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Prebiotic effect of Jerusalem artichoke (*Helianthus tuberosus*) fructans on the growth performance of *Bifidobacterium bifidum* and *Escherichia coli*

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ABSTRACT

Objective: To investigate and compare *in vitro* prebiotic effects of Jerusalem artichoke poly-fructans on the survivability and activity of *Bifidobacterium bifidum* and *Escherichia coli* with high performance-inulin (a high molecular weight fraction of chicory-derived inulin).
Methods: Extracted polyfructose from Jerusalem artichoke tubers and standard inulin were added to the appropriated culture to achieve final concentrations [0.5%, 1.0%, 2.0% and 3.0% (w/v)] to determine the turbidity and pH variations during 48 h incubation.
Results: This study suggested that Jerusalem artichoke tuber fructooligosaccharides had the potential to be used as a prebiotic component. The growth of *Bifidobacterium 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 high performance-inulin. The pH decreased in both media during 48 h incubation time. The specific rate of growth and doubling time determined for *Escherichia coli* demonstrated that the efficacy of various carbon sources in stimulating bacterial growth was influenced by the concentration and 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.

1. Introduction

Lactic acid bacteria are a major part of normal human and animal gut microflora and are 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 and producing antimicrobial agents such as hydrogen peroxide, antimicrobial peptides (bacteriocins), and organic acids such as acetic and lactic acids[1,2]. When used in adequate amounts in diet, they can synthesize 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, oligofructose and inulin) are considered as prebiotics which are composed of β (2-1) linked fructosyl units with or without a terminal D-glucose at the reducing end. They have different degrees of polymerization (DP) and may originate naturally as native components in many plants or derive through biochemical/enzymatic techniques[6-8].

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

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

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

In the present study, the prebiotic potential of poly-fructans extracted from native Jerusalem artichoke tubers on the survivability and activity of *Bifidobacterium bifidum* PTCC1644 (*B. bifidum*) and *Escherichia coli* PTCC 1330 (*E. coli*) was investigated in *in vitro* conditions and compared with prebiotic effects of high performance (HP)-inulin.

2. Materials and methods

2.1. Bacterial strains

Two strains of pure commercial cultures used were *B. bifidum* and *E. coli* obtained from Persian Type Culture Collection.

2.2. Media preparation and growth conditions

The standard prebiotics of HP-inulin was purchased from Orafti (Tienen, Belgium). The fructooligosaccharides of Jerusalem artichoke tubers (JA-Fr) were extracted according to Milani *et al*.[18]. Growth media of *B. bifidum* was deMan Rogosa Sharpe (MRS) 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). Trypticase soy broth (TSB) was used for the propagation of *E. coli*. Both media and all other chemicals were purchased from Merck (Darmstadt, Germany). *B. bifidum* growth was anaerobic (Oxoid Anaerobic System with Gas Pak). The free media of fructooligosaccharides was used as the control and the basal media which were sterilized by autoclaving at 121 °C for 15 min. 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 12% glycerol at -70 °C using basal media. The filter sterilized carbohydrates (JA-Fr and HP-inulin) were added to the basal MRS broth and basal TSB to give final concentrations of 0.5%, 1.0%, 2.0% and 3.0% (w/v).

2.3. Growth measurement

In order to investigate the effects of JA-Fr on the growth and survivability of *B. bifidum* and *E. coli* strains, the bacteria were cultivated overnight in the appropriated basal medium at 37 °C. The activated cultures were centrifuged for 15 min with 2 500 g at 4 °C (sigma centrifuge model 2-16p), then the precipitate was washed twice with phosphate buffer saline [0.1 mol/L phosphate buffer (pH = 7.4) and 0.9% saline], and the final pellet was suspended in phosphate buffer saline 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 into different testing media containing fructans. Then the cultures were incubated at 37 °C for 24 h. The turbidity at 620 nm of each culture was determined every 4 h by subtracting values of bacterial free medium from each test media. All measurements were repeated at least twice.

2.4. Growth kinetic parameters

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

$\mu = (Ln x - Ln x_0)/(t - t_0)$

where, x and x_0 were 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 equation:

$tg = Ln2/\mu$

2.5. pH changes

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

2.6. Statistical analysis

The results obtained were statistically analyzed using 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

B. bifidum and *E. coli* were 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. bifidum* and it had the potential to stimulate the growth of bacteria (Figure 1).

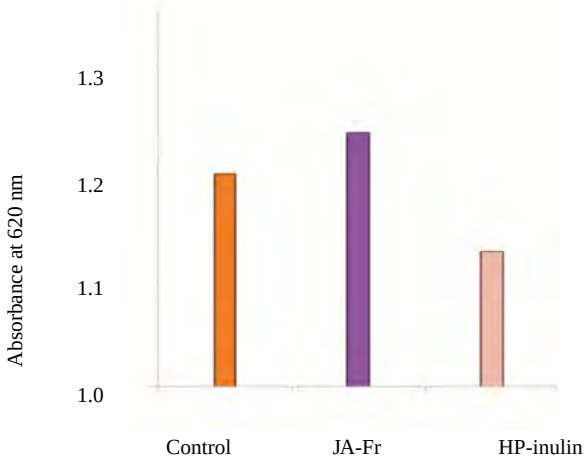

Figure 1. Comparative growth of *B. bifidum* in the presence of JA-Fr, HP-inulin and in control medium after incubation at 37 °C for 48 h.
The data represented the results of a duplicate experiment.

As shown in Figure 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 observe significant differences in the amount of cell growth as the

concentration of JA-Fr rose to 3.0% (w/v). Growth curve of *B. bifidum* showed that the bacteria grew rapidly after about 8 h, with maximum growth observed at about 24 h (Figure 3). 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 *B. bifidum*.

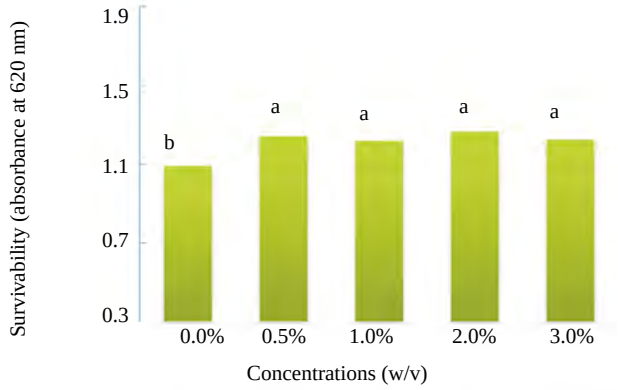

Figure 2. Effect of JA-Fr concentrations on the viability of *B. bifidum*. Different letters meaned statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

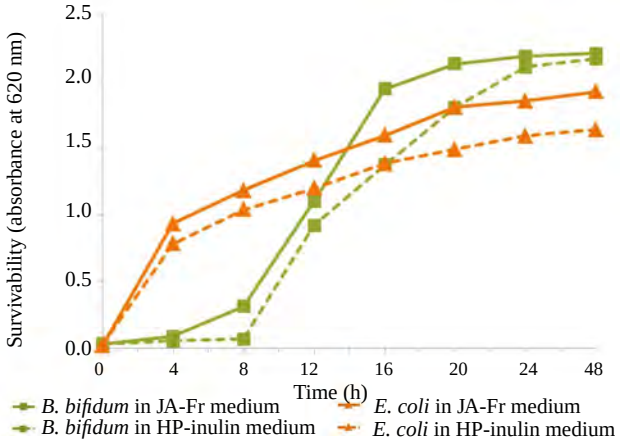

Figure 3. Growth kinetics of *B. bifidum* and *E. coli* in appropriate medium enriched with JA-Fr and HP-inulin at 2.0% (w/v) during 48 h incubation at 37 °C.

Specific rates of growth were determined for the media supplemented with 2.0% (w/v) JA-Fr compared to the control and HP-inulin (Table 1). JA-Fr caused greater *in vitro* growth rate of *Bifidobacterium* than the control and HP-inulin.

Table 1
Comparison of bacterial μ in presence of different fructans.

Treatments	*B. bifidum*	*E. coli*
Control	0.126 ± 0.007	0.902 ± 0.003
JA-Fr	0.142 ± 0.006*	0.973 ± 0.004*
HP-inulin	0.099 ± 0.001	0.905 ± 0.007

All values for μ (h^{-1}) were expressed as mean ± SD of duplicate determination. *: Significant differences at $P < 0.05$ with 95% confidence intervals according to Duncan test in the same column.

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 (Figure 4). Growth promotion of *E. coli* by JA-Fr had dose dependent over the range 0.5% to 3.0% as evidenced by increased turbidity of the bacteria suspensions (Figure 5), indicating that *E. coli* grew faster in the presence of these carbohydrates. Figure 3 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*.

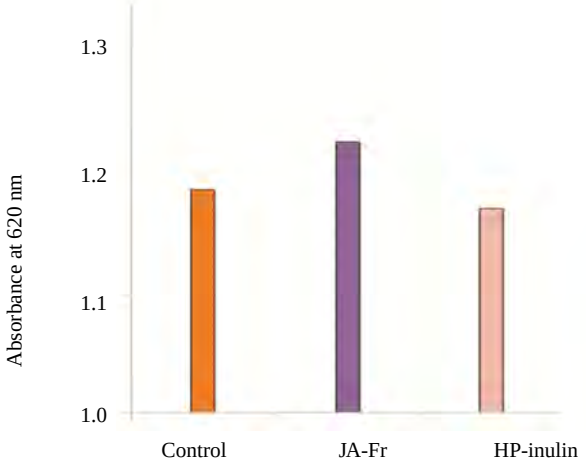

Figure 4. Comparative growth of *E. coli* in the presence of JA-Fr, HP-inulin and in the control medium after incubation at 37 °C for 48 h. The data represented the results of a duplicate experiment.

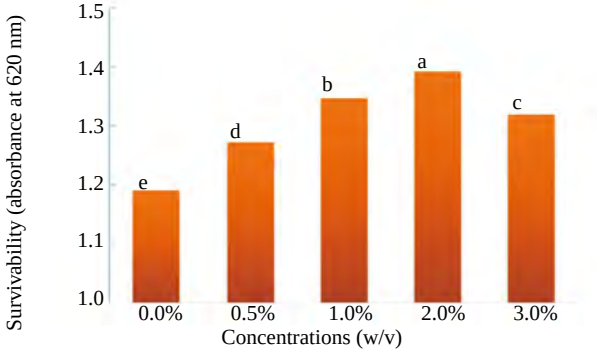

Figure 5. Effect of JA-Fr concentrations on the viability of *E. coli*. Different letters meaned statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

The *tg* of the strain grown in the presence of JA-Fr, HP-inulin and the control medium were compared in Table 2. The *tg* was used as a measure of the efficacy of various carbon sources in modulating growth rate. According to Table 2, *tg* in the medium containing JA-Fr was minimal.

Table 2
The *tg* of the strains in the presence of different fructans.

Treatments	*B. bifidum*	*E. coli*
Control	5.500 ± 0.007	0.760 ± 0.003
JA-Fr	4.880 ± 0.006*	0.710 ± 0.004*
HP-inulin	6.970 ± 0.001	0.770 ± 0.007

All values for *tg* (h) were expressed as mean ± SD of duplicate determination. *: Significant differences at $P < 0.05$ with 95% confidence intervals according to Duncan test in the same column.

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

(Figure 6).

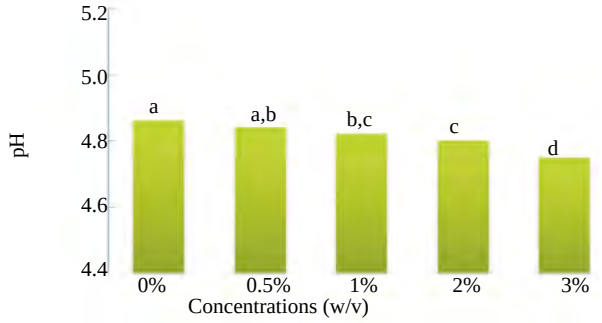

Figure 6. Effects of JA-Fr concentrations on pH changes of media inoculated with *B. bifidum*.
Different letters meaned statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

As shown in Figure 7, the pH continued to decline in a similar way in the presence of both JA-Fr and HP-inulin. After 24 h 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 ($P < 0.05$). Figure 8 demonstrates the changes in TSB medium pH with different concentrations of JA-Fr which occur during fermentation.

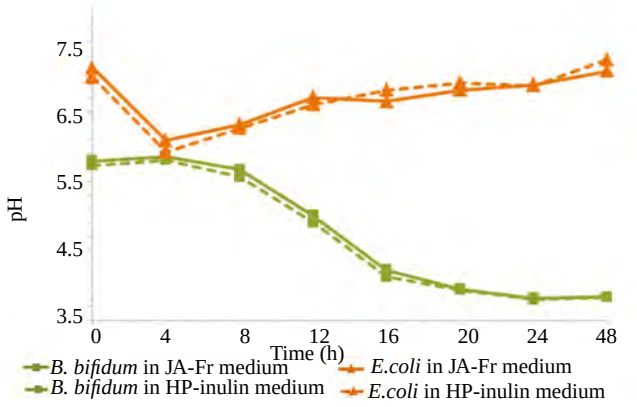

Figure 7. Changes in pH of media inoculated with *B. bifidum* and *E. coli* in appropriate medium enriched with JA-Fr and HP-inulin at 2.0% (w/v) during 48 h incubation at 37 ˚C.

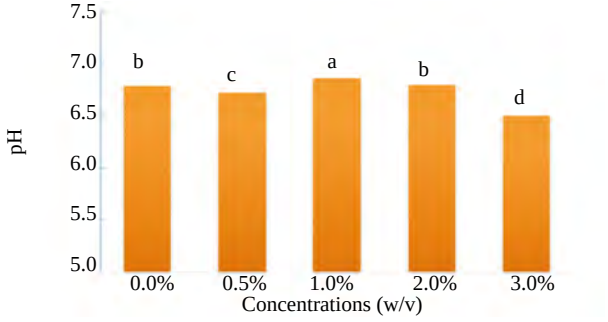

Figure 8. Effects of JA-Fr concentrations on pH changes of media inoculated with *E. coli*.
Different letters meaned statistically significant difference among the values of the same parameter, according to Duncan test ($P < 0.05$).

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 4th hour and at this time, it began to rise (Figure 7). The difference between the pH values of two media was significant ($P < 0.05$).

4. Discussion

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 an index of the fermentability of various carbohydrates in the culture[19]. Biedrzycka and Bielecka reported that the *in vitro* consumption of inulin by bifidobacteria depended on purity and DP of fructooligosaccharides chains[20]. Their research indicated that the majority of *Bifidobacterium* strains studied utilized short chain fructooligosaccharides and oligofructose[20]. Watson *et al*. showed that lactulose, maltodextrin, fructooligosaccharides, galacto oligosaccharides and the galacto oligosaccharides/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, Vigsnæs *et al*. demonstrated that *Bifidobacterium adolescentis* and *Bifidobacterium longum* are able to degrade linear arabino-oligosaccharides (DP 8), whereas *Bifidobacterium breve* is able only to hydrolyze fructooligosaccharides and *B. bifidum* is not able to degrade either fructooligosaccharides or acidified oligosaccharides[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[23]. It was found that inulin had a greater effect on the bacterial growth compared to the oligosaccharides of pitaya, though the difference was not significant[23]. In another study, Wang *et al*. showed that the numbers of *B. 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 fructooligosaccharides[24].

In general, the ability of coliforms to utilize prebiotic oligosaccharides has been a contradictory. Several studies have reported that fructooligosaccharides can support the growth of *E. coli*, *Enterobacter* and *Salmonella*[25,26]. In contrast, others have indicated that *E. coli* is unable to utilize fructooligosaccharides[27]. López-Molina *et al*. studied the utilization of chicory and artichoke inulin (different DP) in mixed cultures of colonic bacteria and showed that the growth of *E. 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 fructooligosaccharides could not do that[29].

Our findings implied that the DP 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, DP, chain length, branched or linear structure and composition of monomer units[20]. Casci *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 DP of fructans from *Helianthus tuberosus* tubers is rather low in comparison with HP-inulin and mainly depends on the variety, climate conditions and time of harvest[15-17]. 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 *E. coli*, casein is a principal nutrient in TSB; so the changes in pH curves are probably due to cells metabolism especially the deamination of amino acids during bacterial growth[19,32].

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 *B. bifidum* and *E. coli* in the media depends on the type and concentration of carbon source. The fructooligosaccharides of Jerusalem artichoke 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.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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