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UTILIZATION OF OLIGOSACCHARIDES BY PREBIOTICS STRAINS FROM COMERSIAL PROBIOTICS PRODUCTS

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Abstract

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The objective of this study was to determine the utilization and growth of *Lactobacillus acidophilus Ts1*, *L. bulgaricus Ts2,* and *L. rhamnosus Ts3* isolated from commercial products in the presence of oligosaccharides – inulin, fructooligosaccharides and raffinose. The results clearly demonstrated that the growth rates of the studied strains showed very different preferences, when were cultivated on oligosaccharides as sole carbon source. Different concentration of prebiotics oligosaccharides (2 and 2.5%) was used. It was found that the higher growth rate was detected in the presence of 2% inulin and 2.5% raffinose As well the effect of the utilization of 2% FOS and 2.5% raffinose was associated with significant decrease in the pH. The antimicrobial activity was studied against *E. coli, St. aureus, E. aerogenes* and *B. cereus* after cultivation of the strains on oligosaccharides. The highest antimicrobial activity was detected against *E. coli.* The system of uptake of unusual sugars influenced the antimicrobial activity in a specific way.

Key words: fermentation, lactic acid bacteria, prebiotics, probiotics

Introduction

Prebiotics are non-digestible food ingredients that beneficially affect the host health by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon and thus improve health (Gibson, 2004, 2011). Inulin (In) and oligofructose (OF) are β (2-1) fructans represented as GFn and Fn, where G is glucose and n is the number of fructose (F) units in the oligosaccharide chain. When In is extracted from the chicory root, it comprises a family of identical linear structures (GFn) that different in their degree of polymerization (DP), ranging from 3 to 60, with an average DP of 10. OF is obtained by partial enzymatic hydrolysis of inulin by an endo-inulinase; it is composed of the same fructose monomer as inulin, but has lower DP, ranging from 2 to 8 (Chi et al., 2011).

Raffinose (Rf) and stachyose are α-galactosides of sucrose comprising three and four monomeric units respectively and are non-digestible in the gut due to the absence of α-galactosidase in the human intestinal mucosa. Consequently, intact OS pass directly into the lower intestine where they are metabolized by bacterial that possess this enzyme, resulting in the production of gases (Tsangalis, 2004). There has been an alternative approach that utilizes lactic acid bacteria (LAB) processing – galactosidase activity which can hydrolysed the raffinose and stachyose during fermentation (Garro et al., 2005).

Prebiotics such as OF, In and Rf provide some beneficial effects, namely improved bioavailability of minerals such as calcium, magnesium, and iron; increased the activity of beneficial live active cultures; and inhibition of harmful bacteria in the digestive tract. In has beneficial effects normally associated with dietary fiber, namely helps in digestion of high-

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protein diets, and decreases fat absorption (Gibson, 1995). Seydin et al. (2005) found that yogurt containing In had a good flavor and a smooth texture. Moreover, the rate of pH decrease of fermented milk products was increased by addition of In (Hardi et al., 2000).

The aims of this work were to determine ability of *Lactobacillus* strains for utilization and growth on different oligosaccharides – fructooligosaccharide, inulin, and raffinose.

Materials and Methods

The strains used this study *L.acidophilus Ts1, L.bulgaricus Ts2 and L.rhamnosus Ts3* were obtained from comersial probiotics products. Initial identification of all the strains was performed by API 59 CHL (API 50 CHL) system (BioMerieux, Craponne, France), according to the manufacturer`s instruction. The fermentation profiles were read after incubation at 37°C in anaerobic condition, for 3 days.

Carbohydrates used in this study is: FOS (Raftilose P95, Orafti, Belgium), Inulin (Raftiline HP), Orafti (Belgium), and Raffinose (Fluka, Switzerland 99% purity). Glucose (Merck, Germany) were used as the control.

Media The strains cultivated in media of MRS (de Mann Rogosa Sharpe, Biolife, Milano, Italia). The basic media was sterilized by autoclaving at 121°C for 20 min, and carbohydrates supplemented were sterilized using 0.22 μm filters (Manisart®). The basic MRS broth was supplemented with 2% and 2.5% glucose, 2% and 2.5% Rf, 2% and 2.5% FOS, and 2% and 2.5% In.

Fermentation. The strains were grown in MRS broth (Merck). Overnigth grown samples were washed twise in MRS and 10% of bacterial suspension (10^7 cfu mL^{-1}) was used to inoculate modified MRS broth and agar medium (pH 6.8) containing eithet 2% and 2.5% glucose, 2% and 2.5% Rf, 2% and 2.5% FOS, and 2% and 2.5% In. The anaerobic fermentations were performed in 5 mL glass bottles at 37°C for 48 h.

Microbial growth. Bacterial growth was measured by a turbidimetric method at 600 nm and calibrated againts cell dry weight using a spectrofotometar (UV/via JENWAY 6315). The OD reading and standard deviations were calculated from duplicate samples from three separate experiments.

Antimicrobial assay was performed as previously described by the well diffusion method (Bertrand-Harb, 2003) by using soft 0.8% agar. After adjusting the pH at 6.5 by NaOH, the activity of the collected samples (100 μl) was checked against *Escherichia coli 3398, Staphylococcus aureus 745, Bacillus cereus 4464* and *Enterobacter aerogenes 3691*.

Statistical analyzis

For each experiment, the data was analized using the Exel statistical package.

Results and Discussion

It was found that the studied strains can utilize different concentration of FOS, inulin and raffinose in mMRS media. The results are presented in Table 1.

Growth was evaluated in terms of maximum optical density at 600 nm. Growth kinetics on glucose were used as control. From the received data is clear thet all examined strains were able to grow in media containing 2% FOS. Only *L. rhamnosus Ts3* was able to grow in the presence of 2.5% FOS. Inulin was fermented by strains *L. bulgaricus Ts2* and *L. rhamnosus Ts3*, in concentration 2%.

Raffinose was metabolized by all strains in the two used concentration.

The acidification ability of studied strains was determine in the presence of the used oligosaccharides. The obtained results are presented in Figure 1 a, b and c).

The pH change during the growth of *L. acidophilus Ts1* and *L. rhamnosus Ts3* (up to 24 hours) reached values lower than 4, when the strains were cultivated in the presence Rf. Decrease in pH was observed for *L. bulgaricus Ts2* when cultivated on FOS. The fermentation pattern depends on the physiological condition of the growing cells. When cultivated on different oligosaccharides, the studied strains can change their fermentation ability towards production of more acidic products (Ignatova et al., 2009).

Table 1

Oligosaccharide utilization of stadied strains

Mikroorganisms isolated from comersial probiotic products	OD 600 nm Glucose	OD 600 nm FOS		OD 600 nm Inulin		OD 600 nm Raffinose	
		2%	2.50%	2%	2.50%	2%	2.50%
L. acidophilus Ts1	3.6±.15	1.9±0.02	0.4±0.04	0.5±0.5	0.4±0.5	1.37±0.5	1.80±0.7
L. bulgaricus Ts2	3.2±0.7	1.38±0.1	0.3±0.02	1.35±0.9	1.1±0.02	1.7±0.5	1.79±0.4
L. rhamnosus Ts3	4.3±0.9	1.7±0.7	0.7±0.06	1.73±0.5	0.8±0.03	1.66±0.7	1.9±0.4

Result are mean ± SEM of three separate trails

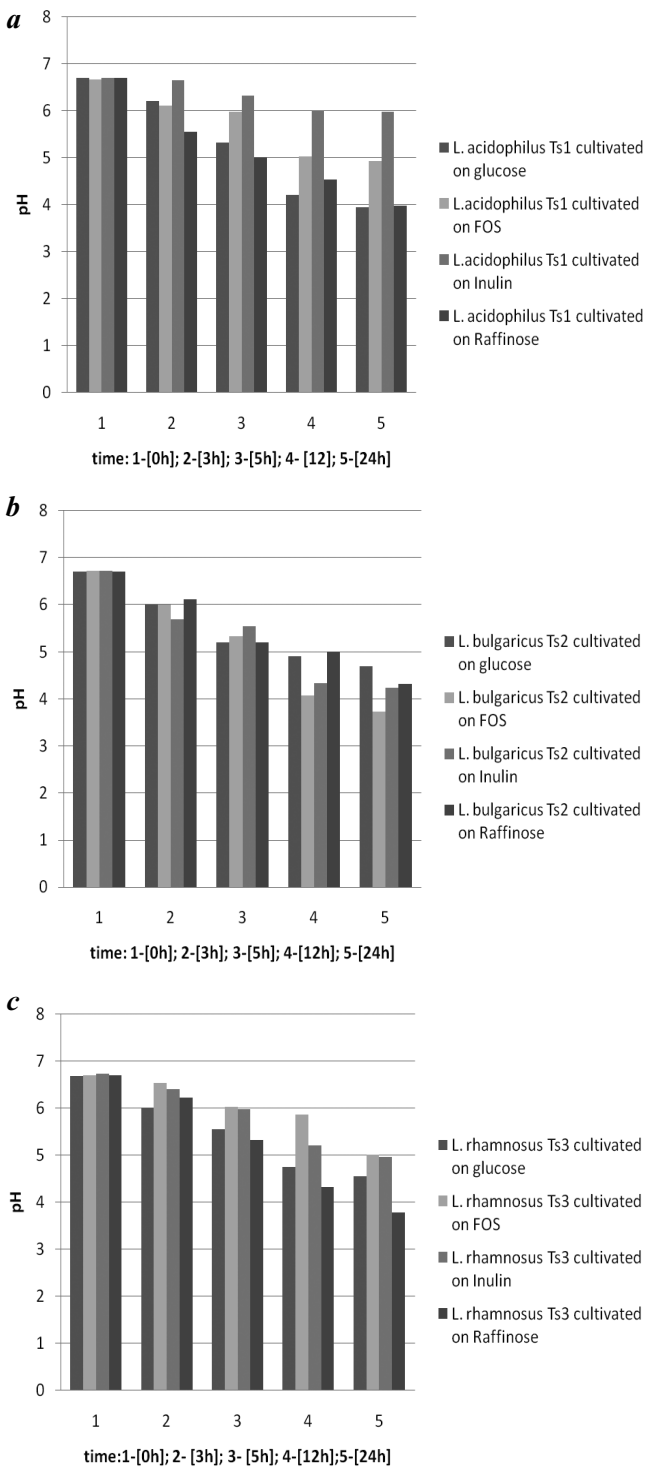

Fig. 1. Dynamic of media acidification by stadied strains in the presences of different carbohydrates Result are mean ± SEM of three separate trails

The supernatants obtained after fermentation in mMRS containing FOS, In and Rf were tested for their antimicrobial activity after pH adjustment to 6.5 against *E. coli 3398, St. aureus 745, B. cereus 4464 and E. aerogenes 3691* (Tables 2, 3 and 4). It could be seen that the inhibition of *E. coli* were considerably higher for strains cultivated in the presences of In. The strains cultivated in the presences on FOS inhibited considerably *E. aerogenes* . Any activity was not detected against test culture *St. aureus* for all tested strains. The found activity indicated that the system of uptake of unusual sugars influenced the production of antimicrobial substances in a specific way. The mechanism of this stimulation remains unclear.

In conclusion, three strains belonging to species *L. delbrueckii subsp. bulgaricus Ts2, L. rhamnosus Ts3* and *L. acidophilus Ts1* were shown to be able to utilize different oligosaccharides.

Table 2

Zone inhibitory activity [mm] of stadied strains cultivated on FOS

Test culture	*L. acidophilusTs1*	*L. bulgaricusTs2*	*L. rhamnosusTs3*
St. aureus	0	0	0
B. cereus	11	12	13
E. coli	15	15	16
E. aerogenes	23	24	22

Result are mean ± SEM of three separate trails

Table 3

Zone inhibitory activity [mm] of stadied strains cultivated on Inulin

Test culture	*L. acidophilusTs1*	*L. bulgaricusTs2*	*L. rhamnosusTs3*
St. aureus	0	0	14
B. cereus	12	10	11
E. coli	21	8	19
E. aerogenes	23	0	22

Result are mean ± SEM of three separate trails

Table 4

Zone inhibitory activity [mm] of stadied strains cultivated on Raffinose

Test culture	*L. acidophilusTs1*	*L. bulgaricusTs2*	*L. rhamnosusTs3*
St. aureus	0	0	0
B. cereus	13	11	9
E. coli	17	18	16
E. aerogenes	15	16	20

Result are mean ± SEM of three separate trails

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