Quantitative response of cell growth and polysaccharide biosynthesis by the medicinal mushroom *Phellinus linteus* to NaCl in the medium

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Abstract The effect of NaCl on cell growth and polysaccharide biosynthesis in the medicinal mushroom *Phellinus linteus* was studied. With the increase of NaCl concentration between 1 g/l and 7 g/l in the culture medium, the cell growth and intracellular polysaccharide (IPS) accumulation were decreased; extracellular polysaccharide (EPS) concentration was enhanced, with an increase of NaCl concentration from 1 g/l to 3 g/l. Under the optimum NaCl concentration of 3 g/l, the maximum EPS and IPS production reached 2.2±0.15 g/l and 53.6±2.45 mg/g DW on day 12, which improved 32.27% and decreased 16.89% compared to the control, respectively. Both EPS and IPS showed new polysaccharide components by fractionation with DEAE-cellulose ion exchange chromatography compared to the control. The results presented in this study are considered helpful for further investigation on the diversity of polysaccharide biosynthesis of this medicinal fungus under NaCl environments.

Keywords EPS · IPS · NaCl environments · *Phellinus linteus*

Introduction

*Phellinus linteus*, a basidiomycete, is one of the most famous traditional Chinese medicines. Its fruiting body is called “Sanghuang” in China. Sanghuang has been well known as a medicinally potent mushroom due to its high anti-tumor activity. The anti-tumor activity of the polysaccharides from the fruit body of this mushroom was first reported in 1968 (Ikekawa et al. 1968), thereafter a wide variety of further reports have been documented by many investigators (Han et al. 1999; Kim et al. 2004a, b). Polysaccharides purified from the mushroom have been capable of potentiating the host immune response without direct cytotoxicity to cancer cells. In particular, the intracellular polysaccharides (IPS) isolated from fruiting-bodies of *P. linteus* have been the most potent material against tumor growth and metastasis (Han et al. 1999; Kim et al. 2004a). The extracellular polysaccharide (EPS) isolated from mycelium culture of *P. linteus* also has activity in preventing hyperglycemia in diabetic patients (Kim et al. 2001).

Polysaccharides produced by medicinal mushrooms usually have multiple components and different distributions of molecular weights, and anti-tumor activity differs greatly with chemical composition and configuration. Although it is difficult to correlate the structure and anti-tumor activity of complex polysaccharides, differences in activity can be correlated with the size of the molecules and their branching pattern. There are some interesting reports that molecular weight distribution and biological activities of polysaccharides, produced from medicinal fungi, vary with the pH of the culture broth and medium composition (Lee et al. 2003; Shu and Lung 2004).

Addition of NaCl will change the medium composition. Some useful metabolites are hyperproduced under NaCl environments, for example the bacterium *Corynebacterium glutamicum* produces more lysine (Ronsch et al. 2003). However, as far as we know, there are no reports that NaCl environments may influence polysaccharide biosynthesis by adding NaCl in submerged fermentation of medicinal fungi. In this work, the effect of NaCl on cell growth, and extracellular polysaccharide (EPS) and intracellular polysaccharide...
(IPS) biosynthesis by submerged fermentation of *P. linteus* are reported for the first time. The study is considered helpful to the further understanding of polysaccharide biosynthesis diversity of the medicinal mushroom in NaCl environments. The information is also beneficial to correlate process parameters such as NaCl concentration on the polysaccharide and the distribution of its components for the development of a fermentation process for high quality polysaccharide production from *P. linteus*.

**Materials and methods**

**Organism and medium**

The strain of *P. linteus* was purchased from the collection bank of Huazhong Agricultural University (Hubei, China). The stock culture was maintained on potato-dextrose-agar slants. For Seed cultures, the medium composition was (g/l): glucose 50, peptone 30, KH$_2$PO$_4$ 3, MgSO$_4$·7H$_2$O 2. For fermentation, the medium consisted of (g/l): glucose 40, peptone 10, yeast extract 10, KH$_2$PO$_4$ 3, MgSO$_4$·7H$_2$O 2, and Vitamin B$_1$ 0.05.

**Culture conditions**

The slants were inoculated with mycelium and incubated at 28°C for 7 days, and then used for seed culture inoculation. The seed culture was grown in a 500 ml shake flask containing 100 ml of liquid medium and incubated at 28°C on a rotary shaker (160 rev/min) for 7 days. The fermentation cultivation was inoculated at 10% (v/v) of the above seed culture medium and kept at 28°C and 160 rev/min.

**Determination of dry weight, EPS and IPS**

Dry cell weight (DW) was measured gravimetrically, and the crude EPS was precipitated by addition of four volumes of 95% (v/v) ethanol, and then separated by centrifugation (10,000×g, 10 min). The insoluble components were suspended in distilled water, and then the content of EPS in the supernatant was measured by the phenol–sulphuric acid method (Dubois et al. 1956).

For the analysis of intracellular polysaccharide (IPS), the dried mycelia (ca. 100 mg) were extracted by using 1 M NaOH at 60°C (1 h), and then the supernatant was assayed by phenol–sulphuric acid method (Dubois et al. 1956).

**Polysaccharide component analysis**

Polysaccharides from samples of medium containing 3 g/l and control were, respectively, collected by the method mentioned above, and then fractionated on ÄKTA primer (Amersham Biosciences Ltd.). 0.5 ml polysaccharide (i.e. EPS 95 mg/l and IPS 32 mg/l) was applied to a HiPrep 16/10 DEAE-cellulose column (1.6 cm×5 cm, Amersham Biosciences Ltd.) in H$_2$O and followed by stepwise elution with 1 M NaCl at a flow rate of 2 ml/min. Each fraction (3 ml) was assayed by the phenol–sulphuric acid method.

**Results and discussion**

Effect of different NaCl concentrations on cell growth and IPS and EPS biosyntheses

The initial culture environment was regulated by adding 1, 3, 5 and 7 g/l (concentration in medium after addition) of NaCl to the culture medium, respectively, while taking the medium without NaCl addition as control. Table 1 shows the biomass, the highest EPS production and IPS content of the cell at different NaCl concentrations. The results indicate that cell growth and IPS content all declined with an increase of NaCl within the range of 1–7 g/l. However, EPS production increased with an increase of NaCl concentration between 1 g/l and 3 g/l, and decreased slightly on raising the NaCl concentration to 7 g/l. A maximum EPS production was obtained at 3 g NaCl/l, which reached 2.2±0.35 g/l. The results indicate that cell growth, and EPS and IPS formation were related to the osmotic stress environment created by adding NaCl to the submerged fermentation by *P. linteus*. A suitable NaCl concentration of 3 g/l was beneficial to EPS formation, but inhibited cell growth and IPS production.

**Effect of NaCl concentration on fermentation kinetics**

Based on the above results, a further study of the cell response to the changes induced by adding NaCl to the medium was performed. Figure 1 shows the time profiles of cell growth, EPS and IPS production and glucose consumption in the culture medium containing 3 g NaCl/l. The cell growth kinetics is shown in Fig. 1a. Compared with the control (normal cultivation), the cell growth was inhibited markedly over the whole course of fermentation. The maximum cell density of 2.3±0.15 g/l was obtained under

**Table 1 Effect of NaCl concentration on the cell growth, EPS and IPS production during submerged fermentation of the medicinal fungus Phellinus linteus in shake flasks**

<table>
<thead>
<tr>
<th>NaCl concentration (g/l)</th>
<th>Biomass (g/l)</th>
<th>EPS production (g/l)</th>
<th>IPS content (mg/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.41±0.5</td>
<td>1.49±0.05</td>
<td>64.5±1.5</td>
</tr>
<tr>
<td>1</td>
<td>3.96±0.45</td>
<td>1.86±0.3</td>
<td>50.7±0.8</td>
</tr>
<tr>
<td>3</td>
<td>2.28±0.34</td>
<td>2.2±0.35</td>
<td>53.6±1.0</td>
</tr>
<tr>
<td>5</td>
<td>2.12±0.31</td>
<td>1.86±0.15</td>
<td>45.62±0.8</td>
</tr>
<tr>
<td>7</td>
<td>1.90±0.21</td>
<td>1.73±0.37</td>
<td>40.23±0.7</td>
</tr>
</tbody>
</table>

*The maximum errors were calculated from three independent samples*