SYNOPSIS

The thesis entitled "**Structural Characterization and Biological Investigation of Mushroom and Plant Polysaccharides**" comprises six chapters.

Chapter-I describes the general introduction to carbohydrate including a short review of the works on mushroom and plant polysaccharides.

Chapter-II deals with the methodologies to isolate, purify and determine the structure of pure polysaccharides and also to study their biological activities.

Chapter-III contains the isolation, purification and structural characterization by chemical, NMR and MALDI-TOF MS analysis and study of immunoenhacing properties of a polysaccharide, isolated from the fruit bodies of an edible mushroom *Volvariella bombycina*. This work has been published in *Carbohydrate Research,* **2008**, *343*, **2258-2265.**

Chapter-IV deals with the structural studies of a dietary fiber of green Chalcumra (*Benincasa hispida*) fruit. This work has been published in *Natural Product Communications,* **2009***, 4(4),* **547-552.**

Chapter-V comprises the structural elucidation along with study of biological activity of a water-soluble heteropolysaccharide, isolated from the aqueous extract of the corm of *Amorphophallus campanulatus*. This work has been published in *Carbohydrate Research,* **2009***, 344,* **2581-2585.**

Chapter VI illustrates the isolation, characterization and study of immunoenhancing properties of a glucan from fruit bodies of somatic hybrid mushroom (*Pflo Vv5 FB*), obtained through intergeneric protoplast fusion between *Pleurotus florida* and *Volvariella volvacea* strains. This work has been published in *Carbohydrate Research,* **2010**, *345*, **974–978.**

Chapter-I: It represents the general introduction of carbohydrates, mushroom and plant polysaccharides along with their biological activities. Carbohydrates are the most abundant and diverse class of organic compounds occurring in nature. Carbohydrates played the key role in establishment and evolution of the life on earth by creating a direct link between the sun and chemical energy. These are classified into monosaccharide,

oligosaccharide and polysaccharide. The great bulk of the carbohydrates in nature are present as polysaccharides, which have relatively large molecular weights.

The term **mushroom** means "*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*". Mushrooms belong to the group of immunoceuticals by their mode of action. The use of medicinal mushrooms in the fight against cancer is known from a very long time in Korea, China, Japan, Russia, USA and Canada. Polysaccharide is the most important component of mushrooms that induces immunomodulation and plentiful medicinal properties including anti-tumor effects. Different polysaccharides from both plant and mushroom showed their immunomodulation and antitumor properties. Mushroom polysaccharides are not only used against cancers of stomach, esophagus, lungs, and colons but also act as anti-inflammatory, antiviral (against AIDS), hypoglycaemic and antithrombotic agents. *Lentinus edodes* (**Lentinan, Japan**), *Schizophyllum commune* (**Schizophyllan**), *Agaricus blazei* (**Agarican, USA**) *Ganoderma lucidum* (**Lingzhi, China**) and *Grifola frondosa* (**Maitake, Japan**) have been used clinically as anti-tumor agents. Different polysaccharides are used as dietary fiber. Chemically, dietary fiber consists of non-starch polysaccharides and several other plant components such as cellulose, lignin, waxes, chitins, pectins, beta-glucans, inulin and oligosaccharides. The biological activities of polysaccharides depend on the size of molecule, branching rate and form. So, it is very important to determine the exact structure of the polysaccharides, isolated either from medicinal plant or from mushroom.

Chapter-II: This chapter illustrates the methodologies of isolation, purification and determination of the structure of pure polysaccharides and also the study of their biological activities. The polysaccharides are purified using different chromatographic techniques. The exact structure of the polysaccharides is determined using two types of methods: [1] Chemical method that includes total acid hydrolysis, methylation analysis, periodate oxidation studies. [2] Spectroscopic method comprising 1D (^{1}H, ^{13}C), 2D NMR (DQF-COSY, TOCSY, NOESY, ROESY, HMQC, and HMBC), and mass spectroscopic experiments (GLC-MS and MALDI-TOF MS).
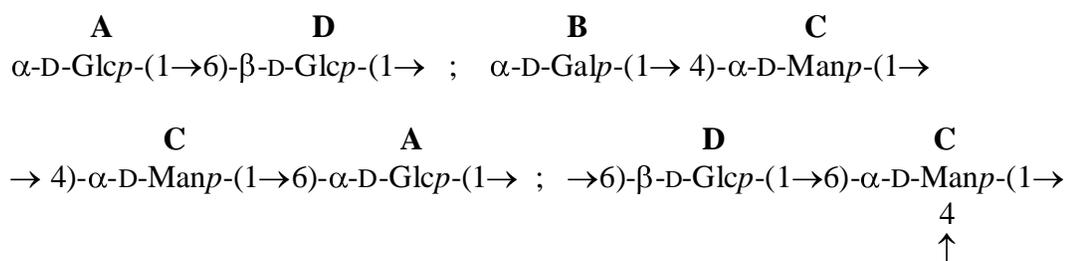
Chapter-III: This chapter consists of the isolation, purification and structural characterization of an immunoenhancing polysaccharide of *V. bombycina* by chemical, NMR and MALDI-TOF MS analysis. The fruit bodies of an edible mushroom, *Volvariella bombycina* is consumed by the local people as delicious food material. Water–soluble polysaccharide was isolated from the hot water extract of the fruit bodies of *V. bombycina*. The crude polysaccharide was dialyzed through cellulose membrane against distilled water to remove low molecular weight materials and then fractionated by passing through Sepharose 6B column to yield one homogeneous fraction. The molecular weight of the polysaccharide (PS) was estimated from a calibration curve prepared with standard dextrans and it was nearly 1.6×10^5 Da. The polysaccharide showed a specific rotation of $[\alpha]^{25}_D + 47.67$ (*c* 0.074, water). The detailed structural studies of this PS was carried out on the basis of total acid hydrolysis, methylation analysis, periodate oxidation, NMR studies (^{1}H, ^{13}C, TOCSY, DQF-COSY, NOESY, ROESY, HMQC, and HMBC), and MALDI-TOF MS analysis. The PS was hydrolyzed by 2M trifluoroacetic acid and the presence of glucose, mannose, and galactose in a molar ratio of 2:1:1 was detected by PC as well as GLC analysis. The absolute configuration of the monosaccharides was determined by the method of Gerwig et al. which showed that each monosaccharide had D-configuration. The PS was methylated using the Ciucanu and Kerek method and then Purdie method followed by hydrolysis and then acetylation. The GLC-MS analysis of the alditol acetates of methylated PS showed the presence of 1,5,6-tri- *O*- acetyl 2,3,4-tri-*O*-methyl-D-glucitol, 1,5-di-*O*- acetyl 2,3,4,6-tetra-*O*-methyl-D-galactitol, and 1,4,5,6-tetra-*O*-acetyl 2,3-di-*O*-methyl-D-mannitol in a molar ratio of nearly 2:1:1. These results indicated that (1→ 6)-linked D-glucopyranosyl, (1→ 4, 6)-linked D-mannopyranosyl and terminal D-galactopyranosyl moieties were present in the PS in a molar ratio of nearly 2:1:1. Thereafter, a periodate oxidation experiment was carried out with the PS. The GLC analysis of the alditol acetates of the periodate-oxidised, reduced, methylated polysaccharide showed that all these sugars were consumed during oxidation. This result supported the mode of linkages of these sugar moieties present in the PS.

The 500 MHz ^{1}H NMR spectrum of this polysaccharide at 27°C showed three anomeric proton signals at 5.12, 4.98 and 4.51 ppm in a molar ratio of nearly1:2:1. The

integral value of the signal at δ 4.98 was almost double of that of the other two signals which indicated that the signal at δ 4.98 consisted of two sugars while each of the other two signals corresponded to one sugar. They were designated as **A**, **B**, **C** and **D** according to their decreasing anomeric proton chemical shifts. In 125 MHz ^{13}C NMR spectrum at 27°C three anomeric carbon signals appeared at δ 103.2, 102.1 and 98.2 ppm in a ratio of nearly 1:1:2. The anomeric carbon signals were readily assigned from the HMQC spectrum. Signal at δ 98.2 ppm was assigned for anomeric carbons of **A** and **B** residues. Signals at δ 102.1 and 103.2 ppm were assigned for anomeric carbons of **C** and **D** residues, respectively. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, HMQC, and HMBC experiments. **A-D** sugar residues were determined on the basis of proton coupling constants, measured from DQF-COSY experiment. The anomeric configuration of the sugar residues was determined from $J_{H-1,H-2}$ and $J_{C-1,H-1}$ coupling constant values. From the above experimental data the residues [A − D] were found to have following linkages;

(1→6)- linked α-D-glucopyranose (**A**), terminal α-D-galactopyranose (**B**),

(1→ 4, 6) - linked-α-D-mannopyranose (**C**), (1→6)-linked-β-D-glucopyranose (**D**)

The sequence of glycosyl residues of the polysaccharide was determined from NOESY as well as ROESY experiments and was found to be as follows;

    A   **D**   **B**   **C**
α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→ ; α-D-Gal*p*-(1→ 4)-α-D-Man*p*-(1→

       C   **A**   **D**   **C**
→ 4)-α-D-Man*p*-(1→6)-α-D-Glc*p*-(1→ ; →6)-β-D-Glc*p*-(1→6)-α-D-Man*p*-(1→
                                                                   4
                                                                   ↑

This sequence was further confirmed by HMBC experiment. The cross peaks of both anomeric protons and carbons of each of the sugar moieties were examined and both inter- and intra-residual connectivities were observed from the HMBC experiment. The cross peaks (**A**H-1, **D**C-6), (**A**C-1, **D**H-6a), (**A**C-1, **D**H-6b), (**B**H-1, **C**C-4), (**B**C-1, **C**H-

4), (**CH**-1, **AC**-6), (**CC**-1, **AH**-6a), (**CC**-1, **AH**-6b) (**DH**-1, **CC**-6), (**DC**-1, **CH**-6a), (**DC**-1, **CH**-6b) were observed.

Based on all these chemical and NMR experimental results, the structure of the polysaccharide was established as;

$$
\begin{array}{ccc}
\textbf{D} & \textbf{C} & \textbf{A} \\
\rightarrow\text{6)-}\beta\text{-D-Glc}p\text{-(1}\rightarrow\text{6)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\text{6)-}\alpha\text{-D-Glc}p\text{-(1}\rightarrow \\
4 \\
\uparrow \\
1 \\
\alpha\text{-D-Gal}p \\
\textbf{B}
\end{array}
$$

The MALDI-TOF MS analysis of this polysaccharide was carried out to support the above structure.

The polysaccharide was found to activate the macrophages. Macrophage activation was studied by NO production in culture supernatant in vitro. An enhanced production of NO was observed at 50 μg/mL which subsequently decreased with further increase in concentration. Hence, the effective dose of the molecule was observed at 50 μg/mL. The molecule also showed the splenocyte and thymocyte activation. 50 μg/mL of the polysaccharide was efficient splenocyte and thymocyte proliferator.

Chapter-IV: This chapter describes the structural studies of dietary fiber of green Chalcumra (*Benincasa hispida*) fruit. *Benincasa hispida* (Thunb) Cogn., locally known as Chalcumra, belongs to *Cucurbitaceae* family. The green fruit body of Chalcumra was extracted with hot water and the polysaccharide was collected by precipitation in ethanol. The precipitated material was freeze dried and boiled with 1% acetic acid solution. The solution was centrifuged, and the filtrate was reprecipitated in EtOH followed by centrifugation to collect solid part. It was then dialyzed through cellulose membrane and freeze dried to yield crude polysaccharide. The purity of the polysaccharide was carried out by gel permeation chromatography through a Sepharose 6B column. The molecular weight of the polysaccharide was determined as ∼186000 Da. This molecule showed a specific rotation of $[\alpha]^{25}_{D}$ +27.92 (C 0.07, water). The pure polysaccharide (PS) was hydrolyzed by 2M CF₃COOH. The paper chromatographic studies of the hydrolyzed

product showed the presence of galactose and galacturonic acid. The GLC analysis of alditol acetates of carboxymethyl- reduced PS showed the presence of galactose only. The absolute configuration of the monosaccharides was determined by the method of Gerwig *et al.* and it was found that all the sugars had D-configuration. The PS was methylated using the Ciucanu and Kerek method. The alditol acetates of the methylated polysaccharide were analyzed by GLC and GLC-MS, which revealed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol. However, the alditol acetate analysis of the methylated carboxyl-reduced PS showed that 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol and 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-D-galactitol were present in a molar ratio of nearly 2:1. These results indicated the presence of (1→4)-linked-D-galactopyranosyl and (1→2)-linked-D-galacturonosyl in the PS. The periodate-oxidised, NaBH$_4$-reduced material of the PS upon hydrolysis showed the absence of D-galactose, which indicated that (1→4)-linked-D-galactose was consumed during oxidation. The paper chromatographic analysis of the periodate-oxidised PS, on hydrolysis, showed the absence of D-galacturonic acid, indicating its destruction during oxidation due to (1→2) - linkage.
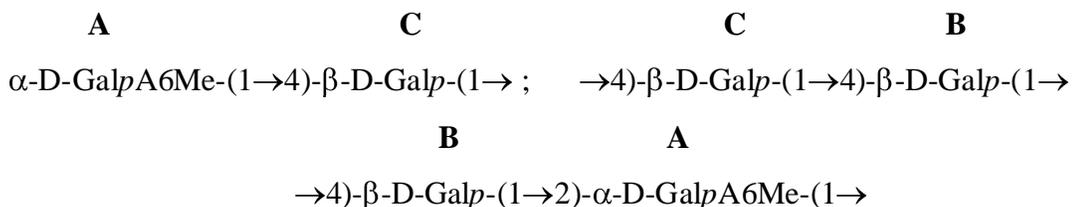
In proton NMR spectrum (500 MHz) two anomeric proton signals were found at δ 4.94 and 4.62 in a molar ratio of almost 1:2. The integral value of the signal at δ 4.62 was almost double to that of the other signal indicating that the later signal consisted of two anomeric protons. The sugar residues were designated as **A**, **B** and **C** according to their decreasing anomeric proton chemical shifts. In ^{13}C NMR spectrum (125 MHz) two anomeric carbon signals appeared at δ 104.7 and 100.8 ppm in a ratio of nearly 2:1. All the proton signals were assigned from DQF-COSY and TOCSY spectra and the carbon signals from HMQC experiment. The signal at 104.7 ppm corresponded to the anomeric carbon of both residues **B** and **C**, and the peak at 100.8 ppm corresponded to the anomeric carbon of residue **A.** Furthermore, the signal at δ 53.2 was assigned for carbomethoxy carbon. From a DQF-COSY experiment the proton coupling constants were measured.

The spin system of residue **A**, which consisted of only five protons with a relatively high chemical shift of the H-5 signal (δ 4.43) and weak coupling of H-4 with H-3 and H-

5, indicated that residue **A** was D-Gal$p$A. The coupling constants, $J_{H-1,H-2}$ (~ 3 Hz) and $J_{C-1,H-1}$ (~ 170 Hz) indicated that it was α- anomer. The downfield chemical shift of C-2 signal (δ 78.6) compared to the standard value of methyl glycosides indicated that it was (1→2)-linked. The appearance of intra-residual cross-coupling between the ester carbonyl carbon and the carboxymethyl proton in the HMBC spectrum confirmed that galacturonic acid residue was methyl esterified. Therefore residue **A** was methyl ester of a (1→2)-linked α-D-galacturonic acid.

In case of residue **B** and **C**, the anomeric proton signal at δ 4.62, large $J_{H-1,H-2}$ (~ 8 Hz) and $J_{C-1,H-1}$ (~ 162 Hz) indicated that those were β- anomer. The large coupling constant between H-2 and H-3 (>5 Hz) and small coupling constant between H-3 and H-4 indicated that residues **B** and **C** were β-D-galactopyranosyl moieties. The downfield chemical shift of C-4 (δ 78.0) with respect to the standard value of methyl glycosides indicated that both the residues, **B** and **C** were (1→4)-β-D-galactopyranose.

The following sequence of glycosyl residues of the PS was determined from ROESY as well as NOESY experiment, followed by confirmation with an HMBC experiment.

    A   **C**   **C**   **B**

α-D-Gal$p$A6Me-(1→4)-β-D-Gal$p$-(1→ ;   →4)-β-D-Gal$p$-(1→4)-β-D-Gal$p$-(1→

                           B   **A**

                  →4)-β-D-Gal$p$-(1→2)-α-D-Gal$p$A6Me-(1→

Long range ^{13}C-^{1}H correlations obtained from the HMBC spectrum supported the following trisaccharide repeating unit.

               C   **B**   **A**

        →4)-β-D-Gal$p$-(1→4)-β-D-Gal$p$-(1→2)-α-D-Gal$p$A6Me-(1→
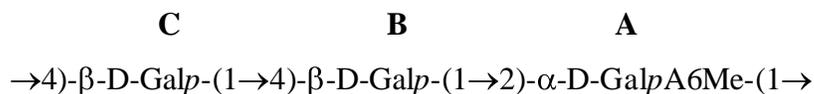
Chapter-V: This chapter illustrates the structural elucidation along with study of biological activity of a water-soluble heteropolysaccharide isolated from the aqueous extract of the corm of *Amorphophallus campanulatus*, an important medicinal plant belongs to *Araceae* family. Its vernacular name is Ol in Bengal. It is largely cultivated through out the plains of India. The corm juice of *Amorphophallus campanulatus*

obtained by boiling with distilled water followed by filtration and centrifugation was precipitated in alcohol. The precipitated material on dialysis followed by freeze drying yielded crude polysaccharide. On fractionation of this water-soluble polysaccharide through Sepharose 6B column yielded single homogeneous fraction. The molecular weight of the PS was estimated from a calibration curve prepared with standard dextrans as ~180000 Da. It showed a specific rotation of $[\alpha]^{25}_{D}$ +44.5 (c 0.6, water). The absolute configuration of the sugar residues was determined by the method of Gerwig et al. taking intact polysaccharide and carboxyl-reduced polysaccharide into consideration. On the basis of paper chromatographic studies, GLC and GLC-MS analysis, it was observed that (1→3,4)-linked-D-glucopyranosyl, terminal arabinofuranosyl, (1→2)-linked-D-galacturonosyl, (1→3)-linked- and (1→4)-linked-D-galactopyranosyl moieties were present in a molar ratio of nearly 1:1:1:1:1.

In the anomeric region of the ^{1}H NMR spectrum (500 MHz) at 27°C, six signals were observed at δ 4.44, 4.93, 5.06, 5.08, 5.12, and 5.22. Among these signals δ 4.44, 4.93, 5.06, 5.08, and 5.22 corresponded to anomeric proton signals, designated as **A**, **B**, **C**, **D**, and **E** according to increasing chemical shifts, whereas, the signal at δ 5.12 corresponded to H-4 of residue **B.** In ^{13}C NMR spectrum (125 MHz) at 27°C, four signals were found in the anomeric region at δ 99.9, 100.8, 104.5, and 109.6. The three anomeric carbon signals at δ 99.9, 104.5, and 109.6 were correlated to three anomeric protons of **D**, **A**, and **E** respectively, whereas, the signal at δ 100.8 was correlated to two anomeric protons of residues **B** and **C**. All the ^{1}H and ^{13}C signals were assigned from DQF-COSY, TOCSY, and HMQC experiments. The ^{13}C NMR spectrum revealed two types of methyl carbons, CH$_3$ of O-acyl group and CH$_3$ of ester group at δ 20.5 and 53.2 respectively. Two types of carbonyl carbons at δ 175.1 and 171.1 were also correlated with O-acyl and ester group respectively. In ^{1}H NMR spectrum the characteristic signals at δ 2.08 was correlated to methyl protons of O-acyl group. From DQF-COSY experiment the proton coupling constants were measured.

On the basis of proton and carbon chemical shifts, proton-proton coupling constants and C-1, H-1 coupling constants, all the sugar residues were assigned as follows;

Residue **A**: →3)-β-D-Gal*p*-(1→

Residue **B**: →2)-α-D-Gal*p*A6Me-(1→
<div align="center">4
↓
OAc</div>

Residue **C**: →3,4)-α-D-Glc*p*-(1→

Residue **D**: → 4)-α-D-Gal*p*-(1→
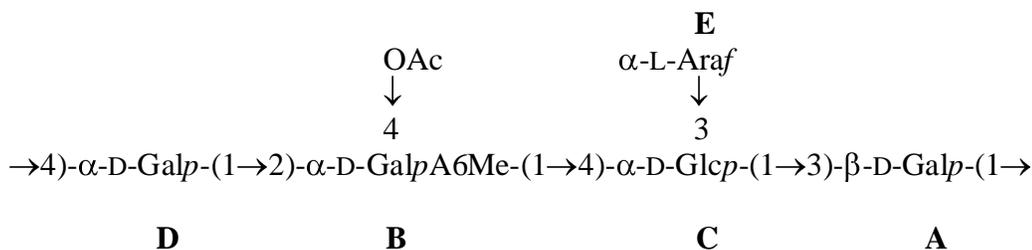
Residue **E**: α-L-Ara*f*-(1→

In NOESY experiment the inter-residual contacts from **A**H-1 to **D**H-4, **D**H-1 to **B**H-2, **B**H-1 to **C**H-4, **E**H-1 to **C**H-3 and also **C**H-1 to **A**H-3 established the following sequences;

<div align="right">OAc
↓
4</div>

β-D-Gal*p*-(1→ 4)-α-D-Gal*p*-(1→   ;   →4)-α-D-Gal*p*-(1→2)-α-D-Gal*p*A6Me-(1→
   A   **D**   **D**

<div>OAc
↓
4</div>

→2)-α-D-Gal*p*A6Me-(1→4)-α-D-Glc*p*-(1→ ;   α-L-Ara*f*-(1→3)-α-D-Glc*p*-(1→
   B   **C**   **E**   **C**

<div align="center">↓
4</div>

→3)-α-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→
   C   **A**
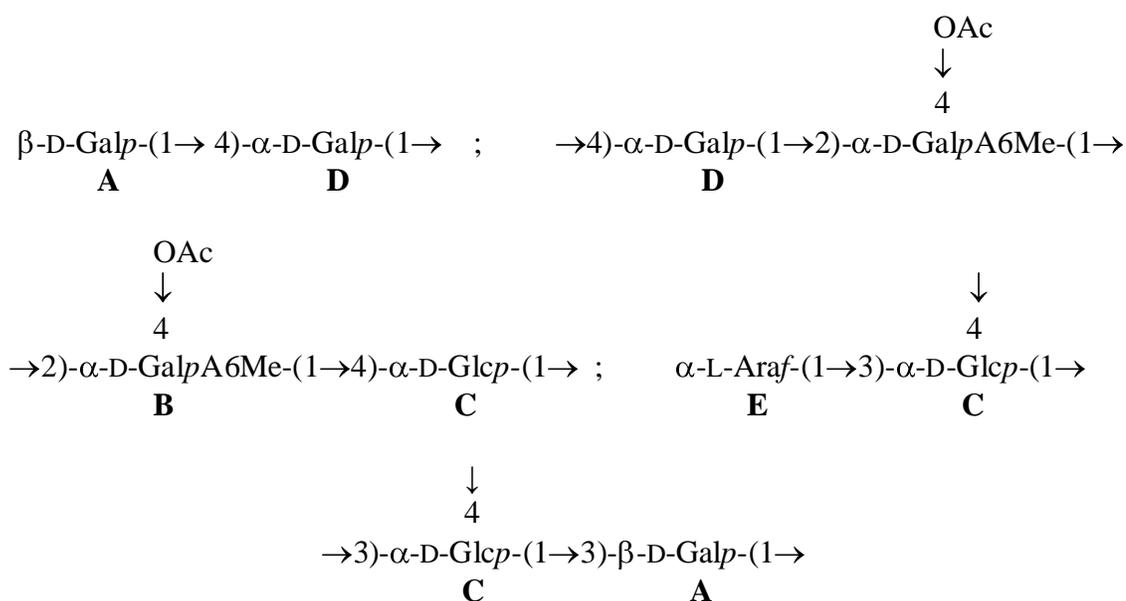
In HMBC spectrum the cross-peaks of both anomeric protons and carbons of each of the glycosyl residues were examined. The cross peaks, (**A**H-1, **D**C-4), (**A**C-1, **D**H-4), (**B**H-1, **C**C-4), (**B**C-1, **C**H-4), (**C**H-1, **A**C-3), (**C**C-1, **A**H-3), (**D**H-1, **B**C-2), (**D**C-1, **B**H-2), (**E**H-1, **C**C-3), and (**E**C-1, **C**H-3) were found. The cross-couplings of carbonyl carbon of *O*-acetyl group with methyl proton and H-4 of residue **B** were observed. Methyl proton and carbonyl carbon of ester group also showed a cross-coupling. Thus, the HMBC and

NOESY connectivities clearly supported the presene of the following pentasaccharide repeating unit in the PS isolated from *A. campanulatus*

$$
\begin{array}{ccc}
 & \mathbf{E} \\
\text{OAc} & \alpha\text{-L-Ara}f \\
\downarrow & \downarrow \\
4 & 3 \\
\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Gal}p\text{A6Me-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow \\
\mathbf{D} \qquad\qquad \mathbf{B} \qquad\qquad\qquad \mathbf{C} \qquad\qquad\qquad \mathbf{A}
\end{array}
$$

The splenocyte activation tests were carried out in mouse cell culture medium with the polysaccharide by the MTT method. Proliferation of splenocytes is an indicator of immunoactivation. Splenocyte proliferation index was maximum at 50 μg/mL, as compared to other concentrations of polysaccharide. Hence, this dose can be considered as efficient splenocyte proliferator.

Chapter-VI: This chapter deals with the isolation, characterization and also study of immunoenhancing properties of a glucan isolated from fruit bodies of somatic hybrid (*Pflo Vv5 FB*), obtained through intergeneric protoplast fusion between *Pleurotus florida* and *Volvariella volvacea* strains. On the basis of acid hydrolysis, methylation analysis, periodate oxidation along with ^{1}H, DEPT-135, and ^{13}C NMR spectroscopy, including two-dimentional TOCSY, DQF-COSY, NOESY, ROESY, HMQC, and HMBC experiments, the structure of the repeating unit of this glucan was determined. The hot water-extract of fresh fruit bodies (500 g) of *Pflo Vv5 FB* was cooled, filtered, and precipitated in   alcohol. The residue was dialyzed, centrifuged and freeze dried to yield 931 mg of crude polysaccharide, which on fractionation through Sepharose-6B using water as eluant yielded only one fraction. The pure polysaccharide (PS) showed specific rotation $[\alpha]^{25}_{D}$ -31.2 (*c* 0.06, water) and the molecular weight-average mass was estimated as ~ 1.85 x 10^5 Da. Acid hydrolysis of the PS followed by using GLC analysis showed the presence of glucose only. Another part of the hydrolyzed product was subjected to paper chromatographic study, which also showed the presence of glucose only. The absolute configuration of glucose residue was determined as D by the method of Gerwig et al. The mode of linkages of PS was determined by methylation analysis

using Ciucanu and Kerek method followed by hydrolysis and alditol acetate preparation. The GLC-MS of alditol acetates of the methylated product showed the presence of only one peak corresponding to 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol. This result indicated the presence of (1→6)-linked glucopyranosyl residue. Alditol acetate of the periodate-oxdised polyasaccharide showed the total disappearance of sugar residues. This result was agreed with the presence of (1→6)-linked glucose residues in the PS.

The anomeric proton signal at δ 4.53 and the coupling constants, $^3J_{1,2}$ (~3.0 Hz) and $J_{C-1, H-1}$ (~161 Hz) indicated that the sugar residue was β-linked. The large $^3J_{2,3}$ and $^3J_{3,4}$ coupling constants (~9.0 Hz) indicated that it had *gluco* configuration. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, and HMQC experiments. The C-6 signal at δ 69.1 showed downfield shift compared to the standard value of methylglucoside indicating (1→6)-linkage of glucose moiety, supported by the strong NOE contacts from H-1 to both H-6a and H-6b. The DEPT-135 NMR spectrum also confirmed the (1→6)-linking. To explain the HMBC results two glucose units were considered as **A** and **A'**. The appearance of cross-peaks, (**A**H-1, **A'**C-6), and (**A**C-1, **A'**H-6a; **A**C-1, **A'**H6b) in HMBC spectrum clearly supported the following repeating unit in the polysaccharide.

$$\rightarrow 6)\text{-}\beta\text{-D-glc}p\text{-}(1\rightarrow$$
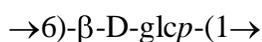
The glucan was found to activate the macrophages. Macrophage activation was studied by NO production in culture supernatant in vitro. Upon treatment with different concentrations of this β-glucan, an enhanced production of NO was observed in a dose dependent manner with optimum production of 17.57 μM NO per 5×10^5 macrophages at 80 μg/mL which subsequently decreased with further increase in concentration. Hence, the effective dose of the glucan was observed at 80 μg/mL. This glucan also stimulated the splenocytes and thymocytes. 50 μg/mL of the polysaccharide was efficient dose in each of the splenocyte as well as thymocyte proliferation.