Viability of *Bifidobacterium bifidum* and *Escherichia coli* in Versus Prebiotic Effects of Jerusalem Artichoke (*Helianthus tuberosus*)

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Abstract

Background: In this study, in vitro prebiotic effects of Jerusalem artichoke poly-fructans on the survivability and activity of *Bifidobacterium bifidum*, *Escherichia coli* were investigated and compared with HP-Inulin (a high molecular-weight fraction of chicory-derived inulin).

Objectives: The prebiotic potential of poly-fructans extracted from native Jerusalem artichoke tubers on the survivability of *B. bifidum* and *E. coli* was evaluated in this study.

Methods: In this experimental study, 24 treatments divided to 2 groups (Extracted poly-fructose from Jerusalem artichoke tubers and standard inulin) randomly. The turbidity and pH variations during 48 hours incubation were determined using final concentrations of each group (0.5%, 1%, 2% and 3% (w/v)). The data were analyzed by MINITAB 14 and MSTATC statistical software, one way ANOVA and Duncan's test.

Results: This study suggests that Jerusalem artichoke tuber fructooligosaccharides (*JA-Fr*) had the potential to be used as a prebiotic component. The growth of *B. bifidum* improved significantly in the presence of Jerusalem *artichoke* fructans compared to the control. There was no significant differences ($P < 0.05$) in *Bifidobacterium* population in different concentrations of Jerusalem artichoke poly-fructans, but the population was significantly higher than the count in the presence of HP-inulin. The pH decreased in both media during 48 hours incubation time. Specific rate of growth and doubling time determined for *E. coli* demonstrated that the efficacy of various carbon sources in stimulating bacterial growth were influenced by the concentration and DP (degree of polymerization) of fructan chains in the media.

Conclusions: *Jerusalem artichoke fructooligosaccharides* can provide the greater stability of probiotics and acid production, so it can be considered as a potential source of high-yielding oligosaccharide for commercial prebiotic production to develop food industry and improve host health.

Keywords: Prebiotic, Jerusalem Artichoke, Inulin, Fructan

1. Background

Lactic acid bacteria is a major part of normal human and animal gut microflora. It is also important for maintaining gastrointestinal health. As a naturally occurring probiotic, *Bifidobacteria* and *Lactobacilli* can modify the metabolic activities in the body by modulating immune system [1], producing antimicrobial agents such as hydrogen peroxide, antimicrobial peptides (bacteriocins), and organic acids such as acetic and lactic acids [2]. When used in adequate amounts in diet, they can synthesis vitamins (such as K and B), stabilize barrier functions and enhance the calcium and other mineral absorption on the gut. Different studies have also demonstrated positive effects of probiotic bacteria on bowel pH, intestinal regularity and the colonization resistance against pathogens [3, 4].

Prebiotics are Non-digestible food ingredients that allow beneficial changes. They may promote the survival or persistence of probiotic strains, enhance defense mechanisms of the host, increase resistance to various health disorders, and modify human gastrointestinal tract troubles [5]. Inulin type fructans fructooligosaccharides (FOS), and inulin are considered as prebiotics which are composed of β (2-1) linked fructosy l units with or without a terminal D-glucose at the reducing end. They have different degree of polymerization (DP) and may originate naturally as native components in many plants or derive through biochemical/enzymatic techniques [6-8].

A number of in vitro and in vivo studies have confirmed that inulin kind fructans are fermented in to lactic and short-chain carboxylic acids. Functional activities of some

commercial prebiotics like NutraFlora P-95 (DP = 2 - 4), Raftilose P95 (DP = 2 - 7), Inulin-S (DP = 2 - 60), Raftiline HP (DP > 23) and GOS (galactooligosaccharides) (DP = 2 - 4) were assessed in in vitro studies [6]. Banuelos et al. tested the capacity of two probiotic strains, Lactobacillus gasseri CECT5714 and Lactobacillus fermentum CECT5716, to use several b (2-1) fructan mixtures as carbon source in in vitro cultures [8].

Jerusalem artichoke (Helianthus tuberosus) and *Chicory (Cichorium intybus)*, belong to *Compositae* family, are two plant species used in commercial production of prebiotics [9, 10].

Jerusalem artichoke tuber contains nearly 13% - 18 % carbohydrates, of which about 80 % are inulin type fructans, 10% - 13 % sucrose, 3.5% - 5 % reducing sugars, 10% - 17 % proteins [11] and 0.8% - 0.9 % important minerals including K, Ca, P, Fe, Zn, Mg, Na, Cu and Mn [12, 13], and traditionally cultivates as food and animal feed [14].

In Jerusalem artichoke tubers the DP of fructans is rather low [15] and mainly depends on the plant source, variety, climate conditions and date of harvest [16, 17].

In the present study, the prebiotic potential of polyfructans extracted from native Jerusalem artichoke tubers on the survivability and activity of *B. bifidum* and *E. coli* was investigated in "in vitro" conditions, and compared with prebiotic effects of HP-inulin.

2. Methods

2.1. Bacterial Strains

Two strains of pure commercial cultures used were *Bifidobacterium bifidumPTCC1644* and *Escherichia coli PTCC 1330*. Both of them obtained from Persian type culture collection.

2.2. Media Preparation and Growth Conditions

The standard prebiotic of HP-inulin purchased from Orafti (Tienen, Belgium).The fructooligosaccharides of *Jerusalem artichoke tubers* (JA-Fr) were extracted according to Milani et al. [18]. B. bifidum growth media were MRS broth (DeMan-Rogosa-Sharpe Broth) supplemented with L-cysteine hydrochloride (0.5 g/L), sodium thioglycolate (0.2 g/L), and $CaCl_2.2H_2O$ (0.1 g/L). TSB broth (Trypticase Soy Broth) was used for propagation of *E. coli*. Both media and all other chemicals purchased from Merck (Darmstadt, Germany). *B. bifidum* growth was anaerobically (Oxoid Anaerobic System with Gas Pak). The fructooligosaccharides free media were used as the control and the basal media which were sterilized by autoclaving at 121°C for

15 minutes. In the case of modified MRS broth, filter-sterilized L-cysteine hydrochloride was added to the autoclaved media. The inoculums were prepared from the standard strains stored in glycerol 12 % at -70°C using basal media. The filter-sterilized carbohydrates (JA-Fr and HP-inulin) added to the basal MRS broth and basal TSB broth to give final concentrations of 0.5 %, 1 %, 2 % and 3 % (w/v).3.3 Growth measurement.

In order to investigate the effects of JA-Fr on the growth and survivability of *Bifidobacterium bifidum* and *Escherichia coli* strains, the bacteria were cultivated overnight in the appropriated basal medium at 37°C. The activated cultures centrifuged for 15 minutes with $2500 \times g$ at 4°C (sigma centrifuge model 2 - 16 p), then the precipitate was washed twice with Phosphate-buffered saline (PBS) (0.1 M phosphate buffer pH = 7.4, 0.9 % saline), and the final pellet was suspended in PBS and diluted to about 10^6 cells/mL for B. bifidum and 10^9-10^{10} cells/mL for *E. coli*. The bacterial suspensions were inoculated at 1 % (v/v) concentration in to different testing media containing fructans. Then the cultures were incubated at 37°C for 24 hours. The turbidity at 620 nm of each culture was determined every 4 hours by subtracting A_620values of bacterial free medium from each test media. All measurements were repeated at least twice.

2.3. Growth Kinetic Parameters

Specific growth rate (μ) was calculated for each microorganism during its exponential growth phase by the formula:

Equation 1.

$$\mu = \frac{Lnx - LnX_0}{t - t_0} \tag{1}$$

Where x and x_0 are absorbance measured at time t and t_0, respectively.

The generation time (tg) was calculated for each culture from the corresponding value of (μ) by the formula:

$$tg = \frac{Ln2}{\mu} \tag{2}$$

2.4. pH Changes

pH value was measured using Inolab pH meter model WTW (Inolab).

2.5. Statistical Analysis

The obtained results were analyzed by MINITAB 14 and MSTATC software and significant differences between groups were determined by the Duncan's multiple range test. Differences were considered significant at P < 0.05.

3. Results

3.1. Effects of Fructans on Microbial Populations

Bifidobacterium bifidum and *Escherichia coli* are the strains associated with human and animal digestive systems. In vitro experiments on the comparative fermentation of selected fructooligosaccharides showed that the *JA-Fr* was fermented by *B. bifidumPTCC1644*, and it had the potential to stimulate the growth of bacteria (Table 1).

As shown in Table 2, supplementation with JA-Fr was found to have significantly better effect on survivability of the bacteria compared with that without *JA-Fr* (P < 0.05). We did not observed significant differences in the amount of cell growth as the concentration of *JA-Fr* rose to 3 % (w/v). Growth curve of *B. bifidum* showed that the bacteria grew rapidly after about 8 hours, with maximum growth observed at about 24 hours (Figure 1). Determined turbidities of the media containing *JA-Fr* were significantly (P < 0.05) higher than the ones containing HP-inulin, indicating that *JA-Fr* was more effective in modifying the growth behavior of *Bifidobacterium bifidum PTCC1644*.
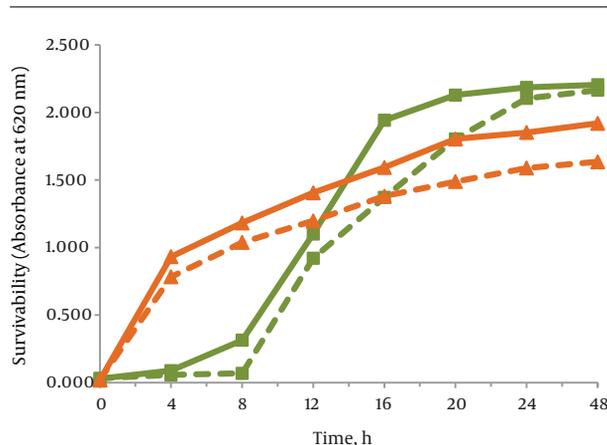

Figure 1. Growth kinetics of *B. bifidum* (■) and E. coli (▲) in appropriate medium enriched with JA-Fr (Rectangle) and HP-inulin (…) at 2 % (w/v) during 48-hour incubation at 37°C

Specific rates of growth were determined for the media supplemented with 2 % (w/v) *JA-Fr* compared to the control and HP-inulin (Table 3). *JA-Fr* caused greater in vitro growth rate of *Bifidobacterium* than did the control and HP-inulin.

To study the effects of different fructans on the growth of *E. coli*, the organism were cultured in the appropriate media supplemented with different concentrations of fructooligosaccharides at 37 °C. Results showed that they were fermented by the microbial flora (Table 4). Growth promotion of *E. coli* by JA-Fr has been dose dependent over the range 0.5 % to 3 % as evidenced by increased turbidity of the bacteria suspensions (Table 5), indicating that *E. coli*

grew faster in the presence of these carbohydrates. Figure 2 shows the growth curves of *E. coli* strain cultured with both carbon sources. HP-inulin was found to be less effective on the viability of *E. coli*.

The doubling time (*tg*) of the strain grown in the presence of *JA-Fr*, HP-inulin and the control medium are compared in Table 3. Doubling time was used as a measure of the efficacy of various carbon sources in modulating growth rate. According to Table 3, *tg* in the medium containing *JA-Fr* was minimal.

3.2. pH Evaluation

The pH was recorded for the cultures with various carbohydrates. In accordance with growth stimulation, acid production by *Bifidobacterium* was also enhanced (P < 0.05) by the presence of *JA-Fr* in the media compared to the control (Table 6).
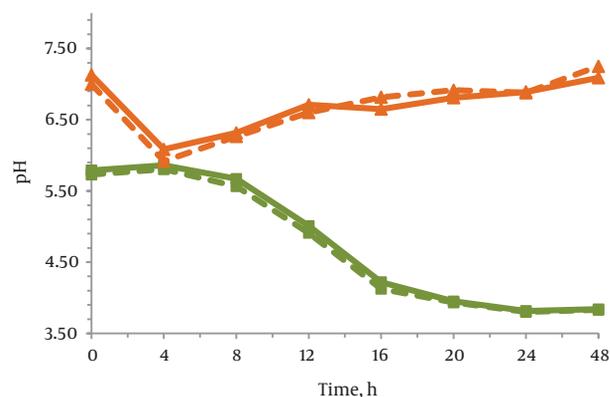

Figure 2. Changes in pH of media inoculated with B. bifidum (■) and E. coli (▲) in appropriate medium enriched with JA-Fr (Rectangle) and HP-inulin (…) at 2 % (w/v) during 48-h incubation at 37°C

As shown in Figure 2, the pH continued to decline in a similar way in the presence of both *JA-Fr* and HP-inulin. After 24 hours of incubation, the pH dropped to 3.76 in *JA-Fr*-containing media, and to 3.87 in media with HP-inulin. The difference of pH values between two fructans was not significant (P < 0.05). Table 7 demonstrates the changes in TSB medium pH with different concentrations of *JA-Fr* which occur during fermentation.

pH has similar behavior in both media containing *JA-Fr* and HP-inulin. It was approximately 7.0 prior to inoculation, then there was a continued fall in medium pH over the first 4 hours and at this time, it began to rise (Figure 2). The difference between the pH values of two media was significant (P < 0.05).

Table 1. Comparative Growth of *B. bifidum* in the Presence of *JA-Fr*, HP-Inulin and in Control Medium[a]

	Bifidobacterium bifidum		
Fructan in the growth media	Control	JA-Fr	HP-Inulin
Absorbance at 620 nm	1.198 ± 0.06[b]	1.237 ± 0.06[a]	1.126 ± 0.08[c]
P-Value	0.004	0.004	0.007

[a] The data represent the results of a duplicate experiment.

Table 2. Effects of *JA-Fr* Concentrations on the Viability of *B. bifidum*[a]

	Bifidobacterium bifidum				
JA-Fr Concentration (w/v)	0%	0.5%	1%	2%	3%
Survivability (Absorbance at 620nm)	1.090 ± 0.003[b]	1.239 ± 0.008[a]	1.217 ± 0.004[a]	1.266 ± 0.009[a]	1.2230.008[a]
P-Value	0.002	0.009	0.005	0.001	0.002

[a] Different letters mean statistically significant difference among the values of the same parameter, according to Duncan test (P < 0.05).

Table 3. Comparison of Bacterial Specific Growth rates (μ) and Doubling Times (tg) in the Presence of Different Fructans[a,b]

	Bifidobacterium bifidum	*Escherichia coli*
Control		
μ	0.126 ± 0.007	0.902 ± 0.003
tg	5.50 ± 0.007	0.76 ± 0.003
JA-Fr		
μ	0.142 ± 0.006*	0.973 ± 0.004*
tg	4.88 ± 0.006*	0.71 ± 0.004*
HP-inulin		
μ	0.099 ± 0.001	0.905 ± 0.007
tg	6.97 ± 0.001	0.77 ± 0.007

[a] All values for μ (h^{-1}) and tg (h) are means from duplicate determination ± SD.
[b] Significant differences at P < 0.05 confidence intervals (according to Duncan test) are shown as *.

Table 4. Comparative growth of *E. coli* in the presence of *JA-Fr*, HP-inulin and in control medium[a]

	Escherichia coli		
Fructan in the growth media	Control	JA-Fr	HP-Inulin
Absorbance at 620 nm	1.192 ± 0.08[b]	1.233 ± 0.05[a]	1.175 ± 0.09[c]
P-Value	0.006	0.001	0.004

[a] The data represent the results of a duplicate experiment.

4. Discussion

Our experiments on the supplementation with *JA-Fr* and HP-inulin showed that the *JA-Fr* had better effect on survivability of *B. bifidumPTCC1644*. Specific rate of growth determined for *E. coli* revealed that the efficacy of various carbon sources in stimulating bacterial growth were influenced by the concentration and DP of fructan chains in the media.

The ability of *Bifidobacteria* to utilize fructooligosaccharides has been reviewed in many studies and the lowering of culture pH as a result of short chain fatty acids production for certain bacterial species has often been used as

Table 5. Effects of *JA-Fr* concentrations on the viability of *E. coli*[a]

	Escherichia coli				
JA-Fr Concentration (w/v)	0%	0.5%	1%	2%	3%
Survivability (Absorbance at 620 nm)	1.192 ± 0.002^e	1.272 ± 0.005^d	1.347 ± 0.007^b	1.392 ± 0.002^a	1.318 ± 0.009^c
P-Value	0.002	0.004	0.001	0.004	0.007

[a] Different letters mean statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

Table 6. Effects of *JA-Fr* Concentrations on pH Changes of Media Inoculated with *B. bifidum*

	Bifidobacterium bifidum				
JA-Fr Concentration (w/v)	0%	0.5%	1%	2%	3%
pH	4.86 ± 0.009^a	$4.84 \pm 0.005^{a,b}$	$4.82 \pm 0.008^{b,c}$	4.80 ± 0.006^c	4.75 ± 0.006^d
P-Value	0.003	0.001	0.003	0.002	0.005

[a] Different letters mean statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

Table 7. Effects of *JA-Fr* concentrations on pH changes of media inoculated with *E. coli*[a]

	Escherichia coli				
JA-Fr Concentration (w/v)	0%	0.5%	1%	2%	3%
pH	6.78 ± 0.005^b	6.72 ± 0.009^c	6.86 ± 0.004^a	$6.79 \pm 0.008b$	6.50 ± 0.003^d
P-Value	0.003	0.005	0.003	0.003	0.002

[a] Different letters mean statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

an index of the fermentability of various carbohydrates in the culture [19]. Our results demonstrated that the viability of *Bifidobacterium bifidum*PTCC1644 and *Escherichia coli*PTCC 1330 in the media depend on the type and concentration of carbon source. Jerusalem artichoke fructooligosaccharides can be considered as a potential source for prebiotic production because it can provide the greater stability of probiotics and acid production.

Biedrzycka and Bielecka reported that the in vitro consumption of inulin by *Bifidobacteria* depended to purity and degree of polymerization of fructo-oligomeric chains. Their research indicated that the majority of *Bifidobacterium* strains studied utilized short chain FOS and OF [20]. Watson et al. showed that Lactulose, maltodextrin, FOS, GOS and the GOS/inulin (9: 1) mixture stimulate the growth performance of *Bifidobacteria* (12 different species), while inulin and polydextrose appeared to be rather poor substrates for *bifidobacterial* growth [21]. Inconsistent findings, Vigsnas et al. demonstrated that *B. adolescentis* and *B. longum* are able to degrade linear arabino-oligosaccharides (DP 8), whereas *B. breve* is able only to hydrolyze FOS and *B. bifidum* is not able to degrade either FOS or AOS [22].

Wichienchot et al. used mixed oligosaccharides obtained from white-flesh dragon fruit (Pitaya) and a reference prebiotic (inulin) as carbon sources for the cultivation of *B. bifidum* NCIMB 702715. It was found that inulin had a greater effect on the bacterial growth compared to Pitaya oligosaccharides, although the difference was not significant [23]. In another study, Wang et al. showed that the numbers of *Bifidobacterium bifidum*ATCC 29521 were greater than those in control medium (P < 0.05) when cultured in the medium supplemented with alginate oligosaccharides. This compound stimulated the growth of *B. bifidum*, more significantly in comparison with fructo-oligosaccharides (FOS) [24].

In general, the ability of coliforms to utilize prebiotic oligosaccharides has been contradictory. Several studies have reported that FOS can support growth of *E. coli*, *Enterobacter* and *Salmonella* [25, 26]. In contrast, others have indicated that *E. coli* is unable to utilize FOS [27]. Lopez-Molina et al. studied the utilization of chicory and Artichoke inulin (different DP) in mixed cultures of colonic bacteria and showed that growth of *Escherichia coli* and total anaerobes was slower but longer-lasting in the presence of both inulins compared to the control with glucose [28].

Van Laere et al. reported that arabino-oligosaccharides could support the growth of *E. coli* but FOS could not [29].

Our findings were implying that the degree of polymerization of fructans was an important factor that decides the accessibility of fructans to the bacteria. According to Biedrzycka and Bielecka, susceptibility of saccharides to fermentation mainly depends on water solubility, chemical structure and degree of polymerization, chain length, branched or linear structure and composition of monomer units [19]. Shetty et al. reported that in "in vitro" fermentation of inulin by human fecal bacteria, molecules with DP > 10 were fermented on the average half as quickly as molecules with DP < 10 [30]. The degree of polymerization of fructans from Helianthus tuberosus tubers is rather low [15] in comparison with HP-inulin and mainly depends on the variety, climate conditions and time of harvest [16, 17]. In body, the lower pH is believed to have additional effects because the production of these acids reduces intestinal pH and restricts or prohibits the growth of many pathogen and putrefactive bacteria. Also it increases mineral uptake [31]. In the case of *Escherichia coli*, casein is a principal nutrient in TSB medium; so the changes in pH curves are probably due to cells metabolism especially the deamination of amino acids during bacterial growth [19, 32].

4.1. Conclusions

Regarding to the concept of synbiotic which is a mixture of probiotics and prebiotics that synergistically enhance equilibrium of the gastrointestinal microflora, finding new natural resources containing various prebiotic components could be an appropriate way to develop food industry and improve host health. Our results revealed that the survival and metabolic activity of *Bifidobacterium bifidum PTCC1644* and *Escherichia coli PTCC 1330* in the media depend on the type and concentration of carbon source. Jerusalem artichoke fructooligosaccharides can provide the greater stability of probiotics and acid production, so it can be considered as a potential source of high-yielding oligosaccharide for commercial prebiotic production; however further investigations are needed with other probiotic strains and in in vivo conditions to optimize the fructans concentration and bacterial growth.

Acknowledgments

Footnotes

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