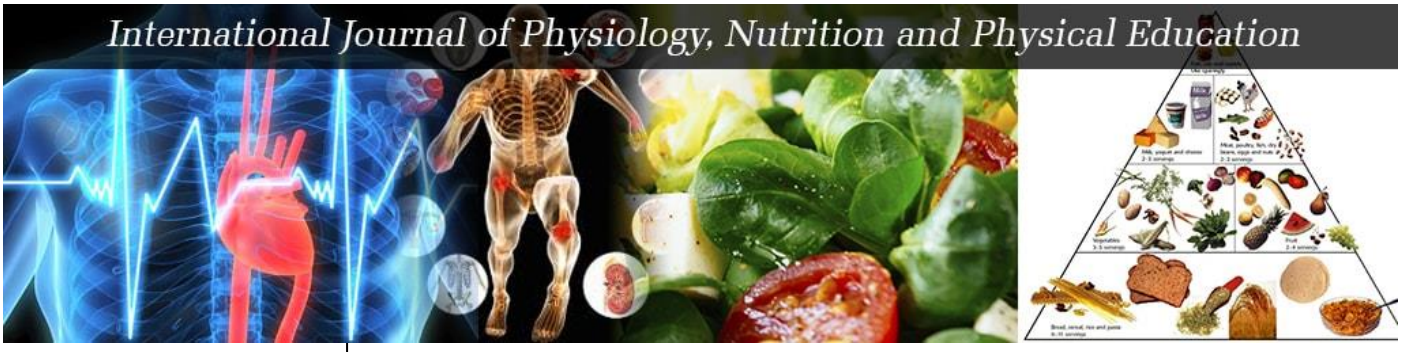

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Polysaccharides of hybrid mushrooms: Structures and biological importance

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Abstract
Water soluble polysaccharides were isolated from the aqueous and alkali extract of fruit bodies of hybrid mushrooms, obtained through protoplast fusion from different mushrooms. The polysaccharides were consisting of different monosaccharide units in different molar ratio. These polysaccharides showed immunoenhancing activity by stimulating the macrophage, splenocyte, and thymocyte. The structural investigations of these polysaccharides have been carried out using acid hydrolysis, methylation analysis, periodate oxidation study, and 1D/2D NMR experiments. In this review, structural characterizations and some biological activities of hybrid mushroom are shown.

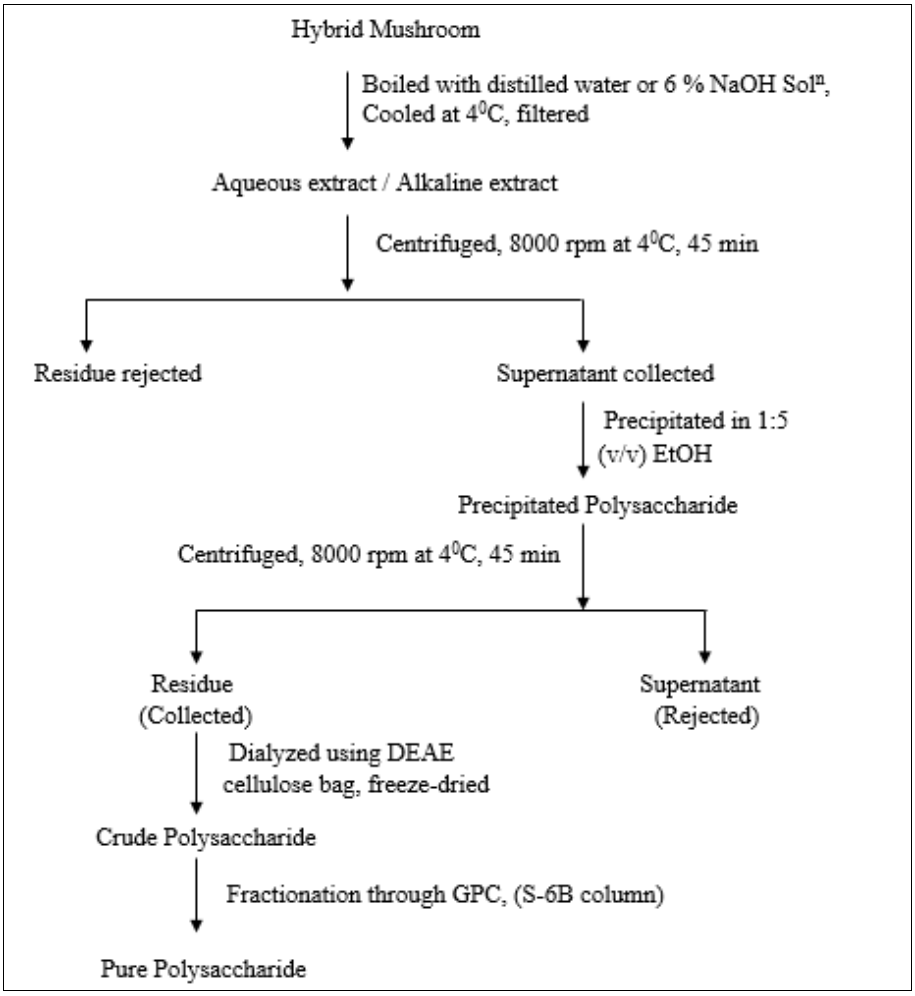
Keywords: hybrid mushroom; polysaccharide; structure; biological activity

1. Introduction
Mushrooms, known as fungi, lack chlorophyll and therefore cannot make its own food. It grows on dead organic matter either parasitically or symbiotically with other living organisms. Since the ancient times, mushrooms have been used as food for their taste, flavor, and for highly nutritive value [1]. Mushrooms are rich in protein, vitamins, minerals, and excellent sources of β-glucan, selenium, thiamine, riboflavin, niacin, pantothenic acid, and folic acid, etc. [2, 3]. Hence, mushrooms are considered to be the best alternative of vegetable protein rich food. They contained high phosphorus, potassium and iron but are low in sodium. Mushrooms are cultivation in major fermentation industry, which involves the bio-conversion of cellulose wastes into edible biomass. Carbohydrates are the most abundant class of organic compounds in the biological world. They are essential constituents of living organisms. The great bulk of the carbohydrates in nature are polysaccharides. Mushrooms contain dietary fibre belonging to β-glucans, chitin, and heteropolysaccharides etc. The dietary fibers absorb carcinogenic substances by physicochemical interactions and thereby prevent their absorption into the intestine and hastening their excretion.
Mushrooms are very important for its medicinal cost [4]. Mushroom polysaccharides have drawn the attention of chemists and immunobiologics because of their immunomodulatory [5], free radical scavenging [6, 7] and antitumor [8, 9] activity. Mushroom extracts, generally polysaccharides enhance immune functions like natural killer cells, T cells, B cells, and macrophage-dependent immune cells [10, 11]. Some mushroom polysaccharides isolated from *Pleurotus ostreatus* [12], *Agaricus blazei* [13], *Grifola frondosa* (Maitake) [14], and *Lentinan* [15] exhibit strong antitumor activity. A water-soluble polysaccharide [16] of an edible mushroom *Calocybe indica* var. APK2 showed immunoenhancing (macrophage, splenocyte, thymocyte, and bone marrow activation) and cytotoxic activity towards HeLa cell lines. Several polysaccharides isolated from various mushrooms, *Lentinus squarrosulus* (Mont.) Singer [17], *Pleurotus florida* blue variant [18], *Pleurotus ostreatus* cultivar [19], and somatic hybrid mushroom [20] of *Pleurotus florida* showed immunoenhancing properties. The protein bound polysaccharide, obtained from turkey tail mushroom (*Trametes versicolor*) is useful in the treatment of stomach cancer [21], colorectal cancer [22], small cell carcinoma of the lungs [23], and non-small cell lung carcinoma [24]. The protein bound polysaccharide (PSK), isolated from turkey tail mushroom (*Trametes versicolor)*, prevents chronic active hepatitis [25], liver cancer [26] and also hepatitis B [27]. The protein containing polysaccharides, extracted from *Pleurotus sajor-caju* and *Tricholoma species* have been found to contain anti-tumor properties.

In this review work structures and biological activities of different polysaccharides isolated from different hybrid mushrooms are reported.

2a. Isolation and Purification of Polysaccharides

The fresh fruit bodies of hybrid mushroom strain were cultivated and collected from Falta Experimental Farm, Bose Institute, Kolkata. The fruit bodies were washed with water and then with distilled water. The mushroom bodies were crushed and boiled with water or 6% NaOH for 6 h. Then, the hot water extract was cooled, filtered, and precipitated. The crude polysaccharide was isolated and purified by gel-permeation chromatography. The fractionation and purification steps are shown below.



2. b. Structural Investigation

A water-soluble glucan [28] was isolated from the aqueous extract of fruit bodies of somatic hybrid (Pflo Vv5 FB), obtained through protoplast fusion between Pleurotus florida and *Volvariella volvacea* strains. It contained only the (1→6)-linked glucopyranosyl residue.

An immunoenhancing water soluble polysaccharide isolated [29] from the aqueous extract of the fruit bodies of somatic hybrid mushroom PCH3FB, obtained through protoplast fusion between the strains Pleurotus florida and Calocybe indica var was found to consist of the presence of glucose, galactose and fucose in a molar ratio of nearly 4:3:1. This polysaccharide contained (1→6)-D-galactopyranosyl, (1→4, 6)-D-galactopyranosyl, (1→3,4)-D-glucopyranosyl, and (1→6)-D-glucopyranosyl residues, and two terminal residues, D-glucopyranosyl and L-fucopyranosyl moieties.

Three polysaccharide fractions (PS-I, PS-II, and PS-III) were isolated from the aqueous extract of a hybrid mushroom obtained through backcross mating of a somatic hybrid mushroom [30] PfloVv12 (Sterile line) with *Volvariella volvacea*. PfloVv12 was obtained through protoplast fusion of Pleurotus florida and V. volvacea. PS-I was identified as 1, 6-b glucan with molecular weight1.88 105 Da. PS-II and PS-III were identified as mannoglucogalactan but differing in molecular weights, PS-II 1.32 105 Da, and PS-III 0.92 105 Da from a calibration curve prepared with standard dextran.

Another water soluble polysaccharide [31] was isolated from the hot aqueous extract of the fruit bodies of the somatic hybrid mushroom (PfloVv1aFB), raised through protoplast fusion between the strains of Pleurotus florida and *Volvariella volvacea*. It was found to consist of D-glucose, D-galactose, and D-mannose in a molar ratio of nearly 4:1:1. The linkage of the sugar moieties were reported as terminal D-glucopyranosyl and D-mannopyranosyl, (1→3)-linked D-glucopyranosyl, (1→6)-linked D-glucopyranosyl, (1→3,6)-linked D-glucopyranosyl, and (1→2,6)-linked D-galactopyranosyl moieties.

A water soluble heteroglycan [32] was isolated from hot aqueous extract of fruit bodies of an edible hybrid mushroom Pfle1r of Pleurotus Florida and Lentinula edodes. It consists of D-glucose, D-mannose, and D-galactose residues in a molar ratio of nearly 1:1:1. It is composed by non-reducing D-mannopyranosyl, (1→6)-linked D-glucopyranosyl, and (1→2, 6)-linked D-galactopyranosyl moieties. It is reported that a water-soluble polysaccharide isolated from hot aqueous extract of the fruit bodies of somatic hybrid mushroom (pfle 1q) [33] obtained through intergenic protoplast fusion between Pleurotus florida and Lentinula edodes consists of D-

galactose and D-mannose in a molar ratio of nearly 2:1. The polysaccharide was found to consist of terminal D-mannopyranosyl, (1→6)-linked D-galactopyranosyl, and (1→2, 6)-linked D-galactopyranosyl moieties respectively. These linkages were analyzed by the methylation, periodate oxidation and NMR experiment.

A heteropolysaccharide (PS-II) [34] with apparent molecular weight 1.65 105 Da, isolated from the fruiting bodies of hybrid mushroom pfle 1p by hot aqueous extraction was found to consist of D-mannose, D-galactose, and 3-*O*-Me-D-galactose in a molar ratio of 1.0:0.99:1.1. The structural investigation of PS-II has been carried out using acid hydrolysis, methylation analysis, periodate oxidation study, and 1D/2D NMR experiments. Based on the results of these experiments, it was established that PS-II contained a main chain of (1→6) linked a-D-galactopyranosyl residues, one of which was substituted at C-2 by a terminal mannopyranosyl residue and also methylated at C-3 position. This heteropolysaccharide (PS-II) exhibited macrophage activation by NO production as well as *in vitro* splenocyte and thymocyte stimulation.

The water soluble heteroglycan, [35] isolated from the alkaline extract of the fruit bodies of the somatic hybrid mushroom (PfloVv1aFB), raised through protoplast fusion between the strains of Pleorutus florida and Volverilla volvacea, was found to consist of (1→3)-, (1→6)-, (1→3,4)-linked, and terminal b-D-Glcp along with (1→2,6)-a-D-Galp and terminal a-D-Manp in a relative proportion of approximately 1:1:1:1:1:1. Structural investigation was carried out using sugar analysis, methylation analysis; periodate oxidation study, and NMR experiments. Another two different glucans [36] (water-soluble PS-I, water-insoluble PS-II) were isolated from the alkaline extract of a novel hybrid mushroom (backcross mating between PfloVv12 and *Volvariella volvacea*). PfloVv12 was initially obtained through intergeneric protoplast fusion between P. Florida and V. volvacea. PS-I was found to consist of only (1→6)-linked b-D-glucopyranose. PS-II was composed of terminal, (1→3,4)-linked, and (1→3)-linked b-D-glucopyranosyl moieties in a molar ratio of nearly 1:1:1.

2c. Biological Studies

Macrophage activation by polysaccharides has been studied by nitric oxide (NO) production in culture supernatant *in vitro*. Upon treatment with different concentrations of PS, an enhanced production of NO was observed in a dose-dependent manner. Splenocytes are the cells present in the spleen that include T cells, B cells, dendritic cells, etc. that stimulate the immune response in living organisms whereas thymocytes are hematopoietic cells present in thymus and the primary function of which is the generation of T cells. Splenocyte and thymocyte proliferations are the measurement of immunoactivation. The activation of splenocyte and thymocyte tests was carried out in mouse cell culture medium with polysaccharides by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] method. It is reported that hybrid mushrooms, (Pflo Vv5 FB), PCH3FB, (PfloVv1aFB), (Pfle1r of Pleurotus florida and Lentinula edodes), (pfle 1q), and pfle 1p isolated from water extract stimulated the macrophages, splenocytes, and thymocytes. The polysaccharides isolated from alkaline extract of hybrid mushrooms, (PfloVv1aFB) and (backcross mating between PfloVv12 and *Volvariella volvacea*) showed immunoenhancing activity by stimulating the macrophage, splenocyte, and thymocyte.

3. Conclusion

Water soluble different heteroglycan and glucan were isolated from aqueous and alkaline extract of different hybrid mushrooms. The water-soluble polysaccharides were purified by gel-filtration chromatography and showed splenocyte, thymocyte and macrophage activation as well as antioxidant property in a particular concentration. The structures of the polysaccharides were determined by chemical and NMR analysis.

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