Mycobiology 37(4) : 286-294 (2009)
© The Korean Society of Mycology

Composition and Partial Structure Characterization of *Tremella* Polysaccharides

Proma Khondkar*

Pharmaceutical Microbiology and Biotechnology Laboratory, Department of Pharmacy, Rajshahi University, Rajshahi- 6205, Bangladesh

(Received August 31, 2009. Accepted September 21, 2009)

Heteropolysaccharides isolated from liquid cultures of nine *Tremella* species contained 0.3 to 1.2% protein, 2.7 to 5% ash, 0.9 to 3.4% acetyl groups, 76.5 to 84.2% carbohydrates and trace amounts of starch. The polysaccharides in aqueous solution were slightly acidic (pH 5.1 to 5.6). They consisted of the following monomeric sugars: fucose, ribose, xylose, arabinose, mannose, galactose, glucose and glucuronic acid. The backbones of the polysaccharide structures consisted of α-(1 $\rightarrow$ 3)-links while the side chains were β-linked.

KEYWORDS : β-linked polysaccharides, Extra-cellular polysaccharides, *Tremella* species, Uronic acid

In recent years, there has been increased interest in natural biopolymers for their potential applications in the food, cosmetics, pharmaceuticals and oil industries as viscosity controlling agents. Traditionally, plant or sea-weed polysaccharides have been used for these purposes (Lewis *et al*., 1988; Margaritis and Pace, 1985), but the identification of new sources of these materials is of interest, particularly if these polymers have novel properties (Sinskey *et al*., 1986). There is a growing interest in the isolation of new microbial polysaccharides with potentially useful applications. A common feature of some bacteria, yeasts and fungi is the production of extra-cellular polysaccharides with unique properties that offer a more attractive alternative to plant derived polymers, as they can be grown under controlled conditions. The advantages of microbial polysaccharides are novel functionality, constant and reproducible chemical and physical properties, a stable cost and reliable supply (MacCormick *et al*., 1996).

Extra-cellular microbial polysaccharides are water soluble macromolecules, which may be ionic or non-ionic in nature, and which increase the viscosity of the medium in conjunction with different physical and chemical agents. Because of their wide diversity in physical structure and properties, they can change the rheological properties and texture of the products in which they are incorporated into (Pavlova and Grigorova, 1999). In recent years, there has been a major emphasis on the search for novel microbial polysaccharides, and a wide variety of microbial strains are reported to produce polysaccharides with varied compositions and useful properties (Yalpani and Sandford, 1987).

The genus *Tremella* (Tremellaceae) belongs to the so-called "Jelly mushroom" group of organisms that form gelatinous fruiting bodies. Among the family Tremellaceae, the genus *Tremella* consists of 120 or more species (Bandoni, 1995). Many of them have been used as food and folk medicines for centuries in Asian countries. The dietary and medicinal properties are assumed to be solely due to the polysaccharides produced by these species (Reshetnikov *et al*., 2000). The chemistry of the extra-cellular polysaccharides produced in liquid culture has only been studied in a limited number of species. for example, *T. mesenterica*, *T. fuciformis* and *T. aurantia* (Fraser *et al*., 1973; Gao *et al*., 1996a, 1996b; Kakuta *et al*., 1979; Kiho *et al*., 1995; Slodki, 1966; Slodki *et al*., 1966; Yui *et al*., 1995). This article describes the basic composition and tentative characterization of polysaccharides from nine *Tremella* species including three of the most commonly studied ones.

Materials and Methods

Microorganisms. Nine *Tremella* species (*T. aurantia* CBS-8213, *T. brasiliensis* CBS-6966, *T. cinnabarina* CBS-8234, *T. encephala* CBS-8235, *T. fuciformis* CBS-8226, *T. foliacea* CBS-8228, *T. mesenterica* CBS-6973, *T. globospora* CBS-6972 and *T. indecorata* CBS-8232) were purchased from Centraalbureau voor Schimmelcultures (CBS), Utrecht.

Isolation of extra-cellular polysaccharides. After incubation in Malt-yeast extract media for 6 days at 27°C, the extra-cellular polysaccharides were isolated from the shake-flask cultures (Khondkar *et al*., 2002). The cells were removed by centrifugation at 48,000 ×g (Beckman, California, USA) for 30 min at 4°C. The slimy cell mass was then re-suspended in water and re-centrifuged as above. Supernatant fractions were collected, mixed together and evaporated to dryness under reduced pressure and a temperature of 35~40°C. This reduced volume was then

*Corresponding author <E-mail : proma_khondkar@yahoo.co.uk>

treated with cold ethanol to precipitate the polysaccharides. To facilitate precipitation, the solutions were kept at 4°C for a further 24 h. They were then re-centrifuged at 4,100 ×g for 15 min at 18°C and the precipitates were collected. Precipitates were then washed with 80~85% (v/v) ethanol followed by dialysis using cellulose tubing (Medicell International Ltd., MWCO-12-14000D, London, UK) against water for 72 h to remove any free sugars and organic solvents, and finally lyophilized.

Compositional analysis of *Tremella* polysaccharides

Determination of the acetyl groups. The acetyl content of the samples was measured by the hydroxamic acid method (McComb and McCready, 1957), which is based on the ability of short chain carboxylic acid esters lactones or anhydrides to react quantitatively with hydroxylamine in an aqueous alkaline solution. The resulting hydroxamic acid derivative reacts quantitatively with ferric chloride to produce an orange-brown/red colour, the intensity of which was measured spectrophotometrically. Pure glucose pentaacetate was used as a standard. Acetyl groups in samples were determined from the standard curve.

Determination of moisture content. The moisture content of air-dried and equilibrated polysaccharides was determined by weight loss of samples after heating in a fan-assisted oven for 1 h at 130°C. Each sample was measured at least in triplicate.

Determination of nitrogen and protein content. The determination of nitrogen content of the *Tremella* polysaccharides was based on standard Kjeldahl methodology (Kirk and Sawyer, 1997). Protein content was also determined by Lowry's method using micro protein determination kit (Sigma 690). Bovine serum albumin (690-10) was used as a protein standard. Absorbance was measured at 725 nm and protein concentration in sample was determined from the calibration curve.

Determination of ash and mineral content. The total ash content was determined after dry ashing in acid cleaned (1% HNO_3) crucibles fitted with lids (79C-1, 24 *ml* capacity, Haldenwanger, Berlin, Germany) at 550°C for 16 h. The samples were ignited over a low flame and then placed in a muffle-furnace (Carbolite, Sheffield, UK) where the temperature was gradually increased to 550°C. The crucibles were cooled in a desiccator and reweighed. The dry ash was qualitatively washed through Whatman No1 (70 mm diameter) filter paper into a 25 *ml* class A volumetric flask with sub-boiled 5% HNO_3, and made up to volume with the 5% HNO_3 before storing in a low-density polyethylene bottle (LDPE, Nalgene 60 *ml* capacity) at room temperature. Determination of the presence of some endogenous minerals (specifically Na, K and Ca) was done with a Perkin Elmer 3110 atomic absorption spectrometer fitted with a Cathoden hollow cathode lamp (Perkin Elmer) against diluted standards (Fisher Scientific UK Ltd, Leics).

Determination of starch content. The starch content was determined in triplicate by the quantitative conversion of starch to glucose according to the method of Karkalas (1985). Starch gelatinisation and dextrinisation was performed at 85°C with a thermostable bacterial α-amylase followed by complete conversion of soluble dextrins to glucose with a fungal amyloglucosidase at 60°C, pH 4.6. Glucose was determined colorimetrically by a glucose oxidase-peroxidase-4-aminophenazone system at pH 7. The absorbance was measured at 505 nm and converted to glucose using a freshly prepared standard (100 μg/*ml*) glucose solution. Starch content was calculated taking into account dilution factors and a factor of 0.9 to convert glucose to starch.

Sugar profiles by gas-liquid chromatography. The individual sugars were determined by gas-liquid chromatography (GLC). Polysaccharide samples were dissolved in 12 molar sulphuric acid at 35°C for 1 h and subsequently hydrolyzed to the monomeric sugars in 1 molar sulphuric acid for 2 h. After neutralization, sugars were converted to the corresponding alditols with sodium borohydride and esterified with acetic anhydride in the presence of 1-methylimidazole (Englyst and Hudson, 1987; Englyst *et al*., 1994). The alditol acetates were analysed by gas-liquid chromatography (Perkin Elmer Auto-system (XL) GC; flame ionisation detector, Norwalk,USA) fitted with a capillary column (SP-2830; Supelco Chromatography Products, PA, USA). The column temperature was 260°C and the injector and detector temperatures were 265 and 270°C, respectively. The carrier gas was helium (30 *ml*/min) with an injected volume of 2.5 μl and split ratio of 100 : 1. Chromatographic data were recorded and stored in a Turbochrom Workstation (Perkin Elmer), where peaks identified on the basis of retention times and allose was used as an internal standard for quantification. Total uronic acid content of the samples was determined spectrophotometrically. The coefficient of variation (CV) of analytical data was about 1%.

Partial Structure characterization of polysaccharides

Fourier Transform Infrared (FTIR) Spectroscopy. Infrared (IR) spectra were recorded as KBr discs on a Mattson Galaxy 5000 FTIR spectrometer at room temperature over the range of 4000-400 cm^{-1}. The polysaccharide samples were prepared by mixing samples with dry KBr fine powders and pressing the resultant mixture into discs. Samples (1 or 2 mg) were mixed with 300 mg of

KBr and tablet was made.

Periodate oxidation. Accurately weighted samples of the polysaccharides were dissolved in 0.2 M aqueous sodium metaperiodate and the mixture was allowed to stand at room temperature in the dark for 72 h. The periodate uptake was measured spectrophotometrically (Aspinall and Ferrier, 1957) and titrimetrically.

Smith degradation. Excess periodate (from above) was destroyed by adding ethylene glycol and the mixture was dialysed against distilled water. Sodium borohydride was added to the dialysed solution and allowed to stand at room temperature for 7 h. The solution was neutralised with 1 N HCl and then concentrated to 50 *ml* under reduced pressure while not exceeding 40°C. Next, 6 N HCl was added to a concentration of 0.5 M and the mixture was kept at room temperature for 8 h. The solution was neutralized with NaOH and dialysed against distilled water and freeze dried (Goldstein *et al.*, 1958).

Statistical analysis. All the experiments were carried out over four times. Results are presented as the mean ± S.D.

Results and Discussion

Proximate composition. In order to characterize a polysaccharide and investigate its fine structure, it is necessary to know the exact composition of the polysaccharide after

Table 1. Composition of *Tremella* polysaccharides

Organism	Moisture content (%)	Ash content (%)	Protein content (%)[a]		Acetyl group content (%)	Carbohydrate content[b] (%)
			a	b		
T. aurantia (TAU)	7.2 (± 0.1)	4.4 (± 0.2)	4.7 (± 0.1)	1.0 (± 0.1)	3.4 (± 0.1)	80.3 (± 0.1)
T. globospora (TGL)	7.8 (± 0.1)	2.8 (± 0.1)	4.4 (± 0.2)	1.1 (± 0.0)	0.9 (± 0.4)	84.1 (± 0.1)
T. mesenterica (TME)	10.8 (± 0.0)	3.7 (± 0.1)	3.8 (± 0.2)	1.2 (± 0.2)	2.1 (± 0.1)	79.6 (± 0.2)
T. foliacea (TFO)	11.2 (± 0.1)	4.5 (± 0.1)	4.1 (± 0.1)	0.9 (± 0.1)	2.3 (± 0.2)	77.9 (± 0.2)
T. fuciformis (TFU)	11.4 (± 0.1)	3.4 (± 0.2)	5.7 (± 0.2)	0.8 (± 0.1)	2.9 (± 0.1)	76.6 (± 0.0)
T. indecorata (TIN)	8.6 (± 0.2)	4.2 (± 0.0)	3.7 (± 0.1)	0.7 (± 0.2)	2.7 (± 0.1)	80.8 (± 0.1)
T. encephala (TEN)	11.6 (± 0.1)	4.9 (± 0.1)	3.4 (± 0.1)	0.9 (± 0.0)	3.3 (± 0.1)	76.8 (± 0.3)
T. cinnabarina (TCI)	8.8 (± 0.1)	3.7 (± 0.1)	4.7 (± 0.0)	0.7 (± 0.3)	3.3 (± 0.1)	79.5 (± 0.1)
T. brasiliensis (TBR)	10.3 (± 0.0)	2.7 (± 0.3)	2.7 (± 0.1)	0.3 (± 0.1)	2.6 (± 0.2)	81.7 (± 0.2)

[a]a = native extra-cellular polysaccharides; b = sodium meta-bisulphite and trypsin treated extra-cellular polysaccharides.
[b] = carbohydrate content by difference (This is supported by the experimental results by Englyst dietary fibre determination method, carbon source-xylose).

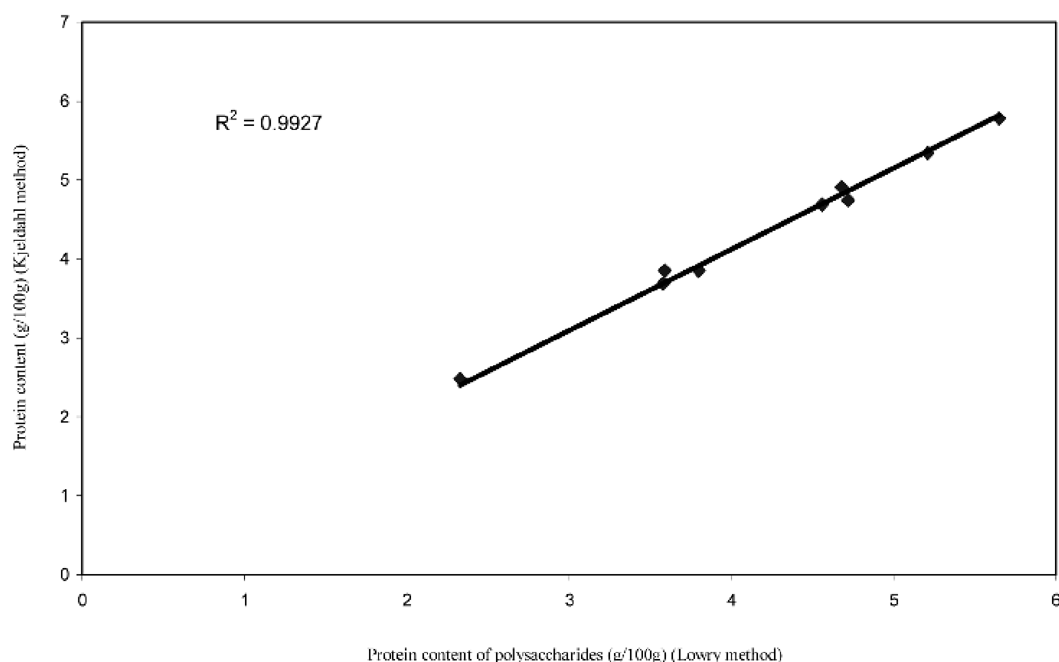

Fig. 1. Comparison of protein content of *Tremella brasiliensis* extra-cellular polysaccharides by Kjeldahl and Lowry method.

removal of interfering substances such as protein or starch (BeMiller and Whistler, 1996). The composition of the purified samples are shown in Table 1. All polysaccharides were composed of glucuronoxylomannan, but also contained variable amounts of protein, ash, minerals and trace amounts of starch.

Protein content. The protein content of native polysaccharides was measured as Kjeldahl nitrogen and by the Lowry method. Both results were highly correlated ($r = 0.9927$, $p < 0.05$, Fig. 1). Among the polysaccharides, the protein content of TBR (*T. brasiliensis*) and TGL (*T. globospora*) found to be 2.7 to 2.8% while TAU (*T. aurantia*), TME (*T. mesenterica*), TFO (*T. foliacea*), TFU (*T. fuciformis*), TIN (*T. indecorata*), TEN (*T. encephala*) and TCI (*T.* cinnabarina) had higher protein content (3.4 to 5.8%). The protein content of the polysaccharides was reduced to 0.3 to 1.2% (Table 1) by the introduction of a sodium meta-bisulphite pre-steep overnight at 40°C followed by treatment with trypsin. These procedures are effective for the removal of tightly bound proteins as the protein matrix is softened by the pre-steep and becomes more susceptible to the protease. The effectiveness of purification with sodium metabisulphite and trypsin was clear in case of these polysaccharides (Table 1). Among these *Tremella* species, only the fruiting bodies of *T. fuciformis*, *T. aurantia* and extra-cellular polysaccharide of *T. mesenterica* have previously been studied for protein content where a range of 0.1 to 8.0% was reported (Wasser and Reshetnikov, 2001). However, Slodki *et al.* (1966) working on *T. mesenterica*, *T. brasiliensis*, *T. aurantia*, *T. encephala* and *T. foliacea* extra-cellular polysaccharides found protein contents ranging from 1.6 to 4.5%.

Moisture and carbohydrate content. The moisture content of the polysaccharides are presented in Table 1. Although all the polysaccharides were air equilibrated under identical conditions, there were variations in the moisture content (7.2 to 11.6%). The carbohydrate content and profile of the polysaccharides was measured by gas-liquid chromatography (Table 1). It ranged from 76.5 to 84.2%. Among the *Tremella* species, polysaccharides of *T. mesenterica*, *T. fuciformis*, *T. brasiliensis*, *T. encephala*, *T. aurantia*, *T. foliacea* and fruiting bodies of *T. mesenterica*, *T. fuciformis* and *T. aurantia* have been investigated by others for carbohydrate content and ranged from 70 to 98% and 48.8 to 101%, respectively (Gao *et al.*, 1998; Reid and Bartnicki-Garcia, 1976; Slodki *et al.*, 1966; Wasser and Reshetnikov, 2001).

Sugar profile. The relationship between the sugar profile of the polysaccharides and the species investigated was clearly not static and depends upon the environment to which the organisms are exposed. The sugar profile of the polysaccharides probably represents an averaging of the different types of polymers produced in these systems. However, the predominant sugars identified in these organisms were xylose, arabinose, mannose, galactose and glucose (Table 2) regardless of the organism investigated and the carbon source (Khondkar *et al.*, 2002). A small amount of fucose and ribose was present in some of the species (Table 2). Previous researchers have isolated neutral and acidic heteroglycans from the fruiting bodies of *T. fuciformis* (Gao *et al.*, 1996a, 1996b, 1997; Yui *et al.*, 1995). They also reported some additional carbohydrates in the heteropolysaccharides of the fruiting bodies of *T. fuciformis*, such as fucose (Gao *et al.*, 1996a, 1996b), xylobiose (Yui *et al.*, 1995), fructose and glucose (Zhang and Hong, 1984). Among these carbohydrates, fucose and glucose were also present in extracellular polysaccharides along with fruiting body polysaccharides of *T. fuciformis*. However, the extracellular polysaccharide purified from the filtrate of *T. aurantia* was neutral and contained only mannose and xylose (Li *et al.*, 1997). In case of *T. mesenterica*, same sugar profile as *T. fuciformis* and *T. aurantia* (Slodki, 1966; Slodki *et al.*, 1966). As previously reported (Gao *et al.*, 1996a, 1996b), this study has also shown that *T. fuciformis* contained fucose. Fucose was also present in *T. globospora* and *T. Cinnabarina*, while ribose was present instead of fucose in *T. brasiliensis*, *T. encephala*, *T. mesenterica*, and *T. aurantia*. Neither fucose nor ribose was present in the extracellular polysaccharides from *T. foliacea* and *T. Indecorata*

Table 2. Sugar profile of *Tremella* extra-cellular polysaccharides (carbon source-galactose, Khondkar *et al.*, 2002)

Name of organism	Fucose (%)	Ribose (%)	Arabinose (%)	Xylose (%)	Mannose (%)	Galactose (%)	Glucose (%)	Glucouronic acid (%)	Total carbohydrate (%)
T. aurantia	0	0.48	0.71	15.36	32.11	12.06	6.52	9.19	76.43
T. globospora	3.01	0	3.05	18.36	31.3	3.59	24.54	6.08	89.93
T. mesenterica	0	1.28	1.23	26.52	24.51	1.06	15.59	11.66	81.85
T. foliacea	0	0	1.46	15.31	27.78	7.44	16.23	7.12	75.34
T. fuciformis	4.12	0	0.39	13.36	31.01	3.62	17.43	9.86	79.79
T. indecorata	0	0	3.54	2.57	25.42	3.75	47.06	3.29	85.63
T. encephala	0	0	0.44	18.43	34.99	4.77	5.58	8.18	72.39
T. cinnabarina	2.52	0	2.33	16.53	41.69	3.63	11.62	8.45	86.77
T. brasiliensis	0	0.34	0.34	36.96	26.49	2.39	5.07	8.76	80.35

(Khondkar *et al*., 2002).

The total uronic acid composition of *Tremella* heteropolysaccharides varied according to species and the carbon source (Khondkar *et al*., 2002). It was observed that the total uronic acid for *T. globospora* and *T. indecorata* was relatively low in comparison to other species while the content was relatively high for *T. cinnabarina*, *T. brasiliensis*, *T. foliacea* and *T. fuciformis*.

Ash content. The ash content of the polysaccharides ranged from 2.7 to 5% as shown in Table 1. The ash content of TAU, TFO, TIN and TEN (Table 1) was found to be higher compared to other polysaccharides. The ash content found in this study was higher (3.37%) in the polymer isolated from *T. fuciformis* compared to the reported value 0.4% (Gao *et al*., 1997). However, the ash content of all *Tremella* polysaccharides was much lower than other commercially available extra-cellular polysaccharides, e.g. xanthan gum (Debon and Tester, 2001). If the pH of the MY medium was about 5 or less, *T. mesenterica*, *T. aurantia*, *T. fuciformis*, *T. brasiliensis*, *T. foliacea*, *T. globospora* and *T. encephala* produced trace amounts of starch (0.1 to 0.8%). The other organisms (*T. cinnabarina* and *T. indecorata*) did not produce any starch at that pH.

Acetyl group content. The acetyl content of the polymers ranged from 0.9 to 3.4% (Table 1) which was greater than the reported values of 0.1 to 2.7% (Gao *et al*., 1998; Slodki *et al*., 1966). The acetyl group content of TAU, TEN and TCI was higher than other polymers (TBR, TFU, TME, TIN and TFO), but for TGL it was relatively low. Kiho *et al*. (1995) reported higher acetyl group contents from the polysaccharide of the *T. aurantia* fruiting body, but no information on the extra-cellular polysaccharide of this organism was available for comparison.

Mineral content. The mineral content of the polysaccharides, as determined by dry-ashing and atomic spectroscopy, are shown in Table 3. The high ash contents in TFO and TEN compared to other *Tremella* polysaccharides were essentially due to the presence of bound monovalent cations potassium and sodium and the divalent cation calcium. A comparison with the literature was difficult since systematic data on the mineral composition of *Tremella* polysaccharides or even on other non-starch polysaccharides were scarce.

Table 3. Mineral content of *Tremella* extra-cellular polysaccharides

Organism	Na (mg/g, db)	K (mg/g, db)	Ca (mg/g, db)
T. aurantia (TAU)	4.1 (± 0.0)	4.9 (± 0.0)	1.7 (± 0.0)
T. globospora (TGL)	2.8 (± 0.1)	3.9 (± 0.0)	0.4 (± 0.1)
T. mesenterica (TME)	5.1 (± 0.1)	5.4 (± 0.0)	2.1 (± 0.0)
T. foliacea (TFO)	5.9 (± 0.1)	8.1 (± 0.1)	3.6 (± 0.1)
T. fuciformis (TFU)	4.9 (± 0.1)	3.2 (± 0.1)	1.1 (± 0.0)
T. indecorata (TIN)	3.3 (± 0.1)	3.6 (± 0.1)	0.3 (± 0.0)
T. encephala (TEN)	5.8 (± 0.1)	9.3 (± 0.1)	2.3 (± 0.1)
T. cinnabarina (TCI)	3.8 (± 0.1)	4.1 (± 0.0)	0.5 (± 0.1)
T. brasiliensis (TBR)	2.4 (± 0.0)	3.7 (± 0.1)	0.4 (± 0.1)

db = Dry weight basis

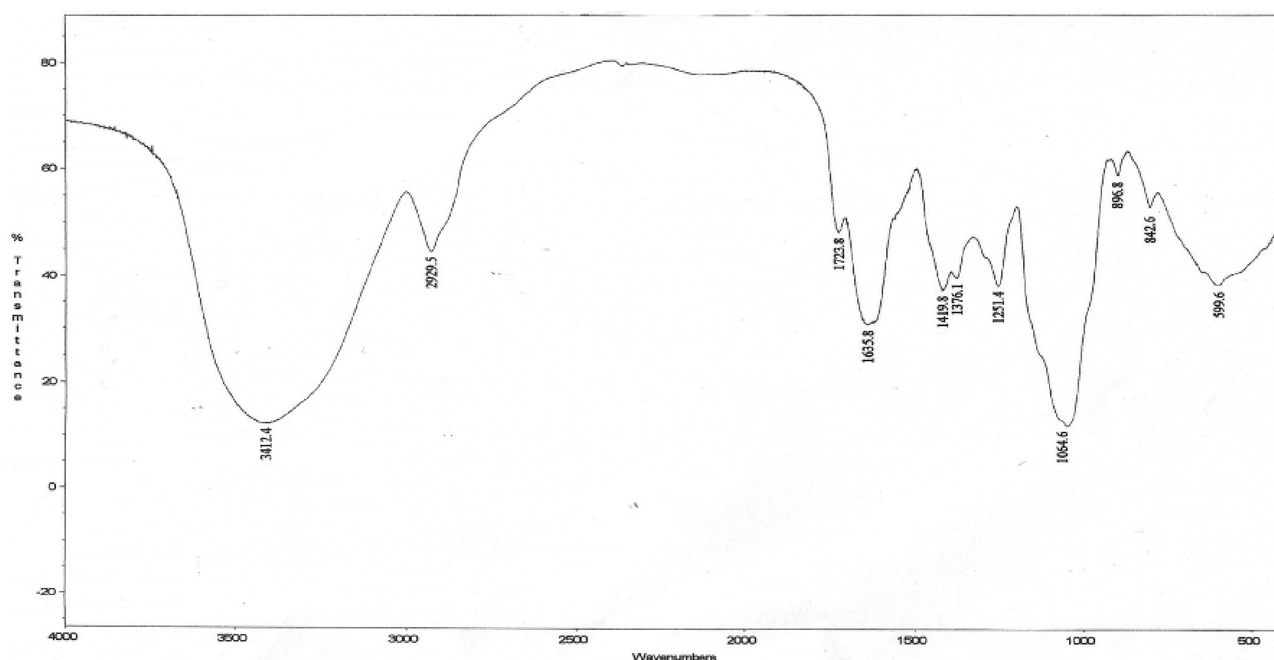

Fig. 2. FTIR spectrum of *Tremella* extra-cellular polysaccharide (TBR).

Partial structural analysis of the *Tremella* polysaccharides

FTIR spectra. The FTIR spectrum of *Tremella* purified polysaccharide (Fig. 2) displayed a broad intense peak at around 3412.4 cm^{-1}, characteristic for hydroxyl groups. An asymmetrical stretching peak at 1723.8 cm^{-1} and a weak symmetrical stretching peak near 1419.8~1376.1 cm^{-1} was seen suggest the presence of carboxyl groups. Specifically, the peaks at 1064.6 cm^{-1} and ~1251.4 cm^{-1} regions ascertain the presence of glucuronic acid and *o*-acetyl groups (Kazy *et al.*, 2002), respectively. It was clear from the IR spectra that the only uronic acid present was glucuronic acid (Williams and Fleming, 1987). This was further confirmed by TLC analysis, where acid hydrolyzates of *Tremella* polysaccharides were compared with standard glucuronic acid (Fig. 3). Glucuronic acid was also previously isolated from *T. mesenterica*, *T. aurantia* and *T. fuciformis* extra-cellular and fruiting body polysaccharides (Gao *et al.*, 1997; Kiho *et al.*, 2000; Slodki *et al.*, 1966). Absorption peaks at 842.6 cm^{-1} and 897.6 cm^{-1} may be taken as evidence for the presence of α- and β-linkages, respectively, in the molecule (Percival, 1962). All *Tremella* polysaccharides presented a similar type of IR spectra.

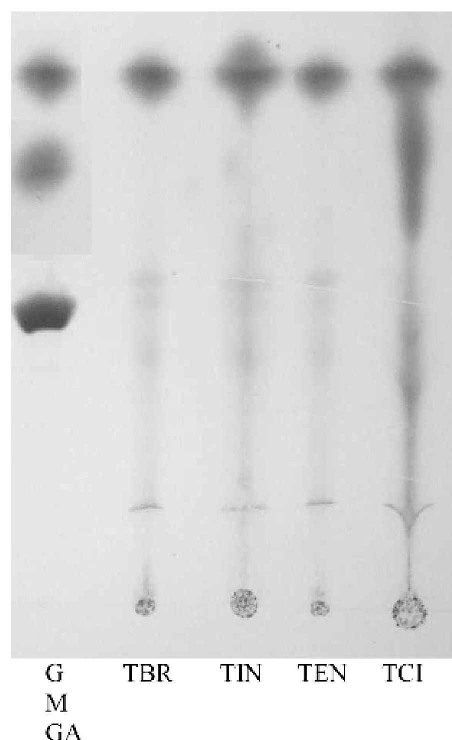

Fig. 3. Chromatographic comparison of *Tremella* polysaccharide hydrolysates (G = glucose, M = mannose and GA = glucuronic acid) using mobile phase of ethylacetate : acetic acid:pyridine:water = 5 : 1 : 5 : 3.

Periodate oxidation and Smith degradation of Tremella polysaccharides. Periodate oxidation of the extra-cellular polysaccharides showed that consumption of periodate by different *Tremella* polysaccharides was approximately in the range 1.25 to 1.68 per mole of sugar (based on an average molecular weight of 162) in 72 hours. The results of TBR polysaccharide are shown in Fig. 4. Periodate oxi-

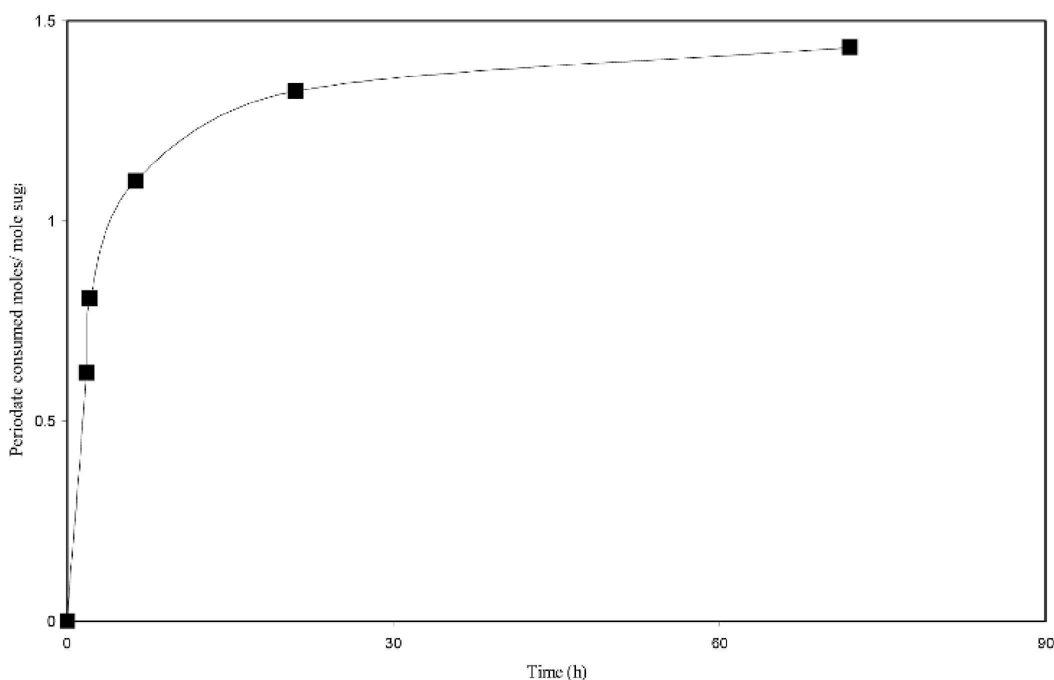

Fig. 4. Periodate oxidation of *Tremella* extra-cellular polysaccharide (TBR). The consumption of periodate is based on an average molecular weight of 162 for a sugar molecule.

dation followed by borohydride reduction and mild acid hydrolysis (Goldstein *et al.*, 1958) resulted in periodate-resistant polysaccharides which were isolated by dialysis and lyophilised. The isolated materials were insoluble in water, but were soluble in DMSO. The oxidation destroyed all the xylose, glucuronic acid and other sugars, leaving a water-insoluble mannan backbone. Acid hydrolysis of the degraded polysaccharides followed by TLC in two solvent systems (ethyl acetate: pyridine: water 10 : 4 : 3 and ethyl acetate : acetic acid : formic acid : water 18 : 3 : 1 : 4, v/v) gave rise to only mannose (Fig. 5). These TLC results were further confirmed by derivatisation to alditol acetates and identification by GLC (Englyst and Hudson, 1987; Englyst *et al.*, 1994) which showed the presence of only one sugar, mannose, indicating that the periodate resistant backbone was a $(1 \rightarrow 3)$-linked mannan (Fig. 6). The optical rotation of extra-cellular polysaccharide was -18° in water and -5° in DMSO : H_2O (3 : 1), which was lower than the reported value (Fraser *et al.*, 1973). Smith-degraded polysaccharide, which contained the mannan backbone, was dissolved in DMSO : H_2O (3 : 1) and had an optical rotation of $+101^{\circ}$, showing that the mannose units were α-linked (Cherniak *et al.*, 1980). When the native polysaccharides were partially hydrolysed, they lost 30% of xylose and compared to native, had a rotation of $+15^{\circ}$ in water indicating that the xylose residues were β-linked (Cherniak *et al.*, 1980). From these observations, it was clear that extra-cellular polysaccharides contained an α-$(1 \rightarrow 3)$-mannan backbone (Fig. 7) and that the side chains were β-linked (in the case of xylose) (Fraser *et al.*, 1973; Gao *et al.*, 1996a, 1996b, 1997). However, how other sugars were linked was not investigated in the present study.

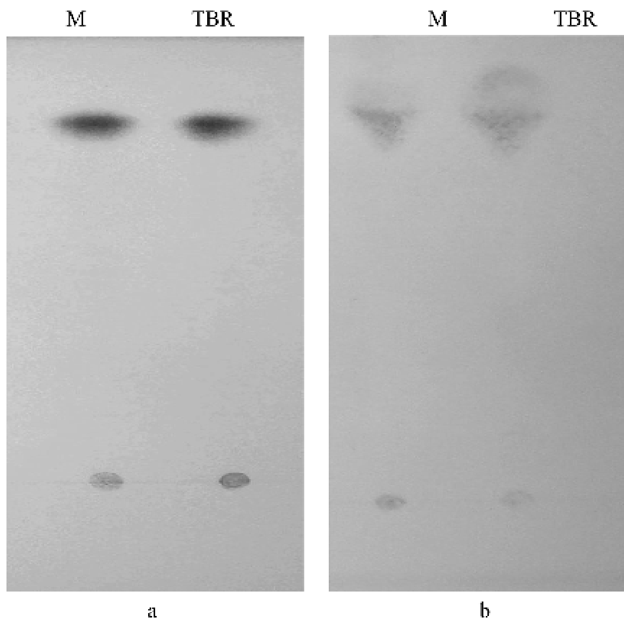

Fig. 5. TLC chromatograph of periodate-resistant polysaccharide (TBR) in two different solvent system (a = ethyl acetate : pyridine : water 10 : 4 : 3 and b = ethyl acetate : acetic acid : formic acid : water 18 : 3 : 1 : 4, v/v, M = mannose).

Conclusions

In comparison to other microbial extra-cellular polysaccharides such as xanthan, which contains 66 to 70% (dry weight basis) carbohydrate, the carbohydrate content of different *Tremella* extra-cellular polysaccharides were relatively high, ranging from 86.4 to 93.2% (dry weight

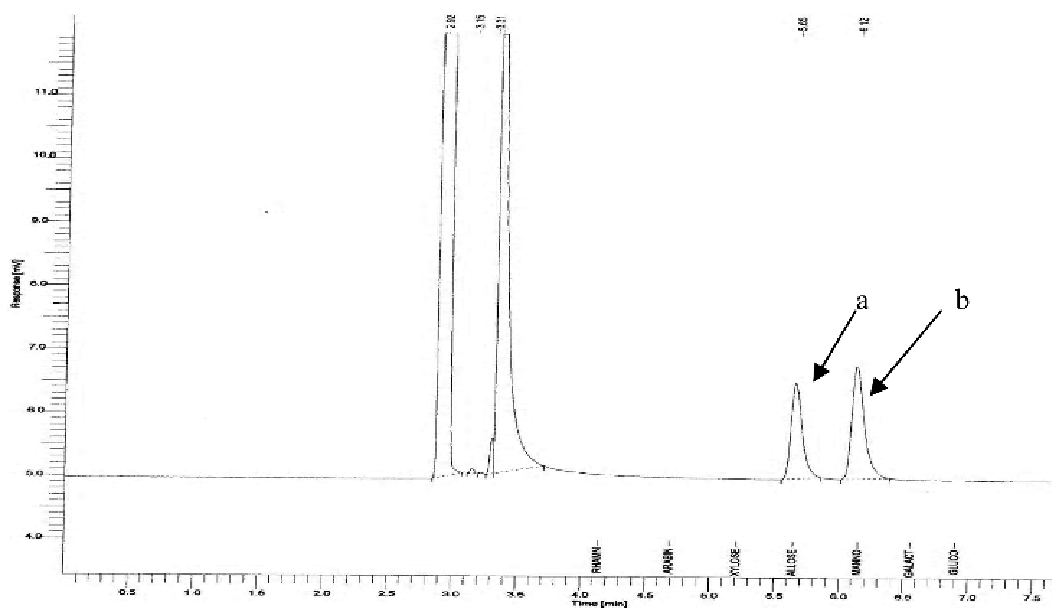

Fig. 6. GC chromatogram of periodate oxidised and Smith degraded polysaccharide (a = allose, b = mannose).

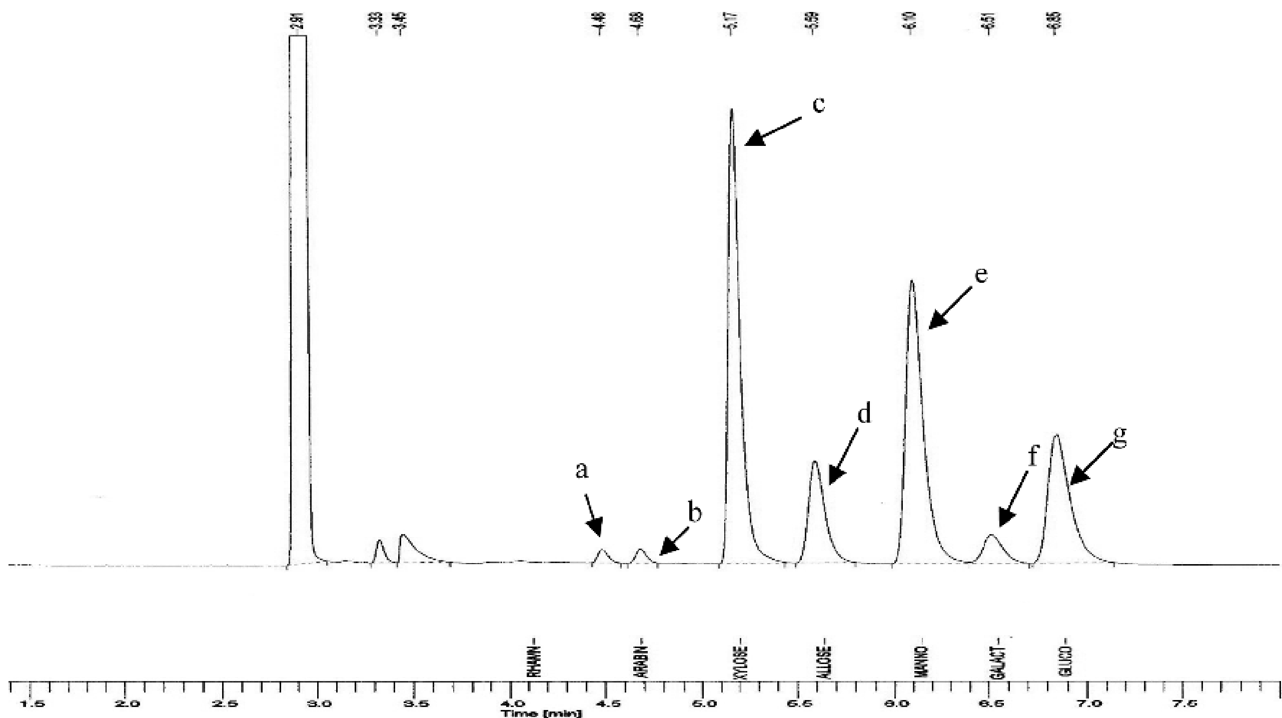

Fig. 7. Gas-liquid chromatogram of TBR (*Tremella brasiliensis* polysaccharide, a = ribose, b = arabinose, c = xylose, d = allose, e = mannose, f = galactose, g = glucose, allose was used as an internal standard).

basis), a unique feature of *Tremella* mushrooms. The protein contents of *Tremella* polysaccharides were relatively lower than commercially available polysaccharides from different sources. Based on the published literature, the polysaccharides extracted in this study were well within the expected range for purified commercially available products and therefore deemed to be sufficiently pure. An area of further study would be the complete linkage analysis of the extra-cellular polysaccharides in the hope that this could be developed further to determine whether or not commercially viable products could be made from these organisms. Possible applications include the food and pharmaceutical industries.

Acknowledgement

The author is grateful to Professor Tester, Professor Aidoo and the late Dr. Karkalas for their valuable advice and guidance throughout this study.

References

Aspinall, G. O. and Ferrier, R. J. 1957. A spectrophotometric method for the determination of periodate consumed during the oxidation of carbohydrates. *Chem. Ind.* (36):1216.

Bandoni, R. J. 1995. Dimorphic heterobasidiomycetes: Taxonomy and parasitism. *Stud. Mycol.* (38):13-27.

BeMiller, J. N. and Whistler, R. L. 1996. Carbohydrates. In: Food Chemistry, 3rd edition, pp. 157-223. Ed. O. R. Fennema. Marcel Dekker Inc., New York.

Cherniak, R., Reiss, E., Slodki, M. E., Plattner, R. D. and Blumer, S. O. 1980. Structure and antigenic activity of the capsular polysaccharide of *Cryptococcus neoformans* serotype A. *Mol. Immunol.* 17:1025-1032.

Debon, S. J. J. and Tester, R. F. 2001. *In vitro* binding of calcium, iron and zinc by non-starch polysaccharides. *Food Chem.* 73:401-410.

Englyst, H. N. and Hudson, G. J. 1987. Colorimetric method for routine measurement of dietary fiber as non-starch polysaccharides: a comparison with gas-liquid chromatography. *Food Chem.* 24:63-76.

Englyst, H. N., Quigley, M. E. and Hudson, G. J. 1994. Determination of dietary fiber as nonstarch polysaccharides with gas-liquid-chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 119:1497-1509.

Fraser, C. G., Jennings, H. J. and Moyna, P. 1973. Structural analysis of an acidic polysaccharide from *Tremella mesenterica* NRRL Y-6158. *Can. J. Biochem.* 51:219-224.

Gao, Q. P., Berntzen, G., Jiang, R. Z., Killie, M. K. and Seljelid, R. 1998. Conjugates of *Tremella* polysaccharides with microbeads and their TNF-stimulating activity. *Planta Med.* 64:551-554.

Gao, Q. P., Jiang, R. Z., Chen, H. Q., Jensen, E. and Seljelid, R. 1996a. Characterization and cytokine stimulating activities of heteroglycans from *Tremella fuciformis*. *Planta Med.* 62:297-302.

Gao, Q. P., Killie, M. K., Chen, H. C., Jiang, R. Z. and Seljelid, R. 1997. Characterization and cytokine-stimulating activities of

acidic heteroglycans from *Tremella fuciformis*. *Planta Med.* 63:457-460.

Gao, Q. P., Seljelid, R., Chen, H. Q. and Jiang, R. Z. 1996b. Characterisation of acidic heteroglycans from *Tremella fuciformis* Berk with cytokine stimulating activity. *Carbohydr. Res.* 288:135-142.

Goldstein, I. J., Lewis, B. A. and Smith, F. 1958. The Structure of the dialdehyde formed by periodate oxidation of methyl α-L-rhamnopyranoside. *J. Am. Chem. Soc.* 80:939-941.

Kakuta, M., Sone, Y., Umeda, T. and Misaki, A. 1979. Comparative structural studies on acidic heteropolysaccharides isolated from Shirokikurage, fruit body of *Tremella fuciformis berk*, and the growing culture of its yeast-like cells. *Agric. Biol. Chem.* 43:1659-1668.

Karkalas, J. 1985. An improved enzymatic method for the determination of native and modified starch. *J. Sci. Food Agric.* 36:1019-1027.

Kazy, S. K., Sar, P., Singh, S. P., Sen, A. K. and D'Souza, S. F. 2002. Extracellular polysaccharides of a copper-sensitive and a copper-resistant Pseudomonas aeruginosa strain: synthesis, chemical nature and copper binding. *World J. Microbiol. Biotechnol.* 18:583-588.

Khondkar, P., Aidoo, K. E. and Tester, R. F. 2002. Sugar profile of extracellular polysaccharides from different *Tremella* species. *Int. J. Food Microbiol.* 79:121-129.

Kiho, T., Kobayashi, T., Morimoto, H., Usui, S., Ukai, S., Hirano, K., Aizawa, K. and Inakuma, T. 2000. Structural features of an anti-diabetic polysaccharide (TAP) from *Tremella aurantia*. *Chem. Pharm. Bull.* 48:1793-1795.

Kiho, T., Morimoto, H., Sakushima, M., Usui, S. and Ukai, S. 1995. Polysaccharides in fungi. 35. Anti diabetic activity of an acidic polysaccharide from the fruiting bodies of *Tremella aurantia*. *Biol. Pharma. Bull.* 18:1627-1629.

Kirk, R. S. and Sawyer, R. 1997. Pearson's Composition and Analysis of Foods, pp. 16-20. Addison Wesley Longman Ltd., London.

Lewis, J. G., Stanley, N. F. and Guist, G. G. 1988. Commercial production and applications of algal hydrocolloids. In: Algae and Human Affairs, pp. 205-237. Eds. C. A. Lembi and J. R. Waaland. Cambridge University Press, Cambridge.

Li, X., Chen, M., Dai, R., Shao, A., Wu, Z. and Lin, S. 1997. A preliminary study on extracellular polysaccharide isolated from fermented *Tremella aurantia* Schw. ex. Fr. *Zhongg. Zhong. Zaz.* 22:745-746.

MacCormick, C. A., Harris, J. E., Jay, A. J., Ridout, M. J., Colquhoun, I. J. and Morris, V. J. 1996. Isolation and characterization of a new extracellular polysaccharide from an *Acetobacter* species. *J. Appl. Bacteriol.* 81:419-424.

Margaritis, A. and Pace, G. W. 1985. Microbial polysaccharides. In: Comprehensive Biotechnology, vol. 3, pp. 1005-1044. Eds. H. W. Blanch, S. W. Drewand and D.I. C Wang. Pergamon Press, Oxford.

McComb, E. A. and McCready, R. M. 1957. Determination of acetyl in pectin and in acetylated carbohydrate polymers: hydroxamic acid reaction. *Anal. Chem.* 29:819-821.

Pavlova, K. and Grigorova, D. 1999. Production and properties of exopolysaccharide by *Rhodotorula acheniorum* MC. *Food Res. Int.* 32:473-477.

Percival, E. G. V. 1962. Structural Carbohydrate Chemistry, 2nd edition, pp. 197-235. Muller, London.

Prosky, L., Asp, N. P., Furda, I., Devries, J. W., Schweizer, T. F. and Harland, B. F. 1984. Determination of total dietary fiber in foods, food-products, and total diets-interlaboratory study. *J. Assoc. Off. Anal. Chem.* 67:1044-1052.

Reid, I. D. and Bartnicki-Garcia, S. 1976. Cell-wall composition and structure of yeast cells and conjugation tubes of *Tremella mesenterica. J. Gen. Microbiol.* 96:35-50.

Reshetnikov, S. V., Wasser, S. P., Nevo, E., Duckman, I. and Tsukor, K. 2000. Medicinal value of the Genus *Tremella* Pers. (Heterobasidiomycetes): review. *Int. J. Med. Mushrooms* 2:169-193.

Sinskey, A., Jamas, S., Easson, D. Jr. and Rha, C. 1986. Biopolymers and modified polysaccharides. In: Biotechnology in Food Processing, pp. 73-114. Eds. S. K. Harlander and T. P. Lapuza. Noyes Publications, Park Ridge.

Slodki, M. E. 1966. Hydrolysis products from an extracellular *Tremella* polysaccharide. *Can. J. Microbiol.* 12:495-499.

Slodki, M. E., Wickerham, L. J. and Bandoni, R. J. 1966. Extracellular heteropolysaccharides from *Cryptococcus* and *Tremella*: a possible taxonomic relationship. *Can. J. Microbiol.* 12:489-494.

Wasser, S. P. and Reshetnikov, S. V. G. 2001. Patent WO, 01/32830 A2.

Williams, D. H. and Fleming, I. 1987. Spectroscopic Methods in Organic Chemistry, 4th edition, p. 49. McGraw-Hill, London.

Yalpani, M. and Sandford, P. A. 1987. Commercial polysaccharides: recent trends and developments. In: Industrial Polysaccharides: Genetic Engineering, Structure/Property Relations, and Applications, pp. 311-335. Ed. M. Yalpani. Amsterdam, Elsevier.

Yui, T., Ogawa, K., Kakuta, M. and Misaki, A. 1995. Chain conformation of a glucoronoxylomannan isolated from fruit body of *Tremella fuciformis* Berk. *J. Carbohydr. Chem.* 14:255-263.

Zhang, Y. J. and Hong, Z. 1984. The study of isolation and properties of *Tremella* polysaccharides. *J. Beijing Med. Univ.* 16:83-87.