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# Physicochemical Property and Pharmacological Activity of Exopolysaccharide From Endophytic Bacterium Bacillus Sp. B3 Isolated From Polygonatum Sibiricum

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# Abstract

*Polygonatum sibiricum* is a widely used traditional Chinese medicine with various functions. In this study, *Bacillus sp*. B3 as its endophytic bacterium, which produced the maximum amount of exopolysaccharide (EPS), was isolated. The EPS-B3 produced by *Bacillus sp.* B3 was purified and identified. As suggested by the analysis of monosaccharide composition, EPS-B3 with an average molecular weight of $1.863\times10^4$ Da, was composed of three main monosaccharides, including galactose, glucose and mannose with a molecular ratio of 15: 3: 1. The monosaccharide component of EPS-B3 was different from *P. sibiricum* polysaccharide (PSP), but they exhibited similar antioxidant and antitumor activity. It is worth to point out that, EPS-B3 exhibited higher Ferric reducing power than PSP. As suggested by the results, the EPS produced by endophytic bacterium from the medicinal plants might have the potential to serve as an alternative of the medicinal herbs.


# 1. Introduction

Traditional Chinese medicine (TCM), which was used by a total of 80% the world's population [1], plays a significant role in people's life and health. As the indispensable and valuable asset of natural and cultural heritages, they are commonly used to treat human and animal diseases. Many components with remarkable bioactivities from TCM have been isolated and characterized, such as saponins, cyrtonema, lectins, polyphenolics, and polysaccharides [2, 3]. An increasing number of evidences indicated that polysaccharides derived from TCM have potent bioactivities, such as immune enhancing property, antitumor activity and antiviral activity [4-6]. Thus, it is urgent to find out the alternatives of the medicinal plants.

*Polygonatumsibiricum* is a common traditional Chinese medicine with various functions widely used in some Asian countries [3] to help facilitate secretion of fluid and quench thirst, treat dryness and cough, and so on [7, 8]. The polysaccharide is one of the main bioactive components from *P. sibiricum* exhibiting remarkable pharmacological applications and biological activities [2, 9, 10], such as antioxidant [3], anti-tumor [11, 12], anti-atherosclerosis activity and immunity enhancement effect [10, 13].

Microbial exopolysaccharides (EPS) with high molecular weight and complex chemical structures are generated and secreted out of the cells by a broad range of microbial species [14]. The EPS have lots of advantages, such as safety, unique biological properties, and the relatively easy preparation [15, 16]. The endophytic bacteria from medicinal plants are a group of microorganisms, which have symbiotic relationship with host medicinal plants. And they normally produce similar or identical metabolite to the host plants, which can be used as an important source of active substances. Therefore, the EPS of endophytic bacteria from medicinal plants with quite significant biological functions could be regarded as the substitute or supplement of polysaccharides from medicinal plants. Due to their diverse functions, an increasing number of researchers are focusing on screening for valuable EPS producing bacteria.

In this study, we aim to screen and identify endophytic strains isolated from *P. sibiricum* producing the highest amount of EPS. Afterwards, the chemical characteristics, monosaccharide compositions, antioxidant and antitumor activity of EPS produced by *Bacillus sp*. B3 (EPS-B3) were investigated. Compared with PSP, the EPS-B3 exhibited similar antioxidant and antitumor activity, which provides evidences for the clinical application of EPS-B3 as an alternative of PSP.

# 2. Materials And Methods

### 2.1 Materials and reagents

*P. sibiricum* was purchased from Hunan and Henan Province of China. Sephacryl-300 HR and DEAE-52 cellulose were bought from GE Healthcare (Gothenburg, Sweden). Taq DNA Polymerase and gel/PCR Extraction Kit were obtained from Tiangen (Beijing, China). The compounds, α-Diphenyl-β-picrylhydrazyl (DPPH), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Shanghai, China). Dulbecco's modified eagle medium (DMEM, high glucose), fetal bovine serum (FBS), trypsin and phosphate buffered saline (PBS) were bought from Thermo Fisher Scientific (Beijing, China). BALA/c mice were purchased from Shandong University laboratory animal center. Bacterial Physiological and Biochemical Identification Reaction Tube were obtained from Binhe Co. Ltd. (Hangzhou, China).

### 2.2 Isolation and identification of the endophytic bacteria from *Polygonatum sibiricum*

After the surface of *Polygonatum sibiricum* root was disinfected, the internal tissues were placed on the separation plate evenly and cultivated in an incubator (37°C, several days). The single colonies were picked according to the colony morphology, amplified and conserved by a conventional method. Specifically, the monoclonal colony was selected in the LB solid medium containing NaCl 10 g/L, yeast 5 g/L, tryptone 10 g/L and agar 15%, respectively. To isolate the targeted strains, plates were incubated in incubator (37°C, 48-72 h). The isolated single colonies were cultivated in LB liquid medium on a shaker (37°C and 200 rpm, 72 h).

The bacterium named as B3 exhibited the highest production of EPS was identified based on morphologic, physiological and biochemical features. After that, the 16S rDNA fragment was synthesized by PCR with two universal primers 9F (5'-GGTTACCTTGTTACGACTT-3') and 1492R (5'-AGAGTTTGATCCTGGCTCAG-3') [17]. Furthermore, it was sequenced (Beijing Genomics Institute, Beijing, China) and compared with the 16S rDNA sequences of BLAST database online. Its evolutionary with other bacteria was analyzed by MEGA (version 6.1) software through the 16S rDNA sequence analysis [18].

### 2.3 Preparation of the EPS-B3

Endophytic bacterium *Bacillus sp*. B3 was cultured on a shaker (200 rpm, 37°C and 72 h). And fermentation broth was centrifuged (8000 *g*, 10 min) to remove cells. The supernatants were precipitated in ethanol (1:4, v/v) at 4°C for 12 h and then centrifuged (8000 *g*, 10 min). Precipitates were gathered and freeze-dried to obtain crude polysaccharides. In addition, the proteinase was added to remove the extra proteins in the supernatant using the savage method [19]. After decoloration by hydrogen peroxide, desalting through dialysis, the sample was further purified with the DEAE column and Sephacryl-300 HR chromatography as previous research [20]. Beyond that, polysaccharide content of the purified sample was analyzed as previously reported [21] and the fractions were combined and lyophilized after desalting.

### 2.4 Molecular Weight Determination of EPS-B3

High-performance gel permeation chromatography (HPSEC) and multi-angle laser light scattering (MALLS) spectrometer (Wyatt, DAWN HELEOS-II) were used to analyzed the homogeneity and average molecular weight (Mw) of EPS-B3 were analyzed as previously reported [20, 22]. Before 250 μL samples (5 mg/mL) was added into MALLS system at flow velocity of 0.5 mL/min, the EPS sample was completely dissolved in 0.2 mol/L $NaNO_2$ solution which contained 0.02% $NaN_3$.

### 2.5 Analysis the Monosaccharide composition of EPS-B3

Before monosaccharide ingredient of the sample was analyzed by ion chromatography (IC) (Dionex, Sunnyvale USA), 10 mg EPS-B3 was hydrolyzed with 3 M of trifluoroacetic acid (TFA) in a total volume of 1 mL at 110°C for 6 h. After the excess acid was discarded, 1 mL acid hydrolyzed sample (10 mg/L) was analyzed by IC using carbopac PA10 column (4 mm × 250 mm, 10 μm) (Dionex) with 10 mM NaOH at a 0.3 mL/min flow rate. Finally, the monosaccharide mixtures

(containing L-fucose, L-rhamnose, D-galactose, D-glucose, D-mannose and D-fructose) were used as standard sugars and the content of each component was analyzed according to the relative area of the peak.

### 2.6 FT-IR determination

Fourier transform infrared (FT-IR) spectral of the purified sample was analyzed by using FT-IR spectrum (Bruker ALPHA-T). The EPS-B3 was mixed with KBr powder (sample: KBr approximately 1:100) and compressed into pellet for FT-IR determination between wave numbers 450 $cm^{-1}$ and 4000 $cm^{-1}$.

### 2.7 Antioxidant property analysis of EPS-B3 and PSP

#### 2.7.1 DPPH free-radical scavenging activity

DPPH free-radical scavenging ability of PSP and EPS-B3 were investigated as previous research [23] with slight modifications [20]. Briefly, 250 μL ethanol solution including 0.02% DPPH was added to the different concentrations (0, 2, 4, 6, 8, and 10 mg/mL) of sample solutions (1 mL). After the mixed solution was kept away from light for 6 min, absorbance of each group at 517 nm was measured. All groups were performed in triplicate and the scavenging activities of all groups were calculated by the formula followed:

DPPH free-radical scavenging activity (%) = $(A_c + A_b - A_s) / A_c \times 100$

Where $A_s$ was the absorbance of experimental group; $A_c$ was the absorbance of the group without samples; $A_b$ was the absorbance of the group without DPPH solution.

#### 2.7.2 Superoxide radical scavenging ability

The superoxide radical scavenging activities of samples were estimated by monitoring the inhibition of pyrogallol autoxidation as previous method [24]. 1 mL sample at different concentrations (0, 2, 4, 6, 8 and 10 mg/mL) was added into the test tube which contains 4.5 mL PBS (0.05 M, pH 8.2) preheated to 25°C. Afterward, pyrogallol solution (0.4 mL, 45 mmol/L) was added into the mixture and test tube was incubated (25°C, 15 min) in water bath. Finally, 3mL HCl solution (8 M) was added to interrupt the reaction and the absorbance of solutions at 320nm was analyzed. The superoxide radical scavenging activity could be calculated by the formula followed:
scavenging effect (%) = $(A_0 - A_s)/ A_0 \times 100\%$

Where $A_0$ was the absorbance of group without the sample, while $A_s$ was the absorbance of the experimental group.

#### 2.7.3 Hydroxyl radical scavenging ability

The ·OH scavenging effect was analyzed via the Fenton's reaction [20, 25]. Firstly, the mixture of 1, 10-phenanthroline (1.0 mL, 0.75 mM), $FeSO_4 \cdot 7H_2O$ (1.0 mL, 0.75 mM), PBS buffer (2 mL, pH 7.4) and various concentrations (0, 2, 4, 6, 8, 10 mg/mL) of samples solution (1.0 mL) was generated. Before the reactions were incubated (37°C, 90 min), 1.0 mL $H_2O_2$ (0.01% v/v) was added into solutions to start the interaction. The absorbance of all groups at 510 nm was recorded. The ·OH scavenging effect was analyzed as follows:

Scavenging effect (%) = $[(A_s - A_0)/ (A_b - A_0)] \times 100$

Where $A_s$ was the absorbance of experimental group; $A_0$ was the absorbance of group containing $H_2O_2$ without samples; $A_b$ was the absorbance of group without $H_2O_2$.

### 2.7.4 Ferric reducing activity

The Ferric reducing abilities of EPS-B3 and PSP were assessed by previous modified method [20]. Briefly, samples (1 mL) at different concentrations (0, 2, 4, 6, 8, and 10 mg/mL) were added into 5 mL PBS (0.2 M, pH 6.6) which contained $K_3Fe(CN)_6$ (5%, w/v) and incubated (50°C, 20 min) in water bath. Next, 2.5 mL TFA (10%, w/v) was added to interrupt the reaction and the mixture was centrifuged at 1200 *g* for 10 min. Then, 3.0 mL $H_2O$ containing $FeCl_3$ (0.0167%, w/v) were added into the supernatant (2.5 mL) and incubated (50°C, 10 min). The absorbance of the mixture was measured at 700 nm. The equal volume of $H_2O$ instead of polysaccharide solution was used as control. The absorbance of reaction solution at 700 nm was positively correlated with the Ferric reducing power.

### 2.8 Determination of antitumor activity

### 2.8.1 Determination of antitumor activity *in vitro*

EPS-B3 and PSP at various concentrations (2.5-80 μg/mL) were incubated with S180 tumor cells, respectively. The blank group was treated with only the CCK8 solution without either polysaccharides or culture medium. The control group was treated with the CCK8 solution in culture medium without polysaccharides. After incubated in an incubator (37°C, 4 h), the absorbance of mixture at 450 nm was measured. The inhibition rate for S180 tumor cells was calculated as follows:

The inhibition rate (%) = $(A_c\text{-}A_e) / (A_c\text{-}A_0) \times 100$

Where $A_e$ signified the absorbance of experimental group; $A_c$ signified the absorption of blank group; $A_0$ signified the absorbance of control group.

### 2.8.2 Determination of antitumor activity *in vivo*

The tumor-bearing mouse model was established by injecting 0.2 mL PBS buffer containing $4 \times 10^5$ S180 cells into the right armpit of the mice. 7 days later model was constructed. The mice were divided randomly into four groups: a control group (PBS), a group of PSP and two groups for EPS-B3 at dosages of 100, 300 mg/kg, respectively. The mice were treated with above solution through single intragastrical administration every day in 24 days.

To monitor growth of tumors, the length and width of the tumor in armpit of S180 tumor-bearing mice were accurately measured every 4 days. The volumes and inhibition rate (TIR) of tumor were analyzed and plotted as formula follows:

Tumor volume = (Tumor length × tumor width × tumor height)/2

TIR (%) = (M−NM) ×100

Where M signified the percentage of tumor weight to mouse weight in control group; and N signified the percentage of tumor weight to mouse weight in the PSP or EPS-B3 treatment groups

### 2.9 Determination anti-polysaccharide antibody titers

Antibody titers of anti-PSP and anti-EPS-B3 in serum of S180 tumor-bearing mice were detected by ELISA. 2.5 μg/mL (100 μL/well) of PSP and EPS-B3 in 0.05 M $Na_2CO_3$ (pH 9.6) buffer was added into ELISA plates and incubated at 4°C overnight. 250 μL/well wash buffer (PBS including 0.1% Tween 20) was used to wash plates for four times and 200 μL/well PBS (containing 1% BSA) was used to block plates at 37°C for 2 h. 100 μL serum dilutions from 1:100 to 1:102400 in PBS (containing 0.05% Tween 20, pH 7.4) were added into the plates and incubated at 37°C for 2 h. 250 μL/well wash buffer was used to wash plates for four times again. 100 μL/well PBS containing Horseradish peroxidase-conjugated polyclonal goat (HRP-Goat) anti-mouse IgG (1:3000, 0.05% Tween 20) were added into the plates and

incubated at 37°C for 1 h. After the plates were washed as mentioned above, 100μL/well 3, 3, 5, 5-tetramethyl benzidine (TMB, Thermo fisher) was added and incubated at 25°C for 15 min in the dark. 100 μL $H_3PO_4$ (1 mol/L) was added to interrupt the reaction, and absorbance of the mixture at 450 nm was measured. The absorbance values of test samples were matched into the linear regression curve of a calibrated control (reference serum) to analyze the titers.

# 3. Results And Discussion

### 3.1 Morphological Analysis of Endophytic strains from Polygonatum

Six rhizobium endophytic strains isolated from the medicinal plant *P. sibiricum* were incubated at 37°C for 48h (**Table S1**). The colony with maximum EPS production (~1.7 g/L, about 67.1% of the total sugar content) strain B3 was round, small, moist, adhesive drawing and opaque (**Table S1**). And it was a gram-negative bacterium, which was positive for D-glucose, citrate salt, malonate, and etc, but negative for arabinose, $H_2S$, catalase, urease, and so on (**Table S2**). Strain B3 was identified as *Bacillus sp.* by combining with the bacterial morphologic, physiological and biochemical features and the phylogenetic analysis results **(Fig.1).***Bacillus sp.* is a kind of microbial species producing EPS with excellently physicochemical and biological properties [6, 20, 26].

### 3.2 Characterization of the Molecular weight (Mw) and the monosaccharide composition of EPS-B3

The exopolysaccharide of *Bacillus sp.* B3 (EPS-B3) was purified with the DEAE cellulose column and Sephacryl-300 column as displayed in **Fig.S1**. The main fraction with 88.9% of the total sugar content was obtained. The homogeneity (Mw/Mn) and average molecular weights of EPS-B3 were assessed by SEC-MALLS. The peak shape in **Fig.S2** has the characteristics of single, narrow, and symmetrical, indicating that EPS-B3 was a homogenized exopolysaccharide. As shown in **Table 1**, the EPS-B3 was a sugar including 103 monosaccharides with the Mw value $1.863\times10^4$ Da. And the distribution coefficient (*M*w/*M*n) of EPS-B3 was 1.24, suggesting that EPS-B3 has a small coefficient dispersion.

The components of EPS-B3 were analyzed and compared with standard monosaccharides by Ion chromatogram. As results shown in **Fig.2,** the EPS-B3 was composed of galactose, glucose, mannose with a molecular ratio of 15:3:1, respectively, which suggested that monosaccharide composition of EPS-B3 was relatively simple. It was interesting that the monosaccharide composition of EPS-B3 was totally different from that of PSP, which consists of five different monosaccharides [3, 27].

### 3.3 The feature peak analysis of EPS-B3

FT-IR spectroscopy was used to measure feature peaks and glucosidic bonds of EPS-B3 (**Fig.3**). The sample had a broad peak at about 3400 $cm^{-1}$, indicating that EPS-B3 showed the polysaccharide characteristic which was identified as the O-H stretching vibration in the sugar ring [28, 29] and the weak peak around 2930 $cm^{-1}$ was attributed to the stretch vibration of C–H bond [28]. The spectrum at around 1650 $cm^{-1}$ was the absorption of C=O bond and attributed to the bound water [30]. Some peaks between 1400 and 1000 $cm^{-1}$ also stand for the characteristics of polysaccharides, such as C-O-C glycosidic bonds and C-O-H link bonds of the pyranose unit are symbol of polysaccharide [20, 31]. Feature peak at 966.48 $cm^{-1}$, 814.33 $cm^{-1}$ and 537.42 $cm^{-1}$ were attributed to the pyranose units. Regarding the spectrum, significant differences were observed between PSP (**Fig.S3**) and EPS-B3, which indicated that EPS-B3 was a different kind of polysaccharide from PSP.

### 3.4 The comparative analysis of antioxidant activities between EPS-B3 and PSP

Antioxidants could effectively delay the development of degenerative disease and ageing by reducing the oxidation free radicals [32]. Herein, DPPH radical scavenging activity, $O_2^{-}\cdot$ scavenging assay, ·OH quenching assay and Ferric reducing

power are used to analyze the antioxidant activities of samples. The comparison of the antioxidant activity of the purified EPS-B3 and the PSP was shown in **Fig.4**.

In the DPPH and superoxide radical ($O_2^{-}\cdot$) assay, EPS-B3 could scavenge DPPH· or $O_2^{-}\cdot$ in a concentration-dependent manner from 2 to 10 mg/mL. The DPPH and $O_2^{-}\cdot$ scavenging ability of EPS-B3 were 39.0 ± 3.21% and 40.1 ± 4.55% at the concentration of 10.0 mg/mL, which were similar with PSP (39.5 ± 3.45% and 39.3 ± 5.21% respectively) **(Fig.4A, B).** In **Fig.4C**, the EPS-B3 could also scavenge ·OH and its scavenging ability increases with increasing EPS-B3 concentration, but its increase rate was slower than that of PSP. The maximum scavenging rate of EPS-B3 was 46.9 ± 4.41% at 8 mg/mL, lower than PSP (66.1 ± 6.12% at 8 mg/mL). Besides, it is interesting that EPS-B3 exhibited higher reducing power than PSP against the Ferric reagents (**Fig.4D**). The maximum reducing power of EPS-B3 (0.56) was observed at the concentration of 8 mg/mL, which is higher than that of PSP (0.35) at 10 mg/mL. The reducing power was a significant indicator of the antioxidant property [33].

Overall, the antioxidant activity of EPS-B3 is relatively poor in its natural neutral form [3]. The chemically modified or degraded polysaccharides exhibited stronger biological activities [3, 27], so further work could be performed to improve the antioxidant activity of EPS-B3.

### 3.5 Analysis of the antitumor activities

It was believed that PSP showed inhibition for the proliferation of cancer cell as a potential candidate for anti-tumor therapy [11, 12]. In this study, S180 tumor cells were exposed to different concentrations (2.5, 5, 10, 20, 40, and 80 μg/mL) of EPS-B3 or PSP to investigate the tumor inhibition effect *in vitro*. As shown in **Fig. 5A**, EPS-B3 can inhibit the growth of S180 tumor cells and the highest inhibition rate was 32.5% (40 μg/mL of EPS-B3), which was a little lower than PSP (48.5% inhibition at 40 μg/mL).

To measure the tumor inhibition effect *in vivo*, tumor bearing mouse model was established using S180 cells and treated with EPS-B3 or PSP. As shown in **Fig.5B**, the tumor weights in PSP group and EPS-B3 group were all significantly declined from the 8th day, compared with that in PBS group. In the same concentration (100 mg/kg), the inhibitory rate of PSP was higher than EPS-B3, while high concentration (300 mg/kg) of EPS-B3 exhibited stronger inhibition. This indicates that EPS-B3 possesses notable anti-tumor effects against tumors in mice. The antibody titers of anti-PSP and anti-EPS-B3 polysaccharides in the serum of treated mice were detected by ELISA. The results showed that PSP and EPS-B3 can significantly stimulate the mice to produce corresponding anti-polysaccharide antibodies (**Fig.5C**). The PSP was found to stimulate autophagy and inhibit the proliferation of tumor [12] mediated by TLR4-MAPK/NF-κB signaling pathways [11], which was affected by many extracellular polysaccharide [16]. However the molecular mechanism of EPS-B3 for tumor inhibition needs further exploration.

## 4. Conclusion

In this study, the exopolysaccharide produced by the endophytic bacterium *Bacillus sp.* B3 isolated from medicinal plant *P. sibiricum* was purified and characterized with an average Mw value $1.863\times10^4$ Da (103 monosaccharides). EPS-B3 was composed of galactose, glucose, mannose with a molecular ratio of 15:3:1, which was different from *P. sibiricum* polysaccharide (PSP). Additionally, EPS-B3 could scavenge DPPH·, $O_2^{-}\cdot$ and ·OH in a concentration-dependent manner as PSP. Afterward, EPS-B3 also showed significant activity in inhibiting the growth of tumor in S180 cells and tumor-bearing mice. These results indicate the potential of EPS-B3 for the clinical drug or functional food application, although the functional chemical optimization and the exploration of the molecular mechanism are further required.

# Abbreviations

PSP: *P. sibiricum* polysaccharide; TCM: Traditional Chinese medicine; ·OH: Hydroxyl radical; DPPH: α-Diphenyl-β-picrylhydrazyl; $O_2^{-}$·: Superoxide radical; DMSO: dimethyl sulfoxide.

# Declarations


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### Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

### Code availability

Not applicable.

### Ethics approval

This work was approved by Shandong University.

### Consent for publication

All authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work is submitted

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# Tables

**Table 1 Main components and molecular weight of EPS-B3**

| Name | Type | Start - End (g/mol) | Limits (%) | Cumulative(%) | Moments (g/mol) | The number of Monosaccharide |
|---|---|---|---|---|---|---|
| Range 1 | molar mass | 8492.1 - 79482.9 | 0.0 - 90.6 | 90.6 | $Mn=1.503\times10^4$<br>$Mw=1.863\times10^4$<br>$Mz=2.560\times10^4$ | 103 |

# Figures

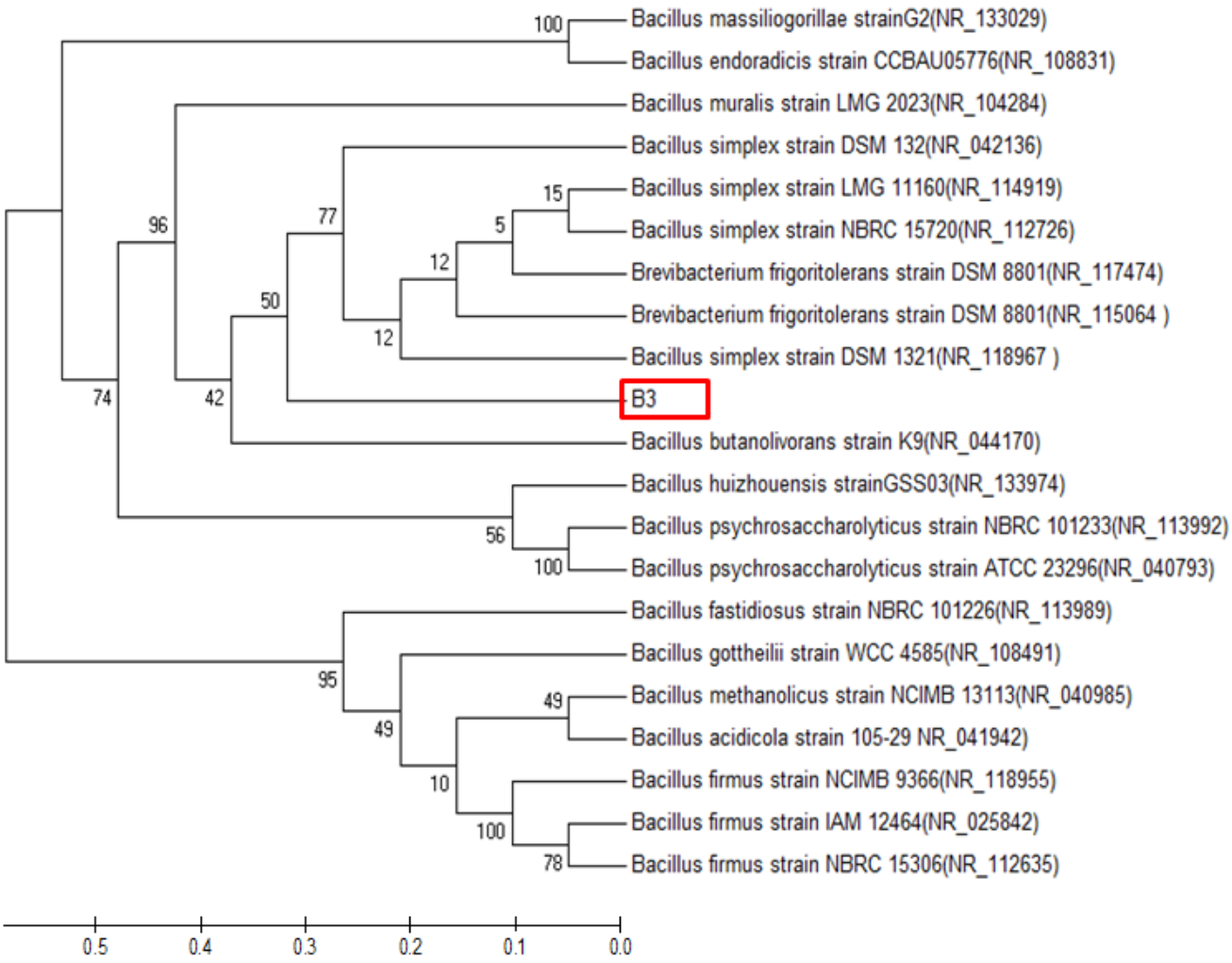



Figure 1

Phylogenetic analysis based on the 16S rDNA genes The numbers at branches represent bootstrap values (above 50%, 1000 replicates) of the neighbor-joining analysis. Bar 0.1 substitutions per nucleotide position.

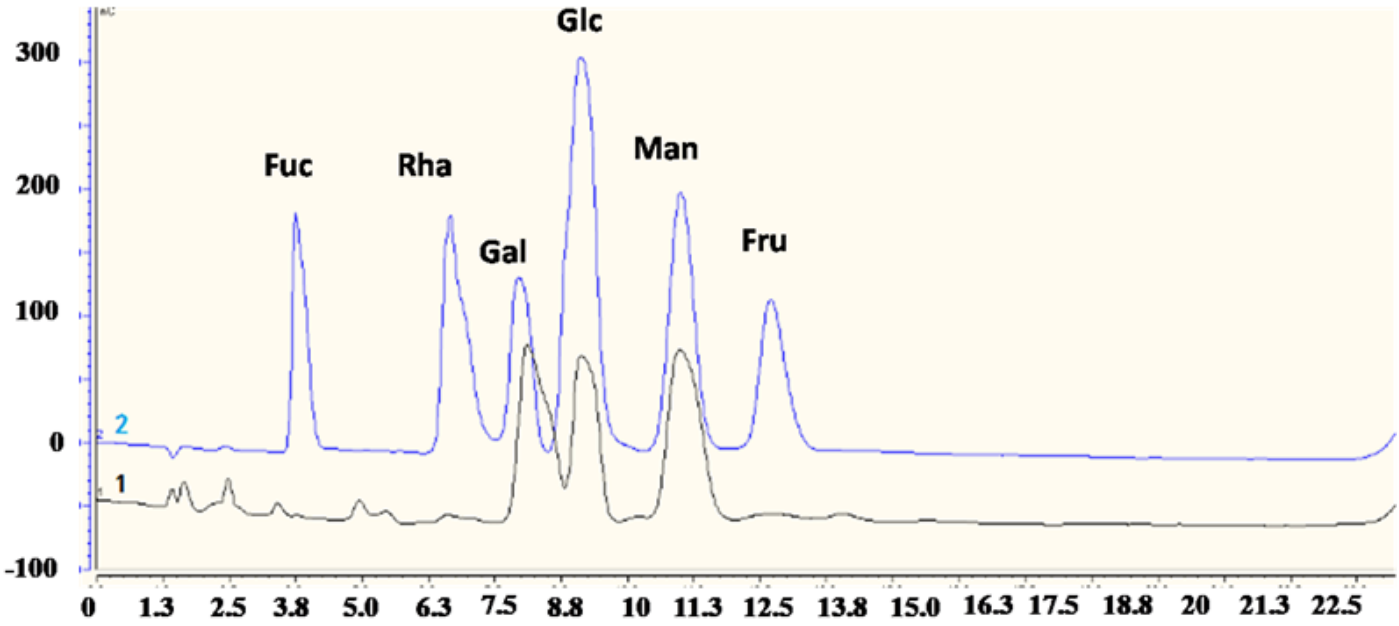


Figure 2

Ion chromatogram analysis of EPS-B3 monosaccharide components Curve 1 is an ion chromatogram analysis of EPS-B3; Curve 2 is an ion chromatogram of six monosaccharides at a concentration of 2 mg/L. Rha: L-rhamnose; Fuc, L-fucose; Man: D-mannose; Gal: D-galactose; Glc: D-glucose; Fru: D-Fructose.

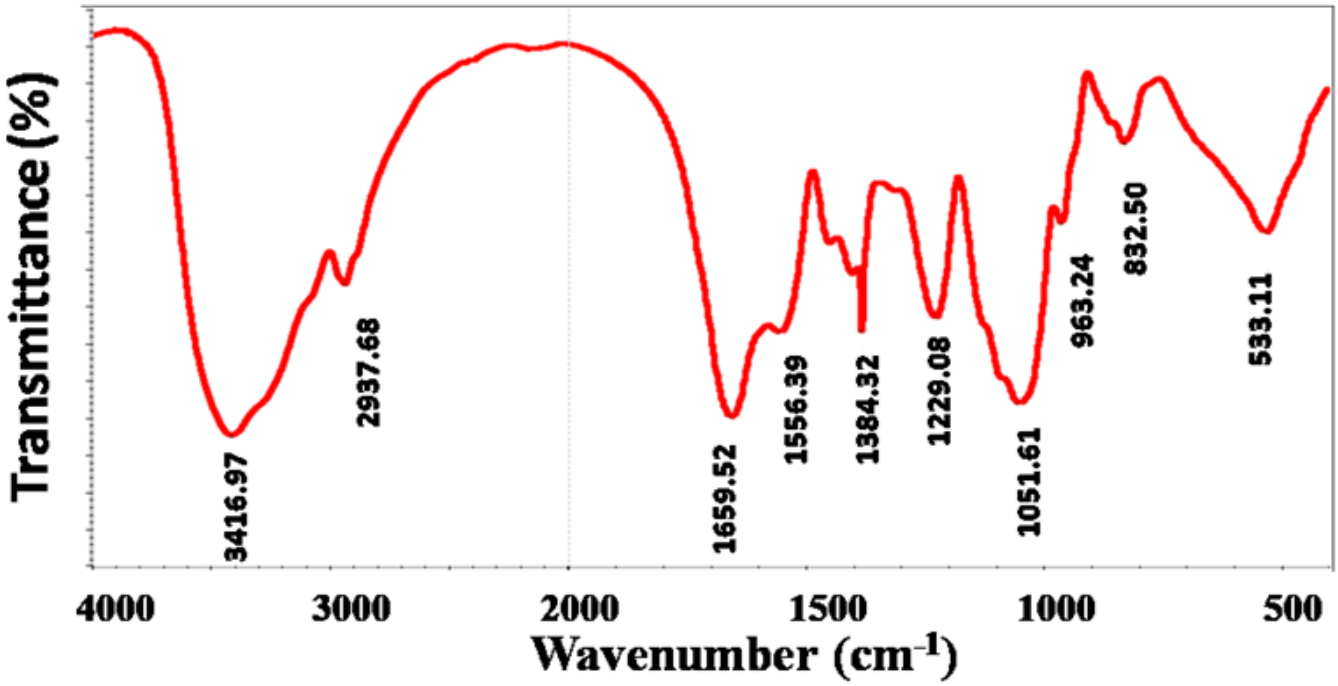
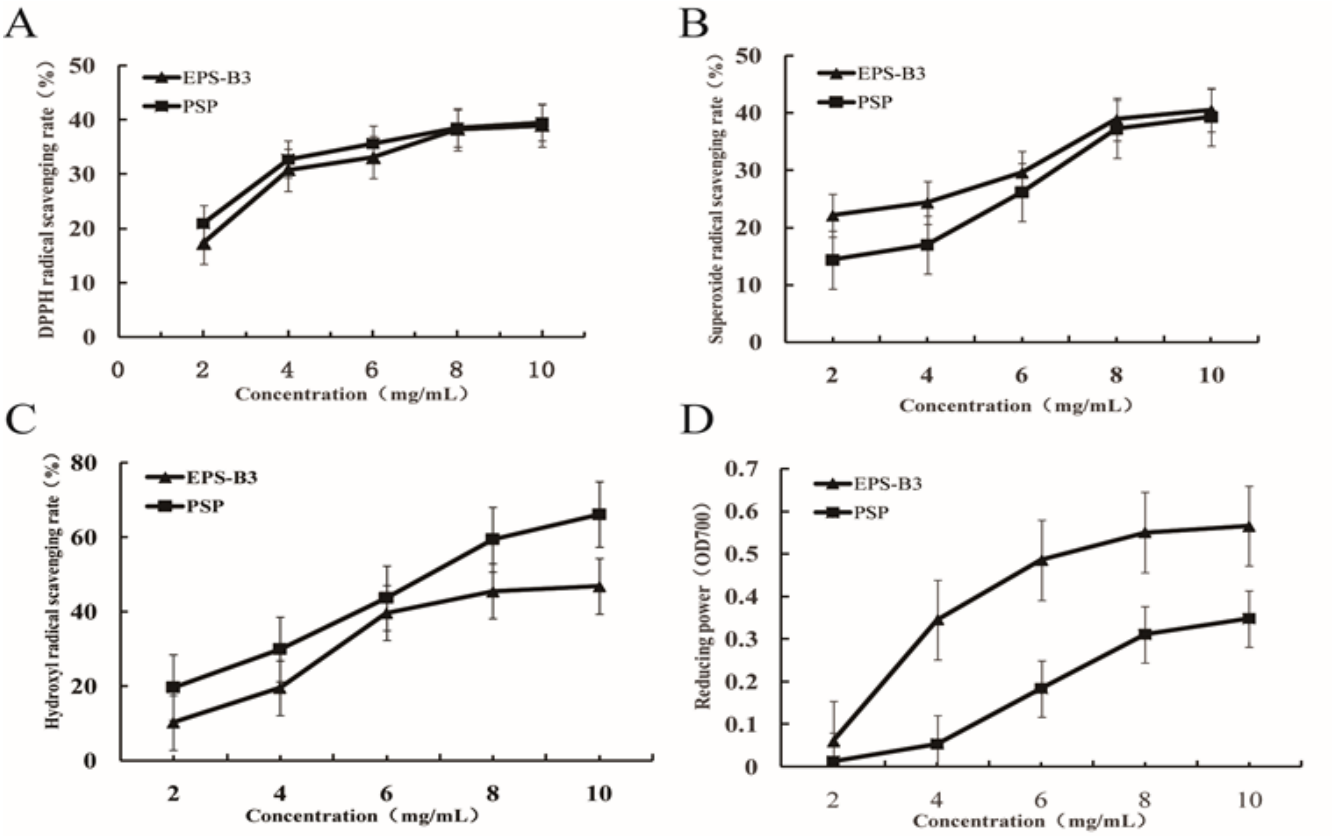



Figure 3

FT-IR spectrum of EPS-B3 Fourier-transform infrared (FT-IR) spectrum (450 cm−1 and 4000 cm-1) was used to measure the characteristic groups of EPS-B3.




## Figure 4

Antioxidant activity of EPS-B3 (A) DPPH· scavenging ability; (B) OH scavenging ability; (C) O2− scavenging ability; (D) Ferric reducing power.

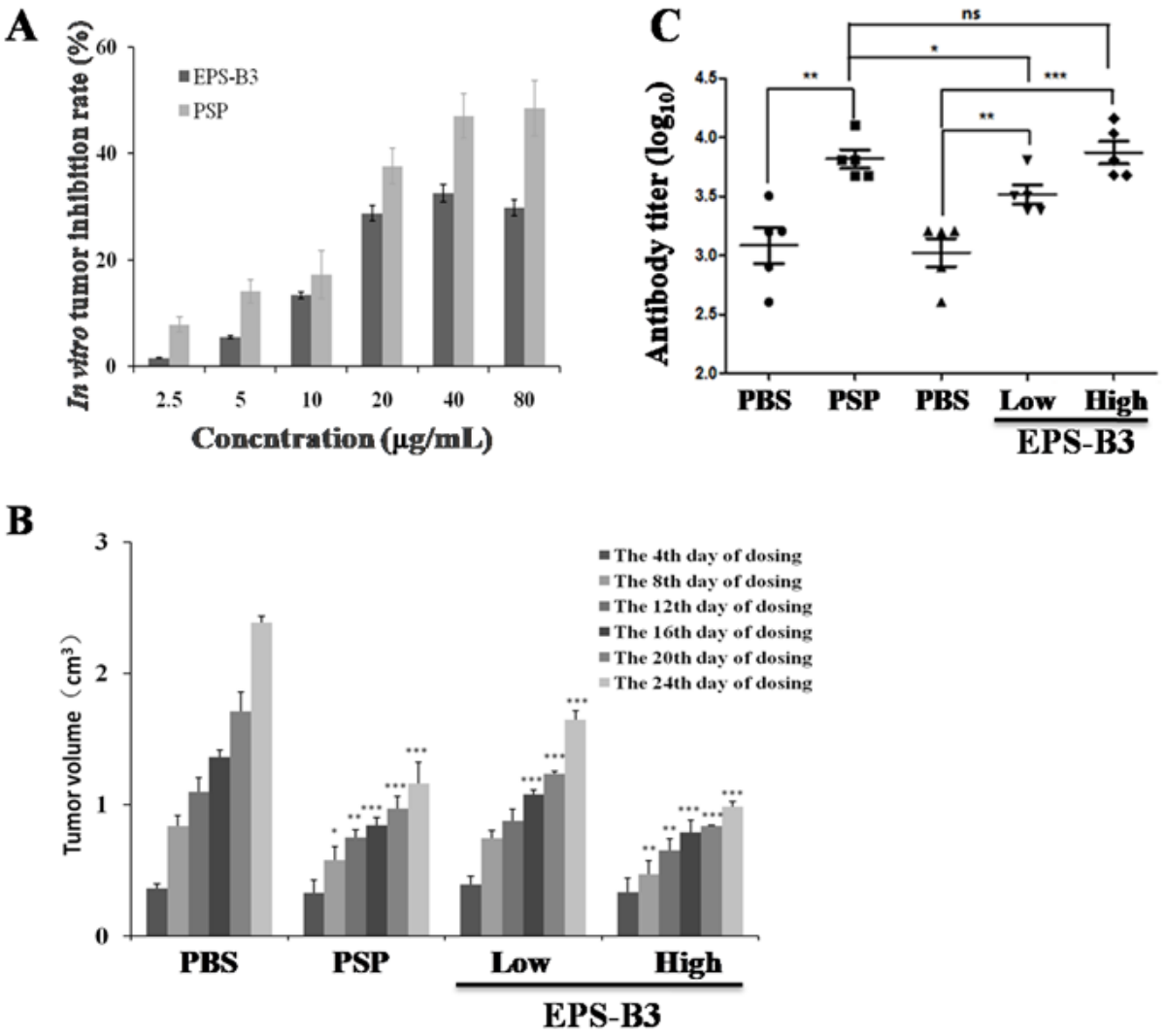



Figure 5

The effects of EPS-B3 and PSP on tumors in vitro or in vivo. (A) Determination of antitumor effects of PSP and EPS-B3 in vitro. (B) Determination of tumor inhibition ability in vivo of PSP and EPS-B3. The PBS, PSP and EPS-B3 in vivo tumor suppressor significance analysis plot, error bars indicate the variance ± SD, the difference between each two groups is expressed by p-value, * p <0.05, ** p <0.01, *** p<0.001. (C) Detection of anti-polysaccharide antibody titer.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportinginformation2021.05.31.docx