

Optimization of Submerged Culture Conditions for Exo-Biopolymer Production by *Paecilomyces japonica*

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Abstract Optimization of submerged culture conditions for the production of exo-biopolymer from *Paecilomyces japonica* was studied. Maltose, yeast extract, and potassium phosphate were the most suitable sources of carbon, nitrogen, and inorganic salt, respectively, for both production of the exo-biopolymer and mycelial growth. The optimal culture conditions in a flask culture were pH 5.0, 25°C, and 150 rpm in a medium containing (as in g/l) 30 maltose, 6 yeast extract, 2 polypeptone, 0.5 K₂HPO₄, 0.2 KH₂PO₄, 0.2 MnSO₄ · 5H₂O, 0.2 MgSO₄ · 7H₂O. Exo-biopolymer production and mycelial growth in the above suggested medium were significantly increased in a 2.5-l jar fermentor, where the maximum biopolymer concentration was 8 g/l. The morphological changes of the mycelium in the submerged culture were observed within pH ranges from 4.0 to 9.0; i.e., growth of the filamentous form was optimal at culture pHs of 5.0 and 6.0, whereas pellet was formed at other pHs.

Key words: *Paecilomyces japonica*, exo-biopolymer, submerged culture

During the past several decades, much interest has been generated in the subject of polysaccharides, which are produced by numerous microorganisms, especially mushrooms, due to their various biological and pharmacological activities [5, 6, 9, 22, 26]. These include immunostimulating activity, antitumor activity together with hypoglycemic activity, applicable as dietary supplements for enhancing stamina, as a medicinal cure of cough and blood circulatory problems, or as tonics to promote longevity and to improve quality of life [5, 6, 9, 22, 26]. A number of reports on microbial polysaccharides from higher fungi like *Ganoderma lucidum*, *Cordyceps* sp., *Lentinus edodes*, and *Pteridium aquilinum* are available. [7, 10, 13, 26, 32]. More recently, *Paecilomyces japonica*, which is one of the cordyceps

species belonging to the ascomycetes family, has been used for medicinal purposes due to its diversified physiological activities [1, 22, 32]. *P. japonica* is identified as a parasite on the larvae of various lepidopteran insects, which forms characteristic fruiting bodies or synnemata. Several investigators attempted to cultivate this organism on a solid artificial media rather than a submerged culture [1, 12, 19, 31]. Submerged culture has potential advantages for higher mycelial production in a compact space and for a shorter incubation time with a lesser chance of contamination. In addition, exo-biopolymers, which have synergistic biological effects with mycelia, can be concurrently produced.

In the present study, submerged culture conditions are optimized for *P. japonica* in order to improve production of the exo-biopolymer. To the best of our knowledge, this is the first report on a submerged culture of *P. japonica*.

MATERIALS AND METHODS

Microorganism and Media

Paecilomyces japonica was cultured in our laboratory. The stock culture was maintained on a PDA slant. Slants were inoculated, incubated at 25°C for 7 days, and then stored at 4°C, where the same PD-broth was used for the inoculum culture. The basal medium was Lilly-Barneet medium which contained 10 g of glucose, 2 g of peptone, 0.5 g of MgSO₄ · 7H₂O, and 1.0 g of KH₂PO₄ in 1-l of distilled water [15].

Flask Cultures

P. japonica was initially grown on PDA medium in a petri-dish, and then transferred to the seed culture medium by punching out 5 mm of the agar plate culture with a sterilized cork borer [24]. The seed culture was grown in a 250-ml flask containing 50 ml of basal medium at 25°C on a rotary shaker at 150 rpm for approximately 5 days. The flask culture experiments were performed in a 500-ml

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flask containing 100 ml of the media after inoculation with 2% (v/v) of the seed culture. To find the optimal culture temperature and pH in the medium, the following factors were studied: 1) Temperature: The inoculated flasks were incubated at 20, 25, and 30°C. 2) pH: The medium was adjusted to different pHs with 1 N HCl or NaOH. The final pHs before sterilization were 4, 5, 6, 7, 8, and 9. [24, 25]. The data presented in the results represent the mean of three independent experiments.

Fermentation Conditions

The fermentation medium was inoculated with 2% (v/v) of the seed culture and then cultivated at 25°C in a 2.5-l jar fermentor (KF-250, Korea Fermentor Co., Seoul, Korea) equipped with a pH electrode. Unless otherwise specified, fermentations were conducted under the following conditions; temperature at 25°C, aeration rate of 0.5 vvm, agitation speed of 150 rpm, initial pH of 5.0, and the working volume of 1.5-l. The seed culture was transferred to the fermentation medium and was cultivated for 5 days at 150 rpm and 25°C.

Analytical Methods

The fermentation broth was filtered through Whatman No. 2 filter paper to mainly separate the mycelium. Dry weight of mycelium after a constant weight at 90°C for 12 h was measured [18, 33]. The filtrate from a membrane filtration (0.45 µm, Millipore) was analyzed by a high performance liquid chromatography (HPLC) for a quantitative analysis of the exo-biopolymer concentration.

The concentrations of exo-biopolymer and maltose were directly analyzed by HPLC using an Aminex HPX-42C column (0.78×30 cm, Bio-rad) equipped with a refractive index detector (Shimadzu Co, Kyoto, Japan).

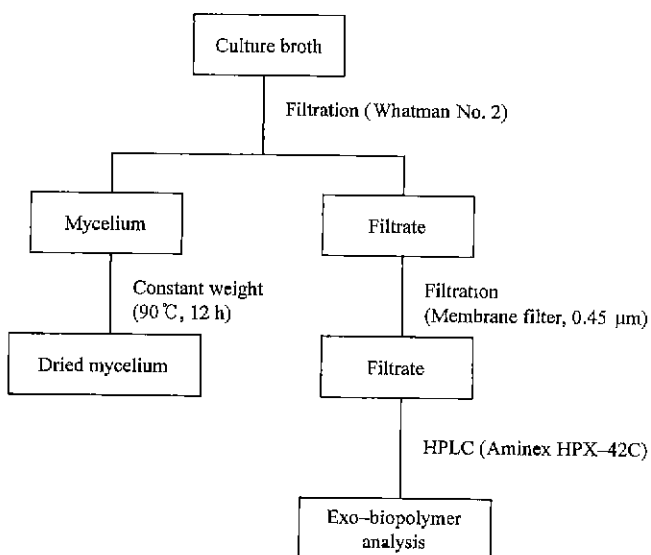


Fig. 1. Fractionation of the exo-biopolymer in *P. japonica*.

Quantitative analysis of mycelium and exo-biopolymer was conducted according to the procedure described in Fig. 1.

Morphological Studies

The effect of pH on morphological changes of mycelium was carefully examined during the submerged culture process within pH ranges of 4.0–9.0 and photographs were taken using an Axiolab microscope (ZEISS, Germany).

RESULTS AND DISCUSSION

Optimal Medium Composition

To find a suitable carbon source for the mycelial growth, *P. japonica* was cultivated in the basal medium containing various carbon sources, and each carbon source was added to the basal medium at a concentration level of 3% (w/v) [13, 18, 23, 25]. Among the 13 carbon sources examined,

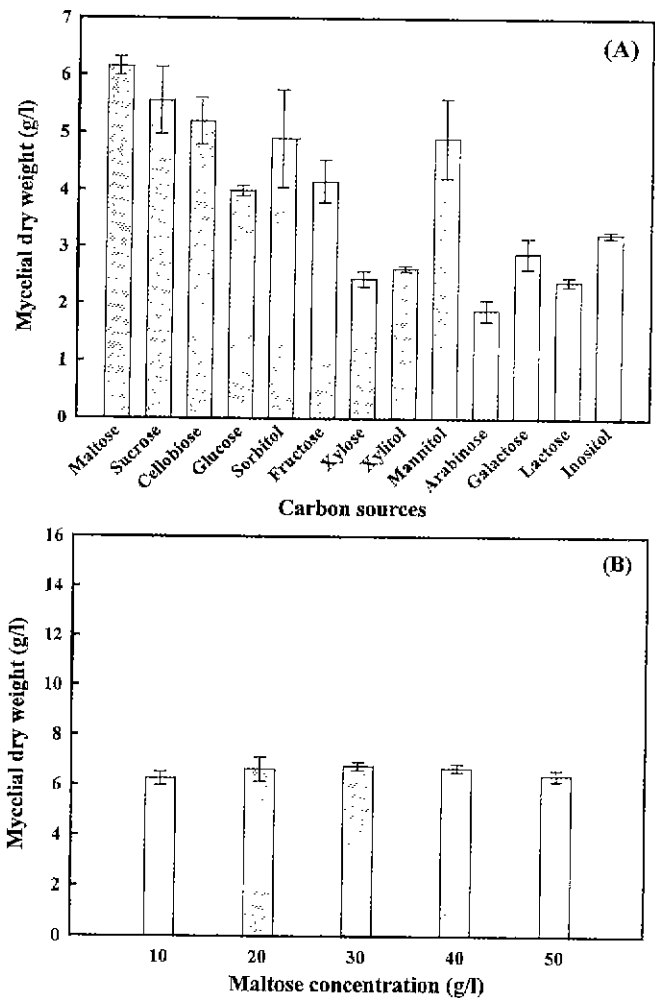


Fig. 2. Effect of carbon sources (A) and maltose concentration (B) on the mycelial growth of *P. japonica*.

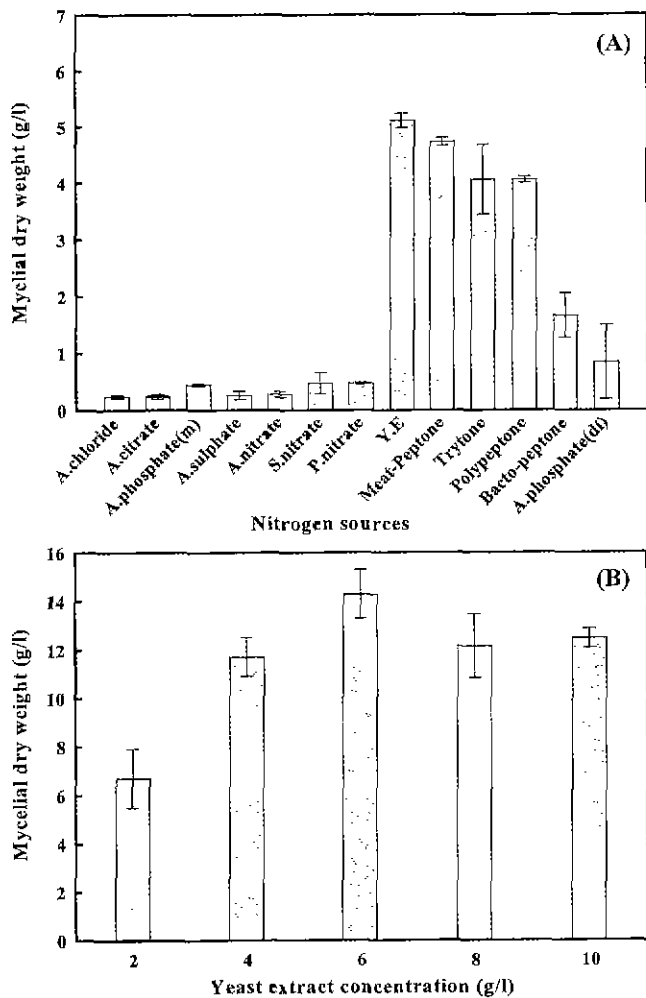


Fig. 3. Effect of nitrogen sources (A) and yeast extract concentration (B) on the mycelial growth of *P. japonica*.

maltose, sucrose, cellobiose, sorbitol, and mannitol were favorable to the mycelial growth of *P. japonica* (Fig. 2A). The maximum mycelial growth (6.85 g/l) was achieved in a 3% (w/v) maltose medium (Fig. 2B). Shim *et al.* [23] reported that maltose was the best carbon source known for stimulating mycelial growth of another higher fungus, *Sparassis crispa*. Organic nitrogen sources gave rise to higher mycelial growth compared to inorganic sources, where maximum mycelial growth (14 g/l) was achieved in a yeast extract medium (0.6%, w/v) (Fig. 3). Mycelial growth was further increased by adding polypeptone (0.2%, w/v) (data not shown). This result is very similar to the observation made by Park and Lee [18] in relations to their two fungal fermentations.

Potassium phosphate (0.05%, w/v) was the most effective inorganic salt for the mycelial growth among 8 different kinds of inorganic salts (0.5 g/l) tested (Fig. 4). $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and KH_2PO_4 were also recognized as favorable bioelements for mycelial growth (data not

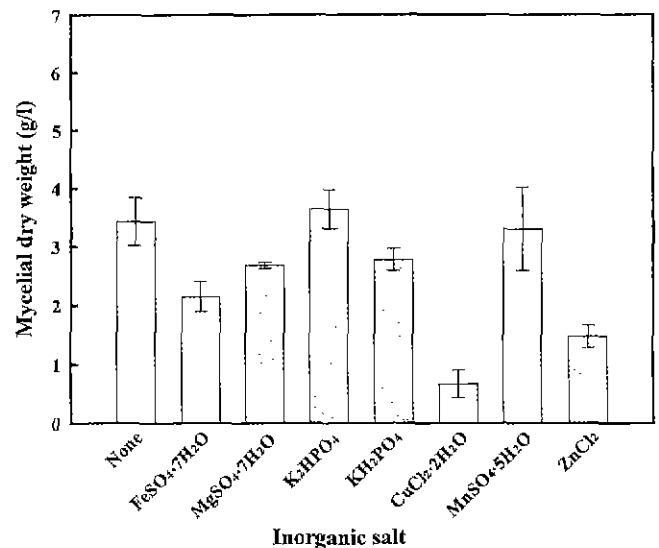


Fig. 4. Effect of inorganic salts on the mycelial growth of *P. japonica*.

shown). The optimal medium composition obtained in this study is summarized in Table 1.

Effects of pH and Temperature

In order to investigate the effect of pH on mycelial growth and exo-biopolymer production, *P. japonica* was cultivated in the optimal medium with different initial pHs in shake culture conditions (Fig. 5A) [8, 13]. The optimal culture pH for both mycelial growth and exo-biopolymer production was 5.0, which is similar to the result obtained by several investigators [13, 27].

To find the optimal culture temperature, *P. japonica* was cultivated under three temperatures of 20°C, 25°C, and 30°C, and the optimal temperature for both mycelial growth and exo-biopolymer production was found to be 25°C (Fig. 5B).

Morphological Changes

A large number of studies on the effect of culture conditions on fungal morphology have been reported [16, 19, 20, 28, 30]. Pirt and Callow investigated the influence of pH on *Penicillium chrysogenum*. Filamentous growth occurred at pH 6.0, while, at a higher pH up to pH 7.4, pellet

Table 1. Suggested medium composition for mycelial growth and exo-biopolymer production by *P. japonica*.

Chemicals	Concentration (g/l)
Maltose	30
Yeast extract	6
Polypeptone	2
K_2HPO_4	0.5
KH_2PO_4	0.2
$\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$	0.2
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2

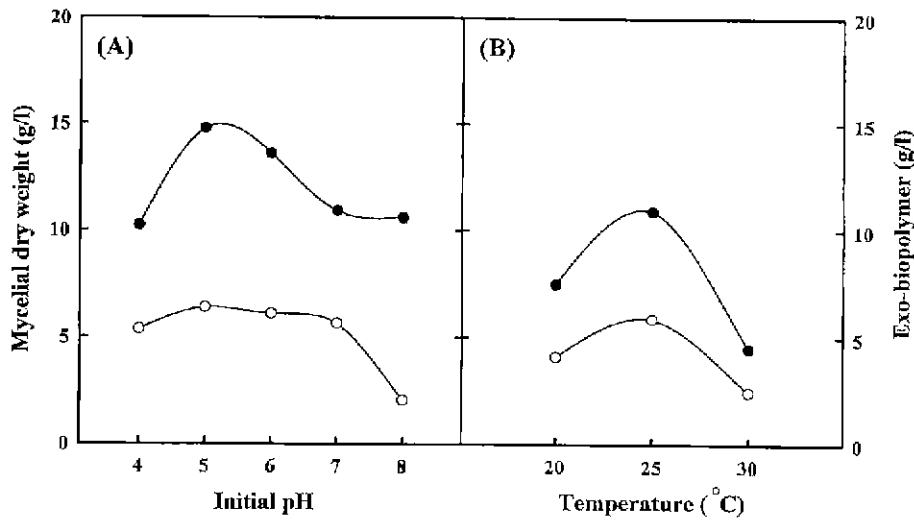


Fig. 5. Effect of temperature (A) and pH (B) on the mycelial growth of *P. japonica* and exo-biopolymer production. Mycelial dry weight (●), and exo-biopolymer (○).

formation took place together with the formation of shorter and thicker hyphae [20]. Whitaker and Long have reported that *Aspergillus niger* grew in a filamentous form at pH 5.0. At pHs 6.0, 7.0, and 8.0, pellets of various types were formed depending on the spore concentration in the inoculum [16, 30]. Submerged growth of *P. japonica* varies from the pellet to the filamentous form depending on the

growth medium, physical environment, aeration and agitation, etc. Of all the factors, culture pH was the most important parameter to significantly affect morphological change. When the organism was grown at pHs 5.0 and 6.0, only the filamentous form was observed, whereas pellet formation occurred at other pH conditions (Fig. 6).

Fermentations under Optimal Culture Conditions

Figures 7 and 8 show various time courses of mycelial growth and exo-polymer production in a flask culture and in a 2.5-l jar fermentor, respectively, under the optimal

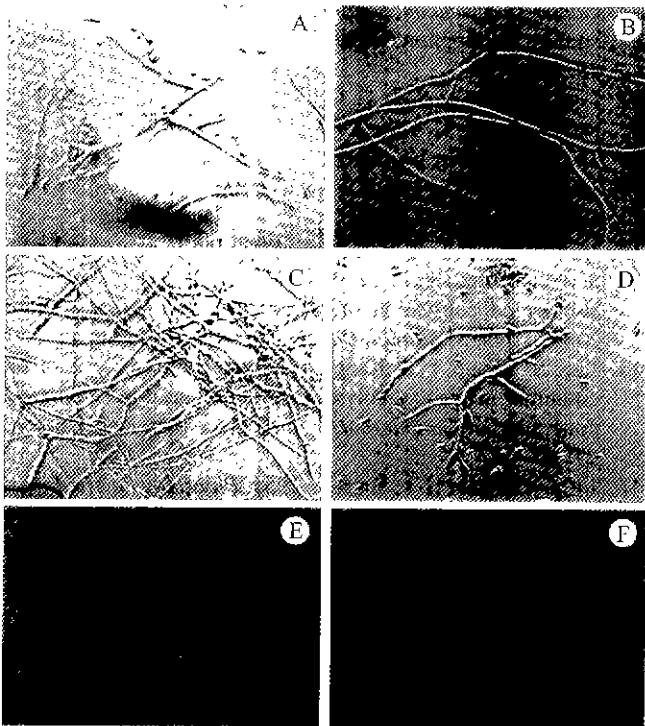


Fig. 6. Morphological change of mycelium during submerged culture of *P. japonica* under different pHs. pH 4.0 (A), pH 5.0 (B), pH 6.0 (C), pH 7.0 (D), pH 8.0 (E), pH 9.0 (F)

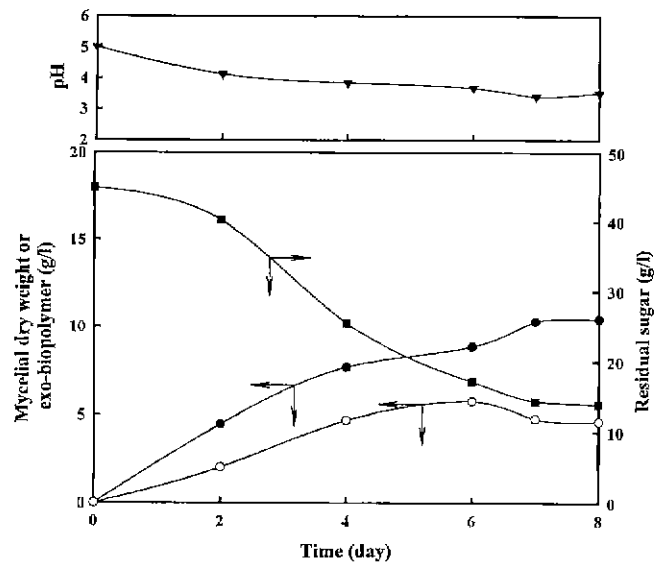


Fig. 7. Typical time course of the mycelial growth and exo-biopolymer production in the suggested medium in the flask culture. pH (▼), residual sugar (■), mycelium (●), exo-biopolymer (○).

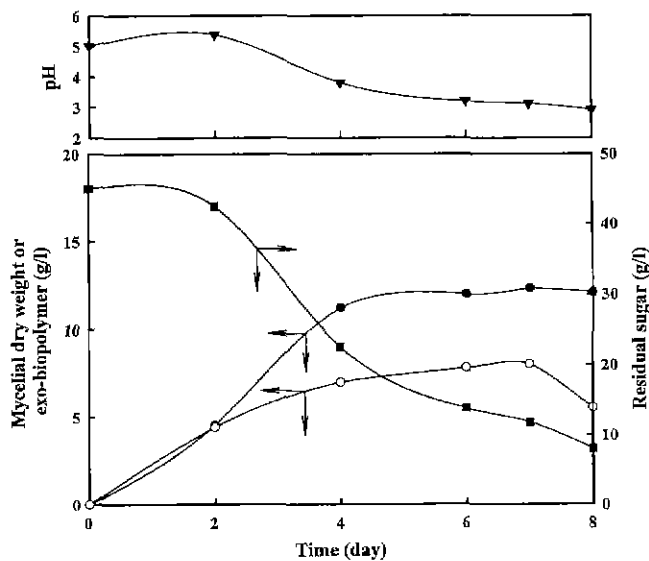


Fig. 8. Typical time course of mycelial growth and exo-biopolymer production in a 2.5-l jar fermenter. pH (\blacktriangledown), residual sugar (\blacksquare), mycelium (\bullet), exo-biopolymer (\circ).

culture conditions. In shake flask cultures, the exo-biopolymer concentration reached a maximum level of 5.75 g/l after 6 days of fermentation, while the mycelial dry weight reached a maximum of 10.4 g/l after 7 days of fermentation (Fig. 7). In a batch bioreactor, the exo-biopolymer concentration (8 g/l) was markedly increased by 40% after 7 days of fermentation, while the maximum mycelial dry weight was also significantly enhanced during the same fermentation period (Fig. 8). For fermentations in both shake culture and fermentor, the growth of microorganism was rapid for the first 4 days with a corresponding depletion in sugar concentration, and then slowly reached a plateau, as shown in Figs. 7 and 8. The initial pHs of the fermentation broth slowly decreased from 5.0 to 3.5, and reached 2.9 after 8 days of fermentation in both shake culture and bioreactor (Figs. 7 and 8). The residual maltose concentration decreased during the entire period of the fermentation process (Figs. 7 and 8). The results strongly suggest that exo-biopolymer production in *P. japonica* is growth-associated.

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