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Metabolism of extracted inulin from *Helianthus tuberosus* by *Lactobacillus* strains isolated from traditional Kordish cheese

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Abstract

The capacity of five probiotic strains, isolated from traditional Kordish cheese, to use inulin extracted from *Helianthus tuberosus* (native inulin) as carbon and energy source in *in vitro* cultures has been tested. Selection criteria were the specific growth rate (μ), generation time (Tg), prebiotic index (PI) and prebiotic activity score (PAS). FT-IR results of native and commercial inulin showed native inulin has more hydrogen and double bond. Microbial results also showed that some strains reached higher growth levels on culture media containing native inulin compared to those obtained by commercial inulin. Based on the results, native inulin was a potential novel source of prebiotics. In addition, probiotic strains tested originated in traditional Kordish cheese are suitable characteristics for use as starters in synbiotic food.

Introduction

Global notice in oligosaccharides has been increasing since the statement of the prebiotic effect of these carbohydrates. Prebiotics are defined as non-digestible but fermentable carbohydrates that pass through to the large intestine and selectively stimulate the proliferation and/or activity of populations of desirable bacteria or a limited the growth of bacteria belonging to fusobacteria and clostridia in the colon, thereby improving the consumer's health (Gibson and Roberfroid, 1995; Kolida *et al*., 2002) . Inulin as oligosaccharide is a mixture of oligomer and polymer chains with a variable number of fructose molecules, joined by β bonds (2 - 1), which also generally contain a glucose molecule at the end of the chain. Native inulin occurs naturally in numerous foods of vegetable origin, such as *Helianthus tuberosus.* It possesses a number of interesting technological properties and is, therefore, used in various foods and drinks as prebiotic (Franck, 2002). However, the prebiotic effect of inulin depends generally on the composition of the intestinal flora in each individual and on the polymerization degree of fructose chains (Abrams *et al.*, 2005).

Lactobacilli are among the intestinal bacteria stimulated by prebiotics and some strains of these bacteria have been added to food products as probiotic cultures (Franck, 2002; Holzapfel and Schillinger, 2002). Due to the potential synergy between prebiotics and probiotics, products containing a mixture of these ingredients are usually referred to as synbiotics (Collins and Gibson, 1995). Therefore, it is necessary to test the prebiotic capacity of different fructans among different probiotic species to find the best synbiotic for food uses. The aim of this study was to determine suitable *Lactobacillus* strains to be included as starters in food with native inulin taking into account the following selection criteria: specific growth rate (μ), generation time (T_g), prebiotic index (PI) and prebiotic activity score (PAS).

Materials and Methods

Extraction of inulin from Helianthus tuberosus

To extract inulin, 3 kg lots of peeled tubers were sliced and dried at 55°C for 48 h. Then they were ground and mixed with water (65°C). The resulting extract was filtered through muslin cloth and then concentrated to 50% of the original volume using evaporator operating under a vacuum of 68 kPa with steam supply at 138 kPa. To remove impurities, the concentrate was mixed with 5% slurry of calcium hydroxide at 50–60°C for 30 min, resulting in the formation of a flocculent precipitate and a brighter yellow supernatant. By this technique, the pH of juice rose from 5–6 to 10–12. After filtration under vacuum using paper filter (Whatman No. 4), 10% phosphoric acid was added to the filtrate with vigorous stirring to adjust its pH to ca. 8–9, causing the precipitation

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of excess calcium and coagulated organic material. The mixture was allowed to stand at 60°C for 2–3 h before re-filtering (Whatman No. 4). The clarification process was repeated twice. Under these conditions, the dry matter of the clarified juice was ca. 7% (w/w). Activated carbon powder was added to the filtrates at 60°C (20 % w/v) and mixed for 15–30 min in order to remove colored materials. The treated syrup was filtered (Whatman No. 1) and the clear syrup obtained was further concentrated by rotary evaporation at 670°C, to obtain syrups with 15 °B. Then, inulin was precipitated in ethanol 99% and was dried with freeze drying (Paseephol *et al*., 2007).

Yield of extracted inulin

Inulin content was measured according to Dubois *et al.* (1956) and inulin yield was determined from the equation:

Yield of extracted inulin (%) = (inulin×volume of extract/ amount of powder)×100

FT-IR spectra acquisition

A Perkin-Elmer Spectrum RXI FT-IR spectrophotometer equipped with a deuterated triglycerine sulphate (DTGS) detector was used to collect FT-IR spectra with a resolution of 8 cm^{-1} at 16 scans. A small quantity of the sample was deposited with the use of a Pasteur pipette between two well-polished KBr disks, creating a thin film. Duplicate spectra were collected for the same sample. All spectra were recorded from 4000 to 400 cm^{-1} and processed with the computer software program Spectrum for Windows (Perkin-Elmer).

Bacterial strains

Native probiotics strains tested were *Lactobacillus plantarum* (LS5, LU5, AF1, and LP3) and *Lactobacillus paraplantarum* which isolated from traditional Kurdish cheese. *Lactobacillus plantarum* ATCC 14917 and *Lactobacillus paraplantarum* DSM 14485 also were used as a reference strains. Enteropathogen used was *Escherichia coli* O157:H7.

Bacterial growth conditions

All bacterial strains after activation in proper medium (MRS broth for probiotic strains and Mueller-Hinton broth for *E. coli*) were grown (10 % (v/v)) in basal medium (30 mL) containing, in g/L, peptone, 2.0; yeast extract, 2.0; NaCl, 0.1; K_2HPO_4, 0.04; KH_2PO_4, 0.04; $CaCl_2.6H_2O$, 0.01; $MgSO_4.7H_2O$, 0.01; $NaHCO_3$, 2.0; Tween 80, 2 mL; hemin (dissolved in three drops 1 M NaOH), 0.05; vitamin K1, 10 µL; bile salts, 0.5 with various carbohydrate sources (0, 0.5, 1% (w/v)) at 37°C.

Specific growth rate (µ), generation time (T_g) and prebiotic index (PI) of strains

After fermentation over night, sampling and dilutions in sterile peptone water were made. *Lactobacillus* viable counts were made on MRS agar (Oxoid) after anaerobic incubation at 37°C using Gas Paks (Anaerobic system BR038B, Oxoid) for 72 h. *E. coli* counts also were made on Tryptone soya agar aerobically at 37°C for 24 h. Specific growth rate (µ) for each culture was calculated using equation:

$$\mu = (\log 10\ X_t - \log_{10} X_0)/ (t_t - t_0),$$

where X_t and X_0 are counts (cfu/mL) at time t_t and t_0.

Generation time (T_g) was calculated as $T_g = \ln(2/\mu)$ (Shin *et al*., 2000).

The PI equation is described below according to Palframan *et al*. (2003) with brief modification:

$$PI = Lac\text{-}Esc$$

where Lac is *Lactobacillus* numbers at sample time/ numbers at inoculation and Esc is *E. coli* numbers at sample time/ numbers at inoculation.

Prebiotic activity score

Prebiotic activity scores were performed according to the procedure established by Huebner *et al.* (2007). Cell densities were determined based on optical density (O.D.) at 600 nm. The prebiotic activity score was determined using the following equation:

PAS = [(probiotic log O.D. on the prebiotic at 24 h - probiotic log O.D. on the prebiotic at 0 h)/(probiotic log O.D. on glucose at 24 h - probiotic log O.D. on glucose at 0 h)]-[(enteric log O.D. on the prebiotic at 24 h - enteric log O.D. on the prebiotic at 0 h)/ (enteric log O.D. on glucose at 24 h - enteric log O.D. on glucose at 0 h)]

Statistical analysis

The results were analyzed using one-way ANOVA, and significant differences between groups were determined by the Duncan's multiple range test and t-test. All statistical analyses were performed using the SPSS package program version 20 and Minitab 16. Differences were considered significant at $P < 0.05$.

Results and Discussion

The yield of inulin extraction was 45.48%. Results of FT-IR (Fig 1) show native inulin in

Table 1. Maximum specific growth rate of microbial strains

	Native inulin+0.05%	Commercial inulin+0.05%	Native inulin +1%	Commercial inulin +1%
*L.plantarum*LP3	0.084± 0.001^{aA}	0.08± 0.001^{aA}	0.182± 0.002^{aB}	0.183± 0.001^{aB}
*L. plantarum*AF1	0.088± 0.001^{bA}	0.092± 0.002^{bcA}	0.187± 0.002^{bB}	0.192± 0.002^{bB}
*L. plantarum*LU5	0.089 ^{bA}	0.093± 0.001^{bcB}	0.186± 0.001^{bC}	0.187 ^{cC}
*L. plantarum*LS5	0.098±0.001 ^{cA}	0.095±0.001 ^{cB}	0.196± 0.001^{cC}	0.194±0.001 ^{bD}
L. paraplantarum	0.092± 0.001^{dA}	0.094 ^{cA}	0.197± 0.001^{cB}	0.192±0.001 ^{bC}
L. paraplantarum DSM 14485	0.093±0.001 ^{dA}	0.092± 0.002^{bcA}	0.178±0.001 ^{dB}	0.181± 0.001^{aB}
L. plantarum ATCC 14917	0.088±0.001 ^{bA}	0.091± 0.001^{bA}	0.171± 0.001^{eB}	0.172±0.001 ^{dB}
E. coli O157 H7	0.044±0.001 ^{eA}	0.042± 0.002^{dA}	0.077± 0.001^{fB}	0.077± 0.002^{eB}

All values are means of three determinations. Values in each column with the same small letter and values in each row with the same capital letter are not significantly different ($p < 0.05$).

Table 2. Generation time of microbial strains

	Native inulin+0.05%	Commercial inulin+0.05%	Native inulin +1%	Commercial inulin +1%
L. plantarum LP3	3.17± 0.018^{aA}	3.21± 0.018^{aA}	2.4± 0.011^{aB}	2.39± 0.005^{aB}
L. plantarum AF1	3.17± 0.011^{bA}	3.07±0.022 ^{bcA}	2.37± 0.009^{bB}	2.34± 0.01^{bB}
L. plantarum LU5	3.17 ^{bA}	3.07± 0.016^{bcB}	2.37± 0.007^{bC}	2.37± 0.003^{cC}
L. plantarum LS5	3.02± 0.01^{cA}	3.05± 0.015^{cB}	2.32± 0.006^{cC}	2.33± 0.008^{bD}
L. paraplantarum	3.08± 0.016^{dA}	3.05±0.022 ^{cA}	2.32± 0.008^{cB}	2.34±0.008 ^{bC}
L. paraplantarum DSM 14485	3.07± 0.016^{dA}	3.07±0.022 ^{bcA}	2.42± 0.005^{dB}	2.4±0.009 ^{aB}
L. plantarum ATCC 14917	3.12± 0.011^{bA}	3.09±0.016 ^{bA}	2.46± 0.011^{eB}	2.45±0.005 ^{dB}
E. coli O157:H7	3.81± 0.034^{eA}	3.86±0.049 ^{dA}	3.26± 0.02^{fB}	3.25± 0.027^{eB}

All values are means of three determinations. Values in each column with the same small letter and values in each row with the same capital letter are not significantly different ($p < 0.05$).

Table 3. Prebiotic index of *Lactobacillus* strains

	Native inulin+0.05%	Commercial inulin+0.05%	Native inulin +1%	Commercial inulin +1%
L. plantarum LP3	0.93± 0.04^{aA}	0.92± 0.04^{aA}	2.55± 0.04^{aB}	2.54±0.02^{aB}
L. plantarum AF1	1.03± 0.03^{bA}	1.21± 0.04^{bcA}	2.66± 0.04^{aB}	2.8± 0.05^{bB}
L. plantarum LU5	1.05± 0.01^{bA}	1.22± 0.04^{bcB}	2.63±0.04^{bC}	2.65± 0.01^{cC}
L. plantarum LS5	1.27± 0.02^{cA}	1.26± 0.04^{cB}	2.89± 0.03^{cC}	2.8± 0.04^{bD}
L. paraplantarum	1.12± 0.04^{dA}	1.26± 0.05^{cA}	2.9± 0.04^{cB}	2.8± 0.04^{bC}
L. paraplantarum DSM 14485	1.14± 0.04^{dA}	1.21± 0.05^{bcA}	2.45±0.02^{dB}	2.5± 0.04^{aB}
L. plantarum ATCC 14917	1.03±0.02^{bA}	1.12±0.01^{bA}	2.78± 0.047^{eB}	2.79±0.05^{dB}

All values are means of three determinations. Values in each column with the same small letter and values in each row with the same capital letter are not significantly different ($p < 0.05$).

Table 4. Prebiotic activity scores of *Lactobacillus* strains

	Native inulin	Commercial inulin
L. plantarum LP3	0.43±0.01 $^{a\ A}$	0.42± 0.02$^{a\ A}$
L. plantarum AF1	0.43± 0.02$^{a\ A}$	0.45± 0.01$^{b\ A}$
L. plantarum LU5	0.41± 0.01^{abA}	0.45 ^{bB}
L. plantarum LS5	0.41± 0.01^{abA}	0.41± 0.01^{cA}
L. paraplantarum	0.41 ^{abA}	0.41± 0.01^{cA}
L. paraplantarum DSM 14485	0.39 ^{bA}	0.41±0.01 ^{cA}
L. plantarum ATCC 14917	0.41± 0.01^{abA}	0.43± 0.01^{abA}

All values are means of three determinations. Values in each column with the same small letter and values in each row with the same capital letter are not significantly different ($p < 0.05$).

comparison to commercial inulin have a more hydrogen bond and double bond. Results also show many single bonds for two inulin types. Table 1 and 2 show specific growth rates (μ) and generation time (T_g) of seven *Lactobacillus* strains and enteric strain. Native strains showed the highest μ followed by standard strains and *E. coli* showed poor growth. Generation time (T_g) was significantly ($P < 0.05$) lower for *E. coli* in comparison to native and reference *Lactobacillus* strains. *Lactobacillus plantarum* LS5 and *Lactobacillus paraplantarum* showed the highest activity for utilizing of native and commercial inulin. In addition, for two mentioned strains native inulin was better carbohydrate source

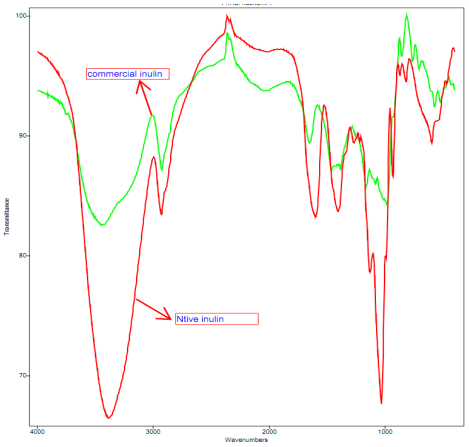

Figure 1. FT-IR of native and commercial inulin

than commercial inulin. However, other strains did not show significantly different for utilizing of two inulin types. All strains demonstrated a positive correlation between growth rate and inulin concentration and Inulin at higher concentration showed better growth of strains.

The averages of prebiotic index for two inulin types are shown in Table 3. These results indicate that the addition of both inulin types to the batch fermenter resulted in a significant increase in the levels of lactobacilli, which are considered to have beneficial properties. In contrast the level of *E. coli* was reduced. The PI values obtained for native inulin compared favorably with those obtained for commercial inulin. The positive PI values were obtained with both substrates suggest they could be used as prebiotic. PI results also indicate that native *Lactobacillus* strains were more active than reference strains for utilizing inulin types. Martinez and Gomez (2007) reported that probiotic strains with higher levels of α-galactosidase activity showed growth and acidification rate enhancement when oligosaccharides from lupins were added, therefore, these substrates could be used as prebiotics. Palframan *et al.* (2003) concluded that fructooligosaccharides and galactooligosaccharides all showed their optimum prebiotic effects at higher concentration. They also suggested the PI gives a quantitative score that describes the prebiotic effect and substrates with higher PI have more prebiotic activity.

In addition, prebiotic activity score is a simple and rapid technique to determine prebiotic activity. These results are listed in Table 4. No statistically significant differences in scores of native strains were observed for local inulin. Furthermore, *Lactobacillus plantarum* LP3 and *Lactobacillus plantarum* AF1 were more active than *Lactobacillus paraplantarum* DSM 14485 for utilizing local inulin. The prebiotic capacity of different compounds is being intensely investigated during last years. Among these

compounds, the non-digestible oligosaccharides and especially the β(2-1) fructans deserve special attention as they constitute the most extensively studied and reported prebiotic group (Roberfroid, 2000; Cummings *et al*., 2001). In the present study, we have analyzed in vitro the capacity of probiotic strains, native and reference, to metabolize native and commercial inulin with the objective of obtaining preliminary data that will allow the selection of a mixture of these oligosaccharides with the highest prebiotic capacity in these microorganisms. The results obtained here demonstrate that the all native strains efficiently metabolize native and commercial inulin in comparison to reference strains. Moreover, from a health perspective, the present results are important due to the growing interest in probiotic bacteria and the perceived benefit of increasing their numbers in the gastrointestinal tract.

Conclusions

In this study, we demonstrated that native inulin was a potential novel source of prebiotics. On the basis of the data obtained through in vitro fermentations, native inulin exhibited a potential to be used as an effective source of prebiotics by increasing the populations of *lactobacillus* strains. Furthermore, results suggest the *lactobacillus* strains tested originated in traditional Kurdish cheese are suitable characteristics for use as starters in synbiotic food.

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