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Antioxidant activities of an exopolysaccharide isolated and purified from marine *Pseudomonas* PF-6

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ABSTRACT

An acidic exopolysaccharide (EPS) was isolated and purified from marine *Pseudomonas* PF-6. The test of physical properties indicated that the EPS was odorless, soluble in water, insoluble in organic solvents, and had a molecular weight of 8.83×10^5 Da. The test of chemical properties of the EPS showed that the EPS could be oxidized into uronic acid. Infrared spectrometry analysis exhibited absorption bands at 927.49 cm^{-1} and 811.76 cm^{-1}, suggesting that the EPS belonged to a β-type heteropolysaccharide with a pyran group. Several *in vitro* assays were applied to evaluate the antioxidant potential of the EPS. Results showed that the acidic EPS had scavenging actions on DPPH·, ·OH and O_2^-· and the antioxidant activities increased with increases in its concentration. The scavenging action of the EPS on ·OH was stronger than that of V_c, and its activity on O_2^-· was almost the same as that of V_c.

1. Introduction

Exopolysaccharides (EPSs) are biological high-molecular-weight polymers that are produced during the metabolic process of microorganisms such as bacteria, fungi, and blue-green algae. They have been used as an important class of bioactive natural products in the biochemical and medical applications due to their specific biological activities such as antioxidant activity (Luo & Fang, 2008), immunostimulating effects (Xu, Wang, Jin, & Yang, 2009), antitumor effects (Tong, Xia, Feng, Sun, & Gao, 2009) and antiviral activity (Wang, Ooi, & Ang, 2007). In recent years, marine microbial EPSs have attracted more attentions, particularly for those originated from marine bacteria, such as *Pseudomonas* (Colliec-Jouault et al., 2004). Microbial EPSs are abundant in the marine environment, for example, in sea ice and ocean particles, where they may assist microbial communities to endure extremes of temperature, salinity and nutrient limitation (Mancuso Nichols, Guezennec, & Bowman, 2005). In fact, most deep-sea bacteria isolated were examined to produce EPSs under these restricted growth conditions (Guezennec, 2002). There has been a growing interest in isolating new bacteria producing EPSs from marine environments (Arena, 2004). Many new marine microbial EPSs with unique chemical compositions, structures and properties have been found to be suitable for potential applications such as thickening agents, stabilizing agents, natural antioxidants, anti-cancer drugs and bio-absorbents (Bozzi, Milas, & Tinaudo, 1996; Cambon-Bonavita, Raguenes, Jean, Vincent, & Guezennec, 2002; Chi & Zhao, 2003; Gorshkova, Nazarenko, & Zubkov, 1993).

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules, which posed damage in the biological system (Clarkson, 1995). They are very active chemically and can be produced by radiation or as by-products of metabolic processes in certain biological systems (Cheung, Cheung, & Ooi, 2003; Kang, Yun, & Lee, 2003). Free radicals also have been widely implicated with etiology of variety of pathological processes, including cancer, diabetes, atherosclerosis, neurodegenerative disorders and arthritis (Emerit, Edeas, & Bricaire, 2004; Kris-Etherton et al., 2002). Besides damage to living cells, free radicals are the major cause of food deterioration, which ultimately affects the organoleptic properties and edibility of foods (Cheung et al., 2003). Thus, intervention of an antioxidant is necessary in both living cells and in food production. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before adverse deterioration occurs. Antioxidants are directly involved in the prevention of cellular damages – the common pathway for cancer, aging and a variety of diseases (Goldfarb, 1993; Witt, Reznick, Viguie, Starke-Reed, & Packer, 1992).

As indicated in the literature, many synthetic antioxidants are strong radical scavengers, however, they usually have side effects (Liu, Ooi, & Chang, 1997). For instance, some have adverse impacts in human organs such as the liver and the kidney upon dietary consumption, some are the sources of allergic reactions, and many of them volatilize in thermal processing of more than

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70 °C, which is a normal practice in food production (Hayashi, Morimoto, Miyata, & Sato, 1993). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroxyquinone (TBHQ) are authorized as synthetic antioxidants for use in food (Abdalla, Tirzite, Tirzitis, & Roozen, 1999). There are some serious problems concerning the toxicity of these compounds. The main concern about the safety of these synthetic compounds is related to their metabolism and possible absorption and accumulation in body organs and tissues (Hayashi et al., 1993). Therefore, it is necessary to find natural antioxidants with same or similar antioxidant effectiveness (Lin, Wang, Chang, Inbaraj, & Chen, 2009; Wu et al., 2007). EPSs isolated from fungi and bacteria can be regarded as natural antioxidants (Jiang, Wang, Lu, & Tian, 2005; Ng et al., 2006). As indicated in the recent findings, certain EPSs have demonstrated an important role as dietary free-radical scavenger for the prevention of oxidative damage, which opens up a potential of using them as therapeutic agents (Kodali & Sen, 2008; Li et al., 2006; Liu et al., 1997; Wang, Zhang, Zhang, & Li, 2008).

Pseudomonas is one of the most widely distributed bacteria in the nature. In this paper, *Pseudomonas* PF-6 was screened and identified by physiological and biochemical properties. We noticed that *Pseudomonas* PF-6 could produce large amounts of viscous exopolysaccharides during liquid fermentation. The EPS was purified by ethanol precipitation, sevage method, ion exchange, dialysis and freeze drying. The physical and chemical properties of the EPS were determined, and functional groups were detected by infrared spectrophotometry. Then antioxidant activities were investigated using various *in vitro* assays such as reducing power, DPPH radical scavenging, hydroxyl radical (·OH) scavenging and superoxide radical($O_2^{-}\cdot$) scavenging, in order to understand the potential usefulness of the EPS from marine *Pseudomonas* PF-6 as a new natural antioxidant.

2. Materials and methods

2.1. Bacterial strain and chemicals

Pseudomonas PF-6 was screened from the marine mud of Dalian HeiShiJiao waters and preserved in our laboratory. Ascorbic acid (vitamin C), hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA), pyrogallol were purchased from Tianjin Kermel Chemical Reagent Company, Tianjin, China. Ferric chloride ($FeCl_3$), potassium ferricyanide ($K_3[Fe(CN)_6]$), safranine T, ferrous sulphate ($FeSO_4$) were purchased from Beijing Aubstadt Star Biotechnology Limited Liability Company, Beijing, China. DEAE–cellulose and 1,1-diphenyl-2-hydrazyl (DPPH) were purchased from Shanghai ShengGong Biological Engineering Technology Service Company, Shanghai, China.

All other chemicals used were all analytical grade and purchased from Tianjin Kermel Chemical Reagent Company, Tianjin, China.

2.2. Production, isolation and purification of the EPS

2.2.1. Liquid fermentation of Pseudomonas PF-6

The culture medium fermented from *Pseudomonas* PF-6 was used to harvest EPSs. The liquid medium for *Pseudomonas* PF-6 mycelial culture was pre-determined according to our previous studies, consisting of (per liter) 60 g sucrose, 6 g yeast extract, 2 g peptone, 1 g $CaCl_2$, 1 g K_2HPO_4, 0.5 g $FeSO_4$, and dissolved in seawater. *Pseudomonas* PF-6 was propagated in 250 mL conical flasks with 50 mL of liquid medium and shaken at 160 rpm for 48 h on a rotary shaker. The fermentation temperature, initial pH value, inoculation proportion were 28 °C, 7.0 and 4.0% (v/v), respectively according to our previous report (Liu, Ye, & Wang, 2010).

2.2.2. Isolation and purification of the EPS

The culture medium was separated by centrifugation (4000 rpm, 15 min). The supernatant was collected and mixed with 3 volumes of 95% ethanol (v/v), and left overnight at 4 °C for polysaccharide isolation. The precipitate in the centrifuging tube was rinsed thoroughly with water, filtered and then dried at 50 °C in an oven to attain the biomass dry weight (Po, Shuna, Kwok, & Jian, 2009).

The crude EPS isolated was dissolved in distilled water and further treated with Sevage reagent (chloroform:n-butanol at 5:1, v/v) for 3 times to remove the residual protein. The EPS, which was in supernatant, was purified again by ethanol and left overnight. The resulting precipitate was re-dissolved in distilled water and dialyzed (MWCO 7000) using running tab water for 48 h and distilled water for another 48 h (Liu et al., 2010). The dialyzed solution was concentrated and used for further EPS fractionation.

The yield of EPS was determined by an anthrone colorimetric method (Po et al., 2009), through sulphuric acid hydrolysis of EPS in the presence of anthrone agent at 100 °C. The absorbance of the sample solution was measured at 620 nm and calibrated using glucose as a standard.

The EPS fractionation was carried out in a DEAE-52 anion-exchange chromatography column (2.6 cm × 50 cm), with successive elution using distilled water and NaCl solutions in a linear gradient of 0–1.0 M, at a flow rate of 1 mL/8 min. Fraction volume was set at 5 mL per each tube. The main fraction was further purified through a Sephadex G-100 column (2.6 cm × 50 cm) and the column was eluted with 0.01 M NaCl solution at a flow rate of 0.8 mL/min. The carbohydrate content was then quantified using the anthrone colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The main fraction was collected, dialyzed, concentrated and lyophilized, resulting in purified EPS which was used for subsequent analyses.

2.3. Analysis of the EPS physical and chemical properties

2.3.1. Appearance and solubility of the EPS

The physical properties of EPSs such as color, smell, texture were investigated, as well as its solubility in several solvents such as ethanol, ether and acetone. The EPS powder was added into different solvents, mixed well and observed if it was soluble.

2.3.2. Purity of the EPS

The EPS solution was placed at −35 °C overnight, thawed rapidly and centrifuged (12,000 rpm, 20 min) to examine the precipitation. If no precipitation occurred, the UV absorption spectrum was recorded using a Lambda 35 spectrophotometer (Perkin Elmer, U.S.) between 190 and 550 nm, in order to examine the existence of proteins and nucleic acids (Wang, Cui, & Xu, 2007).

2.3.3. Molecular weight

The molecular weight of EPS was measured using high liquid chromatography with a TSK-Gel G3000SWXL column (7.5 nm × 300 nm, Tosoh Corp., Tokyo, Japan). The column was eluted with 0.05 M NaCl solution at a flow rate of 0.8 mL/min. The eluent was monitored with a refractive index detector (RID) (Jun et al., 2009). Dextrans with different molecular weights were used as references for determination of the molecular weight of the EPS.

2.3.4. Chemical reactions

Some chemical properties such as structural properties and functional groups of the EPS could be tested by certain chemical reactions. These tests help enhance the understanding of structure identification and relationship. All the assays following were performed according to the reports (Kang et al., 2003; Li, Zhou, Cai, & Zhang, 1999).

For polysaccharides from natural products, the existence of uronic acid in the molecular structure has vital significance, because polysaccharides with high content of uronic acid may be relevant to the radical scavenging activity. The carbazole and sulphuric acid reaction can be used to determine the existence of uronic acid in the EPS (Zhu & Tan, 2008), based on the reaction between uronic acid and carbazole in forming a chromogen, where no color developed in the mixture suggests the absence of uronic acid.

Fehling reagent reaction was usually used to determine the existence of reducing sugar in the molecular structure. The aldehyde group in the reducing sugar could reduce Cu^{2+} in Fehling reagent into Cu^{+} in forming a red-colored compound which precipitates.

The reaction between iodine and starch was used to determine the purity of EPS in terms of the existence of starch residual in the EPS.

Molish reaction was used to identify whether the analyte belonged to carbohydrate. Polysaccharides dehydrated into furfural and other derivatives under the treatment of concentrated sulphuric acid or hydrochloric acid. Furfural and its derivatives react with α-naphthol and form purple compounds containing rings in the structure.

2.3.5. Infrared (IR) analysis

The IR spectrum of EPS was determined using a Fourier transform infrared spectrophotometer (Spectrum One-B, Perkin Elmer, U.S.) for the detection of various functional groups. The purified EPS was ground with KBr powder and pressed into pellets for FTIR measurement in the frequency range of 4000–400 cm^{-1} (Zhbankov, Adnanov, & Marchewka, 1997).

2.4. Antioxidant activities of the EPS

2.4.1. Determination of reducing power

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones. The antioxidant action of reductones is based on its potential to break a free radical chain by donating a hydrogen atom.

The method used in the present study was based on the report of Oyaizu (1986) and Yenhum (1997) with some modifications. The reaction mixtures contained 2.5 mL phosphate buffer saline (pH 6.6, 0.2 M), 2.5 mL potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v) and 0.5 mL EPS sample solution. After incubating at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture, and then centrifuged at 1400 rpm for 10 min. 0.5 mL upper layer of solution was collected and mixed with 2.0 mL deionized water and 0.4 mL $FeCl_3$ (0.1%, w/v). After incubating at room temperature for 10 min, the absorbance of mixture was measured at 700 nm by a spectrophotometer (4501S, Donggang Corp., Tianjin. China). The potential of EPSs in reducing Fe^{3+} to Fe^{2+} was measured by the intensity of a resulting blue-green solution which has a maximum absorbance at 700 nm (Gordan, 1990). Higher absorbance of the reaction mixture indicates greater reductive potential. Ascorbic acid (V_c) was used as a positive control.

2.4.2. Determination of DPPH radical (DPPH·) scavenging activity

DPPH· is a stable radical compound. The DPPH assay used in the study is a quick yet cost-effective method, which has been used frequently for evaluation of antioxidative potentials of various natural products (Molyneux, 2003; Prior, Wu, & Schaich, 2005; Tadhani, Patel, & Subhash, 2007).

The assay was performed according to the reports (Choi et al., 2002; Yang et al., 2006) with some modifications. The reaction mixture contained 3.5 mL DPPH solution (0.2 mM, dissolved in 95% ethanol, v/v) and 0.5 mL EPS sample solution. After incubating at room temperature in the absence of light for 30 min, the absorbance of the mixture solution was measured at 517 nm, and compared to the value measured for the control solution of DPPH in the absence of EPS samples. Ascorbic acid (V_c) was used as a positive control. The DPPH radical scavenging activity was then calculated by the following equation:

$$\text{The scavenging activity } (\%) = \left[\frac{1-(A_{\text{sample}}-A_{\text{blank}})}{A_{\text{control}}}\right] \times 100\%$$

where A_{sample} is the absorbance in the presence of the sample, A_{blank} is the absorbance in the absence of the DPPH solution, and A_{control} is the absorbance in the absence of the sample.

2.4.3. Determination of hydroxyl radical (·OH) scavenging activity

The hydroxyl radical generated in a biological system based on a Fenton reaction is thought to be deterioative, leading to cell damages, where the reaction is known to be the most reactive in the presence of dioxygen (Rollet-Labelle et al., 1998).

The hydroxyl radical scavenging activity of the EPS was measured based on the report of Qi et al. (2005) with some modifications. The reaction mixture contained 1.0 mL phosphate buffer saline (pH 7.4, 0.15 mM), 0.1 mL safranine T (0.52 mg/mL), 1.0 mL EDTA–Fe(II) (6 mM), 0.8 mL H_2O_2 (6%, v/v) and 7.0 mL EPS sample solution. After incubating at 40 °C for 30 min, the absorbance of mixture was measured at 520 nm. The absorbance change of the reaction mixture indicated scavenging ability for hydroxyl radicals. Ascorbic acid (V_c) was used as a positive control. The hydroxyl radical scavenging activity was then calculated by the following equation:

$$\text{The scavenging activity } (\%) = \left[\frac{(A_{\text{sample}}-A_{\text{blank}})}{(A-A_{\text{blank}})}\right] \times 100\%$$

where A_{sample} is the absorbance of the reagent mixture with the sample, A_{blank} is the absorbance of the reagent mixture without the sample, and A is the absorbance of the reagent mixture without the sample and H_2O_2.

2.4.4. Determination of superoxide anion($O_2^{-}\cdot$) scavenging activity

Superoxide anions are free radical precursors that can be produced *in vivo* and can result in the formation of H_2O_2 *via* a dismutation reaction (Blokhina, Virolainen, & Fagerstedt, 2003; Halliwell & Gutteridge, 1984).

The superoxide radical scavenging activity was determined based on the method reported by Kanatt, Chander, and Sharma (2007) with slight modifications. 4.5 mL Tris buffer (pH 8.0, 0.5 mM) solution was added into 2 mL EPS sample solutions at various concentrations. The mixture was incubated at 37 °C for 10 min and mixed with 0.2 mL pre-heated pyrogallol (7 mM). The absorbance was then measured immediately at 320 nm. Ascorbic acid (V_c) was used as a positive control. The superoxide radical scavenging activity was calculated as:

$$\text{The scavenging activity } (\%) = \frac{1-A_{\text{sample}}}{A} \times 100\%$$

where A_{sample} is the absorbance of the reagent mixture with the sample, A is the absorbance of the reagent mixture without the sample.

2.5. Experimental statistics

There were at least three replicates measured for each sample during the above-mentioned assays. All experimental data were presented as mean values ± standard deviations.

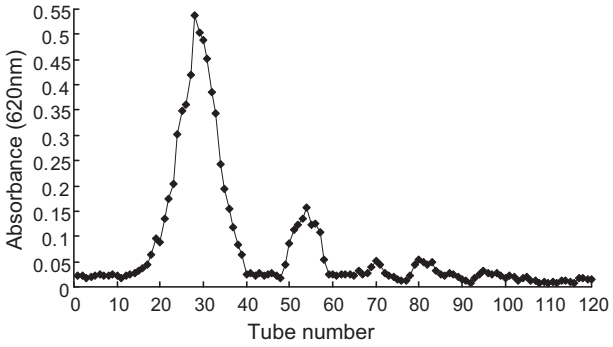

Fig. 1. Fractions of the PF-6 fermentation product by a DEAE–52 cellulose column.

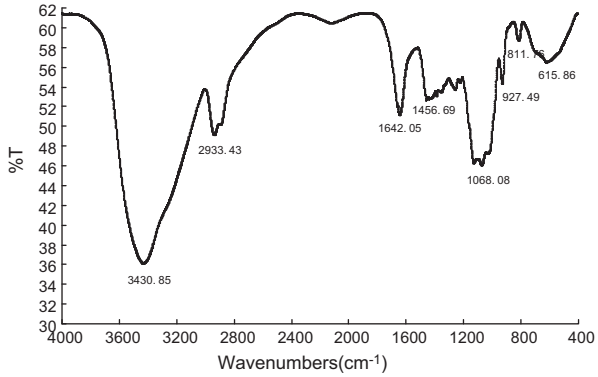

Fig. 3. IR spectrogram of the EPS.

3. Results

3.1. Isolation and purification of the EPS

The purified EPS with a yield of 1.22 mg/mL was from a series of batches fermentation by *Pseudomonas* PF-6. The chromatographic elution profile was shown in Fig. 1, leading to the separation of two polysaccharide subfractions. The major component was collected, concentrated and further purified through a Sephadex G-100 column. Since DEAE is an anion exchanger, positive charged groups and neutral groups in the samples were removed with eluate, whereas negative ions in the samples could exchange with balance ions and combine to the chromatography column. So the purified EPS belonged to acidic polysaccharides (Chang, 1987).

3.2. Physical and chemical properties of the EPS

The purified EPS was light yellow in color, and in a form of odorless powder. It was soluble in water but insoluble in ethanol and other organic solvents, such as ether and acetone, which accorded with general properties of polysaccharides.

The EPS solution was a uniform liquid with a light yellow in color, and no precipitation occurred after centrifugation. So the EPS was thought to be pure. An ultraviolet scan spectrum analysis of the EPS was shown in Fig. 2, indicating an absence of proteins and nucleic acids due to the negative response – no absorption at 260 or 280 nm.

Its molecular weight was measured by HPLC with a size exclusion column. Based on a calibration curve obtained from the elution retention times of dextrans with various molecular weight cut-offs, the average molecular weight of EPS was estimated to be 8.83×10^5 Da.

Responses of the chemical reactions between the EPS and several reagents were illustrated in Table 1, indicating the purified EPS belonged to carbohydrate material, which could be oxidized into uronic acid, while there were no reducing sugar and starch in the EPS structure or in the purified material.

The IR analysis of the EPS was shown in Fig. 3. There were many peaks from 3430.85 to 615.86 cm^{-1}. The band at 3430.85 cm^{-1} region was attributed to the stretching vibration of O–H in the constituent sugar residues (Kanmani et al., 2011). The band at 2933.43 cm^{-1} was associated with the stretching vibration of C–H in the sugar ring. The band at 1642.05 cm^{-1} was due to the stretching vibration of C=O and carboxyl group. The absorptions around 1456.69 cm^{-1} represented CH_2 and OH bonding. The strong absorption at 1068.08 cm^{-1} was dominated by glycosidic linkage ν(C–O–C)-stretching vibration (Sun, Fang, Goodwin, Lawther, & Bolton, 1998). Moreover, the band at 927.49 cm^{-1} indicated the β-pyranose form of the glucosyl residue, and the band at 811.76 cm^{-1} suggested the β-pyranose form of the mannose residue. Therefore, the infrared spectrometry analysis suggested that it was highly likely that the EPS belonged to a β-type heteropolysaccharide with a pyran group (Cheng, Wan, Jin, Wang, & Xu, 2008).

3.3. Antioxidant activities of the EPS

3.3.1. Reducing power

As shown in Fig. 4, the reducing capacity of the EPS increased with increases in its concentration. The perfect linear correlation between the EPS concentration and its reducing capacity suggested a dose and effect relationship. The reducing capacity of the EPS was generally lower than that of V_c.

3.3.2. DPPH radical scavenging activity (DPPH·)

Fig. 5 showed the DPPH radical scavenging activity of the EPS compared to that of V_c. The scavenging effect of the EPS followed a similar increasing trend with an increase in its concentration. At the concentration of 0.1 mg/mL, the scavenging effect of V_c was

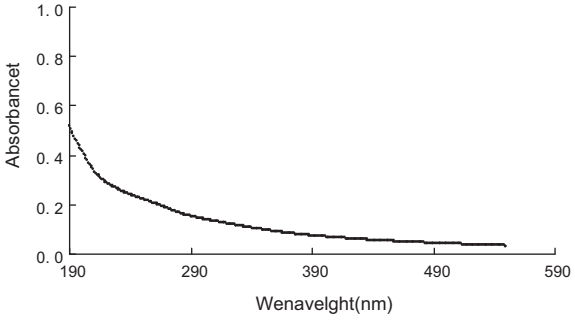

Fig. 2. The ultraviolet scan spectrum of the EPS.

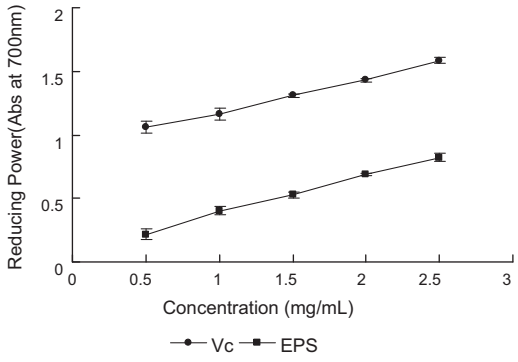

Fig. 4. Reducing power of the EPS and V_c.

Table 1
Responses of chemical reactions between the EPS and several reagents.

Reactions	Results	Analysis
Carbazole and sulphuric acid	+	With an uronic acid group presented in the EPS structure
Fehling reagent	–	No reducing sugar in the EPS structure
I-KI	–	No starch residual in the purified EPS
Molish reaction	+	Carbohydrate materials

"+" means positive; "–" means negative.

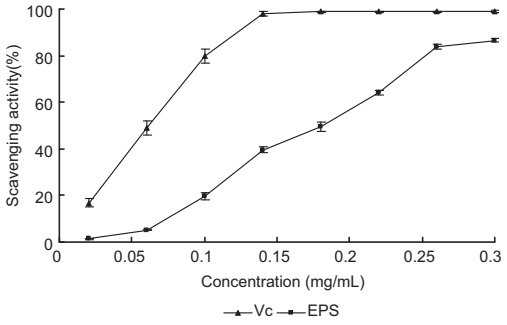

Fig. 5. The scavenging activity of the EPS and V_c to DPPH.

79.81% and arrived almost 100% at a relatively low concentration of 0.15 mg/mL. However, the scavenging activity of the EPS was 19.94% at a concentration of 0.1 mg/mL, which indicates that the EPS had a limited scavenging activity on DPPH· compared to V_c.

3.3.3. Hydroxyl radical scavenging activity

The scavenging activity on hydroxyl radical of the EPS and V_c was shown in Fig. 6. The scavenging effects of both were generally enhanced by the increase in concentrations. At the concentration of 0.6 mg/mL, the scavenging capability of V_c was 47.96%; however, the activity of the EPS reached to 92.12%. This result indicates that the EPS has stronger scavenging activity on hydroxyl radical compared to that of V_c.

3.3.4. Superoxide anion scavenging activity

The scavenging activity of the EPS and V_c on superoxide radicals was shown in Fig. 7. At all of the concentrations tested, the scavenging activity of the EPS and V_c presented an identical increasing trend. The IC_{50} could be obtained by interpolation using the linear equations $y = 338.46x - 0.561$ and $y = 351.8x - 1.786$ for the EPS and V_c, respectively. Hence, the values of IC_{50} were 0.149 mg/mL and 0.147 mg/mL for scavenging effect on $O_2^{-}\cdot$ for the EPS and V_c, respectively. This result clearly showed that the EPS had an equally strong scavenging action on $O_2^{-}\cdot$ compared to that of V_c.

4. Discussion

Many marine bacteria could produce EPSs, such as *Paenibacillus polymyxa*, *Edwardsiella tarda and Alteromonas*. Guo et al. reported that the scavenging ability on hydroxyl radical of an EPS obtained from *Edwardsiella tarda* was about 90% at 8 mg/mL (Guo, Mao, & Han, 2010). But in our study as shown in Fig. 6, the hydroxyl radical scavenging activity of the EPS from *Pseudomonas* PF-6 reached 90% at a much lower concentration level, 0.6 mg/mL. Coincidentally, Jun L et al. reported that the scavenging ability on superoxide anion radical of an EPS from *Paenibacillus polymyxa* EJS-3 was about 80% at 1 mg/mL (Jun et al., 2009). But in the present study as shown in Fig. 7, the scavenging activity of the EPS on superoxide anion radicals was about 80% even at 0.25 mg/mL. Thus, the EPS obtained from *Pseudomonas* PF-6 presented stronger antioxidant activities than those from other marine bacteria. *Pseudomonas* strains might be able to produce high quality EPSs with strong biological activities among the others, however, this requires further investigations.

As indicated in the literature, acidic polysaccharides are likely to contain more uronic acids with negative charges (Duh, Tu, & Yen, 1999). The atoms of polysaccharides with a greater proportion of uronic acid were negatively charged, resulting in less steric hindrance when a superoxide anion radical ($O_2^{-}\cdot$) attacks. This explains the reason for high content of uronic acid in acidic polysaccharides having higher radical scavenging activities, especially for superoxide anion radicals (Duh et al., 1999). Many studies also suggest that the bioactivities of polysaccharides are closely associated with their structures, such as the type of glycosyl units, the configuration of glycosidic bonds, and the substituents of the polysaccharides. In addition, the spatial structure and molecular weight of polysaccharides could also affect the bioactivity (Tsiapali et al., 2001). Therefore, the antioxidative activity of the EPS was not the result of any single factor. It is the result of many factors when combined in the variation of monosaccharide composition, structure configuration, mode of attending glycosidic bonds, molecular weight and other structural characteristics of the EPS. Considering the complexity of antioxidation mechanisms, a comprehensive understanding of these effects is required (Moure, Dominguez, & Parajo, 2006). However, due to the lack of results from a characterization study in understanding the molecular structure of

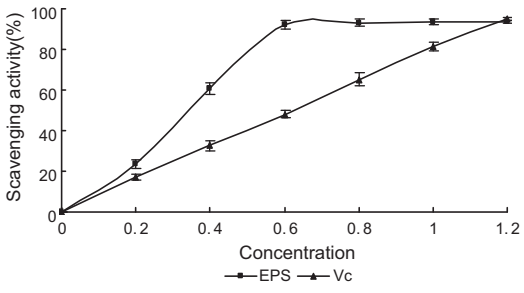

Fig. 6. The elimination rate of the EPS and V_c to ·OH.

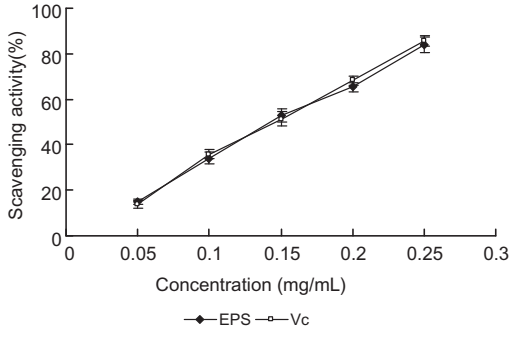

Fig. 7. The elimination rate of the EPS and V_c to O_2^{-}.

the purified EPS in the present study, it is difficult to thoroughly interpret the structure–activity relationship of the EPS during the antioxidation mechanisms. However, this points out a direction for our future studies.

5. Conclusions

In this study, an acidic EPS was extracted and purified from a marine bacterium, *Pseudomonas* PF-6, through a series of processing, i.e., liquid fermentation, ethanol precipitation, sevage purification, ion exchange fractionation, size exclusion, dialysis and freeze drying. The tests of physical properties indicated that the EPS was odorless, soluble in water and insoluble in commonly used organic solvents. It had a molecular weight of 8.83×10^5 Da. Chemical properties of the EPS were tested by chemical reactions with selected reagents, and the results indicated that the EPS belonged to carbohydrate materials and could be oxidized into uronic acid by carbazole and sulphuric acid reagents. Also, the EPS belonged to a β-type heteropolysaccharide with a pyran group based on an infrared spectrometry analysis. *In vitro* assays for its antioxidant activities showed that the EPS had somewhat reducing power and limited scavenging action on DPPH·. However, it presented strong capability in scavenging ·OH and O_2^-, specifically, its scavenging action on ·OH was stronger than that of V_c, and the activity on O_2^-· was identical to that of V_c. Among the factors which may attribute to the strong antioxidant activities, the high content of uronic acid in the EPS might be the main reason. The overall results suggest that the acidic EPS obtained from marine *Pseudomonas* PF-6 has potentials as a natural antioxidant in certain applications, where synthetic chemicals would fail in the requirements of safety and efficiency. The future work is required and will be focused on the understanding of its structure–activity relationship and its *in vivo* antioxidant activities/mechanisms.

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