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Research Article

Effect of Prebiotic Compounds on the Growth and Survival of Bifidobacteria in a Laboratory Medium

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Abstract: This study focuses on the effects of 5 prebiotics (low and high molecular weight dextrans, fructo-oligosaccharides, inulin and lactulose) on 6 bifidobacteria strains (*Bifidobacterium animalis* subsp. *lactis*, *B. animalis* subsp. *animalis*, *B. bifidum*, *B. breve*, *B. longum* subsp. *infantis* and *B. longum*) in a laboratory medium. Two different assays were conducted (a growth experiment-for all the strains-and a survival assay-for *B. animalis* subsp. *lactis* and *B. breve*). Glucose was used as positive control. Growth assay pinpointed that lactulose could be successfully used to promote bifidobacteria and attain a growth index for *B. animalis* subsp. *animalis* and *B. longum* subsp. *infantis* higher than with glucose. Concerning the bifidogenic effect, it is not clear if it could be observed also during the death phase, as we recovered a beneficial effect for *B. breve* but not for *B. animalis* subsp. *lactis*.

Keywords: *Bifidobacterium* spp., bifidogenic effect, growth, prebiotic compounds, survival

INTRODUCTION

According to the definition of Gibson and Roberfroid (1995) a prebiotic is a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activating the metabolism of one or limited number of health promoting bacteria in the intestinal tract, thus improving the host's intestinal balance.

Prebiotics are generally carbohydrates, ranging in the size from small sugar alcohols and disaccharides, to oligosaccharides and large polysaccharides (Guo, 2009), sharing some common criteria:

- A prebiotic should be neither hydrolyzed nor adsorbed in the intestine
- It should be a selective substrate for some probiotic microorganisms
- It alters qualitative composition of intestinal microflora, by promoting beneficial bacteria
- It induces luminal or systemic effects that are advantageous to the host.

Guo (2009) reported some compounds in the list of prebiotic: fructans, inulin, fiber gums, isomalto-oligosaccharides, lactitol, lactosucrose, lactulose, oligofructose, pyrodextrins, soy-oligosaccharides, transgalacto-oligosacharides, xylo-oligosaccharides.

The interest toward prebiotic compounds increased in the last decade, as they were proposed and used as functional compounds in the "symbiotic" products or as ingredients in different foods (dairy products, frozen desserts, table spreads, baked goods and breads, breakfast cereals, fruit preparations, meat products and chocolate) (Bottazzi, 2004; Debon *et al*., 2010; Gonzalez *et al*., 2011; Guo, 2009; Lee *et al*., 1999; Rodrigues *et al*., 2011).

A symbiotic is defined as a mixture of prebiotics and probiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or promoting the metabolism of one or of a limited number of health-promoting bacteria and thus improving the health of the host (Gibson and Roberfroid, 1995).

Several oligosaccharides, like fructo-oligosaccharides (FOS), raffinose and galacto-oligosaccharides (Altieri *et al*., 2013; Dinoto *et al*., 2006a, 2006b; Rossi *et al*., 2005) are known to act as prebiotics towards bifidobacteria. Species from the genus *Bifidobacterium* (*B. animalis* subsp. *lactis*, *B. breve* and *B. longum*) are commonly regarded to as probiotics, for their safety record and effects on human health (Guo, 2009).

This study was aimed to investigate the effects of some prebiotics (fructo-oligosaccharides, inulin, high and low molecular weight dextrans, lactulose) on the growth and survival of some bifidobacteria as a preliminary step for the development of a symbiotic product.

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MATERIALS AND METHODS

Strains: This study focused on six different strains of *Bifidobacterium* spp., purchased from a Public Collection (Deutsche Sammlung von Mikroorganismem und Zellkulturen's collection-DSM, Braunschweig, Germany): *B. animalis* subsp. *lactis* (DSM 10140); *B. animalis* subsp. *animalis* (DSM 20104); *B. bifidum* (DSM 20456); *B. breve* (DSM 20213); *B. longum* subsp. *infantis* (DSM 20088); *B. longum* subsp. *longum* (DSM 20218).

The strains were stored at -20°C in MRS broth (Oxoid, Milan, Italy), added with cysteine 0.05% (w/v) (Sigma-Aldrich, Milan, Italy) and grown before each assay in the same medium, incubated at 37°C for 24-48 h under anaerobic conditions.

Prebiotic compounds and laboratory medium: Five prebiotic compounds were used throughout this research: dextran low molecular weight (9-11 KDa) ($(C_6H_{10}O_5)_n$); dextran high molecular weight (2000 KDa) ($(C_6H_{10}O_5)_n$); fructooligosaccharides (FOS), extracted from chicory ($(C_6H_{10}O_5)_n$, with n>10); inulin, extracted from Dahlia tubers ($(C_6H_{10}O_5)_n$, with n≈36); D-lactulose (4-O-β-D-Galactopyranosyl-D-fructose, $C_{12}H_{22}O_{11}$). All the prebiotic compounds were purchased from Sigma-Aldrich.

The experiments were performed in a laboratory medium, proposed by Liew *et al*. (2005) and Bevilacqua *et al*. (2008) and modified as follows: prebiotic compound, 5.0 g/L; yeast extract, 2.0 g/L; (Oxoid); K_2HPO_4, 2.7 g/L (J.T. Baker, Milan, Italy); $MnSO_4$, 0.05 g/L (J.T. Baker); $MgSO_4*7H_2O$, 0.20 g/L (J.T. Baker); Tween 80 ®, 1.0 mL/L (C. Erba, Milan, Italy).

For each assay the media containing glucose (5 g/L) or without carbohydrates were used as positive and negative controls, respectively.

Growth assay: Aliquots of 20 mL of the laboratory medium, containing prebiotic compounds, glucose (positive control) or without carbon source (negative control), were inoculated separately with ca. 6 log cfu/mL of each strain. The samples were incubated at 37°C for 4 days. Microbial growth was evaluated through the measurement of the absorbance at 600 nm by a spectrophotometer UV-visible Beckman DU 640 (Beckman DU 600, Fullerton, CA).

Data were modeled as Growth Index, as proposed by Blaszyk and Holley (1998), modified by Bevilacqua *et al*. (2009):

$$GI = \left(Abs_s \middle/ Abs_c \right) * 100$$

where, for each time of analysis Abs_s is the absorbance of the samples with different prebiotics and Abs_c the absorbance of the positive control. The experiments were performed at least in duplicate, over two different batches.

Survival assay: This assay was conducted using as test strains *B. animalis* subsp. *lactis* (DSM 10140) and *B. breve* (DSM 20213). Aliquots of the laboratory medium of Liew *et al*. (2005), modified by Bevilacqua *et al*. (2008), containing prebiotics, glucose (positive control) or no carbon source (negative control) were inoculated with ca. 5-6 log cfu/mL of each strain separately. The samples were incubated at 37°C for at least 15 days. The survival of the microbial targets was evaluated periodically through the pour plate method, using MRS agar added with 0.05% cysteine, incubated under anaerobic conditions at 37°C for 48-72 h. In addition, pH of the sample was evaluated periodically by a pH-meter Crison (Crison instruments, Barcelona, Spain).

The experiments were performed in duplicate over two different batches.

Statistical analyses: Data were analyzed through one-way- analysis of variance and Tukey's test ($p<0.05$) through the software Statistica for Windows (Statsoft, Tulsa, OK).

RESULTS AND DISCUSSION

Figure 1 shows the results of the growth assay after 24 h of incubation; data were modelled as Growth Index (GI), referred to the sample containing glucose (positive control). As expected GI value of negative control was ca. 2-9%; the growth was inhibited also in presence of dextrans, FOS and inulin as carbon sources. A partial growth was observed only for the strain 20088 (i.e., *B. longum* subsp. *longum*) with high molecular weight dextran, which resulted in GI of 17.32±13.84%. An interesting result was recovered when lactulose was added to the laboratory medium; in fact, it was able to promote the growth of bifidobacteria and the strains 10140, 20456 and 20213 (*B. animalis* subsp. *lactis*, *B. bifidum* and *B. breve*, respectively) showed GI values of 84.97-92.94%. These values were not statistically different from the growth of the positive control (GI of 100%); concerning the interpretation of GI values, Bevilacqua *et al*. (2009) proposed a simple criterion, as follows: GI<25% stands for a complete inhibition of the microorganism; GI included in the range 25-75% underlines a partial inhibition. Finally GI>75% stands for a growth kinetic similar to that reported for the optimal conditions. Therefore, we could assume that lactulose was able to support the growth of the three strains of bifidobacteria as well as glucose.

Concerning the strains 20104 and 20088 (*B. animalis* subsp. *animalis* and *B. longum* subsp. *infantis*), they showed GI>100% in presence of lactulose; the statistical analysis (data not shown) revealed that these values were different from GI in the positive control, thus underlining a stimulating effect of the prebiotic.

The use of lactulose as an effective prebiotic was proposed by Oliveira *et al*. (2011) and Altieri *et al*. (2013) studied the effect of this compound on the kinetic of fermentation and cell count

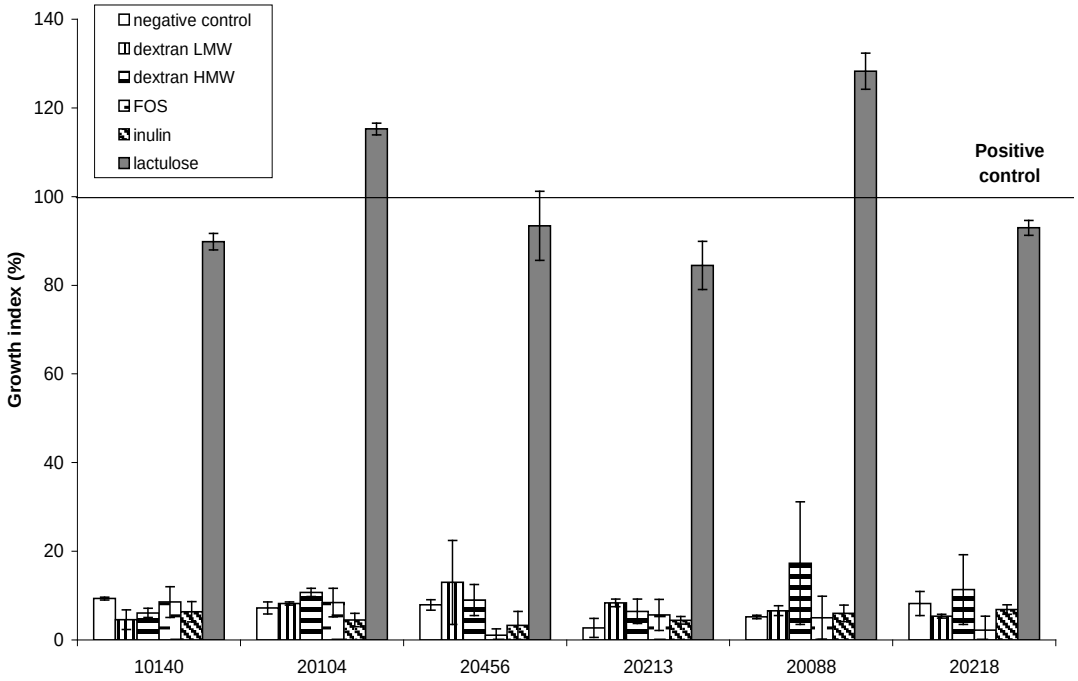

Fig. 1: Growth Index after 24 h at 37°C of *Bifidobacterium* spp. in the medium by Liew *et al*. (2005), modified by Bevilacqua *et al*. (2008); bars represent standard deviation; Negative control, medium without carbohydrate; positive control, medium with glucose; LMW, low molecular weight; HMW, high molecular weight; FOS, fructo-oligosaccharides; 10140, *B. animalis* subsp. *lactis*; 20104, *B. animalis* subsp. *animalis*; 20456, *B. bifidum*; 20213, *B. breve*; 20088, *B. longum* subsp. *infantis*; 20218, *B. longum* subsp. *longum*

Table 1: Cell number (log cfu/mL) of *B. animalis* subsp. *lactis* DSM 10140 and *B. breve* DSM 20123 in the medium of Liew *et al*. (2005), modified by Bevilacqua *et al*. (2008), stored at 37°C for 14 days. Negative control, medium without carbohydrate; positive control, medium containing glucose; LMW, low molecular weight; HMW, high molecular weight; FOS, fructo-oligosaccharides

	Days of storage					
10140	0	1	3	6	10	14
Negative control	6.56^{a}	7.48^{a}	8.59^{a}	7.10^{a}	6.84^{a}	6.65^{a}
Negative control	6.56^{a}	7.48^{a}	8.59^{a}	7.10^{a}	6.84^{a}	6.65^{a}
Positive control	6.56^{a}	8.64^{b}	6.00^{b}	-*	-	-
Dextran LMW	6.56^{a}	7.75^{a}	8.03^{c}	6.79^{a,b}	6.61^{a}	5.06^{b}
Dextran HMW	6.56^{a}	7.78^{a}	6.78^{b,d}	5.51^{c}	5.15^{b}	5.19^{b}
FOS	6.56^{a}	7.78^{a}	7.26^{d}	6.49^{b}	4.78^{b}	4.71^{c}
Inulin	6.56^{a}	7.70^{a}	7.66^{d}	5.05^{c}	4.61^{b,c}	3.57^{d}
Lactulose	6.56^{a}	8.25^{c}	7.60^{d}	-	-	-
	Days of storage					
20213	0	1	3	6	10	14
Negative control	5.64^{a}	7.86^{a}	4.95^{a}	5.60^{a}	3.58^{a}	2.45^{a}
Positive control	5.64^{a}	8.39^{a,b}	5.70^{b}	-	-	-
Dextran LMW	5.64^{a}	7.85^{a}	7.50^{c}	6.00^{a}	4.86^{b}	4.70^{b}
Dextran HMW	5.64^{a}	7.96^{a}	7.79^{c}	4.95^{b}	4.26^{b}	4.15^{b}
FOS	5.64^{a}	8.07^{a}	7.73^{c}	6.04^{a}	4.34^{b}	4.30^{b}
Inulin	5.64^{a}	8.30^{a,b}	6.62^{d}	4.48^{b}	4.00^{a,b}	4.04^{b}
Lactulose	5.64^{a}	8.78^{b}	6.03^{b,d}	5.04^{b}	-	-

$^{a, b}$For each microorganism and time of analysis, different letters indicate significant differences (one-way ANOVA and Tukey's test) ($p<0.05$); *Cell number below the detection limit (1 cfu/mL)

of some probiotics in skim milk (*Lactobacillus acidophilus*, *L. rhamnosus*, *L. delbrueckii* subsp. *bulgaricus* and *B. lactis* in co-culture with *Streptococcus salivarius* subsp. *thermophilus*). The use of prebiotic increased the cell counts of probiotics, with particular concern to *B. lactis* (bifidogenic effects). The data of our research confirmed this result and suggested that the bifidogenic effect of lactulose could be a common characteristics among many species of bifidobacteria.

In a second step, the research focused on the effects of prebiotic compounds on the death kinetic of *B. animalis* subsp. *lactis* (DSM 10140) and *B. breve* (DSM 20213), chosen as they were the most vigorous microorganisms amongst the strains studied in the first step.

The results are reported in Table 1. Concerning the strain 10140, cell number was below the detection limit after 6 days of storage for the samples containing glucose and lactulose; otherwise, the microorganism showed a prolonged viability both in the negative control and in the sample containing the other prebiotics.

B. breve (DSMZ 20213) showed a similar trend, with only one difference; lactulose resulted in a slower death kinetic than glucose, as after 6 days in the sample containing the prebiotic a viable count of 5 log cfu/mL was recovered.

Oliveira *et al*. (2011) reported a beneficial effect of lactulose both in growth and death phases; in the death phase, in fact, skim milk added with the prebiotic showed also higher counts of *B. lactis* than the controls. However, we recovered a bifidogenic effect only for *B. breve* DSMZ 20213 after 6 days, but not for *B. animalis* subsp. *lactis* DSMZ 10140.

Saarela *et al*. (2003) observed that lactulose improved the cold-storage stability of *L. salivarius* at 4°C for 22 days, probably due to splitting of cell-chains. An enhancement by lactulose of β-glucosidase and β-galactosidase activities on intestinal microbiota was also reported (Juśkiewicz and Zduńczyk, 2002; Pham and Shah, 2008). These effects could be responsible of both the beneficial effect of lactulose in the growth and death phases.

Concerning the results obtained for the other prebiotics (FOS, inulin and dextrans), the trend was similar to that observed in the negative control, with a prolonged viability throughout the storage. We can suggest that this result was probably due to the fact that bifidobacteria were not able to use these compounds, as evidenced by the slight decrease of the pH (ca. 0.5-0.7), whereas in the samples containing glucose and lactulose, the pH attained a minimum value of 4.2-4.5 after 2 days (data not shown). This low acidification could be responsible of the prolonged viability of the target strains in presence of FOS, dextrans and inulin; otherwise, the decrease of pH determined the loss of viability in the media containing glucose and lactulose.

CONCLUSION

This study suggests that lactulose could be used successfully to promote the growth of bifidobacteria and attain, for some strains (e.g., *B. animalis* subsp. *animalis* and *B. longum* subsp. *infantis*), a growth index higher than that recovered with glucose. Concerning the bifidogenic effect, it is not clear if it could be observed also in the death phase, as we recovered a beneficial effect for *B. breve* but not for *B. animalis* subsp. *lactis*. These results can be considered as the first step to developing a symbiotic product, containing bifidobacteria and lactulose.

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REFERENCES

Altieri, C., A. Bevilacqua, M. Perricone and M. Sinigaglia, 2013. Using a simplex centroid to study the effects of pH, temperature and lactulose on the viability of *Bifidobacterium animalis* subsp. *lactis* in a model system. Anaerobe, 23: 23-26.

Bevilacqua, A., M. Perricone, M. Cannarsi, M.R. Corbo and M Sinigaglia, 2009. Technological and spoiling characteristics of the yeast microflora isolated from Bella di Cerignola table olives. Int. J. Food Sci. Tech., 44(11): 2198-2207.

Bevilacqua, A., M.R. Corbo, M. Mastromatteo and M. Sinigaglia, 2008. Use of response surface approach to model the biomass production and the acidifying ability of a probiotic strain of *Lactobacillus plantarum*, isolated from table olives, in a batch system. World J. Microb. Biot., 24: 1721-1729.

Blaszyk, M. and R.A. Holley, 1998. Interaction of monolaurin, eugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms. Int. J. Food Microbiol., 39(3): 175-183.

Bottazzi, V., 2004. Latte-fermentati Funzionali Probiotici: Nuove Opportunità Per il Benessere Dell’uomo. Elite Communication, Milano, Italy.

Debon, J., E. Schwinden Prudêncio and J.C. Cunha Petrus, 2010. Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. J. Food Eng., 99(2): 128-135.

Dinoto, A., A. Suksomcheep, S. Ishizuka, H. Kimura, S. Hanada, Y. Kamagata, K. Asano, F. Tomita and A. Yokota, 2006a. Modulation of rat cecal microbiota by administration of raffinose and encapsulated *Bifidobacterium breve*. Appl. Environ. Microb., 72(1): 784-792.

Dinoto, A., T.M. Marques, K. Sakamoto, S. Fukiya, J. Watanabe, S. Ito and A. Yokota, 2006b. Population dynamics of *Bifidobacterium* species in human feces during raffinose administration monitored by fluorescence in situ hybridization-flow cytometry. Appl. Environ. Microb., 72(12): 7739-7747.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125(6): 1401-1412.

Gonzalez, N.J., K. Adhikari and M.F. Sancho-Madriz, 2011. Sensory characteristics of peach-flavored yogurt drinks containing prebiotics and synbiotics. LWT-Food Sci. Technol., 44(1): 158-163.

Guo, M., 2009. Functional Foods: Principles and Technology. Woodhead Publishing, Boca Raton, FL.

Juśkiewicz, J. and Z. Zduńczyk, 2002. Lactulose-induced diarrhoea in rats: Effects on caecal development and activities of microbial enzymes. Comp. Biochem. Phys. A, 133(2): 411-417.

Lee, Y.K., K. Nomoto, S. Salminen and S.L. Gorbach, 1999. Handbook of Probiotics. Wiley, New York.

Liew, S.L., A.B. Ariff, A.R. Raha and Y.W. Ho, 2005. Optimization of medium composition for the production of a probiotic microorganism, *Lactobacillus rhamnosus*, using response surface methodology. Int. J. Food Microbiol., 102(2): 137-142.

Oliveira, R.P.D.S., A.C. Rodrigues Florence, P. Perego, M.N. De Oliveira and A. Converti, 2011. Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. Int. J. Food Microbiol., 145(1): 22-27.

Pham, T.T. and N.P. Shah, 2008. Effects of lactulose supplementation on the growth of bifidobacteria and biotransformation of isoflavone glycosides to isoflavone aglycones in soymilk. J. Agr. Food Chem., 56(12): 4703-4709.

Rodrigues, D., T.A.P. Rocha-Santos, C.I. Pereira, A.M. Gomes, F.X. Malcata and A.C. Freitas, 2011. The potential effect of FOS and inulin upon probiotic bacterium performance in curdled milk matrices. LWT-Food Sci. Technol., 44(1): 100-108.

Rossi, M., C. Corradini, A. Amaretti, M. Nicolini, A. Pompei, S. Zanoni and M. Matteuzzi, 2005. Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. Appl. Environ. Microbiol., 71(10): 6150-6158.

Saarela, M., K. Hallamaa, T. Mattila-Sandholm and J. Mättö, 2003. The effect of lactose derivatives lactulose, lactitol and lactobionic acid on the functional and technological properties of potentially probiotic *Lactobacillus* strains. Int. Dairy J., 13(4): 291-302.