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Screening for exopolysaccharide production from basidiomycetes of chhattisgarh

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Abstract
Polysaccharides extracted from mushrooms have wide applications. Seven species of mushrooms i.e. *Grifola frondosa, Polyporus species 1, Polyporus species 2, Pleurotus sajorcaju, Pleurotus florida, Schizophyllum commune and Jelly species* were collected and each species was tested for their polysaccharide producing ability. Among all seven species, *Pleurotus sajorcaju* produces maximum exopolysaccharide & minimum was produced by Pleurotus *florida 1*. Polysaccharide production was checked by incubating all seven species in shaking and static conditions. Although 28°C at 150 rpm for 7 and 14 days is the best condition for exopolysaccharide production from mushroom species. Total biomass (dry weight and wet weight) was also recorded. Maximum biomass was found to be of *Schizophyllum commune*. Total free glucose produced was also determined and was found to be highest in *Pleurotus florida*. Natural polysaccharides can play a relevant role in biomedical and pharmaceutical applications, particularly in the field of drug delivery, for their intrinsic biocompatibility and potential low cost.

Keywords: Basidiomycetes, exopolysaccharide, biomass and *Pleurotus sajorcaju*

INTRODUCTION

Chhattisgarh have wide diversity in forest, including the mushroom diversity. There are different types of mushroom present in the forest out of which some are reported. While many of them are unexplored, this may possess some medicinal value. Tribal people are using these mushrooms for their traditional treatment system. This study will give information about the medicinally important mushroom present in the forest of the Chhattisgarh. The researchers from various states and other countries have reported several medicinal mushrooms.

Basidiomycetes have been studied extensively for their capacity of degradation. The so-called white rot fungi, which degrade lignin, have this peculiar capacity that leads to research on degradation of xenobiotics. In addition to enzymes, there is evidence that the extracellular polysaccharides produced by these lignocellulolitic fungi play an important role in the process [1&2]. These exopolysaccharides can immobilize the exocellular enzymes. According to Catley [3], the gel formed by these biopolymers prevents the hyphal dehydration, permits cell adherence to other cells or to surfaces and could possibly select molecules from the environment.

A practical aspect of the study and characterization of fungal exopolysaccharide is the availability of data for the investigation of its physiological and ecological importance. In addition, this biopolymer may have potential industrial applications. An example is the exopolysaccharide known as schizophyllan that is produced by the Basidiomycete *Schizophyllum commune.* This polymer is a b- (1 3), (1 6)-glucan, soluble in water, that forms a viscous solution with high thermal stability. It is already used in commercial areas.

Another possible application of these biopolymers is in human health. There is intensive research on fungal polysaccharides as antitumor agents [4 & 5]. The fungal biomass can have various uses, which is an advantage as far as the fermentation is concerned because the process residue is reduced [6]. Possible uses for this biomass are food or feed in the form of protein supplement or source of lipids. It can also be used for the extraction of flavors [7] and other metabolites, such as enzymes and polysaccharides. The most recent utilization of fungal biomass is for wound healing. According to Hamlyn and Schmidt [8], chitin, that has a healing capacity, is already in the fibrous form when extracted from the fungal cell wall. This might facilitate its manipulation.

The aim of this work was to screen seven isolates of Basidiomycetes for exopolysaccharide and biomass production in submerged culture contributing to the study of the potentiality of the Chhattisgarh mycobiota.

MATERIALS AND METHODS

Microorganisms

Seven isolates of native Chhattisgarh Basidiomycetes were screened. The pure cultures came from the Modern Biotech collection center, Raipur, C.G. and are shown in Table 1. All mushroom cultures were maintained on potato dextrose agar plates (200g peeled potato; 20g dextrose; 20g agar; pH 5.6).

Liquid culture medium (g/l)

Peptone 1.0; yeast extract 2.0; K_2HPO_4 1.0; $MgSO_4.7H_2O$ 0.2; $(NH_4)_2SO_4$ 5.0; glucose 39.0; pH 6.0. This medium was selected in preliminary studies as adequate for exopolysaccharide production by Basidiomycetes [9].

Erlenmeyer flasks containing 100 ml of sterilized culture medium were inoculated with the suspension in sterile water of

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fungal mycelium grown on two potato dextrose agar slants. Incubation was done at 28°C on shaker at 150 rpm.

Screening

For the screening, all the isolates were grown in two sets, one set was placed in shaking condition at 150 rpm and another set were placed in static condition at 28°C and incubation times were 7 and 14 days. The culture was filtered to separate fungal biomass, which was washed twice with distilled water and quantified as dry weight (dried at 80º). Isopropanol was added to the culture filtrate (1:1 v/v) and after 24 h at 4°C the precipitated biopolymer was separated by centrifugation (8,000 rpm for 10 minutes) and also quantified as dry weight [10].

Glucose assay

The residual glucose content of the culture filtrate was determined with a spectrophotometric method [11].

RESULTS AND DISCUSSION

Almost all the strains produced exopolysaccharide in different quantities (Table 1 & 2) (Figure 1 & 2). The experiment was carried out in two sets, in one the cultures were kept on shaker (I) at 150rpm and other in incubator (II) at 28ºc for 7 days & 14 days of incubation. After 7 days four of the strains i.e. *Grifola frondosa, Polyporous species (1,2)* (fig: 3.b), *Jelly sp*. (II) (fig: 3.c) produced a yield of 0.6 g dry w/l and *Jelly sp.* (I) produced a yield of exopolysaccharide of 0.5 g dry w/l. After 14 days the best yield was produced by *Pleurotus sajor caju* (I) with 1.1 dry w/l and *Pleurotus florida* and *Grifola frondosa* (I) with 0.8 g dry w/l, whereas *Polyporus sp. (1,2)* (I) with 0.7 g dry w./l, *Pleurotus sajor caju with 0.6 g dry w./l, Shizophyllum commune* (I, II) (fig: 3.a) with 0.4 g dry w/l, and *Pleurotus florida* (II) with the lowest exopolysaccheride yield of 0.3 g dry w./l were obtained.

Table 1. Production of exopolysaccharide in shaking condition

SlNo	Species	Biomass		Polysaccharide (g/l)	
		Wet wt. (g/l)	Dry wt. (g/l)	7 days	14 days
1.	*Grifola frondosa*	94.56	2.87	0.2	0.8
2.	*Polyporus species 1*	23.45	0.44	0.3	0.7
3.	*Polyporus species 2*	62.64	3.35	0.3	0.7
4.	*Pleurotus sajor caju*	26.26	0.49	0.6	1.1
5.	*Pleurotus florida*	73.38	2.00	0.5	0.8
6.	*Schizophyllum commune*	141.80	12.59	0.1	0.4
7.	*Jelly species*	85.11	3.08	0.5	0.5

Table 2. Production of exopolysaccharide in non-shaking condition

SlNo	Species	Biomass		Polysaccharide (g/l)	
		Wet wt. (g/l)	dry wt. (g/l)	7 days	14 days
1.	*Grifola frondosa*	72.36	5.3	0.6	0.6
2.	*Polyporus species 1*	49.34	1.84	0.6	0.6
3.	*Polyporus species 2*	55.68	2.78	0.6	0.6
4.	*Pleurotus sajor caju*	104.88	8.54	0.6	0.6
5.	*Pleurotus florida*	97.5	2.2	0.3	0.3
6.	*Schizophyllum commune*	152.06	18.72	0.4	0.4
7.	*Jelly species*	116.74	6.54	0.6	0.6

Table 3. Free glucose concentration of Basidiomycetes

S.No	Species	Concentration of glucose (mg/ml)	
		Flasks in incubator	Flasks on shaker
1.	*Pleurotus florida*	5.67×10^{-4}	5.94×10^{-4}
2.	*Polyporus species 1*	4.15×10^{-4}	5.93×10^{-4}
3.	*Jelly species*	4.68×10^{-4}	5.59×10^{-4}
4.	*Pleurotus sajor caju*	4.88×10^{-4}	5.56×10^{-4}
5.	*Polyporus species 2*	4.5×10^{-5}	4.29×10^{-4}
6.	*Grifola frondosa*	3.38×10^{-4}	3.77×10^{-4}
7.	*Shizophyllum commune*	6.9×10^{-5}	2.01×10^{-4}

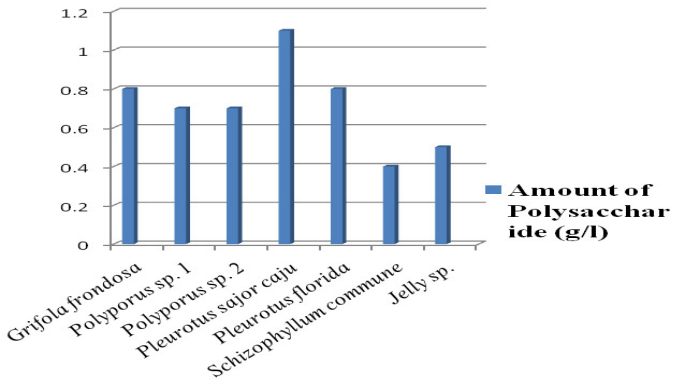

Fig 1. Production of exopolysaccharide in shaking condition.

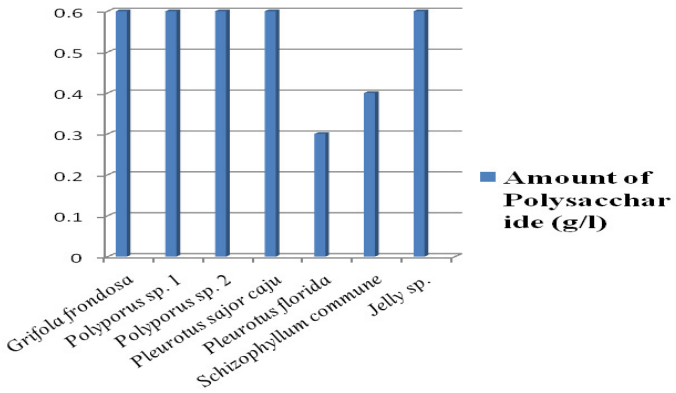

Fig 2. Production of exopolysaccharide in non-shaking condition

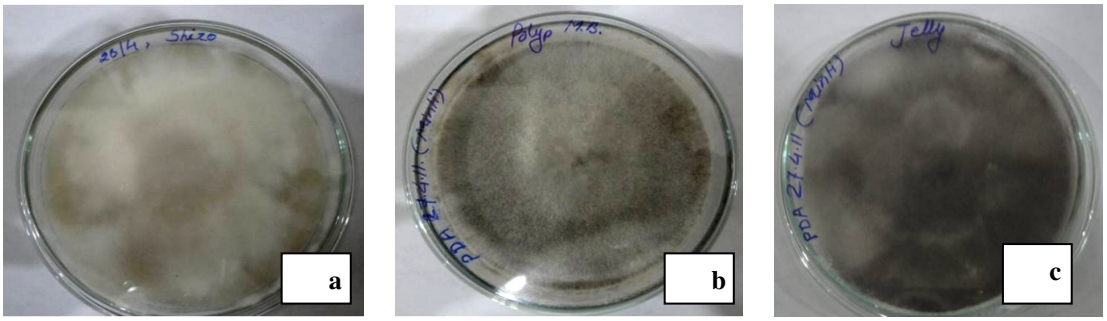

Fig 3. Subculture of mushroom isolates in Potato dextrose agar medium a. Schizophyllum commune; b. Polyporus sp.1; c. Jelly sp.

Fig 4. Polysaccharide production in shaking condition

Maximum exopolysaccharide were produced by 35.71% of the strains in the incubation of 7 days. However 64.29% of the strains produced polysaccharide in 14 days. The best producer of biomass *Shizophyllum commune* with 12.59 g dry w/l (I) and 18.72 g dry w/l (II) in 14 days of incubation. When the culture filtrate was submitted to freezing prior to polysaccharide precipitation a gelatinous fraction was formed. The pellets formed can be regular or irregular in form & size. It may vary in form 1mm to 20mm.

Free glucose content was found highest in *Pleurotus florida* (5.94×10^{-4}) and lowest in *Polyporus* sp. 2 (4.5×10^{-5}) (Table 3).

DISCUSSION

According to R. Maziero et al. [10] the best yield was produced by *Agaricus sp.,* with 6.01 g dry w./l (conversion yield, $Y_{p/s}$= 0.761) and *Oudemansiella canarii* with 3.54 g dry w./l ($Y_{p/s}$= 0.131) after 7 days of incubation. *Tricholoma crassum* had a similar production (3.23 g dry w./l) with conversion yield of 0.131, but after 14 days of incubation. The conversion yield of glucose as polymer varied between 0.020 and 0.100 for 75% of the strains and the best yields were those of *Agaricus sp.* (0.761) and *Calvatia cyathiformis* (0.293). Biomass production ranged from 0.34 to 16.68 g dry w./l. Some strains, such as *Agaricus xanthodermus, Calvatia cyathiformis* and *Climacodon pulcherrimus*, had a slow growth rate in these culture conditions. Others, such as *Schyzophyllum commune, Rigidoporus microporus, Oudemansiella canarii, Irpex lacteus* and *Nothopanus hygrophanus* produced more than 15g dry w./l of biomass. Among the edible strains, those that produced more biomass after 7 days incubation were *Pleurotus sajor-caju* (11.47 g dry w./l), *Pleurotus florida sp.*(11.02 g dry w./l), and *Agrocybe platensis* (10.18 g dry w./l). After 14 days incubation, the best biomass producer was *Lepista sp.* (13.48 g dry w./l).

Y. T. Jeong et al. [12] experimented on Optimal Culture Conditions (pH, temperature, carbon & nitrogen source) for mycelial growth and exo-polymer Production of *Ganoderma applanatum*. They stated that 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. Maximum yield of mycelia (10.03 g/l) and exo-polymer (1.55 g/l) were achieved at 28°C. The maximum yield of mycelial growth and exo-polymer was at 150 rpm. 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 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.

In the present work all the seven strains produced polysaccharide in different quantities. The best producer of biomass was *Shizophyllum commune* with 18.72g dry w/l in 14 days of incubation.

The maximum polysaccharide was produced by *Pleurotus sajor caju* with 1.1 g dry w/l and minimum was produced by *Pleurotus florida* with 0.3 g dry w/l. The optimum condition for culture growth was 28°C in shaker for 150 rpm for 7 and 14 days. The glucose concentration ranged from 3.38×10^{-4} to 6.9×10^{-5} and was found maximum in *Shizophyllum commune*.

Results showed that most of the Basidiomycetes strains screened are potential exopolysaccharide producers. The possibility of using these biopolymers for medical application promises a large opportunity to improve the study of such group of fungi.

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