Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue4-fulltext-9 *RESEARCH ARTICLE*

Effect of isomalto-oligosaccharide and gentio-oligosaccharide on the growth and fatty acid profile of *Lactobacillus plantarum*

Carmen Soto[1,2] ✉

1 Centro Regional de Estudios en Alimentos Saludables, CONICYT-Regional GORE, Curauma, Valparaíso, Chile

2 Pontificia Universidad Católica de Valparaíso, Facultad de Ingeniería, Escuela de Ingeniería Bioquímica, Valparaíso, Chile

✉ Corresponding author: carmensoto@creas.cl
Received March 4, 2013 / Accepted June 4, 2013
Published online: July 15, 2013

Abstract

Background: *Lactobacillus* sp. are probiotic microorganisms, and some of them are able to produce conjugated linoleic acid (CLA) via the bio-hydrogenation of linoleic acid (LA). Both CLA and LA are polyunsaturated fatty acids commonly used in the prevention and control of cardiovascular disease, high cholesterol, and cancer, among other ailments. The carbon source is one variable that can affect the growth and characteristics of these bacteria. Molecules called prebiotics are known to benefit human health by stimulating the growth and activity of probiotic bacteria present in the intestinal microflora. The aim of this study was to evaluate how different oligosaccharides affect the growth and fatty acid profile of *Lactobacillus plantarum* (NRRL - B4496). *L. plantarum* cultivation was performed in Man-Rogosa-Sharpe (MRS) medium, and the original carbon source (glucose) in this medium was partially or totally replaced by an oligosaccharide (isomalto-oligosaccharide (IMO) or gentio-oligosaccharide (GTO)). Then, the biomass concentration and fatty acid profile were determined using spectrophotometry and gas chromatography, respectively.
Results: When 50% of the glucose in the MRS medium was replaced with IMO, the maximum growth was 2.6 g/L at 37ºC. Under the same culture conditions, the incorporation of GTO only produced 2 g/L of biomass. At 45ºC, the growth of the bacterial culture was lower than that observed at 37ºC, reaching only 0.4 g/L. When cultivated at 37ºC in a mixture of glucose and GTO (1:1), CLA (34%, c9t11) was obtained from cells of *L. plantarum*. However, when the cultivation was performed at 45ºC, CLA was not obtained. When IMO was used, differences in CLA content were not observed between *L. plantarum* cultivated with glucose or with IMO present; however, vaccenic acid was produced.
Conclusions: *Lactobacillus plantarum* grow well when a mixture of IMO and glucose is used as the carbon source. However, this mixture does not improve the CLA content, most likely due to high enzymatic activity that promotes the conversion of CLA to vaccenic acid. Additionally, GTO is likely less readily metabolized by this strain. Thus, the enzymatic activity is likely lower and less CLA is converted to vaccenic acid, resulting in an accumulation of CLA.

Keywords: CLA, fatty acid profile, *Lactobacillus plantarum*, oligosaccharides.

INTRODUCTION

Bacteria, such as *Lactobacillus*, are used in the food industry to produce fermented vegetables. These bacteria are known as probiotics (Nowroozi et al. 2004; Maragkoudakis et al. 2006), which according to the FAO and the WHO are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". This type of microorganism has been added to several foods, and its intake has been shown to be beneficial to humans (Oliveira et al. 2009; Reid et al. 2011).

In addition, some of these microorganisms are capable of producing several bioactive molecules, such as antihypertensive amino acids (Hayakawa, 2010) and conjugated linoleic acid (CLA) (Ogawa et al. 2005), among others. CLA is an isomer of linoleic acid that has two double bonds and that can exist in either the *cis* or *trans* configuration. CLA is considered a good fat, and it is used in the prevention and control of conditions such as cardiovascular disease, high cholesterol and cancer (Ip et al. 2003; Bhattacharya et al. 2006; Wahle et al. 2008). Because CLA can be produced by microorganisms, this fatty acid is often found in foods that contain lactic bacteria, such as yogurt, fermented milk and cheese (Prandini et al. 2007).

Ogawa et al. (2005) reported that at least nine strains of *Bifidobacterium*, five strains of *Lactobacillus*, one strain of *Propionibacterium* and one strain of *Megasphaera* have been used for CLA production employing different reaction methods and substrates. Dong and Qi (2006) evaluated the feasibility of producing CLA using *Lactobacillus acidophilus* 11854 strains in cultures containing whole milk and alfalfa seeds. Additionally, *Lactobacillus plantarum*, a microorganism found in many food products as well as in the human gastrointestinal tract (Ningegowda and Gurudutt, 2012), is capable of producing a high amount of CLA (Ando et al. 2003; Kishino et al. 2003; Ogawa et al. 2005) with linoleic and ricinoleic acid as substrates.

The growth of such microorganisms is dependent on several variables. For example, their growth can be affected by the composition of the culture medium, especially the carbon source. Because most lactic bacteria are gut bacteria, they predominantly use foodstuffs that have not been absorbed in the upper gastrointestinal tract, such as resistant starch, dietary fiber, oligosaccharides and proteins, as substrates (Goderska et al. 2008). Oligosaccharides are composed of between two and nine monosaccharides linked through glycosidic bonds, and some of these molecules are known to be prebiotic compounds. Such compounds are non-digestible and beneficially affect the organism and improve its health by stimulating the growth and activity of one or more bacterial strains in its colon (Wrolstad, 2012).

These oligosaccharides affect bacteria in several ways. Hernández-Hernández et al. (2012) evaluated the effect of galacto-oligosaccharides (GOS) on the growth of different *Lactobacillus* strains and their tolerance to a variety of gastrointestinal conditions. In general, they observed the best growth (determined by looking at log CFU/mL) when GOS derived from lactulose or lactose were used. Additionally, galacto-oligosaccharides can function as a protective molecule in the preservation (freeze-dried or dried) of *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tymczyszyn et al. 2011). Furthermore, oligosaccharides derived from soy sauce lees had a dose-dependent effect on the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Yang et al. 2011).

Akalin et al. (2007) evaluated the effect of enrichment with fructo-oligosaccharides (FOS) when yogurt was produced with starter culture (*Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) and different probiotics (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) were added. When yogurt was elaborated in the presence of FOS and *B. animalis*, the CLA content increased from 2.45 and 0.06 mg/g fat to 5.68 and 0.19 mg/g fat for CLA isomers c9t11 and t10c12, respectively, on the first day of storage. In comparison, the total CLA content for yogurt that was conventionally prepared was 2.07 mg/g fat. The incorporation of prebiotics alone increases the production of CLA, but to levels that are much lower than when FOS is also used. Furthermore, the authors indicated that the value for c9t11 content is almost constant with time of storage. In contrast, t10c12 CLA content decreased considerably. Rodrigues et al. (2012) reported the effect of the prebiotic type (fructo-oligosaccharides (FOS) and inulin) on probiotic bacteria (*L. casei* or *B. lactis*). In the free fatty acid (FFA) profile of cheese, they observed that prebiotic inclusion produced FFAs, especially CLA. In other studies, oligosaccharides, such as maltodextrin, polydextrose and oligofructose, had different effects on the growth of *L. acidophilus, L. bulgaricus, L. rhamnosus* and *B. lactis* when these bacteria were used in co-culture with *S. thermophilus* for the fermentation of skim milk. In general, the use of polydextrose and oligofructose resulted in a greater increase of biomass with respect to the control samples. The CLA content was dependent on the prebiotic and the probiotic applied in the skim milk fermentation. They observed that the CLA content was greater than 20% when prebiotics were incorporated (Oliveira et al. 2009).

The objective of this study was to establish the effect of different gluco-oligosaccharides on the growth and fatty acid profile of *Lactobacillus plantarum* and to obtain probiotic biomass with nutraceutical fatty acids.

MATERIALS AND METHODS

Microbial strain and culture medium

The microorganism *Lactobacillus plantarum* NRRL - B4496 was donated by ARS-USDA. Man-Rogosa-Sharpe (MRS) medium was used; this medium is composed of casein peptone (10 g/L), meat extract (10 g/L), yeast extract (5 g/L), glucose (20 g/L), Tween 80 (1 g/L), sodium acetate tri-hydrate (5 g/L), ammonium citrate (2 g/L), di-sodium phosphate (2 g/L), sulphate hepta-hydrate magnesium (0.2 g/L), manganese sulphate mono-hydrate (0.5 g/L) and micro-elements.

This culture medium was modified by replacing the glucose with oligosaccharides (10 or 20 g/L), isomalto-oligosaccharide (IMO) or gentio-oligosaccharide (GTO) (Wako Pure Chemical Industries Ltd., USA). IMO composition, determined by liquid chromatography (HPLC-IR) was 5.2% DP7 (isomaltoheptaose), 1.4% DP6 (isomaltohexaose), 3.6% DP5 (isomaltopentaose), 8.5% DP4 (isomaltotetraose), 18.6% DP3 (isomaltrotriose), 53.2% DP2 (isomaltose), 6.1% glucose, and 3.4% others; whereas in the case of GTO is possible to observe 3.5% DP7 (gentioheptaose), 0.3% DP6 (gentiohexaose), 3.8% DP5 (gentiopentaose), 9.3% DP4 (gentiotetraose), 28.6% DP3 (gentiotriose), 48.7% DP2 (gentiobiose), 5.4% of glucose, and 0.4% of others. It is important to consider that IMO is produced by starch hydrolysis and GTO by synthesis from glucose, so it is the presence of this sugar.

Microbial growth and consumption of carbon source

Lactobacillus cultivation was performed in batch culture in an aerobic environment using 150 rpm agitation. The initial pH was 6.5 and was leave free. To evaluate the effect of the temperature on the growth of *L. plantarum* and its production of CLA, the cultures were maintained at 37ºC, the optimum growth temperature according to the supplier, or at 45ºC to increase the oligosaccharide solubility in the culture medium. Microbial growth was measured by spectrophotometry at 600 nm using a calibration curve developed with the same strain and verified by gravimetry.

The content of the various oligosaccharides was determined using a BP-100 Carbohydrate Ag+ column (Benson Polymerics Inc., USA) in a Perkin Elmer Series 200 HPLC with RI Detector with water as the mobile phase. IMO and GTO components variation was determined using an Oligosaccharide kit (Supelco, USA) as standard, which contains sugars between DP3 and DP7 degree of polymerization. Particularly, in this case maltotriose (DP3), isomaltotriose (DP3) maltotretraose (DP4), maltopentaose (DP5), maltohexaose (DP6) and maltoheptaose (DP7) were used. Also, maltose (DP2) and glucose were used.

Fatty acid profile

The fatty acid profile was determined after a steady state was reached in each experiment. The biomass was harvested, centrifuged and separated from the liquid medium. Briefly, the cells were ground using micro-glass beads and then vortexed for 5 min. Next, 1 mL of methanolic NaOH (0.5 N) was added, and the mixture was vortexed for 1 min. The samples were sealed and placed in a boiling water bath for 10 min. Then, they were cooled and 1 mL of boron trifluoride methanol solution (20%, Merck Chemicals, USA) was added. The mixtures then were placed in a boiling water bath for 20 min and cooled again. Next, 2 mL of a saturated solution of NaCl was added to each sample, and the sample was agitated. Finally, 1 mL of n-hexane was added, and the sample was mixed for 3 min. The samples were centrifuged at 2000 rpm for 5 min. The methyl fatty acids were recovered with n-hexane and injected into a gas chromatograph.

Fatty acid profile determination was performed using a Perkin Elmer Clarus 600 gas chromatograph equipped with a FID detector and a Restek Rtx-2330 column. The injector and detector were maintained at 220ºC and 250ºC, respectively, using the temperature gradient in the furnace. Nitrogen was used as the carrier gas. FAME MIX 37 (Supelco, USA), CLA 10t-12c and 9c-11t (Sigma Aldrich, USA), and the methyl ester of linoleic acid (Supelco, USA) were used as external standards.

RESULTS AND DISCUSSION

Figure 1 shows the effect of IMO incorporation on cultures of *L. plantarum* at two different temperatures (37ºC and 45ºC). At 37ºC, the microorganisms showed similar growth behaviours whether only glucose or a mixture of glucose and the oligosaccharide were used. In both cases, a maximum cell concentration of 2.7 g/L was reached after 30 hrs of treatment; the specific cellular growth rate was 0.204 h^{-1}. When the culture was performed only with IMO, the biomass reached 2.3 g/L with a specific cellular growth rate of 0.149 h^{-1}. IMO is a type of sugar or saccharide composed of glucose monomers linked by α (1-6) glycosidic bonds; types of IMOs include isomaltose, isomaltotriose and isomaltotetraose. *Lactobacillus* strains produce oligo 1-6 glucosidase enzymes, which hydrolyze IMOs, especially isomaltose, to D-glucose. Thus, similar results were obtained whether a mixture of carbon sources or only glucose was used in the culture medium. However, when only the oligosaccharide was used, the results were lower, likely because glucose induces the production of the oligo 1-6 glucosidase enzyme and the monosaccharide is required.

When the culture temperature was increased to 45ºC (Figure 1), the maximum cell concentration was less than that observed at 37ºC, reaching only 1.1 g/L. Furthermore, a different behaviour was observed; better results were obtained for cultures where both IMO and glucose were added. The elevated culture temperature likely results in hydrolysis of the carbon source (glucose monomers) and its release into the medium where it is consumed by *Lactobacillus*. Thus, growth was improved compared to when only glucose was added, and the maximum growth rate only reached values between 0.015 and 0.083 h^{-1}.

These results are in agreement with the pH values. When the culture was performed at 37ºC with only IMO, the pH values decreased from 6.6 to 4.3. When the culture was performed with only glucose or a mixture of glucose and IMO, the end pH values were approximately 3.8. In the case of cultivation at 45ºC, the pH decreases in all cases only reaching a value of 4.4, which is consistent with the slower growth observed under these conditions.

As shown in Figure 2, when the culture was performed with only the oligosaccharide, the disaccharide (isomaltose) content was found to be important and there was a small presence of monosaccharide (observed at higher elution times) that allowed for bacterial growth. Additionally, an increased presence of oligosaccharides of size DP5 (at 8.3 min) was observed at 50 hrs of culture time, probably because hydrolysis of DP7 (isomaltoheptaose) or greater components of IMO, which were consumed in a 33% about. Also, was observed the total disappearance of the monomers (at 14 min in the chromatogram) at 50 hrs of culture time. If a mixture of IMO and glucose was used as the carbon source in the culture of *L. plantarum*, both sugars (IMO components and glucose) were observed and the glucose was completely consumed during the fermentation. In addition, oligosaccharides ranging in size from DP2 to DP5 were observed, producing a DP5 increase due to hydrolysis of DP7, and a small decreased of DP2 - DP3 compounds after 50 hrs of cultivation.

Goderska et al. (2008) established the following trend for the growth of *L. acidophilus* (under anaerobic culture conditions) in MRS with an added carbon source: glucose > sucrose > lactose. The incorporation of other more complex sugars, such as raffinose and Raftilose®, did not produce good results.

When gentio-oligosaccharide (GTO) was used as the carbon source, a lag time of approximately 6 hrs was observed. The growth observed when only the oligosaccharide was used was similar to that observed when a mixture of glucose and GTO was used (Figure 3). In both cases, the maximum biomass obtained was 1.89 ± 0.02 g/L at 50 hrs. Prior to 20 hrs of culture, the growth rates were 0.171 h^{-1} and 0.127 h^{-1}, respectively, lower than those obtained via the addition of IMO. Additionally, two stages of growth were observed. This behaviour is most likely due to early glucose or monosaccharide consumption, which is also observed when commercial GTO formulations and larger saccharides, such as GTO (di-, tri- and tetra-saccharides), are used. At 45ºC, the culture of *L. plantarum* follows the same behaviour independent of which of the three carbon sources are used (only glucose, only GTO and glucose plus GTO). The biomass concentration was lower than that observed when the culture was performed at 37ºC, reaching a maximum of 0.44 g/L when only the oligosaccharide was used. In the same way, the maximum growth rate was low (0.033 h^{-1}).

The decrease in pH was correlated with the *L. plantarum* growth level. When the culture was performed at 45ºC and 37ºC, the pH was 4.3 and 3.8, respectively, further confirming that faster growth occurred under the latter condition. Cultures at lower temperatures (30ºC) were not performed because preliminary studies demonstrated that under these conditions there was a lag period of approximately 20 hrs in which CLA production was not observed.

Both GTO and IMO are composed of glucose monomers (2 to 4 units of glucose mainly), and the hydrolysis of these molecules results in *L. plantarum* growth. Figure 3 shows that at 45ºC, the best growth is observed when only GTO is used. At this temperature, the compound is partially hydrolyzed into glucose monomers. In addition, Figure 4 shows a significant decrease (58%) in the presence of oligosaccharides of a size equal to or greater than DP7, DP2 - DP3 oligosaccharides (69% of decrease) and an appearance of molecules DP5 in size, especially in the case of the culture where only GTO was added. A similar behaviour was observed in the case of cultures where a mixture of saccharides (glucose plus GTO) was used; a decrease (19%) in the presence of oligosaccharides DP7 in size and a total consumption of glucose was observed.

The structure of the oligosaccharide plays an important role in its consumption. In this study, two oligosaccharides composed of between 2 to 4 glucose units were evaluated. As mentioned above, IMOs can be composed of glucose units linked by α (1-6) bonds, while GTOs can be composed of glucose units linked by β (1-6) bonds. Kaplan and Hutkins (2003) reported that *L. paracasei* was able to consume FOS composed of between 2 and 4 fructose units. The microorganisms transport the oligosaccharides inside the cell and then hydrolyze them. This transportation occurs quickly for smaller molecules. Some monosaccharides, such as glucose and fructose, are highly metabolized and inhibit FOS consumption. On the other hand, Gänzle and Follador (2012) indicated that almost all *Lactobacillus* strains are able to metabolize α-glucans. *L. plantarum* produces endo-amylase, which hydrolyzes α (1-6) glycosidic bonds; *L. plantarum* also can hydrolyze β (1-6) glycosidic bonds. According to Saminathan et al. (2011), there are several differences in the growth of *Lactobacillus* strains obtained from the gastrointestinal tracts of chickens (*L. reuteri, L. gallinarum, L. brevi, L. salivarius*). They observed the following trend in growth: IMO > GOS > GTO > FOS. However, the best biomass production was observed when glucose was used alone, suggesting that individual *Lactobacillus* strains each possess specific enzymatic activities and transport systems that allow them to use prebiotic compounds. Barrangou et al. (2003) shows that *L. acidophilus* genome has an operon of the lacc1 family, which expression is induced by sucrose and FOS, but not by glucose or fructose, suggesting some specificity for non-readily fermentable sugars.

The oligosaccharides present in the culture medium of *L. plantarum* not only affect their growth rate and maximum concentration but also their intracellular fatty acid profile. At 37ºC, the production of conjugated linoleic acid (CLA) is higher (34.48% vs. 24.25%) and the production of stearic and linolenic acid is lower when a mixture of glucose and GTO was used compared to when glucose was used alone. Rodrigues et al. (2012) indicated that FOS incorporation in cheese production resulted in a CLA content of 0.4 mg CLA/g_{cheese} after 60 days of ripening. In comparison, when FOS was added to the culture of *Lactobacillus casei* 0.1 mg CLA/g_{cheese} was produced. In addition, gamma-linolenic and alpha-linolenic fatty acids were produced.

When IMO was used as a supplementary carbon source, CLA production was not increased compared to cultures performed with only glucose, but the production of vaccenic acid was observed (Table 1). This result can be explained by an improvement in enzymatic activity, in which sequential reduction steps (isomerization, hydrogenation) convert linoleic acid to CLA and then to vaccenic acid (Khanal and Dhiman, 2004; Aydin, 2005). These reactions are induced by the presence of IMO. Kishino et al. (2003) reported higher CLA production by the *L. plantarum* strain when fructose was used in the culture medium in comparison to maltose or glucose. Li et al. (2013) reported that microorganism growth and CLA production were affected when *Lactobacillus acidophilus* F0221 was fermented using different carbon sources (oligosaccharides GOS, IMO, FOS, XOS and inulin). The best results of growth (measured as absorbance at 600 nm) were observed when glucose and galactose were used as the monosaccharide and GOS was used as the non-digestible carbohydrate. Similarly, for the production of CLA, the best results were obtained with galactose and GOS followed by glucose. In the case of prebiotic carbohydrates, CLA production trended as follows: GOS > IMO> inulin > FOS > XOS. On the other hand, Pan et al. (2009) evaluated the effect of feeding oligosaccharides to mice. They observed that the concentration and profile of short chain fatty acids and the *Lactobacillus* concentration in the cecum of mice changes with the oligosaccharide used.

Table 1. Effect of oligosaccharide incorporation in fatty acid profile of *L. plantarum* cultivated at 37ºC.

Fatty acid	Glucose	Glucose + IMO	Glucose + GTO
Miristic (14:0)	3.68%	7.39%	3.20%
Stearic (18:0)	16.28%	11.72%	11.71%
Vaccenic/Oleic (18:1)	6.31%	10.77%	6.68%
Linoleic (18:2)	28.72%	24.63%	29.70%
Linolenic (18:3)	7.52%	4.92%	5.40%
CLA (18:2)	24.25%	23.7%	34.48%
20:3	6.65%	0	5.33%
Others	6.59%	16.87%	3.5%

Several authors (Pan et al. 2009; Rodrigues et al 2012; Li et al. 2013) reported that the use of different oligosaccharides produces an effect on fatty acid profile, including the use of oligosaccharides with similar structure, as FOS and Inulin. On the other hand, Macfarlane and Macfarlane (2002) report some factors that affect fatty acid production by intestinal bacteria, such as substrate composition and the amount of substrate available. The carbohydrate availability can affect the pyruvate metabolism (Macfarlane and Macfarlane, 2002), and consequently the others metabolic pathways in the bacteria. In addition, it is important to note that, although it is known that *Lactobacillus* strains possess enzymes capable of degrading IMO (oligo alpha 1-6 glucosidase) and GTO (beta 1-6 glucosidase), the expression level and/or enzyme activity of these may be different, and thus, produce differences in the availability of readily metabolizable carbohydrate. Due to these facts, the fatty acid profile could be affected by the type of oligosaccharide used in the cultivation of *Lactobacillus* strains.

In the fatty acid profiles obtained for microorganisms cultivated at 45ºC, CLA was not observed (results not shown). This result suggests that 37ºC is a more appropriate temperature to generate enzymes, such as linoleic acid isomerase, that produce CLA. In comparison to the fatty acid profiles obtained at 37ºC, a high amount of linoleic (approximately 40%) and linolenic acids (approximately 11%) was obtained.

These results are in accordance with those reported by Dong and Qi (2006) for *L. acidophilus*. They found the incubation temperature to have a strong effect on CLA production, and they obtained the best results at 37ºC, observing a decrease of the fatty acid production at other temperatures. Guerzoni et al. (2001) exposed *L. helveticus* strains to sub-lethal conditions of temperature, salt concentration, peroxide concentration and pH and observed that desaturase activation or hyper-induction was a response to heat. The strains produced intracellular oleic, linoleic and palmitic fatty acids as a function of the culture temperature. The temperature also had a significant effect on *Lactobacillus* strains. Matagaras et al. (2003) reported that incubation temperature between 20 and 30ºC did not affect *L. curvatus* growth, but had an effect on bacteriocin production.

CONCLUDING REMARKS

Oligosaccharides, such as IMO, can be used to grow *Lactobacillus plantarum* when they are mixed with glucose. However, using these mixtures did not improve the CLA content, most likely due the activity of enzymes that promote the conversion of CLA to vaccenic acid. Additionally, GTO is less easily metabolized by this strain, and lower enzyme activity results in an accumulation of CLA.

Financial support: Project FONDECYT 11080254; PUCV project 203.769.

REFERENCES

AKALIN, A.S.; TOKUSOGLU, O.; GÖNC, S. and AYCAN, S. (2007). Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. *International Dairy Journal*, vol. 17, no. 9, p. 1089-1095. **[CrossRef]**

ANDO, A.; OGAWA, J.; KISHINO, S. and SHIMIZU, S. (2003). CLA production from ricinoleic acid by lactic acid bacteria. *Journal of the American Oil Chemists Society,* vol. 80, no. 9, p. 889-894. **[CrossRef]**.

AYDIN, R. (2005). Conjugated linoleic acid: Chemical structure, sources and biological properties. *Turkish Journal of Veterinary Animal Science*, vol. 29, no. 2, p. 189-195.

BARRANGOU, R.; ALTERMANN, E.; HUTKINS, R.; CANO, R. and KLAENHAMMER, T.R. (2003). Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 15, p. 8957-8962. **[CrossRef]**

BHATTACHARYA, A.; BANU, J.; RAHMAN, M.; CAUSEY, J. and FERNANDES, G. (2006). Biological effects of conjugated linoleic acids in health and disease. *The Journal of Nutritional Biochemistry*, vol. 17, no. 12, p. 789-810. **[CrossRef]**

DONG, M. and QI, S. (2006). Conjugated linoleic acid production by fermentation. *International Journal of Food Engineering*, vol. 2, no. 4. **[CrossRef]**

GÄNZLE, M.G. and FOLLADOR, R. (2012). Metabolism of oligosaccharides and starch in lactobacilli: A review. *Frontiers in Microbiology*, vol. 3, no. 340, p. 1-15. **[CrossRef]**

GODERSKA, K.; NOWAK, J. and CZARNECKI, Z. (2008). Comparison of the growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* species in media supplemented with selected saccharides including prebiotics. *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 7, no. 2, p. 5-20.

GUERZONI, M.E.; LANCIOTTI, R. and COCCONCELLI, P.S. (2001). Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. *Microbiology*, vol. 147, no. 8, p. 2255-2264.

HAYAKAWA, K. (2010). Synthesis of antihypertensive GABA-enriched dairy products using lactic acid bacteria. In: BAGCHI, D.; LAU, F.C. and GHOSH, D.K. eds. *Biotechnology in Functional Foods and Nutraceuticals*, CRC Press, Taylor and Francis Group, EEUU, p. 349-360.

HERNÁNDEZ-HERNÁNDEZ, O.; MUTHAIYAN, A.; MORENO, F.J.; MONTILLA, A.; SANZ, M.L. and RICKE, S.C. (2012). Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*. *Food Microbiology*, vol. 30, no. 2, p. 355-361. **[CrossRef]**

IP, M.M.; MASSO-WELCH, P.A. and IP, C. (2003). Prevention of mammary cancer with conjugated linoleic acid: Role of the stroma and the epithelium. *Journal of Mammary Gland Biology and Neoplasia*, vol. 8, no. 1, p. 103-118. **[CrossRef]**

KAPLAN, H. and HUTKINS, R.W. (2003). Metabolism of fructooligosaccharides by *Lactobacillus paracasei* 1195. *Applied and Environmental Microbiology*, vol. 69, no. 4, p. 2217-2222. **[CrossRef]**

KHANAL, R.C. and DHIMAN, T.R. (2004). Biosynthesis of conjugated linoleic acid (CLA): A review. *Pakistan Journal of Nutrition*, vol. 3, no. 2, p. 72-81. **[CrossRef]**

KISHINO, S.; OGAWA, J.; ANDO, A.; IWASHITA, T.; FUJITA, T.; KAWASHIMA, H. and SHIMIZU, S. (2003). Structural analysis of conjugated linoleic acid produced by *Lactobacillus plantarum*, and factors affecting isomer production. *Bioscience, Biotechnology and Biochemistry*, vol. 67, no. 1, p. 179-182. **[CrossRef]**

LI, J.; ZHANG, L.; HAN, X.; YI, H.; GUO, C.; ZHANG, Y.; DU, M.; LUO, X.; ZHANG, Y. and SHAN, Y. (2013). Effect of incubation conditions and possible intestinal nutrients on cis-9, trans-11 conjugated linoleic acid production by *Lactobacillus acidophilus* F0221. *International Dairy Journal,* vol. 29, no. 2, p. 93-98. **[CrossRef]**

MACFARLANE, G. and MACFARLANE, S. (2002). Diet and metabolism of the intestinal flora. *Bioscience Microflora*, vol. 21, no. 4, p. 199-208.

MARAGKOUDAKIS, P.A.; ZOUMPOPOULOU, G.; MIARIS, C.; KALANTZOPOULOS, G.; POT, B. and TSAKALIDOU, E. (2006). Probiotic potential of *Lactobacillus* strains isolated from dairy products. *International Dairy Journal*, vol. 16, no. 3, p. 189-199. **[CrossRef]**

MATAGARAS, M.; METAXOPOULOS, J.; GALIOTOU, M. and DROSINOS, E.H. (2003). Influence of pH and temperature on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. *Meat Science*, vol. 64, no. 3, p. 265-271. **[CrossRef]**

NINGEGOWDA, M.A. and GURUDUTT, P.S. (2012). *In vitro* fermentation of prebiotics by *Lactobacillus plantarum* CFR 2194: Selectivity, viability and effect of metabolites on β-glucuronidase activity. *World journal of Microbiology & Biotechnology*, vol. 28, no. 3, p. 901-908. **[CrossRef]**

NOWROOZI, J.; MIRZAII, M. and NOROUZI, M. (2004). Study of *Lactobacillus* as probiotic bacteria. *Iranian Journal of Public Health*, vol. 33, no. 2, p. 1-7.

OGAWA, J.; KISHINO, S.; ANDO, A.; SUGIMOTO, S.; MIHARA, K. and SHIMUZU, S. (2005). Production of conjugated fatty acids by lactic acid bacteria. *Journal of Bioscience and Bioengineering*, vol. 100, no. 4, p. 355-364. **[CrossRef]**

OLIVEIRA, R.P.S.; FLORENCE, A.C.R.; SILVA, R.C.; PEREGO, P.; CONVERTI, A.; GIOIELLI, L.A. and OLIVEIRA, M.N. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acid profiles in nonfat symbiotic fermented milk. *International Journal of Food Microbiology*, vol. 128, no. 3, p. 467-472. **[CrossRef]**

PAN, X.D.; CHEN, F.Q.; WU, T.X.; TANG, H.G. and ZHAO, Z.Y. (2009). Prebiotic oligosaccharides change the concentrations of short-chain fatty acids and the microbial population of mouse bowel. *Journal of Zhejiang University, Science B,* vol. 10, no. 4, p. 258-263. **[CrossRef]**

PRANDINI, A.; SIGOLO, S.; TANSINI, G.; BROGNA, N. and PIVA, G. (2007). Different level of conjugated linoleic acid (CLA) in dairy products from Italy. *Journal of Food Composition and Analysis,* vol. 20, no. 6, p. 472-479. **[CrossRef]**

REID, G.; YOUNES, J.A.; VAN DER MEI, H.C.; GLOOR, G.B.; KNIGHT, R. and BUSSCHER, H.J. (2011). Microbiota restoration: Natural and supplemented recovery of human microbial communities. *Nature Reviews Microbiology,* vol. 9, no. 1, p. 27-38. **[CrossRef]**

RODRIGUES, D.; ROCHA-SANTOS, T.A.P.; GOMES, A.M.; GOODFELLOW, B.J. and FREITAS, A.C. (2012). Lypolisis in probiotic and synbiotic cheese: The influence of probiotic bacteria, prebiotic compounds and ripening time on free fatty acid profiles. *Food Chemistry,* vol. 131, no. 4, p. 1414-1421. **[CrossRef]**

SAMINATHAN, M.; SIEO, C.C.; KALAVATHY, R.; ABDULLAH, N. and HO, Y.W. (2011). Effect of prebiotic oligosaccharides on growth of *Lactobacillus* strains used as a probiotic for chickens. *African Journal of Microbiology Research*, vol. 5, no. 1, p. 57-64.

TYMCZYSZYN, E.; GERBINO, E.; ILLANES, A. and GÓMEZ-ZAVAGLIA, A. (2011). Galacto-oligosaccharides as protective molecules in the preservation of *Lactobacillus delbrueckii* subsp. *bulgaricus. Cryobiology*, vol. 62, no. 2, p. 123-129. **[CrossRef]**

WAHLE, K.W.J.; GOUA, M.; D'URSO, S. and HEYS, S. (2008). Conjugated linoleic acid effects on body composition and clinical biomarkers of disease in animals and man: Metabolic and cell mechanisms. In: DIJKSTRA, A.; HAMILTON, R. and HAMM, W. eds. *Trans Fatty Acids*, Blackwell publishing, p. 54-101.

WROLSTAD, R. (2012). Nutritional roles of carbohydrates. In: WROLSTAD, R. ed. *Food Carbohydrate Chemistry*. Wiley-Blackwell, UK. p. 147-164.

YANG, B.; PRASAD, K.N.; XIE, H.; LIN, S. and JIANG, Y. (2011). Structural characteristics of oligosaccharides from soy sauce lees and their potential prebiotic effect on lactic acid bacteria. *Food Chemistry*, vol. 126, no. 2, p. 590-594. **[CrossRef]**

How to reference this article:

SOTO, C. (2013). Effect of isomaltooligosaccharide and gentiooligosaccharide on the growth and fatty acid profile of *Lactobacillus plantarum*. *Electronic Journal of Biotechnology*, vol. 16, no. 4. http://dx.doi.org/10.2225/vol16-issue4-fulltext-9

Figures

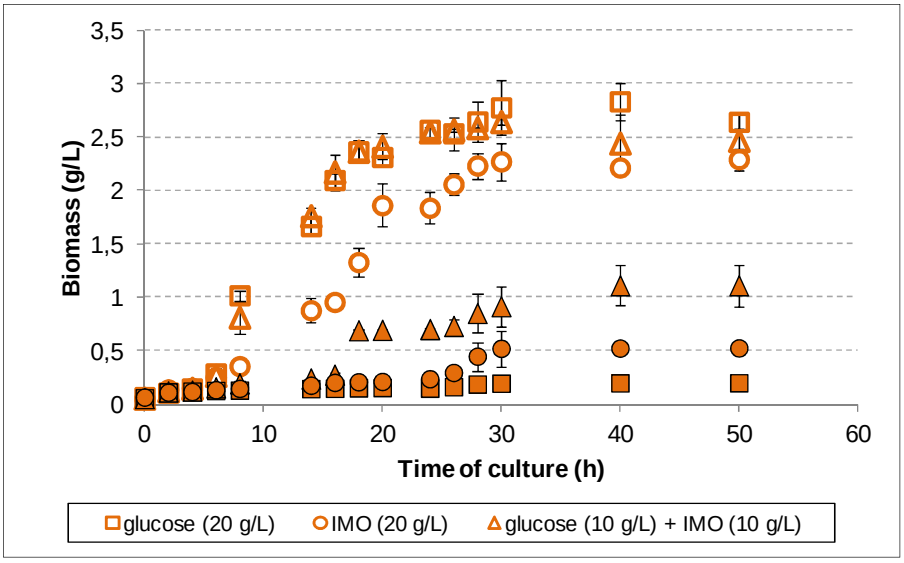

Fig. 1 Growth of *Lactobacillus plantarum* bacteria in media with different carbon sources. Open symbols, 37ºC; filled symbols, 45ºC.

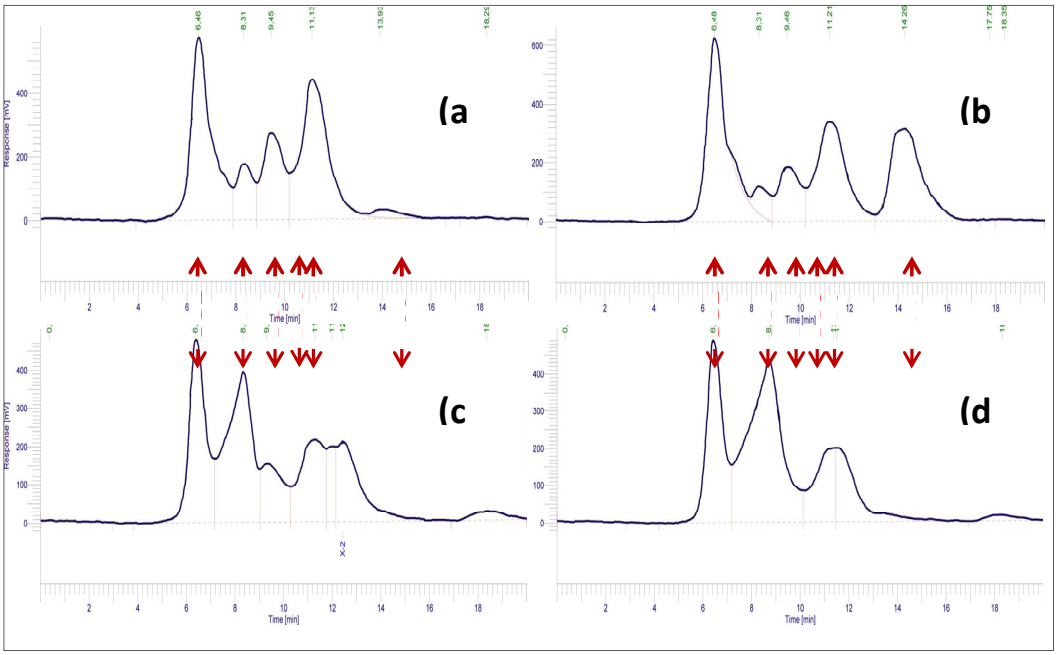

Fig. 2 Profiles of oligosaccharides present in the culture medium of *L. plantarum*. The culture was performed at 37ºC with only IMO or glucose and IMO as the carbon source (a) IMO, culture time 0 hrs; (b) glucose plus IMO, culture time 0 hrs; (c) IMO, culture time 50 hrs; and (d) glucose plus IMO, culture time 50 hrs. DP7: isomaltoheptaose; DP5: isomaltopentaose; DP4: isomaltotetraose; DP3: isomaltotriose; DP2: isomaltose; M: monomer, glucose.

DOI: 10.2225/vol16-issue4-fulltext-9

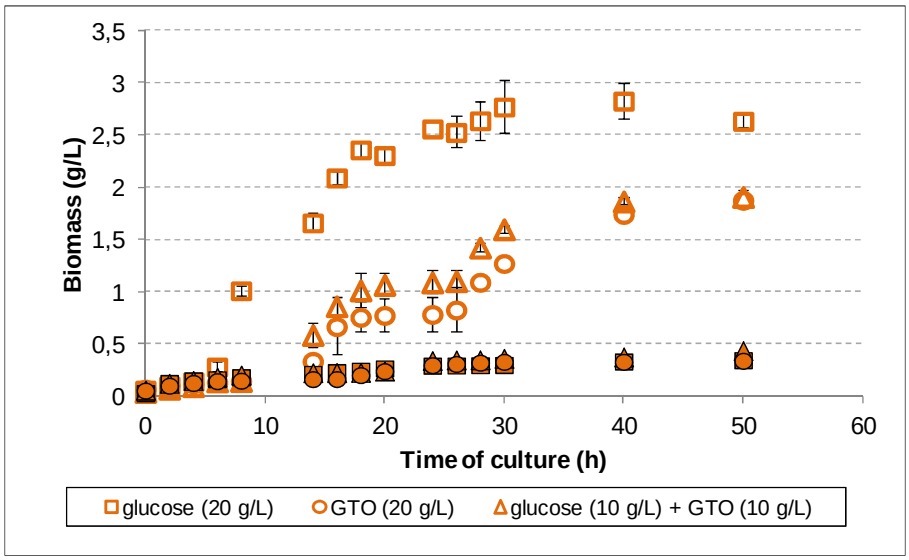

Fig. 3. Growth of *Lactobacillus plantarum* bacteria in media with different carbon sources. Open symbols, 37ºC; filled symbols, 45ºC.

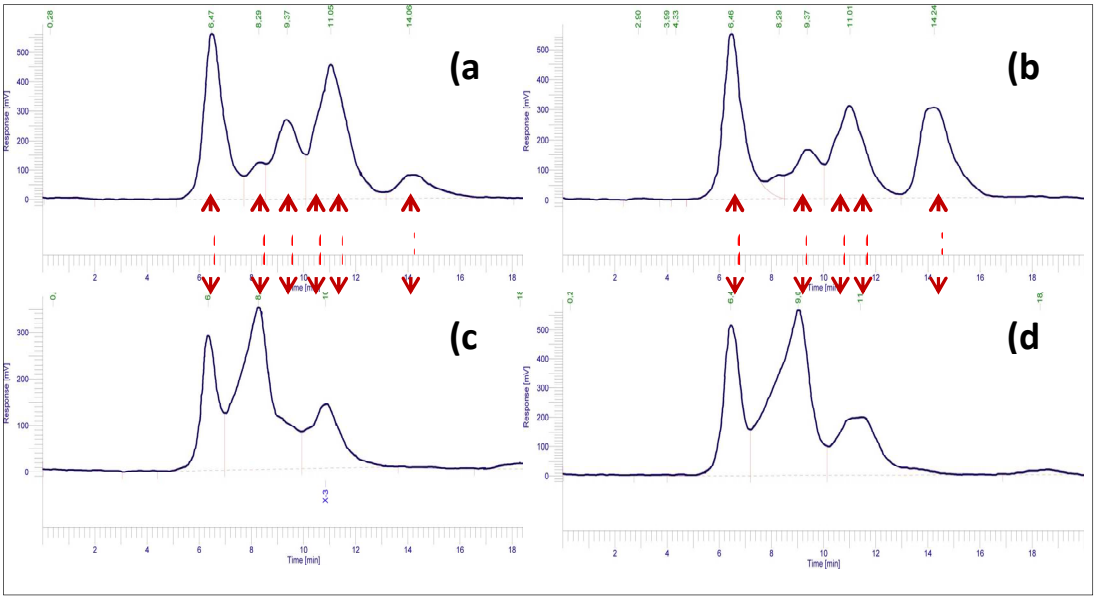

Fig. 4 Profiles of oligosaccharides present in the culture medium of *L. plantarum*. The cultures were performed at 37ºC with only GTO or with glucose and GTO as carbon source (a) GTO, culture time 0 hrs; (b) glucose plus GTO, culture time 0 hrs; (c) GTO, culture time 50 hrs; and (d) glucose plus GTO, culture time 50 hrs. DP7: gentioheptaose; DP5: gentiopentaose; DP4: gentiotetraose; DP3: gentiotriose; DP2: gentiobiose; M: monomer, glucose.

DOI: 10.2225/vol16-issue4-fulltext-9