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Culture conditions and medium components for the production of mycelial biomass and exo-polysaccharides with *Paecilomyces japonica* in liquid culture

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In this study, the liquid culture conditions were optimized for maximal production of mycelial biomass and exo-polysaccharide by *Paecilomyces japonica*. The effects of medium composition, C/N ratio and physical parameters were investigated. From these experiments, 30 g glucose, 20 g yeast extract, 0.5 g KH_2PO_4, and 0.1 g $CuCl_2$ $2H_2O$ in 1-l distilled water were found to be the most suitable carbon, nitrogen, and mineral sources, respectively. The optimal temperature, initial pH, agitation, and aeration were determined to be 27°C, uncontrolled pH, 400 rpm, and 1.0 vvm, respectively. Under these optimal conditions, the maximum mycelial growth and polysaccharides production were 23.1 g/l and 2.5 g/l, respectively.

[**Key words:** *Paecilomyces japonica*; Exo-polysaccharide; Mycelial growth; Carbon-to-nitrogen ratio; Entomopathogenic fungus]

In recent years many natural polysaccharides and polysaccharide–protein complexes, isolated from mushrooms, have been used as therapeutic drugs (1). Many studies have demonstrated that polysaccharides from basidiomycetes mushrooms had highly beneficial therapeutic effects including: (i) preventing oncogenesis after the administration of peroral medications developed these mushrooms or their extracts, (ii) direct anti-tumor activity against various tumors, (iii) synergistic anti-tumor activity in combination with chemotherapy, and (iv) preventive effects on tumor metastasis (2–5). *Paecilomyces japonica*, an entomopathogenic fungus belonging to the class Ascomycetes, has been reported to have beneficial biological activities such as anti-tumor, immunostimulating, and antioxidant activities (6,7). The production of fruiting body is limited and cultivating the fruiting bodies from mycelia in artificial culture medium has proven to be difficult. However, using optimized liquid culture conditions, it may be possible to increase production of mycelial and bioactive molecules in a compact space and shorter time with less chance of contamination. Various studies have been conducted to obtain cellular or extracellular bioactive molecules from the liquid culture of mycelia for use in the formulation of nutraceuticals (8,9). Although the liquid culture of mushrooms for production of bioactive molecules has been studied extensively, the liquid culture of *P. japonica* has only been scarcely studied until recently (10–12). Since nutrient sources, such as carbon and nitrogen, generally play a significant role in cell proliferation and metabolite biosynthesis, the components used in the culture medium are important to the yield of any fermentation products (13–15). Thus, if it is possible to overcome the drawbacks associated with cultivating *P. japonica* in liquid culture, the cultured mycelia and the beneficial substances that it produces may be used in processed foods on a commercial scale. Therefore, in this study, the purpose of this study was to optimize the liquid culture conditions and culture medium components to obtain maximal production of mycelial biomass and exo-polysaccharides (EPS) by *P. japonica*.

MATERIALS AND METHODS

Strain and media The strain used in this study was *P. japonica* KCTC 9817. The basal medium for mycelial growth was YMP, which contained 10.0 g/l glucose, 10.0 g/l yeast extract, 15.0 g/l malt extract, and 10.0 g/l peptone. The composition of the medium for mycelial growth in the 5-l bioreactor was modified and contained 30.0 g/l glucose, 20 g/l yeast extract, 0.5 g/l KH_2PO_4, and 0.1 g/l $CuCl_2$ $2H_2O$.

Inoculum preparation The seed culture was cultivated for 3 days in 50 ml of the YMP media in a 250-ml flask that was inoculated with 8 ml of an activated stock solution frozen at −70°C. For mycelial growth, the mycelia were homogenized with a Heidolph DIAX 600 homogenizer (VWR International, West Chester, PA, USA) and 2% of the seed culture broth was inoculated into 50 ml of the YMK media in a 250-ml flask. They were then cultivated for 8 days at 27°C and 200 rpm in a shaking incubator (Vision Scientific Co., Ltd., Buchun, Korea). Fermentation was carried out in a 5-l bioreactor (Korea Fermentor Co., Seoul, Korea). The culture medium was inoculated with 2% (v/v) of the seed culture. The aeration rate and agitation speed were varied from 0.5 to 1.5 vvm and from 100 to 500 rpm, respectively to assess the effects of these parameters.

Analytical methods After 7 or 8 days of cultivation, the culture broth was centrifuged at 5000 rpm for 20 min. Precipitated mycelia were washed three times with distilled water, and then dried for 24 h at 60°C. The EPS derived from the liquid cultured broth were prepared by the ethanol precipitation method with three times volume followed by standing at 4°C overnight, filtered with 0.45 μm Whatman filter paper and then dried in a drying oven to a constant weight. The dry weight of mycelia and EPS was quantified by subtracting the dry weight of the filter paper from the total weight. The residual glucose in the cultured broth was determined

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using a glucose assay kit (Sigma Diagnostics, St. Louis, MO, USA) and glucose analyzer (YSI Inc., Yellow Springs, OH, USA) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Effects of temperature and initial pH In order to investigate the effect of temperature on mycelial growth and EPS production, *P. japonica* was cultivated at various temperatures ranging from 15 to 33°C in shake flask cultures. The optimum temperature for mycelial growth and EPS production was found to be 27°C (Fig. 1A). Previous studies have reported similar results during mycelial growth and EPS production for most basidiomycetes in liquid culture (16–18). Among the environmental factors, culture pH has been known to significantly influence cellular morphology and metabolites biosynthesis (19,20). To investigate the effect of initial pH on mycelial growth and EPS production, *P. japonica* was cultivated under the different initial pHs (4.0–11.0) in shake flask culture. Initial pH was controlled using 1 N NaOH and 1 N HCl. In these experiments, higher mycelial growth and EPS production were observed at subacid or neutral pH (ranging from 5 to 8) (Fig. 1B). This pH optimum is comparable to many other mushrooms, which have neutral pH optima for both cell growth and EPS production in liquid culture. It has been shown that mycelia produce organic acids, which decrease the medium pH to optimal culture conditions (21–23).

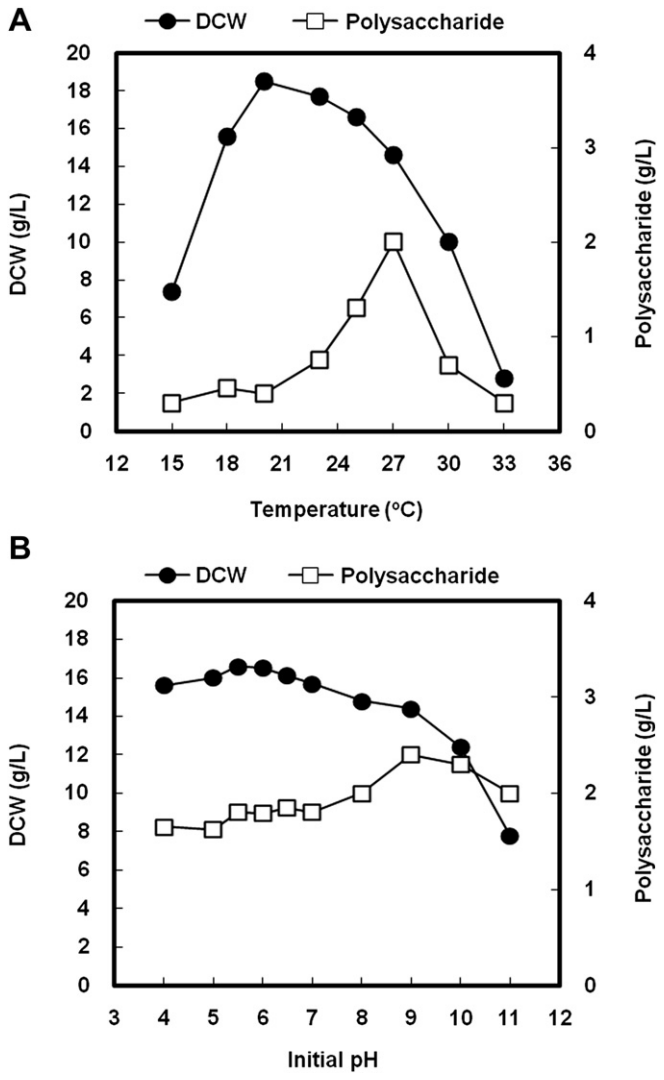

FIG. 1. The effect of temperature (A) and initial pH (B) on mycelial growth and EPS production by *P. japonica* in YMP medium.

Effects of carbon source and nitrogen source Different carbon sources have been shown to maximize mycelial growth in the liquid culture of higher basidiomycetes (24). To find a suitable carbon source for mycelial growth and EPS production in *P. japonica*, various carbon sources at a concentration of 1.0% (w/v) were examined for 4 days in sugar-free basal medium. In these experiments, a high level mycelial growth was observed in medium that contained each carbon source tested relative to the control (sugar-free basal medium). Of all the various carbon sources tested, the highest EPS production was obtained in medium containing glucose, followed by starch (Fig. 2A). Therefore, glucose was selected as the main carbon source due to its low cost and feasibility in handling. The nitrogen sources are the essential factors for mycelial growth and polysaccharide production (25). To investigate the effect of nitrogen sources on mycelial growth and EPS production of *P. japonica*, nitrogen compounds based on inorganic and organic nitrogen were added individually to N-source-free basal medium at a concentration of 0.5% (w/v). Among the 10 nitrogen sources examined, the highest mycelial growth (7.10 g/l) and EPS

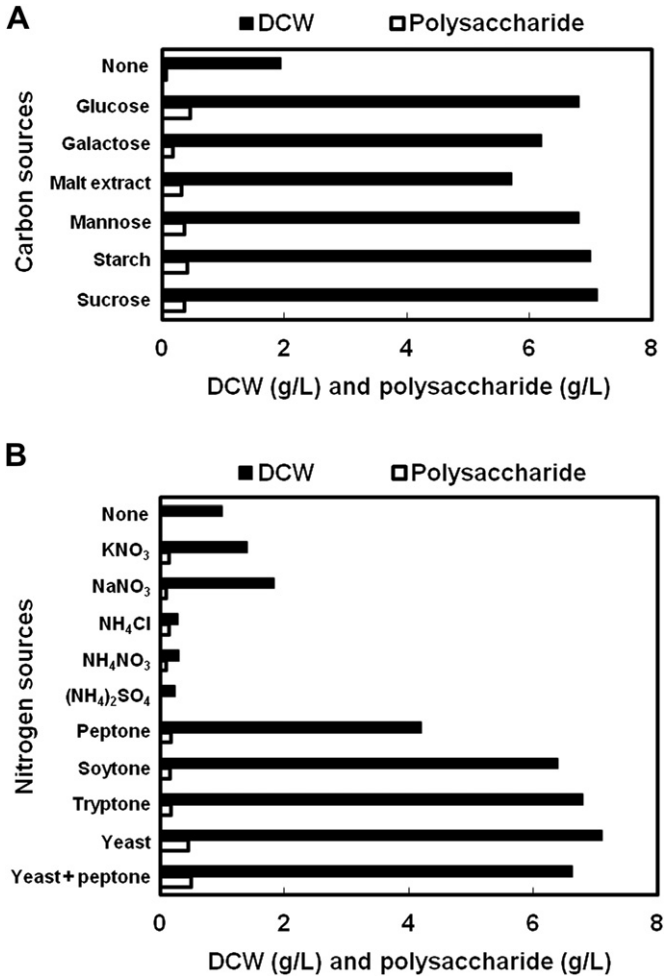

FIG. 2. The effect of different carbon and nitrogen sources on mycelial growth and EPS production by *P. japonica* in YMP medium. The mycelia were cultivated for 4 days at 27°C, 200 rpm, and a 2% inoculum size. (A) Glucose in the YMP was replaced with various other carbon sources. The final concentration of the carbon source was 1.0% (w/v). The control was glucose-free medium in YMP medium. (B) Yeast extract in the YMP was replaced by various other nitrogen sources. The final concentration of the nitrogen source was 0.5% (w/v). Nitrogen source-free YMP medium was used as the control.