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Safety Evaluation of *Lactobacillus paracasei* subsp. *paracasei* LC-01, a Probiotic Bacterium[§]

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The safety of *Lactobacillus paracasei* subsp. *paracasei* LC-01 was evaluated for its use as a potential probiotic. In our *in vitro* study, the antibiotic resistance and the ability to produce biogenic amine were determined. The results showed that the strain was sensitive to all tested antibiotics and did not produce biogenic amine except for tyramine. The oral toxicity of this strain was evaluated in Balb/C mice. One hundred mice were divided into 10 groups. Four groups were administered 0, 10^8, 10^9, or 10^{10} CFU/mouse per day dissolved in saline solution respectively, for 28 days. Three groups were injected intraperitoneally with 10^9 CFU/mouse dissolved in saline solution, and were killed 2, 5, and 10 days after injection. The last 3 groups were injected with the vehicle as controls respectively. The results showed that oral administration of the strain had no adverse effects on mouse body weight and that there was no treatment-associated bacterial translocation. Intraperitoneal administration caused a significant translocation to liver, spleen and kidney. However, this translocation did not cause illness or death throughout the experiment. The results suggest that *L. paracasei* subsp. *paracasei* LC-01 is likely to be safe for human consumption.

Keywords: safety evaluation, antibiotic resistance, biogenic amine, oral toxicity, bacterial translocation

Introduction

Lactic acid bacteria (LAB) are classified as generally recognized as safe (GRAS) for human consumption (Donohue and Salminen, 1996), since they have been consumed in fermented foods for several centuries without any obvious adverse effects (Fuller, 1992). With the increasing interest in the use of these bacteria for nutritional and medical applications, many health-promoting properties of LAB have been researched, including properties of anti-infection (Isolauri *et al.*, 1991), intestinal anti-inflammation (Peran *et al.*, 2005), immunomodulatory activity (Olivares *et al.*, 2006) and prevention of allergic diseases (Furrie, 2005). Thus, a high number of new LAB strains with probiotic characteristics are incorporated into food and pharmaceutical products. These new LAB strains do not necessarily share the GRAS status of traditional LAB strains, and some of them are reported to be associated with clinical pathological conditions, such as bacteraemia, endocarditis or liver abscess (Bayer *et al.*, 1978; Aguirre and Collins, 1993; Gasser, 1994; Rautio *et al.*, 1999). Therefore, the safety assessment of potential probiotics that are aimed at being incorporated into food products is strongly recommended (Conway, 1996; Saxelin, 1997).

Lactobacillus paracasei subsp. *paracasei* LC-01 (LC-01) presents good technological properties for use in functional foods. The strain adhered to human Caco-2 cell line and is resistant to simulated gastrointestinal stress (Fernandez de Palencia *et al.*, 2008). LC-01 also shows the remarkable ferrous iron chelating activity and high antioxidant activity (Kim *et al.*, 2005). Moreover, milk fermented by LC-01 is able to inhibit the growth of MCF7 breast cancer cell line (Biffi *et al.*, 1997). The exopolysaccharides and cell fractions of the strain are able to reduce the cytotoxicity of 4-nitroquinoline 1-oxide (Liu *et al.*, 2011). Therefore, we decided to evaluate the safety of LC-01 for human consumption. Although, up to now, there are no general guidelines on this issue, acute oral toxicity has been proposed as a fundamental test for the assessment of probiotic safety (Stine and Brown, 1996). Moreover, in the report by the Joint FAO/WHO Working Group (2002) (FAO/WHO, 2002), it is stated that Lactobacilli, although generally considered safe, may theoretically be responsible for a number of side effects, and thus, safety assessments in terms of antibiotic susceptibility and virulence properties are recommended. Therefore, in the present study, we analyzed the antibiotic resistance and biogenic amine production of the strain. *In vivo* study, mice were orally administered with different doses of the strain. Oral toxicity, blood haematological and biochemistry parameters, and bacterial translocation was analyzed. To preclude toxicity even in the extreme case of potential translocation, groups of mice were injected intraperitoneally with the strain and the adverse effects of bacterial translocation to different tissues was evaluated.

Materials and Methods

Bacterial strains

LC-01 is supplied in lyophilized powder form by Chr Hansen (Horsholm, Denmark). The strain grew for 24 h at 37°C in

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Man Rogosa and Sharpe broth (MRS; Beijing Land Bridge Technology Co., Ltd, China) and was serially transferred at least three times prior to use in this study.

Sensitivity to antibiotics

Antibiotic susceptibility of LC-01 was determined using antibiotic discs (Tiantan Biological Products Co. Ltd., China). *Staphylococcus aureus* ATCC 25923 was obtained from China General Microbiological Culture Collection Center (Beijing, China) and was used as a quality control organism. LC-01 and *Staphylococcus aureus* ATCC 25923 were incubated on MRS Agar and Mueller Hinton Agar (Beijing Land Bridge Technology Co., Ltd, China) respectively. After drying the surface, antibiotic discs were placed on the agar plate. Zone diameters were recorded after 24 h incubation at 37°C.

Biogenic amine production

Biogenic amine is mainly generated by decarboxylation of amino acid through substrate-specific enzymes of bacteria. For promoting enzyme induction before the test, LC-01 was subcultured 5 times in MRS broth, which contained 0.1% (wt/v) of each precursor amino acid-tyrosine, histidine, ornithine, arginine and 0.005% (wt/v) of pyridoxal-5-phosphate (Sigma-Aldrich Inc., USA). Then the strains were inoculated at 0.1% (wt/v) into a decarboxylase broth (Bover-Cid and Holzapfel, 1999). After incubating at 37°C for 4 days, 5 ml of fermenting broth was extracted with an equal volume of 10% (v/v) trichloroacetic acid for 1 h and was centrifuged at 1,900×g for 10 min. Five standard biogenic amines-putrescine, histamine, tyramine, spermidine, spermine (Sigma-Aldrich Inc.) were prepared (Shukla *et al.*, 2010), and derivatization was carried out by the method described by Li *et al.* (2011). Quantitative analyses of biogenic amines were carried out using a HPLC (SPD-20AT, Shimadzu, Japan) unit consisting of two pumps and a UV/VIS detector. Separation was achieved using a Kromasil C8 column (150 mm × 4.6 mm, 5 μm; Akzo Nobel, Sweden). The mobile phase was water/acetonitrile at a flow rate of 1 ml/min with a gradient elution program for 28 min (Li *et al.*, 2011). The sample was injected (20 μl) and was monitored at 254 nm (Bover-Cid and Holzapfel, 1999).

Animals and experimental design

One hundred female Balb/C mice (Vital River Lab Animal Technology Co. Ltd., China) aged 6–8 weeks, were housed in a plastic cage under standard conditions (temperature 22±2°C, humidity 55%±2%) with a 12 h light/dark cycle. The animals were fed with a basal diet (Rodent Chow Product, Ke Ao Xie Li feeds Co. Ltd., China) and had free access to deionized water. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals and the protocol was approved by the Animal Ethics Committee of China Agricultural University.

After 7 days of acclimation, mice were randomly assigned to 10 groups (n = 10 per group): 3 groups were orally administered with 10^8, 10^9, or 10^{10} CFU/mouse per day (in saline solution), over a period of 28 days. One additional group received the vehicle alone and was used as control. A further 3 groups were injected intraperitoneally with 10^9 CFU/mouse dissolved in saline solution and were killed at day 2, day 5, and day 10 after intraperitoneal injection. The last 3 groups were injected intraperitoneally with the vehicle, and were used as controls. Throughout the experiment, the animal's activity, behaviour and hair lustre of each mouse was observed daily. Body weight (BW) and food intake were recorded weekly. At the end of the experiment, blood was collected from the heart under anaesthesia. After gross observation for abnormalities in all organs and tissues, the liver, spleen, and kidney were collected in sterile conditions and were weighed.

Haematology and blood biochemistry

Red blood cell (RBC), platelet counts, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), hemoglobin, and mean corpuscular hemoglobin concentration (MCHC) of blood were determined by an automatic haematology counter (Hitachi, Japan).

After the haematology assays, plasma was separated from blood samples. The levels of glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) activity, total protein (TP), albumin, glucose (GLU), cholesterol and were determined with an automated biochemical analyzer (Hitachi) of plasma samples.

Bacterial translocation

Bacterial translocation was analyzed in blood, liver, spleen and kidney. 50 μl of blood were directly inoculated onto the surface of MRS and brain heart infusion (BHI) agar plates. The plates were incubated at 37°C during 48 h anaerobically for MRS and aerobically for BHI. Tissue samples were homogenized in buffered saline solution (1 g/ml) and 100 μl of the resulting homogenates were cultured in MRS and BHI agar, as described above. After 48 h, the colonies were

Table 1. Antibiotic susceptibility of tested strain LC-01

Antibiotic	Diameter (mm)	Susceptibility
Penicillin G	33.40±1.82	S
Ampicillin	29.52±0.67	S
Amoxicillin	29.26±0.67	S
Piperacillin	31.88±0.42	S
Ampicillin/Sulbactam	27.59±0.54	S
Amoxycillin/Clavulanate Potassium	30.37±0.74	S
Cefalotin	23.21±1.78	S
Cefoperazone	28.44±0.84	S
Cefazolin	18.80±1.11	S
Cefuroxime Sodium	27.19±1.43	S
Meropenem	20.89±0.69	S
Azithromycin	22.29±0.84	S
Clarithromycin	33.87±0.46	S
Erythromycin	31.09±0.46	S
Doxycycline	33.68±2.07	S
Tetracycline	28.81±0.88	S
Clindamycin	33.72±0.96	S
Chloramphenicol	29.45±0.65	S
Rifampicin	28.10±0.35	S

Data are presented as Mean±SD (n=3). S, sensitive to antibiotics.

Table 2. **Body and tissue weights of mice treated orally with different doses of LC-01**

Items	Control	Dose (CFU/mouse/d)		
		1×10^8	1×10^9	1×10^{10}
Initial body weight (g)	17.21±0.67	17.13±0.93	17.21±0.94	17.27±0.79
Final body weight (g)	18.98±0.56	18.84±0.95	18.98±1.19	18.96±0.97
Food intake (g/mouse/d)	2.82±0.18	2.82±0.16	2.84±0.14	2.92±0.12
Liver weight (mg)	880.3±26.8	871.2±57.1	882.2±67.3	863.2±57.7
Kidney weight (mg)	240.4±14.9	226.9±20.5	225.2±11.4	234.4±15.1
Spleen weight (mg)	81.7±9.7	73.8±11.3	76.2±8.7	79.2±8.4

Data are presented as Mean±SD (n=10).

counted and the results were expressed as incidence of translocation (number of mice where colonies were detected/total number of mice). Positive growth on agar plates was defined by the presence of one or more colonies.

Identification of LC-01

A randomly amplified polymorphic DNA (RAPD) fingerprinting method was used to identify organisms detected in tissue samples. Total genomic DNA was extracted directly from MRS agar colonies using a DNA extraction kit (Tiangen, China). The DNA fragments were then amplified using the RAPD method with the following primer: GCGGAAATAG. The RAPD patterns of suspected strains were compared with the test strain.

Statistical analysis

All results were expressed as the Mean±SD. Differences between means were tested for statistical significance using one-way analysis of variance and post hoc least significance tests. The differences between proportions (bacterial translocation incidence) were analysed with the chi-squared test. All statistical analyses were carried out using SPSS Statistics 17.0 software, with statistical significance set at $P<0.05$.

Results and Discussion

Sensitivity to antibiotics and biogenic amine production

LC-01 was tested with 19 different antibiotics using the disc test method, antibiotic susceptibility levels for the strain were reported as resistant (R), intermediate-resistant (I) or sensitive (S) (Wikler, 2009). According to the zone diameter, LC-01 was sensitive to all tested antibiotics (Table 1), including β-lactam antibiotics (penicillin G and cefoperazone), macrolides (erythromycin), tetracyclines (tetracycline), and other broad-spectrum antibiotics (chloramphenicol and rifampicin), the results suggest that LC-01 is not able to transfer antibiotic resistance to other bacteria.

The capacity of LC-01 to decarboxylate amino acids involved in generating biogenic amines was investigated. For the HPLC analysis, putrescine, histamine, spermidine, and spermine were not detected, while that tyramine was found to be 8.99 mg/L (Supplementary data Table S1). In food, low levels of biogenic amines are not considered a serious risk, but when consumed in excessive amounts, they may cause distinctive pharmacological, physiological and toxic effects (Tassoni *et al.*, 2004). Histamine and tyramine are the most studied biogenic amines due to their toxicological effects, and other BA, such as the diamines and putrescine, may boost

Table 3. **Hematological and biochemical parameters data in mice treated orally with different doses of LC-01**

Items	Control	Dose (CFU/mouse/d)		
		1×10^8	1×10^9	1×10^{10}
RBC count (10^{12}/L)	10.2±0.6	10.6±0.4	10.7±0.8	10.3±0.6
Platelet count (10^9/ L)	1554±274	1497±192	1405±296	1360±191
Haemoglobin (g/L)	162.5±6.3	170.4±5.8	167.9±9.1	163.5±9.0
HCT (%)	53.5±3.3	55.9±2.1	56.1±3.4	54.1±3.7
MCV (fl)	52.3±0.7	52.8±0.6	52.4±1.1	52.4±0.9
MCH (pg)	15.6±0.7	16.1±0.5	15.7±0.4	16.0±0.9
MCHC (g/L)	298.4 ±14.2	305±10	299.2±7.2	304.9±17.5
GPT (U/L)	43.2±11.1	40.2±7.9	48.1±12.1	45.2±7.4
GOT (U/L)	150.4±21.1	164.5±34.8	159.1±24.7	155.1±24.2
TP (g/L)	50.1±2.7	54.5±4.2	51.5±3.6	53.7±3.0
Albumin (g/L)	34.4±0.7	35.6±1.7	34.1±1.6	35.6±1.5
GLU (mmol/L)	6.5±0.5	5.9±0.5	6.0±1.1	6.0±0.5
Cholesterol (mmol/L)	2.1±0.2	2.2±0.2	2.1±0.2	2.2±0.2
Ca (mmol/L)	2.3±0.1	2.3±0.1	2.3±0.1	2.3±0.1

Data are presented as Mean±SD (n=10).

Table 4. Incidence of bacterial translocation to blood, liver, kidney, and spleen in mice orally treated with different doses of LC-01

Items	Control	Dose (CFU/mouse/d)		
		1×10^8	1×10^9	1×10^{10}
Blood				
MRS	0/10	0/10	0/10	0/10
BHI	0/10	0/10	0/10	0/10
Liver				
MRS	1/10	1/10	1/10	0/10
BHI	1/10	1/10	2/10	1/10
Kidney				
MRS	1/10	0/10	1/10	0/10
BHI	1/10	0/10	1/10	1/10
Spleen				
MRS	0/10	1/10	0/10	0/10
BHI	1/10	1/10	0/10	0/10

the toxicity of the above amines (Bover-Cid and Holzapfel, 1999; Ozdestan and Uren, 2010). Both histamine- and tyramine-forming capacity of LAB isolated from different sources are reported (Priyadarshani and Rakshit, 2011). In the present study, tyramine was found to be 8.99 mg/L, which is below the toxic level of 1080 mg/L stipulated by Good Manufacturing Practice (Shalaby, 1996).

Oral administration of different doses of LC-01

During the experimental period, no noticeable activity or behavioural changes were observed in the mice, and no treatment-related illness or death occurred. There was no difference in the animals' aspect between treatment and control groups.

Likewise, there were no significant differences in food intake, BW gain, and organ weights between the control and oral-treatment groups ($P>0.05$, Table 2), indicating that the strain does not exhibit gross oral toxicity effects on the animals' health status, growth and development. In agreement with the absence of clinical symptoms in mice, the haematological and biochemical parameters measured in blood samples did not show statistically significant difference between control and strain-treated group ($P>0.05$, Table 3). Hematological parameters are usually used to represent the deleterious effects of test substances on the blood (Lee *et al.*, 2010), and biochemical assays can be used to detect moderate to mild deficiency of nutrients or an imbalance in nutrient metabolism, and are usually apparent before any clinical symptoms or changes in body weight (Zhou *et al.*, 2000). Our findings suggest that LC-01 do not have adverse effect on murine haematology or blood biochemistry.

Bacterial translocation is an important indicator of probiotic toxicity, since it is the first step in the pathogenesis process for many opportunistic indigenous lumen strains (Steffen and Berg, 1983). As shown in Table 4, no bacteremia was observed in any of the groups, and there were no significant differences in the incidence of translocation to the liver, spleen or kidney between the control and oral-treated groups at any of the tested doses, implying that translocation was not associated with treatment. In addition, colonies found on agar plates were checked by RAPD using specific primer, and no RAPD pattern from the tissue samples matched with the administered strain LC-01 (data not shown). It has been demonstrated in many studies that indigenous gut microflora has the ability to translocate into extra-intestinal tissues (Hale and Hill, 1973; Berg and Garlington, 1979; Swank and Deitch, 1996), and thus, the isolated microorganisms recovered from the tissues may come from the indigenous gut flora rather than from oral intake.

Intraperitoneal administration of LC-01

Although the above data have indicated that LC-01 was not able to translocate from gut to other tissues, we decided to induce this translocation by intraperitoneal administration of LC-01 to preclude adverse effects even in this case. As shown in Table 5, intraperitoneal administration caused a significant translocation of LC-01 to the liver, spleen and kidney at day 2. This increase was probably caused by a transient inflammatory process, and it could be lower over time, since the presence of bacteria in the tissues was lower at day 5 and day 10 after intraperitoneal injection. No bacteremia was shown in any of the mice injected intraperitoneally with LC-01. The presence of bacteria in the spleen was ac-

Table 5. Incidence of bacterial translocation in mice intraperitoneally administered LC-01

Items	Day 2 (CFU/mouse/d)		Day 5 (CFU/mouse/d)		Day 10 (CFU/mouse/d)	
	Control	1×10^9	Control	1×10^9	Control	1×10^9
Blood						
MRS	0/10	0/10	0/10	0/10	0/10	0/10
BHI	0/10	0/10	0/10	0/10	0/10	0/10
Liver						
MRS	2/10	$8/10^a$	3/10	$8/10^a$	2/10	$6/10^a$
BHI	2/10	$10/10^a$	2/10	$8/10^a$	2/10	$6/10^a$
Kidney						
MRS	1/10	$10/10^a$	2/10	$7/10^a$	2/10	$2/10^b$
BHI	1/10	$10/10^a$	2/10	$7/10^a$	2/10	$3/10^b$
Spleen						
MRS	1/10	$10/10^a$	2/10	$10/10^a$	2/10	$3/10^b$
BHI	2/10	$10/10^a$	2/10	$10/10^a$	2/10	$3/10^b$

Data are presented as Mean±SD (n=10). [a] $P<0.05$ vs. control, [b] $P<0.05$ vs. day 2.

Table 6. Body and tissue weights of mice intraperitoneally injected with LC-01

Items	Day 2 (CFU/mouse/d)		Day 5 (CFU/mouse/d)		Day 10 (CFU/mouse/d)	
	Control	1×10^9	Control	1×10^9	Control	1×10^9
Initial body weight (g)	19.09±0.58	18.85±1.05	17.99±0.74	18.85±1.27	19.02±1.39	18.82±0.93
Final body weight (g)	19.11±0.69	18.88±0.89	18.53±0.47	18.99±1.20	19.75±1.50	19.57±0.78
Liver weight (mg)	925.6±87.7	975.1±95.7	916.9±65.3	964.4±69.7	905.4±87.6	956.1±82.1
Kidney weight (mg)	236.8±17.3	248.1±25.2	227.6±15.1	228.8±19.1	241.2±25.8	246.8±15.1
Spleen weight (mg)	77.2±7.0	115.7±12.7[a]	86.2±6.2	133.1±11.2[a,b]	86.7±4.6	109.1±9.8[a]

Data are presented as Mean±SD (n=10). [a] $P<0.05$ vs. control, [b] $P<0.05$ vs. day 2.

companied by a significant increase in spleen weight at day 2, day 5 and day 10 (Table 6), whereas the weight of the liver and kidney did not change. In addition, the platelet count was significantly decreased at day 2, and was then increased to be normal at day 5 (Supplementary data Table S2). In spite of the bacterial translocation to the liver, kidney, and spleen and the increase in spleen weights, no noticeable behavioral and BW changes were observed (Table 6) and there was no illness or death related to LC-01 administration, while intraperitoneal administration of the same dose of other pathogenic microorganisms has been reported to be lethal for mice at day 2 after inoculation (Gras *et al.*, 2006).

Conclusion

In summary, LC-01 was determined to be sensitive to all the antibiotics tested, and were not associated with biogenic amine production other than tyramine (8.99 mg/L). Feeding mice with the potential probiotic strain LC-01 at high doses for 28 days had no deleterious effects on general health status, growth, blood haematology or biochemistry, as examined in this study. The test strain did not cause infection and translocation after feeding for 28 days. Even in the extreme case of translocation, toxicity of the test strain seemed to be negligible. Therefore, it can be concluded that LC-01 is likely to be non-pathogenic and safe for human consumption.

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References

Aguirre, M. and Collins, M.D. 1993. Lactic-acid bacteria and human clinical infection. *J. Appl. Bacteriol.* **75**, 95–107.

Bayer, A.S., Chow, A.W., Betts, D., and Guze, L.B. 1978. Lactobacillemia-eport of nine cases: important clinical and therapeutic considerations. *Am. J. Med.* **64**, 808–813.

Berg, R.D. and Garlington, A.W. 1979. Translocation of certain indigenous bacteria from the gastro-intestinal tract to the mesenteric lymph-nodes and other organs in a gnotobiotic mouse model. *Infect. Immun.* **23**, 403–411.

Biffi, A., Coradini, D., Larsen, R., Riva, L., and DiFronzo, G. 1997. Antiproliferative effect of fermented milk on the growth of a human breast cancer cell line. *Nutr. Cancer* **28**, 93–99.

Bover-Cid, S. and Holzapfel, W.H. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int. J. Food Microbiol.* **53**, 33–41.

Conway, P.L. 1996. Selection criteria for probiotic microorganisms. *Asia Pac. J. Clin. Nutr.* **5**, 10–14.

Donohue, D.C. and Salminen, S. 1996. Safety of probiotic bacteria. *Asia Pacific J. Clin. Nutr.* **5**, 25–28.

Jonit FAO/WHO. 2002. Guidelines for the evaluation of probiotics in food: report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food.

Fernandez de Palencia, P., Lopez, P., Corbi, A.L., Pelaez, C., and Requena, T. 2008. Probiotic strains: survival under simulated gastrointestinal conditions, in vitro adhesion to Caco-2 cells and effect on cytokine secretion. *Eur. Food Res. Technol.* **227**, 1475–1484.

Fuller, R. 1992. Probiotics, pp. 1–8. Chapman & Hall, Wisconsin, USA.

Furrie, E. 2005. Probiotics and allergy. *Proc. Nutr. Soc.* **64**, 465–469.

Gasser, F. 1994. Safety of lactic-acid bacteria and their occurrence in human clinical infections. *Bull. Inst. Pasteur* **92**, 45–67.

Gras, V., Bouffandleau, B., Montravers, P., and Lalau, J.D. 2006. Effect of metformin on survival rate in experimental sepsis. *Diabetes Metab.* **32**, 147–150.

Hale, P. and Hill, A. 1973. The recovery of *Lactobacillus* sp. from the livers of healthy mice. *Lab. Anim.* **7**, 119–124.

Isolauri, E., Juntunen, M., Rautanen, T., Sillanaukee, P., and Koivula, T. 1991. A human *Lactobacillus* strain (*Lactobacillus casei* sp. strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* **88**, 90–97.

Kim, H.S., Chae, H.S., Jeong, S.C., Ham, J.S., Im, S.K., Ahn, C.N., and Lee, J.M. 2005. Antioxidant activity of some yogurt starter cultures. *Asian-Australas. J. Anim. Sci.* **18**, 255–258.

Lee, K.C., Liu, C.F., Lin, T.H., and Pan, T.M. 2010. Safety and risk assessment of the genetically modified Lactococci on rats intestinal bacterial flora. *Int. J. Food Microbiol.* **142**, 164–169.

Li, J., Ren, F., Gu, H., Li, X., and Gan, B. 2011. Safety evaluation *in vitro* of *Enterococcus durans* from Tibetan traditional fermented yak milk. *J. Microbiol.* **49**, 721–728.

Liu, C.T., Chu, F.J., Chou, C.C., and Yu, R.C. 2011. Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from *Lactobacillus casei* 01. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **721**, 157–162.

Olivares, M., Diaz-Ropero, M.P., Gomez, N., Lara-Villoslada, F., Sierra, S., Maldonado, J.A., Martin, R., Rodriguez, J.M., and Xaus, J. 2006. The consumption of two new probiotic strains, *Lactobacillus gasseri* CECT 5714 and *Lactobacillus coryniformis* CECT 5711, boosts the immune system of healthy humans. *Int. Microbiol.* **9**, 47–52.

Ozdestan, O. and Uren, A. 2010. Biogenic amine content of kefir: a fermented dairy product. *Eur. Food Res. Technol.* **231**, 101–107.

Peran, L., Camuesco, D., Comalada, M., Nieto, A., Concha, A., Paz Diaz-Ropero, M., Olivares, M., Xaus, J., Zarzuelo, A., and Galvez, J. 2005. Preventative effects of a probiotic, *Lactobacillus salivarius* ssp. *salivarius*, in the TNBS model of rat colitis. *World J. Gastroenterol.* **11**, 5185–5192.

Priyadarshani, W.M.D. and Rakshit, S.K. 2011. Screening selected strains of probiotic lactic acid bacteria for their ability to produce biogenic amines (histamine and tyramine). *Int. J. Food Sci. Technol.* **46**, 2062–2069.

Rautio, M., Jousimies-Somer, H., Kauma, H., Pietarinen, I., Saxelin, M., Tynkkynen, S., and Koskela, M. 1999. Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. *Clin. Infect. Dis.* **28**, 1159–1160.

Saxelin, M. 1997. *Lactobacillus* GG - A human probiotic strain with thorough clinical documentation. *Food Rev. Int.* **13**, 293–313.

Shalaby, A.R. 1996. Significance of biogenic amines to food safety and human health. *Food Res. Int.* **29**, 675–690.

Shukla, S., Park, H.-K., Kim, J.-K., and Kim, M. 2010. Determination of biogenic amines in Korean traditional fermented soybean paste (Doenjang). *Food Chem. Toxicol.* **48**, 1191–1195.

Steffen, E.K. and Berg, R.D. 1983. Relationship between cecal population levels of indigenous bacteria and translocation to the mesenteric lymph nodes. *Infect. Immun.* **39**, 1252–1259.

Stine, K. and Brown, T. 1996. Principles of Toxicology, pp. 1–10. CRC Press, Boca Raton, FL, USA.

Swank, G.M. and Deitch, E.A. 1996. Role of the gut in multiple organ failure: Bacterial translocation and permeability changes. *World J. Surg.* **20**, 411–417.

Tassoni, A., Germana, M.A., and Bagni, N. 2004. Free and conjugated polyamine content in *Citrus sinensis* Osbeck, cultivar Brasiliano NL 92, a Navel orange, at different maturation stages. *Food Chem.* **87**, 537–541.

Wikler, M.A. 2009. Performance standards for antimicrobial susceptibility testing: fifteenth informational supplement. Clinical and Laboratory Standards Institute.

Zhou, J.S., Shu, Q., Rutherfurd, K.J., Prasad, J., Birtles, M.J., Gopal, P.K., and Gill, H.S. 2000. Safety assessment of potential probiotic lactic acid bacterial strains *Lactobacillus rhamnosus* HN001, *Lb. acidophilus* HN017, and *Bifidobacterium lactis* HN019 in BALB/c mice. *Int. J. Food Microbiol.* **56**, 87–96.