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Fermentative Capacity of Three Strains of *Lactobacillus* Using Different Sources of Carbohydrates: *In Vitro* Evaluation of Synbiotic Effects, Resistance and Tolerance to Bile and Gastric Juices

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Abstract

A fermentation study of three probiotic *Lactobacillus* strains was conducted on individual carbohydrates including glucose (GLU) high methoxy pectin (HMP), sugar beet pectin (SBP), fructooligosaccharide (FOS), galactooligosaccharide (GOS), and inulin agave (IA) as the sole carbon sources. It was observed that *Lactobacillus bulgaricus* (LB), *Lactobacillus casei* (LC) and *Lactobacillus delbruckii* (LD) achieved the highest growth rates when they were grown in the presence of GLU, FOS, and IA, but LB had a slower growth rate in these substrates compared to LC and LD. Only LC had a statistically significantly higher growth rate in GOS than in the basal medium which contained no carbohydrate source. Exposure to bile caused a significant reduction of log colony forming units/ml of all 3 strains, with LD grown in HMP exhibiting the highest survival followed by LC and LD grown in GLU, and LD grown on IA. Although HMP was not fermented by the test organisms, results indicate that HMP may in fact help certain probiotic bacteria to survive exposure to bile. Exposure to simulated gastric juices indicated that the studied *Lactobacilli* are tolerant to simulated gastric juice.

Keywords: *Lactobacillus* spp, probiotic, prebiotic, synbiotic

1. Introduction

1.1 Role of Probiotics in Human Health

The human gastrointestinal (GI) tract contains a variety of microflora including both pathogenic and beneficial bacteria (Manning & Gibson, 2004). Harmful bacteria in the GI tract may cause health problems in the host such as diarrhea, infections, or liver damage, and these bacteria may produce substances that increase the risk of cancer (Mitsuoka, 1982; Salminen et al., 1998). However, beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* can promote host health by inhibiting the growth of these harmful bacteria, stimulating the immune system, and enhancing the digestion and absorption of essential nutrients such as short chain fatty acids (Gibson & Roberfroid, 1995). Certain species of *Lactobacillus* and *Bifidobacteria* have been used as probiotic supplements in food products (Goldin & Gorbach, 1992) to provide better microbial balances in the human GI tract and consequently improve host health (Fuller, 1989). Probiotics are defined as live microorganisms which can improve host health when they are effectively administered. However, probiotic applications are limited because these bacteria are likely to be in a stressed state when they reach the colon due to exposure to diverse barriers in the host such as gastric acid and bile acids (Pochart et al., 1992, Gibson & Roberfroid, 1995). Probiotics should be able to recover and compete with established microflora in the colon for nutrients and colonization sites (Gibson & Roberfroid, 1995).

1.2 Prebiotics and Synbiotics

Hernandez-Hernandez et al. (2012) have shown that prebiotic carbohydrates can enhance the survival of beneficial probiotic bacteria during exposure to gastric conditions. Prebiotics are food ingredients which are indigestible in the human upper GI tract and reach the colon to positively influence the host's health by

selectively promoting the growth and/or activity of certain beneficial bacteria in the colon (Gibson & Roberfroid, 1995). Probiotics and their suitable prebiotics can be designed into food products to improve metabolic activities and stimulate the growth of the probiotic bacteria through their synergistic effects (Zanoni et al., 2008). Prebiotics are normally commercially extracted from fruits and vegetables through methods such as enzymatic hydrolysis of polysaccharides from dietary fibers or starch. Potential prebiotic carbohydrates include fructooligosaccharides (FOS), galactooligosaccharide (GOS), lactulose, lactosucrose, soybean oligosaccharides, isomaltooligosaccharides, palatinose, xylooligosaccharides, and glucooligosaccharides (Gibson & others, 2000; Manning & Gibson, 2004). Pectins are water soluble dietary fibers that also reach the colon (Englyst & Cummings, 1987) and are subsequently fermented by intestinal microbiota but do not necessarily function specifically as prebiotics (Olano-Martin, Gibson, & Rastell, 2002). However, pectins have been reported to be able to reduce serum cholesterol in both rats and humans (Gulfi, Arrigoni, & Amadò, 2005) and are thought to have anti-carcinogenic effects (Scheppach, Bartram, & Richter, 1995). Olano-Martin, Williams, Gibson, and Rastall (2003) have shown that high methoxy (HM) pectin, low methoxy (LM) pectin, and their derived oligosaccharides could help mitigate the toxicity of Shiga-like toxins of *Escherichia coli* O157:H7 against the HT29 cell line of human colonic cells. A study on the fermentation properties of inulin agave (IA) was conducted by Gomez, Tuohy, Gibson, Klinder, and Costabile (2010) and they found an increase in the growth of *Bifidobacteria* and *Lactobacilli* in fecal samples which suggests that inulin derived from Agave plants has a potential prebiotic effect. Using prebiotics and probiotics in combination is often described as a synbiotic, although the United Nations Food & Agriculture Organization (FAO) recommends that the term synbiotic be used only if the net health benefit is synergistic, or that the prebiotic be shown to increase the population and/or function of the probiotic it is paired with (Pineiro et al., 2008).

1.3 Hypothesis and Research Design

In this study we investigated the fermentability of fructooligosaccharides (FOS), galactooligosaccharides (GOS), inulin agave (IA), high methoxy pectin (HMP), and sugar beet pectin (SBP) by three probiotic strains for possible use as suitable prebiotics for potential application as synbiotic mixtures. In addition, we examined the tolerance of the three probiotic strains grown in the presence of different test carbohydrates to bile solution and simulated gastric juices.

2. Method

2.1 Carbohydrate Sources

High methoxy pectin (HMP), low methoxy pectin (LMP) and inulin agave (IA) were obtained from TIC Gums (Maryland, USA). Sugar beet pectin (SBP) was obtained from Herbstreith & Fox KG, Neuenbürg, Germany. Fructooligosaccharides (FOS; FortiFeed, Golden, CO, USA) and galactooligosaccharides (GOS; GTC Nutrition, Golden, CO, USA) were also studied and glucose (GLU; Sigma, USA) was used as a control.

2.2 Bacterial Strains

Lactobacillus bulgaricus ATCC 7517 (LB), *Lactobacillus casei* ATCC 11578 (LC), and *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797 (LD) were selected as probiotic bacteria from the culture collection of the Center for Food Safety, University of Arkansas, Fayetteville, AR, USA. Cultures were stored at -80°C in de Man, Rogosa and Sharpe (MRS) broth (EMD Chemicals, Gibbstown, NJ) supplemented with 20% glycerol. Bacterial strains were each grown independently in MRS broth for 24 h then sub-cultured in fresh BHI broth for 18 h at 37°C before use.

2.3 Growth Experiments

Basal MRS medium supplemented with different carbohydrates was used throughout this fermentation study. The basal MRS medium was prepared by mixing proteose peptone (10 g/L), beef extract (10 g/L), yeast extract (5 g/L), polysorbate 80 (1 g/L), ammonium citrate (2 g/L), sodium acetate (5 g/L) , magnesium sulfate (0.1 g/L), manganese sulfate (0.05 g/L), dipotassium sulfate (2 g/L), and cysteine-HCl (0.05 g/L) in deionized (DI) water and autoclaving at 121^{o}C for 15 min. Carbohydrate sources GLU, FOS, GOS, and IA were dissolved in DI water and filter sterilized by passing through 0.45 µm filters (Carrigtwohill, Co., Cork, Ireland). The HMP, LMP and SBP were dissolved slowly in DI water containing 0.00125 g/L of sodium azide (SA; J.T. Baker Chemical Co., New Jersey, USA) at 55^{o}C while stirring constantly. Carbohydrate solutions were added to the autoclaved basal MRS to the final concentration of 10 g/L and then the pH was adjusted to 6.5 with 0.5 N NaOH or HCl (Fisher Scientific, Fair Lawn, NJ, USA).

All experiments were conducted at 37°C under anaerobic conditions created by flushing argon gas into anaerobic boxes (Mitsubishi Gas Chemical Co., Japan). Overnight cultures (10 ml) were mixed by vortexing for 10 min to disrupt the chains of *Lactobacillus* bacterial cells, and subsequently washed and resuspended in sterile phosphate

buffered saline (PBS) to the original volume. An aliquot of 100 µl of the resuspended culture was inoculated into fresh basal MRS medium containing different carbohydrates and mixed by vortexing; 250 µl of the inoculated medium was placed in each well of a 96-well plate. The growth of bacteria was observed turbidometrically by a microtiter plate reader (Tecan Group Ltd, GrÖdig, Austria) for 48 h. Cultures were diluted and plated for total counts on MRS agar (MRSA; EMD Chemicals, Gibbstown, NJ) with the plates being incubated anaerobically at 37°C for 48 h; pH measurements were taken at time intervals of 0, 6, 11, 14, 24, and 48 h. The specific growth rate (µ) was calculated over the exponential phase of the growth curve by fitting the linear line using Microsoft excel 2010 (Ricke & Schaefer, 1991). Basal MRS medium without any added carbohydrates was included as a negative control in every experiment. Experiments were conducted with duplicate samples and repeated 3 times.

2.4 Effect of Carbohydrate on Probiotic Response to Gastrointestinal Stress

Simulated gastric juices (SGJ) and bile solution were prepared daily before each experiment. The SGJ were prepared by suspending pepsin from porcine stomach mucosa (Sigma, St. Louis, MO, USA) in sterile saline (0.5% w/v) to a final concentration of 0.22%, adjusting pH to 2.0 with HCl and subsequently filtering sterile through 0.45 µm syringe filters (Carrigtwohill, Co., Cork, Ireland) (Charteris, Kelly, Morelli, & Collins, 1998). Bile solution was prepared by dissolving porcine bile extract (Sigma, St. Louis, Missouri, USA) in DI water to a final concentration of 0.33% (Lian, Hsiao, & Chou, 2003) and filtering sterile through 0.22 µm syringe filters (VWR International, USA).

The methods described by Charteris et al. (1998), Lian et al. (2003), and Hernandez-Hernandez et al. (2012) were adopted with some modification for this study. Briefly, probiotic bacteria were grown anaerobically at 37°C in basal MRS medium containing the different respective carbohydrates for 48 h. The cultures were subsequently subjected to vortexing for 10 min to disrupt the *Lactobacillus* chains. One ml aliquots of these cultures were centrifuged at 8,000 rpm for 5 min (Brinkmann Centrifuge 5415C Eppendorf, Westbury, New York, USA), washed two times with PBS and resuspended in PBS to the same volume of 1 ml. An aliquot of 0.1 ml resupended cells was mixed with 0.9 ml of SGJ or bile solution, and incubated anaerobically at 37°C in an anaerobic box flushed with argon gas. The 0 min samples were taken immediately after mixing the cells with bile solution or SGJ. Samples were subsequently taken at time intervals of 30, 90 and 180 min for the bile solution experiment. For the SGJ experiment, the samples were taken only at 0, 120 and 240 min. All samples were plated on MRSA and incubated anaerobically at 37°C for 48 h; blank medium was included in every experiment as a negative control. This study was conducted with duplicate samples and repeated 3 times.

2.5 Statistical Analysis

Tukey honestly significant difference (HSD) was used to determine whether there were any significant differences between means of growth rate and viable counts in the study of kinetic growth and gastric conditions; for the gastric experiment, delta log cfu/ml was calculated by subtracting the final log cfu/ml from log cfu/ml at 0 min of contact with bile solution (3 h) or SGJ (4 h). All statistical analyses were performed using JMP package program ver. 9.0.0 (SAS Institute Inc, NC, USA), and the differences were considered significant if $P < 0.05$.

3. Results

3.1 Growth Kinetics of LB, LC and LD in Different Carbohydrate Substrates

Table 1. Specific growth rates of *Lactobacillus bulgaricus* (LB), *Lactobacillus casei* (LC), and *Lactobacillus delbrueckii* subsp. *lactis* (LD) utilizing different carbohydrates in basal MRS medium

Substrate	Specific growth rates		
	LB	LC	LD
GLU (control)	0.25 ± 0.01^{bA}	0.31 ± 0.01^{aA}	0.32 ± 0.01^{aA}
FOS	0.18 ± 0.01^{cB}	0.30 ± 0.01^{aA}	0.27 ± 0.02^{bB}
GOS	0.04 ± 0.01^{bC}	0.13 ± 0.02^{aB}	0.12 ± 0.02^{aC}
IA	0.18 ± 0.01^{bB}	0.25 ± 0.03^{aA}	0.24 ± 0.00^{aB}
HMP	0.05 ± 0.01^{aC}	0.06 ± 0.02^{aC}	0.03 ± 0.01^{aE}
SBP	0.05 ± 0.02^{bC}	0.05 ± 0.01^{bC}	0.07 ± 0.00^{aD}
Basal medium	0.04 ± 0.00^{bC}	0.06 ± 0.00^{aC}	0.06 ± 0.01^{aD}

Different lowercase letters indicate significant differences ($P \leq 0.05$) within rows, different uppercase letters indicate significant differences within columns. GLU, glucose; FOS, fructooligosaccharide, GOS, galactooligosaccharide; IA, inulin agave; HMP, high methoxy pectin; SBP, sugar beet pectin; WO, basal MRS media.

The maximum growth rates of each probiotic strain in different carbohydrate substrates are shown in Table 1. It was observed that both all strains achieved the highest growth rates when they were grown in the presence of GLU, FOS, and IA, although LB had a slower growth rate in these substrates compared to LC and LD. Only LC when grown in the presence of GOS had a statistically significantly higher growth rate than in the basal MRS that contained no carbohydrate source.

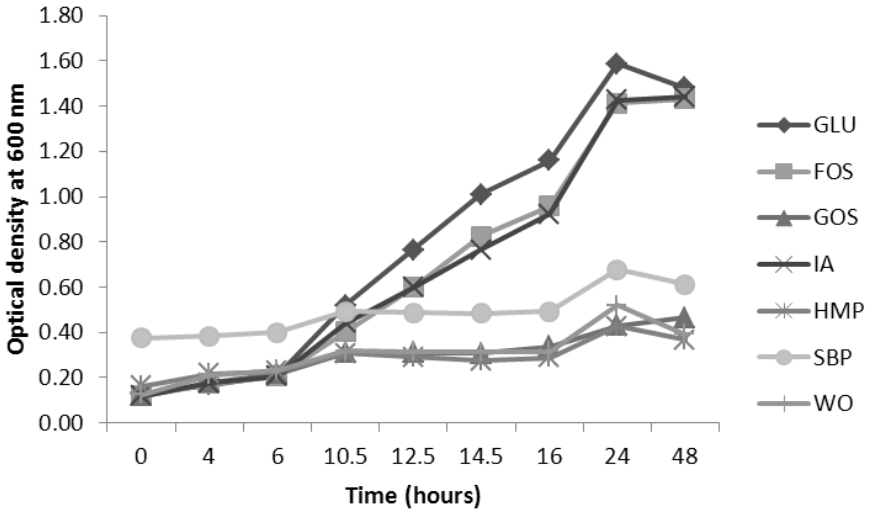

Figure 1. Growth of *Lactobacillus bulgaricus* based on optical density in medium containing different carbohydrates. GLU, glucose; FOS, fructooligosaccharide, GOS, galactooligosaccharide; IA, inulin agave; HMP, high methoxy pectin; SBP, sugar beet pectin; WO, basal MRS medium with no carbohydrate

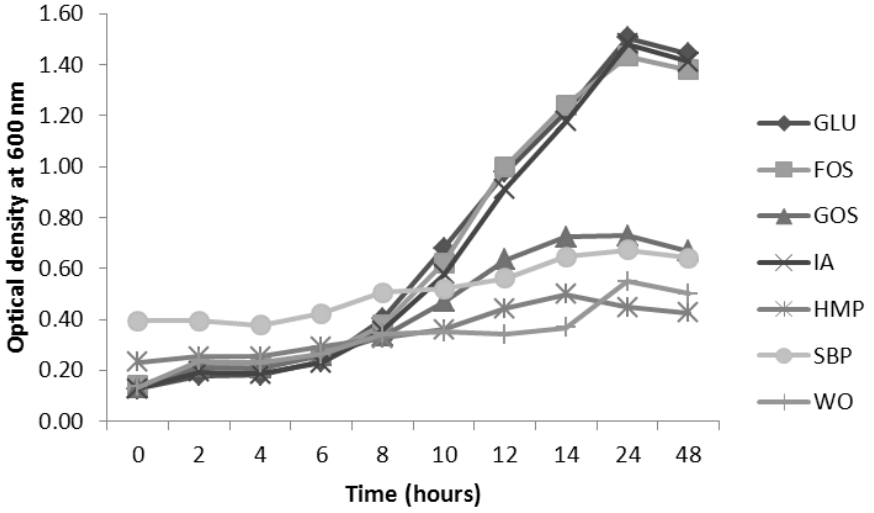

Figure 2. Growth of *Lactobacillus casei* based on optical density in medium containing different carbohydrates. GLU, glucose; FOS, fructooligosaccharide, GOS, galactooligosaccharide; IA, inulin agave; HMP, high methoxy pectin; SBP, sugar beet pectin; WO, basal MRS medium with no carbohydrate

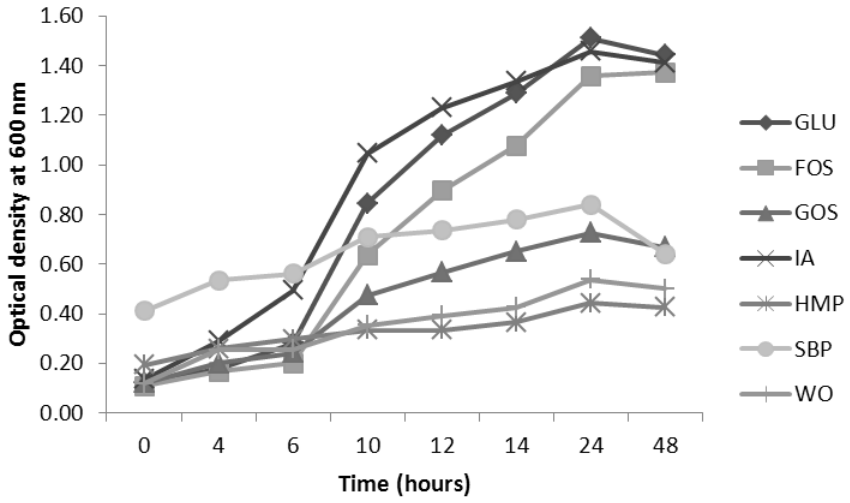

Figure 3. Growth of *Lactobacillus delbrueckii* subsp. *lactis* based optical density in medium containing different prebiotic carbohydrates. GLU, glucose; FOS, fructooligosaccharide, GOS, galactooligosaccharide; IA, inulin agave; HMP, high methoxy pectin; SBP, sugar beet pectin; WO, basal MRS medium with no carbohydrate

Figures 1 through 3 graphically illustrate the growth of the three strains on all carbohydrate sources; all strains exhibited a typical growth curve when grown in GLU, FOS or IA, and LC increased slightly when grown in GOS.

3.2 Survival of LB, LC and LD in Bile Solution and Simulated Gastric Juice

The survival of probiotic strains grown in the presence of different carbohydrates when exposed to bile solution is shown in Table 2. The number of viable cells for each treatment decreased over time. A comparison based on change in log cfu/ml after 3 hours shows that LD grown in HMP exhibited the highest survival followed by LC and LD grown in GLU, and LD grown on IA. The highest reduction of bacteria after exposure to bile solution is found with all strains grown in GOS and with LD in GLU. Table 3 contains the results of the SGJ study. The SGJ did not markedly decrease survival of LB or LD grown in any carbohydrate. However, LC when grown in FOS survived better and when grown in GOS this strain actually grew.

Table 2. Change in log cfu/ml of *Lactobacillus bulgaricus* (LB), *Lactobacillus casei* (LC), and *Lactobacillus delbrueckii* subsp. *lactis* (LD) grown in different carbohydrates and subjected to simulated bile for 3 hours

	LB	LC	LD
GLU (control)	2.50 ± 0.59^{aA}	2.23 ± 0.69^{aB}	ND
FOS	3.13 ± 0.25^{aA}	3.29 ± 0.51^{aAB}	4.05 ± 0.66^{aA}
GOS	3.83 ± 1.05^{aA}	4.79 ± 1.19^{aA}	ND
IA	3.19 ± 0.42^{abA}	3.55 ± 0.21^{aA}	2.90 ± 0.21^{bB}
HMP	3.76 ± 0.50^{aA}	3.48 ± 0.62^{aAB}	1.34 ± 0.43^{bC}

Different lowercase letters indicate significant differences ($P \leq 0.05$) in rows and different uppercase letters indicate significant differences in columns of delta log cfu/ml for treatments GLU, glucose; FOS, fructooligosaccharide, GOS, galactooligosaccharide; IA, inulin agave; HMP, high methoxy pectin. ND = not determined.

Table 3. Change in log cfu/ml of *Lactobacillus bulgaricus* (LB), *Lactobacillus casei* (LC), and *Lactobacillus delbrueckii* subsp. *lactis* (LD) grown in different carbohydrates and subjected to simulated gastric juice for 4 hours

	LB	LC	LD
GLU (control)	0.26 ± 0.35^{aA}	0.43 ± 0.39^{aA}	0.94 ± 0.52^{aA}
FOS	0.12 ± 0.17^{aA}	0.09 ± 0.09^{aB}	0.32 ± 0.28^{aA}
GOS	0.31 ± 0.35^{aA}	-0.19 ± 0.09^{aC}	0.10 ± 0.10^{aA}
IA	0.36 ± 0.43^{aA}	0.25 ± 0.20^{aB}	0.49 ± 0.45^{aA}
HMP	0.43 ± 0.25^{aA}	0.84 ± 0.14^{aA}	0.75 ± 0.77^{aA}

Different lowercase letters indicate significant differences ($P \leq 0.05$) in rows and different uppercase letters indicate significant differences in columns of delta log cfu/ml for treatments GLU, glucose; FOS, fructooligosaccharide, GOS, galactooligosaccharide; IA, inulin agave; HMP, high methoxy pectin.

4. Discussion

Prebiotics are carbohydrates which are not digestible in the upper part of the human intestines. The breakdown of these molecules occurs due to the metabolic activities of certain gut bacteria (Cummings & Macfarlane, 1997), using specific enzymes such as polysaccharidases (amylase, pectinase, and xylanase) and glycosidases (β-D-galactosidase, α-L-arabinofuranosidase, and β-D-glucosidase) to digest these complex carbohydrates (Englyst & others, 1987). Plant polysaccharides such as pectin can also reach the colon where they are degraded by microbial enzymes including hydrolases, esterases, and lyases (Louis, Scott, Duncan, & Flint, 2007). Therefore, to study the fermentability of HMP and SBP it is very important to retain the entire structures of the test pectin molecules to ensure accurate results on whether the probiotic bacteria have appropriate enzymes to break the test pectins.

The study of growth responses revealed the highest and similar growth rates among all test probiotics grown in MRS basal medium containing GLU, FOS or IA. This result suggests that FOS and IA are prebiotic substrates which could promote bacterial growth as well as the control GLU. Similar observations have been reported by Kaplan and Hutkins (2000) who found that FOS can serve as equivalent substrates to GLU in promoting bacterial growth. Specific growth rates of LB, LC and LD utilizing HMP and SBP were not significantly different from that in the basal MRS medium.

The pH of medium containing GLU, FOS or IA was significantly lower at the end of the growth period than that for media in which the strains did not grow (data not shown). Rossi et al. (2005) indicated that the fermentation of FOS and inulin by *Bifidobacteria* resulted in an increase in growth as measured by OD_{600} while the pH of the cultures was reduced, but there was no strong correlation between these two parameters. Also, Gulfi et al. (2005) reported that after 24 h growth, pH of the cultures containing a range of the test HMP and low methoxy pectin (LMP) were only minimally reduced as compared to pH of lactulose based cultures. The ability of *Lactobacilli* to lower the environmental pH is one of their mechanisms in preventing pathogen growth (Vadillo-Rodríguez, Busscher, Norde, de Vries, & van de Mei, 2004). Lowering pH results from the increase in the concentration of acidic by-products from substrate fermentation in the colon and could influence bacterial metabolism and competition (Flint, Duncan, Scott, & Louis, 2007). A study conducted by Walker et al. (2005) on the response of microflora in human feces to pH changes suggests that lowering pH may stimulate the production of butyrate, increase the number of bacteria responsible for its production, and restrict the growth of *Bacteroides* spp. Finally, based on the comparison of growth rates, final biomass, and pH, it is apparent that FOS and IA are fermented by all three strains, but only LC and LD could utilize GOS.

Each day the human stomach secretes approximately 2.5 L of gastric juice with pH approximately 2 (Hill, 1990), which causes a barrier for probiotic applications. When the probiotic bacteria reach the intestinal tract, the presence of bile can further reduce their survival (Kimoto-Nira, Kobayashi, Nomura, Sasaki, & Suzuki, 2009). It

is necessary for probiotic bacteria to survive passage through the stomach and the small intestine to become effectively established in the GI tract of the host. Some studies have shown enhanced survival of probiotic bacteria during their exposure to gastric conditions upon supplementation with milk protein (Charteris et al., 1998), whey cheese matrices (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011), or soymilk (Huang & Adam, 2004) to the medium. Encapsulation of microorganisms by gelatin, soluble starch, skim milk, or gum arabic could also protect probiotic bacteria from gastrointestinal stress as well (Lian et al., 2003). In our study, we have investigated the survival of 3 probiotic strains, LB, LC and LD, grown in MRS basal medium containing different carbohydrates including GLU, FOS, GOS, IA, and HMP. The result illustrates that LD grown in GLU was reduced to an undetectable level from approximately 7 log cfu/ml after 3 h of exposure to bile extract; however, the reduction of LB and LC were approximately 2.5 and 2.23 log cfu/ml respectively. This finding suggests that the differences in bacterial survival are strain-dependent which is in agreement with Begley, Gahan, and Hill (2005). Furthermore, LD when grown in basal MRS containing GOS was also reduced to undetectable levels, but when grown in HMP, LD survived significantly better than did LB and LC. In addition, LD survived better in HMP than in FOS and IA. In contrast, LB and LC grown in GLU could also maintain better survival after exposure to bile extract. Kimoto-Nira, Suzuki, Sasaki, Kobayashi, and Mizumachi (2010) and Hernandez-Hernandez et al. (2012) also reported that the different levels of survival of bacteria exposed to bile extract for 3 h varied according to strains and carbon sources utilized for bacterial growth. Kimoto-Nira et al. (2009) suggested that growth conditions could alter the fatty acid composition of bacterial cells which may enhance the survival of his test bacteria, *Lactococci*, upon exposure to bile extract. The follow-up research of Kimoto-Nira et al. (2010) found that for all bile-sensitive bacteria grown in different carbon sources, only bacteria grown in lactose exhibited different fatty acid composition. They suggested that the higher bile tolerance of bacteria grown in lactose may be achieved due to the alteration of fatty acid composition. However, the mechanisms explaining these survival differences are still not clear.

The results obtained from the simulated gastric juice study demonstrated that the test probiotic bacteria were tolerant to the simulated gastric juices. Zanoni et al. (2008) suggested that although the acidic pepsin solution (pH 2) is often used to simulate gastric transit, it may not be the most appropriate model as they found that bacterial viable counts in human gastric juice were significantly lower than in simulated gastric juices. The experimental conditions to study bacterial tolerance to gastric stress may vary according to the simplicity and complexity of the models, the differences in composition and concentration of components in gastric formulation, and pH ranges (Beumer, de Vries, & Rombouts, 1992; Charteris et al.,1998; Corcoran, Stanton, Fitzgerald, & Ross, 2005; Tamplin, 2005; Corcoran, Stanton, Fitzgerald, & Ross, 2007, Fernández de Palencia, López, Corbí, Peláez, & Requena, 2008; Kimoto-Nira et al., 2010; Madureira et al., 2011). However, previous experiments in our lab conducted by Hernandez-Hernandez et al. (2012) show significant reduction of LB, LC, and LD, which were the same strains as our study, after exposure to low pH (2.5, without pepsin) for only 1 h. Thus, it is conceivable that the sensitivity of probiotic bacteria to gastric conditions may be varied depending on the experimental conditions. A similar question can be raised for the study of bacterial tolerance to bile solution whether the obtained result would truly reflect the ability of test bacteria to tolerate bile *in vivo*. The *in vivo* conditions are very difficult to simulate in the laboratory and the concentration of bile in the intestines may vary by individual hosts. In addition, the tolerance of bacteria to bile can be changed due to bile-adaptation, as pre-exposure of bacteria to low-level bile may enhance their survival when placed in media containing high-level bile (Begley et al., 2005). Cross-adaptation may also occur when pre-exposure of bacteria to certain pH, temperature, or growth conditions may increase either the tolerance or susceptibility of probiotic bacteria (Begley et al., 2005). Thus, our findings may only be of limited use and should be confirmed by appropriate *in vivo* studies.

In conclusion, the results from our study can have an impact on the expansion of probiotic products by incorporating the test prebiotic carbohydrates to enhance the survival of probiotic bacteria through gastric transit and to have synergistic effects in the colon of the human host. Although fermentability of HMP by the test probiotics was not shown, this compound could be still beneficial in probiotic applications because of its ability to enhance the survival of bacteria both in bile solution. However, some studies have demonstrated that certain gut bacteria including *Bifidobacteria* and *Lactobacilli* could grow on HMP and LMP derived from apple and citrus (Olano-Martin et al., 2002, Sirotek, Slovakova, Kopecny, & Marounek, 2004; Gulfi et al., 2005). For the future study of the fermentability of HMP and SBP, human fecal samples or mixed cultures of beneficial bacteria including *Bifidobacteria* and *Lactobacillus* together with the pectin-fermenting strains from Olano-Martin et al. (2002) and Gulfi et al. (2005) should be used because the breakdown of polysaccharides such as pectins are suggested to be the result of cooperative activity of enzymes produced from many different species of colonic bacteria (Cummings & Macfarlane 1991).

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