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Administration of *Lactobacillus fermentum* CRL1446 increases intestinal feruloyl esterase activity in mice

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Keywords
feruloyl esterase, functional food, intestinal esterase activity, *Lactobacillus fermentum*.

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Abstract

Aims: To evaluate the effect of oral administration of *Lactobacillus fermentum* CRL1446 on the intestinal feruloyl esterase (FE) activity and oxidative status of mice.
Methods and Results: Adult Swiss albino mice received *Lact. fermentum* CRL1446 at the doses 10^7 and 10^9 cells per day per mouse for 2, 5, 7 and 10 days. Intestinal FE activity, intestinal microbiota counts, plasmatic thiobarbituric acid-reactive substances (TBARS) percentage and glutathione reductase (GR) activity were determined. Mice that received *Lact. fermentum* CRL1446 at the dose 10^7 cells per day for 7 days showed a twofold increase in total intestinal FE activity, compared to the nontreated group. In large intestine content, FE activity increased up to 6·4 times. No major quantitative changes in colonic microbiota were observed in treated animals. Administration of this strain produced an approx. 30–40% decrease in the basal levels of plasmatic TBARS and an approx. twofold increase in GR activity from day 5 of feeding with both doses.
Conclusions: Oral administration of *Lact. fermentum* CRL1446 to mice increases total intestinal FE activity, decreases the basal percentage of plasmatic lipoperoxides and increases GR activity.
Significance and Impact of the Study: *Lactobacillus fermentum* CRL1446 could be orally administered as a dietary supplement or functional food for increasing the intestinal FE activity to enhance the bioavailability of ferulic acid, thus improving oxidative status.

Introduction

Cinnamoyl esterases (CE) are carboxyl ester hydrolases that degrade hydroxycinnamate esters, which are commonly found in cereals, fruits and vegetables, releasing hydroxycinnamic acids (HA) such as ferulic, sinapic, caffeic and *p*-coumaric acids (Fazary and Ju 2007). HA exhibit *in vitro* chemoprotective and antioxidant properties (Fazary and Ju 2007), and it is suspected that they may contribute toward the beneficial effects of a bran-rich diet (Vitaglione *et al.* 2008). The most abundant HA present in cereals is ferulic acid (FA) (Andreasen *et al.* 2000). Regular ingestion of FA may provide substantial protection against oxidative stress-related ailments like cancer, diabetes, cardiovascular and neurodegenerative diseases, and in ageing (Srinivasan *et al.* 2007). It was also reported that FA induces intrinsic antioxidant mechanisms such as superoxide dismutase, catalase and glutathione reductase (GR) activities (Srinivasan *et al.* 2007). HA may exert their positive effect only if they are free in the gut, thus the hydrolysis of ester bonds and subsequent release of HA is the first step required for the bioavailability and metabolism of hydroxycinnamates. CE that hydrolyse mainly esters of FA are known as feruloyl

esterases (FE) or ferulic acid esterases (FAE). FE activity is commonly found in micro-organisms in the rumen (Faulds and Williamson 1991; Donaghy *et al.* 1998; McSweeney *et al.* 1998) and in different bacterial genera present in the human and animal gut (Couteau *et al.* 2001; Lai *et al.* 2009).

Kroon *et al.* (1997) reported that esters are not digested in the upper digestive tract of rats, and enzymatic secretions of the small intestine do not hydrolyse esters of FA. Nevertheless, there is a partial release of them in the colon. CE activity has been demonstrated in the large intestine microbiota of rats and humans (Buchanan *et al.* 1996; Kroon *et al.* 1997), and levels and specificity of these enzymes are the critical factors influencing the bioavailability of HA (Buchanan *et al.* 1996; Bourne and Rice-Evans 1999).

Andreasen *et al.* (2001a) investigated the distribution of CE activity in the intestine of rats and humans, using synthetic hydroxycinnamoyl esters as substrates. They observed that CE are distributed throughout their intestinal tract. In rats, CE activity in the small intestine was derived mainly from the mucosa, whereas in the large intestine, it was found predominantly in the luminal microbiota. Therefore, intestinal CE activity in rats may have an epithelial and a microbial origin (Andreasen *et al.* 2001a). Additionally, CE may be released into the lumen when cells are exfoliated.

The beneficial intestinal microbiota is represented mainly by *Lactobacillus* and *Bifidobacterium* (Guarner and Malagelada 2003). Several studies demonstrated that the incorporation of these probiotic bacteria into functional foods has beneficial effects on human health. Couteau *et al.* (2001) reported that certain gut bacteria, including species belonging to these genera, are involved in the release of bioactive HA in the human colon.

At present, there is little information about the presence of CE in *Lactobacillus* and their contribution to human health. Bhathena *et al.* (2007) encapsulated cells of *Lactobacillus fermentum* ATCC11976 with CE activity able to produce FA from ethyl ferulate (FE activity), and their administration to diet-induced hypercholesterolemic hamsters produced significant reductions in serum total cholesterol, LDL cholesterol and triglyceride levels (Bhathena *et al.* 2009).

Very few reports describing CE of food-associated bacteria are available (Donaghy *et al.* 1998; Guglielmetti *et al.* 2008). Guglielmetti *et al.* (2008) studied the CE activity of 100 strains of food and human intestinal origin, and selected *Lactobacillus helveticus* MIMLh5 because of its strong activity on chlorogenic acid. This strain was employed for the preparation of a food product containing high concentration of free caffeic acid, and antioxidant and potential probiotic properties demonstrated *in vitro*. However, these authors did not perform *in vivo* trials with lactobacilli administration.

The presence of CE activity, able to release antioxidant compounds that can be absorbed in the gut, could be used as a specific criterion for the selection of probiotic micro-organisms.

The aim of this paper was to evaluate the effect of oral administration of *Lact. fermentum* CRL1446, strain with FE activity *in vitro*, on the intestinal FE activity and oxidative status in mice.

Materials and methods

Bacterial strain and growth conditions

Lactobacillus fermentum CRL1446, strain isolated from an Argentinean goat milk cheese (Oliszewski *et al.* 2007), was obtained from the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA, Tucumán, Argentina). This strain presented CE activity *in vitro*, with higher specificity toward methyl ferulate (FE activity), and was able to tolerate simulated gastrointestinal tract (GIT) conditions (Abeijón Mukdsi 2009). *Lactobacillus fermentum* CRL1446 was maintained in Man–Rogosa–Sharpe broth (MRS; Britania, Buenos Aires, Argentina) containing 20% (v/v) glycerol at −70°C and propagated three times in MRS broth before each experimental use.

Animals and diet

Six-week-old male Swiss albino mice ($n = 156$) were obtained from the closed random-bred colony maintained at CERELA. They were housed in individual cages and acclimated to 22 ± 2°C with a 12 h light/dark cycle. All mice received a conventional balanced diet (60·8% carbohydrates, 25·5% proteins, 3·8% fats, 3·4% raw fibre, 6·5% total minerals; Asociación de Cooperativas Argentinas, Buenos Aires, Argentina) and drinking water *ad libitum*. This diet supplied approx. 0·60 mg of hydroxycinnamates per day per mouse.

All animal protocols were approved by the Animal Protection Committee of CERELA and complied with current Argentinean laws.

Bacterial suspension preparation

Lactobacillus fermentum CRL1446 was cultured in MRS broth for 16 h at 37°C (late exponential phase), harvested by centrifugation (10 000 ***g***, 10 min) and washed twice with phosphate buffered saline (PBS) pH 7·0. Cells were then resuspended in drinking water to the desired concentration: 10^7 or 10^9 cells per ml. Bacterial suspensions were prepared freshly every day and changed every 24 h.

Drinking water was found to maintain the bacterial cell count after 24 h.

Feeding procedures

The experimental protocol comprised two treated groups (n = 48 each) and a nontreated group (NTG) (n = 60):

1. CRL10^7 group, receiving *Lact. fermentum* CRL1446 at the dose 10^7 cells per day per mouse;
2. CRL10^9 group, receiving *Lact. fermentum* CRL1446 at the dose 10^9 cells per day per mouse;
3. NTG, not receiving bacterial administration.

All mice were fasted for 16 h before sacrifice. Twelve animals from NTG were sacrificed by cervical dislocation at day 0 to evaluate the baseline values of intestinal FE activity (n = 6) and intestinal microbiota (n = 6). At days 2, 5, 7 and 10 of feeding, 12 animals from each three groups were sacrificed for FE activity (n = 6) and microbiota (n = 6) determinations.

It was previously confirmed that the administration of *Lact. fermentum* CRL1446 at both doses tested did not produce bacterial translocation (liver and spleen) at the end of each treatment period (Abeijón Mukdsi 2009).

Preparation of intestinal extracts

Small and large intestines were aseptically removed and different intestinal sections (SIM, small intestine mucosa; LIM, large intestine mucosa; SIC, small intestine content; LIC, large intestine content) were obtained according to Andreasen *et al.* (2001b), with slight modifications. Briefly, the content from each intestinal segment was flushed with 1 ml of ice-cold PBS pH 7·0. The diluted gut content was homogenized by vortexing and centrifuging (5000 ***g***, 5 min, 4°C). The supernatant (intestinal content) was removed and kept on ice. The rinsed intestinal segments were everted, the mucosa scraped off and resuspended in ice-cold PBS pH 7·0 (0·1 g mucosa per ml). Samples were homogenized and centrifuged (5000 ***g***, 5 min, 4°C). Supernatants (intestinal mucosa extract) were removed and kept on ice prior to FE activity determination.

Determination of intestinal FE activity

Intestinal FE activity was determined by incubation of intestinal mucosa or content in PBS pH 7·0 containing 1 mmol l^{-1} methyl ferulate as substrate, at 37°C for 18 h. Reactions were stopped by the addition of glacial acetic acid. Controls containing the reaction mixture plus glacial acetic acid were also incubated to test for the presence of background peaks. Samples and controls were centrifuged (13 000 ***g***, 10 min, 4°C) and filtered (0·22 μm) prior to HPLC analysis of released FA. Results were expressed as units (U) of FE activity per gram of intestinal extract. One unit was defined as the amount of enzyme releasing 1 mmol of FA per hour.

Determination of FA by HPLC

Separations were performed on a Knauer system (Berlin, Germany) equipped with an UV detector, using a reverse-phase C-18 column (ReproSil-Pur ODS, 3·5 μm, 250 × 4·6 mm; Dr Maisch GmbH, Ammerbuch, Germany). Twenty microlitres of sample were injected and eluted isocratically with a mixture of water : acetonitrile : acetic acid (69 : 30 : 1, v/v/v) at a flow rate of 1 ml min^{-1}. Compounds were monitored by absorbance at 320 nm. Released FA was quantified from the regression curve (R^2 > 98%) of the standard (Apin Chemicals, Abingdon, UK), using external standard calibration.

Intestinal microbiota counts

Large intestines were aseptically removed, weighted and homogenized in 5 ml of peptone water (0·1%, w/v). Serial dilutions of the homogenized samples were plated onto agarized media (Britania): Reinforced Clostridial (RCA) for total anaerobic bacteria; RCA containing 0·2% (w/v) LiCl, 4 mg l^{-1} colistin, 1% (w/v) aniline blue and adjusted to pH 5·0 with acetic acid after sterilization (RCA pH 5) for bifidobacteria; MRS for total lactobacilli; Brucella containing 20 ml l^{-1} blood and 7·5 μg ml^{-1} vancomycin for *Bacteroides*, and Mac Conkey for *Enterobacteriaceae*. Plates were anaerobically incubated at 37°C for 48–96 h. At the end of the incubation period, colonies were counted and results were expressed as $logarithm_{10}$ of colony forming units (CFU) per g of intestine.

Lipoperoxidation in plasma

Blood was collected by cardiac puncture and transferred into tubes containing anticoagulant EDTA (Wiener Lab, Rosario, Argentina). Plasma was obtained by centrifugation (5000 ***g***, 5 min). Lipid peroxidation was estimated spectrophotometrically by the thiobarbituric acid-reactive substances (TBARS) method of Okawa *et al.* (1979). Results were expressed as nmol of TBARS per mg of protein and presented as percentage, considering the basal TBARS levels in NTG as 100%.

Glutathione reductase activity in plasma

GR activity was determined according to Esterbauer and Grill (1978), by following the rate of NADPH oxidation at 340 nm. Results were expressed as units (U) of GR activity per mg of protein. One unit was defined as the

amount of enzyme producing 1 nmol of oxidized NADP per minute.

Protein determination

Protein concentrations were determined according to the method of Bradford (1976), using a commercial kit (Bio-Rad, Hercules, CA, USA) and bovine serum albumin (Sigma, St Louis, MO, USA) as standard.

Statistical analysis

Results are means of three independent experiments ± standard deviation (SD). After the analyses of variance (ANOVA), Tukey's test was used to identify statistically significant differences ($P < 0{\cdot}05$). These analyses were performed with the software package MINITAB 14 (Minitab Inc., State College, PA, USA).

Results

Effect of *Lactobacillus fermentum* CRL1446 administration on intestinal FE activity

Total intestinal FE activity (mucosa plus content activities) in mice orally administered with *Lact. fermentum* CRL1446 for 2, 5, 7 and 10 days, and nontreated mice (NTG) is shown in Fig. 1. In NTG, no statistically significant changes in total activity were observed between the different time points assayed (Fig. 1). Compared to NTG, mice from CRL10^7 group showed total FE activity increases from day 5, displaying the highest levels at day 7 (14·89 ± 1·23 U g^{-1}). Only at this time point, total activity was significantly different between treated groups. In mice from CRL10^9 group, total activity increased at days 5 and 10, compared to NTG (Fig. 1). Even though no statistically significant difference was observed at day 7 because of large intra-group variations, mean activity in treated mice was higher than in NTG.

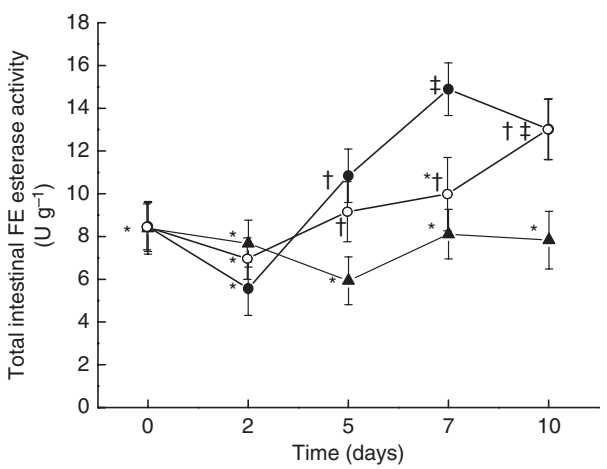

Figure 1 Total intestinal FE activity in mice administered with *Lactobacillus fermentum* CRL1446. (▲) Nontreated group (NTG); (●) dose 10^7 cells per day per mouse (CRL10^7 group); (○) dose 10^9 cells per day per mouse (CRL10^9 group). Each point represents mean ± SD (n = 6/group at each time point). Means for each value without any common symbols (*, †, ‡) differ significantly ($P < 0{\cdot}05$). FE, feruloyl esterase.

FE activity determined in each intestinal fraction is shown in Fig. 2. In NTG, intestinal FE activity was mainly located in mucosa (Fig. 2). Compared to NTG, mice from CRL10^7 group presented FE activity increase in SIM and LIC, and decrease in SIC. No statistically significant changes were observed in LIM (Fig. 2). In mice from CRL10^9 group, activity increase in SIM and LIC, and decrease in LIM and SIC (Fig. 2) was detected. In both groups, the highest activity increases were observed in LIC at day 5 (4- and 6·4-fold, in CRL10^7 and CRL10^9 groups, respectively). Mice from the CRL10^9 group also showed significant activity increases in LIC at days 7 and 10 (4·1- and 5·7-fold, respectively). In mice from CRL10^7 group, increases of 3·6- and 3-fold were observed at day 7 in LIC and SIM, respectively. Even though activity increases detected in LIC at days 5 and 10 were higher in mice receiving the higher dose (Fig. 2), no significant differences in total intestinal FE activity were observed between the treated groups (Fig. 1).

Effect of *Lactobacillus fermentum* CRL1446 administration on intestinal microbiota

To determine whether administration of *Lact. fermentum* CRL1446 quantitatively altered intestinal microbiota, $\log_{10}$ CFU g^{-1} of main intestinal bacterial genera was evaluated in large intestine homogenates at days 2, 5, 7 and 10 of feeding. Counts of main colonic bacterial genera in treated and nontreated mice are shown in Table 1. No significant changes in anaerobic bacteria and enterobacteria populations were observed at the end of each treatment, compared to NTG. At day 10, statistically significant increases (approx. 1 log) in lactobacilli and bifidobacteria populations were detected in mice from both treated groups. A similar increase in *Bacteroides* population was observed in mice from the CRL10^9 group.

Effect of *Lactobacillus fermentum* CRL1446 administration on lipid peroxidation

To assess whether administration of *Lact. fermentum* CRL1446 could affect the oxidative status of mice receiving a conventional balanced diet and this strain, plasmatic lipoperoxide levels were determined as %TBARS in trea-

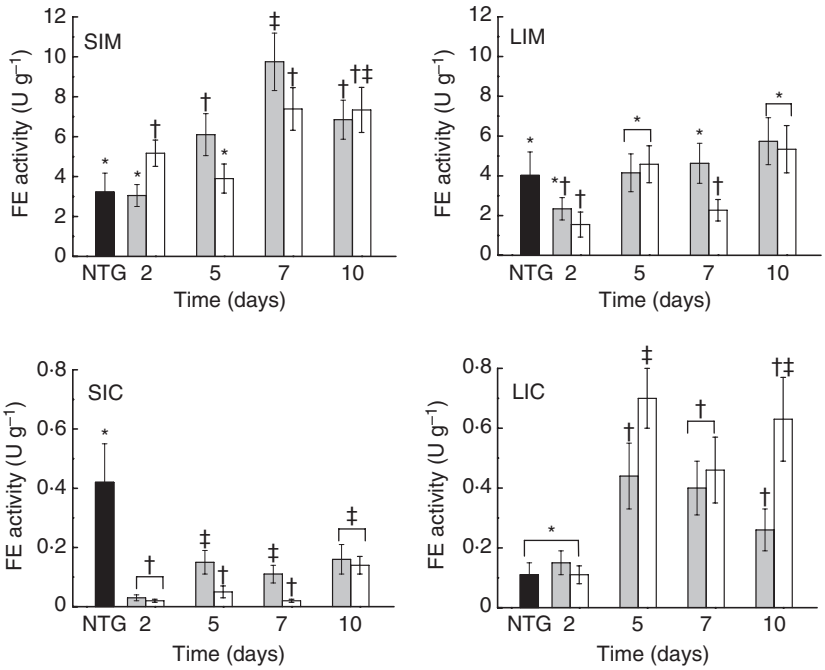

Figure 2 Intestinal FE activity in mice administered with *Lactobacillus fermentum* CRL1446. (■) Nontreated group (NTG); (■) dose 10^7 cells per day per mouse (CRL10^7 group) and (□) dose 10^9 cells per day per mouse (CRL10^9 group). SIM, small intestine mucosa; LIM, large intestine mucosa; SIC, small intestine content; LIC, large intestine content. Data are presented as mean ± SD (n = 6/group at each time point). Means for each value without any common symbols (*, †, ‡) differ significantly. The control group was represented only once because FE activity values at the different time points evaluated were not significantly different (P < 0·05). FE, feruloyl esterase.

ted and nontreated mice (Fig. 3). Compared to NTG (100% TBARS), TBARS percentage decreased from day 5 in both CRL10^7 and CRL10^9 groups. Decreases of approx. 30–40% were observed at day 7. At days 2 and 10, TBARS levels in mice from CRL10^9 group were significantly different from those observed in the NTG and CRL10^7 group.

Effect of *Lactobacillus fermentum* CRL1446 administration on plasmatic GR activity

GR activity determined in plasma of mice administered with *Lact. fermentum* CRL1446 and nontreated mice is shown in Fig. 4. Compared to the NTG, mice from the CRL10^7 group showed a significant increase in GR activity from day 5 (approx. twofold). In mice from the CRL10^9 group, GR activity increased from day 7, reaching the highest levels at day 10 (1·00 ± 0·04 U mg^{-1}).

Discussion

Major advances have been made in the understanding of the absorption of phenolic compounds across the small intestine and the extent to which they are metabolized in the GIT and undergo postabsorption liver conjugation (Andreasen *et al.* 2001b). The majority of those phenolic compounds ingested reach the colon without degradation. Here, they can serve as substrates for the complex indigenous microbiota, which contains species and genera that can exert varying effects on the host health. The colonic microbiota has also been confirmed as the major metabolic site for the release of free HA, because of its CE activity (Couteau *et al.* 2001). This activity has been reported in lactobacilli strains from intestinal and food origin (Donaghy *et al.* 1998; Couteau *et al.* 2001; Bhathena *et al.* 2007; Guglielmetti *et al.* 2008). Nevertheless, the effect of their oral administration on intestinal CE activity levels has not yet been evaluated.

Previous studies demonstrated that *Lact. fermentum* CRL1446 presents FE activity, which is mainly intracellularly located and cell surface-associated. This strain was resistant to GIT conditions, and after incubation in the presence of bile salts, cells showed a twofold increase in FE activity, compared to the control (Abeijón Mukdsi 2009).

In this paper we evaluated the effect of oral administration of the FE-producing strain *Lact. fermentum* CRL1446 on the intestinal FE activity and oxidative status in Swiss albino mice. The determination of basal FE activity levels in nontreated mice showed that this activity was mainly

Table 1 Cell counts of intestinal bacterial microbiota in mice administered with *Lactobacillus fermentum* CRL1446 and nontreated mice

Administration time (days)	Micro-organisms									
	Lactobacilli		Bifidobacteria		*Bacteroides*		Anaerobic bacteria		Enterobacteria	
	10^7	10^9	10^7	10^9	10^7	10^9	10^7	10^9	10^7	10^9
2	8·64 ± 0·56[a]	7·52 ± 0·43[a]	8·25 ± 0·83[a]	7·88 ± 0·53[a]	8·07 ± 0·87[a]	7·85 ± 0·48[a]	8·06 ± 0·48[a]	7·76 ± 0·38[a]	4·99 ± 0·57[a]	5·20 ± 0·49[a]
5	8·79 ± 0·49[a]	7·43 ± 0·48[a]	8·09 ± 0·59[a]	6·95 ± 0·69[a]	8·10 ± 0·60[a]	8·00 ± 0·50[a]	8·15 ± 0·62[a]	7·24 ± 0·47[a]	6·97 ± 0·61[a]	6·80 ± 0·59[a]
7	8·28 ± 0·14[a]	8·53 ± 0·27[a]	7·86 ± 0·21[a]	8·13 ± 0·64[a]	8·03 ± 0·32[a]	8·24 ± 0·20[a]	8·23 ± 0·60[a]	8·71 ± 0·25[a]	6·11 ± 0·50[a]	4·61 ± 0·84[a]
10	9·45 ± 0·27[b]	9·74 ± 0·13[b]	9·20 ± 0·31[b]	9·38 ± 0·22[b]	8·93 ± 0·11[a]	9·37 ± 0·22[b]	9·00 ± 0·54[a]	9·47 ± 0·14[a]	6·34 ± 0·98[a]	6·44 ± 0·94[a]
Nontreated group (NTG)	8·17 ± 0·38[a]		7·72 ± 0·11[a]		8·43 ± 0·55[a]		8·52 ± 0·93[a]		5·81 ± 0·86[a]	

Results are expressed as $\log_{10}$ CFU g^{-1} of large intestine. Data are mean ± SD (n = 6/group at each time point). Means in the same column without a common superscript letter (a–b) differ significantly from NTG ($P < 0·05$). Doses of administration: 10^7 cells per day per mouse (CRL10^7 group), 10^9 cells per day per mouse (CRL10^9 group). For NTG, only results at day 0 are shown, as values at the different time points evaluated were not significantly different ($P < 0·05$).

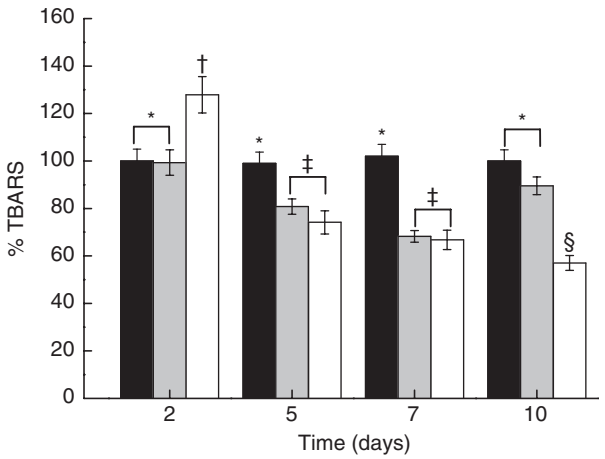

Figure 3 Lipoperoxide levels in plasma of mice administered with *Lactobacillus fermentum* CRL1446. (■) Nontreated group (NTG); (■) dose 10^7 cells per day per mouse (CRL10^7 group) and (□) dose 10^9 cells per day per mouse (CRL10^9 group). Data are presented as mean ± SD (n = 6/group at each time point). Means for each value without any common symbols (*, †, ‡, §) differ significantly ($P < 0·05$). TBARS, thiobarbituric acid-reactive substances.

located in intestinal mucosa, being similar in small and large intestine. To the best of our knowledge, no similar studies in Swiss albino mice have been reported at present. Intestinal CE activity was only evaluated in a Wistar rat model (Andreasen *et al.* 2001a). These authors concluded that intestinal CE activity was mainly located in SIM and LIC.

Our results showed that the effect of *Lact. fermentum* CRL1446 administration on intestinal FE activity is time- and dose-dependent. Compared to NTG, total activity increased in both treated groups from day 5 of feeding. The highest total activity was observed after 7 days in mice receiving 10^7 cells per day, thus this treatment was optimal to increase intestinal FE activity. Regarding FE activity in each intestinal fraction, treated mice showed the highest activity increases in LIC (approx. four- to six-fold). This fact may indicate that *Lact. fermentum* CRL1446 might supply exogenous FE enzymes into the gut, or it could stimulate the FE activity of colonic microbiota via an indirect effect.

The decrease in FE activity in SIC observed in all treated mice from day 2 would suggest that *Lact. fermentum* CRL1446 administration could negatively affect the FE activity of autochthonous luminal microbiota. In contrast, activity increases were generally observed in SIM. Therefore, we hypothesize that *Lact. fermentum* CRL1446 could also stimulate the FE activity of epithelial cells. Similar results were observed in mice receiving *Lact. fermentum* ATCC14932, a reference FE-producing strain (Abeijón-Mukdsi 2009). Nevertheless, FE induc-

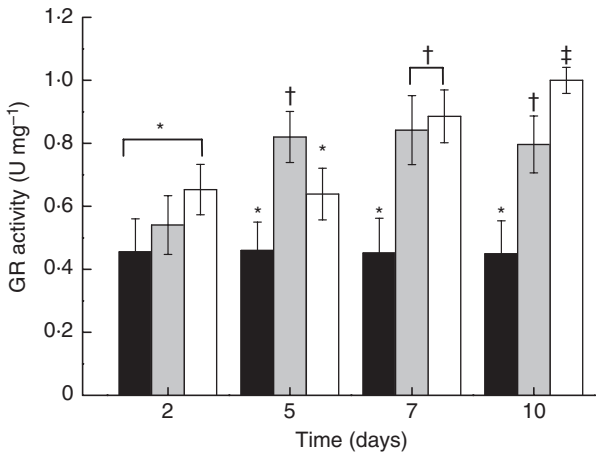

Figure 4 Glutathione reductase (GR) activity in plasma of mice administered with *Lactobacillus fermentum* CRL1446. (■) Nontreated group (NTG); (■) dose 10^7 cells per day per mouse (CRL10^7 group) and (□) dose 10^9 cells per day per mouse (CRL10^9 group). Data are presented as mean ± SD (n = 6/group at each time point). Means for each value without any common symbols (*, †, ‡) differ significantly ($P < 0{\cdot}05$).

tion mechanisms in the gut have not yet been elucidated.

Previous studies of probiotic *Lact. fermentum* RC-14 administration demonstrated its important role in regulating the intestinal microbiota balance (Gardiner *et al.* 2002). Our results showed that there were only significant quantitative changes in colonic microbiota in mice receiving *Lact. fermentum* CRL1446 for 10 days. Statistically significant increases in lactobacilli and bifidobacteria populations were observed in both treated groups. Functional foods targeting the human colon aim to stimulate these beneficial genera either directly by providing growth substrates, which selectively promote the growth of autochthonous bifidobacteria and lactobacilli *in vivo* within the colon (prebiotics), or indirectly by introducing live exogenous bacteria in specially formulated foods (probiotics) (Costabile *et al.* 2008).

In our experimental protocol, mice were fed a conventional balanced diet containing 3·40% fibre, mainly from maize, which is rich in hydroxycinnamates. In maize bran, FA constitutes about 3·10% (w/w), and *p*-coumaric acid is found in significant amounts (0·33%, w/w) (Topakas *et al.* 2007).

HA exhibit *in vitro* antioxidant properties (Vitaglione *et al.* 2008), and low dosage has been related to prevention of oxidative stress and lipid peroxidation (Andreasen *et al.* 2000). *Lactobacillus fermentum* CRL1446 administration at both doses tested produced an approx. 30–40% decrease in basal TBARS levels, and an approx. twofold increase in GR activity from day 5, suggesting a protective effect against oxidative damage. This observation was in accordance with the increase in total intestinal FE activity detected in both treated groups from day 5, compared to NTG. This FE-producing strain would increase the hydrolysis of dietary hydroxycinnamates, releasing FA, which then could be absorbed and exert its *in vivo* antioxidant effect.

On the other hand, GR activity stimulation is of great relevance because GR is involved in the regeneration of reduced glutathione (GSH), which is essential for the endogenous antioxidant defence. Halliwell and Gutteridge (1999) reported that survival in the presence of reactive oxygen species is only possible because GSH protects biomolecules from oxidative damage. GR can maintain GSH concentrations in the cell, which is involved in glutathione peroxidase-catalysed elimination of H_2O_2 and lipoperoxides.

This is the first time that the effect of administration of lactic acid bacteria on the intestinal FE activity was evaluated. According to the results obtained in this study, *Lact. fermentum* CRL1446, FE-producing strain, could be orally administered as a dietary supplement or functional food for increasing the intestinal FE activity to enhance the bioavailability of antioxidant FA, thus improving the consumer's oxidative status.

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