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ORIGINAL PAPER

Effects of water-soluble oligosaccharides extracted from lotus (*Nelumbo nucifera* Gaertn.) seeds on growth ability of *Bifidobacterium adolescentis*

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Abstract Many microorganisms including some probiotic species in human gastrointestinal tract are sensitive to gastric acid secretions. The use of simulated gastrointestinal environments is a suitable approach to study the effects of a single factor on the stability of probiotics in the gastrointestinal tract (GIT). In this study, the growth effects of lotus seed oligosaccharides and purified lotus seed oligosaccharides on *Bifidobacterium adolescentis* were assessed in a simulated gastrointestinal tract environment. Compared to glucose (Glc), xylo-oligosaccharides (XOS), and fructooligosaccharides (FOS), lotus seed oligosaccharides (LOS), and purified lotus seed oligosaccharides (P-LOS) are more effective on promoting the growth of *B. adolescentis*. The final bacterial mass was higher in LOS and P-LOS than in Glc. The viability of *B. adolescentis* incubated at pH 1.5, 2.0, or 3.0 was improved by LOS and P-LOS. Additionally, LOS and P-LOS increased the viability of *B. adolescentis* in bile salts up to 10 g/L and in digestive juices. The results revealed that LOS is an effective growth-accelerating factor of *B. adolescentis*, which improves the viability of *B. adolescentis* in gastrointestinal conditions.

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Introduction

Bifidobacteria, which are important probiotic agents, do not produce endotoxins or exotoxins. Studies have reported that bifidobacteria produce at least 19 amino acids and enzymes, required for the synthesis of pyrimidines, purines, and certain B vitamins [1]. They metabolize monosaccharides and disaccharides through the fructose-6-phosphate dehydrogenase pathway [2]. While all *Bifidobacterium* strains ferment glucose (Glc), galactose, and fructose, certain strains are able to ferment alcohols and other carbohydrates. Bifidobacteria, which play crucial roles in human health, facilitate the digestive system, maintain intestinal microbiota balance [3], suppress allergic responses, enhance the immune system [4], inhibit the growth of pathogenic microorganisms [5], and suppress the growth of certain tumors [6].

Oligosaccharides are carbohydrate polymers that are 3–9 monosaccharides in length. Oligosaccharides, which cannot be digested or absorbed, are utilized by microorganisms in the intestinal tract [7]. The role of oligosaccharides, which have received considerable attention in the past years [8], is involved in a wide range of biological functions and is known to have bifidogenic properties. Carbohydrates, including plant oligosaccharides and mucin [9], can be utilized by bifidobacteria. While studies have focused on the utilization of certain carbohydrates by bifidobacteria [10–12], many studies have been conducted on the bifidogenic effects of plant-derived oligosaccharides [13–15]. Therefore, identifying mechanisms of increasing the number of probiotics, such as bifidobacteria, in the gastrointestinal tract is an area of considerable interest [16, 17].

Lotus (*N. nucifera* Gaertn.) seeds are important plant products in Fujian, China [18]. Due to the presence of bioactive compounds such as polyphenols, protein, and polysaccharides [19–21], lotus seeds have medicinal properties [22], including anti-viral [23], hepato-protective [24], antioxidant [25], memory-enhancing [26], blood-regenerative [27], and anti-inflammatory effects [28]. In mice, fermented milk made from lotus seeds alleviates constipation and promotes intestinal absorption [29], which might be attributed to the presence of oligosaccharides in lotus seeds [30].

Numerous studies have revealed that natural and artificial oligosaccharides have similar effects on the human body; both types of oligosaccharides can significantly increase the growth of bifidobacteria [11, 12, 29]. However, the synthesis of artificial compounds is quite complex and results in some by-products that may be hazardous to human health. Therefore, the objective of this study was to assess the potential health-promoting effects of natural lotus seed oligosaccharides (LOS) and LOS purified with a macroporous resin (P-LOS).

Materials and methods

Lotus seed oligosaccharides extraction

Frozen lotus seeds (Green acres food Co., Ltd, Fujian, China), whose cores had been removed, were mixed with distilled water (3/10, w/v) for 1 h, homogenized (DS-200 electric high-speed tissue homogenizer, Shanghai Precision Instrument, Shanghai, China), and enzymatically hydrolyzed at 65 °C with α-amylase (Sigma, St. Louis, MO, USA) to remove starch [31]; digestion products were determined with iodine. The lotus seed digest was then cooled to 55 °C and treated with papain (Beijing Aoboxing Bio-tech Co., Ltd, China) to remove protein until the protein concentration was stable [32]. Subsequently, 1 % yeast (Guangdong Danbaoli Yeast Co., Ltd, China) was added at the start of the fermentation process (37 °C) to remove monosaccharides from the extraction solution. The fermentation process was terminated when the sugar concentration was stable [33]. The suspensions were heated at 90 °C for 2 h in an autoclave (SYQ-DSX-280, SHENAN Medical Devices, Shanghai, China) to inactivate the enzyme, mixed with five volumes of 95 % ethanol, and allowed to precipitate overnight. Following centrifugation at 3100*g* for 20 min (Hunan Xiangyi Scientific Instrument Corporation, China) to remove polysaccharides from the extraction solution, the resulting supernatant was mixed with 1.5 volumes of 5 % trichloroacetic acid (TCA) (China National Pharmaceutical Group Corporation, China) and allowed to stand at 4 °C for 6 h to remove protein again. The solution was subjected to the same centrifugation procedure described above. Ninhydrin reagent was used to determine the presence of protein [34]. The supernatant was collected to obtain the crude LOS. P-LOS was obtained by purifying LOS using a macroporous resin (3.4 × 30 cm, 1.25 mL/min flow rate, AB-8, Shanghai Mosu Tech. Corp., Ltd.) to remove pigments and proteins [35] and eluted with distilled water. The isolation process was monitored by determining the total sugar content using the phenol–sulfuric acid method. Fractions were collected according to the peaks by the phenol–sulfuric acid method. LOS and P-LOS fractions were freeze-dried.

High-performance liquid chromatography (HPLC) analysis of lotus seed oligosaccharides

P-LOS (10 mg) was dissolved in distilled water (HPLC grade, 10 mL). P-LOS was analyzed on a HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a PL Hi-Plex Na (Octo) 300 × 7.7 mm i.d. ion exchange column (Polymer Laboratories Ltd., UK) and a RID 10A refractive index detector (Shimadzu, Japan). Distilled water was used as the mobile phase at 0.4 mL/min; the column temperature was maintained at 65 °C. Pure standards (sucrose, raffinose, and stachyose) and P-LOS were injected into the HPLC system, and the peak areas were calculated.

Preparation of *B. adolescentis* stock solution

Bifidobacterium adolescentis (Live Bifidobacterium Preparation Oral) was obtained from Livzon Pharmaceutical Group Inc. (Guangdong, China) and maintained at 4 °C in the refrigerator.

The growth medium [36] (pH 7.0) was prepared with soy peptone (0.5 g), Glc (1.0 g), tryptone (0.5 g), yeast powder (1.0 g), Tween 80 (0.1 mL), L-cysteine hydrochloride (0.05 g), deionized water (100 mL), and an inorganic salt solution (4.0 mL). The inorganic salt solution contained 0.2 g $CaCl_2$, 1.0 g K_2HPO_4, 1.0 g KH_2PO_4, 0.48 g $MgSO_4{\cdot}7H2O$, 10.0 g $NaHCO_3$, and 2.0 g NaCl per L. During the preparation, $CaCl_2$ and $MgSO_4$ were first added to 300 mL deionized water and stirred until completely dissolved. Subsequently, the other salts were added to the solution under constant stirring. Finally, the solution was brought to a volume of 1 L with deionized water and stored at 4 °C.

Under sterile conditions, 1 g of freeze-dried bacteria was mixed with 9 mL sterile saline (0.85 g of NaCl in 100 mL of distilled water, moist heat sterilized at 115 °C for 20 min). The growth medium (15 mL) was inoculated with the bacteria (16 %) and anaerobically incubated in anaerobic 2.5 L bags (W-zipper Standing-Pouch, Mitsubishi Gas

Chemical, Tokyo, Japan) at 37 °C for 48 h. After two passages, the bacteria were inoculated in the growth medium for 18 h to obtain bacterial stock solution.

Growth rate of *B. adolescentis* in presence of different carbon sources

All fermentation experiments were conducted in a fermentation medium (pH 6.8 ± 0.2), termed Reinforced Clostridial Medium or RCM (China General Microbiological Culture Collection Center, Beijing, China), containing 1.0 g broth, 0.3 g yeast powder, 0.1 g soluble starch, 0.3 g NaAc, 0.002 g polymyxin B, 0.5 g carbohydrates, 0.5 g peptone, 0.5 g NaCl, 0.05 g L-cysteine hydrochloride, and 100 mL deionized water. The carbohydrates used in the fermentation medium were Glc (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), xylo-oligosaccharides (XOS) (Longlive Biotechnology Technologies Co., Ltd, Shandong, China), fructooligosaccharides (FOS) (Bowling Biotechnology Technologies Co., Ltd, Shandong, China), LOS extracted from lotus seeds, or lotus seed oligosaccharides extracted from lotus seeds and purified with a macroporous resin.

The bacterial stock solution was inoculated into the fermentation medium containing Glc, XOS, FOS, LOS, or P-LOS tested singly as the carbon source and incubated at 37 °C in an anaerobic incubator. The concentration of carbohydrate tested singly was adjusted to 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 g/L following 48 h of incubation. *B. adolescentis* growth curves were obtained by plotting OD_{600nm} and pH values of the fermentation media containing 0.5 g/L each carbohydrate tested singly following 0, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 54, and 60 h of incubation. Each experiment was conducted in triplicate. The pH values of the media were determined using a pH meter coupled to a glass electrode (PB-10, Sartorius, Germany).

The maximum OD value of bifidobacteria was achieved at 600 nm. The number of bifidobacteria was assessed by determining OD_{600nm} and bacterial mass concentration. OD was measured by the following method. First, the bacterial stock solution was inoculated into 20 mL of growth medium at a volume ratio of 16 % and anaerobically incubated at 37 °C. Following centrifugation at 3200*g* for 15 min, the resulting precipitates (bacteria) were washed twice with deionized water, transferred to a tube, brought to a volume of 15 mL, and mixed in rotary shaker for 20 s (Whirlpool oscillator, Qi xin Scientific Instrument Corporation, China) to obtain a bacterial cell suspension. Bacterial cell suspensions with different mass concentrations were prepared, and their absorbance was measured at 600 nm with sterile deionized water as a blank. The absorbance values were used to calculate bacterial mass concentration [37].

Tolerance tests

Tolerance test media, similar in composition to the fermentation media, were prepared using phosphate-buffered saline (PBS), containing 0.5 g/L each carbohydrate tested singly. In the acid tolerance test, *B. adolescentis* was incubated in medium adjusted to pH 1.5, 2.0, 2.5, or 3.0 with 37 % HCl. The viable number and survival rate of *B. adolescentis* were determined following an anaerobic incubation of 0, 1.5, and 3 h [38]. In the bile acid tolerance test, the pH of the medium was adjusted to 7.0; *B. adolescentis* was incubated in medium containing 3, 10, or 20 g/L taurocholic acid sodium salt (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). The viable number and survival rate of *B. adolescentis* were determined following 0 and 12 h of anaerobic incubation [39].

For the gastrointestinal solution tolerance test, pepsin or trypsin was dissolved in 50 mL deionized water and transferred to sterilized plates; the liquid height of the enzyme solutions in each plate was 0.2 cm. The plates were placed under ultraviolet (UV) light (sterilization) for 40 min. Parallel experiments, devoid of random error, were simultaneously performed. Enzymatic activity and number of bacterial colonies were measured [40]. *B. adolescentis* was either incubated in sterilized media (pH 3.0) containing 5 g/L pepsin or in sterilized media (pH 7.0) containing 10 g/L trypsin. The viable number and survival rate of *B. adolescentis* were determined following 0, 3, and 12 h of anaerobic incubation [41].

For each tolerance test, the number of viable bifidobacteria was assessed using de Man Rogosa Sharpe (MRS) agar (Merck, Darmstadt, Germany). The bacterial aliquots (1 mL) were transferred into 0.85 g/L physiological saline (9 mL) to make a serial decimal dilution; 0.1 mL of the diluted aliquots was poured into MRS agar plates, which were incubated anaerobically in anaerobic bags at 37 °C for 48 h. Following the 48-h incubation, plates with 30–300 single bacterial colonies were counted. Survival rate of bifidobacteria was defined as the ratio of viable bifidobacteria number relative to the original bifidobacteria number.

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) using the DPS 7.50 system (Science Press, Beijing, China). Statistical significance was set at $P < 0.05$. The growth rate constant (R) of bifidobacteria in the exponential growth phase [42] was calculated by $R = 3.322 \times (\lg X_2 - \lg X_1)/(t_2 - t_1)$, where t_1 and t_2 are the culture times (h) and X_1 and X_2 are the bacterial cell mass (g) at time t_1 and t_2, respectively. The generation time (G) during the exponential growth phase was calculated by $G = 1/R$. Survival rate was calculated by

$$S = \frac{\lg N_1}{\lg N_0} \times 100\,\% \quad (1)$$

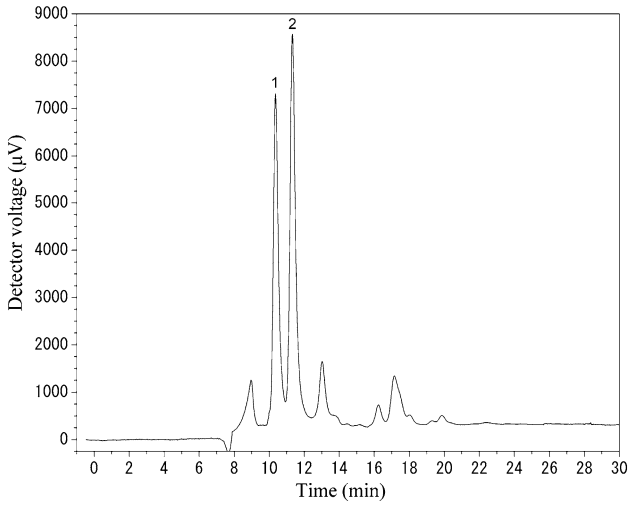

Fig. 1 Analytical high-performance liquid chromatography of P-LOS

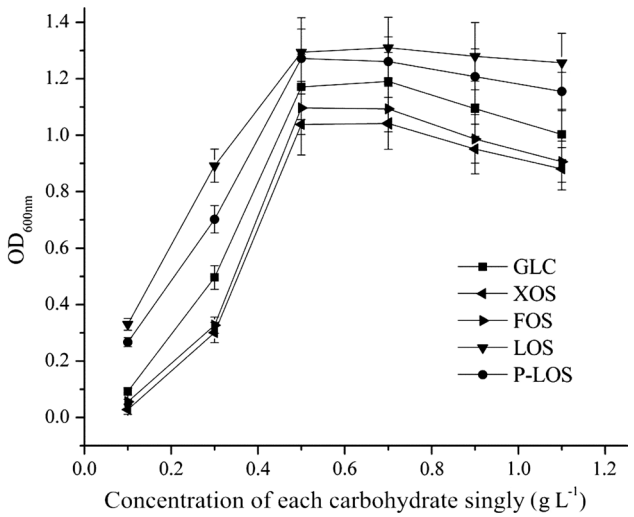

Fig. 2 OD_{600nm} values of media containing Glc, XOS, FOS, LOS, or P-LOS tested singly, after 48 h of fermentation. **Error bars* represent the standard deviation of replicates ($n = 3$)

where S represents the survival rate, N_0 is the post-treatment viable number (CFU/mL), and N_1 is the initial viable number (CFU/mL).

Results and discussion

HPLC analysis of purified lotus seed oligosaccharides

The HPLC chromatogram revealed the presence of two major peaks in P-LOS (Fig. 1), which had identical retention times to those of stachyose and raffinose standards. By comparing the peak areas with those of pure standards,

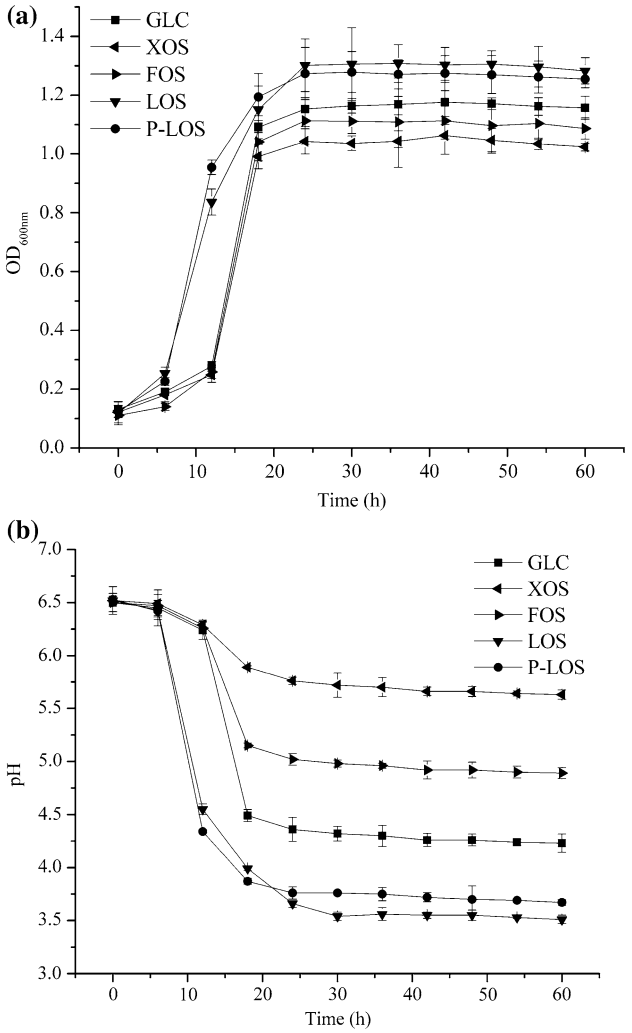

Fig. 3 Growth curves of *B. adolescentis* incubated for 60 h in media containing 0.5 g/L Glc, XOS, FOS, LOS, or P-LOS. **a** OD_{600nm} values; **b** pH values. **Error bars* represent the standard deviation of replicates ($n = 3$)

peaks 1 and 2 corresponded to stachyose (10.378 min) and raffinose (11.315 min), respectively. The proportion of them in P-LOS was 33.36 and 40.43 %, respectively.

Effects of LOS on the growth of *B. adolescentis*

As shown in Fig. 2, with increasing Glc, XOS, FOS, LOS, and P-LOS concentration, OD_{600nm} increased. The maximum OD value was reached adding 0.5 g/L of each different carbohydrate. This value remained substantially stable at 0.7 g/L. This result indicated that higher carbohydrates concentrations would not increase bacterial growth in fermentation medium. Carbohydrates concentrations >0.7 g/L inhibited *B. adolescentis* growth, which could be attributed to dehydration and growth-suppressing effects from high osmotic pressures induced by the high concentration of

Table 1 Effect of pH of the fermentation media containing 0.5 g/L each carbohydrate tested singly on the survival rate of *B. adolescentis*

Carbon sources	Microbial counts (log CFU/mL)[1]						Survival rate (%)			
	0 h		1.5 h		3 h		1.5 h		3 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH = 3.0										
Glc	7.48	0.29	6.88	0.11	6.14	0.07	91.98^{a}	0.38	82.09^{a}	0.25
XOS	7.48	0.29	6.74	0.38	6.12	0.34	90.11^{a}	1.58	81.82^{a}	1.38
FOS	7.48	0.29	6.80	0.09	6.14	0.13	90.91^{a}	0.79	82.09^{a}	0.43
LOS	7.48	0.29	7.04	0.13	6.57	0.22	94.12^{a}	1.15	87.83^{b}	0.76
P-LOS	7.48	0.29	6.92	0.36	6.50	0.27	92.51^{a}	1.66	86.90^{b}	1.58
pH = 2.5										
Glc	7.41	0.17	6.24	0.06	5.40	0.16	84.21^{a}	0.27	72.87^{ab}	0.83
XOS	7.41	0.17	6.18	0.22	5.15	0.04	83.40^{a}	0.68	69.50^{b}	0.14
FOS	7.41	0.17	6.24	0.36	5.26	0.25	84.21^{a}	1.63	70.99^{b}	0.83
LOS	7.41	0.17	6.46	0.04	5.74	0.32	87.18^{a}	0.24	77.46^{a}	2.84
P-LOS	7.41	0.17	6.31	0.13	5.67	0.26	85.16^{a}	0.48	76.52^{a}	1.66
pH = 2.0										
Glc	7.44	0.30	3.94	0.32	1.68	0.28	52.96^{b}	2.16	22.58^{b}	2.85
XOS	7.44	0.30	3.85	0.24	ND	ND	51.75^{b}	1.14	ND	ND
FOS	7.44	0.30	3.76	0.16	ND	ND	50.54^{b}	0.11	ND	ND
LOS	7.44	0.30	4.69	0.52	2.21	0.13	63.04^{a}	4.41	29.70^{a}	0.84
P-LOS	7.44	0.30	4.41	0.29	2.08	0.23	59.27^{a}	1.51	27.96^{a}	1.96
pH = 1.5										
Glc	7.38	0.26	1.56	0.48	ND	ND	21.14^{a}	3.26	ND	ND
XOS	7.38	0.26	ND	ND	ND	ND	ND	ND	ND	ND
FOS	7.38	0.26	ND	ND	ND	ND	ND	ND	ND	ND
LOS	7.38	0.26	2.03	0.47	ND	ND	27.51^{a}	3.70	ND	ND
P-LOS	7.38	0.26	1.88	0.23	ND	ND	25.47^{a}	1.92	ND	ND

Different lower case letters at the same pH with different carbohydrate sources within the same column represent significant difference ($P < 0.05$). Same lower case letters at the same pH with different carbohydrate sources within the same column represent no significant difference ($P > 0.05$)

ND not detected

[1] Values are the mean ± SD; values represent the average of duplicate analyses from three separate experiments ($n = 3$)

carbon sources in the medium. Therefore, the carbohydrate concentration to achieve a growth effect on *B. adolescentis* was 0.5 g/L. Bacterial growth stopped as shown by the steady growth curve.

B. adolescentis growth curves

Compared to Glc, XOS, and FOS, LOS and P-LOS have more stimulatory effect on *B. adolescentis*. In the presence of LOS and P-LOS, *B. adolescentis* entered the exponential growth phase after 6 h (Fig. 3a). In contrast, in the presence of Glc, XOS, or FOS, *B. adolescentis*, which had a longer stationary phase, entered the exponential and steady-state phases after 12 and 18 h, respectively. Bacteria in LOS and P-LOS entered the steady-state phase after 18 h. Subsequently, bacterial growth declined and OD_{600nm} did not change. These results indicate that *B. adolescentis* reached the exponential phase faster with higher OD_{600nm} values in LOS and P-LOS than in Glc, XOS, or FOS. Compared to LOS, P-LOS increased bacterial growth rate by 2.34 % and reduced generation time by 2.29 %. The molecular weight of LOS and P-LOS is lower than that of XOS or FOS, which allows the compounds to readily enter bacteria. Glc, however, might be an exception, because it does not require ATP or extracellular glycoside hydrolases to degrade extracellular oligosaccharides. However, P-LOS had a weaker stimulatory effect on bifidobacteria than LOS, probably due to the presence of other stimulatory factors such as polyphenols in LOS [21, 43]. Polyphenols appear to have potential to exert prebiotic-like effects [44], but scarce information is still available on the influence of phenolic compounds on the growth and viability of bifidobacteria.

Table 2 Effect of bile acid concentration of the fermentation media containing 0.5 g/L each carbohydrate tested singly on the survival rate of *B. adolescentis*

Carbon sources	Microbial counts (log CFU/mL)[1]				Survival rate (%)	
	0 h		12 h		12 h	
	Mean	SD	Mean	SD	Mean	SD
3 g/L Bile salts[2]						
Glc	7.47	0.40	5.53	0.12	74.03[a]	1.66
XOS	7.47	0.40	5.38	0.25	72.02[a]	2.51
FOS	7.47	0.40	5.42	0.17	72.56[a]	1.74
LOS	7.47	0.40	5.69	0.21	76.17[a]	2.76
P-LOS	7.47	0.40	5.54	0.27	74.16[a]	2.68
10 g/L Bile salts						
Glc	7.32	0.26	3.05	0.20	39.89[ab]	2.73
XOS	7.32	0.26	2.76	0.03	37.70[b]	0.75
FOS	7.32	0.26	2.79	0.13	38.11[b]	1.59
LOS	7.32	0.26	3.27	0.07	43.72[a]	0.47
P-LOS	7.32	0.26	3.24	0.10	43.26[a]	1.28
20 g/L Bile salts						
Glc	7.14	0.45	ND	ND	ND	ND
XOS	7.14	0.45	ND	ND	ND	ND
FOS	7.14	0.45	ND	ND	ND	ND
LOS	7.14	0.45	ND	ND	ND	ND
P-LOS	7.14	0.45	ND	ND	ND	ND

Different lower case letters at the same concentration of bile acid with different carbohydrate sources within the same column represent significant difference ($P < 0.05$). Same lower case letters at the same concentration of bile acid with different carbohydrate sources within the same column represent no significant difference ($P > 0.05$)

ND not detected

[1] Values are the mean ± SD; values represent the average of duplicate analyses from three separate experiments ($n = 3$)

[2] Bifidobacteria were incubated for 12 h in media containing 3, 10, or 20 g/L bile acids

Oligosaccharides and polyphenols in LOS may have a synergistic growth effect on *B. adolescentis*. As shown in Fig. 3b, with increasing OD_{600nm} values, the pH values of the media decreased.

With increasing carbohydrates concentration, OD_{600nm} values of *B. adolescentis* increased and pH values decreased, probably due to the acetic acid and lactic acid produced by *B. adolescentis* during fermentation [45]. However, the OD_{600nm} and pH values became almost stable after 24 h of incubation. This result could be attributed to the accumulation of fermentation products, which reduced bacterial growth. When grown in P-LOS- and Glc-containing media, OD_{600nm} values increased from the start of fermentation, accompanied by a decrease in pH. During the lag phase, the changes in OD_{600nm} values were insignificant. This result is consistent with the findings of Kaplan and Hutkins [46].

Tolerance tests

The majority of microorganisms are destroyed by gastric acid secretions. In the jejunum and ileum, microorganisms and digestive juices (e.g., bile, gastric juice, and pancreatic juice) participate in the digestion of foods. Consequently, the selection of acid-resistant and bile-resistant probiotics is crucial for human health. Acid-resistant and bile-resistant bifidobacteria have been screened and incorporated in supplements that can be orally administered [47]. Additionally, oral supplements containing prebiotics, especially oligosaccharides with high resistance to gastric juice and amylase that stimulate the growth of lactobacilli and bifidobacteria in vivo, have been developed [48]. The bioaccessibility results obtained from in vitro digestion studies, which simulate the human gastrointestinal tract, are correlated to those obtained from human studies [49]. Considering that the pH value of gastric juice is 1.5–4.5, the media used in this study were adjusted to different pH values (1.5, 2, 2.5 and 3) to assess the acid resistance of *B. adolescentis* [50]. With increasing incubation, the bifidobacteria number decreased in media at different pH values, which could be attributed to a post-acidification effect during the fermentation process [51].

Following incubation for 1.5 h at pH 3.0 and 2.5, there were no significant differences in survival rates among the

Table 3 Effect of simulated gastrointestinal solutions of the fermentation media containing 0.5 g/L each carbohydrate tested singly on the survival rate of *B. adolescentis*

Carbon sources	Microbial counts (log CFU/mL)[1]				Survival rate (%)	
	Mean	SD	Mean	SD	Mean	SD
Pepsin[2]						
	0 h		3 h		3 h	
Glc	7.41	0.31	5.87	0.25	79.22[a]	1.70
XOS	7.41	0.31	5.76	0.23	77.73[a]	1.37
FOS	7.41	0.31	5.80	0.32	78.27[a]	2.61
LOS	7.41	0.31	6.14	0.15	82.86[a]	1.06
P-LOS	7.41	0.31	6.09	0.17	82.17[a]	1.26
Trypsin						
	0 h		12 h		12 h	
Glc	7.33	0.28	6.23	0.10	84.99[a]	1.85
XOS	7.33	0.28	6.18	0.38	84.31[a]	2.08
FOS	7.33	0.28	6.21	0.26	84.72[a]	1.08
LOS	7.33	0.28	6.46	0.24	88.13[a]	1.65
P-LOS	7.33	0.28	6.42	0.11	87.59[a]	1.76

Different lower case letters at the same concentration of simulated gastrointestinal solutions with different carbohydrate sources within the same column represent significant difference ($P < 0.05$). Same lower case letters at the same concentration of simulated gastrointestinal solutions with different carbohydrate sources within the same column represent no significant difference ($P > 0.05$)

ND not detected

[1] Values are the mean ± SD; values represent the average of duplicate analyses from three separate experiments ($n = 3$)

[2] Bifidobacteria were incubated for 3 h in media (pH 3.0) containing 5 g/L pepsin or 12 h in media (pH 7.0) containing 10 g/L trypsin

different carbohydrate sources. However, at pH 2.0 and 1.5, the survival rate of *B. adolescentis* in LOS and P-LOS was significantly higher than in XOS and FOS ($P < 0.05$; Table 1). Following incubation for 3 h at pH 3.0 and 2.0, the survival rate of *B. adolescentis* in LOS and P-LOS was significantly higher than in the other three carbohydrate sources ($P < 0.05$). There were no significant differences in survival rate between LOS- and P-LOS-containing media ($P > 0.05$). On the other hand, following incubation for 3 h at pH 2.0 and 1.5 h at pH 1.5, no *B. adolescentis* growth was detected in XOS and FOS. Additionally, *B. adolescentis* was not detected following incubation for 3 h at pH 1.5. Therefore, LOS and P-LOS increased the resistance of *B. adolescentis* to low pH values.

Bifidobacteria had high acid resistance; however, the survival rate significantly decreased with increasing incubation time and decreasing pH values. Food ingredients can protect probiotic bacteria from acidic conditions and enhance gastric survival with the pH increased [8]. The discrepancy in the acid-resistant results might be attributed to differences in bacterial strains and culture medium.

As shown in Table 2, the survival rate of *B. adolescentis* in LOS and P-LOS was significantly higher than the one in XOS and FOS ($P < 0.05$; Table 1) with 10 g/L bile salts. Following incubation in medium containing 20 g/L bile salts for 12 h, there were no live bacteria in Glc, XOS, FOS, LOS, or P-LOS.

Bile inhibits the growth of bifidobacteria by affecting bacterial membrane permeability. The composition of human bile is similar to that of bovine bile; therefore, bovine sodium taurocholate was used to assess the bile resistance of bifidobacteria in different carbon sources [52, 53]. Compared to XOS, and FOS, LOS, and P-LOS increased the resistance of *B. adolescentis* to 10 g/L bile salts, thereby favoring the activity of *B. adolescentis* in the gut.

Common sterilization methods include dry heat sterilization, moist heat sterilization, membrane filtration sterilization, and chemical sterilization methods. Among these methods, heat sterilization methods easily inactivate several enzymes. On the other hand, membrane filtration sterilization may not be suitable for proteases due to filtration complications, and chemical sterilization might introduce impurities into samples. Among the non-thermal sterilization methods developed in the last few decades, ultraviolet (UV) light irradiation is one of the most promising methods because of its ease of use and widespread lethality [54]. The penetration of UV light depends on the absorptivity of the liquid, which varies with the color of the compounds, the amount of soluble and/or suspended solids, and the turbidity of the liquid [55]. Therefore, in liquids, UV light penetrates a very short distance. Nevertheless, UV irradiation has been effectively used in fruit juice sterilization [56,

57]. Therefore, UV irradiation was used in this study to sterilize protease solutions.

The results reveal that in simulated gastrointestinal conditions, LOS and P-LOS increased the survival rate of *B. adolescentis* to digestive enzymes; however, this increased survival rate was not significantly different from that obtained with Glc, XOS, or FOS ($P > 0.05$; Table 3). On the other hand, the survival rate of *B. adolescentis* was higher in the presence of LOS and P-LOS than in the other three carbohydrate sources.

The survival rates in the five carbohydrate sources were lower than those in the corresponding media used in acid resistance experiments, which might be attributed to hydrolytic effects, as opposed to protective effects, from pepsin [58]. A high survival rate is indicative of a high tolerance to simulated gastric and pancreatic juices [59]. The results obtained in this study indicate an anti-digestion property of LOS and P-LOS.

In conclusion, LOS and P-LOS enhanced the viability of the assayed strain only in the presence of low pH at pH 2.0, 1.5 for 1.5 h and pH 3.0, 2.5, 2.0 for 3 h, or 10 g/L bile salts for 12 h. The potential of LOS and P-LOS as a growth substrate for probiotics needs to be further studied in relation to bifidobacteria growth.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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