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Protective effects of exopolysaccharide of a medicinal fungus on probiotic bacteria during cold storage and simulated gastrointestinal conditions

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Short running title: **Protective effect of fungal exopolysaccharide on probiotic bacteria**

Abstract

The efficacy of probiotic health products depends on the capability of the constituent probiotic bacteria to survive through long period of cold storage and the gastrointestinal tract. This study was to evaluate the protective effects of a high-molecular weight (MW) exopolysaccharide (EPS) from a medicinal fungus Cs-HK1 on three different bifidobacteria. The EPS had a total dietary fiber content about 70% (w/w), which was close to its total carbohydrate content. It was resistant to artificial gastric acid (pH2) with no more than 4% (w/w) hydrolysis in 6 hours. EPS at 5 g/L significantly increased the survival rate of the probiotic bacteria during cold storage (4 °C) and in simulated gastric acid, reducing the death rate of different bacterial strains by 50% to 70%. The protective effect of EPS was weaker when the concentration was decreased to 3 g/L or when the MW of EPS was reduced by partial degradation with power ultrasound. EPS also showed significantly protective effect on the all bacterial strains in bile juice. The results have demonstrated the potential value of Cs-HK1 EPS as a novel prebiotic fiber for the formulation of synbiotic products with probiotic bacteria.

Keywords: *Bifidobacteria*; Survival; Polysaccharide; Cold storage; Gastrointestinal conditions

1. Introduction

Recent years has witnessed an enormous interest worldwide in the development of probiotic, prebiotic and synbiotic products targeting a healthy human gut microbiota [1, 2]. Human uptake

of probiotic microorganisms has been regarded as an effective strategy for balancing the gut microbiota so as to improve gut health and lower the risk of various diseases such as metabolic syndrome, inflammation the brain and skin [3, 4]. Other potential health benefits of probiotic ingestion include increase of mineral absorption, immunity enhancement, inhibition of tumor formation and hypercholesterolemia prevention [5-7]. *Bifidobacterium* is one of the most common probiotic species used in functional foods and dietary supplements [8]. As an effective probiotic product, the constituent bacteria should be resistant to the harsh conditions in the gastrointestinal tract (GIT), especially gastric acid and bile salt, reaching the large intestine alive to exert an influence on the gut microflora. The number of viable bacteria is an important quality index of probiotic products and is also essential for achieving the desired effects on the host [9]. Various measures have been explored to enhance the survival rate of probiotics, such as screening for high tolerance strains, encapsulation, and supplementation of polysaccharides and oligosaccharides [10-12]. Supplementation of carbohydrate fibers is a relatively simple approach and is also possible to add extra benefits to the products, such as the prebiotic fibers in synbiotic products.

Edible and medicinal fungi or mushrooms provide a rich and diverse source of natural polysaccharides which are recognized as potential prebiotic candidates [13]. In addition to the few well-known β-glucans which have been applied to anticancer and immunotherapy [14], many homo- and hetero-glycans have been isolated from mushrooms or fungal mycelia [15, 16]. Most of the bioactive fungal polysaccharides are resistant to the digestive enzymes in the human GIT and their health benefits and bioactivities such as immunomodulation and anti-inflammation may involve the modulation of gut microbiota [15, 17].

Cordyceps sinensis, the Chinese caterpillar fungus, is a precious medicinal fungus in traditional Chinese medicine (TCM) for treatment of diseases related to the lung, kidney, and respiratory systems and for promotion of health and physical performance [18]. Because the wild caterpillar fungus called Dong Chong Xia Cao in Chinese is very rare and expensive, mycelial fermentation has become the major resort for *Cordycpes sinensis* products. The mycelial culture of a *C. sinensis* fungus Cs-HK1 has been established in our lab for fermentation production of the mycelial biomass and polysaccharides [19]. The exopolysaccharides (EPS) produced by Cs-HK1 fungus in liquid fermentation have shown notable antitumor, immunomodulatory and antioxidant activities in our previous studies [20]. In our recent studies, the high MW fractions of Cs-HK1 EPS (~10^6-10^8 Da) showed significant protective effects on bacterial viability in normal culture conditions [21] and during exposure to antibiotics [22].

The aim of the present study was to further evaluate the potential of the Cs-HK1 EPS as a protective additive during the storage of probiotic bacteria and as a dietary, prebiotic fiber in the human GI environment. Experiments were performed in liquid cultures of some common probiotic strains of *Bifidobacterium*. The original EPS from the Cs-HK1 mycelial fermentation and a partially degraded EPS by power ultrasound were tested together with two well-known prebiotic fibers inulin and galactooligosaccharide (GOS) on the bacterial survival rate during cold storage and in simulated gastrointestinal conditions.

2. Methods and materials

2.1 Cs-HK1 mycelial fermentation and preparation of EPS fractions

Cs-HK1 is a fungus species isolated from a wild *Cordyceps* fruit body by Wu's group several years ago and has been maintained in mycelial culture as reported previously [19]. The liquid medium for Cs-HK1 mycelial culture consisted of 40 g/L glucose, 5 g/L peptone, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$ and 10 g/L yeast extract [23]. The Cs-HK1 liquid fermentation was carried out at 20 °C in a shaking incubator at 200 rpm for 7 days. The mycelial culture broth was then centrifuged at 12,000 rpm (21,612×g) for 15 min and the supernatant medium was collected for recovery of EPS. Ethanol (96%, w/v) was added into the supernatant medium at 5:1 volume ratio and maintained at 4 °C overnight for precipitation. The precipitate was separated by centrifugation at 12,000 rpm (21,612×g) for 15 min and then freeze-dried, yielding the EPS.

Because the original EPS from the Cs-HK1 fermentation had a very high MW and a low water solubility, it was partially degraded with power ultrasound (US) to a lower MW and higher water solubility. As reported previously [21], the power US was generated with a VCX 750 processor with a fixed frequency of 20 kHz and a maximum output power of 750 W (Sonics and Materials Inc., Newton, USA). The Cs-HK1 mycelia culture medium (1 L) was irradiated with a US probe at a fixed US power (80% amplitude) for 30 min, followed by ethanol precipitation, centrifugation and freeze-drying, yielding the US-degraded EPS designated EPS-US.

2.2 Measurement of EPS properties

The molecular weight (MW) of EPS and EPS-US were determined by high-pressure gel permeation chromatography (HPGPC) with the same instruments (Waters 1515 isocratic pump and a 2414 refractive index detector) and conditions as reported previously [21]. The intrinsic viscosity [η] of EPS samples was determined as reported previously by the serial d ilution method

with a Ubbelohde viscometer at 25 °C [24]. The apparent viscosity of EPS and EPS-US solutions was measured by a rheometer (AMETEK Brookfield, USA). The size distribution of EPS and EPS-US aggregates in water was measured at room temperature by a nanoparticle (405 nm) tracking analyzer (Nanosight NS300HSBF). The solubility of EPS and EPS-US in water was determined by the method previously used for starch [25].

2.3 Analysis of dietary fiber content in EPS

The total dietary fiber content was determined by the assay kit (TDF-100A, Sigma-Aldrich, USA). Briefly, EPS samples were first treated by heat stable α-amylase, protease and amyloglucosidase to remove protein and starch. Ethanol (95%, w/v) was then added for precipitation. The residues were filtrated and washed by ethanol and acetone. After drying, the residues were analyzed for the protein by Kjeldahl method and ash at 525 °C. The total dietary fiber was the weight of the residue less the weight of the protein and ash. Calculation equation was provided by the assay kit.

2.4 Degradation of EPS in simulated gastric acid

EPS and EPS-US were tested for acid resistance according to Tingirikari et al. [26] with minor modifications. Simulated gastric acid was prepared using hydrochloric acid buffer containing 8 g/L NaCl, 0.2 g/L KCl, 8.25 g/L $NaHPO_4 \cdot 2H_2O$, 14.35 g/L NaH_2PO_4, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 0.18 g/L $MgCl_2 \cdot 6H_2O$. The pH of the buffer was adjusted to 2 with 5 M HCl. The EPS was dissolve in water at 5 g/L; 1 mL of the EPS solution was mixed with 2 mL simulated gastric acid was incubated in 37 °C for 6 h, during which sample was taken every hour. The

reducing sugar content in the sample was determined by DNS method [27] and total sugar content was determined by anthrone test [28].

2.5 Probiotic bacterial species and culture conditions

Three strains of *Bifidobacterium* were used in this study including *B. adolescentis* (CICC 6070), *B. infantis* (CICC 6069), and *B. infantis* (R33). The first two were obtained from China Centre of Industrial Culture Collection (CICC) and the third was from Biostime Inc. (Guangzhou, China). The conditions for storage and incubation of the bifidobacteria have been described previously [21]. The bacterial culture was initiated by inoculation of each strain taken out of the storage (30% glycerol at -80 ºC) in Reinforce Clostridial Medium (RCM) agar on a petri dish and incubation for about 2 days. A single colony on the agar plate was picked out and inoculated into RCM liquid broth and incubated overnight. The bacterial suspension was then subcultured at 2% (v/v) into RCM broth. All bacterial cultures were incubated at 37 ºC under anaerobic condition in air-tight jars with anaerobic gas generating sachets (AnaeroGen TM, Thermo Scientific Oxoid, USA) [29]. For liquid cultures, the jar was placed on a shaking incubator running at 200 rpm. The CFU of the bacterial growth was determined on selected days over 28 days.

2.6 Cold storage of probiotic bacteria and measurement of H_2O_2

After 24 hours of incubation in broth, the bacterial cells were centrifuged at 5000 rpm for 10 min. The bacterial pellets were washed twice with sterile saline (0.9% w/v NaCl solution) [11] and then were re-suspended in 2 mL centrifuge tube containing 1 mL EPS sample solution (5

g/L) or sterile saline (control). These mixture solutions were stored at 4 °C for 28 d. The viability of the probiotics was determined using RCM agar at 0, 7, 14, 21 and 28 d. Galacto-oligosaccharide (GOS) and inulin (5 g/L) were tested as prebiotic references. According to the results, EPS had the best effect on bifidobacterial viability at 4 °C so that EPS solution at different concentrations (1, 3, 5 g/L) was also tested on bifidobacteria at 4 °C. All the media, sample solutions and centrifuge tubes were sterilized before use by autoclaving at 120 °C for 20 min. The percentage of death rate reduction was estimated by (1–Rd of EPS/Rd of control), where the death rate (Rd) is given by (CFU at time 0 –CFU at time t)/t.

It has been suggested that the oxygen-sensitive bifidobacterial species could produce H_2O_2 in the presence of oxygen which is toxic to the bacterial cells [30]. Therefore the concentration of H_2O_2 in the bifidobacterial culture medium after storage at 4 °C was determined by H_2O_2 assay kit (BC3595, Beijing Solarbio Science& Technology Co., Ltd, China).

2.7 Probiotic bacteria in simulated gastrointestinal conditions

The sample solution suspended with bacteria was prepared in the same way as that described in 2.6. The survival rates of the probiotic strains in sample solutions were examined according to Michida et al. [11] and Chou et al. [31] with slightly modified. 0.5 mL of the bacterial suspended solution was mixed with 1 mL simulated gastric acid (HCl solution buffer, pH = 2) or bile juice (0.3% w/v bile salt in saline, pH = 8). pH was adjusted by HCl and NaOH. The bacteria were maintained at 37 °C for 3 hours, and the viability of the bacteria was determined using RCM agar at selected time points from 0-3 h for evaluation of gastric acid and bile juice tolerance. All the media and sample solution were sterilized before use by autoclaving

at 120 °C for 20 min, and the simulated gastric acid and bile juice were sterilized by membrane filtration.

2.8 Scanning electron microscopy

The morphology of the bacterial cells and EPS samples and their mixture was monitored by scanning electron microscope (SEM). Due to the similar trend of the effect contributed by EPS samples on different bacterial strains, only *B. adolescentis* was applied for SEM detection. *B. adolescentis* was first cultured in RCM broth for 24 h as described in 2.5 and then centrifuged at 5000 rpm for 10 min and the pellets were washed twice with sterile saline and resuspended in EPS sample solution (5 g/L). Inulin was also tested for comparison. The mixtures were immediately frozen by liquid nitrogen, followed by freeze drying. The dried samples were coated with gold. SEM was performed with a JSM 6710 SEM (JEOL Ltd., Tokyo, Japan).

3. Results and discussion

3.1 Physical properties of EPS samples

As shown in Fig. 1A, the original EPS had two overlapping high MW peaks around 1.03×10^8 and 1.33×10^7 Da, respectively. After ultrasonic treatment, the two high MW peaks of EPS-US were shifted to lower MW ranges of around 5.99×10^7 and 7.79×10^6 Da, respectively. Both the intrinsic and apparent viscosity of EPS were decreased by the treatment of ultrasound (Fig. 1B & Table 1) while the solubility of EPS was increased significantly from 8.83 to 20.08 g/L. The particle sizes of EPS and EPS-US were 17.28 nm and 9.85 nm respectively (Fig 1C & Table 1), implying that the EPS samples existed in the form of hydrocolloids in water.

The total dietary fiber content in EPS and EPS-US was 68.9% and 60.1%, respectively. As the total carbohydrate content of EPS was 71%, most of the EPS carbohydrates belong to non-digestible fiber that are resistant to the digestive enzymes in human digestive system. As shown in Fig. 2 EPS and EPS-US were very resistant to simulated gastric acid, with no more than 5% (w/w) hydrolyzed to reducing sugar over 6 h. The higher degradation rate of EPS-US (4.15%, w/w) than EPS (2.64%, w/w) was probably due to the fact that the aggregates of EPS-US were much smaller and looser, and more accessible for the acid in solution. The high intensity ultrasound (US) can cause disruption of the EPS aggregates, reducing the solution viscosity and increasing the water solubility significantly [32]. Inulin, a commercial prebiotic, was hydrolyzed more than 25% (w/w) in the gastric acidic solution [26, 33]. This suggests that the acidic tolerance of EPS samples were much higher than inulin. The high resistance of EPS and EPS-US to the digestive enzymes and simulated gastric acid warrants their function as dietary fibers or prebiotics useful for the gut bacteria in the large intestine.

3.2 Bacterial survival and H_2O_2 accumulation during cold storage

Fig. 3 shows the viability time courses of three bifidobacteria during cold storage at 4 °C. All three strains of bifidobacteria in the control, inulin and GOS groups died out within 28 days or a shorter period. EPS and EPS-US had a significant protective effect on the survival rate of all strains. *B. infantis* (R33) was most susceptible among the bifidobacterial strains at low temperature and died rapidly within two weeks in the control, but retained a survival rate above 10^2 CFU/mL in the presence of EPS or EPS-US. The protective effect of EPS was slightly stronger than that of EPS-US for *B. adolescentis*.

For a more quantitative comparison of the protective effects of various carbohydrates, Table 2 presents the percentage values of death rate reduction that are derived from experimental data in Figs 3&5. Overall, the protective effects of EPS and EPS-US on the probiotic bacteria were much stronger than inulin and GOS. EPS and EPS-US showed significant effect on all bacteria species in cold storage. Although inulin has been previously shown to maintain the viability of probiotics [34], it only increased the survival rate of *B. adolescentis* (CICC 6079) and *B. infantis* (R33) during the cold storage.

A possible cause for the cell death during cold storage is attributed to the oxidative stress produced by the reactive oxygen species such as H_2O_2. Some of the O_2-sensitive bifidobacteria O_2 could produce H_2O_2 in the presence of O_2 which was toxic to the bifidobacteria [35, 36]. To confirm this postulation, the H_2O_2 concentration in the liquid medium of three bifodonacteria at the end of cold storage was measured. As shown in Table 3, *B. adolescentis* and *B. infantis* (R33) produced a much higher level of H_2O_2 than *B. infantis* (CICC6069), suggesting that the first two strains were more sensitive to oxygen. Consistently with the results shown in Fig. 3, the *B. infantis* (CICC6069) strain also survived longer than the first two bifidobacterial strains. Moreover, the addition of EPS and EPS-US significantly decreased the level of H_2O_2 produced by *B. adolescentis* and *B. infantis* (R33) compared to the control. The reduction of H_2O_2 production may be attributable to the high viscosity of EPS, increasing the resistance of oxygen access to the bacteria. Overall, this set of results provided supporting evidence for the postulation that H_2O_2 is a major factor contributing to the cell death during cold storage in previous studies. In this connection, the protective effect of EPS on the bifidobacteria may also be attributable to its antioxidant activity as reported previously [37].

Additional experiments were also performed on the survival of bifidobacteria stored at the normal culture temperature of 37 °C. All three bifidobacterial strains died out much faster than during cold storage at 4 °C. In the EPS solution (without any growth nutrients), three of the bifidobacteria strains died out within a week (data not shown) and only *B. infantis* (CICC 6069) retained a CFU of 413 ± 97 /mL on day 7 but died out in two weeks. In the normal culture medium without EPS, all bifidobacteria died within two weeks (Fig. 4). The less significant protective effect of EPS and EPS-US at 37 °C than at 4 °C was probably attributed to the lower viscosity at the higher temperature.

3.3 Bacterial survival in gastric acid

Fig. 5 shows the viability trend of probiotic bacteria during incubation in simulated gastric acid for three hours. Although *B. infantis* (R33) was very susceptible to oxygen stress and nutrient deficiency, it was relatively tolerant to the acid stress with slower reduction of viability in the control group. EPS, EPS-US and GOS significantly increased the gastric acid survival of all three probiotic bacteria compared to the control group, while inulin showed some positive effect only on *B. adolescentis* (CICC 6070).

For a more quantitative comparison of the protective effects of various carbohydrates, Table 2 presents the percentage values of death rate reduction that are derived from experimental data in Figs 3 & 5. EPS and EPS-US showed significant effect on all bacteria species in both cold storage and gastric acid. Table 4 presents the percentage values of death rate reduction of EPS at three different concentrations, 1, 3, 5 g/L. In most cases, the protection effect (death rate reduction) with EPS was significantly lower at a lower concentration.

Bacterial cells have developed the general stress response (GSR) system to cope with nutrient starvation and various environmental stresses [38]. It has been reported that GSR can be triggered at a high cell density [39, 40]. In this study, the GSR mechanism may be a possible contributor to protective effect of EPS on the probiotic bacteria in various stress conditions as the bacteria cells were agglomerated in the EPS gels to a high density. In addition, the bacterial cell density may also affect their acid tolerance due to the quorum sensing. Besides the GSR, bacterial cells have the acid tolerance response (ATR) system for the better survival in acidic conditions [3, 41]. As reported by Li et al. [42], the high cell density of *Streptococcus mutans* could modulate ATR to gain a significantly higher resistance to lethal pH. This suggests that cell-cell communication plays an important role in bacterial resistance to acid stress.

3.4 Bacterial survival in bile juice

Table 5 shows the survival rate of probiotic bacteria in bile juice with EPS, EPS-US, inulin and GOS. All of the three strains had a very low survival rate in bile juice, which was mainly attributed to the alkaline environment of bile juice (pH 8) plus the antimicrobial activity of bile salt. Bile salts could cause disruption of the cell membrane and DNA damage [43]. After incubation in the bile juice for 3 hours, nearly all bifidobacterial cells died out in the control, GOS and inulin groups, and viable cells were only present in the EPS groups. EPS showed a significant protective effect on the three bacterial strains with a notable survival rate. The protective effect of EPS was weak or negligible as the concentration was lowered to 3 g/L and 1 g/L.

Both inulin and GOS are well-known prebiotic carbohydrates that can support the growth of probiotic bacteria [44, 45]. The increasing viability of some strains by inulin or GOS can be partly attributed to their nutritional effect. As reported previously by our group [21], GOS was a favorable carbon source as glucose, but EPS, EPS-US or inulin was not well utilized for the growth of bifidobacteria in culture. Therefore, the ability of EPS and EPS-US to sustain the bacterial survival in various conditions can be mainly attributed to protective effect. In a recent study [46], a viscous layer formed on the *B. adolescentis* was regarded as a major contributing factor for protecting the bacteria from antibiotic damage and atomic force microscope (AFM) was applied to visualize the layer. Physical interactions of the bacteria cells with the EPS aggregates and gels in the liquid medium might also contribute to the protective effect in this study. Moreover, the formation of biofilm by bacteria may also increase the resistance to detrimental factors and harsh conditions, which is facilitated by an EPS gel matrix surrounding the bacteria cells [47]. In the following experiments, scanning microscopy (SEM) was applied to detect these phenomena possibly occurring to the bifiodobacterial cells with the EPS.

3.5 Interactions between bacterial cells and polysaccharides

Fig. 6 shows the SEM images of *B. adolescentis* cells in three polysaccharide solutions, EPS, EPS-US and inulin. EPS and EPS-US formed planner sheets of aggregates on which rod-shaped bacteria cells were attached. Inulin appeared as clusters of aggregates with relatively few bacteria cells protruding from the outer periphery. Many bacteria cells were attached on the EPS aggregates but very few on EPS-US and inulin. The immobilization or encapsulation of probiotics by polysaccharide gels is recognized as an important factor contributing to the

tolerance of probiotic bacteria to environmental stress, thus increasing their survival rate [48, 49]. The bacterial cells attached to or entrapped by EPS and EPS-US aggregates are less exposed to the stress factors and conditions in their surroundings. Compared to EPS-US, EPS was more viscous in liquid solution and more capable to immobilize the bacterial cells, conferring a stronger protective effects. As the concentration of EPS was decreased, the viscosity of solution decreased and the protective effect was also weaker.

4. Conclusions

The EPS from Cs-HK1 mycelial fermentation has shown notable protective effect on probiotic bacteria in three practical conditions, cold storage, acidic pH and bile salt. The protective effect was mainly associated with the physical properties of EPS, namely the high MW and high liquid viscosity, and was weaker after partial degradation by power ultrasound. The commercial prebiotics such as inulin and GOS with much lower MW showed little or no protective effect. Firstly, the viscous EPS surrounding the bacterial cells may slow or block the access of the stress factors to the bacterial cells. Secondly, the immobilization or attachment of bacterial cells to the EPS gel matrix is also favorable for the bacterial survival under stress. With the protective effect on the probiotic bacteria in various conditions plus its high dietary fiber content and high resistance to gastric acid hydrolysis, EPS is a potential health supplement to be used separately or in combination with probiotic bacteria for improving gut microbiota.

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Table 1 Physical properties of EPS and EPS-US

	Intrinsic viscosity (dL/g)	Solubility (g/L)	Average particle size (nm)
EPS	6.03 ± 0.98	8.83 ± 0.76	17.28 ± 4.93
EPS-US	4.65 ± 0.72	20.08 ± 0.63	9.85 ± 2.53

Table 2 Reduction (%) of bifidobacterial death rate during cold storage and in simulated gastric acid with EPS and other carbohydrate fibres (all at 5 g/L in the culture medium).

Bacterial species	GOS	Inulin	EPS	EPS-US
Cold storage (4°C, 28 days)				
B. adolescentis (CICC 6070)	0.7	25.3	49.9	48.3
B. infantis (CICC 6069)	0.4	-1.3	65.8	47.6
B. infantis (R33)	35.4	34.6	71.8	72.6
Simulated gastric acid				
B. adolescentis (CICC 6070)	33.6	26.5	68.0	50.3
B. infantis (CICC 6069)	36.9	-5.5	49.3	40.3
B. infantis (R33)	30.8	6.7	70.8	70.0

Table 3 Accumulation of H_2O_2 (μmol/mL) by bifidobacteria after cold storage at 4 °C.

Bacterial strains	Control	GOS	inulin	EPS	EPS-US
B. adolescentis	0.47 ± 0.14	0.55 ± 0.12	0.27 ± 0.12	0.14 ± 0.03*	0.23 ± 0.10
B. infantis (R33)	0.74 ± 0.08	0.59 ± 0.16	0.44 ± 0.11	0.36 ± 0.07*	0.52 ± 0.06*
B. infantis (CICC6069)	0.19 ± 0.16	0.16 ± 0.14	0.14 ± 0.04*	0.16 ± 0.08	0.15 ± 0.09

*: significant at $p < 0.05$ by t-test

Table 4 Reduction (%) of bifidobacterial death rate during cold storage and in simulated gastric acid with different concentrations of EPS in the culture medium.

Bacterial species	EPS-1	EPS-3	EPS-5
Cold storage (4°C, 28 days)			
B. adolescentis (CICC 6070)	24.8	23.4	49.0
B. infantis (CICC 6069)	54.2	55.1	67.3
B. infantis (R33)	50.5	72.2	72.4
Simulated gastric acid			
B. adolescentis (CICC 6070)	4.4	76.2	80.9
B. infantis (CICC 6069)	-1.6	32.5	39.2
B. infantis (R33)	16.5	30.6	55.3

Note: EPS-1, EPS-3 and EPS-5 for EPS added to the culture medium at 1, 3, and 5 g/L, respectively.

Table 5 Survival rate (%) of bacterial strains after 3 h incubation in bile juice with EPS and other carbohydrate fibers (all at 5 g/L or specified concentrations in culture).

	B. adolescentis (CICC 6070)	*B. infantis* (CICC6069)	*B. infantis* (R33)
Control	< 0.001	< 0.010	< 0.010
GOS	< 0.001	< 0.010	< 0.010
Inulin	< 0.001	< 0.010	< 0.010
EPS	0.063 ± 0.030	0.67 ± 0.20	0.97 ± 0.18
EPS-US	< 0.001	< 0.010	< 0.010
EPS at different concentrations (EPS-1,-3,-5: EPS at 1, 3, 5 g/L).			
Control	< 0.001	< 0.010	< 0.010
EPS-1	< 0.001	< 0.010	< 0.010
EPS-3	< 0.001	< 0.010	0.47 ± 0.11
EPS-5	0.055 ± 0.020	0.84 ± 0.17	1.01 ± 0.28

<Figure captions>

Fig. 1. Physical properties of EPS and EPS-US.

Fig. 2. Hydrolysis of EPS and EPS-US (to reducing sugar) in simulated gastric acid (37 °C).

Fig. 3. Viability of bifidobacteria with EPS and other carbohydrate fibers (5 g/L) during cold storage at 4°C for 28 d.

Fig. 4. Viability change of bifidobacteria maintained in liquid culture medium (RCM broth) at 37°C and 200 rpm over long period.

Fig. 5. CFU of *Bifidobacteria* in simulated gastric acid with different polysaccharide solution (5 g/L) during 3 h incubation.

Fig. 6. SEM image of *B. adolescentis* trapped by different polysaccharides.

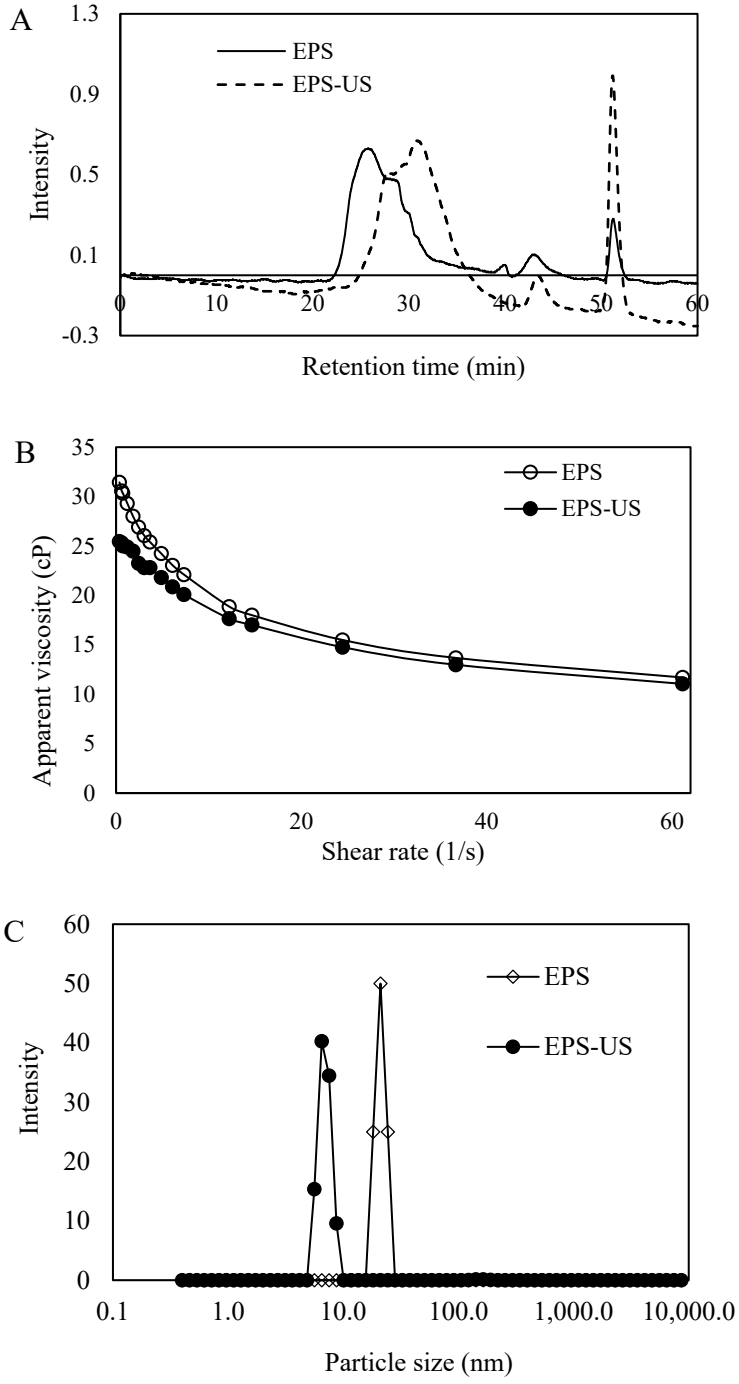
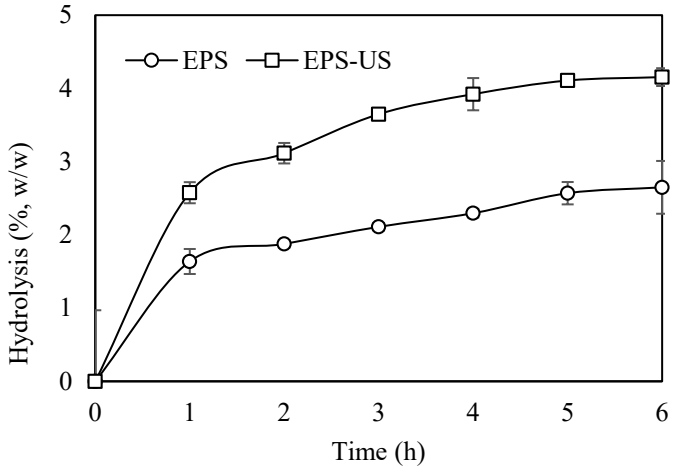


Fig. 1. Song et al.



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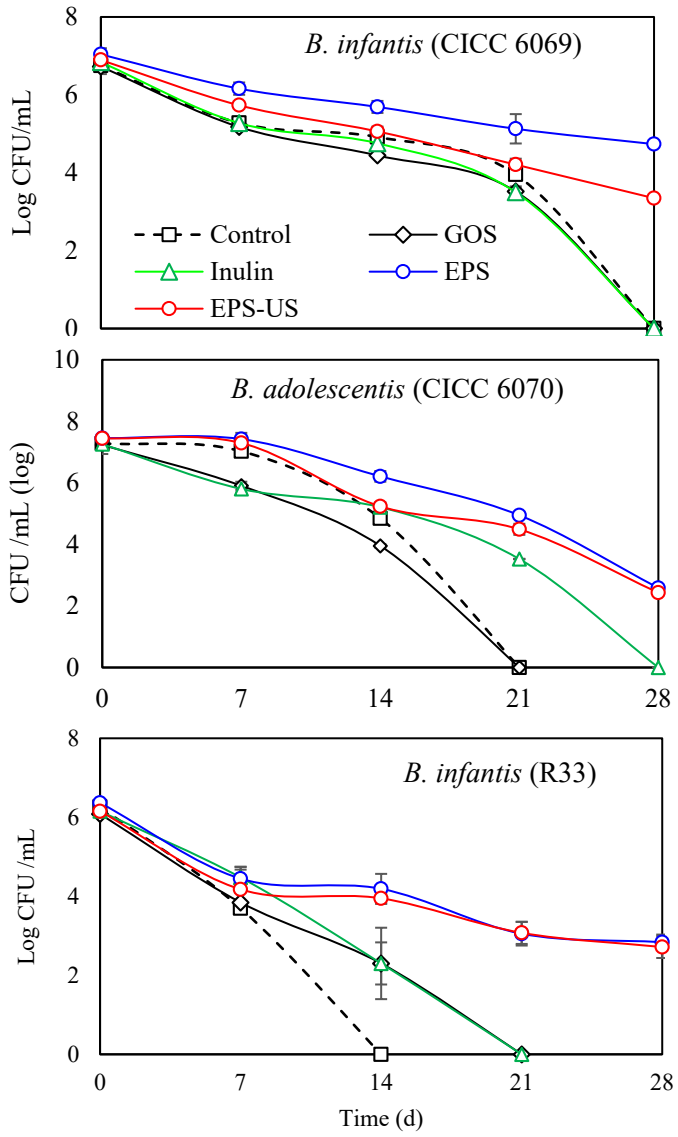

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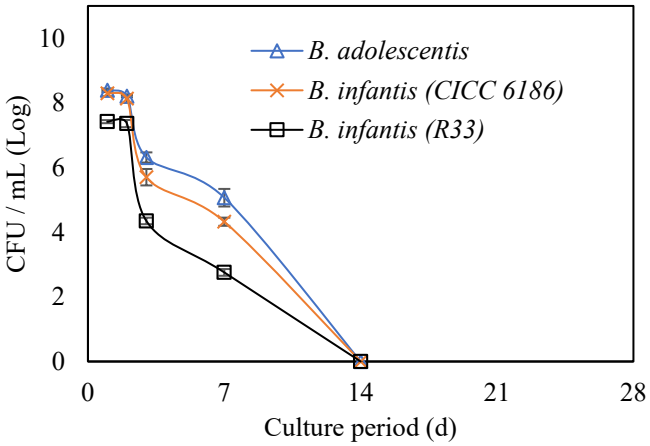

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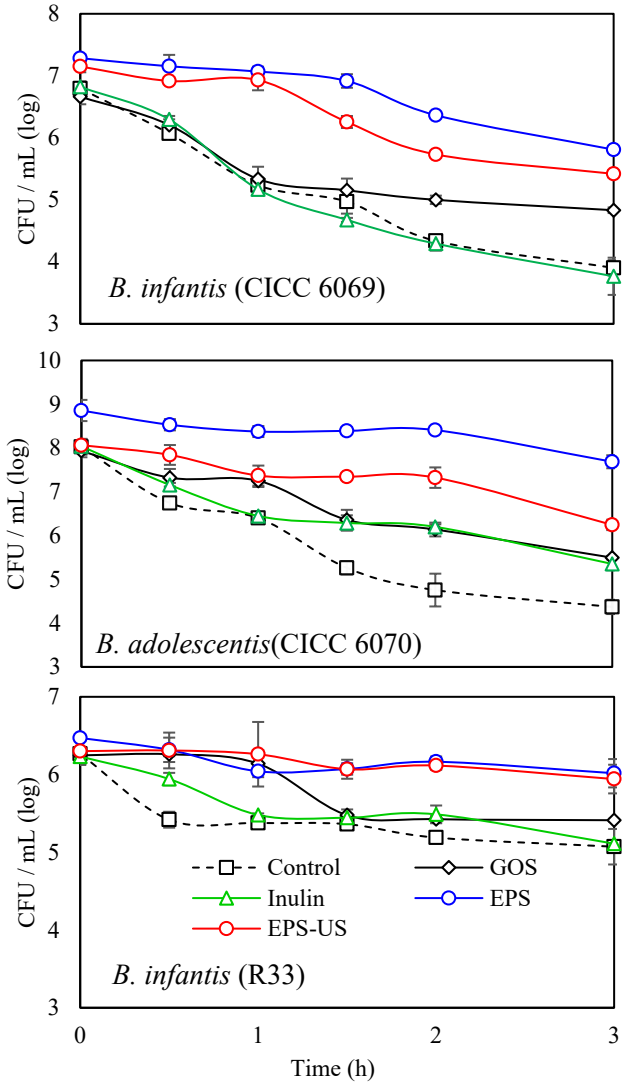

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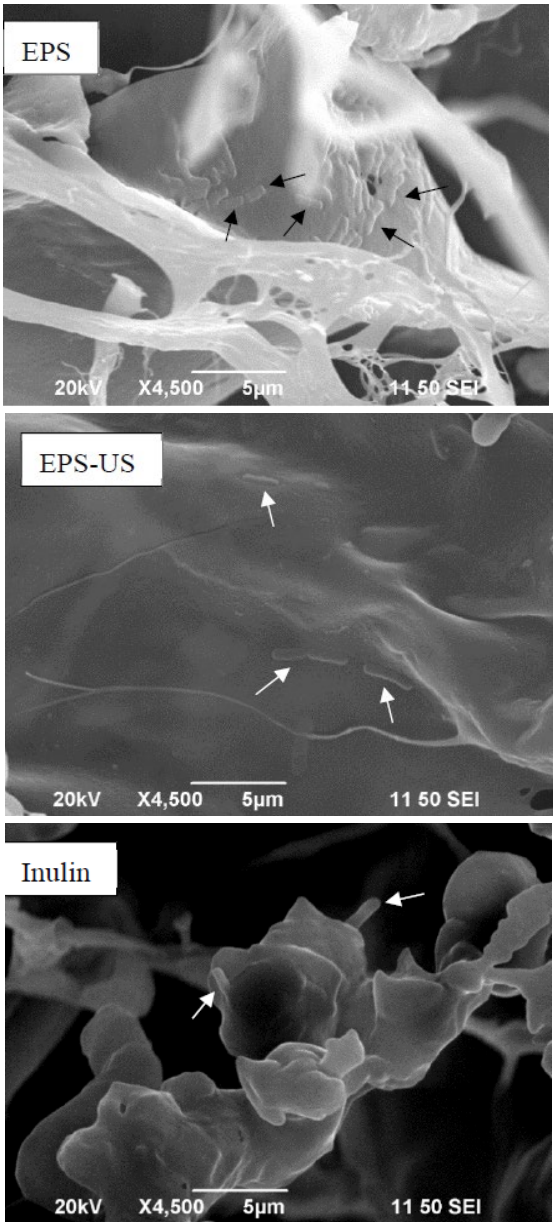

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Fig. 6. Song et al.