Brief Report

Screening of Mushrooms for Polysaccharides

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Background: Mushrooms have been valued as high tasty nutritional and nutraceutical food throughout the world. At present 270 species of mushrooms are known to have various nutritional and therapeutic properties. Polysaccharides are the primary content of mushrooms that are used for their pharmaceutical properties as immunostimulators.
Objectives: Here, Mushrooms were screened for useful polysaccharides.
Materials and Methods: Fungal fruiting bodies were collected in rainy season, isolated and maintained in malt agar medium, and screened for their endo- and exo-polysaccharides.
Results: The mushrooms screened in this study are good producers of exopolysaccharides (EPS) and intracellular polysaccharides (IPS). *Ganoderma lucidum* was the best producer of polysaccharide among all the isolates.
Discussions: Mushroom polysaccharides are well known for their antidiabetic, antitumor and hepatoprotective activities, and so are likely to be used in the preparation of novel drugs. It is suggested that a thorough study on the optimization and characterization of these polysaccharides and biomolecules is essential to exploit this mushroom on an industrial scale.
Keywords: Biomolecules; Immunostimulators; Medicinal properties; Mushrooms; Nutraceutical; Polysaccharides

1. Introduction

"Mushroom is a macrofungus with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand" (1). Many mushrooms are helpful in human ailments because they possess many typical pharmacological features such that of acting as metabolic activators, preventing/controlling intoxication and microbial/viral infections, helping immunomodulation towards greater homeostasis, rejuvenating as antioxidants and energy boosting properties (2). The bioactive compounds of the mushrooms may be useful as starting materials for the development of chemotherapeutic agents in cancer treatment and for other ailments (3).

Attention has been focused on the development of immunotherapeutic compounds that can identify and eliminate cancer cells as foreign matter (non-self), or be able to act on substances such as immunopotentiators, immunoinitiators and biological response modifiers (BRM) (4). Mushroom polysaccharides have proved to enhance body's immune response, instead of direct cytotoxic effect on tumor cells (3). Therefore, these polysaccharides, isolated from edible and medicinal species belonging to Ascomycetes or Basidiomycetes, have the potential to be developed into medicines that can be carcinostatic, anti-inflammatory, antiviral, hypoglycemic, hypotensive and antithrombotic (3, 5, 6). Immunomodulatory effects of mushroom polysaccharides have been demonstrated *in vitro* and *in vivo* in animal studies, and in humans via oral ingestion of glucans (7).

Fungal antitumor polysaccharides have been extensively reviewed elsewhere (2, 8, 9). Many antitumor or immunomodulating fungal polysaccharides have been identified and exploited commercially in far-east Asia. PSK (Krestin) from cultured mycelia of *Coriolus versicolor* (10), Lentinan from *Lentinus edodes* (11), Schizophyllum from *Schizophyllum commune* (12), and polysaccharide produced by the liquid culture of *Ganoderma lucidum* (13) are some examples. Rosana and valeria reported that different strains of *Schizophyllum commune, Pycnoporus sanguinvus* and *Trametes villosa* produce different amounts of biomass and polymer (14). In another report, submerged cultures of strains of *Schizophyllum commune* demon-

strated EPS production (15). Here and in continuation of earlier reports, some potential mushrooms were screened for their useful polysaccharides.

2. Materials and Methods

2.1. Isolation and Maintenance of Macrofungi

The fruiting bodies of basidiomycetous fungi were collected in rainy season from decaying wood, living trees (lignicolous), soil (terrestrial) and decaying leaf litter. A total of ten fungal species were isolated, which were mostly belonging to Polyporales, Aphyllophorales (white rot fungi) or Agaricales. The characteristics of the fruit bodies were corticoid (effused, stereoid, effuoro reflexed, coralloid, dimidiate (shelfs or brackets), cyphelloid (capulate), polyporoid, agaricoid or boletoid. The fruit bodies were photographed before collection and were preserved by sealing in polythene bags. A small piece of fruiting body was dipped in 0.01% mercuric chloride to remove the surface contamination and washed several times with distilled water to remove the traces of mercuric chloride and transferred aseptically on 3% malt agar slants. Slants were incubated for 5 to 7 d and initial growth of mycelium was observed on 5^{th} day. All the species produced white colored mycelium and grew by spreading over the medium for a period of 5 d. The mycelium collected from the growing edge was transferred into new malt agar slant and incubated further for 5 to 7 d. This was repeated 2 to 3 times to get pure isolates that was stored at 4ºC. Approximately 2 mm^2 of mycelial mat was removed from slants and was allowed to grow on malt agar slants for 7 d.

2.2. Screening for Exopolysaccharide and Endopolysaccharide

A liquid culture medium with 1.0 $g.l^{-1}$ peptone; 2.0 $g.l^{-1}$ yeast extract; 1.0 $g.l^{-1}$ K_2HPO_4; 0.2 $g.l^{-1}$ $MgSO_4.7H_2O$; 5.0 $g.l^{-1}$ $(NH_4)_2SO_4$; 20.0 $g.l^{-1}$ glucose 20.0 (pH 6.0) was prepared in preliminary studies. This medium was reported to be ideal for exopolysaccharides (EPS) production by basidiomycetes (16). Erlenmeyer flasks containing 100 ml of sterilized culture media were inoculated with the suspension in sterile water of fungal mycelium grown on malt extract agar slants and were incubated at 27ºC for both still and shake flask cultures. Shake flask cultures were kept on shaker at 150 rpm for 14 d.

Flasks were screened at 7 and 14 d of incubation. Mycelial mat was separated from liquid culture medium, washed with distilled water, lyophilized and quantified as dry weight (105ºC to constant weight). Isopropanol was added to the culture filtrate (1:1 v/v) and after 24 h at 4ºC, the precipitated exopolysaccharide was separated by centrifugation 200 xg for 10 min and also quantified as dry weight (14). The dried mycelium was extracted with 85% (v/v) ethanol to eliminate low-molecular components. The first fraction of endopolysaccharides was extracted with hot water (80ºC), filtered, concentrated and precipitated by 96% ethanol. The precipitated intracellular polysaccharide (IPS) was centrifuged at 150 xg for 20 min, redissolved with distilled water and centrifuged again at 150 xg for 10 min. The IPS was weighed after lyophilization of the supernatant (13).

2.3. Estimation of Carbohydrates

The polysaccharides in the mat of the *Ganoderma lucidum* were estimated at 10 and 20 d interval of incubation period, using anthrone reagent (17), using glucose (10 $mg.100\ ml^{-1}$) as a standard. One gram of dry mat macerated in 10 ml of Tris-Hcl (pH 6.6) and centrifuged at 2500 rpm for 15 min. Trichloroacetic acid (5 ml) was added to the supernatant. The precipitate was used for protein estimation. Anthrone reagent (4 ml) was added to the supernatant and incubated in boiling water for 15 min. The test tubes were cooled by keeping them under running tap water. The optical density was measured at 620 nm using distilled water as a blank. The concentration of soluble carbohydrates in the aliquot was calculated with the help of standard curve.

2.4. Quantification of Total Phenols

The total phenolic content in the mycelial extract of *G. lucidum* was determined by Folin-Ciocâlteau method on 10^{th} and 20^{th} d of incubation period (18). Sample solution of 100 µl was added to 2 ml of 2% (w/v) sodium carbonate, mixed thoroughly and allowed to stand for 2 min. Folin-Ciocãlteau reagent (100 µl of Folin:Methanol, 1:1, v/v) was added and the mixture was mixed well. After incubation for 30 min, the absorbance was measured at 750 nm. A calibration curve was obtained using various concentrations of gallic acid. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per g of dry sample.

3. Results

Based on the fruit body characteristics, the fungi

were identified by Friesian classification system (1874) proposed by Swedish Mycologist Elias Fries (9). All the fungal strains were screened for the production of biomass and polysaccharides (Table 1). The best yield was shown by *Ganoderma lucidum*, which produced a biomass of 6.8 g.dr.wt.l^{-1} after 7 d of incubation and 6.2 g.dr.wt.l^{-1} after 14 d of incubation. Also, *G. lucidum* was found to be the best producer of both exopolysacchride and endopolysaccharide. *Schizophyllum commune, Pycnoporus anguinvus* and *Trametes villosa* showed different results for biomass and polymer production. There is no correlation between biomass and polysaccharides production; in some cases, a considerable decrease of biopolymer was observed after 14 d of incubation. Being a candidate with highest polysaccharides yield, *Ganoderma lucidum* was chosen for further study on quantification of carbohydrates and total phenols. The content of carbohydrates was 300 mg.g^{-1} after 10 d of incubation and reduced to 160 mg.g^{-1} after 20 d of incubation. The reduction was almost half with increase in number of d of incubation time. Total phenolic content of *G.lucidum* increased during incubation period from 220-350 mg.g^{-1}.

4. Discussion

In the present study, *G. lucidum* was found to be the best producer of polysaccharides, which is similar to earlier studies (14, 19). The structure and pharmacological effect(s) of polysaccharides produced by *G. lucidum* is being actively studied (13, 20, 21). The potential role of *G. lucidum* polysaccharide in tumor therapy and the possible mechanisms involved were examined (22). Both *in vitro* and *in vivo* studies suggested that the antitumor activities of the polysaccharides of *G. lucidum* are mediated by its immunomodulatory, antiangiogenic, and cytotoxic effects. Moreover, antitumor activity has been linked to the frequency of polysaccharide branching *in vitro*, which changes with each stage of mycelial growth (23). Apart from carbohydrates, *G. lucidum* was found to contain appreciable quantity of total phenols. The phenolic compounds are known to be powerful chain-breaking antioxidants and they possess scavenging ability due to their hydroxyl groups (24).

In comparison with the earlier work on *P. citrinopileatus*, the total phenol content of *G. lucidum* was much higher. The total phenolic compounds may contribute directly to the antioxidative action (25).

5. Conclusions

Ganoderma lucidum has now become recognized as an alternative adjuvant in the treatment of leukemia,

Table 1. Screening of basidiomycetes for the production of biomass and polysaccharides

Organisms	Incubation Period in d	Biomass g.dr.wt.l^{-1}	IPS mg.g^{-1}	EPS g.l^{-1}
Ganoderma lucidum	7	6.8	551.7	6.8
	14	6.2	482.7	6.2
Schizophyllum commune	7	1.2	325.0	5.2
	14	3.0	309.5	5.8
Daedoleopsis confragosa	7	4.2	500.0	5.0
	14	4.4	200.0	3.6
Pleurotus ostreatus	7	4.6	450.0	5.8
	14	5.0	277.0	3.4
Abortiporus biennis	7	3.2	318.0	5.8
	14	3.6	311.0	5.4
Trichaptum biforme	7	1.6	352.9	5.2
	14	5.0	300.0	3.4
Tephrocybe ambusta	7	1.8	470.0	5.0
	14	1.8	333.0	4.8
Pycnoporus coccineus	7	4.0	142.0	4.6
	14	4.8	360.0	4.6
Calvatia sculpta	7	3.2	181.1	4.6
	14	3.6	142.8	4.0
Antrodiella semisupiforme	7	1.0	500.0	6.6
	14	1.8	200.0	4.6

carcinoma, hepatitis and diabetes. However, it is not available in sufficient quantity for commercial exploitation of vital therapeutic emergencies. Therefore, cultivation of *G. lucidum* on solid substrates, stationary liquid medium or by submerged cultivation has become an essential aspect to meet the driving force towards the increasing market demands. Established nutritional value of mushrooms needs to be identified for proper use, and the results from the present analysis could serve as fundamental data for promotion of cultivation and consumption of universally acclaimed medicinal mushroom, *G. lucidum*.

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