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Full Length Research Paper

Antioxidant properties of polysaccharides from *Laetiporus sulphureus* in submerged cultures

Ming-Yeou Lung* and Wei-Zhong Huang

Department of Chemical and Materials Engineering, Minghsin University of Science and Technology, Xinfeng, Hsinchu, Taiwan 30401, Republic of China.

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Intracellular polysaccharides (IPS) and exopolysaccharides (EPS) are isolated respectively from mycelia and filtrates of submerged culture by *Laetiporus sulphureus* in a 20-L stirred tank bioreactor with corresponding antioxidant properties investigated. Effective productions of IPS and EPS by submerged cultures of *L. sulphureus* are available. Number of average molecular weights (Mn) and protein/polysaccharide ratios are 1.29×10^6 Da and 8.24% for IPS and 3.95×10^6 Da and 2.38% for EPS. IPS shows an excellent antioxidant property in reducing power and scavenging effect on superoxide anion, whereas EPS has a powerful antioxidant activity in reducing power, chelating effect on ferrous ions and scavenging effect on superoxide anion, as evidenced by their quite low EC_{50} values below 5 mg/ml. Confirmed by the result is the potential antioxidant application of fermented *L. sulphureus* polysaccharides in health-promoting functional food and pharmaceutical industries.

Key words: *Laetiporus sulphureus*, intracellular polysaccharide, exopolysaccharide, antioxidant activity.

INTRODUCTION

Oxidation is utilized by living organisms to produce energy to fuel biological processes (Soares et al., 2009; Sun and Kennedy., 2010), but byproducts such as oxygen-derived free radicals and other reactive oxygen species (ROS) are released by physiological processes simultaneously (Shu and Lung, 2008) to make oxidative damages to bring about many diseases and degenerative processes in aging (Liu et al., 1997; Tsai et al., 2007). Many ROS-induced diseases related to pathological effects such as DNA damages, carcinogenesis, cancer, rheumatoid arthritis and cell degeneration associated with aging have been proven to cause human life damage (Sun and Kennedy, 2010; Chattopadhyay et al., 2010). Although almost all organisms can produce antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT) as defense tools against oxidative damages, these enzymes are insufficient to prevent the damage entirely (Shu and Lung., 2008; Turkoglu et al., 2007). Therefore, it is essential to develop some potential antioxidant supplements to reduce oxidative damages. It has been recently reported that many potential natural antioxidants are derived from lots of sources such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, herbs and mushrooms (Luo and Fang, 2008; Soares et al., 2009; Lee and Yoon, 2009).

Laetiporus sulphureus (Aphyllophorales, Polyporaceae) is a wood-rotting basidiomycete mushroom that causes heart-rot disease in deciduous trees and conifers (Imazeki and Hongo, 1998; Rogers et al., 1999). The fruiting bodies of *Laetiporus* species mushrooms contain N-methylated tyramine derivatives (List, 1958; Lee et al., 1975; Rapior et al., 2000), polysaccharides (Alquini et al., 2004; Olennikov et al., 2009a, 2009b), a number of lanostane triterpenoids, laetiporic acids and other compounds (Weber et al., 2004; Davoli et al., 2005; Radic et al., 2009). Many *L. sulphureus* polysaccharides have been isolated not only from fruiting bodies (Alquini et al., 2004; Olennikov et al., 2009a, 2009b), but also from submerged mycelia cultures (Hwang et al., 2008; Hwang and Yun, 2010) with verified and reported therapeutic evidences such as hypoglycemic effect on

*Corresponding author. E-mail: mylong@must.edu.tw. Tel: +886-3-5593142 ext. 3352. Fax: +886-3-6007577.

streptozotocin-induced diabetic rats (Hwang and Yun, 2010) and proliferation insulin secretary function of rat insulinoma RINm5F cells (Hwang et al., 2008).

Submerged culture is an efficient polysaccharide production approach from many mushrooms (Shu and Lung, 2008; Hwang and Yun, 2010). Antioxidant activity is one important bioactivity of bioactive compounds from *L. sulphureus* mushroom sources (Shu and Lung., 2008). It was reported that antioxidant properties of several mushroom poly-saccharides are related to their molecular weights and protein/polysaccharide ratios (Liu et al., 1997; Song et al., 2010). Several researchers have found that many types of polysaccharides produced by submerged cultures of mushrooms possess effective antioxidant properties (Liu et al., 1997; Tsai et al., 2007; Lee and Yoon, 2009). So far, there has been no report on antioxidant properties of polysaccharides from submerged culture of *L. sulphureus* and it is essential to develop possible polysaccharides-related antioxidants or pharmaceutical agents from *L. sulphureus* submerged cultures.

The study is accordingly aimed at the evaluation of *in vitro* antioxidant properties of IPS and EPS from *L. sulphureus* by submerged cultures in a 20-L stirred-tank bioreactor with several complementary test systems including reducing power, scavenging ability on radicals, chelating ability on ions and scavenging superoxide anions activity, to determine the antioxidant properties of *L. sulphureus* polysaccharides.

MATERIALS AND METHODS

Microorganism and seed culture

Seed culture of *L. sulphureus* was carried out as reported previously with some modifications (Lung and Huang, 2011). *L. sulphureus* BCRC 35305 was obtained from the Bioresources Collection and Research Center in Hsinchu, Taiwan. The culture was grown and maintained on a potato dextrose agar (PDA) medium. The sub-culture was conducted by transferring grown mycelia to a fresh nutrient agar medium every month. The one-week-old cells grown on the media agar plate were collected with 25 ml sterilized water mixed by mycelia, and then 20 ml collected mycelia were transferred to 250 ml seed culture flasks containing 100 ml culture medium (g/L) composed of yeast extract, 3; malt extract, 3; peptone, 5 and glucose, 10. The seed culture was incubated at 28°C on a rotary shaker at 125 rpm for 7 days.

Culture conditions of *L. sulphureus*

The products of *L. sulphureus* submerged cultures used in this study were obtained in our laboratory as described earlier (Lung and Huang, 2011). The fermentation of *L. sulphureus* proceeded in a 20-L stirred tank bioreactors filled with 12 L culture medium and 5% (v/v) inoculums derived from seed cultures. The culture medium in the bioreactor was composed of 10.0 g/L glucose, 5.0 g/L yeast extract and 3.0 g/L KH_2PO_4. The stirred tank bioreactor culture was operated at 31°C, 1 vvm (volume of aeration per volume of bioreactor per minute) aeration rate, 150 rpm agitation speed and controlled pH of 4.0 for 15 days. The pH of culture medium was automatically controlled by adding 1 N H_2SO_4 or 1 N NaOH. The mycelia were separated from fermented broth by centrifugation (4°C, 8000 × g for 15 min) and then washed with distilled water. Finally, the mycelia were freeze-dried to powders. Biomass concentration was determined in dry weight per unit volume. Residual sugar in the supernatant was determined by the dinitrosalicylic acid method (Miller, 1959). The polysaccharide concentration in the supernatant was determined by phenol-sulphuric acid assay (Dubois et al., 1956).

Preparation of polysaccharide samples

Preparation of polysaccharide samples was carried out according to the method as described by Lung and Tasi (2009). Intracellular polysaccharide (IPS) was isolated from cultured mycelia of *L. sulphureus*. The cultured mycelia were extracted with boiling water for 1 h, and then filtered through filter paper (Whatman No. 1). Filtrates were precipitated with four volumes of 95% (v/v) ethanol, and left overnight at 4°C. Precipitated polysaccharides were centrifuged at 10,000 × g for 10 min, and then the supernatant was discarded. The precipitate was lyophilized into powders, and afterward the powder was redissolved in deionized water to a concentration of 20 mg/ml and then stored at 4°C for further use.

Exopolysaccharides (EPS) were isolated from the mycelia-free cultured broth of *L. sulphureus* by precipitation with four volumes of 95% (v/v) ethanol. The sequent isolation process was the same as that of IPS. Dried EPS powders were dissolved in deionized water to a solution of the concentration of 20 mg/ml and stored at 4°C for further experiments.

Determination of polysaccharide molecular weight and protein content in polysaccharide

Molecular weight of EPS was determined as described by Lung and Huang (2011) with minor modifications. Molecular weights of poly-saccharides were determined by gel permeation chromatography (GPC) of the Waters (Milford, MA, USA) 600E system equipped with a GPC column (Shodex OHpak SB-804HQ) and a model 410 RI detector. All chromatographic data were processed by Millennium (Milford, MA, USA) software. Polyethylene glycol (PEG) standards (Polymer Laboratories, Church Stretton, UK) with narrow polydispersity and with molecular weights ranging from 1.9 to 1260 kDa constructed a calibration curve. Polysaccharide samples for molecular weight testing were pretreated by membrane filtration (MWCO 8 kDa) before injection. Deionized water was used as the mobile phase at a flow rate of 0.6 ml/min. The method described by Lowry et al. (1951) was used to determine protein contents in polysaccharides.

Reducing power

The reducing power was determined by the approach given by Oyaizu (1986) with minor modifications. Each polysaccharide sample (0 to 20 mg/ml) in methanol and deionized water (2.5 ml) was mixed with 2.5 ml 200 mM sodium phosphate buffer at pH 6.6 and 2.5 ml of 1% (w/v) potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After 2.5 ml of 10% (w/v) trichloroacetic acid was added, the mixture was centrifuged at 200 × g for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% (w/v) ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. EC_{50} value in mg extract/ml expresses the effective concentration at which the absorbance is 0.5 in the reducing power assay and is obtained by linear regression interpolation. Butylated

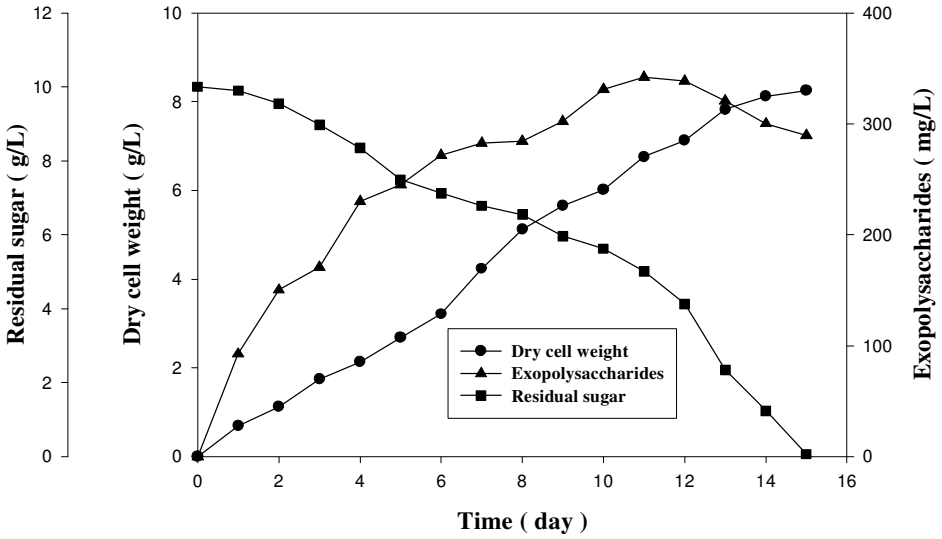

Figure 1. Time–course data of mycelial growth and exopolysaccharide production by *Laetiporus sulphureus* submerged culture in a 20-L stirred-tank fermenter.

hydroxyanisole (BHA), α-tocopherol, and ascorbic acid are used as standards.

DPPH radical scavenging activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was examined with the method of Shimada et al. (1992) with minor modifications. A total of 4 ml polysaccharide samples of various concentrations (0 to 20 mg/ml) were mixed with 1 ml methanolic solution containing 1 mM DPPH radicals and resulted in a final concentration of 0.2 mM DPPH radicals. The mixture was shaken vigorously and left to stand for 30 min in darkness. DPPH radical reduction was determined by measuring the absorbance at 517 nm against a blank. The scavenging ability is expressed as follows:

scavenging ability = [(ΔA_{517} of control - ΔA_{517} of sample)/ ΔA_{517} of control] × 100.

EC_{50} value in mg extract/ml expresses the effective concentration at which DPPH radicals are 50% and are obtained by linear regression interpolation. BHA, α-tocopherol and ascorbic acid are used as standards.

Chelating effects on ferrous ions

Chelating ability was examined according to the method of Dinis et al. (1994) with minor modifications. Each polysaccharide sample (0 to 20 mg/ml, 1 ml) was mixed with 3.7 ml methanol and 0.1 ml 2 mM ferrous chloride. The mixture was then reacted with ferrozine (0.2 ml, 5 mM) for 10 min. Each value was expressed by triplicate measurements with standard deviation. With the absorbance reading at 562 nm (A_{562}), chelating activities on ferrous ions were calculated with the following equation:

Chelating effect (%) = [1-(ΔA_{562} of sample) / (ΔA_{562} of control)] ×100%.

A lower absorbance indicates a higher chelating power. EC_{50} value in mg extract/ml expresses the effective concentration at which ferrous ions are chelated by 50% and are interpolated by linear regression. Ethylenediaminetetraacetic acid (EDTA) and citric acid are used as standards.

Scavenging effects on superoxide anions

The capacity of the polysaccharide sample to scavenge superoxide anions was assayed by the method of Robak and Gryglewski (1988) with minor modifications, in which the reduction of nitro blue tetrazolium (NBT) was measured and superoxide anions were generated in the phenazine methosulphate-nicotinamide adenine dinucleotide (PMS-NADH) system. Identical volumes of the sample, 30 µM phenazine methosulphate (PMS), 338 µM dihydronicotineamidadenine dinucleotide (NADH) and 72 µM nitro blue tetrazolium (NBT) in 0.1 M phosphate buffer of pH 7.4 were mixed and incubated for reaction at ambient temperature for 5 min. The absorbance was measured at 560 nm against blank samples. The scavenging capability to superoxide radicals is calculated as follows: [(ΔA_{56o} of control - ΔA_{560} of sample) / ΔA_{560} of control] × 100. EC_{50} value in mg extract/ml expresses the effective concentration at which the scavenging superoxide anion activity is 50% and is obtained by linear regression interpolation. Ascorbic acid was used for comparison.

RESULTS AND DISCUSSION

Fermentation results of *L. sulphureus* polysaccharides

Shown in Figure 1 are the time-course data on cell, exopolysaccharide and glucose concentrations by *L. sulphureus* submerged cultures in a 20-L stirred tank bioreactor. Moreover, fermentation results of polysaccharides (EPS and IPS) from *L. sulphureus* submerged cultures after 15 days are listed in Table 1. The maximum EPS and IPS products are 342.10 and 300.14 mg/L, respectively, with the corresponding EPS and IPS productivities as 19.29 and 20.01 mg/L/day. The

Table 1. Fermentation results of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus*.

	Q_P[a] (mg/L/day)	P_{max}[b] (mg/L)	P_{max} content[c] (mg/g)	$Y_{P/X}$[d] (mg/g)	$Y_{P/S}$[e] (mg/g)	t[f] (day)
EPS[g]	19.29	342.10	-	35.07	29.12	15
IPS[h]	20.01	300.14	36.38	-	30.21	15

[a]Q_P, Polysaccharide productivity (mg/L/day); [b]P_{max}, maximum polysaccharide product concentration (mg/L); [c]P_{max} content, IPS content in dry cell (mg IPS/g biomass); [d]$Y_{P/X}$, specific product yield (mg EPS/g biomass); [e]$Y_{P/S}$, product yield (mg EPS or IPS/g glucose); [f]t, culture time (day), [g]EPS, exopolysaccharide; [h]IPS, intracellular polysaccharide.

Table 2. Number average molecular weight (Mn) and protein content of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus*.

Polysaccharides	Number average molecular weight (Mn) (Da)	Protein/polysaccharide (%, w/w)
EPS	3.95×10^6	2.38
IPS	1.29×10^6	8.24

production yield ($Y_{P/S}$, mg EPS/g glucose) of EPS is 29.12 mg/g and its specific product yield ($Y_{P/X}$, mg EPS/g biomass) is 35.07 mg/g. In addition, the P_{max} content (mg IPS/g dry mycelia weight) and production yield ($Y_{P/S}$, mg IPS/g glucose) of IPS are 36.38 and 30.21 mg/g, respectively (data not shown). It seems that the level of EPS production is equivalent to IPS. These results indicate that EPS and IPS can be produced effectively by submerged culture of *L. sulphureus* in a 20-L stirred tank bioreactor.

Molecular weight and protein content of *L. sulphureus* polysaccharides

Polysaccharides isolated from higher fungi such as mushrooms are usually associated with protein as complexes. Proteins in polysaccharides seem to play an important role in bioactivities. Biological activities of polysaccharides are closely related to chemical structure and molecular weight (Ohno et al., 2001). Polysaccharides with high molecular weight and protein seem to have relatively high biological activities. Antioxidant properties of poly-saccharide from mushroom sources have recently been studied extensively. Although the antioxidant mechanisms of polysaccharides are not fully understood, many correlations among antioxidant properties of poly-saccharides and the corresponding chemical compo-nents, molecular weights, structures, conformations and protein/polysaccharide ratios have been proposed. The molecular weight and protein content of EPS and IPS are given in Table 2. The number-average molecular weight (Mn) of EPS is 3.95×10^6 Da and IPS 1.29×10^6 Da. Apparently, EPS is higher than IPS in the number-average molecular weight (Mn).

Similar result appears in polysaccharides of other mushrooms such as *Grifola frondosa* (Lee et al., 2003). The protein/polysaccharide ratios (%, w/w) of EPS and IPS are 2.38 and 8.24%, respectively. The protein/ poly-saccharide ratio of EPS is obviously less than that of IPS. The result is inconsistent with our earlier report (4). In this study, both EPS and IPS exhibit high number-average molecular weights and distinct protein/ poly-saccharide ratios. In addition, polysaccharides isolated from sub-merged cultures of *L. sulphureus* are found to have different *in vitro* antioxidant properties (Table 3). Therefore, the difference in antioxidant properties of EPS and IPS may be attributed to their number-average molecular weight (Mn) and protein/polysaccharide ratios.

Reducing power

Figure 2 shows the reducing powers of EPS and IPS from *L. sulphureus* submerged culture determined at 700 nm. Reducing powers of EPS and IPS increase rapidly with their concentration increases. Reducing powers of EPS and IPS are 0.37 and 0.41 at 1.0 mg/ml, and 0.83 and 0.968 at 2.5 mg/ml, respectively. It seems that reducing powers of IPS and EPS are comparable. How-ever, BHA, α-tocopherol and ascorbic acid show excellent reducing power of 1.33, 1.34 and 1.35 at 0.5 mg/ml, respectively. Shimada et al. (1992) reported that reducing powers of various extracts might be due to hydrogen-donating. As a result, our data on reducing capacities of EPS and IPS suggest that reductone-associated and hydroxide groups of polysaccharides can act as electron donors to react with free radicals to form stable products and thereby terminate radical chain reactions. The reducing capacity is a significant index of antioxidant activity (Meir et al., 1995). Various mecha-nisms related to antioxidant activities include chain initiation, binding of transition metal ion catalysts, hydrogen abstraction, reductive capacity and radical scavenging (Gülçin et al.,

Table 3. EC_{50} values of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus*.

Parameter	EC_{50} (mg/ml)						
	Polysaccharides		Standards				
	EPS	IPS	BHA	Ascorbic acid	α-Tocopherol	EDTA	Citric acid
Reducing power	3.08 ± 0.16	1.42 ± 0.08	0.034 ± 0.002	0.030 ± 0.002	0.15 ± 0.03	-	-
Scavenging effect on DPPH radicals	46.95 ± 0.13	6.01 ± 0.22	0.044 ± 0.003	0.056 ± 0.003	0.14 ± 0.02	-	-
Chelating effect on ferrous ions	0.085 ± 0.005	5.51 ± 0.03	-	-	-	0.037 ± 0.004	59.63 ± 2.34
Scavenging effect on superoxide anion	3.22 ± 0.02	1.23 ± 0.11	-	0.43 ± 0.03	-	-	-

EC_{50} value: The effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; the DPPH radicals were scavenged by 50%; the ferrous ions were chelated by 50%; and the superoxide anion were scavenged by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis. BHA, Butylated hydroxyanisole; EDTA, ethylenediaminetetraacetic acid.

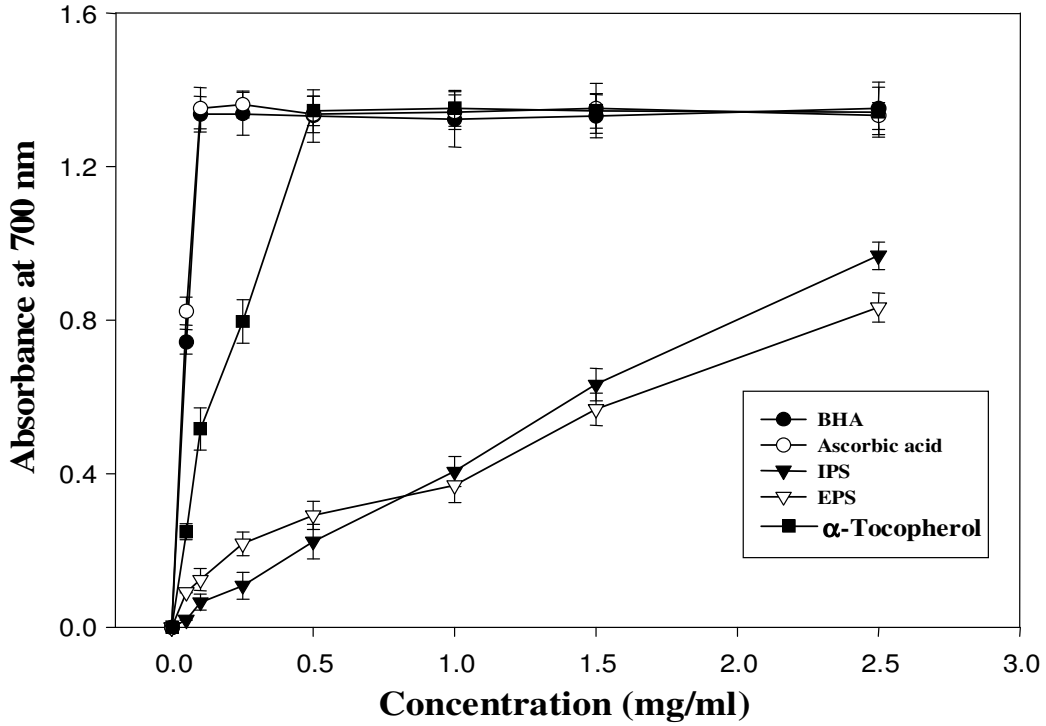

Figure 2. Reducing power of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus*. Each value is expressed as mean ± standard deviation (n = 3). BHA, Butylated hydroxyanisole.

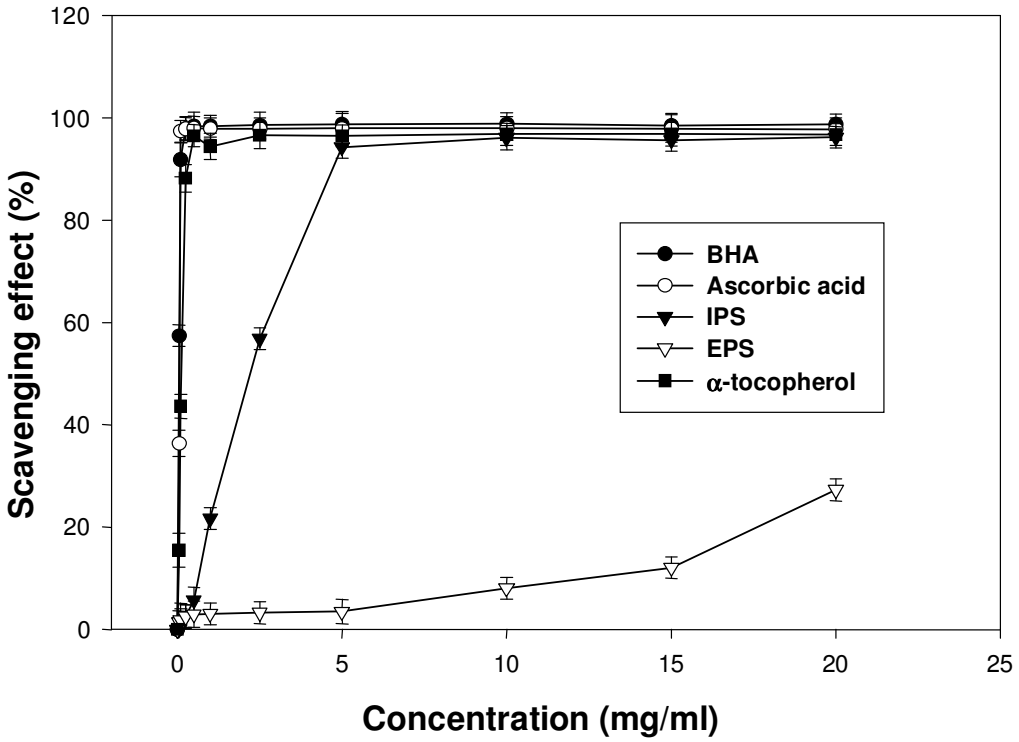

Figure 3. Scavenging effect of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus* on 1,1-diphenyl-2-picrylhydrazyl radical. Each value is expressed as mean ± standard deviation (n = 3). BHA, Butylated hydroxyanisole.

2003). Reducing capacity is one important factor of antioxidant activity. Fermented EPS and IPS of *L. sulphureus* might contain reductones which react with certain precursors of peroxides to prevent peroxide formation.

Scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Free radical-scavenging activities of polysaccharide samples were determined by the method of Shimada et al. (1992). DPPH method is usually used to evaluate anti-oxidant activity of various natural compounds by reducing stable DPPH radicals to yellow-colored diphenylpicryl-hydrazine. DPPH radical scavenging ability is responsible for hydrogen-donating efficiency of antioxidants. As shown in Figure 3, the scavenging DPPH radical activity of IPS exhibits a marked dose-dependent pattern until a concentration of 5 mg/L is reached. DPPH radical scavenging ability of EPS increases gradually with concentration increase. At 1.0 mg/ml, scavenging effects of EPS and IPS are 3.06 and 21.65%, respectively and 3.49 and 94.29% at 5 mg/ml. α-Tocopherol, BHA and ascorbic acid showed scavenging effects of 43.59, 91.82 and 97.40% at 0.1 mg/ml, respectively. IPS was distinctly higher than EPS in scavenging effects. IPS is effective in scavenging effects as compared to α-tocopherol, BHA and ascorbic acid. These results revealed that IPS is a potent scavenger with its antioxidative activity attributed to the proton-donating ability. And IPS may probably contain lots of reductones to react with radicals to stabilize and terminate radical chain reactions.

Chelating effects on ferrous ions

Ferrozine can easily react with ferrous ions to form red Fe^{2+}-ferrozine complexes. With the addition of chelating agents in the reaction solution, formation of complexes is interfered and results in the complex reduction. Chelating abilities of polysaccharides can be estimated by con-centration measurements of the complex. Chelating effect on ferrous ions is one important antioxidant activity mechanism. In addition, chelating abilities are found to be related to the concentration of catalyzing transition metals in lipid peroxidation and this indicates that higher chelating ability brings about lower concentration of catalyzing transition metals. Figure 4 shows the chelating effects of EPS, IPS, EDTA and citric acid on ferrous ions. EPS, IPS and EDTA except for citric acid are found to have more potent chelating abilities on Fe^{2+} in a concentration-dependent manner. At 1.0 mg/ml, the chelating abilities of EPS and IPS are 99.16% and

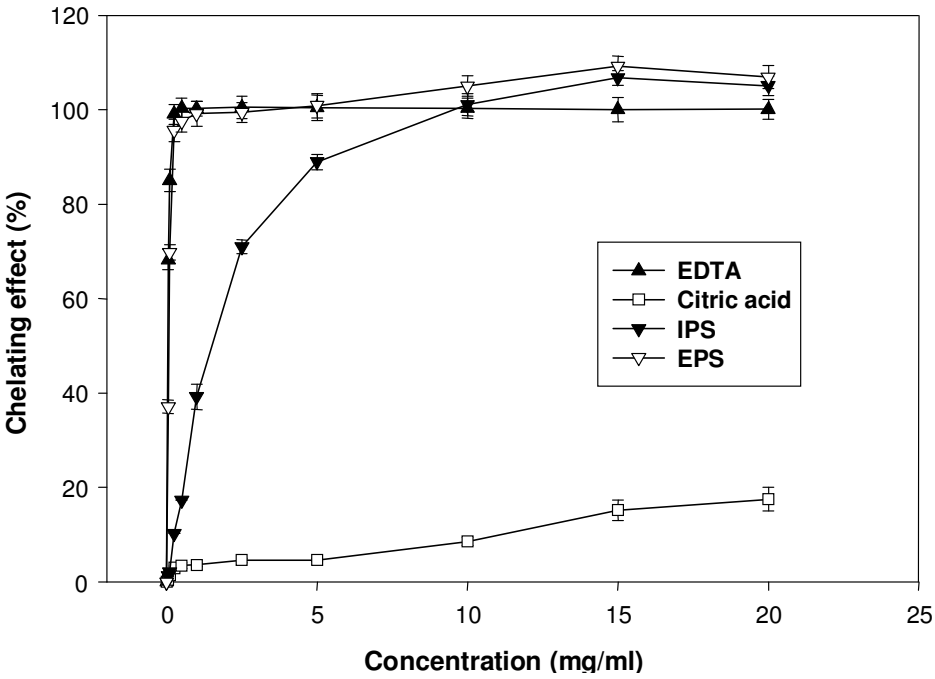

Figure 4. Chelating effect of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus* on ferrous ions. Each value is expressed as mean ± standard deviation (n = 3). EDTA, Ethylenediaminetetraacetic acid.

39.17%, respectively. Apparently, EPS is superior to IPS in chelating ability. However, EPS and IPS are both highly effective in chelating abilities. EDTA exhibits an excellent chelating ability of 85.05% at 0.1 mg/ml and 100.34% at 0.5 mg/ml. Conversely, citric acid shows a weak chelating ability of 5.22% at 10 mg/ml. Therefore, the chelating abilities of EPS and EDTA are comparable. Ferrous ions are known as the most effective pro-oxidant among various species of metal ions due to its high reactivity, which accelerates lipid oxidation by breaking down hydrogen and lipid peroxidase to reactive free radicals by the Fenton type reaction. Metal chelating activity is an antioxidant mechanism, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation (Qiao et al., 2009). Therefore, IPS and EPS from *L. sulphureus* in submerged culture are good chelators for ferrous ions.

Scavenging effects on superoxide radical

Superoxide radicals generated by numerous biological and photochemical reactions (Banerjee et al., 2005) are extremely harmful reactive oxygen species inducing oxidants in biomolecules and are also precursors that convert superoxides and H_2O_2 into more reactive species such as hydroxyl radicals. In this study, superoxide radicals are originated in PMS/NADH system and assayed by the reduction of NBT. As shown in Figure 5, the scavenging abilities of EPS, IPS and ascorbic acid on superoxide radicals rise with concentration increases. IPS leads EPS in superoxide radical scavenging activity for all concentrations. At 500 µg/ml, superoxide radical scavenging activities of EPS and IPS are 15.08% and 26.40%, respectively, but at this concentration, ascorbic acid reaches a considerably high superoxide radical scavenging activity of 50.93%. Apparently, the superoxide radical scavenging activity of IPS is higher than EPS. EPS and IPS reveal moderate superoxide radical scavenging activities. Although superoxide radical is a weak oxidant, it decomposes to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals of higher oxidative and oleophilic abilities than precursors to initiate lipid peroxidation for a longer time (Sun and Kennedy, 2010). Superoxide radicals and its derivatives can cause damage to DNA and cell membranes. These results clearly indicate that antioxidant activities of all polysaccharide samples are related to the abilities of scavenging superoxide radicals. It is of great importance to scavenge superoxide radicals.

EC_{50} values in antioxidant properties

Antioxidant properties assayed herein are summarized in Table 3 with calculated results expressed as EC_{50} values for comparison. High EC_{50} values correspond to weak antioxidant properties and values lower than 10 mg/ml stand for effective antioxidant activities (Liang et al., 2009). EC_{50} values of IPS and EPS for reducing powers

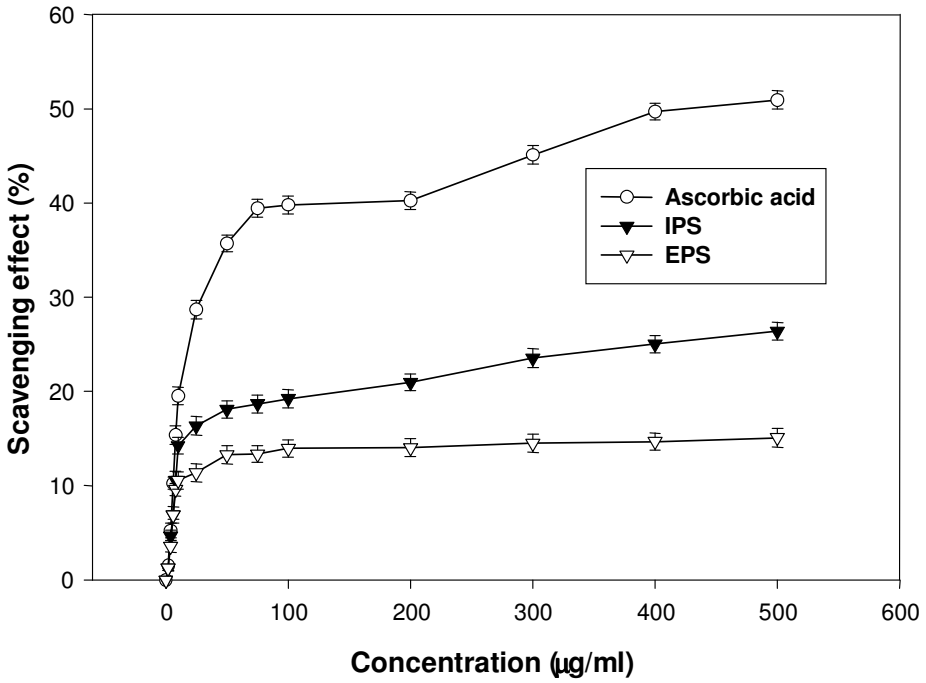

Figure 5. Scavenging effect of exopolysaccharide (EPS) and intracellular polysaccharide (IPS) from submerged culture of *Laetiporus sulphureus* on superoxide anion. Each value is expressed as mean ± standard deviation (n = 3).

are 1.42 and 3.08 mg/ml, respectively. IPS and EPS are highly effective in reducing power as evidenced by their lower EC_{50} values below 5.0 mg/ml. However, EC_{50} values of BHA, α-tocopherol and ascorbic acid for reducing powers are below 0.2 mg/ml. EC_{50} values of IPS and EPS in scavenging DPPH radical ability are 6.01 and 46.95 mg/ml, respectively. The apparent higher EC_{50} value of EPS than IPS indicates that IPS is more effective in scavenging DPPH radicals than EPS. Nevertheless, BHA, α-tocopherol and ascorbic acid exhibit excellent scavenging DPPH radical activities for their exceedingly low EC_{50} values below 0.2 mg/ml. EC_{50} values of IPS and EPS for chelating effects on ferrous ions are 5.51 and 0.090 mg/ml, respectively. Apparently, EPS exhibits powerful chelating effect on ferrous ions and is more effective in chelating effect than IPS. EC_{50} value of EDTA in the chelating effect is 0.037 mg/ml; however, citric acid exceeds 20 mg/ml. Chelating effects of EPS and EDTA are comparable, but ascorbic acid is weak in scavenging DPPH radicals (EC_{50}>20 mg/ml). Furthermore, the EC_{50} values of IPS and EPS on scavenging superoxide radicals are 1.23 and 3.22 mg/ml, respectively but ascorbic acid is excellent in scavenging activity for the EC_{50} value of 0.43 mg/ml. The results suggest that both IPS and EPS show outstanding scavenging activities on superoxide radicals (EC_{50}<5 mg/ml). Except for the scavenging effect on DPPH radicals of EPS, all EC_{50} values of antioxidant properties of IPS and EPS are lower than 10 mg/ml, indicating the high antioxidant properties for both EPS and IPS.

As compared with IPS, EPS with higher number-average molecular weight (Mn) of 3.95×10^6 Da but lower protein/polysaccharide ratios of 2.38% is more effective in reducing power, chelating effect on ferrous ions and scavenging effect on superoxide anions. However, IPS exhibits exceptional reducing power and scavenging effect on superoxide anions for its lower number-average molecular weight (Mn) of 1.29×10^6 Da and higher protein/polysaccharide ratios of 8.24%. It seems that antioxidant properties of polysaccharides from *L. sulphureus* submerged cultures are related to their number-average molecular weights and protein/ polysaccharide rations. Song et al. (2010) pointed out that there appears to be higher antioxidant activities when molecular weights and protein contents increased. The results therefore indicate that polysaccharides isolated from submerged cultures of *L. sulphureus* have different *in vitro* antioxidant properties because of their molecular weights and protein/polysaccharide ratios.

Conclusion

IPS and EPS were isolated from mycelia and filtrates (mycelia-free) of submerged cultures by *L. sulphureus* in a 20-L stirred tank bioreactor. Experimental results clearly demonstrate that IPS and EPS have highly effective antioxidant properties as evidenced by their quite low EC_{50} values. Therefore, IPS and EPS isolated from *L. sulphureus* by submerged cultures can be developed as

new antioxidants potentially applied in pharmaceutical and food industries. However, the antioxidant mechanism of *L. sulphureus* polysaccharides is yet unclear. Hence, future research should be focused on defining the correlation between chemical characteristics and antioxidant properties of *L. sulphureus* polysaccharides.

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