Effects of multistrain lactic acid bacteria with probiotic properties on enhancements of IgA, IgG levels and anti-Salmonella Typhimurium invasion activity

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Abstract

In our study, BALB/c mice were orally administered multistrain probiotics or sterile water for 28 consecutive days. The total serum IgA and IgG antibody levels were significantly higher in the group of mice administered probiotics (\(p < 0.05\)) than in the control group at the 4\textsuperscript{th} week. Consumption of multistrain probiotics for 28 days significantly increased (\(p < 0.05\)) the fecal populations of \textit{Bifidobacterium} spp. and \textit{Lactobacillus} spp. In another animal model, the mice were orally administered for 14 consecutive days. On the 7\textsuperscript{th} day, all the mice were orally challenged with \textit{Salmonella} Typhimurium ISM 50. The mice were sacrificed after 6 days of infection, \textit{Salmonella} counts in the spleen and liver of the mice administered probiotics significantly declined (\(p < 0.001\)) as compared to the control group. We demonstrated that the probiotic mixture could be used to enhance the host serum IgA and IgG levels and prevent \textit{Salmonella} infection.

Key words: Probiotics, immunoglobulin, \textit{Salmonella} invasion
1. Introduction

Salmonella enterica serovar Typhimurium (S. Typhimurium) is one of the causative agents of salmonellosis and an important pathogen in foodborne disease (Swaminathan et al., 2006). S. Typhimurium infection is caused by consumption of inadequately cooked eggs, poultry, and beef or by other interactive human-animal pathogens (Dechet et al., 2006). It is the leading cause of human gastroenteritis, and causes millions of infections and many deaths in the human population annually worldwide (McClelland et al., 2001).

S. Typhimurium is used in mouse models of human typhoid fever, and is a host generalist that occurs in humans and many other mammalian species (McClelland et al., 2001). Although it does not replicate in large numbers in the intestines of mice, it perforates the epithelial barrier by invasion of M cells, transport to dendritic cells, and penetration of enterocytes (Dicks et al., 2010). The cells colonize Peyer’s patches and mesenteric lymph nodes and spread to the liver and spleen, leading to systemic infection (Dicks et al., 2010). Mice that are orally administered S. Typhimurium are considered to serve as a reasonable reflection of S. Typhimurium-induced typhoid fever in humans (Kaufmann et al., 2001).

In recent years, the animal farming industry has initiated supplementing feed with antibiotics or drugs to prevent pathogenic infections in animals. Multi-drug resistant strains of S. Typhimurium are an important concern that should not be ignored (McDonald et al., 2001). However, various researchers have indicated that lactic acid bacteria (LAB) such as Lactobacillus, Bifidobacterium, Enterococcus, Lactococcus, and Streptococcus spp. may possess immune-enhancing properties or bacteriocin that can reduce the occurrence of infection by pathogens (Gill et al., 2001; Herich and Levkut, 2002; Cheikhyoussef et al., 2008; Simova et al., 2009). Lactobacillus spp. have been the most commonly studied organisms for their probiotic properties in controlling Salmonella infections (Tsai et al., 2005; Fayol-Messaoudi et al., 2007; Jain et al., 2008).

Lactic acid bacteria produce lactic acid as the major metabolic end-product of carbohydrate fermentation, and the resultant pH may be sufficiently low to inhibit the growth of other microorganisms including the most common human and animal pathogens (Salminen et al., 2004). Several studies on the mechanism of probiotics against gastrointestinal pathogens infection have been addressed in diverse patent applications, including modification of environmental conditions, competition with pathogens for nutrients and adhesion sites, production of antimicrobial metabolites, and modulation of the immune and non-immune host defense (Cummings and Macfarlane, 1991; Wolowski et al., 2001; Timmerman et al., 2004; Corr et al., 2007).

LAB play a very important role in modulating the human intestinal microflora and also exhibit anti-infection, anti-inflammatory, and antitumor effects (Jones and Versalovic, 2009). Studies have shown that some LAB strains such as Lactobacillus
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*Lactobacillus rhamnosus* GG, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus casei*, and *Lactobacillus reuteri* can enhance immunity and modulate the immune response (Schultz et al., 2003; Pathmakanthan et al., 2004; Bleau et al., 2007; Tsai et al., 2008; Ya et al., 2008; Jones and Versalovic, 2009). However, the probiotic effect of a single strain is limited to the strain-specific properties, and survival depends on the properties of 1 specific strain (Timmerman et al., 2004). Probiotics of different strains having different characteristics may be able to create an anaerobic probiotic niche that enhances colonization and adhesion of the surviving strains (Timmerman et al., 2004). In addition, the additive and synergistic effects of the probiotics are enhanced due to the combination of the specific properties of the different strains, such as colonization, biological activity, and health-promoting properties (Shihata and Shah, 2000; Ouwehand et al., 2000). For example, VSL#3 is a commercialized formulation composed of 8 different strains. Although the mechanism of action of the individual strains was not known, positive clinical outcomes were obtained when used in patients with inflammatory bowel disease (Bibiloni et al., 2005).

In this study, we evaluated 5 LAB strains for their acid/bile salt tolerance, adhering capability to the Caco-2 cells, and antimicrobial susceptibility against the growth of food pathogens in vitro. Then, these LAB strains were mixed and further evaluated for enhancement in the IgA and IgG levels, improvement in the intestinal microflora, and anti-Salmonella invasion activity in vivo. Thus, LAB strains with immunomodulation potential and the capability to prevent pathogenic infections in humans and animals can be obtained and combined for use as multistrain probiotics.

2. Materials and methods

2.1 Bacteria strains and their growth conditions

In this study, the following 5 LAB strains were used: *L. rhamnosus* NBM-01-07-001, *L. acidophilus* NBM-01-07-002, *L. plantarum* NBM-01-07-003, *Bifidobacterium longum* NBM-01-07-004, and *Enterococcus faecium* NBM-01-05-001 from Native Biomedicals Ltd. (Tainan, Taiwan). The stock culture collection within 25% glycerol was maintained at –80°C. LAB strains were propagated twice in *Lactobacilli* MRS broth (DIFCO, Maryland, USA) containing 0.05% L-cysteine each for 24 h at 37°C before experimental use.

The pathogenic strains used in this experiment were pathogenic *Escherichia coli* BCRC10675, *Salmonella enterica* serovar Typhimurium BCRC10747, and *Staphylococcus aureus* BCRC10908. The pathogenic strains were obtained from the Bioresource Collection and Research Center (BCRC), Hsin-Chu, Taiwan. *S. Typhimurium ISM50* isolated from a patient with food-borne diarrhea was obtained from the National Center for Disease Control (NCDC), Taipei, Taiwan. For cultivation of these bacteria, one loopful of the pathogenic culture suspension was inoculated into 5 ml of Luria-Bertani (LB)
broth (DIFCO, Maryland, USA) and incubated at 37°C for 8 h.

2.2 Acid and bile salt tolerance activity of LAB

Acid tolerance was estimated by comparing the counts of viable cells after incubation at pH 2.0 for 3 h (Conway et al., 1987). Culture suspension (1 ml) containing approximately 10⁹ CFU/ml LAB was transferred into 9 ml phosphate-buffered saline (PBS). The pH was adjusted to 2.0 using 0.1 N HCl, and the PBS was incubated at 37°C for 3 h. After incubation, the viable bacterial count was determined by preparing serial dilutions of the culture in PBS (pH 7.2) and plating on MRS agar. The plates were anaerobically incubated with anaerobic jar at 37°C for 48 h. Relative surviving percentage (%) = (Log₁₀ CFU/ml LAB at pH 2.0 for 3 h/Log₁₀ CFU/ml LAB at pH 2.0 for 0 h) × 100.

Tolerance to bile salts was tested by growing the LAB strains in MRS broth with and without bile salts (0.3% oxgall) incubated at 37°C, and monitored for growth at 0, 2, 4, 6, 8, 9, 10, 11, 12 and 15 h by measuring the absorbance at 620 nm (A₆₂₀ₙₐₜ) (Gilliland and Walker, 1990). The growth rate at the logarithmic phase of the bacterial growth curve was calculated. Bile tolerance percentage (%) = (growth rate of LAB in MRS broth with bile salt/growth rate of LAB in MRS broth without bile salt) × 100.

2.3 Antimicrobial activity assay

The antibacterial activity of LAB strains was studied using the agar diffusion test. Strains of LAB were grown 20 h in MRS broth at 37°C. The culture was centrifuged for 5 min at 5,000 × g and the supernatant was recovered. Pathogenic strains were grown in LB broth for 8 h and plated on nutrient agar (NA). Then, wells were hollowed out of the NA plates, each well was filled with 100 μl of untreated LAB supernatant, heat-treated supernatant (100°C, 15 min), and LAB supernatant with pH adjusted to 7.2, and the plates were incubated at 37°C for 14 h. The antimicrobial activity of each sample was determined by measuring the diameter of the clear zone surrounding the wells (Lin et al., 2008).

2.4 Adhesion of LAB to the Caco-2 cell lines

The human cell line epithelial-like Caco-2 was obtained from BCRC. Caco-2 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) (GIBCO) supplemented with 10% (v/v) fetal bovine serum and 1% penicillin-streptomycin (HyClone, Logan, Utah, USA). For the cell culture assays, cells were cultured in a 75-cm² plastic tissue culture flask (Nunc, Denmark). Caco-2 cells were used at the differentiated stage after 21 days of incubation (37°C) in an atmosphere containing 5% CO₂ and 95% air.

The Caco-2 cells were cultured in a 75-cm² plastic tissue culture flask. The cells were washed twice with PBS, and transferred (5 × 10⁸ cells/ml) with 0.05% trypsin to the 24-well plate containing fresh tissue culture medium without 1% penicillin-streptomycin. The mixture was incubated at 37°C in an atmosphere of 5% CO₂ and 95% air until a monolayer was formed in each well. Prior to
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The adhesion test, all the bacterial strains were washed twice with PBS and centrifuged for 5 min at 5,000 × g. Bacterial cells were resuspended in 1 ml DMEM. Then, 100 μl of the suspension was transferred to the 24-well multidish (1 × 10⁸ cfu/ml) containing the Caco-2 monolayer and incubated for 2 h at 37°C in 5% CO₂. The cells were then washed twice with PBS, fixed with 10% formalin for 30 min, washed 4 times with PBS, and stained with crystal violet for 5 min. The adhesion of LAB was determined according to the method of Gopal et al. (2001).

2.5 Preparation of the commercial synbiotic product

The components of the multistrain probiotics in the commercial product included *L. rhamnosus* NBM-01-07-001, *L. acidophilus* NBM-01-07-002, *L. plantarum* NBM-01-07-003, *Bifidobacterium longum* NBM-01-07-004 and *Enterococcus faecalis* NBM-01-05-001. The commercial product also comprising inulin, lactose, maltodextrin, malic acid, glucose, tricalcium phosphate, yeast extract, vitamin B group were used. The commercial product was freeze-dried from Native Biomedicals Ltd. (Tainan, Taiwan) and kept refrigerated at 4°C until tested.

2.6 Mice

Specific pathogen-free mice were used in this study. This animal research was approved by the Institutional Animal Care and Use Committee of HungKuang University, Taichung County, Taiwan (approval No. 96022). Male inbred strains of 8-week-old BALB/c mice weighing 20–22 g were purchased from the National Laboratory for Animal Breeding and Research Center, Taipei, Taiwan. These mice were raised at 20 ± 2°C with a relative humidity of 55 ± 5%, and a 12-h light cycle. Laboratory rodent diet 5001 (Manufacture by PMI Nutrition International Inc., Richmond, Indiana, USA) sterilized by gamma irradiation was used as the feed supplement. The mice were fed freely for 2 weeks, and then used for the study.

2.7 Feeding procedure and ELISA assays of IgA and IgG levels in the serum for animal

Before the animal model experiment (day 0), the mice were randomly divided into 2 groups (n = 10 per group). They were fed a single 0.2-ml dose of a known concentration of multistrain probiotic (3 g dissolved in 20 ml deionized water, 1 × 10⁸ CFU/ml) daily for 4 consecutive weeks. The feed intake and body weight of each mouse was measured weekly. On days 0, 7, 14, and 21, blood samples were obtained from the ophthalmic veins of each mouse for measuring the serum IgA and IgG levels. On day 28, blood samples were collected by cardiac puncture and the sera were stored at −80°C until measurement of the antibody levels.

Cytokines were measured by Mouse IgA (catalog No.E90-103, Bethyl Laboratories, Inc., Montgomery, USA) and IgG (catalog No.E90-131, Bethyl Laboratories, Inc., Montgomery, USA) ELISA Quantization Kit according to the manufacturer’s instructions. Then, 96-well Immuno-Maxisorp plates (Nunc, Roskilde, Denmark) were coated with monoclonal
antibodies for total IgA or IgG, and placed in an incubating buffer overnight at 4°C. The plates were blocked and washed. Sera were added to the plates and incubated for 2 hours at room temperature. The plates were washed again, and biotinylated anti-mouse IgA and IgG along with horseradish peroxidase (HRP)-conjugated streptavidin were added for the detection of IgA or IgG, respectively, and incubated 1 hour at room temperature. The reactions were developed using 3,3′,5,5′-tetramethylbenzidine (TMB) substrate for 30 min at room temperature. The color reactions were stopped using 2N H2SO4 and absorbance was measured at 450 nm. Equivalent levels of IgA or IgG were calculated by comparing the levels with a reference curve generated using IgA and IgG standards. Results were expressed as the concentration of each cytokine in serum (μg/ml) (Tsai et al., 2008).

2.8 Determination of Bifidobacterium and Lactobacillus numbers in fecal bacterial flora

The improvement microflora in the intestine study was conducted in accordance with the guidelines for health food improvement and evaluation of gastrointestinal function from the Department of Health, Executive Yuan, R.O.C. (Taiwan). On days 0, 7, 14, 21, and 28, fecal samples were collected from each mouse for the determination of fecal bacterial flora. Probiotic microorganisms were isolated from feces using the media and methods developed by Liu et al. (2006). The counts of probiotic microorganisms in feces were determined at the 0, 2nd, and 4th week. Approximately 0.5 g (wet weight) of feces was suspended in 4.5 ml anaerobic solution [Gelatin 0.2 g; Distilled water 50 ml; Salts solution (MgSO4·7H2O 10 g, FeSO4·7H2O 0.5 g, MnSO4·2H2O 0.4 g, NaCl 0.5 g, Distilled water 250 ml) 50 ml; Resazurin solution (25 mg/100 ml H2O) 0.4 ml; 0.05 g cysteine], and serially diluted to obtain different concentrations in an Anaerobic Workstation (Forma Anaerobic System, Thermo, USA). After the serial dilutions, 1 ml aliquots were spread onto Bifidobacterium iodoacetate medium-25 (BIM-25) agar for Bifidobacterium spp. and MRS agar with bromocresol green for Lactobacillus spp. The plates were incubated under anaerobic conditions at 37°C for 48 h. The results were expressed as log10 CFU per gram of feces (wet weight).

2.9 Feeding procedure and challenge Salmonella invasion

Mice used for study were male BALB/c mice as described earlier. Each experimental group consisted of 10 mice. The methods put forth by to Hudault et al. (1997) were used with some modifications. Mice were fed with a single 0.2-ml dose of a known concentration of a multistrain probiotic (3 g dissolved in 20 ml deionized water, 1 × 1010 CFU/ml) daily for 7 consecutive days. The 2 mice groups were fed multistrain probiotics and deionized water. On the 8th day, each mouse was challenged with S. Typhimurium ISM 50 by oral administration of a single 0.2-ml dose of culture (1 × 106 CFU/ml).

Viable bacteria counts in the liver and spleen of mice were determined 6 d after the Salmonella
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The mice were sacrificed by cervical dislocation at different time intervals. The spleens and livers were removed aseptically. These organs were mixed with demineralized water to achieve a final volume of 5 ml and then homogenized. Cell suspensions were serially diluted with PBS, and portions were plated on Brilliant Green Agar (DIFCO, Maryland, USA). After incubation for 48 h at 37°C, colonies were enumerated. The results were expressed as logarithmic colony-forming units (log10 CFU) for the liver or spleen.

2.10 Statistical analysis

Data were expressed as mean ± SEM and the statistical significance comparisons were conducted by one-way analysis of variance (ANOVA) followed by Duncan’s New Multiple Range Test using Statistical Package for the Social Sciences (SPSS) 12.0. Statistical significance is considered as p < 0.05.

3. Results

3.1 Acid and bile salt tolerance ability

In order to determine the stability of live LAB strains in the gastrointestinal tract, we evaluated their survival in PBS buffer after 3 h of treatment at pH 2.0 or in MRS broth containing 0.3% oxgall. The results revealed that the survival percentages of L. acidophilus NBM-01-07-002, L. plantarum NBM-01-07-003, L. rhamnosus NBM-01-07-001, B. longum NBM-01-07-004, and E. faecium NMB-01-05-001 were 81, 74, 79, 83, and 72%, respectively (Table 1). The percentages of survival of these organisms in the presence of bile salts were 84, 84, 87, 87, and 83%, respectively (Table 1).

3.2 Adhesion assay and antagonistic pathogen in vitro

We studied 5 LAB strains for their ability to adhere to the human colon cell line Caco-2 (Table 1). E. faecium NBM-01-05-001 and L. plantarum NBM-01-07-003 expressed high levels of adherence to Caco-2 (>200 cfu/10 cells). L. acidophilus NBM-01-07-002 and B. longum NBM-01-07-004 showed medium adherence of 100–200 cfu/10 Caco-2 cells. L. rhamnosus NBM-01-07-001 showed the lowest adhesion ability among all the tested strains, at below 100 cfu/10 Caco-2 cells. E. coli BCRC10675, S. Typhimurium BCRC10747, and S. aureus BCRC10908 were used as indicators, and the supernatant of the 5 tested LAB strains were found to inhibit (+) the growth of these pathogens (Table 2). Diameters of the inhibition zones formed by these LAB strains ranged from 12 (+) to 17 (+ +) mm. After heating (100°C, 15 min), E. faecium NBM-01-05-001 lost the antagonistic activity against these pathogenic bacteria, but the inhibitory activity of L. acidophilus NBM-01-07-002 against the pathogens increased, as evidenced by the increase in the diameter of the zone of inhibition. When the pH of the supernatant was neutralized to 7.2, the antagonistic activity of all tested LAB against the pathogens was lost. It appears that in addition to pH, lactic acid or other unknown substrates against these pathogenic bacteria might be produced by five LAB strains.

3.3 Measurement of antibody levels of IgA
and IgG levels in serum of BALB/c mice

The probiotics that were orally administered to the mice stimulated the immune response in vivo (Fig 1A and 1B). The levels of total serum IgA antibodies were higher in comparison with the control group at the 2nd and 4th week (Fig 1A). The total serum IgG antibody level was also higher in the group administered probiotics than in the control group at 4 weeks (Fig 1B).

3.4 Effect on Fecal bacterial flora

Table 3 presents the results of the fecal microbial analysis. Consumption of probiotics for 28 days significantly increased the fecal population of Bifidobacterium spp. and Lactobacillus spp. ($p < 0.05$). No significant differences in the body weight were observed between the different groups throughout the experiment (data not shown).

3.5 LAB against the invasion of salmonellae to mouse liver and spleen

Statistical analysis revealed that the number of Salmonella in the spleen and liver of the mice fed with probiotics significantly declined ($p < 0.001$) as compared to the corresponding counts in the sterile water control group (Fig. 2A and 2B). Comparison of the results obtained on day 3 and day 6 after the Salmonella challenge revealed that the count of Salmonella in the liver and spleen of mice increased by 3 logarithmic units. Under such conditions, administration of probiotics resulted in a significant antagonistic effect against Salmonella invasion (Fig. 2A and 2B).

4. Discussion

Several mechanisms of LAB have been suggested to inhibit Salmonella invasion. For instance, some metabolic products such as lactic acid and/or bacteriocins produced by LAB may inhibit the growth of pathogenic bacteria (Hudault et al., 1997; Forestier et al., 2001). Some other LAB, such as L. acidophilus strain La1 and B. bifidum strain Bb12, may enhance most of the systematic immune response (Schiffrin et al., 1997). Furthermore, LAB strains that maintain adhesive properties and the ability to colonize the human gastrointestinal tract may hinder the association or invasion between the epithelial cells and the pathogenic bacteria (Hudault et al., 1997; Coconnier et al., 2000; Jankowska et al., 2008). In this study, the adhesion of these 5 LAB strains to colonize the Caco-2 cells may result in the competitive exclusion of adhesion of S. Typhimurium in vivo. This can be explained by the consideration that the entry of Salmonella into a given environment can be prevented if the space is already occupied by probiotic organisms that are better suited to establishing and maintaining themselves in the environment or those that excrete substances that inhibit the growth of Salmonella. In our study, consumption of synbiotic substances such as probiotics, inulin, and maltodextrin for 28 days significantly increased the fecal populations of Bifidobacterium spp. and Lactobacillus spp. in mice. The enhanced growth of probiotic strains may correspond to increased specific bacterial activities, leading to higher antagonistic effects against Salmonella and reduction of intestinal pathogen colonization (Vesterlund et al., 2005). Rishi et al. (2009) found that probiotic L.
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*Probiotic microorganisms* of the gastrointestinal tract stimulate the immune system to respond rapidly to infection with pathogens and antagonize the colonization of harmful or pathogenic bacteria in the intestine (Cebra et al., 1999). IgA plays a critical role in mucosal immunity. In the blood, IgA interacts with an Fc receptor called FcαRI (or CD89), which is expressed on immune effector cells to initiate inflammatory reactions. IgG antibodies are predominantly involved in the secondary antibody response; it can bind to many kinds of pathogens and protects the body against them. The entry of the antigens by the oral route is essential to induce the immune response. This fact was determined in germfree mice receiving an antigen-free diet where it was demonstrated that the serum IgA and IgG levels were diminished (Wostmann and Pleasants, 1991). They concluded that IgA levels depend primarily on the presence of microflora, whereas in the case of IgG levels, diet is the more important factor.

The role played by LAB in various host biological functions has been extensively studied. Frece et al. (2009) indicated that synbiotics enhanced LAB counts and the total serum IgA antibodies in mice. According to Herfás et al. (Herfás et al., 1999), rats colonized with *L. plantarum* had significantly higher total serum IgA antibody levels against *E. coli* than those colonized with *E. coli* alone. Some investigations discovered that the total serum IgG level increased after administration of lactobacilli to Balb/c mice (Tsai et al., 2008; Ya et al., 2008). Jain et al. (2008) demonstrated that pre-feeding of mice with probiotic *L. acidophilus* and *L. casei* for 7 days was more effective than pre-feeding for only 2 days for protection against *S. enteritidis* infection in mice. Therefore, in our study, the mice were fed multistrain probiotics daily for 7 consecutive days before the *S. Typhimurium* challenge. Numerous studies have demonstrated that LAB can prevent intestinal infection; these results imply the active participation of the immune system.

In conclusion, results from our *in vitro* and *in vivo* analyses suggested that the 5 LAB strains studied exhibited properties of acid/bile salt tolerance and adhering capability to the Caco-2 cells and were suitable to serve as probiotic microorganisms. It is likely that these probiotics compete with *S. Typhimurium* for adhesion to intestinal epithelial cells or displace these pathogenic cells from the gastrointestinal tract. The multiple strains of LAB in combination with prebiotic and probiotic properties would increase the probiotic microflora in the intestine, enhance the IgA and IgG levels, and provide protection against *S. Typhimurium*-induced liver and spleen damage. Further investigation is required to determine the mechanisms by which the interaction of the products regulates intestinal immune responses.

**Acknowledgements**

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References


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Kaufmann, S. H., Raupach, B., and Finlay, B.


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Table 1. Adhesion properties, acid tolerance and bile salts tolerance of five lactic acid bacteria strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Adherence to the cells</th>
<th>Acid tolerance</th>
<th>Bile salts tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caco-2 (CFU/10 cells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em> NBM-01-07-002</td>
<td>198 ± 21</td>
<td>81%</td>
<td>84%</td>
</tr>
<tr>
<td><em>L. plantarum</em> NBM-01-07-003</td>
<td>211 ± 11</td>
<td>74%</td>
<td>84%</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> NBM-01-07-001</td>
<td>42 ± 23</td>
<td>79%</td>
<td>87%</td>
</tr>
<tr>
<td><em>B. longum</em> NBM-01-07-004</td>
<td>106 ± 6</td>
<td>83%</td>
<td>87%</td>
</tr>
<tr>
<td><em>E. faecium</em> NBM-01-05-001</td>
<td>321 ± 13</td>
<td>72%</td>
<td>83%</td>
</tr>
</tbody>
</table>

*Each adhesion assay was conducted in duplicate with cells from three successive passages. The table presents mean numbers ± standard deviation of bacteria adhering per 10 epithelial cells.

Table 3. Total counts of *Bifidobacterium* and *Lactobacillus* in feces of mice after oral administration of multistrain probiotics.

<table>
<thead>
<tr>
<th></th>
<th>Bacterial counts (log CFU per g feces)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Bifidobacterium</em> spp.</td>
</tr>
<tr>
<td>Groups</td>
<td>Week 2</td>
</tr>
<tr>
<td>Control</td>
<td>8.51 ± 0.04^a</td>
</tr>
<tr>
<td>Probiotic</td>
<td>8.18 ± 0.15^c</td>
</tr>
</tbody>
</table>

†Data are expressed as means ± S.E.M. (n=7).

a,b Different letters indicate significant difference for each column (p<0.05) by one-way ANOVA.
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Table 2. Antimicrobial activity of supernatant of five lactic acid bacteria strains against *Escherichia coli*,

*Salmonella Typhimurium and Staphylococcus aureus.*

<table>
<thead>
<tr>
<th>Strains</th>
<th>Zones of inhibition (mm)</th>
<th>E. coli BCRC 10675</th>
<th>S. Typhimurium BCRC 10747</th>
<th>St. aureus BCRC 10908</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treatment of supernatant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em> NBM-01-07-002</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em> NBM-01-07-003</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em> NBM-01-07-001</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>B. longum</em> NBM-01-07-004</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>E. faecium</em> NBM-01-05-001</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Heat (100°C, 15 min) treatment of supernatant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em> NBM-01-07-002</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>L. plantarum</em> NBM-01-07-003</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em> NBM-01-07-001</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>B. longum</em> NBM-01-07-004</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. faecium</em> NBM-01-05-001</td>
<td>-</td>
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<td>Supernatant was adjusted to pH 7.2</td>
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<td><em>L. acidophilus</em> NBM-01-07-002</td>
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<td><em>L. plantarum</em> NBM-01-07-003</td>
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<td><em>L. rhamnosus</em> NBM-01-07-001</td>
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<td><em>B. longum</em> NBM-01-07-004</td>
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<td><em>E. faecium</em> NBM-01-05-001</td>
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*Interpretation of zone diameter of inhibition. −, <11 mm; +, 11 ~ 16 mm and ++, 17 ~ 22 mm.*
Fig. 1: Effect of probiotic administration on production of (A) IgA and (B) IgG in serum of BALB/c mice. Data are expressed as means ± S.E.M. (n=7). Mean value with *** was significantly different from control (p<0.001) by one-way ANOVA.
Fig. 2: *Salmonella* Typhimurium ISM50 cells detected in the (A) liver and (B) spleen of the mice which were administrated daily with probiotic product or sterilized demineralized water (control) for 7 consecutive days challenge to mice. Mean value with *** was significantly different from control (*p*<0.001) by one-way ANOVA.
具益生菌特性之多重乳酸菌對提昇IgA, IgG和抗沙門氏菌侵入活性之影響

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摘要

本研究連續28天管餵BALB/c小鼠多重有益菌或無菌水。在第4週，口服有益菌的小鼠，其血清中免疫球蛋白總IgA和IgG抗體濃度顯著高於控制組(p<0.05)。餵食多重有益菌28天後，可明顯增加糞便中雙歧桿菌及乳酸桿菌之菌量(p<0.05)。另一個動物模型為連續餵食小鼠14天。在第7天給予所有小鼠鼠傷寒沙門氏菌ISM50。為期6天的感染後犧牲小鼠，發現餵食過有益菌的小鼠，其脾臟和肝臟中之沙門氏菌菌數與控制組相較有顯著下降(p<0.001)。我們證明多重有益菌可以提高老鼠血清IgA和IgG的含量，並預防沙門氏菌之感染。

關鍵詞：有益菌，免疫球蛋白，沙門氏菌侵入