Mixture of short-chain and long-chain fructans exert a positive effect on the growth of three potentially probiotic *Lactobacillus* strains

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ABSTRACT
Prebiotics are compounds which selectively enhance growth or survivability of certain microbial species in the gastrointestinal tract. The genus Lactobacillus is often used in probiotic preparations; however, probiotic properties are mostly strain specific, as well as the ability to metabolize prebiotics. This study evaluated cellular viability of four Lactobacillus strains after a 24h incubation periodin modified MRS medium containing fructans as main carbohydrate sources. Three MRS broths were used, which differed only regarding their carbohydrate fractions, as follows: A (2% Synergy 1 ® prebiotic mixture); B (2% Glucose) and C (0,2% Glucose). Results showed that all strains grew well in the prebiotic-containing medium (A), and a cellular viability significantly higher (P<0.01) in this group than in the negative control, and equivalent to the positive control. These results showed that fructans supported the growth of these strains and could be employed as prebiotics to increase their survivability in animal hosts.

Keywords: Prebiotics; Fructans; Inulin; Fructooligosacharides; *Lactobacillus*

1. INTRODUCTION

The search for functional foods became very relevant in the last few years, partly due to the industry's extensive advertising pointing out their benefits, and also due to the abundance of clinical evidence and experimental data which support them. The prophylactic use of prebiotics and probiotics is based on their ability to maintain a favorable balance of the microbiota in the gastrointestinal (GI) tract. The earliest definition of probiotics was introduced by Fuller (1989, p.336), which described them as "A live microbial feed supplement which beneficially affects the animal host by improving its intestinal microbial balance". This definition was updated by FAO/WHO (2002, p.8) to "Live microorganisms which when administered in adequate amounts confer a health benefit to the host", which does not restrict probiotics to food applications only.

On the other hand, prebiotics are selective substrates which supports the growth of probiotic microorganisms while is not metabolized by opportunistic and pathogenic microorganisms. Gibson and Roberfroid (1995) defined prebiotics as non-digestible food components which selectively stimulate the growth of beneficial bacteria in the colon.

Both inulin and oligofructose are composed mainly of fructosyl residues linked by β2,1 linkages, and may or not present a terminal glucose residue. Chicory inulin is comprised of a mixture of Fructooligosaccharide (FOS) and fructopolysaccharide, in which the Degree of Polymerization (DP) varies from 2 to 60 units, with an average of 12 units (Roberfroid, 2007).

Generally, prebiotic effects are indirect, andare originated from their positive influenceon beneficial intestinal microbiota (Wang, 2009). *Lactobacillus* species normally present high phenotypical variability regarding substrate utilization (Mcleod et al., 2008), and usually these properties are not correlated with genotypic characteristics. Therefore, substrate consumption capability should be considered individually for each potentially probiotic strain(Axellson, 2004).Therefore it is important to assess strains which are known for their probiotic properties in order to support future studies on the dynamics of symbiotic preparations in complex systems.

In this work, three *Lactobacillus* strains which present probiotic properties were evaluated regarding their ability to grow in modified MRS medium containing long-chain inulin and fructooligosaccharides (FOS).

2. MATERIAL AND METHODS

2.1. MICROORGANISMS

The strains *Lactobacillus acidophilus ATCC 4356, Lactobacillus plantarum ATCC 8014* and *Lactobacillus fermentum ATCC 9338*were obtained from American Type Culture Collection (ATCC)and were kept at -20ºC in de Man-Rogosa-Sharpe (MRS) broth (Man; Rogosa; Sharpe, 1960)containing 20% glycerol (v/v) as a cryoprotectant. Culture activation was undertaken by successively subculturing cell suspensions three times in MRS broth at37ºC/ 24 h.

2.2. PREBIOTIC MEDIA AND VIABILITY EVALUATION

Prebiotic formula Synergy 1® (BENEO-Orafti), kindly donated by Clariant S.A and Orafti S.A/N.V was used as a source of long-chain inulin and FOS. This product is composed by a mixture of long-chain inulin and oligofructose comprising 90-94% of total carbohydrate fraction, and a mixture of sucrose, glucose and fructose in a concentration of up to 10%.

MRS base composition consisted of 10g/L casein peptone, 5 g/L yeast extract, 20 g/L glucose, 5 g/L de sodium acetate, 2 g/L dipotassium phosphate, 2 g/L de ammonium citrate, 0.2 g/L de magnesium

sulphate, 0.05 g/L de manganese sulphate. Evaluation of prebiotic consumption was undertaken by replacing 20g/L of glucosein MRS broth base composition by the Synergy 1® formula (Group A). Two control tests were carried out in order to perform comparative analysis, the positive control (Group B) consisted of MRS broth with the original glucose concentration (20 g/L) (Man; Rogosa; Sharpe, 1960) and a negative control (Group C), containing2g/Lofglucose.

These media were sterilized at 121ºC/15min, and test tubes containing4,95 ml of the respective media were inoculated with 5µL of a 10^8 CFU/ml of an activated cell suspension, which resulted in an initial cell density of 10^6 of each strain, followed by incubation at 37 ºC/ 24 h.

After incubation, the cell suspensions were serially diluted in NaCl solution (0.85%)and the cell population was determined by the pour plate method in MRS medium; followed by incubationat 37º C/ 48 h. All tests were carried out in four repetitions, undertaken in different days.

2.3. STATISTICAL ANALYSIS

Results regarding viable cell counting were converted to Log CFU/ml and statistically analyzed by means of Analysis of Variance (ANOVA) in order to assess the significance of the differences among treatments on each *Lactobacillus* strain. Tukey's test was used to compare paired treatments. The significance level was set to $P<0.05$.

3. RESULTS AND DISCUSSION

Results regarding the effect of the studied treatments on viability of *Lactobacillus plantarum* ATCC 8014 are represented in Figure 1. A significantly higher ($P<0.01$) viable cell population was observed when this strain was grown on Prebiotics and positive control media compared to the negative control. However, there were no significant differences between positive control and prebiotic groups.

Regarding *Lactobacillus acidophilus* ATCC 4356 (Figure 2) a similar behavior was observed concerning prebioticutilization. Therefore, differences in cell populationin prebiotic treatmentand negative control were also statistically significant ($P<0.01$) while no significant differences were observed between positive control and prebiotic media.

Concerning *Lactobacillus fermentum* ATCC 9338 it can be noted in Figure 3 that viable cell counts presented the same pattern observed in the other evaluated strains. The difference between growth in prebiotic MRS medium differed significantly from negative control ($P<0.01$).However, no significant differences were observed between viable cell countsin prebiotic MRS and positive control media.

The high populations observed in the negative control medium, regardless of its low carbohydrate content could be related to the high viable cell concentration present in the inocula. We observed that all strains maintained viability in the negative control medium after 24 hours incubation period. This medium was specifically designed in order to determine if simple fermentable carbohydrates present in the Synergy 1® mixture could have been responsible for the high viable cell concentration observed. However, the significant difference observed between the negative controls and the prebiotic and glucose containing media showed that these fermentable carbohydrates were not responsible for the observed results.

Altieri, Bevilacqua and Sinigaglia (2011) observed a positive influence of glucose in prebiotic preparations containing inulin or FOS for *Lactobacillus plantarum* c19 and DSMZ 2601. This association would contribute to maintain cell viability slowing the rate of consumption of the prebiotics, prolonging shelf life. The authors also proposed that glucose would support biomass production while prebiotics would be used primarily for maintenance, prolonging cell viability.

According to Rurangwa et al. (2009), a strain of *Lactobacillus delbrueckii subsp. lactis*isolated from fermented fish exhibits the same growth patterns in media containing inulin, oligofructose or glucose. This

behavior is similar to the observed in the present work, since there was no significant difference between positive control and prebiotic treatments.

Cebeci and Gürakan (2003) observed different results regarding *Lactobacillus acidophilus* ATCC 4356growing in medium containing purified oligofructose. The authors observed that this strain was not capable of metabolizing short-chain FOS, and attributed growth to L-Cysteine supplementation. However, in the present work, the results show that the prebiotic formula evaluated was used for cellular multiplication without L-Cysteine supplementation. It is also noteworthy that the prebiotic formula used in this work is comprised of both long-chain and short-chain fructans.

The metabolism of *Lactobacillus acidophilus* comprises over 20 PTS systems and six ATP-binding cassette transporter families, and one of them is involved in short-chain FOS transport (Altermann et al., 2005). Transcriptional genomic analysis of *Lactobacillus acidophilus* allowed studying the integration between an ATP-binding cassette transport system and a β-fructosidase, thus forming a gene cluster which can be responsible for FOScatabolism. This system is also responsible for levan (β2,6fructans) catabolism (Barrangou et al., 2003), and β-fructosidase kinetics are similar to that of a hidrolase described by Liebl, Brem and Gotschlich (1998) in the extremophile *Thermotogamaritima*.

Makras et al. (2005) observed that the specific growth rate (μ_{max}) of *Lactobacillus paracasei* 8700:2 in media containing inulin or oligofructose was particularly high. They emphasized that the $\mu_{max}$and cell density values were similar to those obtained in media containing fructose as sole substrate. Therefore, they emphasized that prebiotic enzymatic degradation and metabolism occurred rapidly, which indicated the existence of a high affinity extracellular β-fructosidase in this strain. The authors stated that strains which present the same growth behavior, such as the ones evaluated in the present work, would possibly exhibit the same pattern of enzymatic kinetics, although specific studies are necessary.

Several studies have shown that the use of inulin-type fructans as additives in alimentary products such as bread (Brasil et al., 2011) and ice cream (Kip; Meyer; Jellema, 2006; Paseephol; Sherkat, 2009;Isik et al., 2011) can improve their sensorial characteristics and also act as a fat replacement. These modified products can also be used to modulate the gut microbial composition by improving the population of beneficial bacterial species.

Considering the selective premise of prebiotics, the results of the present work suggest a significant effect of inulin-type fructans on growth and viability of the evaluated strains, however, further studies should be carried out considering the dynamics of substrate consumption and metabolite formation. The observed viable cell counts, which showed similar behavior regarding all strains tested, and the lack of differences between growth on prebiotics and glucose indicate a good growth rate in the presence of the tested prebiotics.

4. CONCLUSION

In conclusion, our results indicate that all evaluated strains were able to metabolize the carbohydrates present in Synergy 1 ® for cell growth, which could not be attributed solely to the presence of simple fermentable carbohydrates present in the mixture. It was also demonstrated that these carbohydrates were rapidly consumed for cell growth, possibly at the same rate as glucose since there was no significant difference between viable populations grown with glucose or the prebiotic mixture.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest which could influence interpretation of the results reported in this study.

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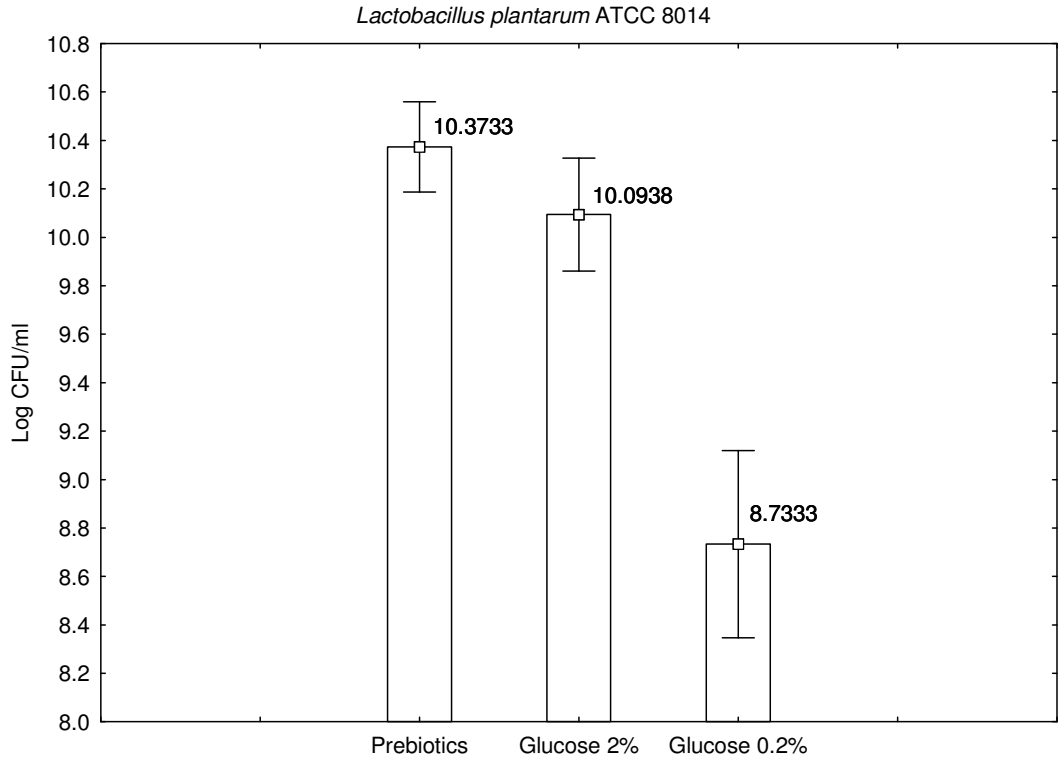

Figure 1 - Values of cell population related to Lactobacillus plantarum ATCC 8014 grown in different media.

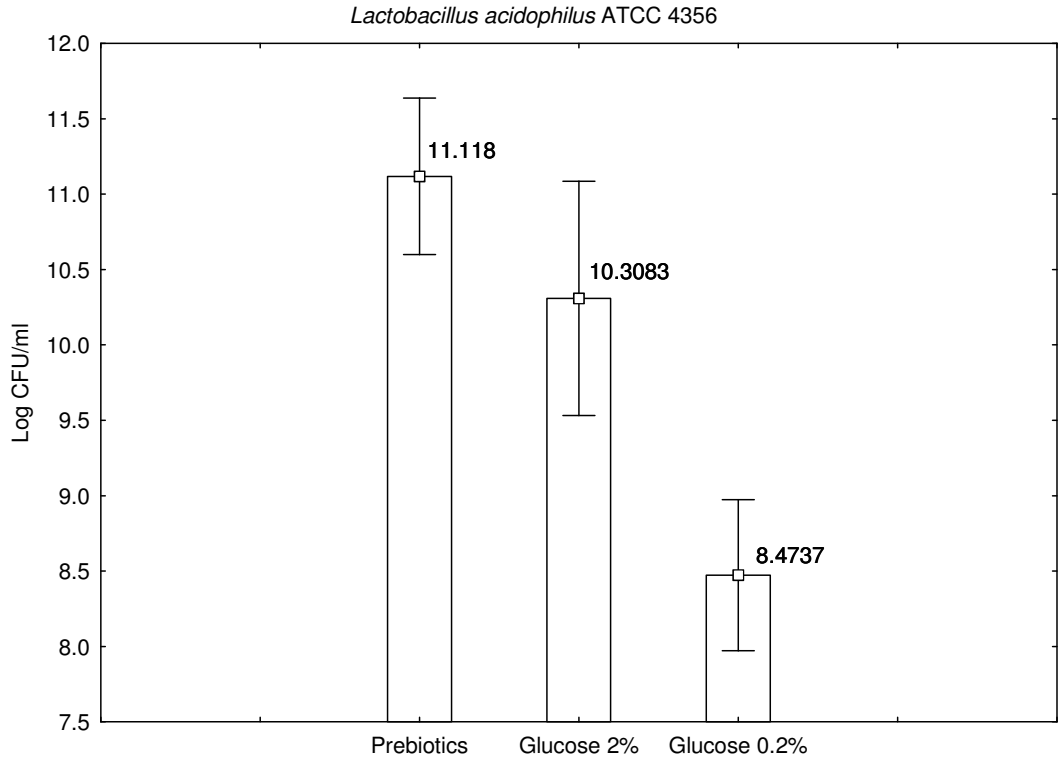

Figure 2 - Values of cell population related to Lactobacillus acidophilus ATCC 4356 grown in different media.

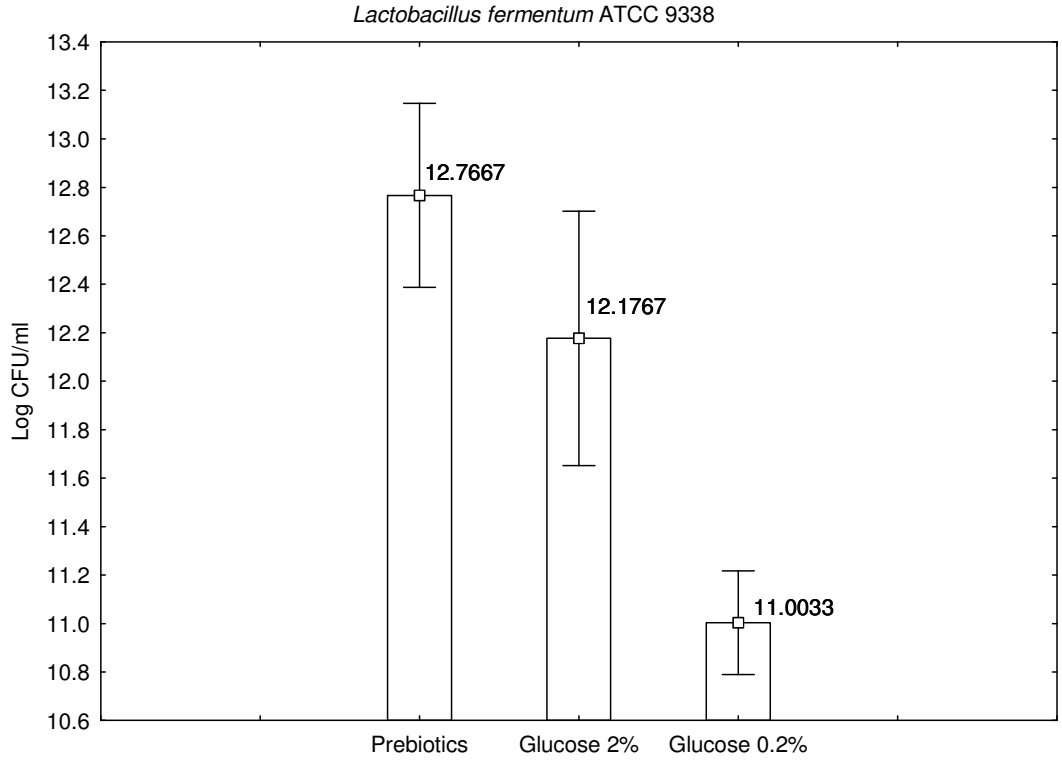

Figure 3 - Values of cell population related to Lactobacillus fermentum ATCC 9338 grown in different media.