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Optimal Culture Conditions for Mycelial Growth and Exo-polymer Production of *Ganoderma applanatum*

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The effect of fermentation parameters and medium composition on the simultaneous mycelial growth and exo-polymer production from submerged cultures of *Ganoderma applanatum* was investigated in shake-flask cultures. The optimum initial pH for mycelial growth and exo-polymer production was 5.0 and 6.0, respectively. The optimum temperature was 25°C and the optimum inoculum content was 3.0% (v/v). The optimal carbon and nitrogen sources were glucose and corn steep powder, respectively. After 12 days fermentation under these conditions, the highest mycelial growth was 18.0 g/*l* and the highest exo-polymer production was 3.9 g/*l*.

KEYWORDS : Exo-polymer, *Ganoderma applanatum*, Mycelia, Submerged culture

Mushrooms are highly nutritious and may exhibit medicinal properties. In recent years, mushrooms have emerged as an important source of bioactive chemicals that have properties such as antitumor (Suzuki *et al*., 1989), immunological (Luettig *et al*., 1989), anti-complementary (Zhao *et al*., 1993), anti-inflammatory (Chihiro *et al*., 1982), anticoagulant (Nishino *et al*., 1991), hypoglycemic (Yamada *et al*., 1989), hypolipidemic activity (Yang *et al*., 2000), mitogenic activity on lymphocyte (Jeon *et al*., 1998) and anti-viral (Rym *et al*., 1999).

Ikekawa *et al*. (1969) published one of the first scientific reports on the antitumor activities of Polyporacease mushrooms. The three kinds of antitumor agent from mushroom polymers were developed: Krestin from cultured mycelia of *Trametes versicolor* (Ohno *et al*., 1976), Lentinan from fruiting bodies of *Lentinus edodes* (Chihara, 1992; Maeda and Chihara, 1971), and Schizophyllan from the liquid cultured broth product of *Schizophyllum commune* (Komatsu *et al*., 1969),. Mushroom polymers are mostly glucans with different types of glycosidic linkages, such as $(1 \rightarrow 3)$, $(1 \rightarrow 6)$-β-glucans and $(1 \rightarrow 3)$-α-glucans, but some are heteroglycans.

Elfvingia applanata is a species of Basidiomycete, also called "*Ganoderma applanata*", which has been used in folk medicine for treating various ailments, including cancer (Makino, 1989). Recently, components of the modulate humoral immune response were detected in a purified fraction obtained from *G. applanatum* (Kim *et al*., 1994c). Later, anti-bacterial and antiviral activities of the aqueous extract of *G. applanatum* was reported (Kim *et al*., 1994b; Rym *et al*., 1999) without any toxicity (Kim *et al*., 1994a).

Biologically active polymers can be obtained not only in fruiting bodies of mushrooms but also in submerged mycelial fermentations. Recently, the production of polymers from submerged mycelial cultures have been extensively exploited because they require fewer steps and because the purification process is simpler (Bae *et al*., 2000; Cavazzoni and Adami, 1992; Jong and Birmingham, 1992; Tseng, 1984).

In the present investigation, therefore, the optimum culture conditions of *Ganoderma applanatum* for the mycelial growth and exo-polymer production were studied.

Materials and Methods

Strain and basal medium. The *G. applanatum* (KACC 50174) was obtained from the Korean Agricultural Culture Collection (KACC). It was maintained on potato dextrose agar (PDA, Difco) slants at 4°C and subcultured every 3 months. The mushroom complete medium (MCM) was used to perform submerged mycelial culture for mycelial growth and exo-polymer production. The composition of MCM (g/*l*) is as follows: glucose (20), $MgSO_4 \cdot 7H_2O$ (0.5), KH_2PO_4 (0.46), K_2HPO_4 (1.0), yeast extract (2) and peptone (2). The pH was adjusted to 4.0 (with HCl) before sterilization.

Preparation of inoculum. The inoculum was prepared using the method described by Song and Cho (1987). The *G. applanatum* was initially grown on potato dextrose agar medium in a petri-dish. The seed culture of *G. applanatum* was grown in 250-*ml* flasks containing 100 *ml* of potato dextrose broth at 25°C on rotary shaker at

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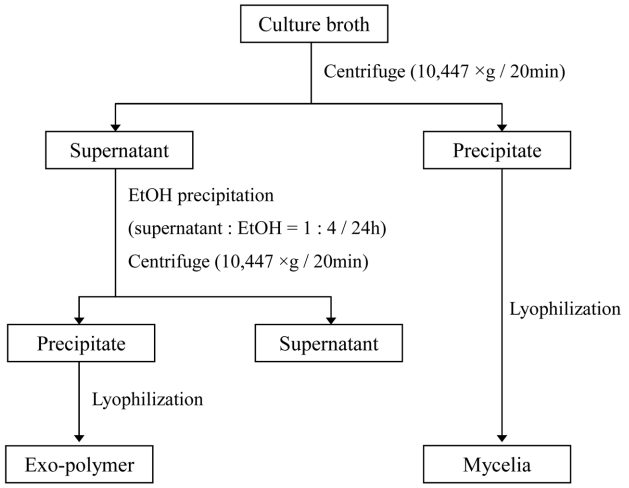

Fig. 1. Schematic diagram of mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum*.

150 rpm. The pH was adjusted to 4.0 before sterilization. After an incubation period of 10 days, 100 *ml* of the culture broth with mycelial pellets were homogenized aseptically in a Sorvall omni-mixer for 3 min in an ice bath. A 2% (v/v) mycelial suspension was used as inoculum for the subsequent experiments.

Submerged culture for mycelial growth and exo-polymer production. The submerged mycelial cultures were carried out in 250-*ml* flasks containing 50 *ml* of the media (MCM) on a rotary shaker (150 rpm). After harvest, the cultured mycelia were collected by centrifugation (10,447 ×g, 20 min). The cell free exo-polymer was precipitated by adding 4 volumes of ethanol to the supernatent. Mycelia and exo-polymer were lyophilized. This recovery process is shown in Fig. 1.

Optimization of culture condition. The submerged mycelial cultures were grown in 250-*ml* culture flasks containing 50 *ml* of MCM medium on a rotary shaker (150 rpm). To determine the optimal medium pH, we grew cultures at 3, 4, 5, 6, 7, and 8 by adding 1 N HCl or NaOH before sterilization. To determine optimal temperature, we grew cultures at 20, 25 and 30°C for 7 days. To determine the optimum concentration of inoculum, we grew cultures from seed amounts of 1, 2, 3, 4, and 5% (v/v).

Carbon and nitrogen source. The optimum carbon source for the mycelial growth and exo-polymer production were determined using either 1, 2, 4 ,6, or 8% (w/v) individually glucose, maltose, arabinose, mannose and molasess. The optimum nitrogen source was determined using medium containing either 0.2, 0.4, 0.6, 0.8, or 1.0% (w/v) individually yeast extract, peptone, malt extract, meat extract, corn steep liquor and corn steep powder.

Results and Discussions

Effect of pH, temperature and inoculum content. The effect of pH on mycelial growth and exo-polymer production is presented in Fig. 2. The growth of *G. applanatum* was highest in a pH of 4 to 6. Maximum mycelia yield (9.76 g/*l*) was achieved at pH 5 and maximum exo-polymer production (1.57 g/*l*) was obtained at pH 6. The effect of temperature on mycelial growth and exo-polymer production is presented in Fig. 3. Maximum yield of mycelia (10.03 g/*l*) and exo-polymer (1.55 g/*l*) were achieved at 25°C. Optimal pH and temperature for the mycelial growth of *Ganoderma* sp. is approximately 4 and 25 to 30°C, respectively (Yang and Liau, 1998). The effect of inoculum content on mycelial growth and exo-polymer production is presented in Fig. 4. The maximum yield of

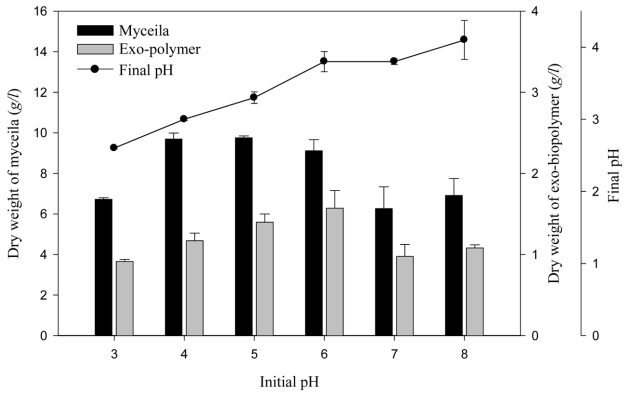

Fig. 2. Effect of pH on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, 7 days and 2% inoculum.

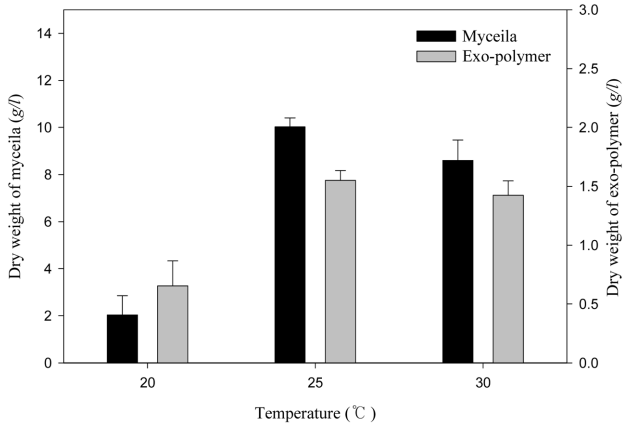

Fig. 3. Effect of temperature on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, pH 5.0, 7 days and 2% inoculum.

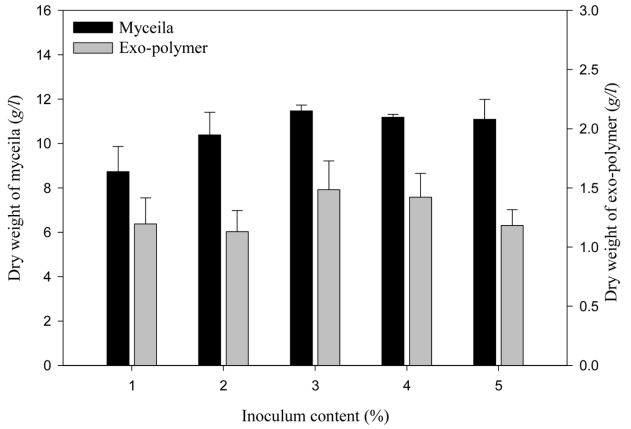

Fig. 4. Effect of inoculum content on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, pH 5.0, 7 days.

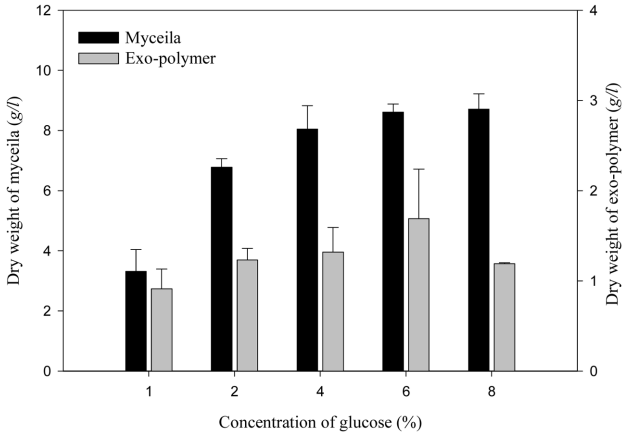

Fig. 6. Effect of various concentrations of glucose on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, pH 5.0, 7 days and 3% inoculum.

mycelia (11.47 g/*l*) and maximum exo-polymer production (1.48 g/*l*) were achieved using a seed content of 3% (v/v).

Effect of carbon source. The effect of carbon source on mycelial growth and exo-polymer production were determined using medium containing various carbon sources. When five different carbon sources (glucose, maltose, arabinose, mannose and molasess) were examined (Fig. 5), the highest mycelial growth (10.65 g/*l*) and exo-polymer production (1.65 g/*l*) were obtained using glucose as a carbon source. The optimum concentrations of glucose are shown in Fig. 6. The highest mycelial growth (8.71 g/*l*) was obtained using 8% (v/v) glucose and the maximum exo-polymer production (1.69 g/*l*) was obtained using 6% (v/v) glucose. When glucose concentration exceeded 8% (v/v), exo-polymer production decreased. Lee *et al.* (2007) also reported that exopolysaccharide production in *G. applanatum* was highest in cultures with glucose by air-lift bioreactor, and exopolysaccharide production was enhanced by an increase in glucose concentration.

Effect of nitrogen source. The effect of nitrogen source on mycelial growth and exo-polymer production were

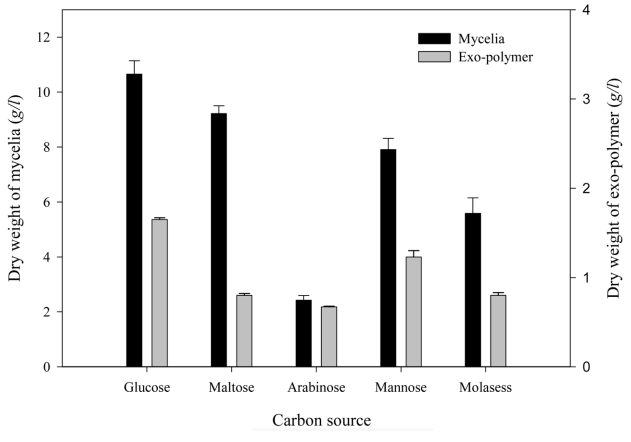

Fig. 5. Effect of various carbon sources on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, pH 5.0, 7 days and 3% inoculum. Each carbon source was supplemented to 2% in basal medium.

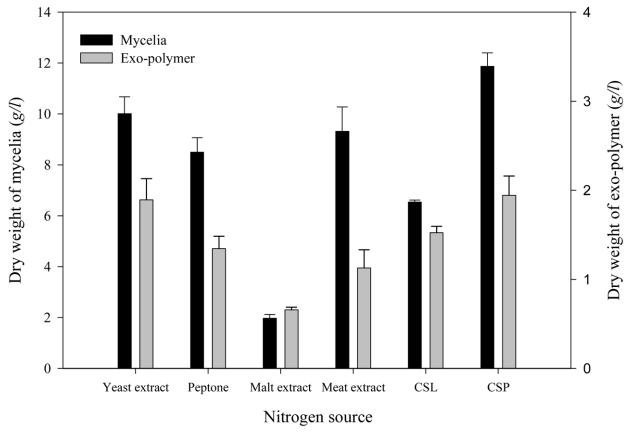

Fig. 7. Effect of various nitrogen sources on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, pH 5.0, 7 days and 3% inoculum. Each nitrogen source was supplemented at a rate of 0.4% in basal medium (CSL: Corn steep liquor, CSP: Corn steep powder).

determined using medium containing various nitrogen sources (yeast extract, peptone, meat extract, malt extract, corn steep liquor and corn steep powder; Fig. 7). The highest mycelial growth (11.87 g/*l*) and exo-polymer production (1.94 g/*l*) were obtained using corn steep powder (CSP) as a nitrogen source. Kim *et al.* (1997) reported a higher production of mycelia and exo-polysaccharides using organic nitrogen than inorganic nitrogen.

The optimum concentration of CSP for mycelial growth and exo-polymer production is shown in Fig. 8. The highest mycelial growth (11.87 g/*l*) and exo-polymer production (2.58 g/*l*) were obtained using 1.0% (v/v) CSP.

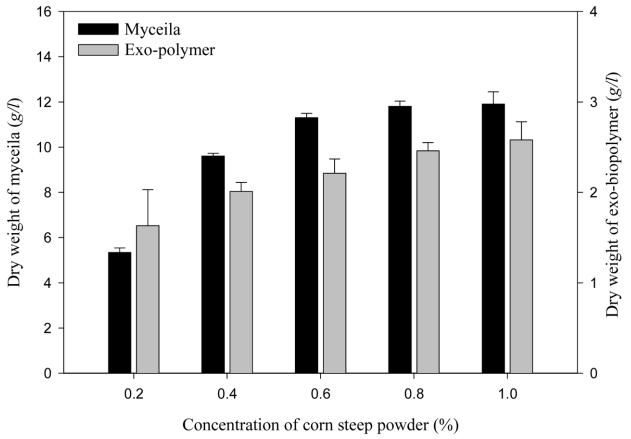

Fig. 8. Effect of various concentrations of corn steep powder on mycelial growth and exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, pH 5.0, 7 days and 3% inoculum.

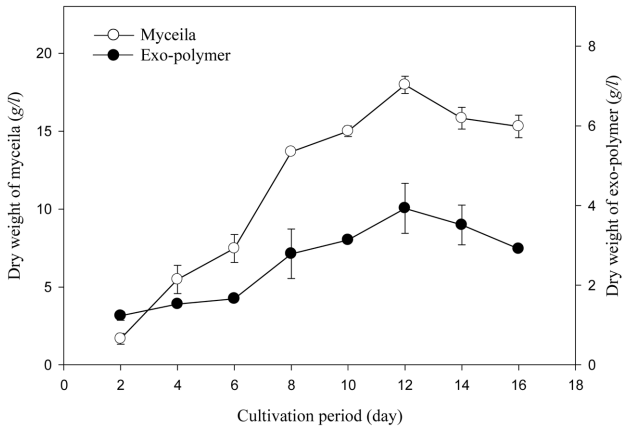

Fig. 9. Time profiles of mycelial growth and the exo-polymer production in submerged mycelial cultures of *G. applanatum* in shake flasks. All experimental data are mean ± SD of three replicates. Culture conditions: 150 rpm, 25°C, pH 5.0, 7 days and 3% inoculum. Medium composition (g/*l*): glucose (60), $MgSO_4$ (0.5), KH_2PO_4 (0.46), K_2HPO_4 (1.0), corn steep powder (10).

Effect of culture period for exo-polymer production and mycelial growth. The time courses for the mycelial growth and the exo-polymer production in shake flasks are shown in Fig. 9. The maximum yield of mycelia (18.0 g/*l*) and exo-polymers (3.9 g/*l*) was achieved at the end of log phase (12 days), with the specific growth rate (μ) of 0.01 h^{-1}. The pH value decrease to pH 3.7 (data not shown). This decrease in pH seems to be due to the production of organic acid as metabolites.

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