J Ind Microbiol Biotechnol (2011) 38:291–298
DOI 10.1007/s10295-010-0772-z

ORIGINAL PAPER

Enhancement of exo-polysaccharide production and antioxidant activity in submerged cultures of *Inonotus obliquus* by lignocellulose decomposition

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Received: 10 May 2010 / Accepted: 29 June 2010 / Published online: 14 July 2010
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Abstract We reported that lignocellulose decomposition can be used to facilitate the production of bioactive polysaccharides from submerged culture of *Inonotus obliquus*. Exo-polysaccharide (EPS) production and antioxidant activity by *Inonotus obliquus* was enhanced by employing lignocellulose decomposition in a corn straw-containing submerged fermentation. A significant increase in the EPS production and hydroxyl radical scavenging activity from 1.09 ± 0.01 g/l and 72.3 ± 1.9% in a basal medium to 1.38 ± 0.02 g/l and 82.7 ± 0.5% in a corn straw-containing medium was obtained. A synchronized effect between lignocellulose decomposition and malondialdehyde presenting hydroxyl radical concentration in the fermentation broth was identified. The adding of thiourea, a hydroxyl radical-scavenging reagent, suppressed malondialdehyde generation and lowered the lignocellulose decomposition rate. Correspondingly, the EPS production and hydroxyl radical scavenging activity decreased to 1.26 g/l and 74%. The EPS obtained from the corn straw-containing medium also presented the strongest superoxide radical scavenging activity. The monosaccharide components of the EPS from the corn straw-containing medium are rhamnose, arabinose, xylose, mannose, glucose, and galactose with molar proportions at 3.0, 3.0, 0.9, 46.6, 11.4, and 35.1%, respectively, which are largely different from the molar proportions of the EPS from the basal medium.

Keywords *Inonotus obliquus* · Exo-polysaccharides · Antioxidant activity · Submerged fermentation · Lignocellulose decomposition

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Introduction

The medicinal mushroom *Inonotus obliquus* (*I. obliquus*) belongs to the family Hymenochaetaceae, Basidiomycetes and has long been a folk remedy in Russia and the northern latitudes [1–3]. Many triterpenoids, steroids, and phenolic compounds from *I. obliquus* have various biological activities [1, 4–6]. Particularly, polysaccharides from the *I. obliquus* fruit body, mycelia, and fermentation broth exhibit strong immunomodulating, anti-tumor, and antioxidant activities [7–10].

Submerged cultures offer a promising alternative to obtain large quantities of polysaccharides and are fast, cost-effective, and easy to control. Many medicinal mushroom polysaccharides are being commercially produced by submerged cultures [11–13] because of the higher productivity compared to production from fruit bodies. Submerged fermentation is an effective process for the production of mycelial biomass and bioactive compounds, especially exo-polysaccharides (EPS). In the last few years, an increasing number of studies have been reported on mycelial fermentations of *I. obliquus* for EPS and endo-polysaccharides (IPS) [4, 7, 12–16]. Recently, we demonstrated a submerged fermentation optimization for bioactive polysaccharide production from *I. obliquus* using the response surface methodology (RSM) method combined with hydroxyl radical-scavenging activity screening [17]. With the RSM optimized medium, the hydroxyl radical-scavenging activity per unit of the exo-polysaccharides (EPS) was significantly enhanced compared to that from either the

basal fermentation medium or the single variable optimization of fermentation medium [17].

I. obliquus is a kind of white rot fungi. In nature, white rot fungi produce lignin-degrading enzymes that degrade the lignin to carbon dioxide and water, exposing the hemicellulose and cellulose in the wood matrix [18]. Because enzymes are too large to penetrate sound wood, most researchers believe that white rotters attack wood polymers by producing small, diffusible, extracellular oxidants that operate at a distance from the hyphae. One of these oxidants is the hydroxyl radicals [19–21]. To protect the fungus itself from hydroxyl radical damage, production of antioxidant phenolic compounds by *I. obliquus* is a defense response induced by such oxidative stress [6]. Several questions arise concerning *I. obliquus* submerged fermentation: do hydroxyl radicals play an important role when *I. obliquus* in submerged fermentation decomposes lignocellulose, and are there effects of the lignocellulose decomposition on the production or bioactivity of phenolic compounds and polysaccharides from *I. obliquus* cultures as a defense response?

In this study, we investigated the production and antioxidant activity of exo-polysaccharide from *I. obliquus* in submerged fermentation with corn straw as a source of lignocellulose. The decomposition rates of cellulose, hemicellulose, and lignin in corn straw during the fermentation were detected and related to the concentration of malondialdehyde (MDA), which presents the concentration of hydroxyl radicals to some degree [22]. Our purpose was to improve active polysaccharide production by *I. obliquus* with lignocellulose decomposition during submerged fermentation.

Materials and methods

Microorganism and culture conditions

I. obliquus (CBS314.39) was purchased from the Centraal Bureau voor Schimmelcultuur, Utrecht, The Netherlands. It was maintained on malt extract agar slants containing (%w/v) malt extract 3, peptone 0.3, and agar 1.5 at pH 5.6 ± 0.2. The fungi were cultivated at 25°C for about 2 weeks, then stored at 4°C and sub-cultured every 3 months.

Seed culture preparation

The seed culture was prepared by incubating mycelia on a malt extract agar slant in a 250-ml Erlenmeyer flask with 100 ml of medium. Then 1 cm^2 of malt extract agar with mycelia was chipped off and transferred to the Erlenmeyer flask. The medium contained (% w/v) glucose 2, peptone 0.3, yeast extract 0.2, KH_2PO_4 0.1, $MgSO_4$ 0.15, and $CaCl_2$ 0.01. Cultures were incubated for 4–5 days in a rotary shaker (150 rpm) at 28°C.

Shake-flask culture

The cultures were incubated at a rate of 10% (v/v) at 28°C. They were maintained for 288 h on a rotary shaker at 150 rpm to produce exo-polysaccharides.

The harvested seed culture was added to 250-ml Erlenmeyer flasks containing 100 ml of liquid fermentation medium. The three kinds of fermentation media used in this study were as follows.

1. Basal fermentation medium (w/v): corn flour 5%, peptone 0.3%, KH_2PO_4 0.1%, $ZnSO_4 \cdot 2H_2O$ 0.001%, K_2HPO_4 0.04%, $FeSO_4 \cdot 7H_2O$ 0.005%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CuSO_4 \cdot 5H_2O$ 0.002%, $CoCl_2$ 0.001%, and $MnSO_4 \cdot H_2O$ 0.008%. pH = 6.0. The medium was designed by Stepanova et al. [18] and optimized by response surface methodology (RSM) in our previous work [17].
2. Lignocellulose fermentation medium contained (w/v): corn flour 3.5%, corn straw 3%, and all of the other components were the same as the basal fermentation medium.
3. Lignocellulose + thiourea medium contained (w/v): thiourea 10 mmol/l, and all of the other components were the same as in the lignocellulose fermentation medium.

The starch contained in corn flour, as a sort of polysaccharide, can be precipitated by ethanol in the initial phase of fermentation. It causes significant interference in the quantitative analysis of the EPS from early fermentation of *I. obliquus*. In order to remove the interference of the starch, the corn flour contained in the three kinds of media was drastically hydrolyzed by α-amylase and glucoamylase into glucose. After having been hydrolyzed, the corn flour suspension was filtered, and the filtrate, mainly containing glucose and other water-soluble nutrients such as biotin and thiamine [17], was used to prepare the fermentation medium.

The main components of the corn straw were measured as follows (dry weight basis): cellulose 36.9%, hemicellulose 20.3%, lignin 21.4%, and others 21.4%.

Extraction and purification of exo-polysaccharides

The fermentation broth was harvested after incubation and filtered in a vacuum to separate the mycelia and the corn straw from the broth. The filtrate was concentrated under vacuum to one quarter of the original volume. The concentrate was mixed with four-times volume of absolute

ethanol, stirred vigorously, and left overnight at 4°C. The precipitated extract was repeatedly washed with 95% ethanol to remove adherent sugar residue and other small molecules, then centrifuged (6,500×*g* for 10 min), and lyophilized [13].

The Sevag method was employed to remove protein after neutrase treatment with some modification [23]. The extract water solution was mixed with Sevag reagent [chloroform: butanol = 5:1 (v/v)] at the proportion 5:1 (v/v) and oscillated intensively. The supernatant was dialyzed against water (48 h), and then concentrated, precipitated, and lyophilized as described above to produce crude EPS.

Chemical analysis and GC determination of monosaccharides

The crude EPS obtained from the liquid medium at 288 h was used for chemical characteristics and hydroxyl radical-scavenging activity measurement. The total sugar content was determined by the phenol-sulfuric acid method [24]. Protein content was determined by the method of Bradford with bovine serum albumin as a standard [25]. The total phenolic content was determined according to the Folin-Ciocalteu colorimetric method [26].

The exo-polysaccharides obtained from the different media were hydrolyzed [27]. Ten milligrams of crude EPS was added to a 10-ml screw-capped tube with 4 M trifluoroacetic acid. The mixture was reacted in a 110°C oven for 12 h and then evaporated to dryness under a stream of nitrogen. Ten milligrams of hydroxylamine hydrochloride and 0.5 ml pyridine were added to the former three tubes separately, followed by oximization in a 90°C water bath for 30 min, and cooled to room temperature. Then 0.5 ml acetic anhydride was added, and the mixture was shaken homogeneously. The solution was subjected to acetylation in a 90°C water bath for 30 min and cooled to room temperature for the formation of aldonitrile peracetylated derivatives. The derivative solution was evaporated to dryness under a stream of nitrogen, and 1 ml chloroform was added to dissolve the separated substance.

The monosaccharide compositions of the crude EPS were analyzed by gas chromatography (GC) [23, 27]. The derivatized monosaccharide solution was injected into a Varian CP-3800 (Varian Inc., Palo ALto, CA) equipped with a Varian CP-Sil 5 CB capillary chromatography column (25 m × 0.53 mm, 0.25-μm film thickness) and a flame-ionization detector for analysis. The GC operation was performed under the following conditions: injection temperature: 270°C; detector temperature: 250°C. The temperature in the oven was programmed as follows: 110°C in the beginning, maintained for 5 min, increased to 180°C at 3°C/min and maintained for 5 min, and increased to 220°C at 5°C/min and maintained for 10 min. The monosaccharide components were identified by matching the GC retention time with the six standard compounds. The relative amount of each monosaccharide was calculated as the proportion of the compound peak area to the internal standard (myo-inositol) peak area.

Determination of scavenging activity of hydroxyl radicals

The hydroxyl radical (·OH) scavenging activity of the crude EPS was measured with the salicylic method [28] with some modifications. The reaction mixture (4 ml) contained: 1 ml H_2O_2 (8.8 mmol/l), 1 ml $FeSO_4$ (9 mmol/l), 1 ml salicylic (9 mmol/l), and 1 ml crude EPS samples obtained from the three kinds of fermentation media in the same concentration gradient (0.2, 0.5, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml). The H_2O_2 was added to the mixture and the reaction was started up at last. The reaction mixture was incubated at 37°C for 60 min and then centrifuged at 15,000×*g* for 6 min. The absorbance (A) of the reaction solutions at 510 nm was measured. The scavenging rate was calculated according to the equation:

$$\text{scavenging rate}\,(\%) = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100\% \quad (1)$$

where A_0 is the absorbance for the control (double distilled water), A_x is the absorbance for the reaction mixture with the EPS sample solution, and A_{x0} is the absorbance for background, i.e., the reaction mixture without H_2O_2.

Determination of superoxide radical ($O_2^{-\cdot}$) scavenging activity

The EPS samples were dissolved in 50 mmol/l Tris-HCl buffer (pH = 8.2) at varying concentrations (20, 40, 60, 80, and 100 μg/ml). The four reaction systems contained 5 ml of the EPS solution at different concentrations and 10 μl of 50 mmol/l pyrogallol solution. The absorbance variation was detected within 2 min at 325 nm. The scavenging rate was calculated according to the equation:

$$\text{scavenging rate}\,(\%) = \left(1 - \frac{\Delta A}{\Delta A_0}\right) \times 100\% \quad (2)$$

where ΔA is the absorbance variation for the EPS solution at different concentrations within 2 min. ΔA_0 is the absorbance variation for the control.

Analysis of chemical compositions of corn straw

The cellulose and hemicellulose were determined according to van Soest's method [29] with some modification. Lignin was determined by the 72% H_2SO_4 method. The decomposition rates of cellulose, hemicellulose,

and lignin were calculated by the percentage content remaining.

Determination of the MDA concentration

The MDA concentration in the fermentation broth was determined with a MDA commercial kit (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions.

Statistical analysis

Experimental results recorded were the means ± standard deviation (SD) of triple determinations. The data were analyzed by one-way analysis of variance (ANOVA). Tests of significant differences were determined by Duncan's multiple range tests at $P = 0.05$ or independent sample *t* test ($P = 0.05$). The IC50 values were calculated by using median-effect analysis and origin 7.5 software (OriginLab Corp., Northampton, MA).

Results

Production of exo-polysaccharides in fermentation media

Figure 1 shows the typical time course of the EPS production in *I. obliquus* submerged culture in the basal medium, the corn straw-containing medium, and the corn straw-containing medium + thiourea, respectively. There was no significant difference in the EPS concentrations in the three culture media in the initial 96 h. The EPS

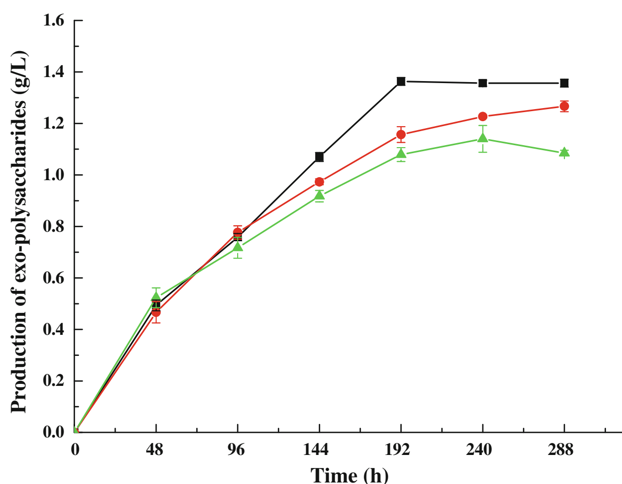

Fig. 1 Production of *I. obliquus* exo-polysaccharides obtained from the lignocellulose medium (*filled square*), the lignocellulose + thiourea medium (*filled circle*), and the basal medium (*filled triangle*). Each point is the mean ± SD of triplicates

concentration from the corn straw-containing medium increased much more rapidly between 96 and 192 h and reached a maximum of about 1.38 g/l. After 288 h, the maximum EPS concentrations from the lignocellulose and lignocellulose + thiourea media were 1.38 and 1.26 g/l, while that from the basal medium was 1.09 g/l.

Decomposition of lignocellulose and generation of MDA in cultures

The decomposition rates of cellulose (Fig. 2a), hemicellulose (Fig. 2b), and lignin (Fig. 2c) in the corn straw were measured by the percentage content remaining (Fig. 2). After 144 h of incubation, the remaining content percentages of cellulose, hemicellulose, and lignin in the corn straw were 93.1 (97.1), 95.4 (96.6), and 95.2 (96.2)% in the lignocellulose medium (the lignocellulose + thiourea medium), respectively. The decomposition rates began to increase dramatically from the 144th h. After 288 h of incubation, the remaining rates of cellulose, hemicellulose, and lignin in the corn straw-containing cultures were 79.1, 82.1, and 80.2%, respectively, which were lower than the remaining rates of 81.3, 84.9, and 84.1% in the medium with the presence of thiourea (Fig. 2).

As shown in Fig. 3, the MDA concentration was low in the fermentation broth of *I. obliquus* obtained from the basal medium, the lignocellulose medium, and the lignocellulose + thiourea medium before 144 h. The MDA in the corn straw-containing cultures increased rapidly between 144 and 240 h of incubation and reached a maximum at 240 h. The generation of MDA in the corn straw-containing culture was inhibited significantly by thiourea, an ·OH scavenger. A fairly constant low level of MDA generation was detected in the control flasks lacking corn straw throughout the course of the incubation.

Hydroxyl radical-scavenging activity

As is shown in Fig. 4, the EPS samples obtained from the three kinds of fermentation media exhibited a hydroxyl radical-scavenging activity in a dose-dependent manner (0.2–5.0 mg/ml). The scavenging activity of the EPS from the lignocellulose medium was significantly stronger than that from either the lignocellulose + thiourea medium or the basal medium in the same concentrations. The highest scavenging rates of the EPS samples from the lignocellulose medium, the lignocellulose + thiourea medium, and the basal medium were 82.7, 78.2, and 72.3%, respectively. The IC50 (the half maximal inhibitory concentration) value (1.08 mg/ml) of the EPS from the lignocellulose medium was significantly lower than those from the lignocellulose + thiourea medium (1.23 mg/ml) and the basal medium (1.29 mg/ml), respectively ($P < 0.05$).

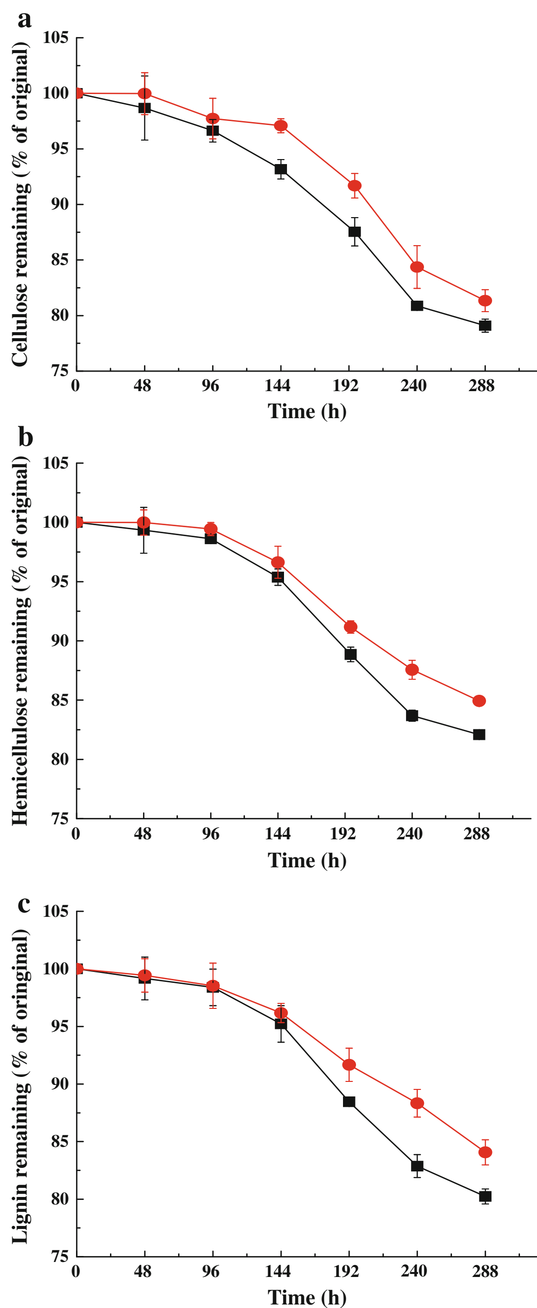

Fig. 2 Remaining rates of cellulose (**a**), hemicellulose (**b**), and lignin (**c**) in the corn straw during submerged fermentation of *I. obliquus*. The lignocellulose medium (*filled square*), the lignocellulose + thiourea medium (*filled circle*)

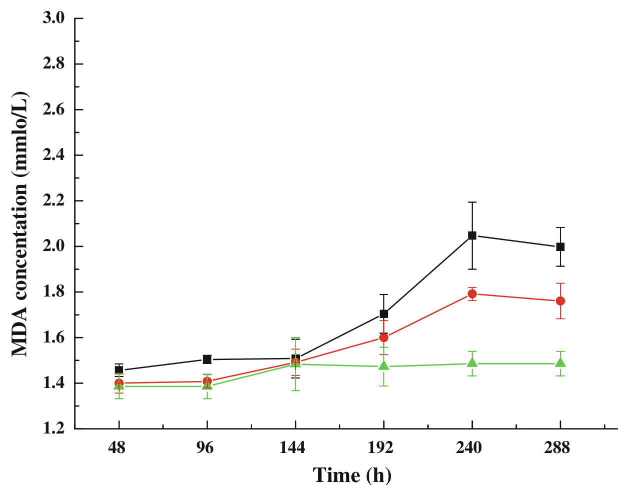

Fig. 3 MDA concentrations of the fermentation broth from the lignocellulose medium (*filled square*), the lignocellulose + thiourea medium (*filled circle*), and the basal medium (*filled triangle*). Each point is the mean ± SD of triplicates

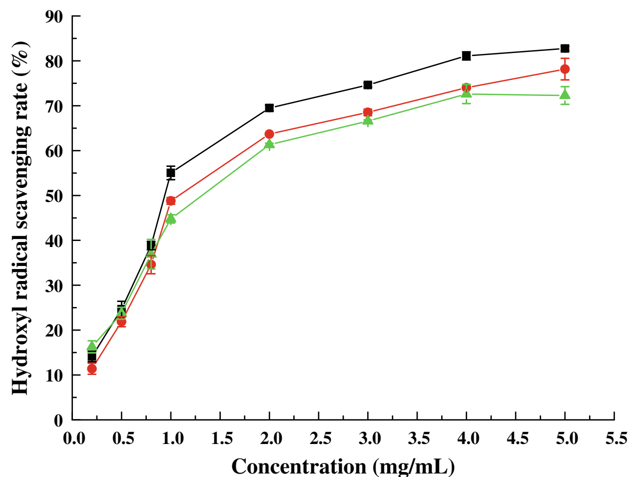

Fig. 4 Hydroxyl radical-scavenging rates of the exo-polysaccharides obtained from the lignocellulose medium (*filled square*), the lignocellulose + thiourea medium (*filled circle*), and the basal medium (*filled triangle*). Each point is the mean ± SD of triplicates

Superoxide radical-scavenging activity

As is shown in Fig. 5, the EPS samples obtained from the three kinds of fermentation media had a scavenging activity toward superoxide radicals in a dose-dependent manner (20–100 μg/ml). The scavenging activity of the EPS from the lignocellulose medium was significantly stronger than that from either the lignocellulose + thiourea medium or the basal medium in the same concentrations. The highest scavenging rates of the EPS samples from the lignocellulose medium, the lignocellulose + thiourea medium, and the basal medium were 32.1, 27.9, and 23.9%, respectively. The IC50 value (174.1 μg/ml) of the EPS from the lignocellulose medium was significantly lower than those from the lignocellulose + thiourea medium (217.8 μg/ml) and the basal medium (312.2 μg/ml), respectively ($P < 0.05$).

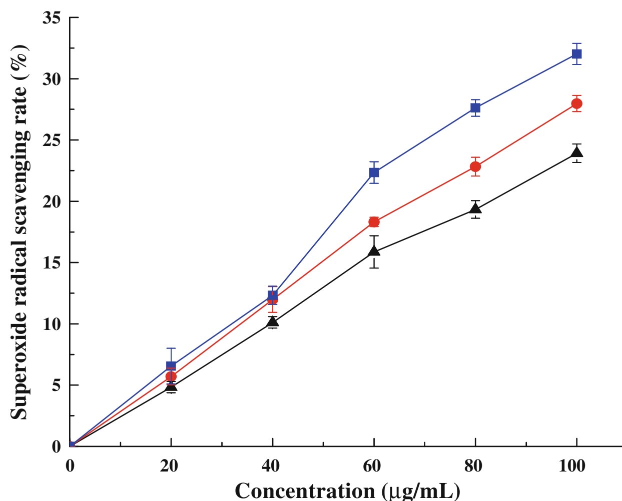

Fig. 5 Superoxide radical scavenging rates of the exo-polysaccharides obtained from the lignocellulose medium (*filled square*), the lignocellulose + thiourea (*filled circle*), and the basal medium (*filled triangle*). Each point is the mean ± SD of triplicates

Chemical analysis and GC determination of monosaccharides

Table 1 is a summary of the major chemical contents of the EPS samples from the basal medium, the lignocellulose medium, and the lignocellulose + thiourea medium. The three samples were all composed of sugars with very low polyphenols. No proteins were detected.

Table 2 shows the monosaccharides of the three samples. The EPS from the lignocellulose medium contained rhamnose, arabinose, xylose, mannose, glucose, and galactose with molar proportions at 3.0, 3.0, 0.9, 46.6, 11.4, and 35.1%. The EPS from the lignocellulose + thiourea medium was composed of rhamnose, arabinose, xylose, mannose, glucose, and galactose with molar proportions at 2.6, 2.9, 1.5, 36.5, 16.5, and 40.3%. The EPS from the basal medium was composed of rhamnose, arabinose, xylose, mannose, glucose, and galactose with molar proportions at 1.5, 3.6, 2.2, 15.9, 50.0, and 26.8%. The results clearly demonstrated that mannose, glucose, and galactose were the dominant monosaccharides in the three EPS samples, but the molar proportions were different. The proportion of mannose of the EPS obtained from the

Table 1 Chemical characteristics of the exo-polysaccharides

Medium	Main components (%)		
	Polysaccharides	Protein	Polyphenol
Basal medium	99.90	nd	0.10
Lignocellulose + thiourea medium	99.88	nd	0.12
Lignocellulose medium	99.92	nd	0.08

Table 2 Monosaccharide components of the exo-polysaccharides

Medium	Constitutive sugar (unit, mol%)					
	Rha	Ara	Xyl	Man	Glu	Gal
Basal medium	1.45	3.63	2.17	15.94	50.00	26.81
Lignocellulose + thiourea medium	2.62	2.91	1.15	36.48	16.54	40.30
Lignocellulose medium	2.98	2.97	0.91	46.65	11.39	35.1

Rha rhamnose, *Ara* arabinose, *Gal* galactose, *Glu* glucose, *Xyl* xylose, *Man* mannose

lignocellulose medium was much higher than that from the basal medium. On the contrary, the proportion of glucose was much lower. The composition difference may contribute to the stronger antioxidant activity of the EPS from the corn straw-containing medium.

Discussion

An evident increase in the EPS production and antioxidant activity in the presence of corn straw was obtained (Figs. 1, 4 and 5). The higher EPS production might be linked to the lignocellulose decomposition in the corn straw in this experiment. Lignocellulosic biomass, which includes agricultural residues, paper wastes, and wood chips, is an ideal inexpensive, renewable, abundantly available resource. Lignicellulose is composed of cellulose, hemicellulose, and lignin [30]. In the basal medium, corn flour was used as a carbon source. The reducing sugars produced by the corn flour hydrolysis were consumed easily. Lignocellulose was a kind of carbon source that was difficult to use. *I. obliquus* did not decompose lignocellulose until the hydrolysis-reducing sugars from the corn flour were exhausted. In our previous investigation, we found that the exo-polysaccharides produced by *I. obliquus* show some characteristics of secondary metabolities, which are synthesized rapidly when the fungi entered the stable phase [17]. In the first stage, the fungi used glucose from the corn flour hydrolysis to reproduce largely without high production of exo-polysaccharides as catabolic repressions. In the following stage, *I. obliquus* began to decompose lignocellulose because of the exhaustion of the reducing sugars to survive and accelerated the production of exo-polysaccharides. This happened after the 144th h of liquid cultivation (Fig. 2). After the time point, the three main components of lignocellulose (lignin, cellulose, and hemicellulose) were decomposed dramatically (Fig. 2a, b, c). In other words, the lignocellulose decompositions in the corn straw provided reducing sugars for the EPS production when the reducing sugars from the corn flour hydrolysis were exhausted. Synchronously, it was found that the MDA concentration increased rapidly after 144 h (Fig. 3). The

MDA concentration of the fermentation broth from the corn straw-containing medium was significantly higher than that from the basal medium, which stayed at a low level over the fermentation course. MDA is the result of lipid peroxidation, which is initiated by hydroxyl radicals [31]. It is able to indicate the concentration of hydroxyl radicals [22]. The time of 96–144 h was a key transition phase, after which the MDA concentration increased rapidly, following with the dramatic lignocellulose biodegradation and the EPS production.

This can be explained in that *I. obliquus* mycelia in the corn straw-containing medium were stimulated to form a defense system of antioxidation to prevent damage when lignin biodegradation occurred [32]. The cellular defense against destructive oxidative stress is based on increased formation of non-enzymatic components, which may reduce certain free radicals [33]. It could be assumed that EPS might be one of the compounds produced by the mycelia in response to oxidative damage. The addition of thiourea, a hydroxyl radical-scavenging reagent, in the corn straw-containing medium suppressed MDA generation and lowered the lignocellulose decomposition rates (Figs. 2, 3). The production of the EPS was lower than that from the lignocellulose medium (Figs. 1, 4 and 5). It should be noted that the exo-polysaccharide production of 1.38 g/l in lignocellulose medium is not far from the 1.26 g/l in the medium containing lignocellulose + thiourea. This result further demonstrates the roles of lignocellulose decomposition in the production of EPS because thiourea as a hydroxyl radical scavenger did affect the efficiency of lignocellulose decomposition, but it did not deter lignocellulose decomposition.

The higher EPS production and activity from the corn straw-containing medium were attributed to the hydroxyl radical generation due to the lignocellulose decomposition during submerged fermentation.

In our previous work, we reported that the RSM optimized medium could significantly improve the production and antioxidant activity of the EPS [17]. In this study, we used the RSM optimized medium as a basal medium and added corn straw into the basal medium as the lignocellulose medium. The results show that the lignocellulose decompositions largely changed the monosaccharide compositions of the EPS (Table 2). The main monosaccharide of the EPS obtained from the basal medium is glucose (50.0%), while that from the lignocellulose medium is mannose (46.7%). The high molar proportion of mannose may contribute to the further enhancement of antioxidant activity over the RSM optimization method. There are many factors affecting the bioactivity of exo-polysaccharides such as monosaccharide components, molecular weight, average DP (degree of polymerization), linkage, etc. Studies on the other factors are under way.

After 288 h of incubation, the decomposition rates of cellulose, hemicellulose, and lignin in the corn straw-containing cultures were 20.9, 17.9, and 19.8%. In addition, as shown in Fig. 2a, b, and c, the decomposition rates slowed down after 240 h fermentation. The reason for the results may be that the exo-polysaccharides synthesized by the fungi gathered around the mycelia and blocked the interaction between the mycelia and the corn straw [34]. If the culture time is prolonged and the exo-polysaccharides are removed by some measures during fermentation, the lignocellulose would further be decomposed, and the production of exo-polysaccharides would further increase.

Although this study focused on the exo-polysaccharides in *I. obliquus* cultures, it should be noted that the polyphenols in *I. obliquus* mycelial cultures, as known antioxidants [6, 35], should be considered to be one of the defense compounds, too. A study on improvement of polyphenol production of *I. obliquus* mycelia stimulated by lignocellulose decompositions is under way.

In this study, we successfully established an easy and cost-effective method that utilizes inexpensive corn straw to enhance the production of active exo-polysaccharides of *I. obliquus* in submerged fermentation. It significantly outperforms the RSM optimization method. The exo-polysaccharide production was increased by 126.6%, and antioxidant activity was increased by 114.4% with lignocellulose biodegradation. It can be concluded that the lignocellulose consumption was an effective stimulant of extracellular polysaccharide biosynthesis.

Acknowledgments This research was supported by a research grant from the Science and Technology Department of Zhejiang Province, China (2010C34016).

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