged at 2000 g for 10 min. The enzyme activity was measured by monitoring the increase in absorbance of the supernatant at 405 nm in a Beckman DU 800 spectrophotometer.

Evaluation of colon segments. At the end of the 10 week experiment, the colons were immediately removed from their proximal end to the beginning of the rectum of each animal by ventral incision. They were washed with physiological saline (0.9 % NaCl) to remove the faeces, slit longitudinally and laid open on blocks of expanded polystyrene. These were immersed in 10 % buffered formaldehyde solution for 24 h and then transferred to 70 % aqueous ethanol (Alves de Lima *et al.*, 2007). The fixed colon segments were stained in 0.1 % methylene blue solution for 10 min. Starting at the distal end, 25 consecutive fields were examined at $10 \times$ magnification under a microscope coupled to an image-capture system (Nikon), and the images were analysed to identify and count the ACF. ACF were identified as elevated focal lesions having one to multiple abnormal aberrant crypts (ACs) with thickened epithelial linings and enlarged luminal openings compared with the normal adjacent mucosa (Bird, 1987).

Statistical analysis. Statistical analyses were performed using SPSS (Version 11.0), and all data are expressed as means $\pm$ SD. Statistically significant differences between the groups were found using a one-way analysis of variance (ANOVA) (Garófalo *et al.*, 2004). Duncan's multiple-range test was then performed to identify significant differences between the mean values of each treatment group at $P < 0.05$.

RESULTS

General observations

There were no significant differences in the initial body weight and food intake among the experimental groups (data not shown). After 6 weeks, the HFCL group had reduced food intake compared to the HF and HFC groups ($P < 0.05$; data not shown). During the experimental period, no clinical signs of toxicity were observed in any of the groups. Although DMH injection (HFC group) and feeding *L. acidophilus* KFRI342 (HFCL group) induced a statistically significant reduction in food consumption, no significant differences in food efficiency ratio (FER) were observed among the groups ($P < 0.05$; Table 1).

Table 1. Weight gain, net food intake, FER and relative organ weights in the three experimental groups

HF, high-fat diet; HFC, high-fat diet + DMH; HFCL, high-fat diet + DMH + *L. acidophilus* KFRI342. Values are the means $\pm$ SD of 15 rats in each group. Values in the same row that do not share a common superscript are significantly different at $P < 0.05$.

	HF	HFC	HFCL
Growth efficiency			
Net weight gain (g)	190.1 $\pm$ 15.4 ^a	169.2 $\pm$ 15.9 ^b	171.8 $\pm$ 14.1 ^b
Net food intake (g)	1400.6 $\pm$ 135.9 ^a	1367.7 $\pm$ 125.1 ^b	1355.7 $\pm$ 117.9 ^b
FER (%) [*]	13.6 $\pm$ 0.6 [†]	12.4 $\pm$ 0.5 [†]	12.7 $\pm$ 0.8 [†]
Organ weight [g (100 g body wt) ⁻¹]			
Liver	2.98 $\pm$ 0.23 ^a	2.87 $\pm$ 0.26 ^b	2.90 $\pm$ 0.31 ^{ab}
Kidney	0.78 $\pm$ 0.01 ^a	0.60 $\pm$ 0.02 ^b	0.61 $\pm$ 0.02 ^b
Spleen	0.22 $\pm$ 0.02 [†]	0.18 $\pm$ 0.04 [†]	0.20 $\pm$ 0.04 [†]

^{*}FER (%), food efficiency ratio = weight gain (g)/food intake (g) $\times$ 100.

[†]Not significantly different between groups.

Gross evaluation of visceral organs

Organ (liver, kidney and spleen) weights per 100 g body weight are shown in Table 1. DMH injection (HFC group) and administration of *L. acidophilus* KFRI342 (HFCL group) resulted in a slight reduction in relative organ weights, except for the spleen, compared to the HF control group. Colon weight and length were not affected by injection with DMH, but were increased in the HFCL group. The HFCL group had higher mean weight and length of the colon, at 540.43 $\pm$ 7.02 mg and 62.85 $\pm$ 6.16 cm, respectively, than the other two groups ($P < 0.05$; Fig. 2).

Fig. 2. Colon weight (a) and length (b) in groups HF (white bars), HFC (grey bars) and HFCL (black bars). For each group, columns that do not share a common letter are significantly different ($P < 0.05$).

Biochemical analyses of plasma

The levels of plasma TC, HDL, LDL and TG were examined at 10 weeks (Fig. 3). Plasma TC and HDL concentrations were significantly lower in the HFCL group compared with the HF group ($P < 0.05$). LDL concentration was lower in the HFCL group, though not significantly. Plasma TG concentration was significantly influenced by dietary *L. acidophilus* KFRI342: HFC (129.8 $\pm$ 9.3 mg dl⁻¹) > HFCL (118.4 $\pm$ 10.3 mg dl⁻¹) ($P < 0.05$).

Faecal pH and microbial analyses

Faecal samples of the rats were collected to compare pH, TPC and EC between the control (HF group) and the two treatment groups. At the end of the 10-week experiment,

Fig. 3. Blood lipid profiles in groups HF (white bars), HFC (grey bars) and HFCL (black bars). Groups not sharing a common superscript letter (a–c) differ significantly at $P < 0.05$.

the HFCL group had lower pH, TPC and EC than the HF and HFC groups (Fig. 4). A comparison of pH among groups showed that the pH of the faecal samples started changing after 2 weeks (Fig. 4a). The pH of the faecal samples in the *L. acidophilus* KFRI342-fed group (HFCL) declined until week 4, while the pH of the faecal samples in the other groups remained constant. The pH of the faecal samples after 10 weeks was 7.8 in the HFCL group and 8.4 in the non-fed groups (HF and HFC). The microbial counts obtained from the HFCL group showed a significant ($P<0.05$) decrease after 10 weeks (Fig. 4b, c). The HF group's TPC value increased from 6.7 to 8.0 $\log_{10}(\text{c.f.u. g}^{-1})$ during the first 2 weeks and there were no further changes (Fig. 4b). The TPC in the HFC group reached 6.7–7.4 $\log_{10}(\text{c.f.u. g}^{-1})$ and did not change during the 10-week observation period. However, the TPC of the HFCL group after 10 weeks was 2 logs lower than those of the other groups. The EC in the HFCL group declined from week 3 and was 4 and 2 logs lower ($P<0.05$) than in the HF and HFC treatments after 10 weeks (Fig. 4c).

Faecal bacterial enzyme assays

The activities of β -glucuronidase and β -glucosidase after 10 weeks were reduced by the administration of *L.*

Fig. 4. Faecal pH and bacterial counts. (a) pH, (b) total plate count of aerobic bacteria (TPC), (c) *E. coli* count. ●, HF group; ▲, HFC group; □, HFCL group.

acidophilus KFRI342 (Fig. 5). Activities of both enzymes declined in the first 2–3 weeks; this may have been due to adaptation following the switch from the standard AIN-76 diet to the high-fat diet. The enzyme levels in the HF and HFC groups were lowest after 2 and 3 weeks, respectively, and then increased until week 10. In the HFCL group the activities were lowest for both enzymes between 2 and 4 weeks, and then increased until week 10. At the end of the 10 week experiment, the β -glucuronidase level was 40 % lower in the HFCL group (A_{405} 0.7) as compared to the HF and HFC groups (A_{405} 1.2) (Fig. 5a). The β -glucosidase level was 30 % lower in the HFCL group (A_{405} 1.1) as compared to the HFC group (A_{405} 1.8) and 20 % lower than the HF group (A_{405} 1.5) (Fig. 5b).

Evaluation of colon segments

ACF developed in rats treated with or without DMH. ACF were counted 1 week after the last DMH administration. Lesions (ACF) can be classified as foci containing three or more ACs, which are characterized by their larger size and wider pericryptal zones with thickened epithelial linings and enlarged luminal openings. Data on the incidence of ACF and multiplicity of ACs in the different groups are summarized in Table 2. At the end of the 10 week experiment, there were significant differences in the frequency of ACF between the treatments. The total numbers of ACs in the colon were significantly ($P<0.05$) decreased, by 41.1 % in the HFCL group (179.1 ± 92.8) as compared to the HFC (435.9 ± 198.4) and were similar to the control group (HF, 169.3 ± 81.7). The number of ACF containing one or two crypts in the rats with *L. acidophilus* KFRI342 treatment was the same as in the HF control

Fig. 5. Activity of (a) β -glucuronidase and (b) β -glucosidase in faecal samples. ●, HF group; ▲, HFC group; □, HFCL group.

Table 2. Effects of *L. acidophilus* KFRI342 on development of ACs and ACF induced by DMH in the rat colon

Values are the means $\pm$ SD of 15 rats in each group. Values in the same row that do not share a common superscript are significantly different at $P < 0.05$.

Group*	No. of ACF with			Total no. of		AC/ACF
	1 crypt	2 crypts	≥ 3 crypts	AC	ACF	
HF	48.4 $\pm$ 15.7 ^a	20.2 $\pm$ 10.5 ^a	10.8 $\pm$ 7.4 ^b	169.3 $\pm$ 81.7 ^a	80.6 $\pm$ 33.1 ^a	2.1 $\pm$ 0.3 ^a
HFC	94.1 $\pm$ 41.8 ^b	31.3 $\pm$ 19.6 ^b	19.2 $\pm$ 11.2 ^c	435.9 $\pm$ 198.4 ^b	145.3 $\pm$ 83.8 ^b	3.0 $\pm$ 0.3 ^b
HFCL	56.3 $\pm$ 21.5 ^a	18.3 $\pm$ 4.2 ^a	5.8 $\pm$ 1.4 ^a	179.1 $\pm$ 92.8 ^a	81.4 $\pm$ 10.9 ^a	2.2 $\pm$ 0.4 ^a

*HF, high-fat diet; HFC, high-fat diet + DMH; HFCL, high-fat diet + DMH + *L. acidophilus* KFRI342.

group ($P < 0.05$), but there were significantly fewer ACF with three or more crypts.

DISCUSSION

There have been few investigations on the possible correlations between colon cancer phases and probiotic consumption. Our results showed that the ingestion of *L. acidophilus* KFRI342 inhibited the development of ACF. This indicates that this LAB strain was able to impede the clonal proliferation of cells initiated by DMH in the intestinal mucosa, under these experimental conditions. This study was designed to assess whether the ingestion of *L. acidophilus* KFRI342 might have a short-term effect on carcinogenesis induced in rats by DMH injection. The dietary supplements were administered with a diet that conformed to the nutritional composition of the AIN-76A purified rodent diet, supplemented with 7.5 % corn oil and 7.5 % lard.

Rats fed with *L. acidophilus* KFRI342 showed no treatment-associated signs of adverse effects in the clinical appearance of the animals. Although DMH injection (HFC group) and administration of *L. acidophilus* KFRI342 (HFCL group) induced a statistically significant reduction in food consumption ($P < 0.05$), no significant differences in FER were observed among the groups (Table 1). This would suggest that the DMH was affecting appetite rather than the FER. Feeding *L. acidophilus* KFRI342 reduced lesions but did not reduce food consumption. The levels of TG were lower ($P < 0.05$) in rats injected with the carcinogen DMH, and were further lowered ($P < 0.05$) by feeding the carcinogen-injected rats with *L. acidophilus* KFRI342. The reduction in TG with the injection of the carcinogen was unexpected, as several epidemiological studies have suggested that there is a positive association between elevated levels of serum TG and risk of colorectal cancer (Yamada *et al.*, 1998; Manus *et al.*, 1997). An experimental model with mice also provided convincing evidence that polyp formation in the intestine is positively correlated with serum TG levels, suggesting a pivotal role of TG in tumorigenesis (Niho *et al.*, 2003). Currently, the mechanism is largely unknown, although several studies have

suggested that the association between TG and colon cancer risk might be ascribable to increased secretion of faecal sample bile acids or hyperinsulinaemia (McKeown-Eyssen, 1994; Giovannucci, 2001). The present study demonstrated that *L. acidophilus* KFRI342 inhibited the development of colonic precancerous lesions *in vivo* and reduced serum TG (Fig. 3). When compared to the other groups, the *L. acidophilus* KFRI342-fed group (HFCL) had an increase in the mean length and weight of the colon (Fig. 2). Sung *et al.* (2004) found that a rat group that was fed with probiotics showed increased colon weight and length compared to the control group. They also observed a change in the microflora in the rat colon when prebiotics were added to the diet. The increase in length and weight of the colon in the rats fed with LAB in our study may be due to a similar effect.

L. acidophilus KFRI342 reduced the colonization of *E. coli* and aerobic bacteria (TPC) in the colon, perhaps by colonizing the gut itself, and reduced the pH in the gastrointestinal tract. *Lactobacillus plantarum* 299v reduced *E. coli*-induced injury and intestinal permeability in rats (Mangell *et al.*, 2002). A culture condensate of *Bifidobacterium longum* inhibited *E. coli* translocation from the gastrointestinal tract in antibiotic-decontaminated specific-pathogen-free and germ-free mice (Suzuki *et al.*, 1997). Evidence from a wide range of sources supports the view that the colonic microflora may play a significant role in the aetiology of colon cancer (George *et al.*, 2004). Dietary factors can modify the metabolic activity of intestinal microflora, such as affecting the conversion of bile acids and neutral sterols to form reactive metabolites that can act as promoters of cancer (Reddy *et al.*, 1974).

In this study, *L. acidophilus* KFRI342 reduced the activity of β -glucuronidase and β -glucosidase (Fig. 5); this may have been due to the reduction in numbers of *E. coli*. Faecal bacterial enzymes are known to be associated with the conversion of pro-carcinogens into proximal carcinogens. Glucuronidation and sulfation are the commonest forms of conjugation observed during metabolic processes in mammals, and they also represent the major pathway for detoxification (Rod & Midtvedt, 1977). Moreover, β -glucuronidase has been considered as a key enzyme for the

activation of DMH metabolites to carcinogens in the colonic lumen (Reddy *et al.*, 1974). Bacterial β -glucuronidase is a hydrolase that catalyses the cleavage of terminal glucuronic acid, which is believed to be largely responsible for the hydrolysis of glucuronide conjugates in the colon. Thus, this is important in the generation of toxic and carcinogenic substances (Chipman, 1982). Effects of dietary compounds on DMH-metabolizing (microbial) enzymes such as β -glucuronidase, β -glucosidase, β -galactosidase and mucinase may influence tumour development (Reddy *et al.*, 1977; Robertson, 1993). A number of studies have shown that gut microbial enzymes play a significant role in the aetiology of colon cancer (Onoue *et al.*, 1997; George *et al.*, 2004). These enzymes are involved in DMH metabolism, causing the release of the reactive metabolite methylazoxymethanol (MAM) from its conjugate in the colon; therefore a decrease in β -glucuronidase and β -glucosidase may be protective. The underlying molecular mechanisms remain to be elucidated. The reduction in enzyme activity associated with feeding *L. acidophilus* KFRI342 in this study may be implicated in the reduction in DMH-induced ACF. In several human intervention studies, LAB strains were shown to influence the activity of β -glucosidase (Goldin & Gorbach, 1984; Goldin *et al.*, 1992; Benno & Mitsuoka, 1992; Bouhnik *et al.*, 1996). To achieve a decrease in enzyme activity, a continual intake of LAB was necessary.

Our results, combined with those of Chang *et al.* (2010), indicate that the LAB strain *L. acidophilus* KFRI342, selected for attributes associated with intestinal colonization, bile resistance and acid tolerance, can inhibit DMH-induced precancerous lesions in rats. Further studies are needed to determine the reason for the decrease in microbial enzyme activity observed during the initial period on the high-fat diet, and to examine the ability of strain KFRI342 to penetrate the gastric barrier and potentially colonize the host. Such investigations should lead to better understanding of the complex interaction between the host, LAB and the aetiology of colon cancer. With improved information on these interactions, LAB-based intervention strategies to beneficially modify intestinal flora composition could potentially be devised.

ACKNOWLEDGEMENTS

We are very grateful to Dr Mary Leggett (Novozymes Biologicals, Canada) for helpful discussion and comments. This work was supported by the Agriculture Development Fund, South Korean agriculture (Project GE051002-06032).

REFERENCES

Alves de Lima, R. O., Bazo, A. P., Salvadori, D. M., Rech, C. M., de Palma Oliveira, D. & de Aragão Umbuzeiro, G. (2007). Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source. *Mutat Res* 626, 53–60.

- Benno, Y. & Mitsuoka, T. (1992). Impact of *Bifidobacterium longum* on human fecal microflora. *Microbiol Immunol* 36, 683–694.
- Bird, R. P. (1987). Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett* 37, 147–151.
- Bird, R. P. & Good, C. K. (2000). The significance of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Toxicol Lett* 112–113, 395–402.
- Bouhnik, Y., Flourie, B., Andrieux, C., Bisetti, N., Briet, F. & Rambaud, J.-C. (1996). Effects of *Bifidobacterium* sp. fermented milk ingested with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans. *Eur J Clin Nutr* 50, 269–273.
- Chang, J.-H., Shim, Y. Y., Cha, S.-K. & Chee, K.-M. (2010). Probiotic characteristics of lactic acid bacteria isolated from kimchi. *J Appl Microbiol* 109, 220–230.
- Chipman, J. K. (1982). Bile as a source of potential reactive metabolites. *Toxicology* 25, 99–111.
- Choudhary, G. & Hansen, H. (1998). Human health perspective on environmental exposure to hydrazines: a review. *Chemosphere* 37, 801–843.
- Commene, D., Hughes, R., Shortt, C. & Rowland, I. (2005). The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutat Res* 591, 276–289.
- de Roos, N. M. & Katan, M. B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *Am J Clin Nutr* 71, 405–411.
- Demarzo, M. M. & Garcia, S. B. (2004). Exhaustive physical exercise increases the number of colonic preneoplastic lesions in untrained rats treated with a chemical carcinogen. *Cancer Lett* 216, 31–34.
- Femia, A. P., Luceri, C., Dolara, P., Giannini, A., Biggeri, A., Salvadori, M., Clune, Y., Collins, K. J., Paglierani, M. & Caderni, G. (2002). Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* on azoxymethane-induced colon carcinogenesis in rats. *Carcinogenesis* 23, 1953–1960.
- Fodde, R. (2002). The APC gene in colorectal cancer. *Eur J Cancer* 38, 867–871.
- Garófalo, A., Avesani, C. M., Camargo, K. G., Barros, M. E., Silva, S. R. J., Taddei, J. A. A. C. & Sigulem, D. M. (2004). Dieta e câncer: um enfoque epidemiológico. *Rev Nutr* 17, 491–505.
- George, S. E., Wolf, D. C., Brooks, L. R., Bailey, K. C., Hooth, M. J. & Nelson, G. M. (2004). Changes in cecal microbial metabolism of rats induced by individual and a mixture of drinking water disinfection by-products. *Cancer Lett* 204, 15–21.
- Giovannucci, E. (2001). Insulin, insulin-like growth factors and colon cancer: a review of the evidence. *J Nutr* 131 (Suppl.), 3109S–3120S.
- Goldin, B. R. & Gorbach, S. L. (1984). The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. *Am J Clin Nutr* 39, 756–761.
- Goldin, B. R., Gorbach, S. L., Saxelin, M., Barakat, S., Gualtieri, L. & Salminen, S. (1992). Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig Dis Sci* 37, 121–128.
- Goldin, B. R., Gualtieri, L. J. & Moore, R. P. (1996). The effect of *Lactobacillus* GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. *Nutr Cancer* 25, 197–204.
- Maldonado Galdeano, C. M., de Moreno de LeBlanc, A., Vinderola, G., Bonet, M. E. & Perdigón, G. (2007). Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. *Clin Vaccine Immunol* 14, 485–492.
- Mangell, P., Nejdfor, P., Wang, M., Ahrné, S., Weström, B., Thorlacius, H. & Jeppsson, B. (2002). *Lactobacillus plantarum* 299v

inhibits *Escherichia coli*-induced intestinal permeability. *Dig Dis Sci* 47, 511–516.

Manus, B., Adang, R. P., Ambergen, A. W., Brägelmann, R., Armbricht, U. & Stockbrügger, R. W. (1997). The risk factor profile of recto-sigmoid adenomas: a prospective screening study of 665 patients in a clinical rehabilitation centre. *Eur J Cancer Prev* 6, 38–43.

McKeown-Eyssen, G. (1994). Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? *Cancer Epidemiol Biomarkers Prev* 3, 687–695.

Niho, N., Takahashi, M., Shoji, Y., Takeuchi, Y., Matsubara, S., Sugimura, T. & Wakabayashi, K. (2003). Dose-dependent suppression of hyperlipidemia and intestinal polyp formation in Min mice by pioglitazone, a PPAR gamma ligand. *Cancer Sci* 94, 960–964.

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.

Reddy, B. S., Weisburger, J. H. & Wynder, E. L. (1974). Fecal bacterial β -glucuronidase: control by diet. *Science* 183, 416–417.

Reddy, B. S., Mangat, S., Weisburger, J. H. & Wynder, E. L. (1977). Effect of high-risk diets for colon carcinogenesis on intestinal mucosal and bacterial β -glucuronidase activity in F344 rats. *Cancer Res* 37, 3533–3536.

Roberton, A. M. (1993). Roles of endogenous substances and bacteria in colorectal cancer. *Mutat Res* 290, 71–78.

Rod, T. O. & Midtvedt, T. (1977). Origin of intestinal β -glucuronidase in germfree, monocontaminated and conventional rats. *Acta Pathol Microbiol Scand [B]* 85, 271–276.

Rowland, I. R., Rumney, C. J., Coutts, J. T. & Lievense, L. C. (1998). Effect of *Bifidobacterium longum* and inulin on gut bacterial

metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis* 19, 281–285.

Sequeira, J. L., Kobayasi, S. S. & Rodrigues, M. A. (2000). Early and late effects of wound healing on development of colon tumours in a model of colon carcinogenesis by 1,2-dimethylhydrazine in the rat. *Pathology* 32, 250–252.

Soylemez, G., Brashears, M. M., Smith, D. A. & Cuppett, S. L. (2001). Microbial quality of alfalfa seeds and sprouts after a chlorine treatment and packaging modifications. *J Food Sci* 66, 153–157.

Sung, H.-Y., Jeong, H.-J. & Choi, Y.-S. (2004). Effects of fructans and isomaltooligosaccharide on large bowel mass and plasma and fecal immunoglobulin A in rat. *Nutr Sci* 7, 196–200.

Suzuki, T., Itoh, K., Kaneko, T. & Suzuki, H. (1997). Inhibition of bacterial translocation from the gastrointestinal tract of mice by oral administration of a culture condensate of *Bifidobacterium longum*. *J Vet Med Sci* 59, 665–669.

Takayama, T., Katsuki, S., Takahashi, Y., Ohi, M., Nojiri, S., Sakamaki, S., Kato, J., Kogawa, K., Miyake, H. & Niitsu, Y. (1998). Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 339, 1277–1284.

Wargovich, M. J., Chen, C. D., Harris, C., Yang, E. & Velasco, M. (1995). Inhibition of aberrant crypt growth by non-steroidal anti-inflammatory agents and differentiation agents in the rat colon. *Int J Cancer* 60, 515–519.

Yamada, K., Araki, S., Tamura, M., Sakai, I., Takahashi, Y., Kashiwara, H. & Kono, S. (1998). Relation of serum total cholesterol, serum triglycerides and fasting plasma glucose to colorectal carcinoma in situ. *Int J Epidemiol* 27, 794–798.