

Characterization and Biological Activity of a Novel Exopolysaccharide Produced By *Pediococcus Pentosaceus* SSC-12 From Silage

Yang Fan

South China Agricultural University

Xinqin Li

South China Agricultural University

Rong Tian

South China Agricultural University

Ruxue Tang

South China Agricultural University

Jianguo Zhang (✉ zhangjg@scau.edu.cn)

South China Agricultural University <https://orcid.org/0000-0003-0126-0069>

Research

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Abstract

Background: The exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) are widely used in various fields because of their safety and various biological activities. In this study, we extracted and characterized the composition as well as antioxidant and antibacterial activities of EPS from *Pediococcus pentosaceus* SSC-12 isolated from the silage.

Results: The LAB strain SSC-12 was screened and identified as *Pediococcus pentosaceus*, based upon 16S rDNA gene sequencing and Neighbor Joining (NJ) phylogenetic analysis. The analysis of the EPS production kinetics results of SSC-12 showed that the EPS production reached the maximum at 20 h of culture. High-performance anion exchange chromatography (HPAEC) analysis showed that the EPS produced by SSC-12 was a heteropolysaccharide comprising glucose (42.6 %), mannose (28.9 %), galactose (16.2 %), arabinose (9.4 %) and rhamnose (2.9 %). The EPS had good antioxidant activity, especially hydroxyl radical scavenging activity. When the concentration of the EPS produced by SSC-12 (SSC-12 EPS) was 10 mg/mL, its 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability, hydroxyl radical scavenging ability, superoxide scavenging ability, and reduction ability were 77.4 %, 97.5 %, 77.5 % and 1.3, respectively. At 10 mg/mL SSC-12 EPS completely killed *Staphylococcus aureus* GDMCC 1.1220 and substantially inhibited the growth of *Salmonella enterica* subsp. *enterica* GDMCC 1.345; however, it had a weak inhibitory effect on *Listeria monocytogenes* GDMCC 1.347.

Conclusions: Due to its strong antioxidant and antibacterial properties, EPS produced by LAB strain SSC-12 have potential application as a bioactive product in the feed, food, and pharmaceutical industries.

Background

Exopolysaccharide (EPS) is a carbohydrate secreted by microorganisms during growth and metabolism. It is produced by several types of microorganisms that produce EPS, such as *Lactobacillus* [1], *Bacillus* [2], *Bifidobacterium* [3] and *Leuconostoc* [4]. Among them, lactic acid bacteria (LAB) are generally recognized as safe (GRAS) and food-grade microorganisms. Several studies have shown that most LAB, such as *Lactobacillus Kefiranofaciens* [5], *L. plantarum* [1], *L. confusus* [6] and *L. acidophilus* [7], can produce EPS.

EPS can be divided into homopolysaccharides (HoPSs) and heteropolysaccharides (HePSs) based on its composition [8]. HoPSs are composed of repeating units of monosaccharide—primarily glucose, galactose, or rhamnose [9]; contrarily, HePSs are polysaccharides comprising three or more monosaccharides, such as arabinose, rhamnose, galactose, and mannose [10].

The EPS produced by different LAB have different bioactive functions. It has been identified that EPS produced by LAB has immunomodulatory properties, antioxidant activity, antibacterial activity, anti-tumor, anti-cancer, hypoglycemic and other biological properties [11]. EPS produced by LAB could enhance cellular defense mechanisms and prevent diseases by reducing reactive oxygen species and free radicals [12]. Therefore, it is a safe and harmless biologically active substance that has potential antioxidant

property. Moreover, EPS produced by LAB could inhibit the growth of harmful microorganisms and even kill them, thereby preventing the occurrence of microbial infections [5]. Therefore, EPS can be used as an alternative to antibiotics to reduce drug resistance. With the increasing health hazards caused by food spoilage or bacterial infections, the search for EPS with antioxidant and antibacterial activities has attracted the attention of researchers.

Currently, the sources of EPS-producing LAB are relatively limited; hence, the identification of new sources and the determination of their functional activities have become the focus of several studies. Silage is a kind of roughage obtained through the fermentation of LAB under anaerobic conditions to inhibit the reproduction of various miscellaneous bacteria [13]. Silage is rich in a variety of LAB, and hence, it is potentially a good source of LAB. This study primarily aimed to screen out LAB with high EPS yield from silage, analyze the relationship between LAB growth and EPS production, and determine monosaccharide composition and biological activity of EPS produced. In this study, we not only identify a new source of EPS-producing LAB, but also provide theoretical support for EPS application in the feed, food, and pharmaceutical industries.

Results And Discussion

EPS-producing LAB and EPS yield

In this experiment, 22 EPS-producing LAB strains were screened out after the observation of colony morphology, Gram stain test, and EPS yield determination. As shown in Table 1, the EPS yield of 22 strains screened from silage ranged 30.5–276.6 mg/L. Of all the strains, 14 were considered as high-yield EPS strains (EPS yield > 100 mg/L), based on Smitinont [14]. SSC-12 exhibited the highest yield of crude EPS, producing 276.6 mg/L in De Man, Rugose, and Sharpe (MRS) broth; therefore, this strain was selected for further studies. The EPS yield of SSC-12 selected in this experiment was higher than that of *Pediococcus pentosaceus* F3 (99.53 mg/L) [15], but was similar to that produced by *P. pentosaceus* NR 042058.1 (263.6 mg/ L) [16]. The 16S rDNA results indicated that SSC-12 was closely related to *P. pentosaceus* (> 99 % identity); it clustered apart from other species of this genus, and thus, was identified as *P. pentosaceus* (Fig. 1). *Pediococcus pentosaceus* is a homofermentative LAB with physical characteristics and biological functions, which can be used in the production of fermented food [17]. Previously, it has been demonstrated that the EPS produced by *P. pentosaceus* had good antioxidant activity and could be used as food preservative and therapeutic agent [15].

Table 1 The sources and EPS yield of 22 suspecting LAB strains isolated from silage.

Source silage	Strains	Yield (mg/L in MRS broth)
Napier grass	SPP-1	59.3 ± 2.9 ^l
	SPP-2	67.9 ± 4.9 ^{kl}
	SPP-3	62.4 ± 5.1 ^l
	SPP-5	87.5 ± 6.5 ^{jk}
	SPP-6	202.5 ± 17.9 ^{bc}
	SSP-9	172.6 ± 27.2 ^{def}
Corn	SSC-1	51.0 ± 5.0 ^l
	SSC-3	200.5 ± 16.3 ^{bc}
	SSC-12	276.6 ± 12.1 ^a
	SSC-16	110.6 ± 6.3 ⁱ
	SSC-23	212.3 ± 10.5 ^b
	SSC-48	143.7 ± 16.7 ^{gh}
Orchardgrass	SDJ-3	30.5 ± 5.2 ^m
	SDJ-6	188.5 ± 9.0 ^{cd}
	SDJ-8	203.7 ± 8.2 ^{bc}
	SDJ-16	131.1 ± 10.0 ^h
Stylo	SS-9	90.5 ± 4.9 ^j
	SS-17	181.7 ± 10.2 ^{cde}
Soybean	SGM-9	202.9 ± 11.5 ^{bc}
	SGM-16	164.8 ± 15.8 ^{ef}
	SGM-18	176.8 ± 19.9 ^{de}
	SGM-20	153.2 ± 7.3 ^{fg}

Data with different letters within a column were significantly different at P < 0.05.

The EPS yield in the table was mean \pm SD of three replicates.

Strain Growth and EPS Production

With increase in culture time, the number and EPS production of SSC-12 gradually increased and both reached the peak at 20 h of culture (Fig. 2). However, after 20 h, SSC-12 entered a decline stage, because its life activities slowed down, leading to a gradual decrease in its count and EPS production. SSC-12 had the strongest activity in the exponential phase because EPS was the secondary metabolite produced by bacterial activities, and EPS production was the highest at this time. SSC-12 exhibited fast growth during first 8 h of incubation. According to previous studies, *L. reuteri* SHA101 and *L. vaginalis* SHA110 reached maximum EPS production in 48 h [18] and *L. plantarum* WLPL04 in 24 h [1]. However, SSC-12 achieved maximum EPS production in 20 h, suggesting that SSC-12 would have a great application value.

Monosaccharide composition of EPS

High-performance anion exchange chromatography (HPAEC) determination showed that the EPS produced by SSC-12 (SSC-12 EPS) was a heteropolysaccharide. The total sugar content of SSC-12 EPS was 73.6 %, comprising glucose (42.6 %), mannose (28.9 %), galactose (16.2 %), arabinose (9.4 %), and rhamnose (2.9 %) (Table 2). According to the number of monosaccharide and the proportion of each monosaccharide, EPS produced by SSC-12 was different from that produced by *P. pentosaceus* as previously reported. For example, the EPS produced by *P. pentosaceus* M41 consisted of glucose (79.0 %), mannose (9.5 %), arabinose (6.2 %), and galactose (5.2 %) [19], and the EPS produced by *P. pentosaceus* DPS comprised glucose, mannose, and fructose in different ratios [20]. Therefore, SSC-12 produced a novel type of EPS.

Table 2
The monosaccharide composition of EPS produced by SSC-12.

Monosaccharide name	Molar ratio (%)
Glucose	42.7 \pm 0.28
Mannose	28.9 \pm 0.39
Galactose	16.3 \pm 0.06
Arabinose	9.4 \pm 0.36
Rhamnose	2.9 \pm 0.02
The values are represented as mean \pm SD (n=3).	

Antioxidant activity of EPS

Free radicals, such as reactive oxygen species, combine with biological macromolecules in the body to cause tissue damage and induce different diseases [21]. Certain EPSs can scavenge active oxygen free radicals *in vivo* and reduce the incidence of diseases. EPS from *L. kimchi* SR8 significantly improved the

liver index, serum superoxide dismutase activity, and the survival rate of mice [22]. Moreover, some EPSs have antioxidant properties and can slow aging and deterioration by fighting off excess free radicals *in vitro*; for example, EPS produced by *P. pentosaceus* has good antioxidant activity and could prolong the shelf life of bananas [15]. The EPS produced by LAB is a natural and safe antioxidant, which could have a good application prospect in food preservation and health product industry.

DPPH radicals can accept free electrons into stable molecules, thus attacking cells and causing lesions [23]. Hydroxyl free radical is the most active free radical, which could cause oxidative damage to neighboring biological molecules and induce diseases [24]. Superoxide free radicals cause severe tissue damage by inducing lipid peroxidation and oxidative damage [25]. Antioxidants provide electrons to scavenge free radicals through their reducing action, and a high reducing power indicates a strong antioxidant power.

In this study, the antioxidant activity of SSC-12 EPS was assessed in terms of its ability to inhibit the formation of free radicals and its reducing ability. The antioxidant capacity of SSC-12 EPS was concentration-dependent and the gap between them at the same concentration became smaller and smaller. At 10 mg/mL, the DPPH scavenging ability (77.4 %), hydroxyl radical scavenging ability (97.5 %), superoxide radical scavenging ability (77.5 %), and reducing ability (1.3) of SSC-12 EPS reached maximum values. Rajoka et al [18] observed lower values than that of SSC-12 EPS (56.72 %) at a concentration of 4 mg/mL, with the superoxide radical scavenging rate of 40.5 % and 25.5 % for the EPS produced by *L. reuteri* SHA101 and *L. vaginalis* SHA110, respectively. Seo et al [26] also evaluated the antioxidant capacity of an EPS produced by *L. plantarum* YML009 and reported a lower DPPH radical scavenging (7.24 %) and reduction (0.15) activities at 10 mg/mL, compared with those of SSC-12 EPS.

As shown in Fig. 3(c), the hydroxyl radical scavenging ability of SSC-12 EPS was higher than that of ascorbic acid, with 2 mg/ml of EPS (86.6 %) exhibiting roughly 1.4-times higher scavenging activity than ascorbic acid (63.7 %) at the same concentration. At low concentrations (1.0–4.0 mg/mL), the hydroxyl radical scavenging ability of SSC-12 EPS was substantially stronger than that of ascorbic acid. At 4 mg/mL, the hydroxyl radical scavenging ability of SSC-12 EPS (87.33 %) was also higher than the EPS produced by *L. helveticus* MB2-1 (56.30 %) [27]. However, the hydroxyl radical scavenging ability of SSC-12 EPS (95.30%) was similar to that of EPS produced by *L. kimchi* SR8 (96.58%) [22] at 8 mg/mL.

Our results indicated that EPS produced by SSC-12 had good antioxidant capacity and might serve as a good alternative to ascorbic acid. The degree of antioxidant capacity of EPS produced by different LAB differed, which may be attributed to the varying composition and structure, such as glycosidic linkages embodiment, functional group, and molecular weight [11]. For example, it has been reported that the EPS produced by *L. delbrueckii* ssp. *bulgaricus* SRFM-1 had more carboxyl functional groups than those produced by other LAB, which could provide an acidic environment to promote its hydrolysis and expose more hemiacetal hydroxyl groups for excellent antioxidant activity [28]. Moreover, low molecular weight of EPS indicates that few hemiacetal hydroxyl groups are exposed at the same mass concentration and

consequently high antioxidant activity [11]. The strong antioxidant activity of SSC-12 EPS might be due to the presence of hydroxyl groups and other functional groups.

Antibacterial ability

Pathogens can cause food to decay *in vitro*, while pathogenic bacteria in the gastrointestinal tract can cause gastrointestinal infections *in vivo* [11]. Antibiotics are widely used for the control of bacterial infections. However, as the phenomenon of drug resistance becomes a growing concern, the search for safe and effective antibacterial drugs has also become a focus for researchers. It has been reported that some EPSs from LAB have good antibacterial activity. For example, the EPS of *L. plantarum* HM47 isolated from human breast milk had a strong inhibitory effect on pathogenic *Escherichia coli* and *Salmonella typhimurium in vitro* [29]. The EPS of *L. fermentum* S1 isolated from traditional fermented Fuyuan pickle had good antibacterial activity against *E. coli* and *Staphylococcus aureus*, with the highest inhibition rates of 32 % and 43 %, respectively [30].

The absorbance of a bacterial liquid indicates its turbidity degree. The higher the absorbance, the more the number of bacteria, and the determination of absorbance facilitated the qualitative analysis of the antibacterial property of SSC-12 EPS in this study. The inhibition rate of SSC-12 EPS was quantitatively analyzed by measuring the number of harmful bacteria cultivated in the culture medium after adding SSC-12 EPS. The inhibitory effect on *Staphylococcus aureus* and *Salmonella enterica* subsp. *enterica* increased with increase in SSC-12 EPS concentration, but the inhibitory effect on *Listeria monocytogenes* was not significant (Fig. 4). Although the inhibitory effect of SSC-12 EPS on the three harmful bacteria was concentration-dependent, SSC-12 EPS had a considerable inhibitory effect on *S. aureus*, *Salmonella enterica* subsp. *Enterica*, and *Listeria monocytogenes* at 2, 6, and 8 mg/mL respectively. At 10 mg/mL, the inhibitory effect of SSC-12 EPS on *Staphylococcus aureus*, *Salmonella enterica* subsp. *Enterica*, and *Listeria monocytogenes* reached the maximum, which were 100 %, 71.9 % and 14.9 %, respectively. These results suggest that the SSC-12 EPS has the strongest inhibitory effect on *Staphylococcus aureus*, followed by *Salmonella enterica* subsp. *enterica*, and the worst inhibitory effect on *Listeria monocytogenes*. Liu et al [1] observed that the EPS produced by *L. plantarum* WLPL04 had good inhibitory effect on *Staphylococcus aureus* and *Listeria monocytogenes* and its inhibitory ability increased gradually with increasing concentrations of EPS. At 2 mg/ml, the inhibitory rate of EPS produced by *L. fermentum* S1 [30] against *Staphylococcus aureus* did not exceed 12 %, while SSC-12 EPS reached 17.8 % at the same concentration. When the EPS produced by *P. pentosaceus* M4 [19] was 5 mg/ml, its inhibitory effect on *Staphylococcus aureus* reached 56.5 %, which was similar to that of SSC-12 EPS.

Different types of EPS have varying inhibitory effects on harmful microorganisms. According to previous studies, the antibacterial mechanism of EPS might be attributable to the prevention of biofilm formation or destruction of membrane integrity and fluid soluble protein, which are mediated by signal molecules or sugar receptors [31, 32]. SSC-12 EPS had good antibacterial activity (Fig. 4), providing a theoretical basis for its application in feed production and clinical treatment. However, the associated mechanism is unclear and needs further exploration.

Conclusions

In this study, a novel SSC-12 EPS isolated from silage was identified as *P. pentosaceus*. SSC-12 had strong vitality and fast EPS production rate. EPS produced by *P. pentosaceus* SSC-12 was a heteropolysaccharide and consisted of glucose (42.6 %), mannose (28.9 %), galactose (16.2 %), arabinose (9.4 %), and rhamnose (2.9 %). The EPS had strong antioxidant ability and antibacterial ability. The present study identified a new strain of EPS-producing LAB, and the EPS showed good biological activity, which can potentially be applied in the feed, food, and pharmaceutical industries as well as in the development of new natural antibiotic substitutes.

Materials And Methods

Screening and identification of EPS-producing LAB

LAB was isolated from silage and purified by culturing on MRS agar medium under anaerobic condition for 24–48 h at 37 °C [1]. After morphological observation, a single colony, having milky white color, sticky surface, surrounding diffusion phenomenon, protruding round shape, and obvious viscosity when picked by inoculation ring [11], was suspected to be the EPS-producing LAB.

The above strains were inoculated into 50 ml MRS liquid medium and statically cultured at 37 °C for 24 h. Next, the broth was collected, centrifuged at 8000 r/min for 10 min, and the supernatant was collected, followed by the addition of 95 % ethanol (3×volume) and incubation at 4 °C overnight [1]. The mixture was centrifuged at 8000 r/min for 10 min, and the precipitate was collected and dissolved in distilled water, followed by dialysis for 3 days to obtain crude EPS solution [33]. The EPS production was measured by phenol sulfuric acid method, and the strains with the highest EPS production were identified.

The screened strains were purified and cultured for two generations to obtain bacterial suspension, and total DNA was extracted from cell precipitate using TIANamp Bacteria DNA Kit. The 16S rDNA was amplified with primer pair 27F / 1492R using polymerized chain reaction (PCR) procedure [34]. Purified PCR products purified from each strain were sequenced by ABI3730-XL DNA Analyzer, and we used the Blast (<http://www.ncbi.nlm.nih.gov/blast>) to compare the spliced sequence file with the data in the NCBI 16S database [5]. The MEGA-X software was used to construct phylogenetic evolutionary tree, and the species with more than 99% similarity were identified. The identified LAB was stored in MRS liquid medium with 20% glycerol at -80 °C [35].

Analysis of strain growth and EPS production

The identified strains were inoculated into MRS liquid medium and cultured for two generations. After the LAB was cultured for different periods, the number of LAB was counted by the plate counting method and EPS production was determined by using the phenol sulfuric acid method. The peak time for EPS production was determined, and the extraction and purification of EPS was carried out. The purification

steps of EPS were repeated to obtain EPS aqueous solution. The EPS solution was freeze-dried (Thermo Modulyo Freeze Dryer, Thermo Fisher Scientific, USA) at low temperature, and the flocs were stored at ambient temperature in a sealed state [35].

Determination of EPS monosaccharide composition

After the EPS samples were acid hydrolyzed, their monosaccharide composition was analyzed by high performance anion exchange chromatography (HPAEC) (DIONEX ICS-6000, USA) equipped with Dionex™ Carbopac™ PA-20 anion exchange chromatography column (3 × 150nm) and electrochemical detector. NaOH (5 mmol) was used as the mobile phase with a flow rate of 0.4 mL/min at 30 °C [36]. Established calibration curves of mannose, rhamnose, glucose, galactose, and arabinose were used for quantitative analysis [37].

Assessment of EPS antioxidant activity

DPPH radical scavenging ability

The DPPH radical scavenging ability of EPS was measured according to the method described by Rajoka et al [18], with minor modifications. The reaction solution consisted of 2.0 mL of water, 1.0 mL of EPS sample (1.0–10.0 mg/mL), and 0.4 mL of DPPH ethanol solution (0.5 mM). The mixture was agitated and incubated in the dark for 30 min, followed by the measurement of the absorbance at 517 nm. Different concentrations of ascorbic acid in same volume (1.0–10.0 mg/mL) were used as a positive control. Each treatment was carried out three times. The DPPH radical scavenging ability of EPS was calculated as follows (1):

$$\text{DPPH radical scavenging ability (\%)} = \left[1 - \frac{(A_{11} - A_{01})}{A_{10}} \right] \times 100 \quad (1)$$

where "A₁₁" is the absorbance of the sample, "A₀₁" is the absorbance of the control group, and "A₁₀" is the absorbance of the blank. Ethanol (0.4 mL) and sample solutions of different dilutions (1.0 mL) were used as the control, and DPPH radical-ethanol (0.4 mL) and water (1.0 mL) were used as the blank.

Hydroxyl radical scavenging ability

The hydroxyl radical scavenging ability of EPS was measured according to the method described by Xu et al [34] with few modifications. Briefly, 50 µL PBS solution (20 mM, pH 7.4), 25 µL 1,10-phenanthroline solution (12.5 mM), 25 µL FeSO₄ solution (2.5 mM), and 25 µL H₂O₂ solution (20 mM) were added consecutively in a test tube. Next, 100 µL EPS of different concentrations (1.0–10.0 mg/mL) was added to the mixture, followed by incubation at 37 °C for 1 h and measurement of absorbance at 536 nm. Different concentrations of ascorbic acid in equal volume (1.0–10.0 mg/mL) were used as positive control. The experiment was carried out in triplicate. The hydroxyl radical scavenging ability of EPS was calculated as follows (2):

$$\text{Hydroxyl radical scavenging ability (\%)} = \left[\frac{A_{\text{sample}} - A_{\text{black}}}{A - A_{\text{black}}} \right] \times 100 \quad (2)$$

where " A_{sample} " was the absorbance when the sample contains different concentrations of EPS, " A_{black} " was the absorbance of the sample when EPS and H_2O_2 were replaced by water, and " A " was the absorbance of the sample when EPS and H_2O_2 were replaced by water.

Superoxide radical scavenging activity

The superoxide radical scavenging activity of EPS was measured according to the method described by Xu et al [34]. The superoxide radical was generated in 50 μL of Tris-HCl buffer (pH 8.0, 150 mM) containing 25 μL of pyrogallol (1.50 mM, dissolved in 10 mM HCl) and 100 μL of EPS samples (1.0–10.0 mg/mL). Next, the mixture was incubated at 25 $^\circ\text{C}$ for 30 min and the absorbance was measured at 325 nm. Different concentrations of ascorbic acid in equal volumes (1.0–10.0 mg/mL) were used as a positive control. Each experiment was carried out in triplicate. The superoxide radical scavenging activity of EPS was calculated as follows (3):

$$\text{Superoxide radical scavenging activity (\%)} = \left[1 - \frac{(A_s - A_c)}{A_b - A_0} \right] \times 100 \quad (3)$$

where " A_s " is the absorbance of samples containing EPS and pyrogallic acid, " A_c " is the absorbance of samples containing EPS but were used 10 mM HCl was used instead of pyrogallic acid, " A_b " is the absorbance of deionized water instead of EPS but containing pyrogallic acid, " A_0 " is the absorbance of EPS sample and pyrogallic acid sample replaced with deionized water and 10 mM HCl, respectively.

Reducing ability

The reduction ability of EPS was measured according to the method of Rajoka et al [18]. A solution containing 1.5 mL sodium phosphate buffer (0.2 M, pH 7.3), 1.5 mL $\text{K}_3\text{Fe}(\text{CN})_6$ (1 %, w/v), then 1.5 mL EPS samples (1.0–10.0 mg/mL) was incubated at 50 $^\circ\text{C}$ for 25 min. After cooling, 1.5 ml trichloroacetic acid (12 %, w/v) was added, and the mixture was centrifuged at 6000 $\times g/\text{min}$ for 10 min. Next, 0.5 ml FeCl_3 (0.2 %, w/v) was added to the supernatant, and the absorbance was measured at 700 nm. Different concentrations of ascorbic acid in equal volume (1.0–10.0 mg/mL) were used as a positive control. The experiment was carried out in replicates. The reducing ability of EPS was calculated as follows (4):

$$\text{Reducing ability} = A_1 - A_0 \quad (4)$$

where " A_1 " is the absorbance of EPS sample, " A_0 " is the absorbance of FeCl_3 replaced by deionized water.

Measurements of EPS antibacterial ability

In this experiment, *Staphylococcus aureus* GDMCC 1.1220, *S. enteritidis subsp. enteritidis* GDMCC 1.345, *Listeria monocytogenes* GDMCC 1.347 were selected as indicator bacteria. These bacteria were

separately inoculated into LB broth medium and cultured at 37°C for 24 h with shaking, which was repeated twice to obtain the second-generation strain [1]. Next, the bacterial suspension was adjusted to an estimated concentration of 10^5 – 10^6 CFU/mL based on the absorbance at 600 nm. The purified EPS was diluted with deionized water into EPS solutions of different concentrations (1.0–10.0 mg/mL), followed by filtration and sterilization with a 0.45- μ M microporous filter. Next, 1 mL of the EPS solutions of different concentrations and 10 μ L of indicator bacteria solution were added to 1 mL of fresh LB broth medium and cultured for 24 h at 37 °C with shaking [5], with equal amount of sterile water used as a blank instead of EPS. The inhibition rate and bacterial count indicated the antibacterial activity of EPS. The experiment was carried out in triplicate. The formula for the inhibition rate is as follows (5):

$$\text{Inhibition rate (\%)} = \left[1 - \frac{\log A_{\text{EPS}}}{\log A_{\text{Blank}}} \right] \times 100 \quad (5)$$

where " A_{EPS} " is the number of colonies of EPS sample, " A_{Blank} " is the number of colonies of positive control.

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics 22 software for Windows (IBM Corp, New York, USA), and means were compared for significance by Duncan's multiple range method.

Abbreviations

EPS: exopolysaccharides; LAB: lactic acid bacteria; NJ: Neighbor Joining; HPAEC: High-performance anion exchange chromatography; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GRAS: generally recognized as safe; SSC-12 EPS: EPS produced by SSC-12; HoPSs: homopolysaccharides; HePSs: heteropolysaccharides; PCR: polymerized chain reaction.

Declarations

Acknowledgments

Not applicable.

Authors' contributions

Authors' contributions were introduced in order of name. FY was responsible for experimental design, experiment execution, data analysis, and writing (manuscript). XL performed the experiment and collected data. RT contributed to experiment execution and data analysis. RT was participated in the experiment operation. JZ contributed to experimental design and writing (review and editing).

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Availability of data and materials

All data generated or analysed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

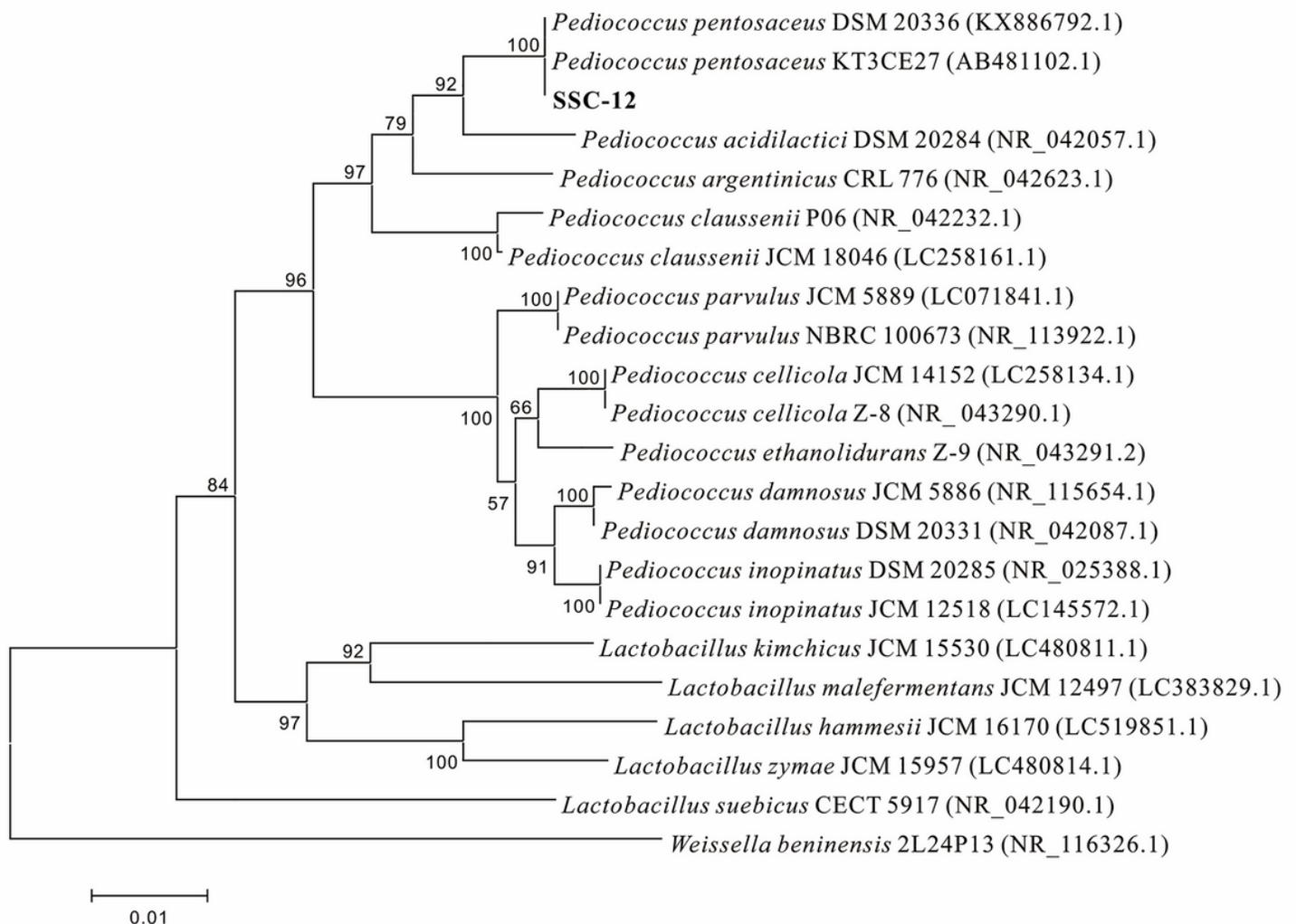


Figure 1

Neighbor-joining phylogeny of SSC-12 with closely related *Lactobacillus* strains according to 16S rRNA gene sequence.

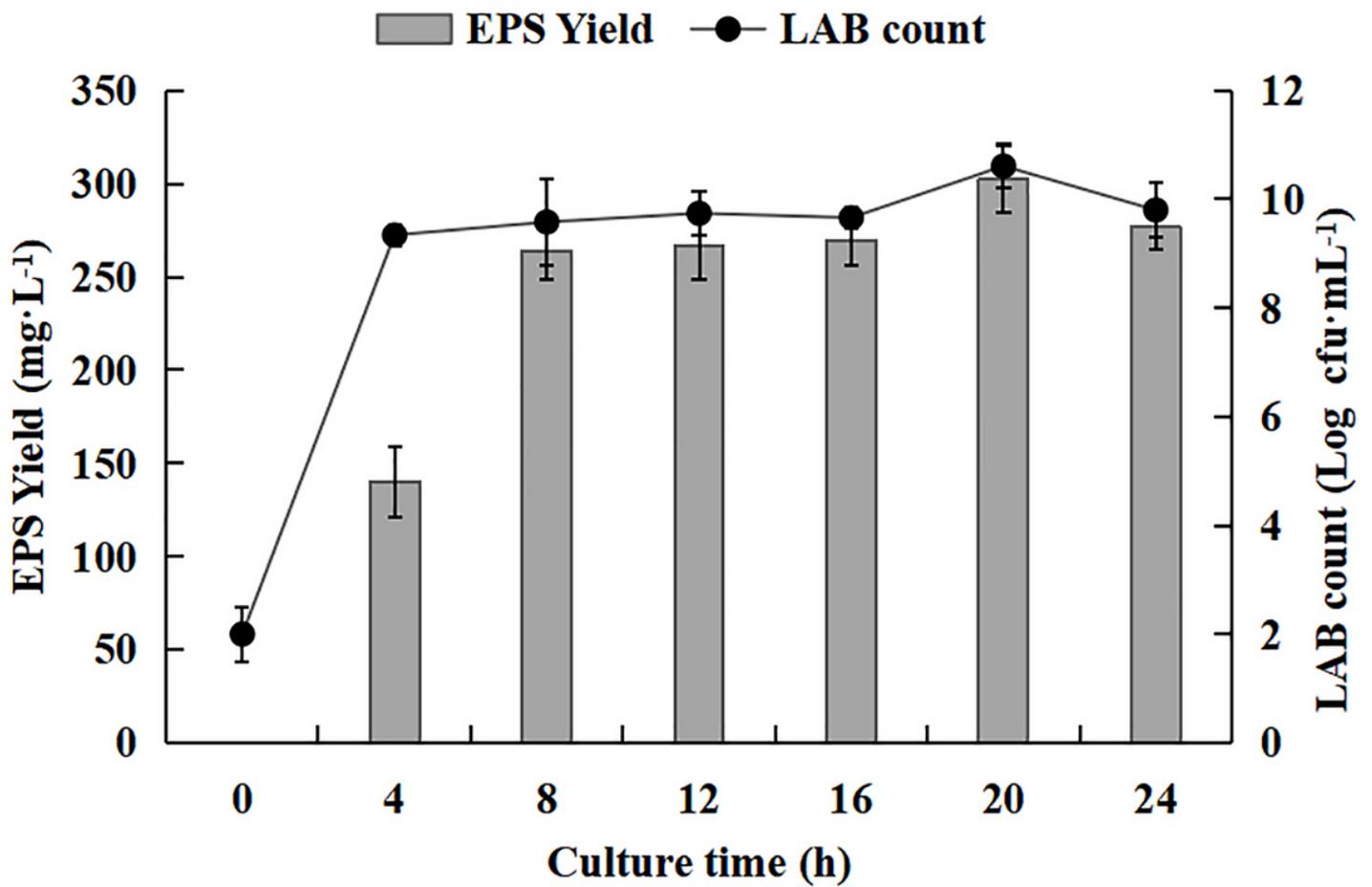


Figure 2

The dynamic relationship between the SSC-12 counts and its EPS yield. The values are expressed as mean \pm SD of three independent analyses.

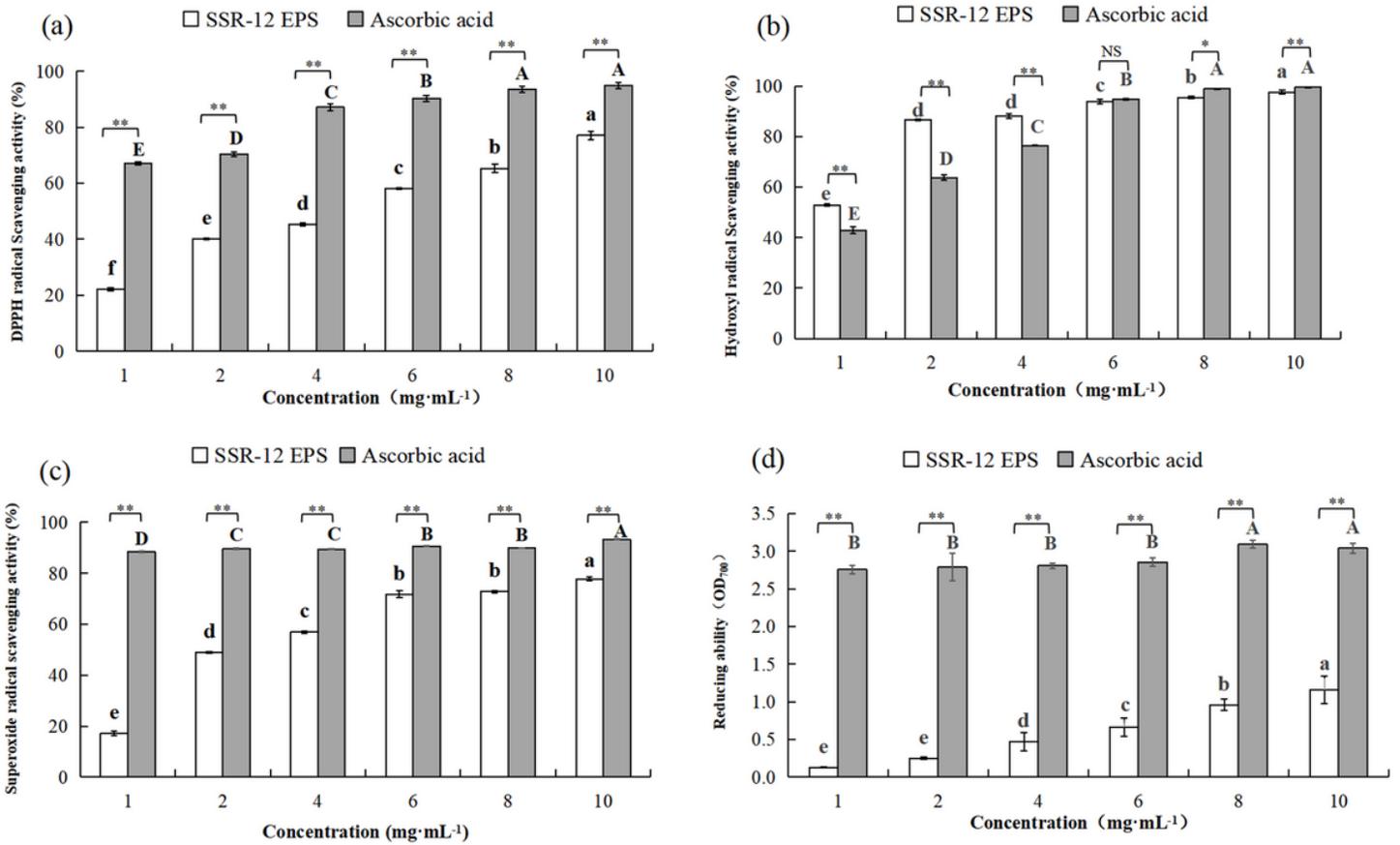


Figure 3

Antioxidant ability of SSC-12 EPS and standard antioxidant reagents ascorbic acid. SSC-12 EPS, EPS produced by SSC-12. Antioxidant ability includes DPPH radical scavenging activity (a), Hydroxyl radical scavenging activity (b), Superoxide radical scavenging activity (c) and Reducing power (d). The values are expressed as mean \pm SD of three independent analyses. Different lowercase letters indicate significant differences among SSC-12 EPS concentrations ($P < 0.05$). Different capital letters indicate significant differences among ascorbic acid concentrations ($P < 0.05$). The differences between SSC-12 EPS and ascorbic acid at the same concentration was represented by "** $P < 0.01$, * $P < 0.05$, NS $P > 0.05$ ".

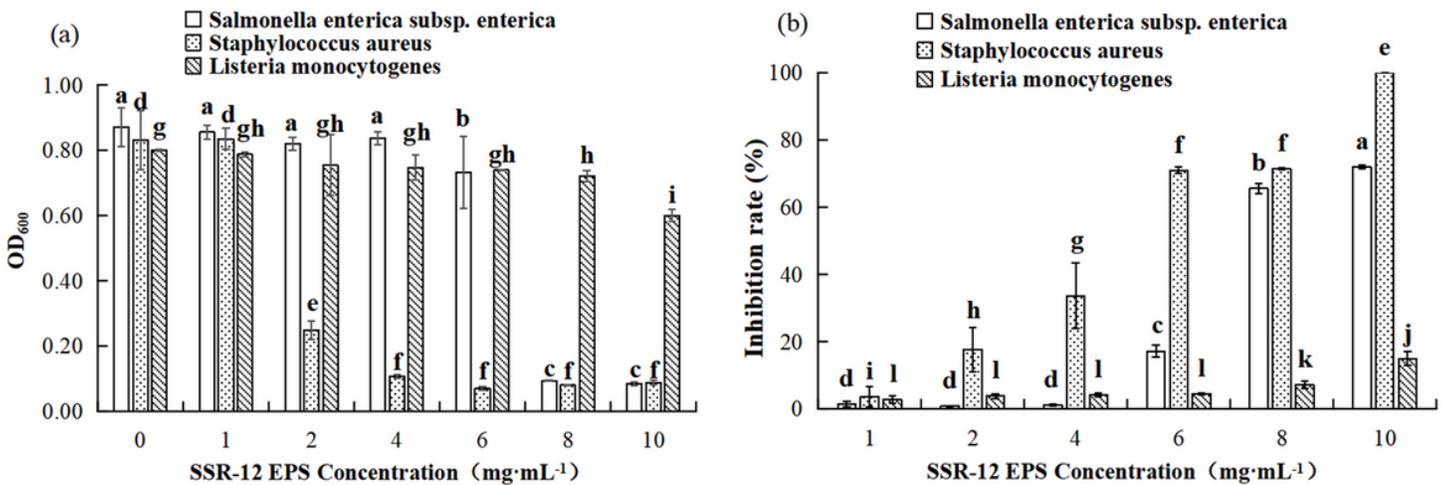


Figure 4

Antibacterial ability of SSC-12 EPS at different concentrations. SSC-12 EPS, EPS produced by SSC-12. The inhibitory ability on *Staphylococcus aureus*, *S. enteritidis* subsp. *Enteritidis* and *Listeria monocytogenes* are determined by OD600 (a) and inhibition rate (b). The values are expressed as mean \pm SD of three independent analyses. Different lowercase letters indicate significant differences among SSC-12 EPS concentrations of same indicator bacterial ($P < 0.05$).