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Full Length Research Paper

Effect of prebiotic oligosaccharides on growth of *Lactobacillus* strains used as a probiotic for chickens

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In the present study, the effect of 10 commercially available oligosaccharides on the growth of 11 *Lactobacillus* strains, which were isolated from the gastrointestinal tract of chickens and have been used as a multistrain probiotic for chickens, was evaluated *in vitro*. The utilization of oligosaccharides was highly variable among the 11 *Lactobacillus* strains and considerable strains differences ($P < 0.05$) were observed. Isomaltooligosaccharides (IMO) supported good growth for all the 11 *Lactobacillus* strains, followed by galactooligosaccharides (GOS), gentiooligosaccharides (GTO) and fructooligosaccharides (FOS). Oligosaccharides such as Raftilose L60, Raftilose P95, Raftiline LS, and mannanoligosaccharides (MOS) were poorly utilized by all the *Lactobacillus* strains. Growth kinetics study also showed variations in the specific growth rates and growth patterns of four representative *Lactobacillus* species on four selected oligosaccharides. The highest specific growth rate was demonstrated by *Lactobacillus salivarius* I 24 on FOS. The results showed that the ability of the 11 probiotic *Lactobacillus* strains to utilize oligosaccharides could be both strain and substrate specific, which demonstrates the importance of selecting suitable prebiotic oligosaccharides for the preparation of synbiotics.

Key words: Probiotic, prebiotic, synbiotic, *Lactobacillus*, oligosaccharides.

INTRODUCTION

The exploitation of gastrointestinal microflora as probiotics to replace antibiotic growth promoters for animals has received increasing interest due to the growing concern on the development of antibiotic-resistant bacteria. Probiotic is defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989). Bacteria belonging to the genus *Lactobacillus* are the predominant lactic acid bacteria used as probiotic feed supplements for animals, particularly poultry. By altering the colonization of beneficial bacteria in the avian alimentary tract, probiotics have shown to confer health benefits to the host such as in improving the growth rate, feed efficiency and immune response, suppressing the growth of pathogenic bacteria, and reducing cholesterol level (Jin et al., 1996, 1998a, b, 2000; Zulkifli et al., 2000; Kalavathy et al., 2003, 2005, 2009; Murry et al., 2006). At present, considerable attention is focused on determining ways to increase the number of probiotic microorganisms that colonize the gastrointestinal tract.

Prebiotics are substances that act as microbial modulators and are defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited

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number of bacteria in the colon and thus improve host health" (Gibson and Roberfroid, 1995). This definition was revised in 2004 and prebiotics are now defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (Gibson et al., 2004). Intake of prebiotics can either significantly modulate the colonic microbiota by increasing the number of specific probiotic bacteria such as lactobacilli and bifidobacteria (Rycroft et al., 2001a) or reducing undesired intestinal colonization of pathogenic bacteria by mimicking their attachment sites on the intestinal mucosa (Iji and Tivey, 1998). Complex dietary prebiotics, such as fructooligosaccharides (FOS), have the ability to reduce accessibility of *Salmonella* to colonization in poultry as well as increase *Bifidobacterium* level in chicken cecum (Nurmi and Rantala, 1973; Bailey et al., 1991). Additional beneficial effects of prebiotics on physiological aspects include enhancement of mineral absorption, reduction in serum lipid levels, reduction in production of putrefactive substances and inhibition of gut pathogens (Cummings and Macfarlane, 2002; Marteau and Boutron-Ruault, 2002). There are several types of nondigestible oligosaccharide substances, such as FOS, galactooligosaccharides (GOS), glucooligosaccharides, mannanooligosacharides (MOS), Raftilose P95, isomaltooligosaccharides (IMO), xylooligosaccharides (XOS), gentiooligosaccharides (GTO) and inulin, which have been reported to possess prebiotic effects (Playne and Crittenden, 1996; Rycroft et al., 2001b; Desai et al., 2004; Pennacchia et al., 2006).

A combination of probiotic and prebiotic, which is termed synbiotic, could have a synergistic effect in promoting growth of existing strains of beneficial bacteria in the colon as well as improving the survival and growth of newly added probiotic strains (Schrezenmeir and de Vrese, 2001). The functional benefits of synbiotic, such as resistance to gastrointestinal bacterial infection, antimicrobial activity, and improvement of immune system are envisaged in the development of synbiotic products. In recent years, studies have shown that synbiotic has the potential to be an alternative to antibiotic as a growth promoter for broilers (Mohnl et al., 2007; Jung et al., 2008; Awad et al., 2009). In our previous studies, a mixture of 11 *Lactobacillus* strains has been used as a multi-strain probiotic for chickens and results showed that it could significantly improve the growth performance (Jin et al., 1998a, b, 2000; Zulkifli et al., 2000; Kalavathy et al., 2003, 2005, 2009) and immune response (Zulkifli et al., 2000), as well as reduce the fat and cholesterol contents (Jin et al., 1998a; Kalavathy et al., 2003) of chickens. In the current investigation, the effects of 10 commercially available oligosaccharides on the growth of these 11 probiotic *Lactobacillus* strains were studied *in vitro* to determine suitable oligosaccharides which could serve as effective prebiotics for the strains, particularly for the preparation of synbiotic containing a mixture of the 11 probiotic *Lactobacillus* strains.

MATERIALS AND METHODS

Lactobacillus strains and growth conditions

The 11 probiotic *Lactobacillus* strains (*Lactobacillus reuteri* C 1, C 10 and C 16; *Lactobacillus gallinarum* I 16 and I 26; *Lactobacillus brevis* I 12, I 23, I 25, I 218 and I 211, and *Lactobacillus salivarius* I 24) used in this study were originally isolated from the gastrointestinal tract of local chickens and were the same as those identified and described by Jin et al. (1996). These strains were recently re-classified by sequencing the 16S rRNA gene and 16S-23S rRNA gene intergenic spacer region (Lee et al., 2008). The cultures were maintained by routine subculturing in de Man-Rogosa-Sharpe (MRS) broth (Oxoid Ltd, Basingstoke, UK) using a 1% (v/v) inoculum from an overnight culture, and incubated under anaerobic conditions in anaerobic jars (Oxoid Ltd) with gas generating kits (Oxoid Ltd) at 37°C. The stock cultures were stored in MRS broth containing 15% (v/v) sterilized glycerol at -80°C.

Basal growth media

To study the growth of *Lactobacillus* strains in various oligosaccharides, carbohydrate-free MRS broth was used as a basal growth medium. The basal MRS broth medium contained the following components (L^{-1}): Peptone (Oxoid Ltd), 10.0 g; yeast extract (Oxoid Ltd), 5.0 g; sodium acetate (Sigma-Aldrich, St Louis, MO, USA), 5.0 g; $K_2HPO_4 \cdot 3H_2O$ (Sigma-Aldrich), 2.0 g; $(NH_4)_3C_6H_5O_7 \cdot 2H_2O$ (Sigma-Aldrich), 2.0 g; $MgSO_4 \cdot 7H_2O$ (Sigma-Aldrich), 0.2 g; $MnSO_4 \cdot 4H_2O$ (Sigma-Aldrich), 0.05 g; and Tween 80 (Merck, Darmstadt, Germany), 1 ml. The pH of medium was adjusted to 6.2 and sterilized at 121°C for 15 min (Saarela et al., 2003).

Oligosaccharides substrates

The 10 different types of commercially available oligosaccharides that were used in the present study were obtained from various producers: GOS (Yakult, Japan), lactulose (Wako, Japan), GTO (Wako, Japan), XOS (Wako, Japan), FOS (Wako, Japan), IMO (Wako, Japan), Raftilose P95 (Orafti, Belgium), Raftilose L60 (Orafti, Belgium), Raftiline LS (Orafti, Belgium) and MOS (Lanospharma, China). Stock solutions of these oligosaccharides were prepared in de-ionized water and filter sterilized with 0.2 µm filters (Minisart filters, Sartorius AG, Germany). The sterile oligosaccharides solutions were added to autoclaved basal MRS medium to obtain a final oligosaccharide concentration of 1% (w/v). Glucose (Sigma-Aldrich) (1%, w/v), which was the favorable carbohydrate source for all the *Lactobacillus* strains, was used as control.

Screening of ten oligosaccharides on growth of eleven *Lactobacillus* strains

The 10 oligosaccharides were screened for their effects on the growth of the 11 probiotic *Lactobacillus* strains. Basal MRS medium supplemented with 1% (w/v) of the test oligosaccharides as the sole carbon source was inoculated with 1% (v/v) inoculum of an overnight *Lactobacillus* culture. The inoculated media were incubated under anaerobic conditions using anaerobic jars with gas generating kits at 37°C. After 24 h of incubation, the cultures were

vortexed for 30 s to disperse the bacterial cells, and the growth of each strain was determined by measuring the optical density (OD) at 620 nm using a spectrophotometer (Biomate 3, Thermo, USA). Three replications were made for the experiment.

Determination of relative growth of *Lactobacillus* strains

The growth response of each *Lactobacillus* strain on each oligosaccharide relative to its growth response on glucose was calculated using the method of Kneifel et al. (2000). The calculation was performed using the following formula in which the growth on glucose = 100%:

Relative growth of *Lactobacillus* strain on an oligosaccharides substrate = (A/B) × 100%

where A is the mean OD_{620} value of a strain on oligosaccharides substrate and B is the mean OD_{620} value of the same strain grown on glucose. In this way, the results would reflect the growth of a specific *Lactobacillus* strains on a particular oligosaccharides substrates relative to its growth on a glucose substrate which was fixed at 100%.

Growth kinetics of four representative *Lactobacillus* species on selected oligosaccharides

From the results of the screening of 10 oligosaccharides on the growth of the 11 *Lactobacillus* strains described, the oligosaccharides which supported good growth of most of the strains were selected for growth kinetic study of four *Lactobacillus* strains representing the four *Lactobacillus* species present within the 11 strains. The four representative strains, chosen for their good growth activities, were *L. reuteri* C 1, *L. gallinarum* I 16, *L. salivarius* I 24 and *L. brevis* I 25. Basal MRS medium containing 1% (w/v) of an oligosaccharide substrate was inoculated with 1% inoculum (v/v) of a *Lactobacillus* strain and incubated at 37°C under anaerobic conditions as described. The growth responses of the strains in media with the test oligosaccharides and control were determined at 0, 3, 6, 9, 12, 15, 18 and 24 h by measuring the OD of the cultures at 620 nm. The growth study was performed in three replications and the mean values were plotted to generate growth curves for each *Lactobacillus* strain. The specific growth rate (μ) was calculated using the following formula:

$$\mu = (\ln x - \ln x_0)/ (t - t_0)$$

where x and x_0 are absorbances measured within the exponential phase of growth at time t and t_0, respectively.

Statistical analysis

Statistical analysis of data was performed using the SPSS statistical package program, version 16.0 (SPSS Inc., Chicago, IL, USA). For the growth (OD_{620nm}) study, significant differences in the effect of the different substrates, probiotic *Lactobacillus* strains and their interaction (substrate × strains) were tested using two-way analysis of variance (ANOVA). Statistical significance of specific growth rates of *Lactobacillus* strains on different oligosaccharides was evaluated by one-way ANOVA. Significant differences between means were analysed using post hoc tests at $P < 0.05$.

RESULTS

Screening of oligosaccharides on growth of probiotic *Lactobacillus* strains

The growth densities (OD_{620}) of the 11 *Lactobacillus* strains in basal MRS media containing oligosaccharides or glucose (control) are shown in Table 1. Significant ($P < 0.05$) effects of different *Lactobacillus* strains, substrates and their interaction were observed on the growth activities of the 11 *Lactobacillus* strains. *Lactobacillus reuteri* C 1 demonstrated the best growth ($P < 0.05$) on GOS followed by IMO, lactulose, and GTO, but it did not grow well on the remaining oligosaccharides. *Lactobacillus reuteri* C 16, like *L. reuteri* C 1, also grew best ($P < 0.05$) on GOS, followed by IMO, lactulose and GTO. Growth was poor on Raftiline LS, XOS, FOS or MOS. Growth of *L. reuteri* C 10 was particularly good ($P < 0.05$) on GOS and lactulose. It showed moderate growth on IMO and to a lesser extent on GTO. Poorer growth was observed on the remaining oligosaccharides.

Lactobacillus salivarius I 24 demonstrated significantly high ($P < 0.05$) growth on lactulose and GOS (Table 1). Growth was also good on FOS. However, growth on Raftiline LS, IMO and Raftilose L60 was moderate and was not significantly different ($P > 0.05$) among the three oligosaccharides. Growth on the rest of the oligosaccharides was poor.

The two *L. gallinarum* strains, I 16 and I 26, showed quite similar growth responses on most of the oligosaccharides (Table 1). They both grew best on GTO and oligosaccharides such as IMO, FOS and GOS also supported good growth of these two strains. Moderate growth of both strains was observed on Raftilose L60. However, on Raftiline LS, growth of *L. gallinarum* I 16 was moderate, but growth of *L. gallinarum* I 26 was poor. In contrast to *L. reuteri* C 1, C 10 and C 16, and *L. salivarius* I 24, which exhibited very good growth on lactulose, growth of *L. gallinarum* I 16 and I 26 was very poor on this oligosaccharides. For both strains, growth on Raftilose P95 or XOS was also poor.

Lactobacillus brevis I 25, I 12, I 23, I 211 and I 218 showed similar growth activities on the test oligosaccharides (Table 1). All five *L. brevis* strains showed the highest growth on XOS and the growth was significantly ($P < 0.05$) more than or same as that on glucose. The strains also grew well on IMO, but the growth was lower ($P < 0.05$) than that on glucose. Moderate growth of these strains was observed in the presence of GOS, FOS and GTO. Like the two *L. gallinarum* strains, all five *L. brevis* strains exhibited very poor growth on lactulose.

Lactobacillus strain-oligosaccharides combinations

From the results of the screening study, it is evident that

Table 1. Growth (OD_{620} ± SD) of *Lactobacillus* strains on 1% glucose or oligosaccharides after 24 h of incubation.

Strain	Growth (OD_{620})[1,2]										
	Substrate										
	Glucose	GOS	P95	L60	LS	Lactulose	XOS	IMO	FOS	GTO	MOS
L. reuteri C 1	1.56 ±0.02^{CDa}	1.47±0.02 ^{Cb}	0.39 ±0.01^{Cgh}	0.64 ±0.04^{De}	0.35 ±0.01^{Eh}	1.36 ±0.02^{Cc}	0.36 ±0.00^{Dh}	1.37 ±0.03^{Cc}	0.44 ±0.03^{Gg}	1.28 ±0.02^{Bd}	0.48 ±0.00^{Cf}
L. reuteri C 16	1.60 ±0.02^{BCa}	1.51 ±0.01^{Bb}	0.82 ±0.01^{Ae}	0.71 ±0.01^{Cf}	0.41 ±0.00^{Dh}	1.24 ±0.00^{Dd}	0.34 ±0.01^{Di}	1.38 ±0.01^{Cc}	0.54 ±0.00^{Fg}	1.23 ±0.01^{Cd}	0.42 ±0.01^{Dh}
L. reuteri C 10	1.55 ±0.01^{CDa}	1.45 ±0.02^{Cb}	0.25 ±0.01^{DEg}	0.57 ±0.02^{Ee}	0.24 ±0.00^{Fg}	1.44 ±0.00^{Bb}	0.19 ±0.01^{Eh}	1.02 ±0.03^{Dc}	0.43 ±0.02^{Gf}	0.69 ±0.00^{Dd}	0.41 ±0.02^{Df}
L. salivarius I 24	1.85 ±0.02^{Aa}	1.77 ±0.01^{Ab}	0.25 ±0.01^{DEf}	0.61 ±0.02^{DEd}	0.65 ±0.00^{Bd}	1.81 ±0.01^{Ab}	0.19 ±0.00^{Eg}	0.63 ±0.01^{Fd}	1.55 ±0.01^{Ac}	0.45 ±0.01^{Fe}	0.22 ±0.01^{Fg}
L. gallinarum I 16	1.52 ±0.01^{Da}	1.18 ±0.01^{Ed}	0.36 ±0.01^{Ch}	0.85 ±0.01^{Be}	0.77 ±0.00^{Af}	0.14 ±0.00^{Fi}	0.42 ±0.01^{Cg}	1.47 ±0.02^{Bb}	1.24 ±0.01^{Cc}	1.56 ±0.02^{Aa}	0.74 ±0.01^{Bf}
L. gallinarum I 26	1.65 ±0.02^{Ba}	1.30 ±0.02^{Dd}	0.45 ±0.00^{Bg}	0.96 ±0.01^{Ae}	0.42 ±0.01^{Dg}	0.14 ±0.00^{Fh}	0.40 ±0.01^{Cg}	1.51 ±0.01^{Ac}	1.31 ±0.00^{Bd}	1.56 ±0.02^{Ab}	0.86 ±0.05^{Af}
L. brevis I 25	1.41 ±0.02^{Eb}	0.81 ±0.00^{Fd}	0.28 ±0.01^{DEi}	0.34 ±0.01^{Fh}	0.45 ±0.00^{Dg}	0.22 ±0.01^{Ej}	1.46 ±0.01^{Ba}	0.96 ±0.02^{DEc}	0.59 ±0.00^{DEf}	0.68 ±0.00^{DEe}	0.31 ±0.01^{Ehi}
L. brevis I 12	1.40 ±0.01^{Ea}	0.80 ±0.01^{Fc}	0.27 ±0.01^{DEh}	0.37 ±0.00^{Fg}	0.50 ±0.00^{Cf}	0.21 ±0.00^{Ei}	1.44 ±0.01^{Ba}	0.98 ±0.01^{DEb}	0.58 ±0.01^{Ee}	0.71 ±0.00^{Dd}	0.35 ±0.01^{Eg}
L. brevis I 23	1.36 ±0.01^{Eb}	0.81 ±0.01^{Fd}	0.23 ±0.00^{Eh}	0.37 ±0.01^{Fg}	0.46 ±0.00^{Df}	0.19 ±0.00^{Ei}	1.49 ±0.02^{Aa}	0.93 ±0.01^{Ec}	0.65 ±0.01^{De}	0.63 ±0.00^{Ee}	0.31 ±0.00^{Eh}
L. brevis I 211	1.38 ±0.01^{Eb}	0.81 ±0.00^{Fd}	0.28 ±0.01^{DEh}	0.34 ±0.01^{Fg}	0.53 ±0.00^{Cf}	0.18 ±0.00^{Ei}	1.44 ±0.00^{Ba}	0.92 ±0.01^{Ec}	0.56 ±0.01^{EFf}	0.68 ±0.00^{DEe}	0.34 ±0.01^{Eg}
L. brevis I 218	1.38 ±0.02^{Eb}	0.80 ±0.02^{Fd}	0.30 ±0.00^{Dh}	0.35 ±0.01^{Fg}	0.45 ±0.02^{Df}	0.21 ±0.00^{Ei}	1.46 ±0.02^{ABa}	0.98 ±0.01^{DEc}	0.63 ±0.01^{De}	0.65 ±0.01^{DEe}	0.31 ±0.00^{Egh}

[1]Results are mean values from three replications ± standard deviations;[2]Strain effect ($P < 0.05$), substrate effect ($P < 0.05$), strain × substrate ($P < 0.05$). $^{A-G}$ Means in the same column with different uppercase letters are significantly different ($P < 0.05$); $^{a-i}$ means in the same row with different lowercase letters are significantly different ($P < 0.05$). IMO = isomaltooligosaccharides, FOS = fructooligosaccharides, GOS = galactooligosaccharides, GTO = gentiooligosaccharides, XOS = xylooligosaccharides, MOS = mannanoligosaccharides, L60 = Raftilose L60, P95 = Raftilose P95 and LS = Raftiline LS.

Table 2. Summary of relative growth of 11 probiotic *Lactobacillus* strains on 10 prebiotic oligosaccharides to illustrate the preference of the strains for specific oligosaccharides.

Lactobacillus strain	Relative growth[1]									
	IMO	GOS	GTO	FOS	XOS	Lac	L60	MOS	P95	LS
L. reuteri C 1	****	****	****	*	*	****	*	*	*	*
L. reuteri C 16	****	****	***	*	*	***	**	*	**	*
L. reuteri C 10	***	****	**	*		****	*	*		
L. salivarius I 24	**	****	*	****		*****	*			*
L. gallinarum I 16	****	***	*****	****	*		***	**	*	**
L. gallinarum I 26	****	***	****	***	*		***	**	*	*
L. brevis I 25	***	**	**	**	*****		*	*		*
L. brevis I 12	***	**	**	**	*****		*	*		*
L. brevis I 211	***	**	**	**	*****		*	*		*
L. brevis I 218	***	**	**	**	*****		*	*		*
L. brevis I 23	***	**	**	**	*****		*	*		*

[1]Relative growth (growth of strain on oligosaccharides relative to its growth on glucose): no symbol represents relative growth of ≤ 20%; * relative growth of 21- 40%; ** relative growth of 41- 60%; *** relative growth of 61 to 80%; **** relative growth of 81- 100%; ***** relative growth of > 100%; values were calculated from values shown in Table 1. IMO = isomaltooligosaccharides, FOS = fructooligosaccharides, GOS = galactooligosaccharides, GTO = gentiooligosaccharides, XOS = xylooligosaccharides, MOS = mannanoligosaccharides, L60 = Raftilose L60, P95 = Raftilose P95, LS = Raftiline LS, Lac = lactulose.

the *Lactobacillus* strains exhibited oligosaccharide preference. A simplified summary of the growth responses of the 11 *Lactobacillus* strains on the 10 oligosaccharides, relative to the growth of the strains on glucose which was designated as 100% was produced to illustrate the preference of the strains for specific oligosaccharides (Table 2). From the summary, it can be seen that the low polymerization degree (DP) oligofructose substrates, Raftilose L60 and Raftilose P95, and the higher DP and highly-pure (> 99.5) inulin-based substrate, Raftiline LS, as well as the oligosaccharides MOS supported poor growth of most of the *Lactobacillus* strains. Only the five strains of *L. brevis* showed high growth densities in the presence of XOS, and only the three *L. reuteri* strains and *L. salivarius* I 24 grew well on lactulose. In contrast, IMO was generally well utilized by all the *Lactobacillus* strains, followed by GOS, GTO and FOS. These four oligosaccharides have the potential to be suitable prebiotic substrates for the *Lactobacillus* strains in synbiotic combinations.

Growth kinetics of four representative *Lactobacillus* species on IMO, GOS, GTO and FOS

The growth curves of the four *Lactobacillus* species in MRS basal media supplemented with GOS, IMO, GTO, FOS or glucose are shown in Figures 1A to D. Generally, the growth patterns of *L. reuteri* C 1 (Figure 1A) and *L. salivarius* I 24 (Figure 1B) were quite similar, in which after a lag phase of 3 h, their exponential growth phase in glucose and the four oligosaccharides occurred very was rapidly, between 3 to 6 h of incubation, after which growth more gradual and then plateaued with the onset of stationary growth phase. However, the amount of growth on various oligosaccharides varied significantly between the two *Lactobacillus* species. For instance, on IMO and GTO, the growth of *L. reuteri* (C 1) was significantly more than that of *L. salivarius* (I 24) and the reverse was found on FOS. The lag phase of *L. gallinarum* I 16 (Figure 1C) on the four oligosaccharides and glucose was 3 h, but the exponential phase varied on different oligosaccharides and glucose. The exponential phase on FOS, GOS and glucose occurred between 3 to 9 h of incubation, whereas on IMO and GTO it occurred between 3 to 12 h of incubation. For *L. brevis* I 25, the lag phase was also 3 h, but the exponential phase was more gradual and longer (between 3 to 18 h of incubation) than those of the other three *Lactobacillus* species (Figure 1D).

Table 3 shows the specific growth rates of the four representative *Lactobacillus* species on the four oligosaccharides and glucose. *L. salivarius* I 24 demonstrated high growth rates on FOS and GOS (0.68 and 0.63 h^{-1}, respectively), which indicated that high rate of cell proliferation occurred on these oligosaccharides within a short period of incubation. For *L. reuteri* C 1, high specific growth rates were obtained on IMO and GOS (0.46 and 0.44 h^{-1}, respectively). As for *L. gallinarum* I 16, FOS was the most effective oligosaccharides in increasing its cell concentration (0.31 h^{-1}). The specific growth rates of *L. brevis* I 25 on the four oligosaccharides were the slowest among the four *Lactobacillus* species; its highest specific growth rate, which was on GTO, was only 0.23 h^{-1}.

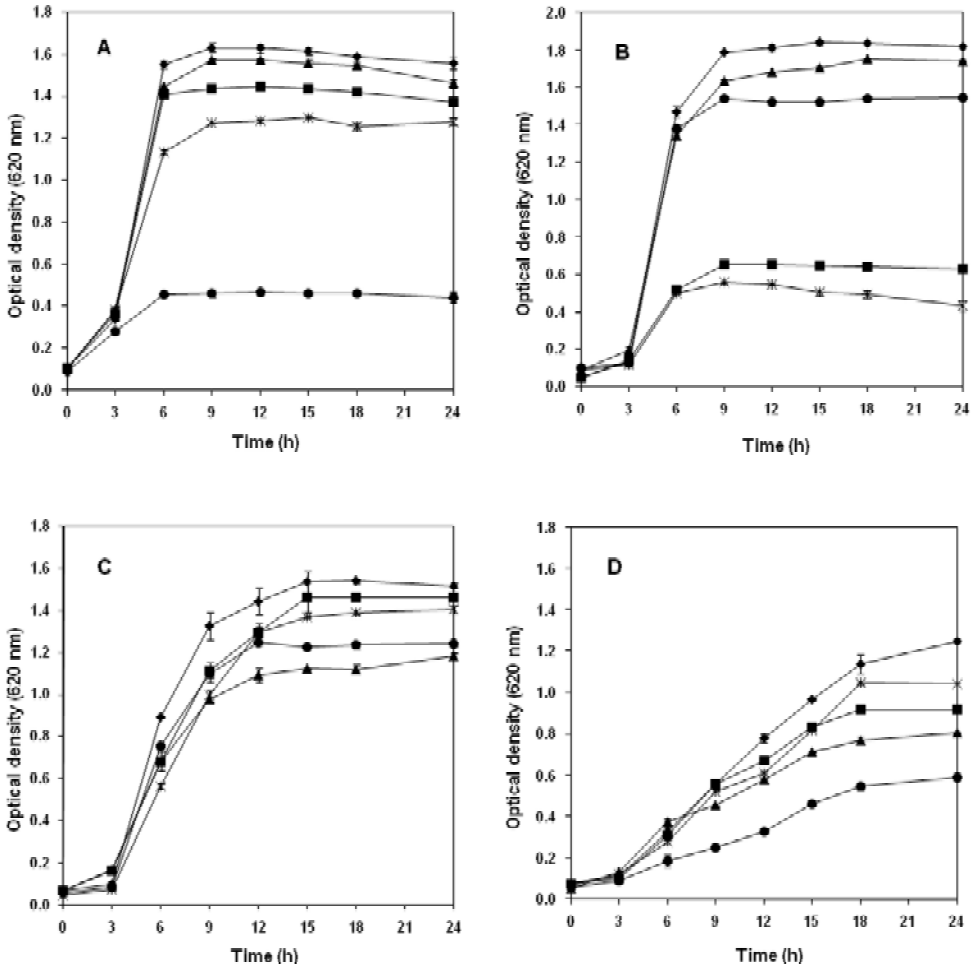

Figure 1. Growth curves of probiotic *L. reuteri* C 1 (A), *L. salivarius* I 24 (B), *L. gallinarum* I 16 (C) and *L. brevis* I 25 (D) in basal MRS medium supplemented with 1% (w/v) glucose (control) (♦), IMO (■), GOS (▲), FOS (●) or GTO (×). Results are the means from three experiments. Vertical lines represent standard deviations (SD).

Table 3. Specific growth rates (h^{-1}) of four representative *Lactobacillus* species in media with selected oligosaccharides.

Substrate	Specific growth rates (h^{-1})[1]			
	***L. reuteri* C 1**	***L. gallinarum* I 16**	***L. salivarius* I 24**	***L. brevis* I 25**
Glucose (control)	0.51±0.03[a]	0.36±0.02[a]	0.70±0.09[a]	0.26±0.00[a]
IMO	0.46±0.02[b]	0.18±0.01[c]	0.44±0.00[c]	0.20±0.02[b]
FOS	0.29±0.01[d]	0.31±0.03[b]	0.68±0.00[a]	0.10±0.03[d]
GOS	0.44±0.03[b]	0.20±0.05[c]	0.63±0.01[b]	0.16±0.07[c]
GTO	0.36±0.04[c]	0.19±0.02[c]	0.40±0.04[c]	0.23±0.06[ad]

[1]Results are mean values from three replications ± standard deviations, [a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.05$), IMO = isomaltooligosaccharides, FOS = fructooligosaccharides, GOS = galactooligosaccharides, GTO = gentiooligosaccharides.

DISCUSSION

Several oligosaccharides, such as IMO, FOS, GOS, inulin, and lactulose have been reported to significantly enhance the growth of desirable bacteria such as bifidobacteria and lactobacilli (Kneifel et al., 2000; Pennacchia et al., 2006). The results of the present study showed that the 11 probiotic *Lactobacillus* strains were capable of utilizing all the 10 oligosaccharides examined but the growth varied among the species, strains and

substrates.

The three *L. reuteri* and two *L. gallinarum* strains showed strain differences ($P < 0.05$) within the same species based on their different growth activities on various oligosaccharide substrates (Table 1). Differences were also shown among the four different species, (*L. reuteri*, *L. gallinarum*, *L. salivarius* and *L. brevis*) as demonstrated in the growth profiles of their representative strains on different oligosaccharides such as GOS, IMO, GTO and FOS (Figures 1A to D). Similar results were reported by Pennacchia et al. (2006), who studied the growth of lactobacilli on GOS, IMO, FOS and lactulose, and found species-related fermentation behaviour shown by the *Lactobacillus* species.

The results of the present study also showed that among the 10 oligosaccharide substrates, IMO, GOS, GTO and FOS could support the growth of all the 11 *Lactobacillus* strains, but lactulose was only well utilized by the *L. reuteri* strains and *L. salivarius* I 24. The other prebiotic substrates such as Raftilose L60, Raftilose P95, Raftiline LS and MOS were poorly utilized by the majority of the strains. Earlier, Chung and Day (2004) reported that IMO had the capability to stimulate the growth of *Lactobacillus* strains originated from chicken gut, but it was not utilized by pathogens such as *Salmonella* or *Escherichia coli*. In another study, Pennacchia et al. (2006) found that *L. brevis* strains grew well in the presence of IMO or GOS, but not inulin or lactulose. Similar growth responses were also shown by the five *L. brevis* strains in the present study. Rastall and Maitin (2002) had reported that the increase in lactobacilli was higher on FOS than on lactulose, which is similar to the growth behavior of the two *L. gallinarum* and five *L. brevis* strains in the current study.

Although, there has been much commercial and research interest in the beneficial effects of probiotics and prebiotic oligosaccharides, and the possible twofold synergistic effects of synbiotics, very little is known on the mechanisms of metabolism of prebiotic oligosaccharides by probiotic bacteria. Some studies that have been carried out on the metabolism of FOS by *Lactobacillus* strains suggest that the bacteria might have specific enzymatic activities and substrate transport systems that allow them to use the specific prebiotic oligosaccharides (Kaplan and Hutkins, 2003; Saulnier et al., 2007). In the molecular perspective, the role of specific enzymatic activities and oligosaccharide transport mechanisms depend on the presence of specific gene codes in bacterial cells resulting in various patterns of growth in different prebiotic substrates (Kaplan and Hutkins, 2003; Goh et al., 2007; Saulnier et al., 2007). Therefore, the effectiveness of a prebiotic depends on its ability to be selectively be fermented by specific organisms. The current finding in which all the strains could utilize IMO, GOS and GTO well probably reflect the activities of substrate transport systems in these strains, which seem to be more efficient with these oligosaccharide substrates.

Most of the current oligosaccharides are of rather low molecular weight and they are generally rapidly fermented by probiotic microbes in the proximal colon (Rastall and Maitin, 2002). In the present study, oligosaccharides with lower degree of polymerization (mixture of mono- di-, tri- and tetrasaccharides), such as GOS, FOS, IMO and GTO, were fermented by all the *Lactobacillus* strains. A study by Kaplan and Hutkins (2000) on the fermentation of individual oligomers of FOS by *L. plantarum* and *L. rhamnosus* showed that the two lactobacilli were only capable of metabolizing the lower molecular weight substances, such as trisaccharide (DP3) and tetrasaccharide (DP4) fractions, and pentasaccharides (DP5) were not metabolized by these strains, which suggests that there may be specific transport systems for trisaccharides and tetrasaccharides in these organisms. In another study carried out by Chung and Day (2004), two poultry cecal isolates, which were grouped as lactic acid bacteria, used isomaltotriose (DP3) oligomers from IMO primarily, and were also able to use prebiotic larger than DP3 but with lower efficiency. Gopal et al. (2001) also reported that in the metabolism of GOS by *L. rhamnosus*, the monosaccharide and disaccharide fractions were well utilized as growth substrates; other oligosaccharides with longer chain (DP > 10) such as inulin were less utilized by the strain.

Results of the current study showed that in some *Lactobacillus* strains, high specific growth rates could occur even though the overall growth was low. For example, the specific growth rates of *L. salivarius* I 24 on GTO and IMO were higher than those of *L. brevis* I 25 (Table 3), although the maximal growth was lower than *L. brevis* I 25 (Table 1). As suggested by Hopkins et al. (1998), this phenomenon may be due to the rapid growth of the *Lactobacillus* strain within a very short period during the incubation, giving rise to a misleading assessment of the substrate effectiveness. Therefore, care should be taken when assessing information concerning bacterial growth rates; results on cell yields should also be taken into consideration.

Saarela et al. (2003) have reported that the determination of suitable synbiotic pairs, in which the prebiotic would benefit the specific probiotic strain, is not a simple task. According to Kneifel et al. (2000), the development of synbiotic to exploit a twofold potential by combining probiotic and prebiotic will only be sensible if both components fit well together. The present study demonstrated that, of the 10 oligosaccharides investigated, only IMO, GOS. FOS and GTO were favorably suitable for the growth of the 11 probiotic *Lactobacillus* strains and were therefore considered as suitable prebiotics for the strains. These prebiotics would have the potential to increase the population and activity of these probiotic strains in the gastrointestinal tract of chickens when administered in the form of synbiotic, and thus enhance their beneficial effects on the host. Further

investigation *in vivo* (in chickens) is needed to determine the efficacy of the synbiotic and whether it could provide a twofold synergistic effect on the host.

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