Effect of Prebiotic Carbohydrates on the Growth and Tolerance of Lactobacillus

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ABSTRACT

Resistance to gastrointestinal conditions is a requirement for bacteria to be considered probiotics. In this work, we tested the resistance of six different *Lactobacillus* strains and the effect of carbon source to four different gastrointestinal conditions: presence of α-amylase, pancreatin, bile extract and low pH. Novel galactooligosaccharides synthesized from lactulose (GOS-Lu) as well as commercial galactooligosaccharides synthesized from lactose (GOS-La) and lactulose were used as carbon sources and compared with glucose. In general, all strains grew in all carbon sources, although after 24 h of fermentation the population of all *Lactobacillus* strains was higher for both types of GOS than for glucose and lactulose. No differences were found among GOS-Lu and GOS-La. α-amylase and pancreatin resistance was retained at all times for all strains. However, a dependence on carbon source and *Lactobacillus* strain was observed for bile extract and low pH resistance. High hydrophobicity was found for all strains with GOS-Lu when compared with other carbon sources. However, concentrations of lactic and acetic acid were higher in glucose and lactulose than GOS-Lu and GOS-La. These results show that the resistance to gastrointestinal conditions and hydrophobicity is directly related with the carbon source and *Lactobacillus* strains. In this sense, the use of prebiotics as GOS and lactulose could be an excellent alternative to monosaccharides to support growth of probiotic *Lactobacillus* strains and improve their survival through the gastrointestinal tract.

Keywords: probiotic, prebiotic, lactobacillus, lactulose, galactooligosaccharides, tolerance

1. INTRODUCTION

Probiotics are live microorganisms (mainly lactobacillus and bifidobacteria) which administered in adequate amounts confer a health benefit to the host (FAO/WHO, 2003). The *Lactobacillus* genus is distributed in various ecological niches and is an important constituent of the human and animal gut microbiota (Charteris et al., 1997).

Lactobacilli are currently added to a variety of functional foods and several studies have demonstrated their beneficial properties in human health (Reid et al., 2011). However, an important requirement is that these bacteria should be able to survive gastrointestinal conditions (amylases in the oral cavity, low pH in the stomach, bile secretions and pancreatic juice in the duodenal section of the small intestine). Several *in vivo* (Jain et al., 2004; Reid, 2008; Park et al., 2008) and *in vitro* (Charteris et al., 1998; Fernandez et al., 2003; Pitino et al., 2010) studies have indicated that some *Lactobacillus* strains only partially survive the passage through gastrointestinal tract and it is said that generally a population of 10^7 – 10^9 CFU per mL of bacterial cells should be present in foods in order to colonize, at least temporally, the intestine (Lee & Salminen, 1995). Nevertheless, it has been observed that only specific strains can survive these conditions. In this sense, Fernández et al. (2003) reported that *L. acidophilus* and *L. gasseri* strains were resistant to low pH and to the presence of different gastrointestinal enzymes. Similarly, Pitino et al. (2010) observed that six different strains of *L. rhamnosus* were resistant to a simulated human digestion process and Charteris et al. (1998) studied the survival of seven different *Lactobacillus* species where *L. fermentum* KLD was considered intrinsically resistant; additionally, these authors found that the addition of milk protein improved the tolerance of the probiotics to gastrointestinal conditions. Similar results have been found by Chavarri et al. (2010) and Madureira et al. (2010) using microencapsulation with alginate-chitosan and whey cheese matrix, respectively.

Kimoto-Nira et al. (2010) have recently studied the resistance of *Lactococcus lactis* G50 grown in six different non-prebiotic carbohydrates (fructose, glucose, galactose, xylose, lactose and sucrose) under simulated gastrointestinal stress. The survival behaviour of G50 strain was found to be dependent on the carbon source where they were grown. However, to the best of our knowledge the resistance to gastrointestinal conditions of *Lactobacillus* strains grown in prebiotic carbohydrates has rarely been considered. Valerio et al. (2006) reported the protective effect of artichokes on different probiotics strains in the gastrointestinal tract could be hypothetically attributed to the presence of prebiotic carbohydrates and to the physical structure of the vegetable matrix.

Prebiotics are defined as “nondigestible food ingredients that beneficially affects host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson et al., 2004). Some prebiotic carbohydrates are currently available in the market, such as fructooligosaccharides, lactulose, inulin and galactooligosaccharides from lactose (GOS-La) (Rastall, 2010). However, currently there is considerable interest in the discovery of new carbohydrates with potential prebiotic properties. Among them, galactooligosaccharides from lactulose (GOS-Lu) have recently been studied (Cardelle-Cobas et al., 2008; Martinez-Villaluenga et al., 2008). GOS-Lu can be obtained by transgalactosylation reaction of the lactulose by the action of β-galactosidases from different bacterial sources (Cardelle-Cobas et al., 2008; Martinez-Villaluenga et al., 2008). Recently, it has been reported that GOS-Lu have the ability to promote the growth of bifidobacteria using *in vitro* fermentation systems with human fecal cultures in a similar manner as the more highly recognised prebiotic GOS-La (Cardelle-Cobas et al., 2009).

Therefore, the aim of this study was to investigate the growth of six *Lactobacillus* strains, normally used in fermented food, with different prebiotics (lactulose, GOS from lactose and GOS from lactulose) as carbon sources and to determine their resistance to different gastrointestinal conditions (amylases, low pH, bile extract and pancreatin). Hydrophobicity as a measure of potential adhesion of lactobacillus, as well as lactic and acetic acid concentrations produced during incubation were also evaluated.

2. MATERIALS AND METHODS

2.1.Chemicals

Glucose, lactulose, bile extract, pancreatin and α-amylase (1440 units/mg protein) from porcine pancreas, β-galactosidase from *Aspargillus oryzae* (8.0 units/mg protein) and *n*-hexadecane was purchased from Sigma-Aldrich (St. Louis, USA). The bacteriological growth media supplements were obtained from EMD Chemicals, Gibbstown, NJ. The galactooligosaccharide from lactose (GOS-La) was obtained from Vivinal-GOS®, kindly provided by Friesland Foods Domo (Zwolle, The Netherlands). This product has a 73 wt% dry matter, the composition of which was 60 wt% GOS, 20 wt% lactose, 19 wt% glucose and 1 wt% galactose, as stated by the supplier. Duphalac® (Solvay Pharma, Brussels, Belgium) was used to obtain the galactooligosaccharides from lactulose (GOS-Lu).

2.2.Preparation of galactooligosaccharides

In order to purify the GOS-La, the industrial product Vivinal-GOS® was fractionated using size exclusion chromatography, following the method reported by

Hernandez et al. (2009) with some modifications. In brief, 80 mL of Vivinal-GOS® (25 % w/v) were injected in a Bio-Gel P2 (Bio-Rad Hercules, CA, USA) column (90 x 5 cm) using water as mobile phases, at 1.5 mL min^{-1}. Sixty fractions of 10 mL were collected, after the elution of void volume. The fractions degree polymerization (DP) was determined by electrospray ionization mass spectrometry (ESI-MS) at positive mode, ranging from monosaccharides to octasaccharides. Fractions with DP ≥ 3 were pooled and freeze dried.

GOS from lactulose were obtained following the method previously described (Clemente et al., 2011). A solution (450 g L^{-1}) of lactulose (Duphalac®) was dissolved in 50 mM sodium phosphate buffer and 1 mM $MgCl_2$, pH 6.5, after addition of 8 U mL^{-1} of β-galactosidase from *Aspergillus oryzae* (Sigma, St. Louis, MO USA), and incubation at 50 ºC for 20 h under continuous agitation at 300 rpm. After incubation, the mixtures were immediately immersed in boiling water for 5 min to inactivate the enzymes. The DP of initial GOS-Lu mixture contained from monosaccharides to octasaccharides. Subsequently, the GOS-Lu mixture was fractionated using size exclusion chromatography in order to remove mono- and disaccharides, following the previous methodology applied to Vivinal-GOS®.

2.3.Bacterial Strains

Lactobacillus bulgaricus ATCC7517 (LB), *Lactobacillus casei* ATCC11578 (LC), *Lactobacillus delbrueckii* subsp. *lactis* ATCC4797 (LD), *Lactobacillus plantarum* ATCC8014 (LP1), *Lactobacillus plantarum* WCFS1 (LP2) and *Lactobacillus sakei* 23K (LS) were purchased in lyophilized form and maintained at -80 ºC for long-term storage. All these strains are considered as probiotics as previously reported previously in different studies (Jain et al., 2004; Reid, 2008; Park et al., 2008).

Freeze-dried strains were grown in Lactobacilli MRS broth or in Lactobacilli MRS agar (EMD Chemicals, Gibbstown, NJ) at 37 °C in an anaerobic chamber (10% CO_2: 5% H_2: 85% N_2) (Coy Laboratory Products, Ann Arbor, MI) after transfer through an airlock with two exchanges of N_2 gas followed by one exchange of the oxygen-free mixed gas of the same composition as within the chamber.

2.4.*Growth conditions*

Bacteria were grown in MRS basal media carbohydrate free containing: 10 g L^{-1} protease peptone, 10 g L^{-1} beef extract, 5 g L^{-1} yeast extract, 1 g L^{-1} Tween 80, 2 g L^{-1} ammonium citrate, 5 g L^{-1} sodium acetate, 0.1 g L^{-1} magnesium sulphate, 0.05 g L^{-1} manganese sulphate, 2 g L^{-1} dipotassium sulphate and 0.5 g L^{-1} cysteine-HCl. Glucose, lactulose, GOS-La and GOS-Lu were dissolved in water (10 % w/v) and sterilized by filtration, this solution was added to MRS basal media to a final concentration of 1% w/v. The incubation was carried out under anaerobic conditions at 37 ºC. Inoculum was prepared from 48 h MRS grown *Lactobacillus* cells and approximately 1 x 10^7 CFU per mL of each *Lactobacillus* strain (individually) was added to the MRS basal media containing 1% w/v of glucose, lactulose, GOS-La or GOS-Lu and incubated under anaerobic conditions, at 37ºC during 24, 48, 72 and 120 hours. Viable count was carried out by plating on MRS agar in duplicate. All experiments were carried out in triplicate.

2.5.Lactic and acetic acid analyses

The incubated samples at 24, 48, and 72 h were centrifuged at 13,000 *g* for 10 min to remove all insoluble particles and the lactic and acetic acid fermentation products were quantified using a BioRad HPX-87H HPLC column (Watford, UK) at 50 ºC, with a 0.005

mM H_2SO_4 as the mobile phase, in isocratic mode, at a flow rate of 0.6 mL min^{-1} (Sanz et al., 2005). The analyses were carried out in triplicate.

Since minor levels of acetic acid were initially present in the MRS broth, this value was quantified and subtracted from the amounts calculated for the samples subjected to incubation.

2.6.Tolerance to different gastrointestinal conditions

One mL aliquots of cultures was taken after 48 h of fermentation as outlined previously and then centrifuged for 15 min, at 4 ºC and 8,000 rpm. The cells were washed twice using PBS buffer. The cell pellet was re-suspended in 1 mL of PBS pH 7.0 with: (i) bile extract (0.3 % w/v), or (ii) α-amylase (100 U mL^{-1}) or (iii) pancreatin (0.2 % w/v; a mixture of digestive enzyme secreted by the pancreas and commonly used to simulate the pancreatic juice present in the intestinal digestion), or (iv) 1 mL of saline solution adjusting the pH with HCl 0.1 M (0.85 % w/v; pH 2.5) for low pH studies. The percentage of survival was calculated from triplicate experiments using the following formula:

$$\% \text{ survival} = (\beta / \alpha) * 100$$

Where α is the CFU per mL of the assayed strain at 48 h and β the CFU per mL of the same strain after incubation with the different gastrointestinal conditions.

2.7.Hydrophobicity of bacteria

Hydrophobicity was determined following the method proposed by Kimoto-Nira et al. (2010) with some modifications. After 48 h of incubation the bacteria grown on the different substrates (glucose, lactulose, GOS-La and GOS-Lu) were washed and suspended in PBS in order to obtain an OD_{620} of 1.0. One millilitre of *n*-hexadecane was added to 1.0

mL of cell suspensions. The solution was incubated during 10 min at 30ºC, mixed during 60 s and then left to stand for 15 min. The aqueous phase was removed and the OD_{620} determined. The percentage of hydrophobicity was calculated using the following equation: 100 x [1-(Initial OD_{620}/OD_{620} after incubation with *n*-hexadecane]. The analyses were carried out by triplicate.

2.8.Statistical analyses

Statistical analyses were performed using Statistica for Windows version 6 (2002) by Statsoft Inc. (Tulsa, OK, USA). Differences between bacterial survival, % of hydrophobicity and lactic and acetic acid concentrations were tested using one-way ANOVA test, followed by a least significant difference (LSD) test as a post hoc comparison of means ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1.Growth of Lactobacillus strains with prebiotic sources

The growth profiles of six different *Lactobacillus* strains in the presence of lactulose, GOS-La, GOS-Lu are shown in Figure 1. Glucose was also included in this study for comparative purposes. All *Lactobacillus* strains grew during the first 24 h for all the substrates. Higher growth rates were observed for LC and LD with glucose and lactulose than with GOS-La and GOS-Lu substrates, whereas for LP1, LP2 and LS the initial growth rates were similar for all carbohydrates tested, and for LB the lowest initial growth was obtained with glucose. However, after this time, growth rates of all *Lactobacillus* strains decreased quickly when they were grown with glucose and lactulose, whilst all strains kept constant or were slightly modified with GOS-Lu and GOS-La. This response could be attributed to different reasons. It is known that carbohydrates with longer chain lengths are fermented more slowly (Cummings et al., 2001) which is in agreement with the fermentation kinetics of lactobacillus strains exhibited in presence of GOS-La and GOS-Lu (Figure 1). Likewise, this could also explain the initial higher growth observed for LC and LD with glucose and lactulose at 24 hours of incubation. However, no notable differences were detected between GOS-La and GOS-Lu for all fermentation times and strains. Similar behaviour has previously been reported in some bifidobacteria species, using fructooligosaccharides and inulin as the carbon sources, where the oligomers with high molecular weight promoted a higher bacterial growth than other substrates with lower molecular weight (Vernazza et al., 2006).

Conversely the metabolism of large carbohydrate molecules requires the use of glycosidases and specific transport mechanisms for the hydrolysis products (Vernazza et al., 2006). In *Lactobacillus* genus, the β-galactosidases are specifically located in the cytoplasm (Fortina et al., 2003) which implies that for the metabolization of GOS, *Lactobacillus* strains need a transport system in order to hydrolyze these oligosaccharides into the cell by β-galactosidases. This could explain the slower growth of LC and LD strains at 24 h with GOS-Lu and GOS-La compared with glucose and lactulose; however, for LP1, LP2 and LS, the similar values for initial growth provide evidence for a strain-dependence on the assimilation of carbon source.

Furthermore, it has been previously observed that the monomeric composition, polymerization degree and type of glycosidic linkages can affect the growth of probiotic strains (Rastall et al., 2005). GOS-La obtained from Vivinal-GOS® primarily consist of β-(1-4) linkages (Coulier et al. 2009; Rastall, 2010) and GOS-Lu consist of β-(1-6), being the most abundant trisaccharide 6´-galactosyl-lactulose (Hernandez-Hernandez et al., 2011). Cardelle-Cobas et al. (2011) when studying the effect of different trisaccharides isolated from GOS-Lu and GOS-La mixtures on different bacteria strains, including *Lactobacillus*, reported a preference for linkages β-(1-6) instead of β-(1-4); however, the results obtained in our work showed no differences in growth responses of *Lactobacillus* strains using GOS-Lu or GOS-La.

3.2.Lactic and acetic acid production

In general, for all strains and carbon sources tested, concentrations of lactic acid were higher than that of acetic acid (**Table 1**). *Lactobacillus* strains grown in glucose and lactulose generated higher concentrations of lactic acid than GOS-La and GOS-Lu, whilst similar levels of acetic acid were found for all assayed carbohydrates. The low amount of lactic acid produced in GOS grown culture could be due to the slower and prolonged fermentation by the bacterial strains. This could also have an influence on the higher survival rate of *Lactobacillus* strains grown in GOS substrates (Figure 1), as a lower acid production leads to less acidic pH values. No significant differences were, in general, detected among the different incubation times either for each carbohydrate or between GOS-La and GOS-Lu. Lactic and acetic acids are fermentation products of lactic acid bacteria (Lindgren and Dobrogosz, 1990). These acids decrease the pH and consequently can prevent the over growth of pathogenic bacteria in the intestine (Roy et al., 2006). Short chain fatty acids (SCFA) such as acetic and lactic acids are involved in multiple beneficial effects on the host. Acetic acid is metabolised by different human tissues representing a route to obtain energy from non-digestible carbohydrates (Roy et al., 2006; Roberfroid et al., 2010); however, lactic and acetic acids are assimilated by different species present in the gut microbiota, producing butyric acid which can be involved in multiple positive effects such as the reduction of colon cancer risk (Roy et al., 2006; Falony et al. 2009; Roberfroid et al., 2010).

These results support that *Lactobacillus* strains are able to hydrolyze GOS synthesized from lactose and lactulose, as well as lactulose to produce beneficial metabolites as final products.

3.3.Tolerance to different gastrointestinal conditions

The survival responses of the *Lactobacillus* strains, previously grown in the different carbohydrates tested, after 1 and 3 hours of being exposed to different gastrointestinal conditions are shown in **Tables 2** and **3**.

All the strains survived after 1 and 3 h of exposure to α-amylase and pancreatin treatments **(Table 2)**, although a significant decrease in survival of LS incubated with lactulose in the presence of amylase was observed. Survival rate values were greater than 100 % in some cases which could be due to the presence of low molecular weight carbohydrates in the commercial enzymatic preparations. Pitino et al. (2010) reported an increase on the survival of some strains of *L. rhamnosus* during simulation of duodenal digestion, due to the presence of a carbon source in the MRS broth used as the vehicle for digestion of the cells. Similar data were found by Kimoto-Nira et al. (2009) for *Lactococcus lactis* in media containing bile salts and lactose as carbon source.

Survival to bile extract appeared to be dependent on the carbon source and the *Lactobacillus* strain at both tested times (**Table 3**). After 1 hour a general decline in bacteria numbers was detected for all strains and carbon sources, with the exception of LB grown on glucose and lactulose and LP1 on lactulose. This decrease was greater at 3 hours of treatment. LC and LD exhibited the lowest survival rates for all carbohydrate sources, whereas LP1 was the most resistant strain.

Regarding LB, its survival after bile treatment was higher when it was incubated with glucose and lactulose, whereas LC survived better when it was incubated with GOS-Lu and GOS-La as compared to non-survival in the presence of glucose and lactulose after 3 hours of fermentation. LD grown on lactulose exhibited its highest survival rate in the presence of the bile extract. Lower significant differences in bile tolerance were detected for LP1, LP2 and LS grown on the different carbohydrate sources.

Charteris et al. (1998) reported that a level of survival higher than 30% would be considered intrinsically tolerant to gastric transit when using simulated gastric and pancreatic juices. Although the results presented here are based on resistance to bile extracts, this value could be considered to classify the *Lactobacillus* strains, tested for the different gastrointestinal conditions, as being as tolerant or not tolerant. Following this premise, most of the strains grown in the different carbohydrates used could be considered as bile tolerant, with the exception of LC using glucose and lactulose. Similarly, Fernandez et al. (2003) and Koll et al. (2010) reported tolerance to bile salts at 0.15 and 2% w/v, respectively, of different *Lactobacillus* strains grown in MRS agar.

Tolerance to gastric pH (2.5) expressed as % survival is shown in **Table 3**. In general, after 1 h of exposition, significant survival decreases were observed for all assayed strains. LB and LS grown on prebiotic carbohydrates exhibited a higher resistance to pH conditions than the strains grown on glucose, whereas LC and LD grown on glucose were more tolerant. LP1 and LP2 grown on lactulose or glucose exhibited higher resistance to low pH values. Although gastric emptying is strongly influenced by volume and composition of gastric contents, type of meal and/or gastrointestinal disorders (Bolondi et al., 1985), the average time for 50% of gastric emptying has been estimated to be approximately 1.2 hours (Read et al., 1986). This means that physiologically relevant levels of most of the studied *Lactobacillus* strains could be able to reach further down the gastrointestinal tract. Finally, at extreme exposure times to treatment (3 h), only LP2 grown on lactulose, GOS-La or GOS-Lu, LD grown on glucose and LS grown on GOS-Lu could be detected.

3.4.Hydrophobicity of bacteria

The percentage of hydrophobicity of all strains after 48h of fermentation is shown in Table 4. It is worth noting that LB, LC and LD grown with GOS-Lu exhibited the highest values of hydrophobicity, whereas hydrophobicity of LP1 and LS was higher when they were grown on GOS-La. Both prebiotic carbohydrates also contributed to the higher hydrophobicity values of LP2. Hydrophobic index of bacteria is related to their adhesion capacity to intestinal cells (Wadstrom et al., 1987). This capacity is necessary for the bacteria to colonize, at least temporally, the intestine and consequently, they may be considered as probiotics. Therefore, LB, LC and LD strains grown on GOS-Lu and LC, LP1 and LS strains grown on GOS-La could exhibit the higher adhesion capacity. It has also been reported that hydrophobicity index varies depending on the strain and the carbon source used (Kimoto-Nira et al., 2010) which is in good agreement with our results.

In conclusion, resistance to gastrointestinal conditions (mainly to bile extracts and gastric pH values) and bacterial hydrophobicity depend highly on carbohydrates used as carbon source and the *Lactobacillus* strain. Growth of some *Lactobacillus* strains on different prebiotics could help to increase their resistance to gastrointestinal conditions, thus, enhancing their survival through the gastrointestinal tract, as well as to promote their adhesion capacity. Additionally, food matrix effects may also contribute to the ability of a probiotic to survive through the gastrointestinal tract (Sanders and Marco, 2010). Thus, several studies have previously shown that the inclusion of milk-based products improved the resistance to gastrointestinal conditions of different probiotics including some *Lactobacillus* strains (Charteris et al, 1998; Fernández et al, 2003; Madureira et al, 2010; Martinez et al, 2011). A possible explanation for this response is that milk proteins could act as buffering agents and/or inhibitors of digestive proteases (Charteris et al., 1998). On the basis of these studies, it could be expected that the combined use of milk-based products and GOS-La or GOS-Lu might increase the survival of the assayed *Lactobacillus* strains. These findings may help to expand the applications of lactulose, and galactooligosaccharides derived from lactulose and lactose in synbiotic products with important applications in the design of new functional food ingredients.

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Table

Table 1. Lactic acid and acetic acid concentrations (mM) after 24, 48 and 72 h of fermentation using glucose, lactulose, GOS from lactulose (GOS-Lu) and from lactose (GOS-La). LB (*L. bulgaricus* ATCC7517), LC (*L. casei* ATCC11578), LD (*L. delbrueckii* subsp. Lactis ATCC4797), LP1 (*L. plantarum* ATCC8014), LP2 (*L. plantarum* WCFS1), LS (*L. sakei* 23K).

Carbon Source	Acid	Time (h)	LB	LC	LD	LP1	LP2	LS
Glucose	Lactic	24	209.70 (0.20)*^{efg}	178.54 (14.71)^{efg}	203.52 (1.92)^{efg}	184.33 (23.85)^{efg}	203.18 (5.57)^{efg}	189.12 (6.32)^{efg}
		48	228.67 (27.53)^{g}	197.93 (29.10)^{efg}	203.09 (0.44)^{efg}	184.55 (29.74)^{efg}	208.12 (0.40)^{efg}	192.89 (16.52)^{efg}
		72	214.89 (5.69)^{fg}	189.86 (23.11)^{efg}	199.74 (0.40)^{efg}	230.39 (41.87)^{g}	199.79 (3.62)^{efg}	198.20 (9.37)^{efg}
	Acetic	24	36.76 (2.89)^{abcde}	34.34 (2.17)^{abc}	33.61 (1.15)^{abc}	32.68 (4.37)^{ab}	32.82 (0.49)^{ab}	37.37 (4.84)^{abcde}
		48	42.65 (0.91)^{abcde}	31.71 (1.34)^{a}	31.97 (1.13)^{a}	33.67 (3.10)^{abc}	34.87 (0.22)^{abc}	35.42 (0.44)^{abcd}
		72	38.52 (3.94)^{abcde}	31.04 (0.14)^{a}	39.16 (14.93)^{abcde}	40.65 (8.53)^{abcde}	31.35 (0.89)^{a}	37.11 (2.68)^{abcde}
Lactulose	Lactic	24	180.67 (25.27)^{efg}	154.68 (3.47)^{de}	165.38 (3.50)^{efg}	199.46 (1.36)^{efg}	201.05 (6.86)^{efg}	158.91 (1.39)^{def}
		48	215.54 (26.31)^{fg}	208.51 (13.35)^{efg}	203.18 (7.44)^{efg}	204.92 (3.37)^{efg}	206.51 (1.45)^{efg}	195.12 (7.39)^{efg}
		72	202.73 (10.70)^{efg}	200.28 (3.02)^{efg}	204.26 (0.51)^{efg}	227.11 (36.28)^{g}	203.36 (6.14)^{efg}	202.85 (2.10)^{efg}
	Acetic	24	42.38 (0.90)^{abcde}	38.39 (1.57)^{abcde}	37.95 (1.93)^{abcde}	49.11 (16.90)^{abcde}	46.66 (1.25)^{abcde}	48.70 (5.52)^{abcde}
		48	49.46 (3.45)^{abcde}	37.95 (4.93)^{abcde}	32.41 (5.85)^{ab}	42.19 (1.08)^{abcde}	37.99 (6.26)^{abcde}	45.07 (3.18)^{abcde}
		72	42.96 (6.43)^{abcde}	35.03 (2.19)^{abc}	31.84 (0.56)^{a}	48.42 (6.61)^{abcde}	35.33 (3.20)^{abcd}	44.12 (7.82)^{abcde}
GOS-Lu	Lactic	24	69.20 (1.52)^{abc}	183.87 (29.23)^{efg}	42.90 (6.90)^{ab}	63.61 (0.94)^{abc}	66.72 (6.73)^{abc}	67.42 (4.53)^{abc}
		48	80.73 (4.40)^{abc}	40.42 (2.05)^{ab}	35.56 (9.37)^{ab}	75.06 (5.23)^{abc}	66.87 (6.21)^{abc}	65.58 (6.10)^{abc}
		72	77.09 (2.50)^{abc}	43.80 (0.77)^{ab}	44.51 (0.99)^{ab}	87.71 (12.00)^{abc}	67.30 (6.55)^{abc}	71.26 (8.22)^{abc}
	Acetic	24	43.02 (5.75)^{abcde}	42.49 (6.04)^{abcde}	42.04 (0.94)^{abcde}	63.07 (2.84)^{e}	31.61 (2.12)^{a}	58.52 (2.72)^{bcde}
		48	54.13 (2.07)^{abcde}	44.37 (2.05)^{abcde}	43.64 (3.40)^{abcde}	51.95 (0.09)^{abcde}	44.42 (13.69)^{abcde}	53.11 (0.12)^{abcde}
		72	48.56 (10.26)^{abcde}	42.32 (5.80)^{abcde}	52.51 (13.54)^{abcde}	59.21 (11.69)^{cde}	42.42 (14.22)^{abcde}	54.94 (2.83)^{abcde}
GOS-La	Lactic	24	65.66 (2.90)^{abc}	33.06 (11.05)^{a}	54.12 (1.89)^{abc}	46.91 (1.55)^{abc}	76.81 (2.04)^{abc}	77.96 (3.09)^{abc}
		48	83.71 (9.03)^{abc}	33.20 (10.09)^{a}	36.14 (12.14)^{ab}	102.97 (22.16)^{cd}	82.53 (0.36)^{abc}	80.25 (0.28)^{abc}
		72	76.73 (7.55)^{abc}	32.87 (9.92)^{a}	33.52 (10.84)^{a}	91.42 (16.87)^{bc}	85.21 (2.95)^{abc}	84.91 (1.25)^{abc}
	Acetic	24	45.22 (0.25)^{abcde}	40.21 (12.37)^{abcde}	46.47 (2.11)^{abcde}	57.36 (1.97)^{abcde}	61.59 (10.40)^{de}	64.06 (3.85)^{e}
		48	52.99 (2.45)^{abcde}	47.05 (1.43)^{abcde}	42.46 (1.66)^{abcde}	59.38 (9.52)^{cde}	52.35 (0.34)^{abcde}	52.84 (1.89)^{abcde}
		72	51.69 (0.32)^{abcde}	47.69 (1.31)^{abcde}	46.59 (2.88)^{abcde}	57.10 (10.38)^{abcde}	50.01 (1.82)^{abcde}	53.46 (1.79)^{abcde}

*Standard deviation in parentheses
Different letters indicate significant differences ($P \leq 0.05$) for each acid

Table 2. Survival (%) of strains grown in glucose, lactulose, GOS from lactulose (GOS-Lu) and from lactose (GOS-La) in the presence of α-amylase and pancreatin after 1 and 3 hours of fermentation.

Strain	% Survival			
Carbon source	α-amylase 1h	α-amylase 3h	Pancreatin 1h	Pancreatin 3h
LB				
Glucose	95.74 (2.02)$^{\S a}$	99.53 (2.59)^{a}	107.86 (2.95)^{b*}	115.95 (2.95)^{c*}
Lactulose	109.45 (1.97)^{b*}	99.31 (3.54)^{a}	119.96 (0.22)^{cd*}	125.06 (14.91)^{d*}
GOS-Lu	89.72 (0.84)^{c}	98.23 (11.03)^{a}	95.20 (2.82)^{a}	114.45 (1.46)^{c*}
GOS-La	105.55 (1.43)^{b*}	104.90 (3.74)^{b}	106.73 (3.82)^{b*}	108.00 (3.41)^{b*}
LC				
Glucose	100.78 (6.59)^{ab}	101.66 (6.48)^{a}	108.66 (5.95)^{b*}	104.60 (1.59)^{abc}
Lactulose	96.58 (0.23)^{a}	99.06 (2.12)^{a}	106.53 (4.72)^{b*}	105.58 (3.26)^{bc*}
GOS-Lu	108.20 (4.61)^{b*}	110.45 (2.46)^{*b}	120.02 (6.26)^{d*}	107.27 (6.98)^{b*}
GOS-La	105.22 (12.03)^{b*}	106.81 (10.80)^{a}	103.56 (0.21)^{abc}	100.54 (0.85)^{ab}
LD				
Glucose	105.66 (1.55)^{c*}	107.70 (3.15)^{c}	106.60 (2.23)^{b*}	111.23 (0.28)^{cd*}
Lactulose	103.99 (1.08)^{b}	106.48 (2.56)^{b}	106.71 (3.48)^{bc*}	107.65 (0.68)^{bc*}
GOS-Lu	93.91 (10.28)^{a}	93.05 (10.62)^{a}	105.81 (9.42)^{bc*}	113.19 (2.63)^{d*}
GOS-La	95.66 (5.18)^{a}	93.77 (3.93)^{a}	97.27 (3.54)^{a}	108.46 (0.56)^{bc*}
LP1				
Glucose	99.00 (3.13)^{a}	96.87 (2.47)^{a}	91.33 (5.80)^{b*}	91.28 (3.48)^{a*}
Lactulose	106.43 (0.17)^{bc*}	101.92 (0.22)^{a}	97.99 (13.74)^{ab}	99.73 (0.48)^{a}
GOS-Lu	105.09 (0.00)^{cd*}	97.80 (0.46)^{a}	100.75 (6.74)^{a}	101.05 (9.82)^{a}
GOS-La	106.98 (4.30)^{d*}	100.77 (1.35)^{a}	99.27 (4.05)^{ab}	97.63 (6.64)^{a}
LP2				
Glucose	98.09 (4.42)^{b}	92.63 (6.64)^{a*}	97.01 (2.52)^{a*}	95.82 (5.24)^{c*}
Lactulose	102.64 (3.15)^{c}	101.56 (3.08)^{bc}	98.60 (1.06)^{abcd}	98.00 (0.66)^{acd}
GOS-Lu	100.15 (3.20)^{bc}	100.72 (0.93)^{bc}	99.00 (1.67)^{abd}	101.02 (0.68)^{b}
GOS-La	101.34 (4.10)^{bc}	100.06 (3.09)^{bc}	99.87 (2.55)^{ab}	100.49 (1.53)^{ab}
LS				
Glucose	96.80 (0.98)^{b}	99.86 (5.95)^{b}	101.55 (3.13)^{ab}	104.64 (0.57)^{ab}
Lactulose	76.71 (0.87)^{a*}	87.68 (4.92)^{a}	100.16 (0.28)^{a}	101.85 (0.40)^{a}
GOS-Lu	108.21 (4.59)^{c*}	101.90 (2.18)^{b}	106.31 (4.40)^{b*}	108.58 (5.16)^{b*}
GOS-La	104.23 (6.56)^{c}	100.18 (2.23)^{b}	101.67 (7.82)^{ab}	103.05 (6.00)^{a}

[§]Standard deviation in parentheses
Different letters indicate significant differences ($P \leq 0.05$) for each strain and treatment
[*]Significant differences with 0 hours for each strain and treatment

Table 3. Survival (%) of strains grown in glucose, lactulose, GOS from lactulose (GOS-Lu) and from lactose (GOS-La) in the presence of bile extract and low pH after 1 and 3 hours of fermentation.

Strain	% Survival			
Carbon source	Bile extract 1h	Bile extract 3h	Low pH 1h	Low pH 3h
LB				
Glucose	100.50 (1.41)$^{\S a}$	96.94 (1.16)^{a}	28.87 (1.16)^{b*}	ND
Lactulose	98.36 (0.02)^{a}	98.45 (2.83)^{a}	72.90 (12.23)^{a*}	ND
GOS-Lu	78.41 (4.96)^{d*}	43.19 (6.04)^{b*}	74.61 (1.61)^{a*}	ND
GOS-La	87.95 (1.36)^{e*§}	62.01 (0.82)^{*c}	72.34 (0.86)^{a*}	ND
LC				
Glucose	29.84 (5.77)^{a*}	ND	29.85 (8.73)^{a*}	ND
Lactulose	30.97 (4.83)^{a*}	ND	21.91 (3.44)^{b*}	ND
GOS-Lu	45.08 (6.69)^{c*}	38.15 (4.27)^{b*}	ND	ND
GOS-La	44.60 (4.57)^{c*}	33.31 (0.85)^{ab*}	ND	ND
LD				
Glucose	55.84 (0.38)^{b*}	38.78 (11.65)^{a*}	60.63 (0.41)^{d*}	28.03 (3.00)^{e*}
Lactulose	72.93 (6.77)^{d*}	63.45 (7.77)^{c*}	42.05 (0.29)^{b*}	ND
GOS-Lu	37.63 (1.26)^{a*}	35.88 (1.40)^{a*}	55.21 (6.35)^{c*}	ND
GOS-La	52.02 (0.13)^{b*}	33.22 (1.41)^{a*}	37.94 (1.08)^{a*}	ND
LP1				
Glucose	95.51 (3.69)^{de*}	90.48 (2.30)^{bc*}	77.58 (2.48)^{a*}	ND
Lactulose	99.69 (0.36)^{a}	99.57 (0.36)^{ae}	73.82 (3.41)^{a*}	ND
GOS-Lu	92.68 (6.73)^{cd*}	90.07 (4.30)^{bc*}	22.08 (0.01)^{c*}	ND
GOS-La	89.93 (4.46)^{bc*}	87.66 (1.92)^{b*}	25.76 (0.12)^{b*}	ND
LP2				
Glucose	84.45 (2.04)^{ab*}	77.81 (0.99)^{c*}	69.56 (7.01)^{f*}	ND
Lactulose	85.29 (0.73)^{ab*}	81.13 (1.04)^{de*}	77.39 (5.92)^{f*}	29.81 (2.42)^{cd*}
GOS-Lu	85.96 (2.51)^{b*}	79.14 (5.15)^{cd*}	52.87 (2.36)^{e*}	19.58 (0.13)^{ab*}
GOS-La	82.45 (1.18)^{ae*}	73.51 (4.04)^{f*}	42.75 (13.00)^{de*}	33.92 (0.79)^{bc*}
LS				
Glucose	80.36 (3.32)^{a*}	81.58 (7.00)^{a*}	ND	ND
Lactulose	82.30 (1.37)^{a*}	82.08 (2.82)^{a*}	ND	ND
GOS-Lu	87.09 (3.56)^{b*}	87.54 (1.38)^{b*}	42.04 (0.22)^{b*}	23.68 (0.03)^{a*}
GOS-La	83.66 (0.23)^{a*}	83.31 (1.69)^{a*}	46.05 (5.46)^{b*}	ND

[§]Standard deviation in parentheses
Different letters indicate significant differences ($P \leq 0.05$) for each strain and treatment
[*]Significant differences with 0 hours for each strain and treatment
ND no detected

Table 4. Hydrophobicity (%) of strains grown in glucose, lactulose, GOS from lactulose (GOS-Lu) and from lactose (GOS-La).

Carbon source	**% Hydrophobicity**					
	LB	*LC*	*LD*	*LP1*	*LP2*	*LS*
Glucose	46.76 (8.40)[h§]	0.00 (0.00)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	13.35 (1.29)[cd]
Lactulose	0.00 (0.00)[a]	6.65 (1.44)[abc]	0.00 (0.00)[a]	0.00 (0.00)[a]	21.55 (8.91)[e]	29.76 (11.97)[f]
GOS-Lu	64.05 (14.11)[i]	79.47 (6.47)[j]	80.09 (0.73)[j]	0.00 (0.00)[a]	28.75 (5.19)[f]	15.40 (5.29)[d]
GOS-La	3.73 (0.17)[ab]	62.72 (1.50)[i]	0.00 (0.00)[a]	66.38 (4.45)[i]	27.90 (2.38)[f]	48.57 (2.76)[h]

*Standard deviation
[§] Different letters indicate significant differences ($P \leq 0.05$) for each acid
VL: Vivinal-GOS purified and GOS: galactooligosaccharides from lactulose purified.

Figure captions

Figure 1. Growth of lactobacillus strains in MRS containing different carbohydrates carbon source. (♦) Glucose, (■) Lactulose, (▲) GOS from lactulose, (X) GOS from lactose. LB (*L. bulgaricus* ATCC7517), LC (*L. casei* ATCC11578), LD (*L. delbrueckii* subsp. Lactis ATCC4797), LP1 (*L. plantarum* ATCC8014), LP2 (*L. plantarum* WCFS1), LS (*L. sakei* 23K).

Figure 1. Hernandez-Hernandez et al.