BIOCELL
2005, 29(1): 15-24.

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Reduction of β-Glucuronidase and nitroreductase activity by yoghurt in a murine colon cancer model

A. de Moreno de LeBlanc[1,2] and G. Perdigón[1,2]

1. Centro de Referencias para Lactobacilos (CERELA). Chacabuco 145, (4000) Tucumán. Argentina.
2. Cátedra de Inmunología. Instituto de Microbiología. Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán, Argentina.

Keyword: colon cancer, yoghurt, starter bacteria, β-glucuronidase, nitroreductase

ABSTRACT: Yoghurt feeding inhibits induced colon cancer in mice. Several studies showed the immunomodulatory effect of yoghurt which can explain this inhibition. It is possible that yoghurt bacteria can also affect gut flora enzymes related to colon carcinogenesis as reported for other probiotics in different animal tumours**.** The aim of this work was to evaluate the role of yoghurt starter bacteria and their cell-free fermentation products on the reduction of procarcinogen enzyme activities (β-glucuronidase and nitroreductase). Mice injected with 1,2-dimethylhydrazine (DMH) and fed with yoghurt were used for this study. Mice given milk or yoghurt supernatant (cell free extract) were used to evaluate if the yoghurt antitumour effect is due to the starter bacteria or other components released during fermentation, that could inhibit these enzymes. We determined that yoghurt by itself maintained enzymes activities similar or lower than non-treatment control group, and the enzyme activity was also lower than milk or yoghurt supernatant groups. DMH increased the activity of the enzymes. Mice injected with DMH and fed cyclically with yoghurt presented lower enzymes activities than the tumour control group. Feeding yoghurt decreased procarcinogenic enzyme levels in the large intestine contents of mice bearing colon tumour. The results of this study provide another mechanism by which yoghurt starter bacteria interact with the large intestine of the mice and prevent colon cancer.

Introduction

The intestinal microflora is a complex ecosystem of multiple microorganisms. It is estimated that over 400 species of bacteria inhabit the human gastrointestinal tract. This complex bacterial flora can exert negative and positive effects (Gorbach, 1986). They can be separated into two categories: those considered to be beneficial (i.e., *Bifidobacterium* and *Lactobacillus*) and those considered detrimental (i.e., Enterobacteriaceae and *Clostridium* spp.). The influences of the digestive tract normal microflora on the animal host had been demonstrated by comparing the characteristics of animals with conventional microflora with animals that are germ free, showing that the biochemistry, physiology and immunology of animal is modified by the presence of normal microflora.

One group of beneficial microorganisms traditionally used in food fermentation, lactic acid bacteria, have attracted the most attention and are the microorganisms most commonly used as probiotics (de Ross and Katan, 2000). Probiotics are defined as live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1992).

Address correspondence to: Dra. Gabriela Perdigón. Centro de Referencias para Lactobacilos (CERELA). Chacabuco 145, (4000) Tucumán, ARGENTINA. FAX: (+54-381) 400-5600.
E-mail: perdigon@cerela.org.ar
Received on February 28, 2004. Accepted on August 18, 2004.

When lactic acid bacteria enter the digestive tract, they have contact with different intestinal cells and other bacteria already present. Although a variety of health benefits have been attributed to probiotics, the stimulation of the immune system and their anticarcinogenic, hypocholesterolemic, and antagonistic actions against enteric pathogens and other intestinal organisms have recieved particular attention (Kato, 2000; Perdigón *et al.,* 2001).

The interaction of lactic acid bacteria with the immune cells associated with the intestinal tissue was studied by Perdigón *et al.* (1998, 2000, 2001) using BALB/c mice. They observed that this interaction was different for each bacterial strain studied. Some bacteria antigens were only seen associated to immune cells in Peyer's patches of small intestine, whereas other interacted with cells of lamina propria of small and large intestine (Perdigón *et al.,* 2000). Hughes and Rowland (2003) reviewed the relationship between several probiotics, prebiotics and animal and human cancers. Probiotics such as lactobacilli and bifidobacteria in fermented or culture-containing dairy foods (i.e., yoghurt) may play a role in reducing the risk of colon cancer (Braddy *et al.,* 2000; Wollowski *et al*., 2001). This effect could be due to the fact these cultures can alter the activity of fecal enzymes (i.e., β-glucuronidase, azoreductase, nitroreductase) that can play a role in the development of colon cancer. The increase of the immune cells activity in the prevention of cancer by lactic acid bacteria (LAB) consumption was also described (Kato *et al*., 1984; Hayashi and Ohwaki, 1989).

The consumption of *L. acidophilus* in experimental animal models decreased enzyme fecal activities such as β- glucuronidase, azoreductase and nitroreductase (Goldin and Gorbach, 1976; 1980). The activities of these enzymes have been well correlated to the number of LAB in the intestine. These enzymes form products which are known to be mutagenic and carcinogenic. Goldin and Gorbach (1976) showed the relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. Furthermore, the effect of the diet on cancer at various sites has been the subject of many reviews (Cummings and Bingham, 1998; Reddy, 1998).

Research has demonstrated that a specific strain of *lactobacilli*, *Lactobacillus* GG, reduces the incidence and number of tumours in carcinogen-treated laboratory rats, particularly during the early promotional stages of carcinogenesis and when the animals are fed with a high fat diet (Goldin *et al*., 1996). Likewise, studies indicate that another bacteria, *Bifidobacterium longum*, inhibits cell proliferation and the development of colon tumours in laboratory rats treated with a chemical carcinogen and fed with a high fat diet (Singh *et al*., 1997). Sreekumar and Hosono (2000) showed the effect of *L. acidophillus* on flora and fecal enzymes of rats: *L. acidophillus* decreased fecal β-glucuronidase activity and was related to the decreased number of bacteria in the intestinal tract. Studies in humans indicate that when healthy female adults supplemented their diet with yoghurt containing viable *Lactobacillus* GG for four weeks, fecal bacterial enzyme activities decreased (Ling *et al.,* 1994).

There are many reports about beneficial effects of yoghurt in large intestine disorders. In France, consumption of yoghurt was associated with reduced risk of large adenomas in a human case-control study (Boutron *et al.,* 1996). Perdigón *et al*. (2000 and 2001) showed the inhibition of a chemically induce colon cancer in mice fed with yoghurt.

The immune system stimulation exerted by yoghurt in the large intestine of mice has also been studied (de Moreno de LeBlanc and Perdigón, 2004b). That study demonstrated that yoghurt bacteria exerted a direct interaction with the large intestine cells, reinforcing or increasing the response produced in the immune inductor site (Peyer's patch).

In an experimental model of colon cancer using BALB/c mice, it was demonstrated that cyclical yoghurt feeding inhibited tumour development (Perdigón *et al*. 1998).

This carcinogenesis inhibition was related with important modulation of the immune response observed in the mice fed with yoghurt, such as decreased inflammatory response induced by the carcinogen. It was shown that the stimulation of the immune cells in the intestine is related to high cytokine levels. The enhancement of cell apoptosis was also reported in mice treated with carcinogen and fed with yoghurt (Perdigón *et al*., 2002; Rachid *et al.,* 2002).

Furthermore, studies in mice showed that long term yoghurt consumption increases the large intestine cell activity with increased cytokine levels and immune response modulation without detrimental effect to the host (de Moreno de LeBlanc *et al*., 2004) and that yoghurt inhibited the tumour progression and promotion but not its induction (de Moreno de LeBlanc and Perdigón, 2004a).

Since it is know that different lactic acid bacteria have shown effects on the intestine microflora and to better understand other possible mechanisms of inhibition of colon cancer by yoghurt in mice, the aims of this work were to evaluate the role of yoghurt on procarcinogenic enzyme activities in an induced colon

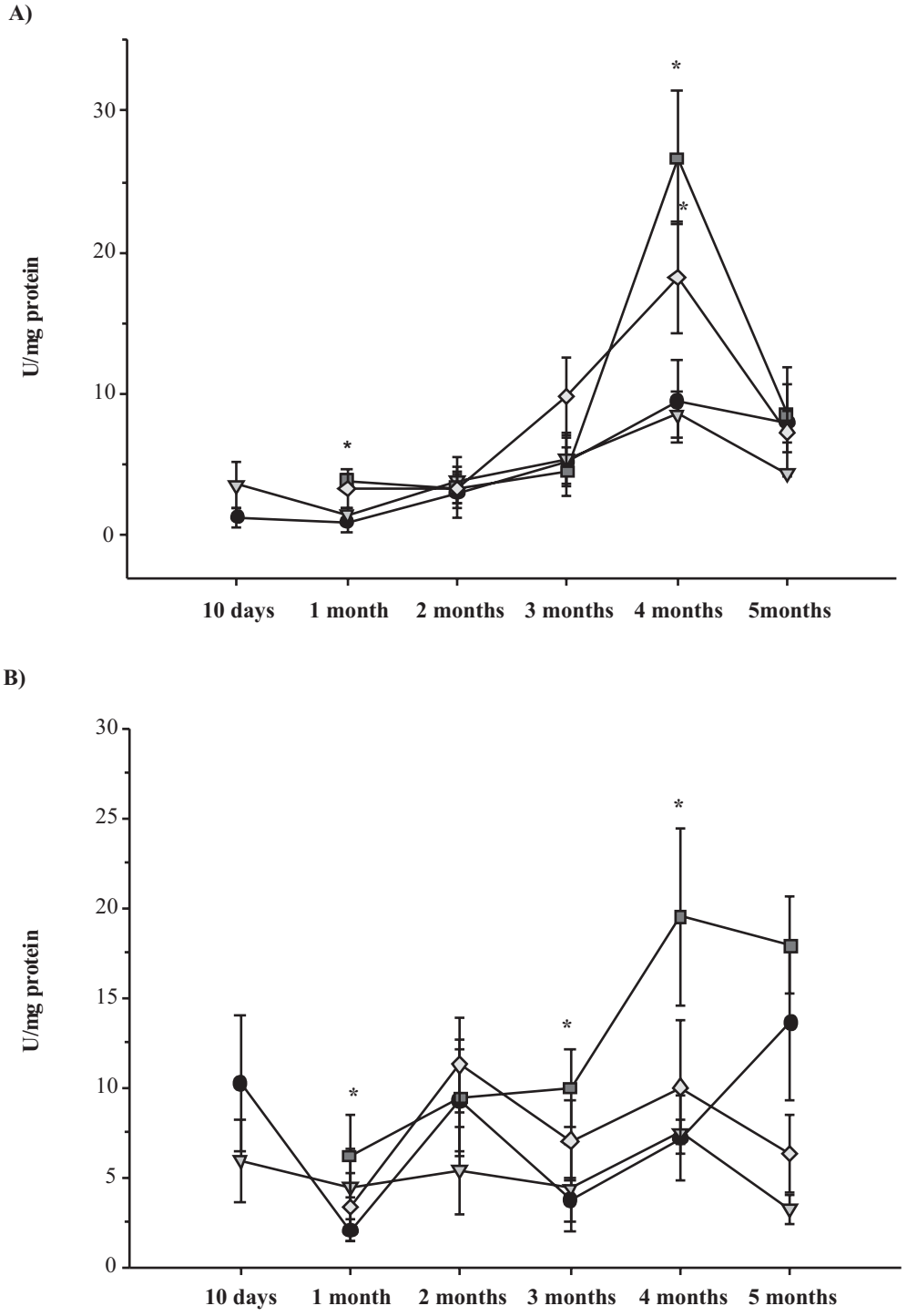

FIGURE 1. Effect of yoghurt feeding and DMH carcinogen on the specific activity of β-glucuronidase in large intestine content of mice.
Specific activity is expressed as the μg of phenolphthalein released per minute per mg large intestine content protein. Values are means for n = 5 ± SD. Significant difference were calculated in comparison to the non-treatment control group for each month of the study. * = $P < 0.01$.
A) Sample obtained before glass beads breaking (BGBB). B) Sample obtained after glass beads breaking (AGBB).
• non-treatment control; ▼ yoghurt; ■ DMH; ♦ yoghurt-DMH-yoghurt

cancer model and to compare the effect of the long term administration of yoghurt and of cell free yoghurt supernatant on the reduction of procarcinogenic enzymes.

Material and Methods

Animals and diets

BALB/c mice, weighting 25-30 g were obtained from the random-bred closed colony kept in our Microbiology Department. The mice were separated into six experimental groups: 1) **DMH group**: Mice treated with carcinogen 1-2 dimethylhydrazine (DMH) to induce colon tumour. 2) **yoghurt-DMH-yoghurt group**: Mice fed with yoghurt for ten days (basal yoghurt), injected with DMH and then fed again cyclically with yoghurt. 3) **Yoghurt group**: Mice fed cyclically with yoghurt from the eighth week until the sixth month to analyze the effect of long term yoghurt administration. 4) **Yoghurt supernatant group**: Mice fed cyclically with yoghurt supernatant in the same form as the yoghurt group. 5) **Milk group**: Mice received cyclically non-fat-milk as control. 6) **Non-treatment control group**: Mice not given any special treatment. All mice had free access to water and were fed *ad libitum* with a balanced diet. Each experimental group consisted of 45-50 mice.

Yoghurt preparation

Simulated commercial yoghurt was prepared freshly in our laboratory and controlled everyday to avoid variations due to storage. Strain pools of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were used (CERELA culture collection). The total number of bacteria in the fermented product was 2 x 10^8 cells/ml.

Yogurt supernatant preparation

Yoghurt was centrifuged (600 *g*), for 20 min at 4ºC. Lactobacilli growth was determined by counting colony-forming units (C.F.U) after plating serial dilutions on LAPTG agar and incubation at 37ºC for 48 hs. The C.F.U. counted were always less than 1.0 x 10^2 per millilitre when cell-free supernatant was used.

Tumour induction and feeding periods

To induce colon cancer, mice were injected with the carcinogen DMH dihydrochloride (Sigma, St. Louis, USA). Each mouse received per week, subcutaneously, 20 mg DMH/kg body weight in 0.1 ml of saline solution containing 1.5 g/l of EDTA, pH 6.4. The tumour developed five or six months after first injection (tumour control or DMH group).

In other test groups, mice were given a diet supplemented with yoghurt for 10 consecutive days (basal yoghurt) before carcinogen injections. At the end of the feeding period they were separated into two groups: 1) Mice that were injected with DMH as in the tumour control group, but in the eighth week, yoghurt was added to the diet for ten consecutive days, one week break and they again received yoghurt feeding for ten days. Feeding was given in this manner cyclically until the sixth month (yoghurt-DMH-yoghurt group). 2) Mice that were not injected with DMH; yoghurt feeding was repeated cyclically from the eighth week until the sixth month (yoghurt control group).

For the yoghurt supernatant or milk groups, the mice were given the product of yoghurt centrifugation or nonfat rehydrated milk (10 % wt/vol) respectively, during the same periods as the other groups.

Collection of samples for enzyme assays

Five mice from each group (DMH, yoghurt-DMH-yoghurt, yoghurt, yoghurt supernatant, milk and non-treatment control groups) were sacrificed monthly by cervical dislocation. The large intestine was removed and cut in two parts (caecum and colon-rectum). Each portion was washed with 300 µl of cold buffer solution depending on enzyme to analyze. The samples were centrifuged at 9,000 *g* for 15 min at 4ºC. The supernatant was collected and maintained on ice (sample before glass beads breaking, BGBB). Glass beads (0.25-0.30 mm diameter) and buffer were added to the pellets; the suspension was agitated for five min on a vortex mixer. The suspensions were centrifuged at 9,000 *g* for 15 min at 4ºC and the supernatants were removed (sample after glass beads breaking, AGBB). Both fractions (BGBB and AGBB) were used for the enzyme assays. The samples were used immediately after collection.

β-glucuronidase assay

Potassium phosphate buffer (0.1M, pH 7.0) was used for sample collection (BGBB and AGBB). The enzyme reaction was run at 37°C (pH 6.8) as described by Goldin and Gorbach (1980). The total volume of the reaction mixture was 200 µl, containing a final concentration of 0.02M potassium phosphate buffer, 0.1 mM EDTA, 1 mM phenolphthalein-glucuronic acid (Sigma, St Louis, USA) and 20 µl of intestinal sample.

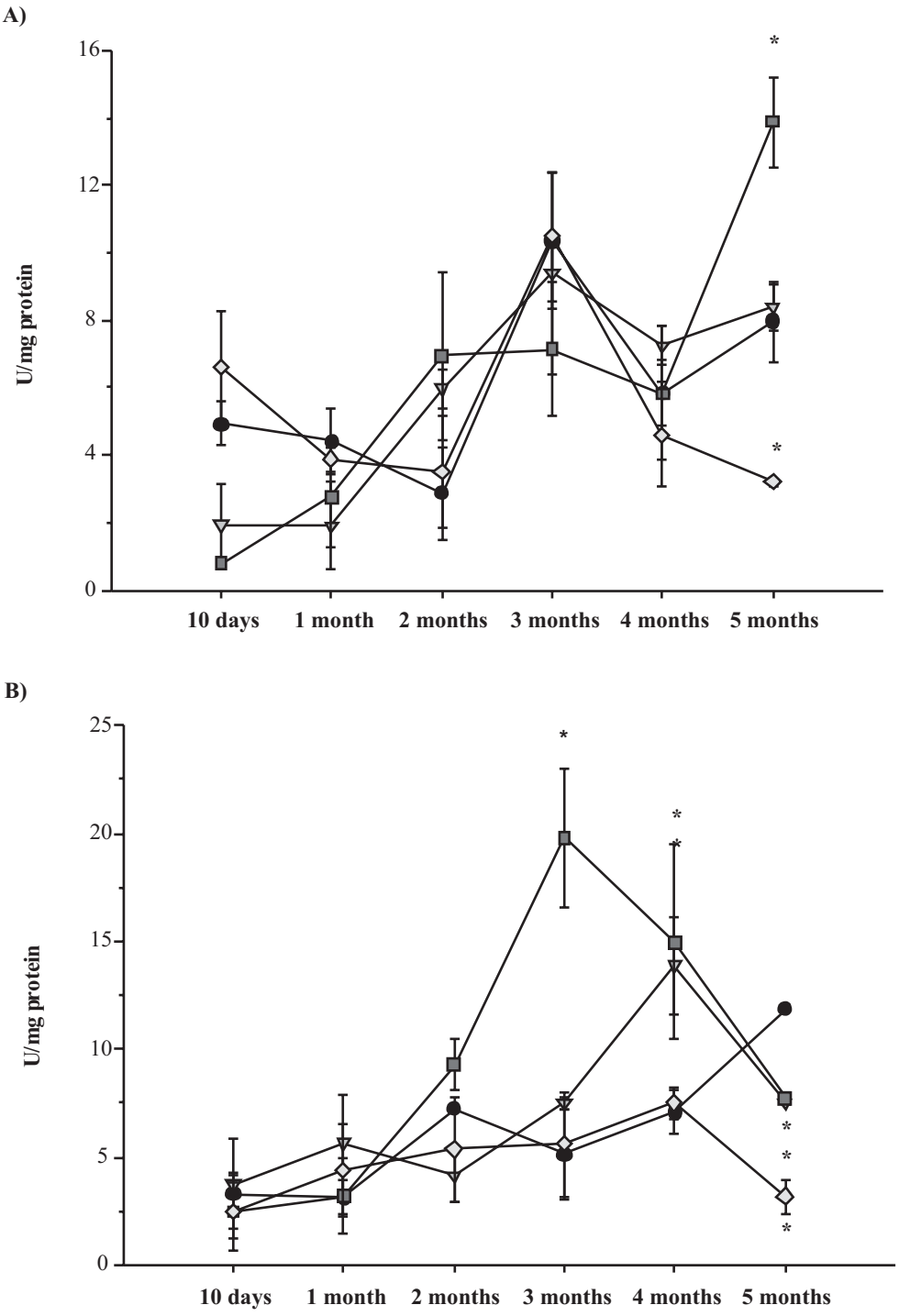

FIGURE 2. Effect of whole yoghurt compared with yoghurt supernatant and milk on the specific activity of β-glucuronidase in large intestine content of mice.
Specific activity is expressed as the μg of phenolphthalein released per minute per mg protein. Values are means for n = 5 ± SD. Significant difference were calculated in comparison to the non-treatment control group for each month of the study.* = $P < 0.01$.
A) Sample obtained before glass bead breaking (BGBB).
B) Sample obtained after glass bead breaking (AGBB).
• non-treatment control; ▼ milk; ■ yoghurt supernatant; ♦ yoghurt

The reaction was stopped after 15 min by addition of 1ml 0.2M glycine buffer (pH 10.4) containing 0.2 M NaCl. Absorbance was read at 540 nm using a VERSA Max Microplate Reader (Molecular Devices, USA). The amount of released phenolphthalein was determined and compared to standard. One unit of enzyme was defined as the amount of enzyme necessary to release 1 μg of phenolphthalein at 37°C in 1 min.

Nitroreductase assay

Cold prereduced 0.2 M Tris-HCl buffer (pH 7.8) was used for sample collection and maintenance (BGBB and AGBB). All manipulations and additions (including intestinal content removal) were performed under anaerobic conditions (flow oxygen-free, CO_2, filled anaerobic chamber). The enzyme reaction was run anaerobically at 30°C (pH 7.8) using a modification of the technique described by Goldin and Gorbach (1980). The total volume of reaction mixture was 200 μl, containing a final concentration of 0.08M Tris-HCl buffer, 0.35 mM m-nitrobenzoic acid (Aldrich Chemical Co., Milwaukee, USA), 0.5 mM NADPH, 1 mM NADH, and 80 μl of intestinal sample. The reaction was stopped by addition of 300 μl of 1.2 N HCl. The amount of *m*-aminobenzoic acid produced was then measured using a diazotation reaction. Sodium nitrite (final concentration, 0.007 %) was added to the mixtures to form a diazonium salt with *m*-aminobenzoic acid. The reaction tubes were incubated at room temperature (21ºC) for 20 min followed by the addition of 5M sodium hidroxide (100 μl in each tube), then 20 mM β-naphtol solution (15 μl in each tube) was added to form diazonium salt with *m*-aminobenzoic acid in acidic solution and red-purple azo dye was produced. Absorbance at 540 nm was measured using a VERSA Max Microplate Reader (Molecular Devices, USA). The amount of *m*-aminobenzoic acid produced was calculated comparing with standard *m*-aminobenzoic acid (Sigma, St Louis, USA) using the same diazotation reaction. One unit of enzyme was defined as the amount of enzyme necessary to produce 1μg of *m*-aminobenzoic acid at 30°C under anaerobic condition in 1 h.

Protein determination

Enzyme levels were expressed in term of units of activity per milligram of total soluble protein. Intestinal content proteins (before and after glass beads treatment) were determined using the Bio-Rad Protein Assay which is based on the method of Bradford (1976).

Statistical analyses

Results are expressed as means ± standard deviation (SD). Student's *t*-test was used to asses the statistical significance of the differences between each group and the non-treatment control in each month.

Results

β-glucuronidase activity in the large intestine fluid (BGBB)

The enzyme levels increased with mice age in the non-treatment control. The enzyme activity for different groups of mice can be seen in Figs. 1A and 2A.

Long term whole yoghurt administration maintained the enzyme activity lower or similar to the non-treatment control. Mice recieved yoghurt supernatant or milk showed β-glucuronidase levels similar to the non treatment control group and only these values were lower than control in the first months.

DMH carcinogen increased β-glucuronidase activity in the intestinal content compared to the non treatment control, significant differences ($P<0.01$) were seen in the first and fourth month.

Whole yoghurt administration to DMH injected mice (yoghurt-DMH-yoghurt group) showed lower enzyme activity levels compared to DMH group in the intestinal fluid BGBB at the fourth and fifth month.

β-glucuronidase activity after samples rupture (AGBB)

Enzymatic activity in the non-treatment control group showed fluctuations during the experiment. In the same form as BGBB sample, the most elevated values were obtained in the last month. (Figs. 1B and 2B).

Whole yogurt administration maintained enzyme activity similar or lower than the non-treatment control; the last observation was evident in the fifth month.

Yoghurt supernatant and milk administration produced different levels compared to the whole yoghurt. They only decreased enzyme activity in the fifth month, compared to the non-treatment control, but the mice administered with them had elevated enzymatic levels at other periods.

Mice treated with carcinogen maintained elevated β-glucuronidase activity. The most significant increases were obtained from the third month until the end of the experiment.

Yogurt-DMH-yoghurt group showed enzyme

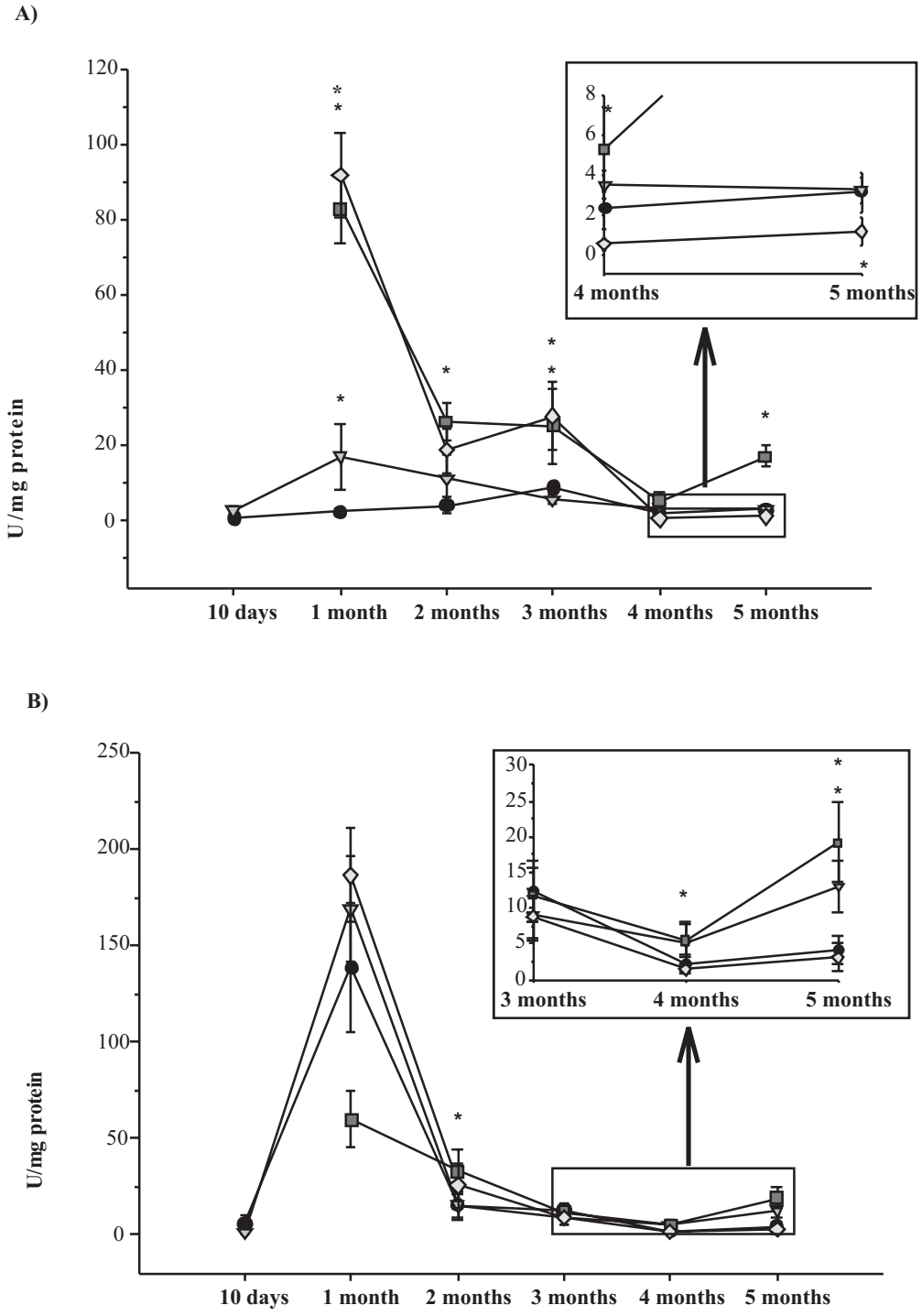

FIGURE 3. Effect of yoghurt feeding and DMH carcinogen on specific activity of nitroreductase in large intestine content of mice.
Specific activity is expressed as mg of *m*-aminobenzoic acid formed per hour per mg protein. Values are the means for n = 5 ± SD. Significant difference were calculated in comparison to the non-treatment control group for each month of the study . * = $P < 0.01$.
A) Sample before glass beads breaking (BGBB).
B) Sample after glass beads breaking (AGBB).
• non-treatment control; ▼ yoghurt; ■ DMH; ♦ yoghurt-DMH-yoghurt

activity lower than DMH group (except for the second month). Also, β-glucuronidase activity was lower than the non-treatment control in the fifth month.

Nitroreductase activity in large intestine fluid (BGBB)

Figs. 3A and 4A show nitroreductase activity for the different mice groups.

The groups were compared with non-treatment control group, which showed fluctuations. Whole yoghurt feeding decreased or maintained enzyme levels similar to the non-treatment control in the BGBB samples obtained from the third month of study onwards (Fig. 3A); these mice were fed with yoghurt cyclically again after eight week. The intestinal fluid from mice given yoghurt supernatant or milk showed enzyme activities higher than non-treatment control. Only the sample of the fifth month had activity similar to the control.

DMH injections increased nitroreductase activity compared to the control group. The animals from yoghurt-DMH-yoghurt group showed lower nitroreductase activity than in DMH group. Enzymatic activity was significant reduced in the last group ($P<0.01$) compared to the non-treatment control in the fourth and fifth month.

Nitroreductase activity in the large intestine content after glass beads breaking (AGBB)

Nitroreductase activity was variable throughout the feeding period in the mice given whole yoghurt cyclically (Figs. 3B and 4B).

Yoghurt supernatant or milk administration increased enzyme activities not only compared to non-treatment control but also in comparison with yoghurt group (Fig. 4B).

DMH injections increased nitroreductase activity begining at the second month, but the animals from yoghurt-DMH-yoghurt group showed enzyme activity levels lower than in the DMH group also begining at the second month (Fig. 3B).

Discussion

The enzymes selected in this study are known to be potential mediators of colon carcinogenesis. β-glucuronidase is an enzyme responsible for the hydrolysis of glucuronides in the lumen of the gut. This reaction generates toxic and carcinogenic substances which are detoxified by glucuronide formation in the liver and then enter the bowel via bile. In this way, toxic aglycones can be regenerated *in situ* in the bowel by bacterial β-glucuronidase. In humans, fecal β-glucuronidase activity was shown to be higher in colorectal cancer patients as compared to healthy controls suggesting a role of this enzyme in carcinogenesis (Kim and Jin, 2001). Another enzyme which is important in colon cancer prevention is nitroreductase. This enzyme is responsible for reducing nitrocompounds to aromatic amines. The end products and the highly reactive intermediates derived from these reactions such as reactive nitroso- and N-hydroxy-intermediates and aromatic amines are mutagenic and carcinogenic (Gillette *et al*., 1968). The reduction of aromatic nitro- and azo- compounds result from the activity of the intestinal flora (Zachariah and Juchau, 1974; Peppercorn and Goldman, 1972).

Reddy *et al.* (1974 and 1975) studied the effect of the gut flora on the rate of intestinal carcinogenesis when various intestinal carcinogens were used as tumour initiators, because carcinogens such as DMH require deconjugation in order to induce carcinogenesis. They observed that when DMH was used, the rate of tumour formation was faster in rats with conventional microflora than in germ-free rats; the intestinal flora thus, had a tumour promoting effect when DMH was used as tumour initiator.

In this work we demonstrated that long term yoghurt feeding maintained β-glucuronidase and nitroreductase activities similar or lower than in the non-treatment control group (Figs. 1 and 2), and were different than the values obtained with the carcinogen DMH, which were increased, contributing in this way to its mutagenic power.

Mice injected with DMH which were fed cyclically with yoghurt presented enzyme activity levels lower than the tumour control group. This last observation could explain previously reported histological differences between both groups (Perdigón *et al*., 1998). It is important to note that the decrease of these enzyme activities was observed in the samples before and after that the cells were broken with the glass beads, showing that yoghurt feeding decreases the levels of the enzymes in the intestinal fluids and prevents their induction in the interior of the cells.

It was important to compare the effect of yoghurt and the non-bacterial fraction of this fermented food on the large intestine enzymes, because yoghurt possesses not only lactic acid bacteria but also other substances released during milk fermentation. These products can

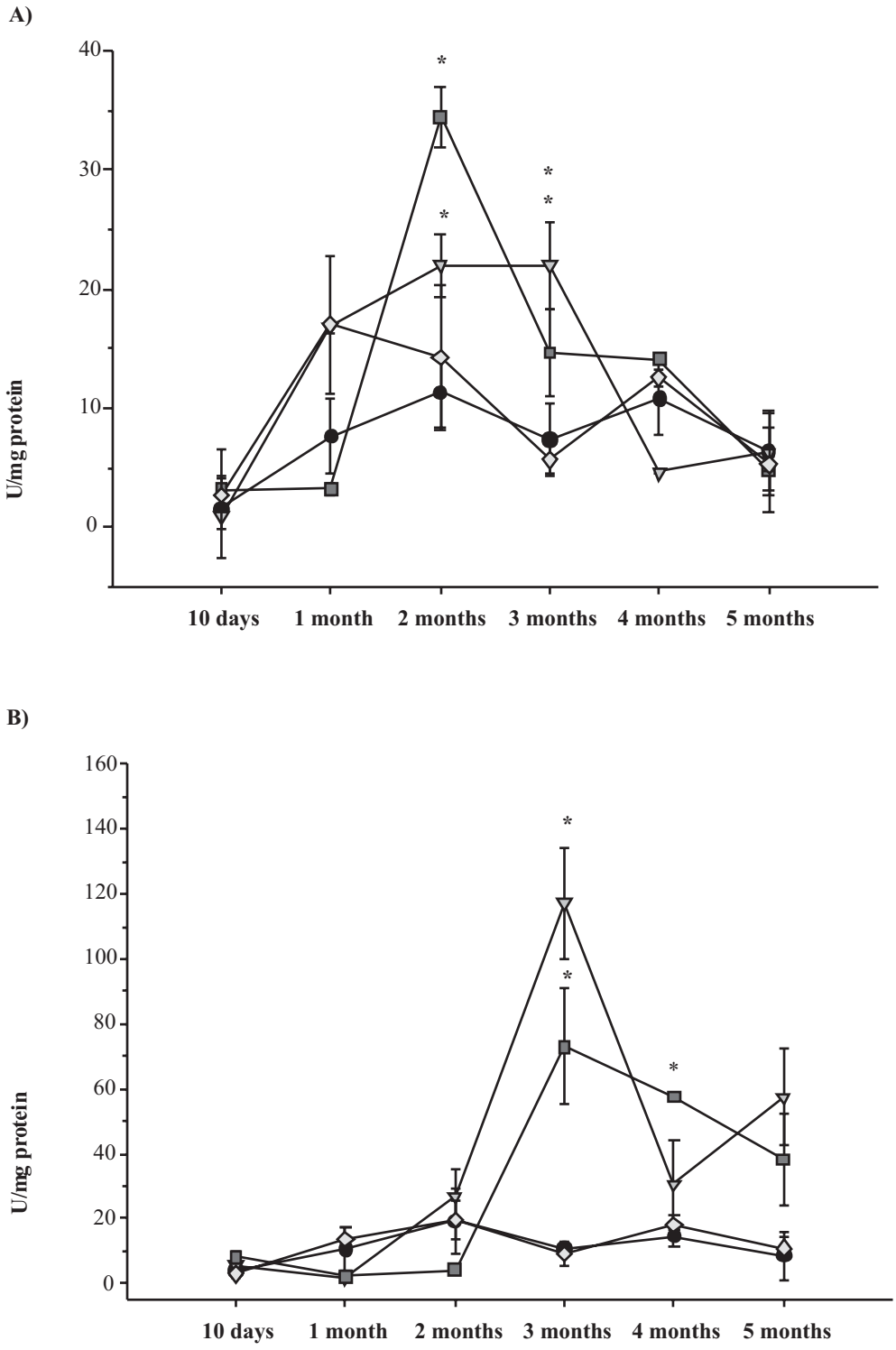

FIGURE 4. Effect of whole yoghurt feeding in comparison with yoghurt supernatant and milk on specific activity of nitroreductase in large intestine content of mice.
Specific activity is expressed as mg of *m*-aminobenzoic acid formed per hour per mg protein. Values are the means means for n = 5 ± SD. Significant difference were calculated in comparison to the non-treatment control group for each month of the study . * = $P < 0.01$.
A) Sample before glass beads breaking (BGBB).
B) Sample after glass beads breaking (AGBB).
• non-treatment control; ▼ milk; ■ yoghurt supernatant; ♦ yoghurt

inhibit enzyme activities showing another cancer preventing mechanism of yoghurt. In the present work, the variations of the β-glucuronidase and nitroreductase activities observed in mice fed whole yoghurt were not observed in the animals given yoghurt supernatant. Similar effects to those obtained with milk were observed, where the enzyme activities were higher than the non-treatment control in some periods of the cyclical feeding. The last observation allowed the speculation that yoghurt bacteria would be involved in the diminution of the procarcinogenic enzyme levels reported in this paper.

Even when there are many evidences of antiinflammatory and immune modulator properties of the yoghurt, the results of this study implicate another mechanism by which yoghurt could exert the antitumour activity observed in our murine model: at the level of normal intestinal flora, by reducing of the gut enzyme activities which are involved in the colon carcinogenesis.

Acknowledgements

This work was financially supported by grant from Consejo de Investigaciones Universidad Nacional de Tucumán (CIUNT) 26/D231- 2001/2003, Argentina and from PIP 02176 (CONICET), Argentina.

References

Boutron MC, Faivre J, Marteau P, Couillaut C, Senesse P, Quipourt V (1996). Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case-control study. *Br J Cancer* 74: 145-151.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.

Brady LJ, Gallaher DD, Busta FF (2000). The role of probiotic cultures in the prevention of colon cancer. *J Nutr* 130: 410S-414S.

Cummings JH, Bingham SA (1998). Diet and the prevention of cancer. *BMJ* 317: 1636-1640.

de Moreno de LeBlanc A, Valdéz J, Perdigón G (2004). Regulatory effect of yoghurt on intestinal inflammatory immune response. *Eur J Inflammation* 2(1): 21-31.

de Moreno de LeBlanc A, Perdigón G (2004a). Yoghurt feeding inhibits promotion and progression of experimental colorectal cancer. *Medical Science Monitor* 10(4): BR96-BR104.

de Moreno de LeBlanc A, Perdigón G (2004b). Papel del yogur en el cáncer de colon. Mecanismos involucrados. In: Actualización en Nutrición, inmunidad e infección. *A. Marcos Ed. Médica Panamericana, Madrid, España*. pp. 93-100.

de Roos NM, Katan MB (2000). Effects of probiotic bacteria on diarrhoea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *Am J Clin Nutr* 71(2): 405-411.

Fuller R (1992). History and development of probiotics. In: Probiotics. *R. Fuller Ed.: Chapman & Hall, New York*. pp. 1-8.

Gillette JR, Kamm JJ, Sasame HA (1968). Mechanism of p-nitrobenzoate reduction in liver: the possible role of cytochrome P-450 in liver microsomes. *Mol Pharmacol* 4: 541-548.

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.

Goldin BR, Gorbach SL (1976). The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *J Natl Cancer Inst* 57(2): 371-375.

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.

Gorbach SL (1986). Biochemical methods and experimental models for studying the intestinal flora. *Ann Ist Super Sanita* 22(3): 739-747.

Hayashi K, Ohwaki M (1989). Antitumour activity of *Lactobacillus casei* (LC 9018) in mice: T cell subset depletion. *Biotherapy* 3: 1568-1574.

Hughes R, Rowland I (2003). Nutritional and microbial modulation of carcinogenesis. In: Gut flora, nutrition, immunity and health. *R. Fuller and G. Perdigón Eds. London: Blackwell Publishing, UK*, pp. 208-220.

Kato I (2000). Antitumor activity of lactic acid bacteria. In: Probiotics 3: Immunomodulation by the gut microflora and probiotics. *R. Fuller and G. Predigón Eds. London: Kluwer Academic Publishers, UK*, pp. 115-138.

Kato I, Yokokura T, Mutai M (1984). Augmentation of mouse natural killer cell activity by *Lactobacillus casei* and its surface antigens. *Microbiol Immunol* 28: 209-217.

Kim DH, Jin YH (2001). Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. *Arch Pharm Res* 24: 564-567.

Ling WH, Korpela R, Mykkanen H, Salminen S, Hannine O (1994). Lactobacillus strain GG suplementation decreases colonic hydrolitic and reductive enzyme activities in healthy female adults. *J Nutr* 124: 18-23.

Peppercorn MA, Goldman P (1972). The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. *J Pharmacol Exp Ther* 181: 555-562.

Perdigon G, de Moreno de LeBlanc A, Valdez J, Rachid M (2002). Role of yoghurt in the prevention of colon cancer. *Eur J Clin Nutr* 56(suppl 3): S65-68.

Perdigon G, Fuller R, Raya R (2001). Lactic acid bacteria and their effect on the immune system. *Curr Issues Intest Microbiol* 2: 27-42.

Perdigón G, Medina M, Vintiñi E, Valdéz JC (2000). Intestinal pathway of internalization of lactic acid bacteria and gut mucosal immunostimulation. *Int J Immunopathol Pharmacol* 13: 141-150.

Perdigón G, Valdez JC, Rachid M (1998). Antitumour activity of yogurt: study of possible immune mechanisms. *J Dairy Res* 65: 129-138.

Rachid MM, Gobbato NM, Valdéz JC, Vitalone HH, Perdigón G (2002). Effect of yogurt on the inhibition of an intestinal carcinoma by increasing cellular apoptosis. *Int J Immunopathol Pharmacol* 15(3): 209-216.

Reddy BS (1998). Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. *Br J Nutr* 80: S219-S223.

Reddy BS, Narisawa T, Maronpot R, Weisburger JH, Wynder EL (1975). Animal models for the study of dietary factors and cancer of the large bowel. *Cancer Res* 35: 3421-3426.

Reddy BS, Weisburger JH, Narisawa T, Wyder EL (1974). Colon carcinogenesis in germ-free rats with 1,2-dimethylhydrazine and N-methyl-n-nitrosoguanidine. *Cancer Res* 34: 2368-2372.

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.

Sreekumar O, Hosono A (2000). Immediate effect of *Lactobacillus acidophilus* on the intestinal flora and fecal enzymes of rats and the in vitro inhibition of *Escherichia coli* in coculture. *J Dairy Sci* 83: 931-939.

Wollowski I, Rechkemmer G, Pool-Zobel BL (2001). Protective role of probiotics and prebiotics in colon cancer. *Am J Clin Nutr* 73(2): 451S-455S.

Zachariah PK, Juchau MR (1974). The role of gut flora in the reduction of aromatic nitro-groups. *Drug Metab Dispos* 2: 74-78.