

Prebiotic Potential of Xylooligosaccharides Derived from Corn Cobs and Their *In Vitro* Antioxidant Activity When Combined with *Lactobacillus*

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In the present work, the *in vitro* prebiotic activity of xylooligosaccharides (XOS) derived from corn cobs combined with *Lactobacillus plantarum*, a probiotic microorganism, was determined. These probiotics exhibited different growth characteristics depending on strain specificity. *L. plantarum* S2 cells were denser and their growth rates were higher when cultured on XOS. Acetate was found to be the major short-chain fatty acid produced as the end-product of fermentation, and its amount varied from 1.50 to 1.78 mg/ml. The antimicrobial activity of XOS combined with *L. plantarum* S2 was determined against gastrointestinal pathogens. The results showed that XOS proved to be an effective substrate, enhancing antimicrobial activity for *L. plantarum* S2. *In vivo* evaluation of the influence of XOS and *L. plantarum* S2, used both alone and together, on the intestinal microbiota in a mouse model showed that XOS combined with *L. plantarum* S2 could increase the viable lactobacilli and bifidobacteria in mice feces and decrease the viable *Enterococcus*, *Enterobacter*, and *Clostridia* spp. Furthermore, in the *in vitro* antioxidant assay, XOS combined with *L. plantarum* S2 possessed significant 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis, and superoxide anion radical-scavenging activities, and the combinations showed better antioxidant activity than either XOS or *L. plantarum* S2 alone.

Keywords: Xylooligosaccharide, *Lactobacillus plantarum*, prebiotic, probiotic, antioxidant activity

Introduction

A prebiotic is defined as a “non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” [26]. Many oligosaccharides have been reported to possess prebiotic properties, such as fructooligosaccharides, galactooligosaccharides, and inulin [12]. Among them, xylooligosaccharides (XOS) derived from a variety of xylan-containing raw materials have been proposed as excellent candidates for new-generation prebiotics [15]. XOS are indigestible by gastric or pancreatic enzymes but can be utilized by a select group of beneficial gut microflora for their many beneficial physiological

properties, including improving bowel function, increasing mineral absorption, reducing the risk of colon cancer, and helping to control type 2 diabetes mellitus [22]. In addition, diets supplemented with XOS provide antioxidant activity, antibacterial properties, antiallergic activity, anti-inflammatory properties, and positive effects on the immune system [29, 30].

Both *in vitro* and *in vivo* studies have shown XOS to be capable of imparting prebiotic effects when fermented by specific beneficial bacteria, such as lactic acid bacteria and *Bifidobacterium* spp. [25]. These microorganisms are known as “probiotics,” and have been reported to have health-promoting properties, such as stimulating the immune system, balancing intestinal flora, reducing serum cholesterol,

and reducing the risk of tumors. In addition, they are known to act as antioxidants and to slow the aging process [20].

Corn cobs are a major byproduct of maize processing and are available in enormous quantities worldwide. Recent studies have shown that an important part of the hemicelluloses present in corn cobs could be recovered as prebiotic XOS by enzymatic or chemical methods [11]; however, XOS derived from corn cobs as active prebiotic components have not yet been effectively exploited. Very few reports mention that XOS derived from corn cobs appear to have a potential prebiotic effect on lactobacilli [14, 21]. The aim of this work was to determine the *in vitro* prebiotic activity of XOS derived from corn cobs using the probiotic microorganism *Lactobacillus plantarum*. In addition, the potential *in vitro* antioxidant activities of combining XOS with *L. plantarum* were also determined.

Materials and Methods

Materials

Commercial XOS derived from corn cobs were purchased from Qingdao Century Longlive International Trade Co., Ltd. (Shandong, China) and was >90% pure. The degrees of polymerization of the XOS mixture ranged from xylobiose to xylohexaose. All chemicals were purchased from Sigma-Aldrich Co., Inc. (St. Louis, MO, USA). Bacterial culture media and other additives were obtained from Oxoid (Poland).

Bacterial Strains and Culture Conditions

Lactobacillus strains Lb1, B72, QH25-1, C88, Sc52, YNF-5, S62, S2, S56, and MI 12-2-2 were used in the present study. All strains were isolated from traditional Chinese fermented foods and identified as *L. plantarum* by API 50 CHL kit (bioMérieux Inc., Marcy l'Etoile, France) and 16S rDNA sequencing analysis. Stock cultures of each strain were maintained in MRS medium with 20% glycerol and stored at -80°C .

Before the fermentation trials, *L. plantarum* strains were precultured in semi-defined medium (SDM) broth that contained the following (in grams per liter): 20.0 g glucose, 2.0 g ammonium citrate, 5.0 g sodium acetate, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g MnSO_4 , 2.0 g K_2HPO_4 , 5.0 g yeast nitrogen base (Difco), 10 g Bacto casitone (Difco), and 1.0 ml of Tween 80, adjusted to pH 6.6 with 1.0 M acetic acid [5]. Testing media were made by supplementing sterilized carbohydrate-free SDM with XOS (filter-sterilized using 0.2 mm filters) at final concentrations of 0.5% (w/v). SDM with glucose (0.5% (w/v)) was used as a positive control, and carbohydrate-free SDM served as a negative control.

The bacterial pathogens used were *Escherichia coli* CMCC44825, *Salmonella typhimurium* CMCC50115, *Shigella flexneri* CMCC51061, and *Staphylococcus aureus* CMCC2607. All bacterial pathogens were reactivated in Luria-Bertani (LB) broth.

Prebiotic Effect on *L. plantarum*

To study the prebiotic activity of XOS on *L. plantarum* strains, 50 μl of overnight cultures of all strains (1×10^6 cells/ml) were inoculated into 5.0 ml of different testing media. The bacteria were cultured anaerobically at 37°C for 12 h in anaerobic jars. Growth was assessed by determining the OD_{600} values at 2.0 h intervals. The pH changes were determined using a Sartorius PB-10 pH meter (Germany). All measurements were performed twice in triplicate parallels.

Short-Chain Fatty Acid Analysis

The concentrations of short chain fatty acids (SCFAs) were determined using a modification of the gas chromatography (GC) method described by Schneider *et al.* [23]. Samples of supernatants (2.0 ml) from the fermentation broths were run through 0.22 mm filters. Then, 0.4 ml of 50% (v/v) sulfuric acid and 2.0 ml of diethyl ether were added to the samples. The samples were mixed at room temperature for 45 min with an orbital shaker (250 rpm), and centrifuged at $10,000 \times g$ for 5 min at 4°C , after which the supernatants were dehydrated with anhydrous calcium chloride. After centrifugation, the supernatants were analyzed by GC using a DB-FFAP column (60 m \times 0.25 mm, ID 0.25 mm; Supelco, Inc., Bellefonte, PA, USA). To perform GC, helium was used as the carrier and the makeup gas, with a flow rate of 2.0 ml/min. The injection temperature was 250°C . The SCFA (acetic, propionic, butyric, isobutyric, butyric, valeric, isovaleric, and lactic acids) concentrations were automatically calculated from the areas of the resulting peaks using the Star Chromatography Work Station program (ver. 5.5), which was connected online to the flame ionization detector.

Antibacterial Activity

The modified method of Pan *et al.* [18] was used for screening antimicrobial activity against a variety of bacterial pathogens. The selected *L. plantarum* S2 strain was grown in MRS broth containing 0–6 g/l XOS at 37°C for 12 h. The cultures were centrifuged at $10,000 \times g$ for 10 min at 4°C , and the supernatants were neutralized to pH 6.2 with 1.0 M NaOH, added to a sterile solution of catalase (1,000 U/ml; Sigma-Aldrich, Co., Inc., St. Louis, MO, USA), and filter-sterilized through a 0.22 mm millipore membrane filter (Millipore, Billerica, MA, USA). Aliquots (50 μl) of the cell-free supernatants were poured into 8 mm wells previously made on LB agar plates coated with the test pathogens. The agar was then cultured overnight at 37°C under anaerobic conditions. The diameter of the inhibition zone surrounding the wells was then measured and scored as follows: a diameter of 8 mm equals no inhibition (–); between 0 and 3 mm, weak (+); between 3 and 6 mm, good (++); and >6 mm, strong (+++). All assay experiments for inhibitory activity were performed in triplicate.

Regulatory Effect on the Intestinal Flora in a Mice Model

To evaluate the regulatory effect of XOS and the *L. plantarum*

strain on the intestinal flora in mice, 5-week-old Kunming male mice were used. Mice were divided randomly into five groups of 20 mice each and fed by oral gavage. Mice in group 1 (control) received 0.4 ml of PBS (phosphate-buffered saline), those in group 2 were fed 0.4 ml of PBS containing 5.0 g/l XOS, and those in groups 3 were given 0.4 ml of PBS containing 5.0 g/l XOS mixed with 0.4 ml of *L. plantarum* S2 at a cell concentration of 1.0×10^9 CFU/ml. After 14 days, oral gavage was stopped and the mice were fed a basal diet for another 7 days. At 0, 7, 14, 16, and 21 days, four mice in each group were killed by cervical dislocation, and fresh stool samples from the rectum were collected, serially diluted, and plated on LBS, MRS + NNLP, VRBDA (violet red bile dextrose agar), BEA (bile esculin azide), and TSC (tryptose sulfite cycloserine) agar to estimate the number of viable lactobacilli, bifidobacteria, *Enterobacter* spp., enterococci, and *Clostridium perfringens*, respectively [19]. The plates were incubated at 37°C for 24 or 48 h. The results are reported as \log_{10} CFU/g stool sample. All measurements were performed twice in triplicate parallels.

Assay of *In Vitro* Antioxidant Activity

Scavenging of 2,2-diphenyl-1-picrylhydrazyl free radical. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method described by Kao and Chen [4] with some modifications. Briefly, 1.0 ml of XOS samples at different concentrations (0.5–4 mg/ml) containing the same volume of *L. plantarum* S2 (10^{10} CFU/ml) cell pellet was added to 2.0 ml of ethanolic DPPH radical solution (0.05 mM). The mixture was shaken vigorously and incubated at room temperature in the dark for 30 min. The controls comprised only deionized water and DPPH solution. The blanks contained only ethanol and the cells. The absorbance of the resulting solution was measured in triplicate at 517 nm after centrifugation at 8,000 \times g for 10 min. The scavenging ability was defined as

$$\text{Scavenging activity (\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100.$$

Scavenging activity on ABTS radical. The ABTS (2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonate) radical scavenging activity of the samples was assessed using a reported procedure [28] with some modifications. The ABTS radical cation (ABTS^{•+}) was chemically produced by mixing 2.45 ml of ABTS solution (7 mM) with an equal volume of potassium persulfate (15 mM) and incubating the mixtures in the dark at room temperature for 16 h. After incubation, the ABTS^{•+} solution was diluted to an absorbance of 0.7 ± 0.01 at 734 nm. Then, 2.5 ml of ABTS^{•+} solution was added to 0.5 ml of XOS sample solution at various concentrations (0.5–4.0 mg/ml) containing the same volume of *L. plantarum* S2 (10^{10} CFU/ml) cell pellet. After reaching room temperature and remaining there for 6 min, the absorbance readings were taken at 734 nm. Ascorbic acid was included as a positive control. Then, scavenging activity on the ABTS radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1 - (\text{Abs. of sample} - \text{Abs. of blank}) / \text{Abs. of control}] \times 100$$

Chelating Effect on Ferrous Ion

The ferrous ion chelating ability was assessed according to the method reported by Ma et al. [8] with minor modifications. One-milliliter XOS samples of different concentrations (0.5–4.0 mg/ml) containing the same volume of *L. plantarum* S2 (10^{10} CFU/ml) cell pellet were mixed with FeCl₂ (0.05 ml, 2 mM) and ferrozine (0.2 ml, 5.0 mM), shaken well, left to rest for 10 min at room temperature, and centrifuged at 8,000 \times g for 10 min. The absorbance of the resulting supernatant was determined at 562 nm. The ferrous ion chelating activity was calculated using the following equation:

$$\text{Chelating ability (\%)} = [1 - (A_o - A_s) / A] \times 100$$

where A_o is the absorbance of the reaction solution, A_s is the absorbance of the solution (1.0 ml sample and 2.5 ml distilled water), and A is the absorbance of the solution with distilled water instead of a sample.

Statistical Analysis

Analysis of variance using the Statistica 6.0 software was used to assess the data. Mean values were compared using the Tukey's test ($p < 0.05$). All data are presented as the mean \pm standard deviation.

Results and Discussion

Prebiotic Activity

The growth profiles of 10 *L. plantarum* strains in SDM supplemented with an XOS-rich fraction generated from a byproduct of the corn processing industry are shown in Table 1. All the bacterial strains tested readily used XOS derived from corn cobs, as indicated by the increase in turbidity of the culture broth and the liberation of SCFAs. When grown with 0.5 g/l XOS, the OD₆₀₀ of all strains exceeded 0.6 after 12 h of fermentation, whereas in the medium without any carbohydrate, the OD₆₀₀ never exceeded 0.2 (data not shown). *L. plantarum* S2 was the probiotic whose growth was the most stimulated by the presence of the prebiotic XOS, although the growth of strains S56 and Lb1 was also stimulated; hence, it was concluded that XOS was the preferred substrate, which is consistent with the reports of several authors, who observed a clear beneficial effect of this prebiotic on the viability of *Lactobacillus* strains [14, 18]. Similar results were also reported that XOS derived from water-extractable polysaccharides in Bengal gram husk and wheat bran stimulated the growth of lactobacilli [9]. In addition, our present studies indicated that different strains of *L. plantarum* possessed various growth profiles, which was probably a result of the differences in xylanase activity in the hydrolysis of XOS.

Table 1. Prebiotic effect of xylooligosaccharides derived from corn cobs on strains of *L. plantarum*.

Strains	Growth (OD ₆₀₀)						
	0 h	2 h	4 h	6 h	8 h	10 h	12 h
B72	0.20 ± 0.15	0.32 ± 0.23	0.59 ± 0.19	0.64 ± 0.26	0.65 ± 0.39	0.66 ± 0.48	0.66 ± 0.34
Lb1	0.21 ± 0.32	0.31 ± 0.46	0.60 ± 0.42	0.68 ± 0.32	0.69 ± 0.25	0.72 ± 0.42	0.73 ± 0.17
QH25-1	0.21 ± 0.45	0.34 ± 0.28	0.50 ± 0.35	0.57 ± 0.25	0.59 ± 0.27	0.60 ± 0.26	0.61 ± 0.35
C88	0.21 ± 0.36	0.32 ± 0.45	0.51 ± 0.34	0.58 ± 0.34	0.60 ± 0.13	0.61 ± 0.47	0.64 ± 0.23
Sc52	0.18 ± 0.38	0.35 ± 0.24	0.48 ± 0.22	0.56 ± 0.26	0.57 ± 0.19	0.61 ± 0.18	0.62 ± 0.46
YNF-5	0.21 ± 0.25	0.37 ± 0.19	0.61 ± 0.46	0.64 ± 0.18	0.66 ± 0.22	0.68 ± 0.42	0.69 ± 0.22
S62	0.18 ± 0.27	0.33 ± 0.26	0.49 ± 0.37	0.59 ± 0.24	0.67 ± 0.19	0.70 ± 0.16	0.78 ± 0.39
S2	0.19 ± 0.42	0.34 ± 0.19	0.68 ± 0.18	0.74 ± 0.41	0.80 ± 0.26	0.87 ± 0.39	0.94 ± 0.41
S56	0.21 ± 0.26	0.36 ± 0.27	0.64 ± 0.26	0.72 ± 0.42	0.75 ± 0.37	0.76 ± 0.26	0.79 ± 0.38
ML12-2-2	0.20 ± 0.33	0.37 ± 0.43	0.56 ± 0.22	0.63 ± 0.35	0.65 ± 0.29	0.67 ± 0.38	0.67 ± 0.42

Short-Chain Fatty Acids

Fermentation of non-digestible oligosaccharides by select colonic bacteria results in the production of lactate and SCFAs such as acetate, propionate, and butyrate. Intestinal generation of SCFAs lowers the pH, increases the bioavailability of calcium and magnesium, and prevents the overgrowth of pathogenic bacteria in the intestine. Table 2 shows the concentration of lactic acid and SCFAs in the medium at the end of the fermentation process. The *L. plantarum* strains produced SCFAs in different proportions in all the cultures inoculated with XOS as a carbon source after 12 h of fermentation. The amount of total SCFAs produced varied for individual strains. Among the lactobacilli, *L. plantarum* S2 produced the highest concentration of total organic acids (5.64 mg/ml), whereas *L. plantarum* B72 produced the lowest (4.42 mg/ml). Acetate was the chief SCFA produced and its amount varied from 30% to 35%.

L. plantarum S2 produced the highest amount of acetate and lactic acid (1.78 and 2.18 mg/ml, respectively). Similar results were reported by Moura *et al.* [13, 14], who assessed the acetogenic potential of various types of XOS in media inoculated with an intestinal disease from porcine gut; whereas Connolly *et al.* [1] observed that acetic acid was the dominant SCFA produced in fecal fermentation using konjac glucomannan hydrolysate as a carbon source. Among SCFAs, butyrate is particularly important because it provides energy for colonocytes, stimulates colon epithelial cells (increasing their absorptive capacity), and inhibits the growth of colonic carcinoma cells *in vitro* and *in vivo* [3]. Our results are consistent with those of previous studies. Minor amounts of propionic acid, isobutyric acid, valeric acid, and isovaleric acid were found in the fermentation media containing other strains; therefore, after consideration of the results of the fermentation and

Table 2. Short-chain fatty acid production by *L. plantarum* strains after xylooligosaccharide fermentation.

Strains	Short-chain fatty acids (mg/ml)						
	Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Valeric acid	Isovaleric acid	Lactic acid
B72	1.31 ± 0.14	0.36 ± 0.06	0.73 ± 0.08	0.23 ± 0.03	0.12 ± 0.04	0.05 ± 0.01	1.62 ± 0.15
Lb1	1.63 ± 0.13	0.37 ± 0.08	0.68 ± 0.05	0.09 ± 0.02	0.11 ± 0.02	0.04 ± 0.02	1.97 ± 0.09
QH25-1	1.50 ± 0.07	0.43 ± 0.03	0.73 ± 0.10	0.21 ± 0.06	0.06 ± 0.03	0.03 ± 0.01	1.75 ± 0.08
C88	1.59 ± 0.09	0.41 ± 0.06	0.84 ± 0.07	0.11 ± 0.01	0.13 ± 0.04	0.04 ± 0.02	1.95 ± 0.13
Sc52	1.54 ± 0.11	0.46 ± 0.04	0.63 ± 0.03	0.25 ± 0.05	0.16 ± 0.02	0.01 ± 0.03	1.86 ± 0.06
YNF-5	1.71 ± 0.06	0.39 ± 0.05	0.79 ± 0.06	0.23 ± 0.02	0.15 ± 0.02	0.04 ± 0.02	1.91 ± 0.12
S62	1.68 ± 0.12	0.33 ± 0.07	0.80 ± 0.04	0.14 ± 0.03	0.17 ± 0.03	0.03 ± 0.01	1.99 ± 0.15
S2	1.78 ± 0.08	0.47 ± 0.05	0.83 ± 0.05	0.20 ± 0.01	0.13 ± 0.02	0.05 ± 0.02	2.18 ± 0.11
S56	1.44 ± 0.12	0.41 ± 0.08	0.55 ± 0.11	0.17 ± 0.02	0.09 ± 0.01	0.01 ± 0.01	1.76 ± 0.07
ML12-2-2	1.65 ± 0.13	0.23 ± 0.05	0.67 ± 0.09	0.19 ± 0.04	0.10 ± 0.01	0.04 ± 0.01	1.83 ± 0.05

Table 3. Antibacterial activity of *L. plantarum* S2 grown in MRS broth containing xylooligosaccharides, measured by well-diffusion assay against pathogens.

	Pathogen bacteria			
	<i>Shigella flexneri</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Staphylococcus aureus</i>
S2	++	++	+	+
S2 + 2.0 (g/l) XOS	+++	++	+++	++
S2 + 4.0 (g/l) XOS	+++	+++	++	++
S2 + 6.0 (g/l) XOS	++	+++	+++	++

Note: Symbols refer to the size of the inhibition zone diameter observed with growing cells: +, 0–3 mm; ++, 3–6 mm; +++, larger than 6 mm.

SCFA profiles, *L. plantarum* S2 was chosen for further investigation.

Antimicrobial Effects of XOS

The antimicrobial activity of the cell-free *L. plantarum* S2 supernatant fermented with XOS was detected using the well-diffusion method. As shown in Table 3, *L. plantarum* S2 grown in MRS broth alone was found to have potent inhibitory effects against the bacteria tested in this study. After addition of XOS in the MRS broth, the antimicrobial activity of *L. plantarum* S2 increased remarkably against the four pathogens. The addition of cell-free *L. plantarum* S2 supernatant with corn cob XOS showed strong antibacterial activities against *S. flexneri* and *E. coli* compared with those of *S. aureus* and *S. typhimurium*. *L. plantarum* S2 grown in MRS broth containing XOS exhibited inhibition zones when the pH values were adjusted to 6.6 and catalase solutions were added, indicating that the antagonistic activities of this strain originated from alternative or simultaneous acid and hydrogen peroxide (H₂O₂) inhibition. These results suggested that XOS promoted the proliferation of beneficial bacteria and increased the production of the antimicrobial substances in the cell-free supernatant, including organic acids, H₂O₂, bacteriocins, low-molecular-mass peptides, and other composites [10].

Modulation of the Intestinal Microbiota

Evidence from human feeding trials, animal models, and *in vitro* modeling systems has shown that prebiotics affect the composition of gut microbiota, leading to an increase in health-promoting organisms, such as bifidobacteria and lactobacilli [7, 20]. Using the experimental model in this study, the administration of XOS from corn cobs and its combination with *L. plantarum* S2 selectively increased the number of both lactobacilli and bifidobacteria in the fecal samples of mice within 2 weeks (Figs. 1A and 1B), whereas the number of *Enterococcus*, *Enterobacter*, and *Clostridia* spp. (Figs. 1C, 1D, and 1E) was significantly reduced. The addition of *L. plantarum* S2 increased total probiotic bacteria

throughout the administration ($p < 0.05$). After 2 weeks, the number of lactobacilli and bifidobacteria in the fecal samples decreased, while the number of *E. coli* and *Enterococcus* and *Clostridia* spp. increased. In mice at 21 days, no difference was observed in the number of *E. coli* and *Enterococcus* spp., or between the group with XOS or the group with a combination of XOS with *L. plantarum* S2 and the control group.

A recent study assessed the combined effects of a prebiotic and *Bacillus subtilis* on improving disease resistance by enhancing the immunity of fish [31]. Likotrafiti et al. [7] also showed that the elderly gut microbiota can be modulated *in vitro* with the appropriate pro-, pre-, and synbiotics. The presence of XOS combined with *L. plantarum* S2 increased *Bifidobacterium* spp. and *Lactobacillus* spp. in the mouse gut. Higher counts of intestinal bifidobacteria and lactobacilli in the groups containing XOS combined with *L. plantarum* S2 might be explained by the fact that XOS could not be digested by human enzymes, preventing it from being used by most intestinal microbiota except probiotic species. In contrast, XOS combined with *L. plantarum* S2 significantly decreased *E. coli* and *Clostridium* spp. counts; the *Enterococcus* spp. counts showed only a slight decrease. Although XOS consumption decreased pathogenic bacteria in the mice colons, XOS did not have any antibacterial effect on either *E. coli* or *E. faecalis* during the *in vitro* assay (data not shown), which suggests that other components of the gut environment helped increase the beneficial bacteria that consumed XOS by altering the acidity of the environment.

In Vitro Antioxidant Activity

The DPPH radical scavenging activity of XOS, *L. plantarum* S2, and their combination is shown in Fig. 2A and compared with ascorbic acid as the control standard. Their scavenging abilities correlated well with increasing concentrations. Among the tested samples, XOS combined with *L. plantarum* S2 had better DPPH radical scavenging activity than XOS

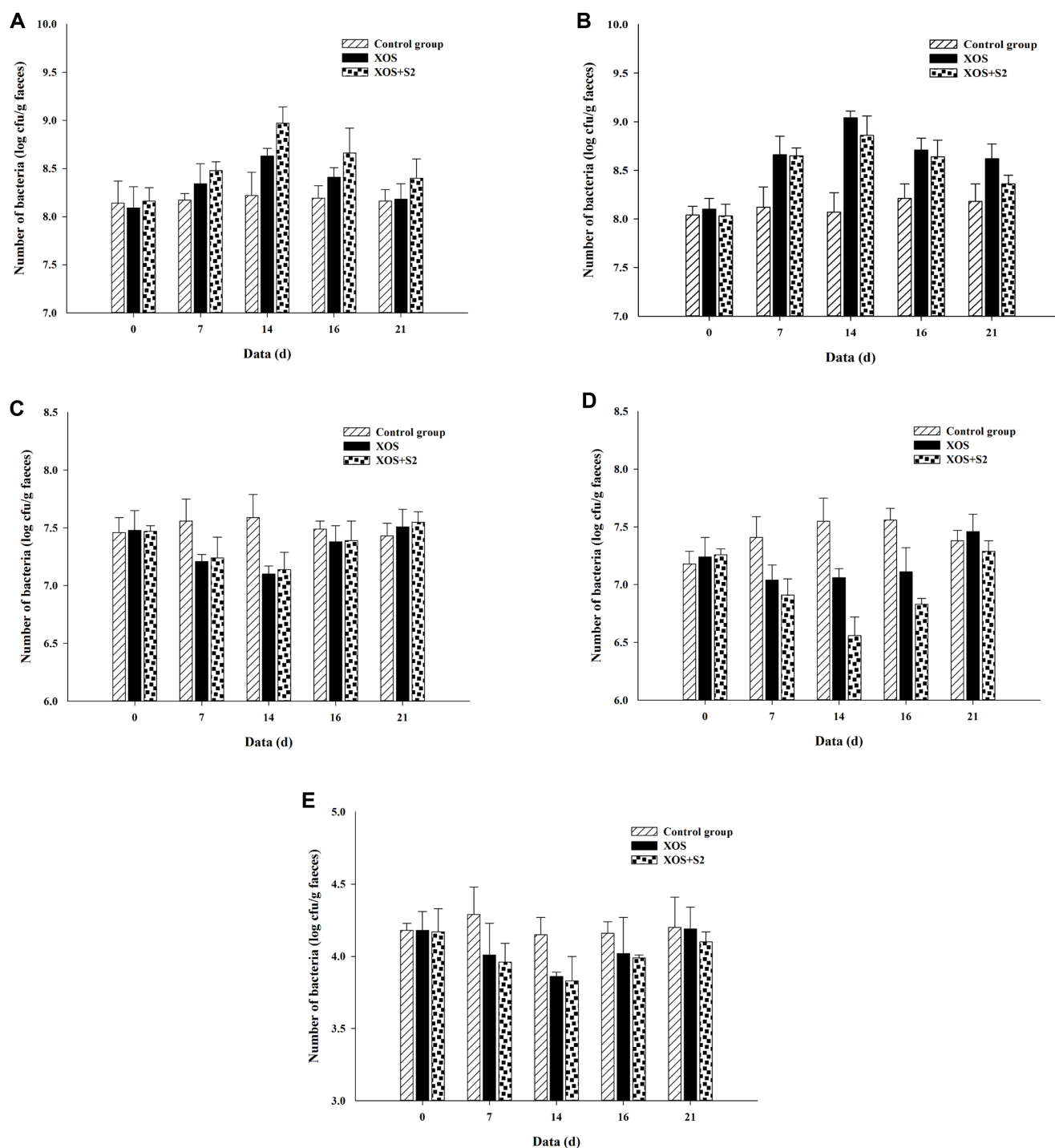


Fig. 1. Effects of xylooligosaccharides (XOS) derived from corn cobs, *L. plantarum* S2, and their combination on the intestinal flora of mice, including lactobacilli (A), bifidobacteria (B), *Enterobacter* (C), enterococci (D), and *Clostridium perfringens* (E). Control group means the control; XOS means xylooligosaccharides; XOS+S2 means xylooligosaccharides + *L. plantarum* S2.

or *L. plantarum* S2 alone. Both XOS and *L. plantarum* S2 could scavenge DPPH radical, and the maximum inhibition values of XOS and *L. plantarum* S2 were 65.29% and 77.34%,

respectively. *L. plantarum* S2 has a greater scavenging capacity than XOS at all the test concentrations.

The ABTS radical scavenging activity of XOS, *L. plantarum*

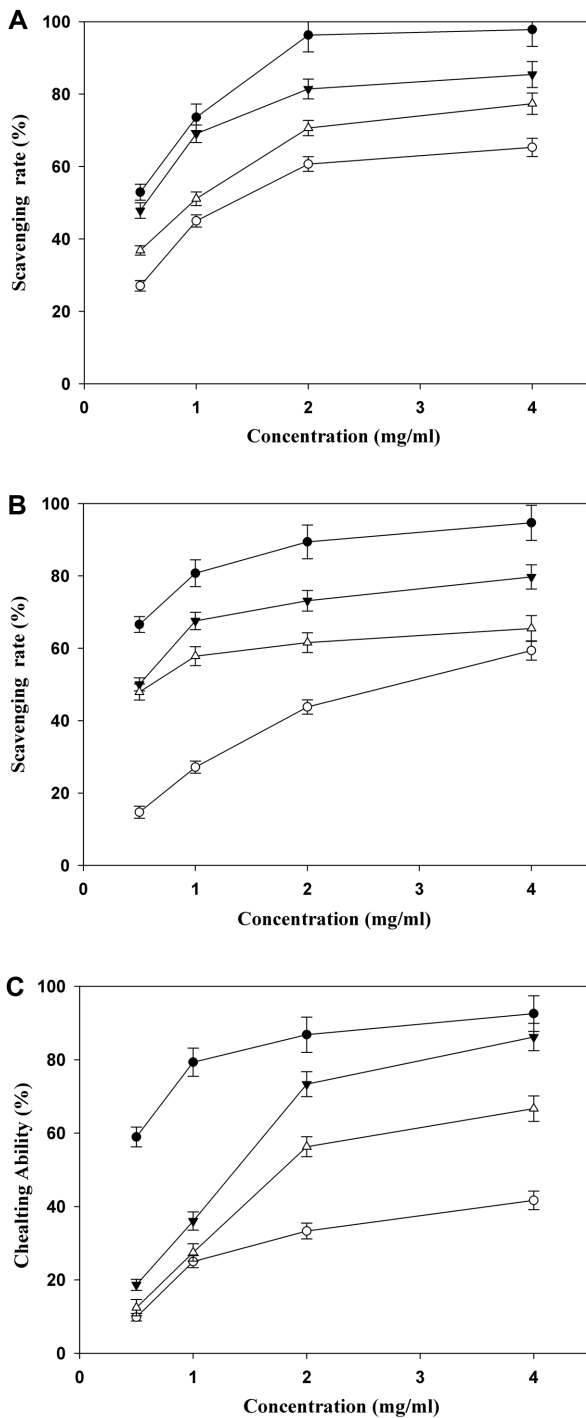


Fig. 2. Antioxidant activities of xylooligosaccharides derived from corn cobs, *L. plantarum* S2, and their combination on DPPH radical scavenging activity (A), ABTS radical scavenging activity (B), and chelating effect on ferrous ions (C).

-●- Control group: ascorbic acid; -○- XOS means xylooligosaccharide, -▼- XOS+S2 means xylooligosaccharide + *L. plantarum* S2; -△- S2 means *L. plantarum* S2.

S2, and their combination was evident at all test concentrations, but lower than that of ascorbic acid (Fig. 2B). The scavenging effect increased with the increase in concentration up to 4.0 mg/ml. The highest ABTS radical scavenging activities were 59.38%, 65.48%, and 79.71% for XOS, *L. plantarum* S2, and their combination, respectively, at a concentration of 4.0 mg/ml. XOS combined with *L. plantarum* S2 showed stronger ABTS radical scavenging activity than XOS or *L. plantarum* S2 alone at the range of 0.5–4.0 mg/ml.

As shown in Fig. 2C, the metal chelating activity of XOS, *L. plantarum* S2, and their combination increased with increasing concentration. The chelating ability of the XOS on ferrous ion was weaker than that of ascorbic acid. XOS combined with *L. plantarum* S2 showed higher chelating abilities with chelation of 86.19% at a concentration of 4.0 mg/ml containing *L. plantarum* S2 (10^{10} CFU/ml), but it was lower than that of ascorbic acid.

Antioxidant activities have had different reaction mechanisms, including reductive capacity, total antioxidant capacity, scavenging free radicals, and inhibiting lipid peroxidation [17]. In this experiment, XOS, *L. plantarum* S2, and their combination were assayed by various methods such as DPPH, metal chelating ability, and ABTS radical scavenging activity, which was compared with a control of ascorbic acid. The antioxidant properties of XOS, *L. plantarum* S2, and their combination were better than the control activity; however, XOS combined with *L. plantarum* S2 showed higher DPPH, metal chelating ability, and ABTS radical scavenging activity compared to the use of XOS or *L. plantarum* S2 alone. The results support a synergistic antioxidant effect of XOS derived from corn cobs in combination with *L. plantarum* S2 (Figs. 2A–2C). The results suggest that the antioxidant properties of this combination were substantially superior to the sum of the individual antioxidant effects, and these interactions can enhance the antioxidant effectiveness of natural antioxidants. Previous studies showed that natural antioxidants exist in nature in combination, and a combination of different antioxidants might act additively and even synergistically. For example, Li *et al.* [6] reported that the ethyl acetate extract of herb pairs showed a more significant synergistic effect in antioxidant capacity than the extract of single herbs. Wang *et al.* [27] also found that food mixtures in the *in vitro* antioxidant systems had a significant synergistic effect on attenuating H_2O_2 -mediated cell damage under co-incubation conditions. In the present study, we found a similar effect resulting from differences in the hydrophilic/hydrophobic characterization of the antioxidants; however, the synergistic

effect of an antioxidant was shown to be dependent on the type of antioxidant and its concentration [2, 16, 24].

In conclusion, XOS derived from corn cobs exhibited prebiotic properties, which included antimicrobial effects, the generation of SCFAs, regulatory effects on intestinal flora, and the capability to stimulate the growth of *L. plantarum*. Furthermore, a combination of XOS and *L. plantarum* S2 exhibited strong *in vitro* antioxidant activities. Thus, XOS derived from corn cobs are a potential source of prebiotics that could be used as an ingredient in functional foods and nutraceutical products.

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