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Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity

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ABSTRACT

Cultivated oyster mushrooms (genus *Pleurotus*) are interesting as a source of biologically active glucans. Partially, β-glucan from *Pleurotus* sp. (pleuran) has been used as food supplements due to its immunosuppressive activity. Like other dietary fibre components, oyster mushroom polysaccharides can stimulate the growth of colon microorganisms (probiotics), i.e. act as prebiotics. Specific glucans were isolated from stems of *Pleurotus ostreatus* and *Pleurotus eryngii* by subsequent boiling water and alkali extraction. Obtained water soluble (*L1*), alkali soluble (*L2*) and insoluble (*S*) fractions were characterised by various analytical methods. Spectroscopic analysis detected glucans in all the fractions: branched 1,3-1,6-β-D-glucan predominated in *L1* and *S*, while linear 1,3-α-D-glucan in *L2*. Fractions *L1* also contained marked amount of proteins partially in complex with glucans; protein content in *L2* was insignificant. Effective deproteinisation of *L1* and separation of α- and β-glucans in *L2* was achieved by the treatment with phenolic reagent. Small amount of chitin was found in *S* as a component of cell wall chitin–glucan complex. Potential prebiotic activity of extracts *L1* and *L2* was testing using nine probiotic strains of *Lactobacillus*, *Bifidobacterium* and *Enterococcus*. These probiotics showed different growth characteristics dependently on used extract and strain specificity due to the presence of structurally diverse compounds. The extracts *L1* and *L2* can be applied to synbiotic construction only for carefully selected probiotic strains. This exploitation of fruit body extracts extends the use of mushrooms *P. ostreatus* and *P. eryngii* for human health.

1. Introduction

For millennia, humankind has been valued mushrooms as an important edible and medical resource. Currently mushroom-derived substances with antitumour and immunomodulating properties are used as dietary supplements or drugs. The most of cultivated mushrooms such as *Ganoderma lucidum*, *Lentinus edodes* (Shiitake), *Schizophyllum* sp. and many others have been collected and used for centuries in Korea, China, Japan, and eastern Russia (Wasser, 2002). The dry matter of mushroom fruit bodies is about 5–15%, they have a very low fat content and contain 19–35% proteins. Mushroom fruit bodies are plentiful of vitamins, mainly B_1, B_2, C and D_2 (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999; Mattila, Suonpää, & Piironen, 2000). The content of carbohydrates, which are mainly present as polysaccharides or glycoproteins, ranges 50–90%. Most abundant mushroom polysaccharides are chitin, hemicelluloses, β- and α-glucans, mannans, xylans and galactans. The average molecular mass M_w of them varies according to the source and ranges from 5 to 2000 kDa (Bohn & BeMiller, 1995). Mushroom polysaccharides are present mostly as linear and branched glucans with different types of glycosidic linkages, such as (1 → 3),(1 → 6)-β-glucans and (1→3)-α-glucans, but some are true heteroglycans containing glucuronic acid, xylose, galactose, mannose, arabinose or ribose (Wasser, 2002). Like polysaccharides originated from other food products, they contribute to the digestion process as soluble or insoluble dietary fibres depending on their molecular structure and conformation (Cheung & Lee, 1998; Johansson et al., 2006; Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004; Manzi & Pizzoferrato, 2000; Prosky, Asp, Schweizer, DeVreis, & Furda, 1988).

Mushroom polysaccharides with antitumour action differ greatly in their chemical composition and configuration, as well as in their physical properties. Antitumour activity is exhibited by a wide range of glycans extending from homopolymers to highly complex heteropolymers (Ooi & Lui, 1999). The differences in their activity can be correlated with solubility in water, size of the molecules, branching rate and form (Wasser, 2002). A triple-helical tertiary conformation

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of medicinal mushroom β-(1 → 3)-glucans is known to be important for their immune-stimulating activity (Maeda, Watanabe, Chihara, & Rokutanda, 1988). Beside the well-known antitumour β-(1 → 3)-glucans, a wide range of biologically active glucans of other structure have been described. There are linear or branched polysaccharides chains with a backbone composed of α- and/or β-linked glucose units, and their various side chains can be attached in different ways. The main source of biologically active polysaccharides appears to be fungal cell walls consisting mainly of chitin–glucan complexes. However, fungal chitin has no antitumour activity (Mizuno, Sakai, & Chigara, 1995).

Commercial importance of fungal polysaccharides has attracted much attention in the field of functional foods. Partially, commonly cultivated mushrooms of genus *Pleurotus* are interesting because of its β-glucans demonstrating great immunomodulation, antioxidant, antiinflammatory and analgesic properties (Bobek & Galbavy, 2001; Smiderle et al., 2008). Mushrooms of genus *Pleurotus* are cultivated in several countries because of their high adaptability. Annual production of these mushrooms is more than 900,000 tons. There is a lot of different species in genus *Pleurotus* that have pharmacological properties, for example *P. florida, P. tuber-regium, P. pulmonarius, P. ostreatus* and *P. eryngi* (Ragunathan, Gurusamy, Palaniswamy, & Swaminathan, 1996). Biologically active glucans itself or their complexes with proteins as well as other polysaccharides isolated from fruit bodies of these species are interesting for the preparation of novel food supplements. Water solubility is an important factor in enhancing some biological activities of fungal glucans. It can be easy achieved by the introduction of charged groups. Carboxymethylated derivative of pleuran, an alkali-insoluble β-glucan isolated from *Pleurotus* sp. (Karácsonyi & Kuniak, 1994), exhibited immunomodulatory effects including stimulation of phagocytic activity (Paulik et al., 1996). Highly branched β-glucans isolated from the sclerotia of *Pleurotus tuber-regium* and their sulfated derivatives showed potent antitumor activities *in vivo* and *in vitro* (Tao, Zhang, & Cheung, 2006), the latter exhibited relatively higher *in vitro* antitumor activity against human hepatic cancer cell line HepG2 than the native polysaccharide. Another sulfated β-glucan from this source exhibited strong antiviral activity against herpes simplex virus, while corresponding native polysaccharide was inactive (Zhang, Cheung, Ooi, & Zhang, 2004). Low-molecular-weight α-glucan isolated from the mycelium of the liquid cultured mushroom *P. ostreatus* inhibited colon cancer cell proliferation via induction of apoptosis (Lavi, Friesem, Geresh, Hadar, & Schwartz, 2006). Water-soluble α-(1 → 6)-glucan obtained from fruit bodies of *P. florida* showed significant macrophage activation through the release of nitric oxide (Rout, Mondal, Chakraborty, Pramanik, & Islam, 2005, 2004).

Fungal polysaccharides as well as products of their partial hydrolysis have potential prebiotic function like other ones. The term *prebiotic* has been introduced by Gibson and Roberfroid (1995) who exchanged "pro" for "pre", which means "before" or "for". They defined prebiotics as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". This definition partially overlaps that of *dietary fiber* with the exception of digestibility by certain probiotic strains (Gibson, Beatty, Wang, & Cummings, 1995; Gibson & Roberfroid, 1995; Hidaka, Eida, & Takizawa Teta, 1986; Schrezenmeir & De Vrese, 2001; Su, Henriksson, & Mitchell, 2007). The selectivity of prebiotics has been shown for bifidobacteria, which may be promoted by the ingestion of substances such as fructooligosaccharides and inulin (Gibson & Roberfroid, 1995; Gibson et al., 1995; Hidaka et al., 1986), transgalactosylated oligosaccharides (Tanaka, Takayama, & Morotomi, 1983; Ito et al., 1993; Rowland & Tanaka, 1993) and soybean oligosaccharides (Hayakawa et al., 1990; Saitô, Tanaka, & Rowland, 1992). Polysaccharides, mainly cereal β-glucans (Su et al., 2007), and especially their oligomeric fragments (Grootaert et al., 2007; Kontula et al., 1998; Lee, Park, Jung, & Shin, 2002; Manderson et al., 2005; Olano-Martin, Gibson, & Rastall, 2002) are also used as prebiotics. Hydrolysates of oat β-glucan have been reported to stimulate the growth of three *Bifidobacterium* strains and *Lactobacillus rhamosus* GG (Kontula et al., 1998). Prebiotic potency of arabinoxylan oligosaccharides is reviewed by Grootaert et al. (2007). Chitosan oligosaccharides have been demonstrated prebiotic effect on the *Bifidobacterium bifidium* and *Lactobacillus* sp. (Lee et al., 2002). Prebiotic character of pectins and pectic oligosaccharides has been proved by Olano-Martin et al. (2002) and Manderson et al. (2005).

The aim of this study was (a) isolation and characterisation of glucans from fruit bodies of mushrooms *P. ostreatus* (four strains) and *P. eryngii* cultivated in the Czech Republic and (b) testing of water and alkali extracts from these sources for their prebiotic potential in relation to the selected probiotic strains.

2. Experimental

2.1. Samples of fruit bodies

Mushrooms *P. ostreatus* (strains 70, 77, L22, 137) and *P. eryngii* (non-specified strain) were cultivated under controlled conditions by mushrooms producer Rudolf Ryzner in the region South Moravia, the Czech Republic. Stems were separated from pilei and homogenized with the laboratory dispenser DI 25 basic equipped with the dispersing element S25 N-25 G (IKA-Werke GmbH, Germany). The homogenized samples were kept at −20 °C.

2.2. Isolation and purification of polysaccharides

Fractions of water and alkali soluble and insoluble polysaccharides were isolated from the stems of the two species of *Pleurotus* according to modified Freimund's method (Freimund, Sauter, Käppeli, & Dutler, 2003) as it is shown in the Fig. 1. The homogenates were washed with 80% (w/w) ethanol, then washed with distilled water and extracted with boiling water for 6 hours. Extracts were incubated with α-amylase from *Bacillus* sp. (1:500 v/v) at pH 7 for 30 min to remove α-glucans. Sevag reagent (chloroform/butanol 4:1, v/v) was applied to deproteinization. Then deproteinized supernatants were dialyzed and lyophilized to give water soluble fractions (*L1*). The insoluble parts were extracted with 1 M solution of sodium hydroxide containing 0.05% of sodium borohydride. Solid parts were signed as the insoluble fraction (*S*). The supernatant was adjusted in the same way as the previous supernatant to give the alkali soluble fraction (*L2*).

Boiling water and alkali extracts originated from glucan-rich strains 70 and 77 were additionally treated with phenolic reagent to remove reminder proteins (Westphal & Jann, 1965). Purified fractions according to separated layers were assigned as *L1a* and *L2a* (phenolic phase) and *L1b* and *L2b* (aqueous phase).

2.3. Analytical methods

All isolation products were analysed for their carbon, hydrogen and nitrogen content using the Elementar vario EL III (Elementar Analysensysteme GmbH, Germany). High-performance gel permeation chromatography (HP-GPC) analysis of the extracts was carried out at room temperature by using a PL aquagel–OH Guard column, followed by PL aquagel–OH Mixed column (Polymer laboratories Ltd., UK), high pressure pump LCP 4100 (ECOM, Czech Republic) and a refractive index detector Shodex RI 71 (Dionex Softron GmbH, Germany). Samples were eluted at a flow rate of 0.5 ml min^{-1} with salt solution (0.05 M NaH_2PO_4, 0.05 M Na_2HPO_4, 0.2 M $NaNO_3$ and 0.02% NaN_3) as a mobile phase.

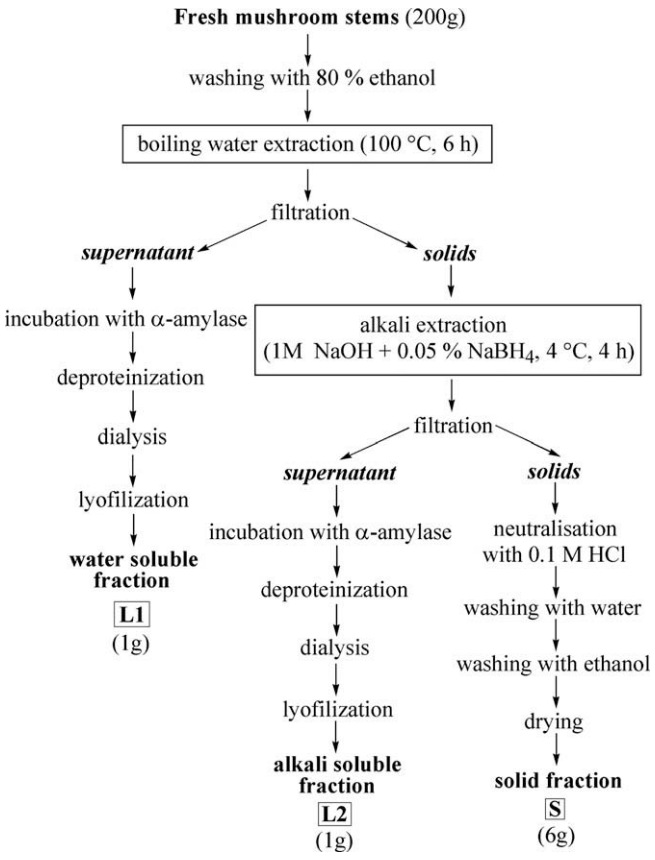

Fig. 1. Scheme of isolation and purification of polysaccharide fractions from stems of *Pleurotus ostreatus* and *Pleurotus eryngii*.

Neutral sugars were released by Saeman hydrolysis (Selvendran, March, & Ring, 1979) and analysed as alditol acetates by GC using a GC 07 chromatograph (Labio, Czech Republic). A 30 m capillary column DB-1 Agilent (J and W) with i.d. 0.25 mm and 0.1 μm film thickness was used. The temperatures of injector and detector were, respectively, 220 and 230 °C. The oven temperature program was following: 120 °C for 1 min, then rose to 130 °C (2.5 °C/min) for 30 min, and finally rose to 220 °C (5 °C/min).

FT-IR spectra (spectral region 4000–400 cm^{-1}, resolution 2 cm^{-1}) of the solid samples in the form of KBr tablets were recorded on FT-IR spectrophotometer 1760 X (Perkin Elmer, USA). FT-Raman spectra of powder samples were recorded by using Bruker FT-Raman (FRA 106/S, Equinox 55/S) spectrometer equipped with a quartz beam splitter, a liquid nitrogen cooled germanium detector and excitation at 1064 nm from a Nd:YAG laser. The laser power was set at 100 mW, and 256 scans were accumulated with a spectral resolution of 2.0 cm^{-1}. Vibration spectra were 10-point filtered and baseline corrected using Origin 6.0 (Microcal Origin) software. The second derivatives of the spectra were used for wavenumber determination of overlapped bands. High-resolution ^{13}C CP/MAS (cross polarization/magic angle spinning) NMR spectra were measured by using Bruker Avance 500 spectrometer in 4 mm ZrO_2 rotors at the frequency 125.8 MHz. Spinning frequency was 10–11 kHz, contact time 2 ms, repetition delay 3 s, spectral width 37.5 kHz and number of scans 2100–5400. ^{13}C chemical shifts were calibrated with glycine standard (a low field carbonyl signal at 176.0 ppm from TMS) by sample replacement.

Small amounts of the fractions (~8 mg) were methylated according to Ciucanu and Caprita (2007) to give a product showing no IR absorption for hydroxyl. The methylated samples were hydrolysed with 2 mol l^{-1} formic acid (1 ml) for l h at 100 °C. Obtained products were reduced with 1 mol l^{-1} sodium borohydride, then converted into their alditol acetates and analysed by GLC–MS system (5890 Series II, 5972 Series Mass Selective Detector, HP-5: 5% phenyl, 95% dimethylpolysiloxane). Obtained data were assigned using library NITO2.L.

Contents of total and α-1,4-glucans were determined in the obtained fractions according to the Mushroom and Yeast β-glucan Assay Procedure K-YBGL 10/2005 (Megazyme, Ireland). Estimation of non-starch glucans is based on the difference between glucose contents after the total acidic hydrolysis of glucans and specific enzymatic hydrolysis of α-1,4-glucans. The samples were previously dried under a stream of nitrogen to ensure the right acid concentration important for the hydrolysis. The values of glucan contents were calculated in dry matter.

2.4. Prebiotic activity

Water (*L1*) and alkali (*L2*) extracts of *P. ostreatus* strain 77 and *P. eryngii* were tested on potential prebiotic activity. The strains selected for tests were *Lactobacillus* ssp. (4 strains: Lac A–D), *Bifidobacterium* ssp. (3 strains: Bifi A–C) and *Enterococcus faecium* (2 strains: Ent A and B). These strains were chosen from laboratory collection of probiotic strains. Cultivation tests were performed at 37 °C in the medium based on MRS (oxoid) commonly used for lactic acid bacteria cultivation. The medium was prepared without glucose and mixed with different portions of liquid extract and water (the final premix contains 50% of MRS medium). The medium was supplied with 0.05% cysteine. The growth characteristic was compared to the medium mixed with water only. Maximum growth rate, maximum biomass concentration and final acid production (as pH decrease) were observed in four independent cultivations.

3. Results and discussion

3.1. Isolation and purification of polysaccharides

The suitable procedures for isolation of polysaccharides from fruit bodies (stems) summarised in Fig. 1 were worked out. The homogenized samples were subsequently washed with 80% ethanol and cold water to remove small molecules including mono and oligosaccharides. Then polysaccharides were extracted with boiling water and supernatants were isolated by filtration. Analysis of solid residues, however, confirms that water-insoluble fractions still contained a high amount of glucans, which can be isolated. That is why an alkali extraction step was included into the isolation procedure. Water and alkali extracts were submitted to purification procedures to remove protein and ballast polysaccharides (starch). Starch was fragmentised by α-amylase to release maltose, which was removed by dialysis. Multiply application of Sevag reagent led to partial removing of proteins, while more complete deproteinisation was achieved by the treatment of extracts and/or dissolved lyophilisates with phenol reagent. Thus, three fractions were gained: water soluble (*L1*), alkali soluble (*L2*) and insoluble (*S*) ones.

3.2. Content of glucans in obtained fractions

Table 1 describes the composition of isolated fractions *L1*, *L2* and *S*. These products contained 86.7–91.5% of dry matter and showed a wide range of non-starch glucan (NSG) contents (44.2–90.1% for *P. ostreatus* and 33.6–66.4% for *P. eryngii*). In all cases fractions *L1* contained less NSG than corresponding fractions *L2* and *S*. The level of NSG in the fractions significantly varied for the strains of *P. ostreatus*. The highest amount of these polysaccharides (90.1%) was observed in *L2* fraction of strain L22; the lowest ones in the

Table 1
Characterisation of fractions obtained from stems of *P. ostreatus* and *P. eryngii*.

Sample specification			Dry matter (%)	Contents (% in dry matter)					
Specie	Strain	Fraction		NSG	Starch	Glc	Gal	Man	GlcN
P. ostreatus	70	*L1*	93.6	56.4	traces	55.6	0	4.8	0
		L1b	90.3	78.9	0	87.5	0	0	0
		L2	89.9	71.2	0	70.1	0.7	0	0
		L2b	91.2	84.3	0	85.7	0	0	0
		S	90.7	73.4	2.2	66.2	0	2.0	1.8
	77	*L1*	88.5	72.0	traces	74.0	0	0	0
		L1b	87.9	85.0	traces	82.0	0	0	0
		L2	89.2	72.1	traces	77.6	0	0	0
		L2b	87.6	89.2	traces	92.1	0	0	0
		S	91.5	86.9	1.2	80.4	0.9	1.3	3.7
	L22	*L1*	86.8	56.5	0	54.1	5.1	3.4	0
		L2	87.4	90.1	0	81.0	3.7	6.5	0
		S	88.0	86.5	traces	85.5	2.0	1.5	1.8
	137	*L1*	89.8	44.2	traces	43.3	1.2	7.3	0
		L2	87.9	45.9	0	43.0	0	4.1	0
		S	90.2	65.8	traces	63.1	0.6	1.2	3.7
P. eryngii	NS[a]	*L1*	86.8	33.6	traces	27.4	0	2.6	0
		L2	87.4	55.4	traces	50.4	5.0	10.9	0
		S	86.7	66.4	2.6	61.4	0	0	2.5

[a] NS, non-specified.

strain 137 (44.2–65.8%) and *P. eryngii* (33.6–66.4%). The content of starch was very low for *L1* and *L2* due to the α-amylase treatment, while some fractions *S* contained marked amounts of starch (1.2–2.6%).

3.3. Carbohydrate composition and linkages

Carbohydrate composition of the fractions obtained by GC is summarised in Table 1. D-Glucose is the main sugar in all cases, while small amounts of D-galactose and D-mannose were also found, so galactomannans are present in some fractions as minor component. D-Glucosamine was observed only in *S* fractions indicating chitin in complex with glucans. The contents of D-glucose obtained by GC well correlated with enzymatic determination of total glucans (Fig. 2), so these polysaccharides are the main source of D-glucose in the fractions. Methylation analysis of fractions *L1* revealed the presence of 2,3,4,6-tetra-, 2,4,6-tri-, 2,3,4-tri- and 2,4-di-*O*-methyl-glucose (molar proportions 1:4:3:1), while the fractions *S* contained much less 2,3,4-tri-*O*-methyl-glucose (molar proportions 1:4:0.2:0.8) (Table 2). These results confirm that *L1* and *S* are highly branched 1,3-1,6-glucans. In contrast, fractions *L2* contained only 2,4,6-tri- *O*-methyl-glucose, so these are linear 1,3-glucans.

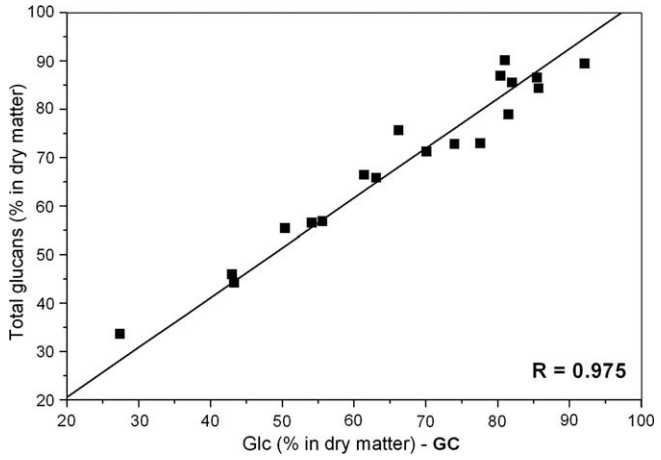

Fig. 2. Correlation between the values of Glc contents obtained by GC and total glucans obtained by enzymatic assay.

3.4. Removing of proteins

The amount of nitrogen (% m/m) in the isolated fractions obtained by elemental analysis is represented in Table 3. This value varied for different species and strains. Usually, glucan-rich samples (>85%) did not contain nitrogen or showed only small amounts of this element (up to 1%). Based on sugar analysis we assume that nitrogen is originated from proteins in fractions *L1* and *L2* (no D-glucosamine) and from chitin in fractions *S* (1.8–3.7% of D-glucosamine). Significant amounts of nitrogen (1.80–2.71% for *P. ostreatus* and 6.26% for *P. eryngii*) were observed in *L1* fractions even after the treatment with Sevag reagent. Thus these fractions were contaminated with proteins removed from fruit bodies by first boiling water extraction step. These proteins are probably bound to soluble glucans, and the Sevag method was not able to separate these compounds completely. On the other hand, fractions *L2* showed much lower nitrogen contents (0.81–1.76% for *P. ostreatus* and 2.06% for *P. eryngii*). Moreover, marked decrease of protein contents established as an amount of nitrogen was evident for the extracts purified with phenol reagent: fractions *L2b* originated from strains 70 and 77 contained no nitrogen. Therefore, the treatment with phenol led to complete removing of proteins only from *L2* fractions, so alkali soluble glucans are not strongly bound to proteins like water soluble glucans.

3.5. Gel permeation chromatography

The GPC chromatograms of typical water and alkali extracts obtained from stems of *Pleurotus* sp. are shown in Fig. 3. Molecular weight of the main glucan fraction was in the range of 2900–2200 kDa, while smaller molecules (110–10.5 kDa) were also detected. These low molecular fractions are probably proteins and/or partially degraded polysaccharide molecules. Water extract obtained from stems of *P. ostreatus* strain 77 had two peaks indicating fractions of high and low molecular weights of 2200 and 10.5 kDa, respectively. Corresponding alkali extract contained polysaccharides of significantly higher molecular weights, 2900 and 110.3 kDa. High and low molecular fractions were also detected for water (2200 and 12 kDa) and alkali (2300 and 20.3 kDa) ex-

Table 2
Glycosyl linkage compositions of the polysaccharide fractions from *P. ostreatus* and *P. eryngii*.

O-methyl-d-glucose	*t* (min)	Molar ratios			Linkage
		L1	L2	S	
2,3,4,6-tetra	29	1	1	1	D-Glc*p*-(1 →
2,4,6-tri-	35	4	0	4	→ 3)-D-Glc*p*-(1 →
2,3,4-tri-	37	3	0	0.2	→ 6)-D-Glc*p*-(1 →
2,4-di-	40	1	0	0.8	→ 3,6)-D-Glc*p*-(1 →

Table 3
Nitrogen contents (% m/m) in fractions obtained from stems of *P. ostreatus* and *P. eryngii*.

Sample specification		Fraction						
Specie	Strain	*L1*	*L1a*	*L1b*	*L2*	*L2a*	*L2b*	*S*
P. ostreatus	70	1.89	4.70	0	0.43	ND	0	1.44
	77	1.68	3.22	traces	0.62	ND	0	0.98
	L22	2.35			0.71			0.78
	137	1.62			1.55			1.94
P. eryngii	NS[a]	5.43			1.80			1.64

[a] NS, non-specified; ND, non-determined.

tracts obtained from stems of *P. eryngii*. It is evident in most cases that the fractions of alkali extracts showed something higher molecular weights than those of corresponding water extract.

3.6. Infrared and Raman spectroscopy

FT-IR spectra of all fractions obtained from stems of *P. ostreatus* strain 77 are shown in Fig. 4. The spectra of corresponding fractions isolated from stems of the other species/strains of genus *Pleurotus* were similar to those presented in this figure. Spectra of fractions *L1*, *L2* and *S* showed several intense highly overlapped IR bands in the region of 950–1200 cm^{-1} (mainly CC and CO stretching vibrations in pyranoid rings) indicating the presence of polysaccharides as the major component. Partially, the intense band at 1150–1160 cm^{-1} was assigned to COC stretching of glycosidic bonds. The weaker band near 894 cm^{-1} found in the spectra of *L1* and *S* is specific for β-glycosidic bonds and, therefore, indicates the presence of β-glucans. Other bands and shoulders assigned to β-glucans were found near 1376, 1317, 1162, 1100, 1080, 1040 and 990 cm^{-1} (Gutiérrez, Prieto, & Martínez, 1996; Šandula, Kogan, Kačuráková, & Machová, 1999). In contrast, the spectrum of *L2* has several bands at 1367, 930, 853, 822, 548 and

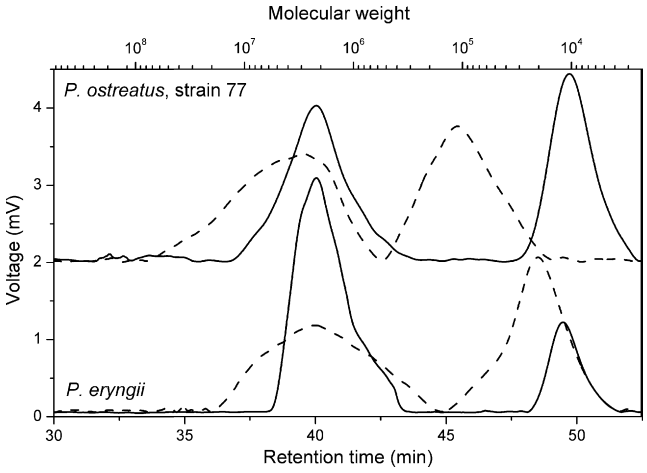

Fig. 3. HP-GPC chromatograms of *L1* (solid) and *L2* (dash) extracts obtained from stems of *P. ostreatus* strain 77 and *P. eryngii*.

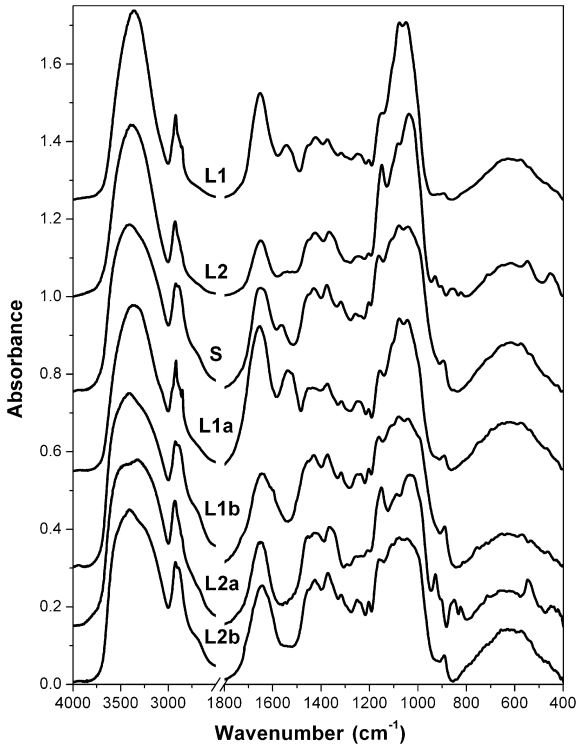

Fig. 4. FT-IR spectra of the fractions obtained from stems of mushrooms *P. ostreatus* strain 77 (*L1* and *L2*, lyophilisates obtained, respectively, from boiling water and alkali extracts; *S*, solid parts; *L1a*, *b* and *L2a*, *b*, phenolic (*a*) and aqueous (*b*) parts after treatment of corresponding fractions with phenolic reagent).

454 cm^{-1} indicating α-1,3-glucan (Chen, Zhou, Zhang, Nakamura, & Norisuye, 1998; Unursaikhan, Xu, Zeng, & Zhang, 2006; Wang, Deng, Li, & Tan, 2007; Zhang, Zhang, & Chen, 1999; Šandula et al., 1999). Two bands at 1650 and 1540 cm^{-1} (*L1* and *L2*) were assigned, respectively, to amide I and amide II vibrations of proteins (Belton et al., 1995). The amide I bands are overlapped by in-plane deformation of water near 1640 cm^{-1} and, therefore, cannot be used itself for identification of amide bonds. The amide II band of *L2* is much less pronounced than the corresponding band of *L1*, so the former fraction contained significantly more proteins

than the latter one. Broad band of *S* at 1649 cm^{-1} (amide I), which is composed from two components at 1667 and 1630 cm^{-1} according to second derivative, together with the band at 1562 cm^{-1} (amide II) indicate the presence of α-chitin (Focher, Naggi, Torri, Cosani, & Terbojevich, 1992). Sharp bands at 2924 and 2856 cm^{-1} were assigned to CH_2 stretching vibrations of lipids. These bands are pronounced for *L1*, so small amount of lipids is extracted with boiling water together with polysaccharides and some proteins.

Precipitates from phenolic layers *L1a* and *L2a* showed strong polysaccharide bands in the region of 1200–950 cm^{-1}. The former fraction also showed intense protein bands at 1654 and 1538 cm^{-1} more pronounced than those of initial *L1*. In contrast, the spectrum of *L1b* showed marked features of β-glucan and no protein bands. Therefore, the treatment with phenol led to remove of proteins together with some amount of polysaccharides. Fraction *L2a* showed the bands of α-1,3-glucan at 930, 850, 822, 542, 448 and 421 cm^{-1}. These bands are more pronounced then those of corresponding bands of *L2*. In contrast, the spectrum of *L2b* is very similar to that of *L1b* and contains no protein or β-glucan markers, so both water layer precipitates contained β-glucans of similar structure. Sample *L1b*, however, have several bands at 1600, 1580 (shoulder) and 812–692 cm^{-1} that could be owing to protein degradation products bound to polysaccharide; more intense CH_2 stretching bands together with smaller features at 1738 cm^{-1} (C=O stretching), 1675 cm^{-1} (CH_2 bending) and 720 cm^{-1} (CH_2 rocking) indicate the presence of lipids. Therefore, only *L2b* fraction can be identified as pure glucan.

FT-Raman spectra of fractions *L1*, *L2* and *S* obtained from stems of *P. ostreatus* strain 77 are shown in Fig. 5. Bands at 1458–1464, 1363–1371, 1258–1267, 1118–1131, 1074–1084 and 1040–1048 cm^{-1} indicate the presence of polysaccharides (Edwards, Russell, Weinstein, & Wynn-Williams, 1995). Bands at 894–894 cm^{-1} (*L1* and *S*) and 865 cm^{-1} (*L2*) are sensitive to anomeric structure around glycosidic bonds. These wavenumbers confirmed, respectively, β- and α-configuration of the major polysaccharide in the corresponding fractions. The band at 423–425 cm^{-1} found in all the fractions is indicative for β-1,3-glucan, whereas intense and sharp bands of *L2* at 950 and 554 cm^{-1} could be assigned to α-1,3-glucan. Weak and broad band at 1667 cm^{-1} (amide I) together with weak features at 1348–1354 cm^{-1} (amide III) indicated the presence of proteins in *L1* and *L2*; bands at 1403–1408 cm^{-1} (*L1*, *L2*) and 1009 cm^{-1} (*L1*) probably arise, respectively, from Glu and Asp carboxylates and from Phe rings in proteins (Li-Chan, 1996; Wong, Phillips, & Ma, 2007; Zhao, Ma, Yuen, & Phillips, 2004). Raman features of *S* at 1657, 1417 and 940 cm^{-1} arise from chitin (Focher et al., 1992).

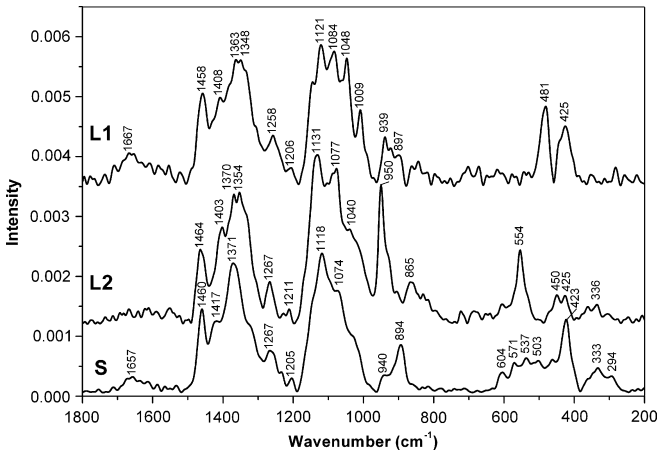

Fig. 5. FT-Raman spectra of the fractions *L1*, *L2* and *S* obtained from stems of mushrooms *P. ostreatus* strain 77.

3.7. ^{13}C CP/MAS NMR

^{13}C CP/MAS NMR spectra of fractions *L1*, *L2* and *S* obtained from stems of *P. ostreatus* strain 77 and *P. eryngii* are shown in Fig. 6. The spectral features of *L1* are quite similar to those of *S*, while *L2* showed evident difference in the position of polysaccharide carbon signals. The β configuration of the D-Glc residues was indicated by the C-1 resonance at 103.3–103.6 ppm; branching at C-6 was confirmed by C-6 signals at 68.9–69.1 ppm (*O*-substituted units) and at 62.0–62.3 ppm (unsubstituted units) (Chenghua et al., 2000; Saitô, Yoshioka, Yokoi, & Yamada, 1990; Yoshioka, Tabeta, Saitô, Uehara, & Fukcoka, 1985; Saitô, Yokoi, & Yoshioka, 1989; Saitô, Onki, & Sasaki, 1979; Stipanovic & Stevens, 1981). The broad multiply C-3 signal at 86.6–85.4 ppm could be ascribed to the presence of 1,3-linked residues (Chenghua et al., 2000). The most intense peak at 74.1–74.4 ppm corresponds to C-2, the shoulder near 76.0 ppm to C-5; the other carbons make a contribution to the whole envelope at 65–80 ppm. Resonance signals at 173.5 ppm (C=O), 51.1 ppm (C-2) and 22.6–23.1 ppm (CH_3) indicate the presence of chitin in fractions *S* (Focher et al., 1992; Heux, Brugnerotto, Desbrières, Versali, & Rinaudo, 2000; Tanner, Chanzy, Vincendon, Roux, & Gaill, 1990). In contrast, the C-1 resonance at 100.9–101.0 ppm indicates α-configuration of the D-Glc residues in *L2*, and the broad signal at 84.5–82.2 ppm is characteristic for α-1,3-linkages (Unursaikhan et al., 2006; Wang et al., 2007; Zhang et al., 1999). Intense and sharp peak near 71.5 ppm and smaller one at 60.5 ppm corresponds, respectively, to C-2 and C-6 carbons of α-1,3-glucan. The spectra of *L1* and *L2*, however, showed several peaks and shoulders corresponding, respectively, to α and β-glucans, so the separation of these polysaccharides by water and alkali extraction was not complete. The intense and broad peak of carbonyl carbons as well as several peaks in the regions of aromatic (120–160 ppm) and aliphatic (10–60 ppm) carbons indicated the presence of protein in *L1* fractions, more pronounced in the case of *P. eryngii* (Belton, Gil, Grant, Alberti, & Tatham, 1998; Belton et al., 1995).

3.8. Prebiotic activity

The difference between the values of analysed parameters (maximum growth rate, maximum biomass concentration and acid production) measured for the medium without and with the extract is compared for the specific strains and extracts (Fig. 7a–d). Positive value shows stimulating effect of extract to related growth characteristic of selected strain, negative value show inhibition effect. Nine probiotic strains showed different growth characteristics in dependence on used extract and strain specificity. In most cases the extracts *L1* and *L2* from *P. ostreatus* and *P. eryngii* support probiotic bacteria growth rate, biomass and SCFA (short chain fatty acid) production for *Lactobacillus* strains. Extracts from *P. eryngii* proved better growth source than those from *P. ostreatus*. *Lactobacillus* strain Lac A grew with the same rate as control as with water extract *L1* of *P. eryngii*, but alkaline extract L2 increased this rate twice. Strains Lac B, C, D utilized both extracts with similar results. *Bifidobacterium* strains showed marked differences. Strain Bifi A grew with *P. ostreatus* extracts better than with those of *P. eryngii*. Strain Bifi B grew only with *P. eryngii* extract, while *P. ostreatus* was not able to stimulate its growth. Strain Bifi C was slightly inhibited with higher concentration of the extracts. *Enterococcus* strains grew slower than *Lactobacillus* strains with all tested extracts; also SCFA production was lower. The physiology of *Enterococcus* strains is in accordance with these results. Growth rate of *Enterococcus* strains (Ent A and B) was stimulated better with the alkaline ex-

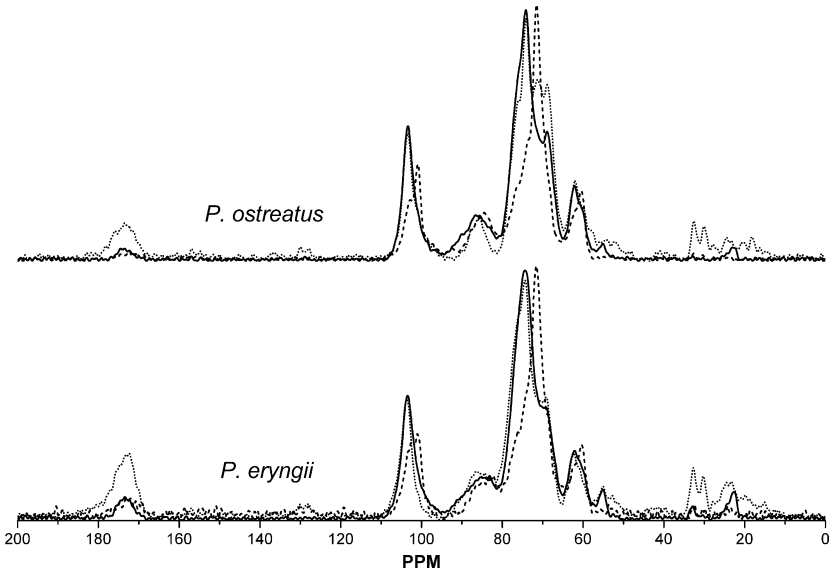

Fig. 6. ^{13}C CP/MAS NMR spectra of the fractions *S* (solid), *L1* (dot) and *L2* (dash) obtained from stems of mushrooms *P. ostreatus* strain 77 (top) and *P. eryngii* (bottom).

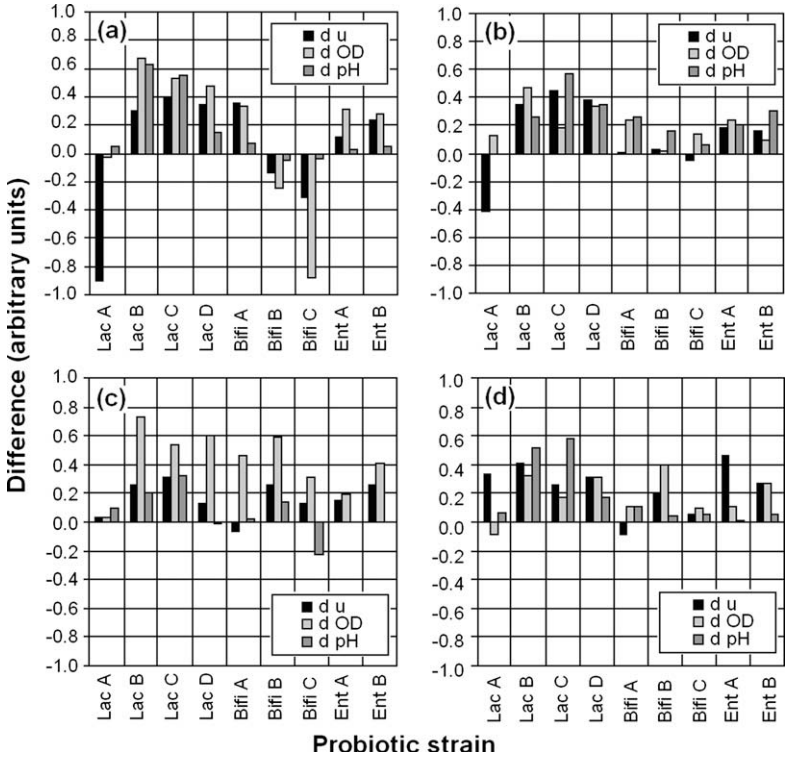

Fig. 7. The plot of difference between the values of maximum growth rate (d u), maximum biomass concentration (d OD) and final acid production (d pH) measured for the medium without and with the water *L1* (a and c) and alkali *L2* (b and d) extracts obtained from stems of mushrooms *P. ostreatus* strain 77 (top) and *P. eryngii* (bottom).

tracts. However, water extracts stimulated biomass concentration to higher values than alkaline extracts. All extracts had a positive effect on both strains.

SCFA production is an important characteristic of probiotic strains (Juśkiewicz et al., 2007). Acid production was established as the difference between the pH of fermented medium control (MRS with water) and the sample (MRS with extract). Naturally, *Lactobacillus* strains show the highest production of SCFA including lactic acid as the major product of their carbohydrate metabolism. In this work the highest SCFA production was registered for strains Lac B and Lac C, while strain Bifi B produced the lowest amount of SCFA. Moreover, in case of the latter strain the SCFA production decreased with increased concentration of the extract (data not shown). The other strains produced comparable amount of SCFA.

4. Conclusions

Fruit bodies of *P. ostreatus* and *P. eryngii* contain branched β-1,3-1,6-glucan and linear α-1,3-glucan as the major components of cell walls. Subsequent water and alkali extraction led to evident separation of these polysaccharides: both *L1* and *S* fractions consist mainly of β-1,3-1,6-glucan, while *L2* contained α-1,3-glucan as

the major polysaccharide component. The fractions *L1* and *L2* (extracts) were purified with the phenolic reagent. As a result, a protein–β-1,3-1,6-glucan complex (*L1a*) and α-1,3-glucan (*L2b*) were separated from pure β-1,3-1,6-glucan (respectively L1b and *L2b*). Other polysaccharides (α-1,4-glucans, chitin and galactomannans) are present in small amount in some cases; chitin only as a minor component of chitin–glucan complexes in *S*.

Probiotics utilization *L1* and *L2* extracts from *P. ostreatus* and *P. eryngii* by different manner affirm different chemical structure of the polysaccharides. Obtained results support the conclusion that least two types of glucans and proteoglucan complexes from *Pleurotus* can be used for synbiotic construction with selected probiotic strains. Such synbiotic construction may be successful with selected *Lactobacillus* strains for both types of the extracts. *Bifidobacterium* strains may be combined especially with extracts from *P. eryngii*. All combinations of probiotics and prebiotics must be verified *in vivo* before the final confirmation synbiotic character. This new exploitation of fruit body extracts extends the understanding of mushrooms value *P. ostreatus* and *P. eryngii* for human health.

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