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ORIGINAL ARTICLE

Production and partial characterization of the exopolysaccharide from *Pleurotus sajor caju*

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Abstract

Purpose Microbial exopolysaccharides (EPSs) are very important because they are used in biotechnological applications in different industrial areas. The aim of the study was to determine the best EPS producer *Pleurotus* sp., to optimize EPS production and to perform partial purification and characterization of the produced EPS.

Methods After the production conditions were optimized, the EPS was isolated and partially purified. EPS was characterized by HP-TLC, ^{1}H-NMR, FT-IR, and TGA. Hydroxyl, superoxide, and DPPH radical scavenging activities of the EPS were also investigated spectrophotometrically.

Result The best EPS producer and its incubation period in submerged fermentation were determined as *Pleurotus sajor caju* and on 5 days, respectively. Culture conditions to increase EPS production were optimized as follows (in per liter): 90 g of glucose, 10 g of yeast extract, 10 g of peptone, and 100 mM of Mg^{2+}. The optimal initial pH, temperature, and an agitation rate of culture were determined as 5.0, 25 °C, and 150 rate min^{-1}, respectively. The highest EPS production was determined as 33.32 ± 1.6 g L^{-1}. After isolation of EPS, one active fraction was obtained by gel filtration chromatography. EPS is composed mainly of glucose according to HP-TLC analysis.

Conclusion To the results, EPS had a complex structure by having carbohydrate and protein contents. The produced EPS had high degradation temperature as well as high antioxidant activity.

Keywords *Pleurotus sajor caju* · Submerged fermentation · EPS production · EPS characterization · Antioxidant activity

Introduction

Polysaccharides, a group of valuable biopolymers, have applications at a wide range of industrial fields, like food and pharmaceuticals. In recent years, extracellular polysaccharide (EPS) production has been prominent with numerous fungal submerged cultures since it has biological and pharmacological activities such as anti-tumor, antioxidant, and hypoglycemic activities (Han et al. 2006; Song et al. 2008; Li et al. 2010; Zhao et al. 2012; Cao et al. 2018). Polysaccharides are important components that determine the hardness and morphological characteristics of the fungal cell wall and which can be excreted into the culture medium depending upon the culture conditions (Gern et al. 2008). Many microorganisms synthesize EPS in the form of amorphous loam that binds to the cell surface or is present extracellularly. EPSs help the cell in a wide variety of functions. EPSs provide protection against biotic stress and protection against abiotic stresses that may include nutrient limitation, temperature, light intensity, or pH. For example, in acidophilic or thermophilic species, EPSs help to adapt to extreme conditions (Antón et al. 1988).

The genus *Pleurotus* is widely cultivated and commercialized in the world. It involves many biologically active components, such as polysaccharides, proteins, enzymes, dietary fibers, and vitamins (Wang et al. 2001). Large amounts of biomass and EPS production, which can be used for biotechnological areas, occur in the submerged cultures of the *Pleurotus* species. For instance, water-soluble polysaccharide of *Pleurotus ostreatus* could be a potent immune stimulant for use in nutraceuticals or medicine against both pathogens and

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cancer (Ooi and Liu 2000). However, EPS of *Pleurotus pulmonarius* showed in vitro antioxidant activity (Shen et al. 2013). Likewise, it was observed that EPS produced via *Pleurotus eryngii* has antioxidant and antitumor activities (Jing et al. 2013). A few works on EPS production have also been carried out by submerged fermentation (SmF) of *Pleurotus* spp. (Silveira et al. 2015). To improve EPS production, some nutritional factors have been investigated on the SmF of fungi.

The purpose of this study is to achieve high efficiency EPS production in SmF using white-rot fungi *Pleurotus* spp. In the first step of this study, the best producer *Pleurotus* strain and its incubation period were determined. In the next step, effects of carbon and nitrogen sources, and concentrations, Mg^{2+} ion concentration, pH, temperature, and agitation rate were investigated to optimize EPS production conditions in SmF. After production at an optimum condition in SmF, EPS was extracted and partially purified by Sepharose CL-6B column. In the last step of the study, partial purified EPS was characterized by chromatographic (TLC), spectroscopic (FT-IR and ^{1}H-NMR), and TGA analyses. Also, antioxidant features, like hydroxyl, superoxide, DPPH radical scavenging activities, reducing power, and chelating activity of the partial purified EPS, were investigated.

Materials and methods

Maintenance of microorganisms

Four different *Pleurotus* strains were used in the study: *Pleurotus djamor* (Rumph. Ex Fr.) Boedijn (MCC15), *P. ostreatus* (Jacq.) *Pleurotus* Kumm. (MCC16), *Pleurotus sajor caju* (Fr.) Singer (MCC29), and *P. eryngii* (DC.) Gillet (MCC58). *Pleurotus djamor*, *P. ostreatus*, and *P. sajor caju* were maintained on potato dextrose agar (PDA) (39 g L^{-1}, pH 5.6) at 25 °C for 7 days; *P. eryngii* was grown on malt extract:peptone:agar (MPA) (30:3:15 g L^{-1}, pH 5.6) at 25 °C for 14 days (Akpinar and Ozturk Urek 2017).

Production, optimization, and isolation of EPS

Pleurotus spp. were grown on PDA and MPA slants and then transferred to basal medium by 1 cm^2 of the five agar plates with a sterilized cutter. Submerged fermentation was performed in 250-mL Erlenmeyer flasks including 100 mL of basal medium. The basal medium composition was as follows in (g L^{-1}): glucose, 10; NH_4NO_3, 0.724; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 1.0; KCl, 0.5; yeast extract, 0.5; $FeSO_4.7H_2O$, 0.001; $ZnSO_4.7H2O$, 0.0028; $CaCl_2.2H_2O$, 0.033; peptone, 10 (Bazalel et al. 1997). The medium pH was adjusted to 6.0 and sterilized at 121 °C for 20 min then cooled. Inoculated flasks were incubated on the rotary shaker at 150 rate min^{-1} and 25 °C for 2 weeks.

To increase EPS production, sucrose, glycerol, and sorbitol as 10 g L^{-1} were used as carbon source instead of glucose. The optimum concentration was determined after glucose was identified as the best carbon source. The effects of varying nitrogen sources and concentrations, such as peptone, yeast extract, ammonium nitrate, and urea, on EPS production were investigated. Then, different concentrations of Mg^{2+} ion (4, 7, 10, 30, 50, 100, 250 mM), various pH levels (3, 4, 5, 6, 7, 8, 9), different fermentation temperatures (20, 25, and 30 °C), and agitation rate (100, 150, and 180 rate min^{-1}) were investigated. All samples were harvested on the 5th day of incubation and analyzed for EPS production.

EPS samples taken from the flasks were centrifuged at 10000×*g* for 20 min, and then, the upper phase was filtered using a 0.45-μm membrane filter. The supernatant was used for analysis below. The dry weight of cell (CDW) was measured after washing the mycelial pellet with distilled water and drying at 70 °C to obtain invariable value. For EPS isolation, the obtained culture filtrate was mixed with four volumes of absolute cold ethanol, admixed vigorously, and put for 24 h at 4 °C. The precipitated crude EPS was obtained by centrifugation at 10000×*g* for 20 min and then dried at 60 °C overnight to obtain constant value.

Protein content of cell-free supernatant of production media was determined by Bradford (1976) using bovine serum albumin standard. The content of reducing sugar and nitrogen was determined by DNS method and phenol-hypochlorite assay, respectively (Miller 1959; Weatherburn 1967).

Partial purification and characterization of produced EPS

EPS precipitated with ethanol was handled with Sevag reagent (1:4 n-butanol/chloroform, *v*/*v*) (Staub 1965). After centrifugation, water phase was ultra-filtered (10 kDa) and filtrate was admixed with absolute cold ethanol; the precipitated EPS was centrifuged and dried. The EPS (100 mg) was disintegrated in 0.2 M NaCl buffer, and fulled onto a Sepharose CL-6B column (1.6 cm × 90 cm). The column was eluted with this buffer at a flow rate of 0.6 mL min^{-1}. Five dextran molecules (25000, 80000, 270000, 670000, and 1100000 Da) were used as molecular-weight standards. The dextran standards (2.5 mg) were prepared in 0.2 M NaCl buffer, and loaded. The protein level in the gel fractions was measured at 280 nm, while the carbohydrate level was recorded at 490 nm.

The total carbohydrate, protein, uronic acid, and pyruvate contents of the EPS were investigated by the phenol-sulfuric acid method, Bradford method, borate-sulfuric acid-carbazole assay, and 2,4-dinitrophenyllhydrazine assay, respectively (Dubois et al. 1956; Healy et al. 1996; Friedemann and Haugen 1943).

To determine monosaccharide composition of the partial purified EPS, standard sugars (glucose, mannose, galactose, rhamnose, and xylose) were analyzed by HP-TLC (CAMAG). Ethyl acetate:acetic acid:water (2:2:1) was used as mobile phase on TLC silica gel 60 F254 plates (MERCK, Germany) with solution as staining reagent (2% naphtoresorcinol in 10% ethanolic phosphoric acid). EPS (50 mg) was hydrolyzed with 2 N H_2SO_4 (4 mL) at 100 °C in oil bath for 2 h, then it was neutralized with NaOH (1 g) and loaded on plates and analyzed.

For spectroscopic analysis, EPS, FT-IR, and ^{1}H-NMR were used. The FT-IR spectrum was monitored on the Perkin Elmer Spectrum BX, in the 4000–400 cm^{-1} spectral region. For ^{1}H-NMR spectroscopy, the sample was recorded, as solution in dimethyl sulfoxide, on a MERCURY Plus-AS 400 spectrometer at 400 MHz and 30 °C. The chemical shifts were expressed in ppm.

A thermal gravimetric analysis of EPS was carried out with Perkin Elmer-Diamond TG/DTA. About 3–5 mg of dry EPS sample was loaded on a platinum pan and its energy level was scanned in the ranges of 30–600 °C under a nitrogen atmosphere with a temperature gradient of 10 °C min^{-1}.

Evaluation of antioxidant activity of partial purified EPS

Hydroxyl and superoxide radical scavenging activities of EPS were measured according to the methods of Halliwell et al. (1987) and Liu et al. (1997), respectively. DPPH and chelating activity methods were based on Kumaran (2006) and Decker and Welch (1990), respectively. The reducing power of EPS was detected to the method of Oyaizu (1986).

Statistical analysis

All experiments were carried out three times ($n = 3$) and repeated three times. Each value is the average of three parallel experimental studies. Data were given as mean ± standard deviation (SD). The data were assayed by analysis of variance (ANOVA) to identify the significantly different groups at $P < 0.05$ by one-way ANOVA test utilizing SPSS software statistical program (SPSS for windows ver. 21.00, USA).

Results and discussion

Optimization of fermentation conditions

EPS production capacities of four different *Pleurotus* spp. were investigated in order to determine the best EPS producer in the basal medium of SmF and the incubation day of maximum EPS production. They were incubated on the shaker incubator at 150 rates min^{-1} and 25 °C for 2 weeks. As shown in Fig. 1a, our results indicated that *P. sajor caju* was the best strain for the EPS production (2.62 ± 0.16 g L^{-1}, on the 5th day of incubation period). The maximum EPS productions by *P. eryngii*, *P. djamor*, and *P. ostreatus* were achieved on the 6th, 3rd, and 7th days of incubation period as 2.1 ± 0.35, 1.4 ± 0.3, and 1.2 ± 0.25 g L^{-1}, respectively. Our results showed that EPS production depends on microorganism's strain. Likewise, Nehad and El-Shamy (2010) reported that the yield of EPS produced by different fungal strains was different.

After the determination of the best EPS producer as *P. sajor caju*, the effects of incubation time and pH of the medium on EPS production were investigated. Figure 1 b shows that maximum EPS production was determined as 2.62 ± 0.16 g L^{-1} on the 5th day of incubation, while maximum CDW was obtained as 4.06 ± 0.4 g L^{-1} on the 7th day. pH value of SmF medium dependent on incubation period was not a significant change up to 7 days, and then it slightly increased to 8.17 ± 0.07 on the 12th day.

Chemical composition analyses of SmF medium were carried out during the incubation period (Fig. 2). Maximum protein and nitrogen levels were detected as 78.09 ± 3.2 ppm and 459.9 ± 17 ppm on the 9th and12th days of incubation, respectively. Level of reducing sugar on the 2nd day of incubation was determined as 593.4 ± 29 ppm and limited conditions were reached on the 5th day.

After the determination of the best EPS producer as *P. sajor caju* and the incubation day (5th) for EPS production

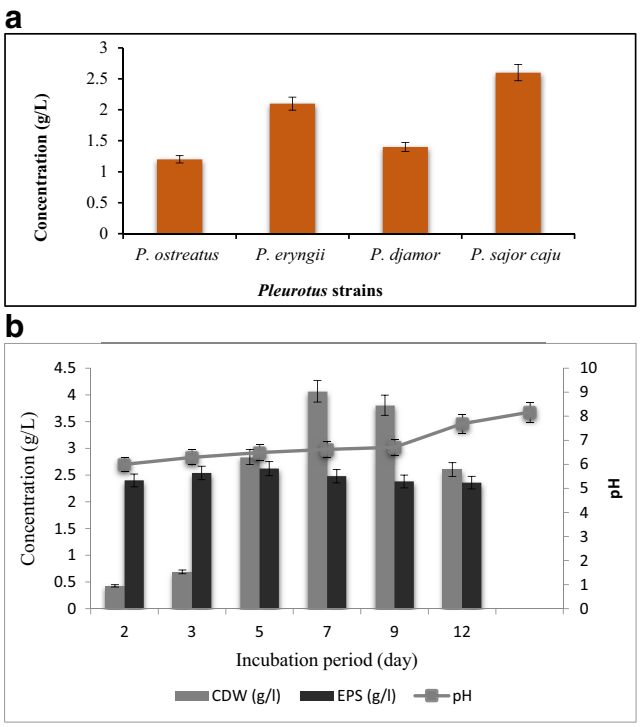

Fig. 1 **a** Production of EPS by *Pleurotus* strains. **b** Production of EPS by *P. sajor caju* and CDW levels during incubation period. The values are mean ± SD of three separate experiments

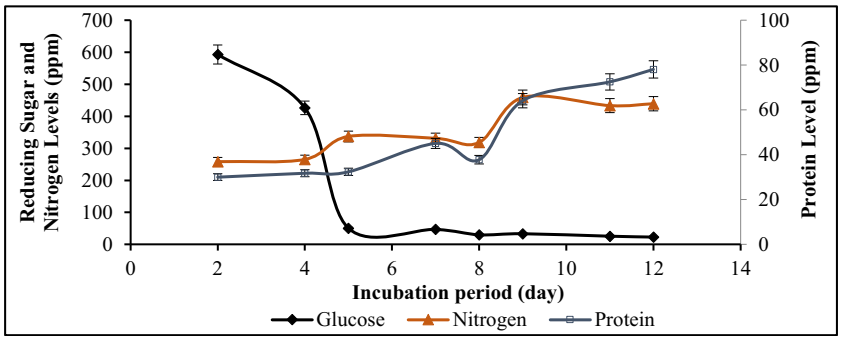

Fig. 2 Glucose, nitrogen, and protein levels of medium during incubation period of *P. sajor caju*. The values are mean ± SD of three separate experiments

according to the yield of EPS, components of the SmF medium and fermentation conditions were optimized. To find out the most appropriate carbon source in EPS production by *P. sajor caju*, three different carbon sources named sorbitol, sucrose, and glycerol were separately provided at 10 g L^{-1} instead of glucose employed in the basal medium. Betwixt the investigated carbon sources, maximum EPS production and CDW were obtained in glucose medium as 2.62 ± 0.16 g L^{-1} and 2.83 ± 0.14 g L^{-1}, respectively (Fig. 3a).

After selecting glucose as the best carbon source for EPS production, the optimum concentration of glucose was determined for EPS production (Fig. 3b). For this purpose, various glucose concentrations were used in medium and the results showed that optimum glucose concentration for EPS production (3.97 ± 0.15 g L^{-1}) and CDW (6.03 ± 0.32 g L^{-1}) was 90 g L^{-1}.

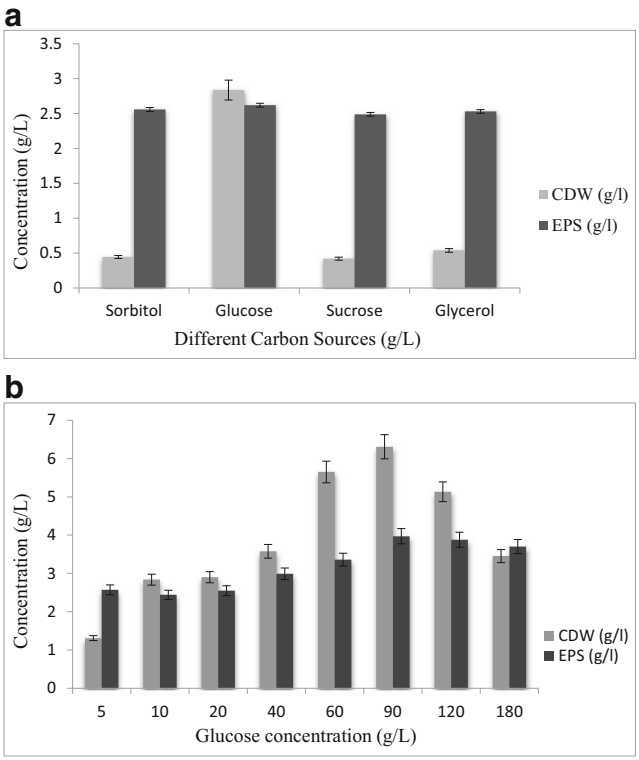

Fig. 3 **a** Effect of carbon sources on EPS production by *P. sajor caju*. **b** Effect of glucose concentration on EPS production by *P. sajor caju*. The values are mean ± SD of three separate experiments

The carbon source is required for energy and structural molecules. Therefore, the concentration and type of carbon source are important for all bioprocesses. The carbon source and its concentration have a major impact on the production and yield of EPS. Glucose, sucrose, maltose, lactose, fructose, galactose etc. are generally used as carbon sources in the culture medium (Mahapatra and Banerjee 2013). In most studies, glucose, sucrose, and maltose have been chosen as the most necessary carbon source for the fungal EPS production (Kim et al. 2005; Zhang and Cheung 2011). These results suggest that various carbon sources may have some catabolic repression effects in various EPS syntheses. It is also an indication that different fungal strains have different carbon source intake characteristics or that these carbon sources can be easily utilized by fungi. Furthermore, the structure and concentration of the carbon source used can regulate secondary metabolism, such as catabolic repression (Görke and Stülke 2008).

In this study, the capability of *P. sajor caju* to use various nitrogen sources for EPS production was analyzed. For this purpose, *P. sajor caju* cells were grown in basal medium containing glucose (90 g L^{-1}) as a carbon source, and four diverse nitrogen sources, namely, peptone, yeast extract, ammonium nitrate, and urea, were added at various concentrations. Figure 4a shows that maximum EPS production was achieved in 10 g L^{-1} peptone, 10 g L^{-1} yeast extract, and 0.724 g L^{-1} NH_4NO_3 as 4.01 ± 0.38 g L^{-1}, 3.56 ± 0.3 g L^{-1}, and 3.09 ± 0.27 g L^{-1}, respectively. Figure 4b indicates that maximum CDW was determined in 12 g L^{-1} peptone and 10 g L^{-1} yeast extract medium as 5.93 ± 0.5 g L^{-1} and 8.51 ± 0.77 g L^{-1}, respectively. Our findings are similar to those of other researchers, which show comparatively low mycelial growth and EPS production with inorganic nitrogen sources compared with organic nitrogen sources (Yang et al. 2003). Similarly, nitrogen concentrations between 1 and 10 g L^{-1} are sufficient for fungal EPS production (Yuan et al. 2012).

Mg^{2+} concentration effects on EPS production and mycelial growth were investigated (Fig. 5). Addition of Mg^{2+} to the culture medium may increase EPS production. The maximum amount of 31.08 ± 3.0 g L^{-1} EPS was obtained with the

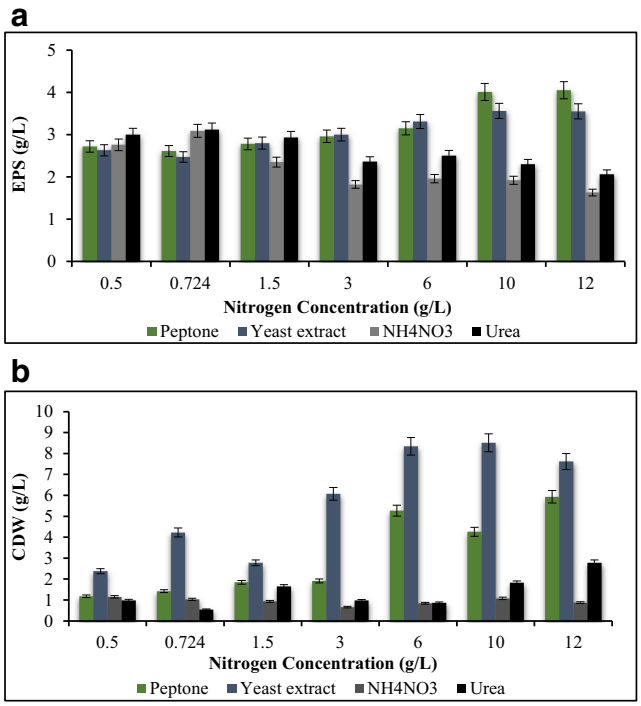

Fig. 4 **a** Effect of nitrogen sources and concentrations on EPS production and **b** CDW by *P. sajor caju*. The values are mean ± SD of three separate experiments

supplementation of 100 mM $MgSO_4.7H_2O$. The maximum CDW (19.25 ± 1.89 g L^{-1}) was achieved in 50 mM Mg^{2+}. Therefore, 100 mM $MgSO_4.7H_2O$ was selected as the best concentration for the EPS production by *P. sajor caju*. This can be explained by the fact that sulfates may also have effects on EPS production (Liu and Miao 2017). Raposo et al. (2014) expressed that the highest yield of EPS was observed when 21 mM $MgSO_4$ was added to the culture medium of *Porphyridium cruentum*; the total yield of EPS in microalgae cultivation suggests that it may be affected by varying $MgSO_4$ concentrations. Many researchers have deemed this bio-element to be suitable for mycelial growth and EPS production, usually in liquid cultures of several basidiomycetes (Chardonnet et al. 1999; Hwang et al. 2003). It is known that cations affect EPS synthesis both qualitatively and quantitatively. Mg^{2+} is required for all fungi and used as a cofactor in many enzymatic reactions. It is also involved in the

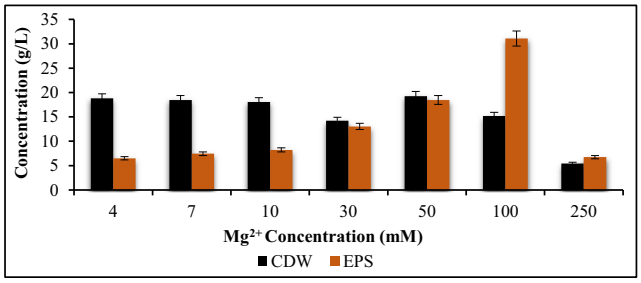

Fig. 5 Effect of different Mg^{2+} ion concentrations on EPS production by *P. sajor caju*. The values are mean ± SD of three separate experiments

stabilization of the plasma membrane, and its uptake into the cell is also dependent on ATP.

To study the effect of the initial culture pH on EPS production, *P. sajor caju* was cultivated in SmF with various starting pH (3.0–9.0); after 5 days, it was analyzed for EPS production (Fig. 6a). The results showed that the optimum pH for EPS production (33.32 ± 1.6 g L^{-1}) and CDW (7.49 ± 0.5 g L^{-1}) was 5 and 7, respectively. In general, fungi preferred low pH for EPS production with a range between pH 3.0 and 6.5 (Feng et al. 2010). In order to determine the optimum temperature, *P. sajor caju* was cultivated at different temperatures in the shaking incubator for 5 days; the optimal temperature for EPS production was detected at 25 °C (Fig. 6b). The maximum CDW was achieved at 30 °C. Similarly, many microorganisms produce maximal amounts of EPS at temperatures slightly lower than optimal growth as observed for *Klebsiella* sp. (Farres et al. 1997). Many researchers have reported different optimal temperature values for EPS production; changes in environmental factors appear to give rise to

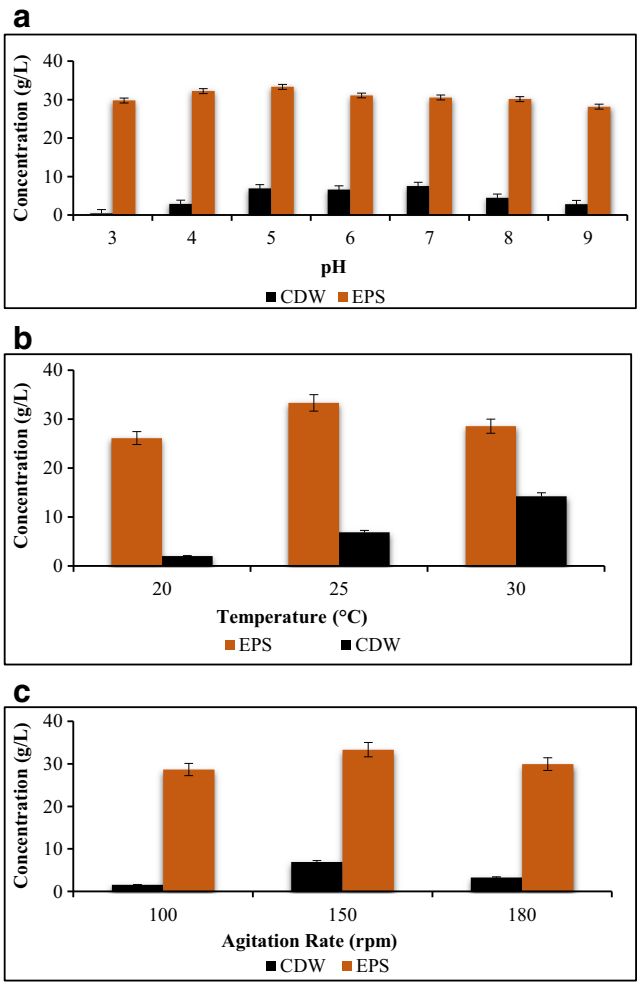

Fig. 6 The effect of **a** initial pH, **b** temperature, and **c** agitation rate on EPS production and CDW by *P. sajor caju*. The values are mean ± SD of three separate experiments

some changes in EPS efficiency, contingent upon the type of fungus (Ruas-Madiedo et al. 2002). To find the agitation rate effect on the EPS production, *P. sajor caju* was cultivated in a medium with various agitation rates (100, 150, 180 rate min^{-1}). Figure 6c shows that maximum EPS production and CDW were obtained at an agitation rate of 150 rate min^{-1}. Additionally, aeration and agitation are important factors to ease the oxygen transfer and enable nutrient transport to microorganisms. The presence of oxygen and the rate at which the culture medium is admixed might have a direct effect on the production polysaccharide, and an increase in EPS production often results from the increase in oxygen supply (Dassy et al. 1991; Bayer et al. 1990). The EPS surrounding the cell can serve as a barrier for oxygen and nutrient transfer. Admixing culture at high rates can increase the availability of both nutrients and oxygen.

The obtained results showed that the EPS yield was the highest when the concentrations of glucose, yeast extract, peptone, and Mg^{2+} were 90, 10, 10 g L^{-1}, and 100 mM, respectively. Thus, the yield of EPS in the optimized culture medium was found to be about 13.33 times higher than that obtained when the basal culture medium was used. According to the optimized conditions, the highest EPS was 33.32 ± 0.3 g L^{-1}. The EPS obtained in this study is 6.45 times more than that produced by *P. eryngii* and 5.24 times more than *P. pulmonarius* (Jing et al. 2013; Shen et al. 2013). To Osińska-Jaroszuk et al. (2015), the maximum produced EPS yield from Ascomycota and Basidiomycota fungi ranges from 0.12 to 42.24 g L^{-1}, which is mostly dependent on the strain and culture conditions used.

Recently, it is important to investigate the chemical composition and physicochemical properties of EPS produced since the introduction of natural polymers for industrial applications increases the interest in EPS production. After optimization of EPS production conditions, the produced EPS was isolated and its chemical characterization was assayed. The chemical composition of 1 mg isolated EPS was formed by 2.26 ± 0.41 μg protein, 219.75 ± 10 μg total carbohydrate, 28.59 ± 0.32 μg reducing sugar, 15.73 ± 1.1 μg nitrogen, 1.01 ± 0.01 μg pyruvate, and 6.7 ± 0.5 μg uronic acid. Results of chemical composition analyses demonstrated the complex structure of produced EPS.

Isolation and partial purification of EPS

The produced EPS in optimum SmF conditions by *P. sajor caju* of 90 g glucose, 10 g peptone, 10 g yeast extract, 100 mM Mg^{2+} per liter, pH 5, at 25 °C, and 150 rpm was isolated. Isolated EPS was mixed with Sevag reagent then ultrafiltrated and applied on gel filtration chromatography (Sepharose CL-6B). Active one fraction of EPS (Fr-I), which consisted of polysaccharides and proteins, was co-eluted. Fr-I was revealed to be glycoprotein based on preliminary data. A similar result was reported by Shen et al. (2013), and one fraction was isolated by purification of polysaccharide from *P. pulmonarius*. Many research findings indicated that in EPS produced by fungi, glycoprotein generally occurs (Hwang et al. 2003; He et al. 2012).

The molecular weight of the produced EPS was detected as the low-molecular-weight EPS of approximately 25 ± 3 kDa. Similar observations were made by Oh et al. (2007) and Cheng et al. (2011) in other fungi fermentations. The molecular weight of EPS produced by *P. sajor caju* (Fr.) Singer (CCB 019) was determined as 6.4×10^4 g mol^{-1} according to Silveira et al. (2015). Differences in Mw may indicate differences in the methods used in isolation and production procedures, or for the determination of Mw.

Partial characterization of EPS

HP-TLC is an effective chromatographic method for detecting monosaccharide composition. On HP-TLC analysis, the Rf values of the standards were as follows: glucose 0.456 ± 0.04, galactose 0.445 ± 0.05, mannose 0.503 ± 0.05, xylose 0.551 ± 0.06, and rhamnose 0.563 ± 0.04 (Fig. 7a). Our results showed that Fr-I mainly included glucose. Many fungal EPSs are composed of glucose monosaccharide (Lin and Chen 2007). The monosaccharide composition was very important to determine the produced EPS function.

The FT-IR spectrum is a method used to detect functional groups and characterize covalent binding information. Typical FT-IR spectrum for Fr-I is presented in Fig. 7b. Fr-I displayed a stretching intense distinctive peak at approximately the region of 3388 cm^{-1} for the carbohydrate ring. Small band at 2948 cm^{-1} was assigned to the stretching vibration of the methylene group (C-H), commonly found in hexoses. A distinguishing peak absorption band emerged at 1638 cm^{-1} was ascribed to the stretching vibration of the carboxyl group (C=O). A meager peak about 1110 cm^{-1} corresponding to the glycosidic link bond (C-O-C) was determined in the spectrum of the polysaccharide (Copikova et al. 2001). The stretching vibration peak of 1040 cm^{-1} implied the presence of C-H-O linkage position. The weak absorption peak at 985 has coincided to N-H of primary amine. The FT-IR spectrum showed that proteins and polysaccharides were present in EPS composition. Additionally, it was determined that $MgSO_4$ did not bear an impact on the structure of EPS in the FT-IR spectrum. This data was also supported by Liu and Miao (2017).

According to the ^{1}H-NMR spectrum of Fr-I, signals at 2.2–2.8 ppm were ascribed to peaks of C-H (data not shown). The C-C signals were established in the region 3.0–4.2 ppm, which were ascribed to protons of the C-2-C-6 glycoside ring of hexoses. The NH signals at 7.1–7.5 ppm demonstrated amino acid and peptide structure. The obtained results demonstrated that EPS produced by *P. sajor caju* had functional groups, bonds, and structures which are present in

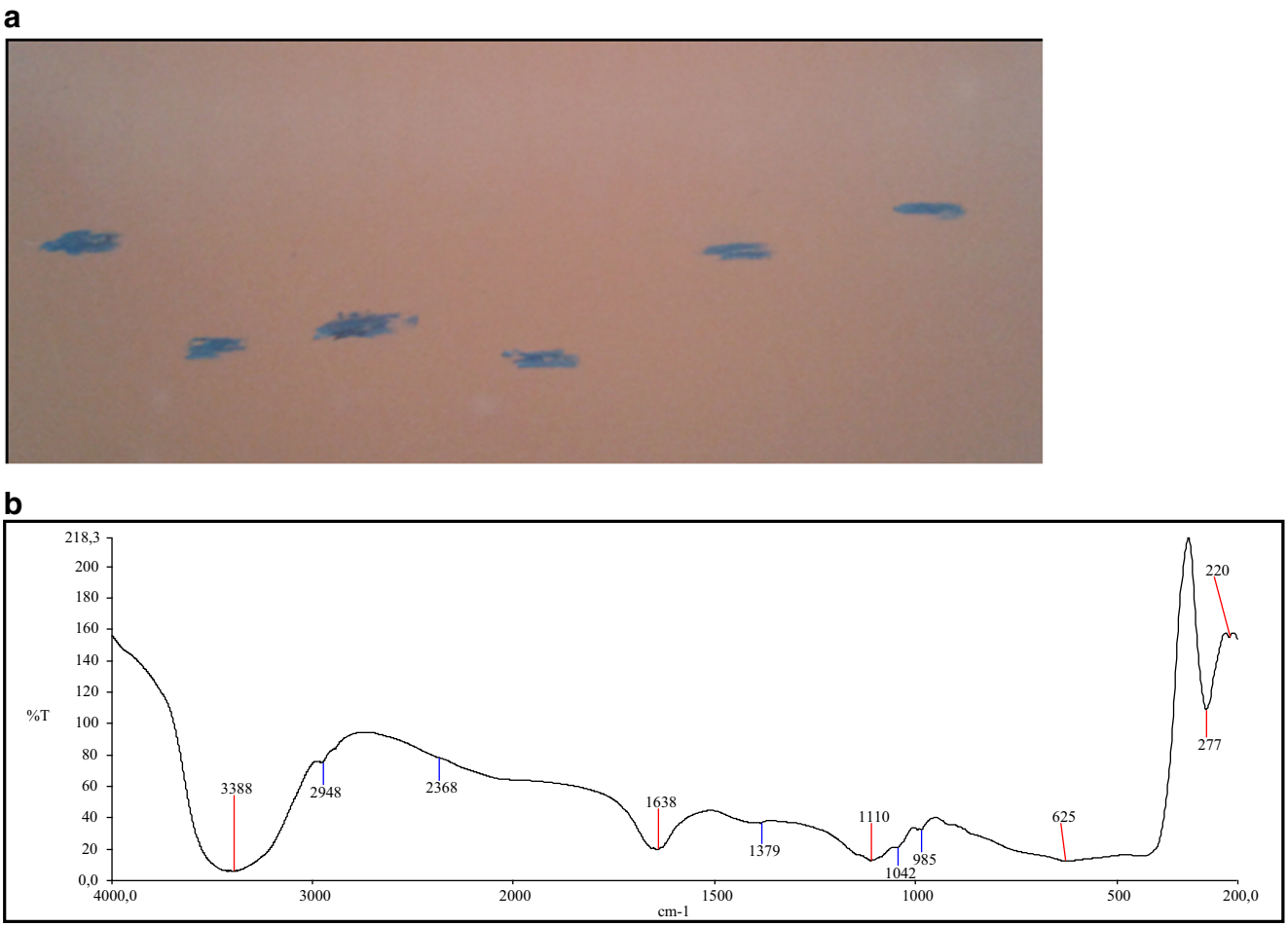

Fig. 7 **a** HP-TLC analysis of EPS and standards. Solvent system-ethyl acetate butanol:acetic acid:water (2:1:1), spray reagent; 2% naphtoresorcinol in 10% ethanolic phosphoric acid. Spot (2) Fr-I; (1), (3), (4), (5), and (6) mannose, glucose, galactose, xylose, and rhamnose, respectively. **b** FT-IR spectrum of EPS. The values are mean ± SD of three separate experiments

glycoprotein-type polysaccharide. It was deduced that EPS possessed a complicated structure with carbohydrate and protein contents.

The presence of thermal durability of the polysaccharide is a paramount property for its applications. TGA measures the mass change of the sample by temperature change and is a very effective system for analyzing samples with mass attain or loss throughout heating. Degradation of Fr-I occurred by two stages as noticed in TGA. At first part, 6.769% of weight decrement was documented from 38.16 to 78.74 °C owing to the loss of alcohol molecules and humidity. At the second step of degradation, 11.196% weight loss was observed between 169.94 and 369.44 °C. The decomposition temperature of Fr-I was around 276.91 °C. Degradation temperatures of the purified EPSs by *P. pulmonarius* and *Lactobacillus plantarum* MTCC 9510 were 217 and 260 °C, respectively (Shen et al. 2013; Ismail and Nampoothiri 2010). The complex structure of the EPS produced has been shown to provide high degradation temperature.

Antioxidant activities of partial purified EPS from *P. sajor caju*

Free radicals, especially hydroxyl radicals and other derived radicals, are highly potent oxidant molecules capable of reacting with many biomolecules in living cells and leading to significant biological detriment and lipid peroxidation.

As seen from Fig. 8, the high hydroxyl free radical scavenging activity of EPS increased from 10 ± 1.08 to 61.3 ± 5.4% in terms of concentration, which was higher than that of 50.8 ± 4.8% for *Boletus edudis*, 49.4 ± 4.2% for *Pholiota adipose*, and 26.2 ± 2.4% for *Antrodia camphorate* at 5 mg mL^{-1} (Lin et al. 2012). DPPH free radical is largely used to assess the free radical scavenging activities of the usual substance (Leong and Shui 2002). As shown in Fig. 8, the EPS had an obvious DPPH scavenging ability increase from 7.4 ± 0.3% to 41.83 ± 4.0% in terms of concentration. Radical scavenging activity of EPS was much higher than 20 ± 0.3% of *Boletus aereus* EPS (Zhen et al. 2014). The scavenging activities of superoxide radical of EPS produced by *P. sajor*

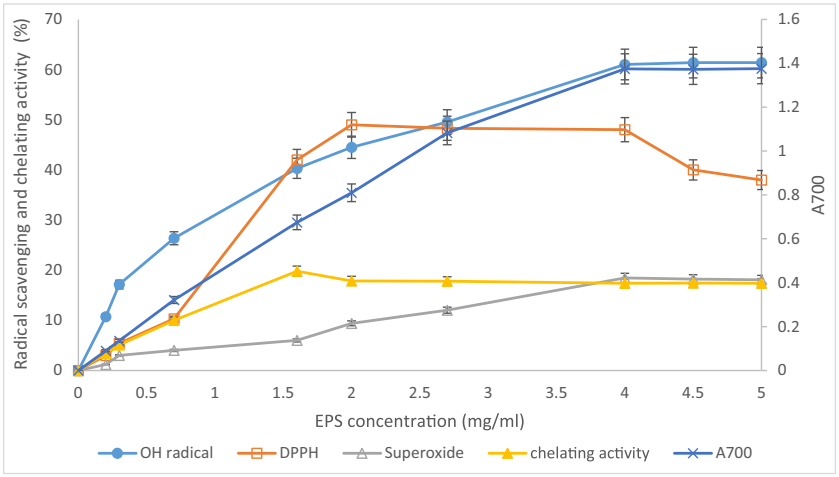

Fig. 8 OH, DPPH, O_2 radicals scavenging (%), and chelating activities (%) and reducing power (A_{700}) of produced EPS by *P. sajor caju*. The values are mean ± SD of three separate experiments

caju are shown to be dose-dependent in Fig. 8. The superoxide radical scavenging impact of EPS ranged from 1.31 ± 0.2% at 0.25 mg mL^{-1} to 18.83 ± 1.5% at 5 mg mL^{-1}, which was similar to *Cordyceps militaris* SU5-08 and *Bacillus edudis*. It has also been indicated that the decomposition energy of O-H bonds may be related to the superoxide anion scavenging mechanism (Tsiapali et al. 2001). The reducing power of compound may serve as a substantial sign of its potential antioxidant activity (Kumar et al. 2004). As shown in Fig. 8, our results indicated that EPS had greater reducing power. Compared with other studies, the reducing power of Fr-I at 3.5 g mL^{-1} was also higher than some researcher's data (Ma et al. 2013; Li et al. 2013; Gao et al. 2013). The iron-chelating ability of EPS was associated with sample concentration. The iron-chelating ability of EPS was determined as 19.8 ± 1.7% at 1.5 mg mL^{-1}. The obtained results show that the EPS has promising antioxidant capacities. This can be ascribed to the hydroxyl and other related functional molecules in EPS obtained from *P. sajor caju*. These groups can donate electrons to reduce radicals to a more stable form or react with free radicals to terminate the radical chain reaction (Nakiboglu et al. 2007).

Conclusion

In this study, the optimization of submerged culture conditions for EPS production by *P. sajor caju* was investigated. Production of high EPS of 33 ± 0.3 g L^{-1} was carried out in SmF. In addition, the concentration of produced EPS was also considerably higher than some of the results determined in other researches. One active fraction of EPS was obtained by gel filtration chromatography and characterized by HP-TLC, FT-IR, 1H-NMR, and TGA. According to the results, EPS had a complex structure by having carbohydrate and protein contents. The produced EPS had high degradation temperature as well as high antioxidant activity. The produced EPS was able to scavenge superoxide anion, hydroxyl, and DPPH radicals, and showed great potential as an antioxidant in vitro. This research contributes important knowledge about EPS production, which can be used as functional food, antioxidant, or a valuable compound for biotechnological applications, such as cosmetics, medicine, and pharmaceutics, from *P. sajor caju*.

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Compliance with ethical standards

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

Research involving human participants and/or animals Not applicable.

Informed consent Not applicable.

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