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ORIGINAL PAPER

Optimization and Characterization of an Antioxidant Exopolysaccharide Produced by *Cupriavidus pauculus* 1490

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Abstract

In the present study, exopolysaccharides (EPS) production by *Cupriavidus pauculus* 1490 was optimized by response surface methodology. The results showed that sodium gluconate (4.15 g/L), NH_4Cl (0.52 g/L), and $Na_2HPO_4{\cdot}12H_2O$ (0.04 g/L) were the optimal medium components and concentrations. The actual EPS yield of 293.2 m g/L in the optimized medium was in close agreement with the predicted value of 283.35 m g/L. Analysis of fourier transform infrared spectroscopy indicated the EPS contained abundant functional groups, such as –OH, C=O and C–O–C, and all of them were attributed to the characteristics of polysaccharides. Mannose, glucuronic acid, glucose and xylose were detected as the main monosaccharide composition of EPS. Rheological analysis suggested that the rheogram of EPS has similar trend with Xanthan and presented the property of non-Newtonian fluid. Moreover, the addition of NaCl and KCl would partly weaken the shear stress of EPS. Three in vitro assays were conducted to evaluate the antioxidant potential of the EPS. Results demonstrated that the EPS possessed scavenging capacity on hydroxyl radical, DPPH radical and superoxide anion radical in a dose-dependent way. As indicated by above results, the EPS isolated from *C. pauculus* 1490 might serve as a potential antioxidant agent to be applied in nutraceutical and pharmaceutical industries.

Keywords Exopolysaccharides · *Cupriavidus pauculus* 1490 · Monosaccharide · Antioxidant activity

Introduction

Microbial exopolysaccharide (EPS) is a kind of extracellular high-molecular-weight polymer excreted into the surroundings by microorganisms during growth, such as fungi, blue-green algae and bacteria [1, 2]. EPS has received considerable attention because of their stabilizing, gelling and texturizing properties, as well as low toxicity and environmental friendliness. Moreover, microbial EPS possesses potential biological function in antioxidant, anti-inflammatory and antitumor activity [3–6]. Up to now, a variety of commercial microbial EPSs, such as Xanthan, Gellan, Curdan, Pullan, Dextran and Alginate, have been explored in industrial applications [7–9].

Cupriavidus is a gram-negative bacterium of the class beta-proteus, which exists widely in the environment [10]. Some of *Cupriavidus* species were reported to have the characteristics of producing EPS [11–13]. Genome analysis of *C. alkaliphilus* ASC-732 suggested it possessed a total of four putative gene clusters related to EPS production, including *epsI*, *epsII*, *epsIII* and *epsIV*. Depending on their rheological and viscoelastic properties, EPS produced by ASC-732 strain could be used in various applications, such as emulsifiers, food additives thickeners and so on [11]. *C.* necator IPT 027 produced novel EPS from glucose and crude glycerol, which was mainly constituted of monosaccharides (glucose, mannose, arabinose and fucose), uronic acid and amine group. It also exhibited pseudoplastic non-Newtonian fluid behavior and potential applicability in food industry [12]. EPS produced by *C. pauculus* KPS 201 was positively affected by the increase of nitrogen and phosphate in the

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medium, and it was identified as a homopolymer of rhamnose [13].

Response surface methodology (RSM) is a widely used statistical tool to optimize biomass cultivation, enzymes production, spore generation and so on [14, 15]. In this study, single factor experiments were conducted firstly to select the key medium components affecting EPS production by *C. pauculus* 1490, and further optimization was carried out by RSM. Thereafter, Fourier transform infrared spectroscopy and monosaccharide composition of EPS were analyzed to reveal its basic structure feature. Rheological property and antioxidant activity of EPS were also characterized to explore its potential application value. This preliminary study focused on improving EPS yield and recognizing the correlation between structure, rheological property and antioxidant activity. This information will be conductive to the subsequent modification at molecular level targeting at EPS structure.

Experimental Section

Microorganism and Culture Conditions

Cupriavidus pauculus 1490 used in this work was purchased from China General Microbiological Culture Collection Center, CGMCC. The strain was originally preserved in the form of freeze dried powder and firstly activated in LB medium (peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, pH 7.0). Thereafter the enriched bacterial strain was inoculated into 100 mL Tris-buffered minimal medium, composed of Tris-base 6.06 g/L, NaCl 4.68 g/L, KCl 1.49 g/L, NH_4Cl 1.07 g/L, $NaSO_4$ 0.43 g/L, $MgSO_4{\cdot}6H_2O$ 0.2 g/L, $CaCl_2{\cdot}2H_2O$ 0.03 g/L, $Na_2HPO_4{\cdot}12H_2O$ 0.02 g/L and sodium gluconate 0.04 g/L, with an initial pH of 7.0 in 250 mL Erlenmeyer flasks for EPS production. All the flasks were incubated in the rotary shaker at 30 °C and 150 rpm.

Single Factor Experiments

To find out the critical medium components affecting EPS production, different concentrations of sodium gluconate (1, 2, 3, 4, 5, 6, 7, 8 g/L), NH_4Cl (0.267, 0.535, 0.802, 1.07, 1.337, 1.605, 1.872, 2.14 g/L), $Na_2HPO_4{\cdot}12H_2O$ (0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 g/L) and $MgSO_4{\cdot}6H_2O$ (0.107, 0.215, 0.322, 0.43, 0.537, 0.645, 0.752, 0.86 g/L) were selected as carbon source, nitrogen source, phosphorus source and sulfur source, respectively, to explore their influence in EPS yield. 1% (v/v) of seed culture from Tris-buffered minimal medium was inoculated into 100 mL fresh medium with initial pH of 7.0 in 250 mL Erlenmeyer flasks. All the flasks were incubated in the rotary shaker at 30 °C and 150 rpm. EPS yield was tested after 72 h.

Central Composite Design (CCD)

The most significant variables from single factor experiments were further optimized by RSM using a CCD to maximize EPS yield. Three components (sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$) that significantly influenced EPS production were optimized by RSM using a 3-factor-3-level CCD. The actual levels of variables are displayed in Table 1. In order to correlate the response variable (i.e., EPS yield) to the independent variables, the yield was fit according to the following second-order polynomial model Eq. (1):

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} {x_i}^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} b_{ij} x_i x_j, i \neq j \quad (1)$$

where Y represents the response variable, b_0 is the interception coefficient, b_i is the coefficient of the linear effect, b_{ii} is the coefficient of quadratic effect, b_{ij} is the coefficient of interaction effect when $i < j$, and k is the numbers of involved variables.

Statistical analysis of the data was performed by Design-Expert software (version 8.0.6.1). The model and the second-order polynomial equation were validated by performing the EPS production under the conditions predicted by the model.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

1 mg of dry sample was mixed with 100 mg KBr powder and pressed into fine powder for FTIR analysis. FTIR spectrum was obtained by a Nicolet Nexus 670 FTIR spectrometer at a resolution of 2 cm^{-1} between the range of 4000–400 cm^{-1}.

Analysis of Monosaccharide Composition

5 mg of EPS was hydrolyzed with 1 mL 2 M TFA at 121 °C for 2 h. After derivatization by 1-phenyl-3-methyl-5-pyrazolone (PMP), the product was analyzed by high performance liquid chromatography (HPLC). The standard monosaccharide did not need acidolysis by TFA, but was derivatized directly. The products were analyzed using a HPLC (1260 Infinity II, Agilent, America) equipped with

Table 1 Factors and level value of central combination experiment design

Variables	Symbol	Coded variable level		
		−1	0	1
Sodium gluconate (g/L)	A	3	4	5
NH_4Cl (g/L)	B	0.267	0.535	0.802
$Na_2HPO_4{\cdot}12H_2O$ (g/L)	C	0.02	0.04	0.06

a diode array detector (DAD) and Agilent Eclipse XDB-C18 column (5 µm, 4.6 × 250 mm). The eluent was composed of 0.1 M phosphate buffer (pH 6.7) and acetonitrile at a ratio of 83:17 (v/v, %) with a flow rate of 1.0 mL/min. The UV detection wavelength was 254 nm. The monosaccharide composition was identified by comparing with the retention time of standard sugars, including mannose (Man), glucuronic acid (GluUA), glucose (Glc), xylose (Xyl), araose (Ara) and fucose (Fuc).

Rheological Analysis

For preliminary rheological analysis, 1% (w/v) EPS sample was prepared. Shear stress of the sample solution under different shear rates (0–500 s^{-1}) was analyzed at 25 °C by a super rotary rheometer (Kinexus Pro MAL1082692, Malvern, England). Commercially available xanthan gum was used as a contrast (Aladdin Biochemical Technology Co., Ltd., Shanghai, China). The effect of salts (NaCl and KCl) on the shear stress of EPS solution was also studied.

Antioxidant Activities Test

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was measured according to the reported method [16] with some modifications. A total of 1 mL of 0.75 mM phenanthroline, 2 mL of 0.2 M phosphate buffer (pH 7.4) and 1 mL of 9 mM $FeSO_4$ was added to the sample solution (1 mL). Then 1 mL of 8.8 mM H_2O_2 was added, followed by incubation at 40 °C for 30 min. The absorbance of the mixture was measured at 520 nm, with ascorbic acid (Vc) used as positive control. The capability to scavenge the hydroxyl radical was calculated according to the following equation (Eq. (2)):

$$\text{Hydroxyl radical scavenging ability (\%)} = \left[\left(A_1 - A_0\right)/\left(A_2 - A_0\right)\right] \times 100\% \quad (2)$$

where A_0 is the absorbance of the blank (deionized water instead of EPS sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the control solution (deionized water instead of H_2O_2 and sample).

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was tested based on the method reported by Wang et al. [17] with some modifications. A total of 2 mL of DPPH ethanol solution (0.004%, w/v) was added to 2 mL of EPS sample. The mixture was shaken and kept in dark at room temperature for 30 min. The absorbance of the supernatant was measured at 517 nm. Vc was used as a positive control. The capability to scavenge the DPPH radical was calculated according to the following equation (Eq. (3)):

$$\text{DPPH scavenging ability (\%)} = \left[A_0 - \left(A_1 - A_2\right)\right]/A_0 \times 100\% \quad (3)$$

where A_0 is the absorbance of the control (deionized water mixed with DPPH solution), A_1 is the absorbance of the sample mixed with DPPH solution and A_2 is the absorbance of the sample with deionized water instead of DPPH solution.

Superoxide Anion Radical Scavenging Activity

The superoxide anion radical scavenging activity was determined according to the method reported by Li et al. [14] with slight modifications. 4.5 mL Tris–HCl buffer (0.05 M, pH 8.2) was added into 2 mL EPS sample. The mixture was incubated at 37 °C for 10 min and mixed with 0.2 mL pre-heated pyrogallol (30 mM). The absorbance was then measured immediately at 320 nm. Vc was used as a positive control. The superoxide radical scavenging activity was calculated according to the following equation (Eq. (4)):

$$\text{Superoxide anion radical scavenging ability (\%)} = \left(1 - A_1\right)/A_0 \times 100\% \quad (4)$$

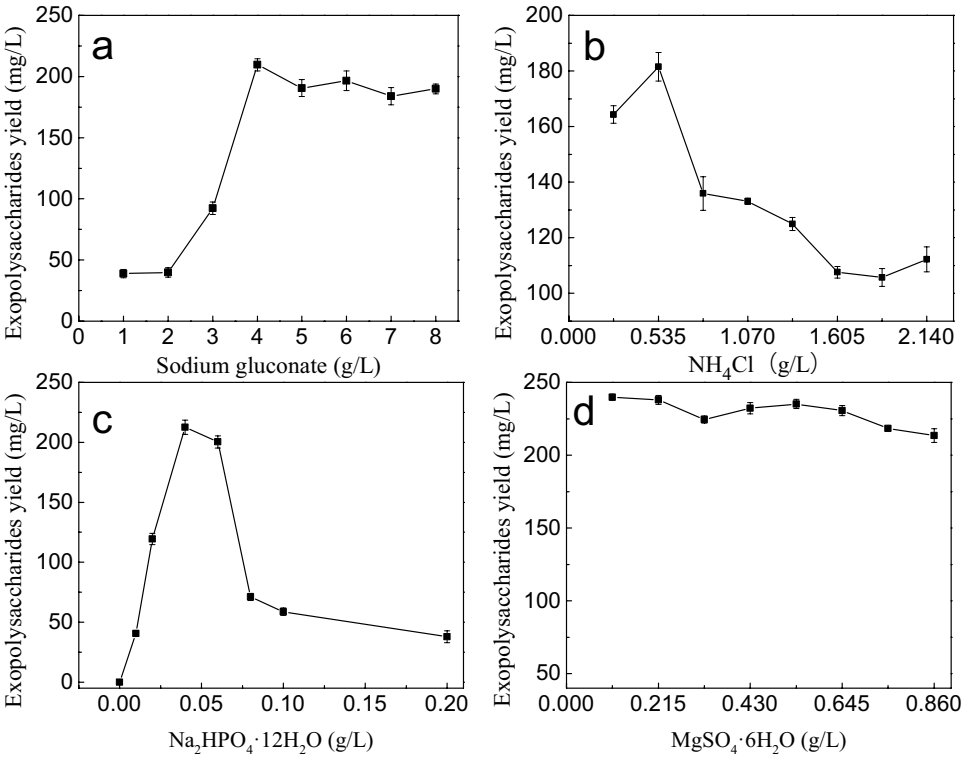

Fig. 1 Single factor experiment: **a** sodium gluconate as carbon source, **b** NH_4Cl as nitrogen source, **c** $Na_2HPO_4{\cdot}12H_2O$ as phosphorus source, **d** $MgSO_4{\cdot}6H_2O$ as sulfur source

where A_0 is the absorbance without the sample, A_1 is the absorbance with the sample.

Results and Discussion

Variables Affecting EPS Produced by *C. pauculus* 1490

It is supposed that all the medium components will more or less affect the cell growth and activity. In this study, single factor experiments were carried out to identify critical variables influencing EPS production by *C. pauculus* 1490. As displayed in Fig. 1, the concentration gradients of sodium gluconate (Fig. 1a), NH_4Cl (Fig. 1b) and $Na_2HPO_4{\cdot}12H_2O$ (Fig. 1c) all caused remarkable fluctuation in EPS yield. While the concentration of $MgSO_4{\cdot}6H_2O$ did not obviously take effect on the production of EPS (Fig. 1d). So the optimized concentration of sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$ was set as 4 g/L, 0.535 g/L and 0.04 g/L, respectively, and applied in the subsequent optimization.

Optimization of Screened Variables by RSM

Based on the results of single factor experiments, sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$ as the significant factors were further optimized by RSM. A 3-factor-3-level CCD with seventeen experiments was conducted (Table 2). After regression analysis, the following second-order polynomial equation was fit to the experimental EPS yield as a function of sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$:

$$Y = 281.81 + 16.43 \times A - 6.46 \times B - 4.46 \times C - 8.72 \times AB + 9.72 \times AC + 6.30 \times BC - 55.05 \times A^2 - 47.65 \times B^2 - 57.49 \quad (5)$$

where Y is the response (i.e., EPS yield), and A, B and C are the concentrations of sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$, respectively.

To validate the regression coefficient for EPS production, the results of the second-order response surface model fitting in the form of ANOVA are given in Table 3.

Table 2 Design scheme of central combination experiment and response value of investigation indicator

No	A (g/L)	B (g/L)	C (g/L)	Yield (mg/L)
1	5.00	0.27	0.04	213.44
2	4.00	0.27	0.06	169.54
3	4.00	0.53	0.04	286.06
4	4.00	0.27	0.02	193.67
5	3.00	0.27	0.04	161.56
6	3.00	0.53	0.06	140.74
7	4.00	0.53	0.04	267.58
8	4.00	0.53	0.04	286.49
9	3.00	0.53	0.02	166.50
10	5.00	0.53	0.06	191.48
11	5.00	0.53	0.02	178.34
12	3.00	0.80	0.04	162.23
13	5.00	0.80	0.04	279.22
14	4.00	0.80	0.02	171.21
15	4.00	0.53	0.04	289.56
16	4.00	0.80	0.06	172.28
17	4.00	0.53	0.04	279.37

The developed model had an acceptable p value (<0.0001) and R^2 value (0.9921), which explained 99.21% of the response variability. The high value of the adjusted R^2 (0.9819) further supported the accuracy of the model. As shown in Table 3, the model was statistically valid given an F-test with a low probability value ($P_{model} < 0.0001$). The lack-of-fit value (0.9022) was not significant, indicating that the equation was adequate for predicting EPS yield. The low coefficient of variation (CV = 3.43%) suggested the model was precise and reliable.

Three dimensional response surface plots were drown by Design-Expert V8.0.6.1 software to identify the effect of medium components on EPS yield. Figure 2a–c illustrate the pair-wise interaction of the three variables (sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$). The result from Fig. 1a indicated that the interaction between sodium gluconate and NH_4Cl was significant (P = 0.0432). When sodium gluconate was from 3 to 4.15 g/L and NH_4Cl was from 0.267 to 0.52 g/L at a fixed $Na_2HPO_4{\cdot}12H_2O$ (0.04 g/L), the yield of EPS increased. Figure 2b displays 3D response surface plot of the influence of sodium gluconate and $Na_2HPO_4{\cdot}12H_2O$ on EPS yield when NH_4Cl concentration was 0.535 g/L. When sodium gluconate and $Na_2HPO_4{\cdot}12H_2O$ were 4.15 and 0.04 g/L, respectively, the maximum EPS yield was obtained. Figure 2c represents the interaction between NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$, which was not significant (P = 0.1184) and indicated the N/P ratio did not play a critical role in producing EPS. According to the established second-order response surface model, the optimal concentration of sodium gluconate, NH_4Cl, and $Na_2HPO_4{\cdot}12H_2O$ was 4.15 g/L, 0.52 g/L and 0.04 g/L, respectively. The maximum EPS production was estimated to be 283.35 mg/L, and the actual production obtained in the optimized medium was 293.2 mg/L, suggesting that the mathematical model could predict the experimental results.

Table 3 Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of exopolysaccharides yield

Source	Sum of Squares	Degrees of freedom	Mean square	*F*-value	*p*-Value
Model	43,975.61	9	4886.16	97.44	< 0.0001
A	2159.10	1	2159.18	43.06	0.0003
B	354.65	1	354.65	7.07	0.0352
C	158.95	1	158.59	3.17	0.1182
AB	304.40	1	304.40	6.07	0.0432
AC	377.91	1	377.91	7.54	0.0278
BC	158.77	1	158.77	3.17	0.1184
A^2	12,760.88	1	12,760.88	72.71	< 0.0001
B^2	9558.74	1	9558.74	190.62	< 0.0001
C^2	13,916.27	1	13,916.27	277.52	< 0.0001
Residual (error)	351.02	7	50.15	–	–
Lack of fit	42.54	3	14.18	0.18	0.9022
Pure error	308.47	4	77.22	–	–
Total	44,326.63	16	–	–	–
R^2	0.9921				
Adj. R^2	0.9819				
CV	3.43%				

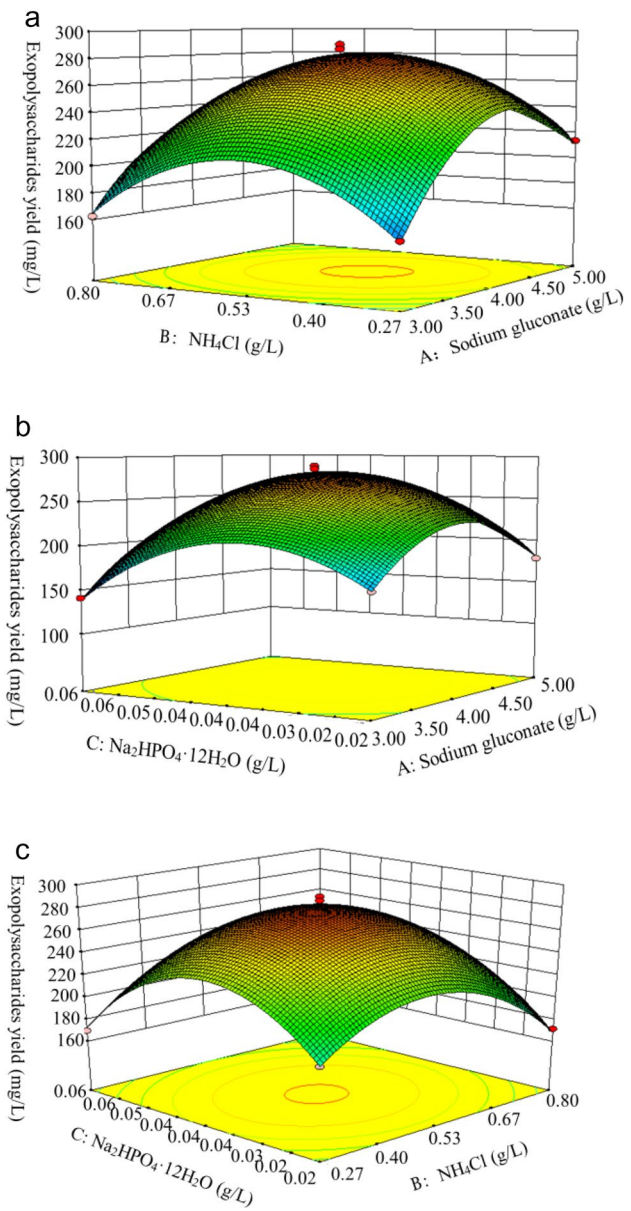

Fig. 2 3D response surface plots demonstrating the effect of **a** NH_4Cl and sodium gluconate, **b** $Na_2HPO_4{\cdot}12H_2O$ and sodium gluconate and **c** $Na_2HPO_4{\cdot}12H_2O$ and NH_4Cl on the exopolysaccharides yield from *Cupriavidus pauculus* 1490

FTIR Spectrum Analysis

The FTIR spectrum of EPS produced by *C. pauculus* 1490 was shown in Fig. 3. A strong and broad peak at 3424.13 cm^{-1} corresponds to stretching vibration of –OH, which was mainly caused by the glycoside hydroxyl in polysaccharides [18]. The peak at 2921.81 cm^{-1} represents C–H stretching vibration [19]. The bands in the region of 1734.20 and 1607.40 cm^{-1} are assigned to C=O stretching vibration, implying the existence of uronic acid [20]. The peak at 1420.75 cm^{-1} is attributed to C-H bending

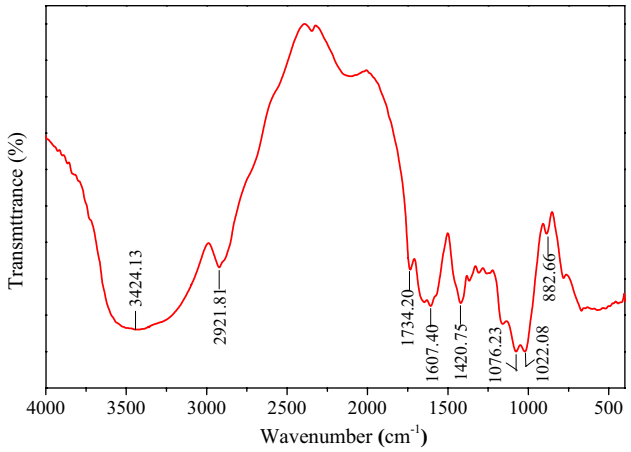

Fig. 3 FTIR spectrum of exopolysaccharides produced by *Cupriavidus pauculus* 1490

vibration. The peaks at 1076.23 and 1022.08 cm^{-1} are associated with the dissymmetry stretching vibrations of C–O–C from pyranose ring [21]. The C–H bond in β-style has an absorption peak nearby 882.66 cm^{-1} [22].

Monosaccharide Composition

Monosaccharide composition analysis was conducted by HPLC–DAD through comparing with standard monosaccharides. As displayed in Fig. 4, six standard monosaccharides were separated totally within 30 min. In the same condition, the sample from *C. pauculus* 1490 was analyzed by matching their retention time with standard monosaccharides. The results indicated that EPS were composed by Man, GlcUA, Glc and Xyl. Glucose was always found to be the most abundant monosaccharide even from different species in literatures [23–25]. Xylose commonly existed in plant and fungi, like *porphyra haitanensis* [15], *Trichoderma kanganensis* [26] and so on. There are still a very few reports about xylose appearing in bacteria. For example, two purified polysaccharides components from *Paenibacillus mucilaginosus* GIM1.16 both contained xylose [27]. These dissimilarities reflect the species specific production and biotechnological potential of polysaccharides [28].

Rheological Characterization

The shear stress as a function of the shear rate of 1% Xanthan gum and EPS solution is shown in Fig. 5a. The rheogram of EPS solution presented a similar trend comparing with Xanthan, which was non-Newtonian fluid and displayed the characteristics of peudoplastic fluid [29, 30]. While the shear stress of EPS solution at all given shear rates was

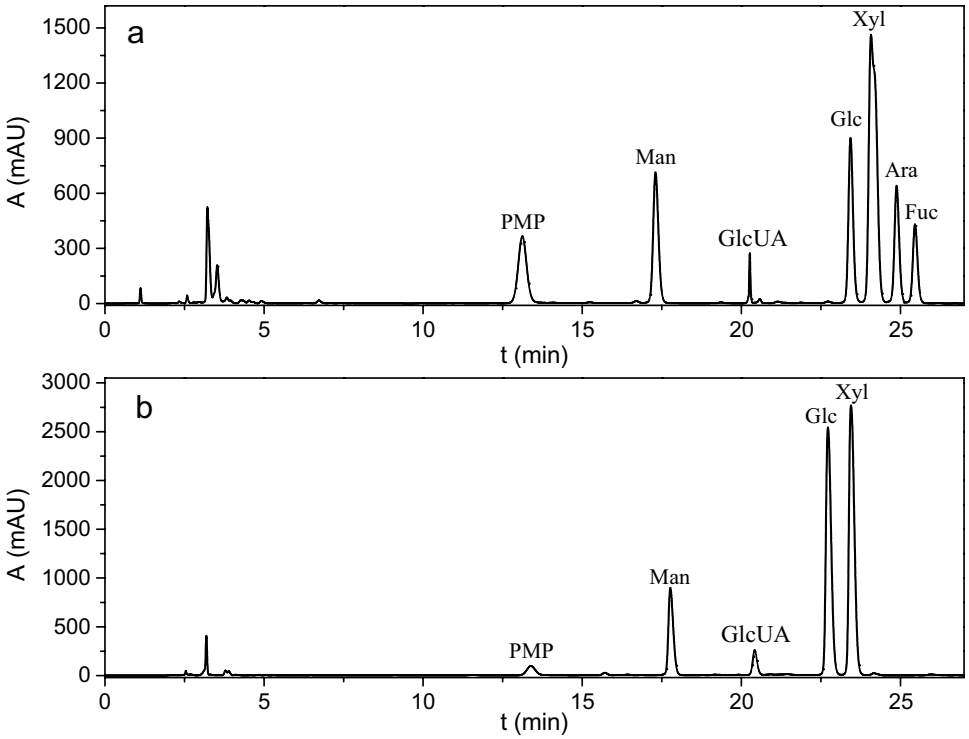

Fig. 4 The chromatogram of PMP derivatives of the standards monosaccharide (**a**) and exopolysaccharides from *Cupriavidus pauculus* 1490 (**b**) by HPLC–DAD

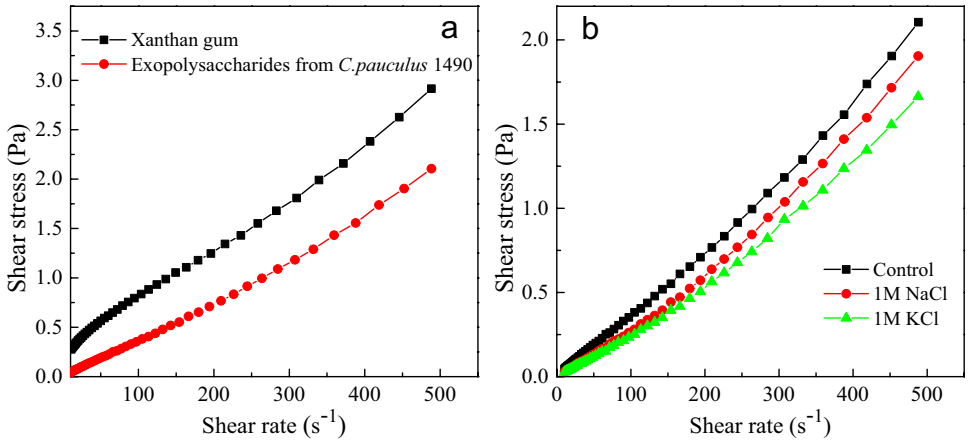

Fig. 5 Rheological test: Shear stress versus shear rate curve of 1% Xanthan gum and exopolysaccharides from *Cupriavidus pauculus* 1490, **b** Shear stress versus shear rate curve of 1% exopolysaccharides after treated by NaCl and KCl, respectively

lower than Xanthan, suggesting the viscosity of EPS solution was still weaker than Xanthan. The treatment by NaCl and KCl both weakened the shear stress of EPS solution, that is, its rheological property was affected because of the addition of salts (Fig. 5b). According to Diaz's report, the influence of salts depended on the bacterial species, the cultivation and the type of salts, for example, the viscosity of xanthan increased in some case, in others the viscosity decreased [31].

Antioxidant Activities

Hydroxyl radical (OH·) is a highly reactive oxygen-centered radical and it attacks all proteins, DNA, polyunsaturated fatty acid in membranes, as well as almost any biological molecules it touches [32]. Therefore, removing hydroxyl radical is important for antioxidant defense. As illustrated in Fig. 6a, EPS showed dose-dependent hydroxyl radical scavenging activity in the tested concentrations. At the concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/mL, the scavenging activity of EPS was 8.2%, 26.4%, 38.4%, 40.9% and 52.3%, respectively. The scavenging efficiency of polysaccharides from *Ganoderma lucidum* on hydroxyl radical only reached 15.79% at 0.5 mg/mL [33]. EPS from *Neopestalotiopsis* sp. strain SKE15 exhibited scavenging effect of 35.45% and 43.3% at 2 and 4 mg/mL, respectively [34]. The data presented here imply that EPS produced by *C. pauculus* 1490

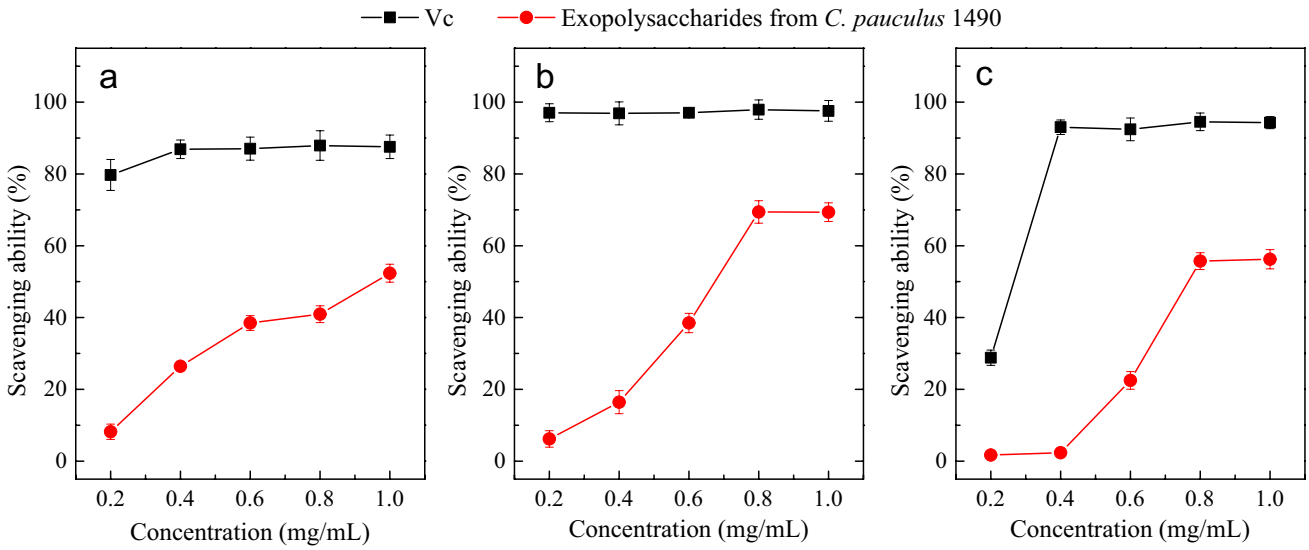

Fig. 6 Antioxidant activity of exopolysaccharides from *Cupriavidus pauculus* 1490 with various methods: **a** scavenging activity of hydroxyl radicals, **b** scavenging activity of DPPH radicals, **c** scavenging activity of superoxide anion radicals

have the potential to be exploited as prospective radical scavengers.

DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants [35]. As displayed in Fig. 6b, the scavenging effect of Vc on DPPH radical kept high level in all tested concentrations. EPS gradually took effect on scavenging DPPH radical with the increase of dosage. The maximum DPPH radical scavenging activity was reached at 0.8 mg/mL for EPS (69%) and Vc (97.9%), respectively. It's speculated that the antioxidant activity of polysaccharides may be originated from the ratio of monosaccharide and their side chain linkages [36]. A water-soluble polysaccharide (SSPP11) from *Schisandra sphenanthera* showed 45% DPPH radical scavenging activity at the concentration of 1.5 mg/mL, and consisted of Man, Glu and Gal with molar ratio of 1:1.77:1.29 [25], which was very different from our EPS component.

Superoxide radical is a highly toxic species generated by abundant biological and photochemical reactions [37]. Scavenging activities of EPS and Vc on superoxide anion radical was shown in Fig. 6c. The scavenging ability of EPS increased significantly from 2.4% at 0.4 mg/mL to 55.7% at 0.8 mg/mL. Vc has reached 93% at 0.4 mg/mL and maintained high level throughout the subsequent concentrations. EPS secreted by *Bacillus* sp. S-1 also possessed quenching ability on superoxide in a dose-dependent way from 0.5 to 7 mg/mL [38]. It has been reported that polysaccharides may act as hydrogen donor to superoxide anion radicals due to weak dissociation energy of O–H bond [39].

Conclusions

Response surface methodology was carried out to optimize EPS yield from *C. pauculus* 1490. Sodium gluconate, NH_4Cl and $Na_2HPO_4{\cdot}12H_2O$ were selected as the significant factors firstly based on single factor experiments. Thereafter, the individual optimal concentration was further obtained as 4.15 g/L, 0.52 g/L and 0.04 g/L, respectively, by a central composite design. FTIR spectrum displayed that abundant functional groups, such as –OH, C=O and C–O–C, existed in EPS. Monosaccharide analysis indicated the EPS mainly consisted of mannose, glucuronic acid, glucose and xylose. The EPS also exhibited antioxidant activity on hydroxyl, DPPH and superoxide anion radicals in vitro in a concentration-dependent manner, implying that EPS secreted by *C. pauculus* 1490 could be regarded as a potential candidate for antioxidant agent. Understanding the molecular structure of EPS and revealing the relationship between structure and activity to further improve its antioxidant capacity will be the focus of future work.

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Author Contributions MCX, SSZ: conceptualization, experimental investigation, writing, reviewing, and editing; LS, RLY, YDL, JKL, XLW, MC, WMZ: reviewing and editing; LS, GZQ, WMZ: funding acquisition.

Data Availability The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All authors approved.

Consent to Participate All authors approved.

Consent for Publication All authors approved.

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