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Exopolysaccharide Production by Some Mushroom Fungi in Wheat Flour Liquid Culture Medium

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Abstract

Polysaccharide production at extracellular as well as intracellular level in wheat flour medium by five mushroom mycelial liquid cultures of *R. lepida, R. brevipes, R. nigricans, L. tuberregium* and *C. indica* was evaluated in the present study. All strains are found to produce both extra- and intra-cellular polysaccharides efficiently in wheat flour broth medium at pH 6.0. Among all the strains, *R. lepida* and *L. tuberregium* are found to be potent producer of exopolysaccharide i.e 440 mg/gm and 431 mg/gm of crude extract. C. indica followed by R. lepida are the best for intracellular polysaccharide production and R. brevipes contributed least.

Keywords: Fungus; Mushroom; Mycelium; Flour broth; Polysaccharides

Abstract

Introduction

Mushrooms are good source of bioactive compounds with versatile applications in different bio-productive field [1-7]. Mushroom mycelial culture can also be an alternative source of these bioactive compounds as mushroom grows seasonally in limited way. However, liquid medium or submerged culture condition was the most reliable technique researchers are using now days, giving more fruitful products i.e both use of mycelium along with secreted compounds in comparison to solid culture technique which doesn't support effective product production [8,9]. Polysaccharide production under liquid culture is also affected by the media types, composition, pH, temperature [10-12]. In view to this, we have used wheat flour as main medium component and observed the effect on exopolysaccharide (EPS) & intra-cellular polysaccharide (IPS) production by mushroom mycelial cultures.

Materials and Methods

Flour broth medium was prepared at a concentration of 20 g per 1000 ml of distilled water. pH of the medium maintained at 6.0. Inoculation of five mycelial cultures were done in 50 ml of medium and kept for 14 days of incubation period. Un inoculated flour broth medium was taken as control.

Mycelial culture of 5 edible mushrooms (*Russula lepida, Russula brevipes, Russula nigricans, Lentinus tuberregium* and *Calocybe indica*) was prepared in wheat flour medium of pH 6.0 and incubated at 28 ºC for 14 days. The mycelial biomass produced in each treatment was harvested by filtration to separate the culture broth and the fungal biomass was washed several times with distilled water, then air dried at room temperature in order to get the intracellular polysaccharide.

The crude exopolysaccharide produced in the culture filtrate was obtained through precipitation method [13] by adding isopropanol at the rate of 1:1 v/v. The solution was shaken at 60 rpm for 5 hrs followed by incubation at 4 ºC, overnight. The samples were centrifuged at 4500 rpm for 15 minutes and the precipitate (Crude EPS) & supernatant was separated. The extracted EPS was lyophilized and 10 mg of each crude EPS sample was used for the estimation using Phenol Sulphuric acid method [14]. The intracellular polysaccharide present in the mycelia was extracted by heating crushed paste of fungal culture at 100 ºC for 3 hrs. After cooling at room temperature, left overnight at 4 ºC and centrifuged at 2000 rpm for 5 minutes to get the extract. Isopropanol

was added at 1:1 v/v to the extract and shaken for 5 hrs at 60 rpm, 28 ºC and left overnight at 4 ºC. Precipitation was obtained after centrifuging at 4500 rpm for 15 minutes. 10 mg of each crude intracellular polysaccharide sample was used for estimation by phenol sulphuric acid method [14]. Similar procedure was followed to get the EPS from control flour medium

Results and Discussion

Mushrooms have wide variety of medium adaptability; hence can be grown in wide range of medium composition. Also mushroom culture secretes different concentration of EPS when grown in different medium. Much work has been done using different media showing positive effect on EPS productivity. General fungi including *Mucor rouxii* produces good amount of exopolysaccharide using beet molasses as medium under low pH [15]. Also Mushroom complete medium, Tien and Kirk medium, Trametes defined medium, Yeast malt extract medium, Glucose yeast extracts peptone medium, Potato dextrose broth medium used to study the EPS productivity by *Coriolus versicolor* [10], Mushroom complete medium for *Plerotus ostreatus* by [16] and Potato dextrose broth medium for EPS productivity by *Pleurotus citrinopileatus* [17]. However using flour broth medium for EPS production by mushroom mycelial culture has not been evident to the best of our knowledge. And it shows good contribution towards growth and exopolysaccharide productivity by mushroom mycelial cultures.

As wheat flour itself is a source of carbohydrate, to get the polysaccharide from the strain of interest we have deducted the polysaccharide of control from the test sample and got the amount to be secreted by the organism by Phenol sulphuric acid method.

Results obtained on the analysis of the EPS and IPS production in culture filtrate and fungal biomass, respectively are depicted in (Figure 1 & 2). *Russula lepida* and *Lentinus tuberregium* are the best exopolysaccharide producers in wheat flour medium (440 mg/gm and 431 mg/gm). However, *R. brevipes, R. nigricans* and *C. indica* also have EPS producing ability i.e 140 mg/gm, 114 mg/gm and 134 mg/gm, respectively. *C. indica* produced 428 mg/gm of the product (intracellular polysaccharide) followed by *R. lepida* and *L. tuberregium* (350 mg/gm and 335 mg/gm) respectively.

It was observed that the wheat flour medium is quite simple and cost effective technique for obtaining crude polysaccharide is good amount. Further addition of some carbon and nitrogen sources along with minerals and vitamins may enhance its productivity.

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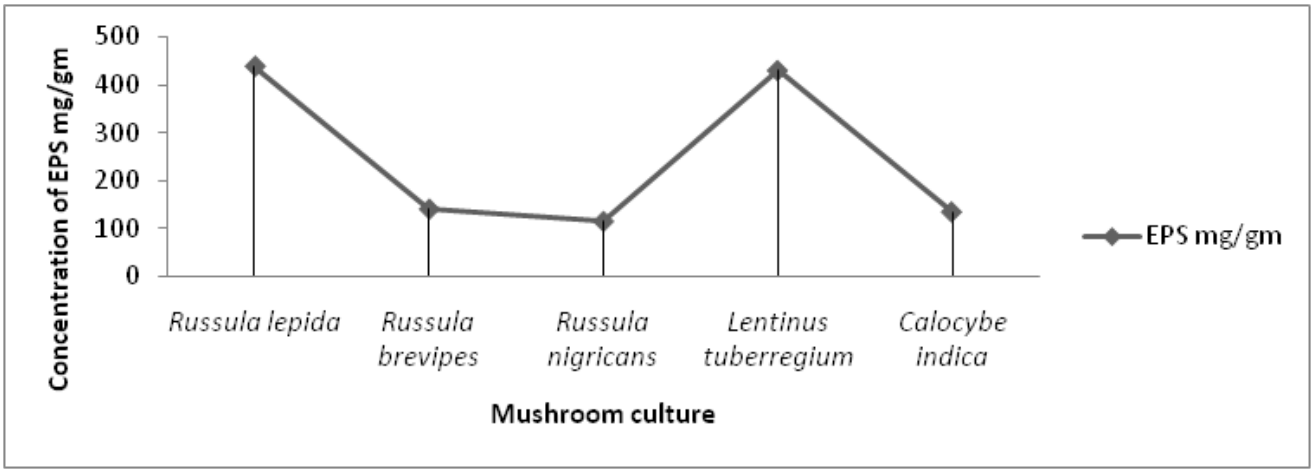

Figure 1: Exopolysaccharide produced by five edible mushroom cultures in wheat flour broth medium at 14 days of incubation period

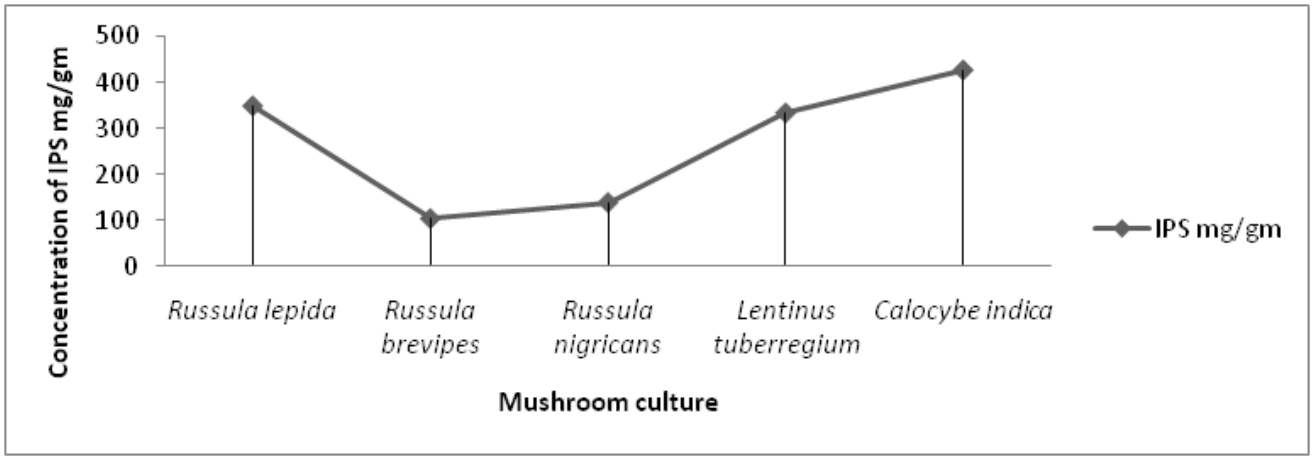

Figure 2: Intracellular polysaccharide produced by five edible mushroom cultures in wheat flour broth medium at 14 days of incubation period.

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