Effect of Salt Stress on the Production and Properties of Extracellular Polysaccharides Produced by *Cryptococcus laurentii*

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The composition, main structural features and molecular properties of exopolysaccharides (EP) produced by *Cryptococcus laurentii* var. *laurentii* CCY 17-3-16 under optimal (EPo) and NaCl-stress conditions (EPs) as well as their subfractions isolated by gel chromatography were studied using chemical, FT-IR and NMR spectroscopy methods. The results showed that under stress conditions the yeast produced EP with a lower content of protein and phosphorus. In comparison to EPo, the EPs exhibited a substantially larger proportion of high molecular mass populations. NMR analysis of EPs revealed a higher degree of branching with single xylose side chains of the heteromannan components. The increase of the molecular mass and degree of branching of the macromolecular chains of the heteromannan components might in part be related to the function of EPs to protect the yeast cells from water loss and maintain growth conditions under the salt stress.

Key words: Cryptococcus laurentii, Salt Stress, Exopolysaccharides

Introduction

Different types of environmental and physiological stress conditions constantly challenge all living organisms. To cope with the deleterious effects of stress, cells have developed rapid molecular responses to repair the damage and protect against further exposure to the same and other forms of stress. The exopolysaccharides (EP) produced by species of *Cryptococcus laurentii* are of interest because of their potential industrial use (Cadmus *et al*., 1962) and immunochemical characteristics (Helms *et al*., 1969; Fraser *et al*., 1973). In our laboratory, they found application as a cryoprotective substance (Breierová *et al*., 1996).

The outstanding characteristic of the *Cryptococcus* genus is the existence of capsules representing slimy extramural layers, which may serve in protecting cells from physical and biological stresses encountered in their natural habitats. They are suggested to prevent water loss from cells and enhance acquisition of trace levels of nutrients in oligotrophic environments (Golubev *et al*., 1971). From the culture medium of *C. laurentii* exocellular protein-rich polysaccharides have been isolated and characterized (Abercombie *et al*., 1960; Cohen and Feingold, 1967; Ankel *et al*., 1970; Ross and Taylor, 1981). They contain mannose, galactose, xylose, glucose, arabinose, and glucuronic acid. However, the proportion of monosaccharide constituents in these polymers may vary. Until now, one acidic polysaccharide, the glucuronoxylomannan (GXM) (Perry and Webb, 1982; Bartek *et al*., 2001), and two neutral polysaccharides, the galactoglucoxylomannan (GalGXM) (Bartek *et al.,* 2001; Matulová *et al.,* 2002) and glucomannan-protein (GM) (Kolarová *et al.,* 1997), produced by various *C. laurentii* strains have been isolated from the culture medium and structurally characterized in detail. Recently, Tikhomirová *et al*. (1998) confirmed the compositional similarity between capsular glucuronoxylomannans – acidic heteropolysaccharides of the soluble cellular fraction and crylan – an exocellular heteroglycan produced by *C. laurentii.* At very low pH value, starch-like polymers, identified as amylose, have been detected and isolated from the culture medium of *C. laurentii* as well (Abercombie *et al.,* 1960; Foda *et al.,* 1973).

Growth conditions are known to affect the production and composition of the exocellular and capsular material (Foda *et al*., 1973; Ross and Taylor, 1981). For the growth and metabolism of yeasts, water is an essential component. After its reduction in the extracellular environment, for instance by increasing the concentration of NaCl, the water activity decreases resulting in a lower

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culture production. The yeast cells as a response to the hyper-osmotic pressure create an adaptation mechanism which involves the production of extracellular glycoproteins, changes in their amino acid and sugar composition and hydration behaviour (Breierová *et al.,* 1997).

The aim of the present work was to compare the extracellular polysaccharides produced by *C. laurentii* CCY 17-3-16 under optimum and salt-stressed cultivation conditions and to study possible changes in composition, structure and properties of their carbohydrate components.

Materials and Methods

Microorganism

The strain *Cryptococcus laurentii* CCY 17-3-16, isolated from soil (Sláviková and Vadkertiová, 2000), has been stored at the Culture Collection of Yeasts (CCY, Institute of Chemistry, Bratislava, Slovakia) at 4 °C on slant agar. All chemicals used were of analytical grade. The wall mannan isolated from *Candida utilis* CCY 29-38-18 for the FT-IR measurement was kindly supplied by Dr. G. Kogan from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava.

Cultivation conditions and separation of exopolysaccharides (EP)

The *C. laurentii* strain was grown at laboratory temperature in 500 ml flasks with 250 ml cultivation medium on the orbital shaker (80 cycles min^{-1}). The basal-optimal medium used for cultivation contained ($g\,l^{-1}$): yeast extract (4), $(NH_4)_2SO_4$ (10), glucose (20), KH_2PO_4 (1), $K_2HPO_4 \cdot 3H_2O$ (0.2), NaCl (0.1), $CaCl_2$ (0.1), $MgSO_4 \cdot 7H_2O$ (0.5), and 1 ml microelement solution ($mg\,l^{-1}$): H_3BO_4 (1.25), $CuSO_4 \cdot 5H_2O$ (0.1), KI (0.25), $MnSO_4 \cdot 5H_2O$ (1), $FeCl_3 \cdot 6H_2O$ (0.5), $(NH_4)_2Mo_7O_{24} \cdot 4H_2O$ (0.5), and $ZnSO_4 \cdot 7H_2O$ (1). The stress conditions were realized by addition of 10% (w/v) NaCl into the basal-optimal medium. When the culture reached the late exponential phase, the cells were separated by centrifugation. The EP was isolated by precipitation from the supernatant with two volumes of 96% (v/v) ethanol, and subsequent centrifugation. The precipitate was dispersed in distilled water and freeze-dried. The crude EP was used for analysis.

Fractionation methods

Fractionation of the EP produced by *C. laurentii* at optimum (EPo) and stress conditions (EPs) was performed on a FPLC instrument (Pharmacia, Sweden) using gel permeation chromatography on a Superose 12™ (10 × 300 mm) column in 0.05 M phosphate buffer (pH 7.0) containing 0.15 M NaCl. The flow rate was 0.5 ml min^{-1}. The column was calibrated with standards: ribonuclease (13.7 kDa), chymotrypsinogen A (25 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), aldolase (158 kDa), catalase (232 kDa), ferritin (440 kDa), thyroglobulin (669 kDa), and blue dextran (2000 kDa). EPs was separated into two subfractions by pooling test tubes No. 15–17 (EPs-I) and No. 18–24 (EPs-II). EPo was separated into three subfractions by pooling test tubes No. 15–17 (EPo-I), No. 18–24 (EPo-II), and No. 25–28 (EPo-III). The individual fractions were isolated by repeated chromatography on the same column.

Analytical methods

Protein was calculated from the nitrogen content (N% × 6.25) assayed using an elemental analyzer (Perkin-Elmer, Model 240) and total phosphorus was assayed according to Breierová *et al.* (1996). The carbohydrate content was determined by the phenol-sulfuric acid assay (Dubois *et al.,* 1956) using glucose as standard. After hydrolysis with 2 M trifluoroacetic acid under reflux for 2 h the constituent monosaccharides were qualitatively determined by paper chromatography, and the neutral saccharides in form of their alditol trifluoroacetates by gas chromatography as previously described (Ebringerová *et al.,* 1997). Amylose was detected by the KI/I_2 test.

Fourier-transform infrared (FT-IR) spectra were obtained on a NICOLET Magna 750 spectrometer with a DTGS detector and OMNIC 3.2 software using 128 scans at a resolution of 4 cm^{-1}. The samples (2 mg) were pressed into pellets of KBr (200 mg). ^1H, ^{13}C and ^{31}P NMR spectra were recorded at 25 °C on a Bruker DPX AVANCE-300 spectrometer operating at 300 MHz for ^1H and 75.46 MHz for ^{13}C NMR. The samples (80 mg ml^{-1}) were dissolved in D_2O.

Results and Discussion

The exopolysaccharides of the tested *C. laurentii* produced at different conditions as EPo (optimal) and EPs (stress) (10% NaCl) were isolated by

ethanol precipitation from the cultivation medium. Their main analytical characteristics are summarized in Table I. The both preparations contained protein and phosphorus, but in lower proportions in the case of EPs. The most striking are the differences in the neutral sugar composition of EPo and EPs. As seen, the neutral sugar constituents of EPo comprised mainly mannose, xylose and galactose, present in the molar ratio 10:3.4:1.3, next to glucose and arabinose. A similar Xyl/Man ratio (~ 3:10) has been reported for GGX produced by *C. laurentii* NRRL Y-1401 (Perry and Webb, 1982). In EPs the relative proportions of mannose, xylose, and galactose changed to 10:6.1:1.1 indicating a substantial increase of the xylose constituent. Paper chromatography showed the presence of glucuronic acid in both polysaccharides. The positive KI/I_2 test indicated that amylose was trace, in a higher proportion in EPs. It is known that *Cryptococcus* species produce polysaccharides of the amylase type under certain growth conditions (Abercombie *et al.,* 1960; Foda *et al*., 1973).

The both EPo and EPs were fractionated by FPLC on a Superose column and the elution was monitored by the phenol-sulphuric acid assay. The chromatograms, illustrated in Fig. 1, indicated the presence of three molecular populations in EPo, *e.g.* I (~ 1000 kDa), II (400–60 kDa), and III (60–25 kDa) present in the ratios 10:57:33 (calculated from the peak areas). In EPs the higher molecular mass populations (I and II) prevailed and were present in the ratio 26:70, whereas, the proportion of III was very small (4%). By repeated chromatography on the same column, the corresponding fractions EPo-I–III and EPs-I–II were separated.

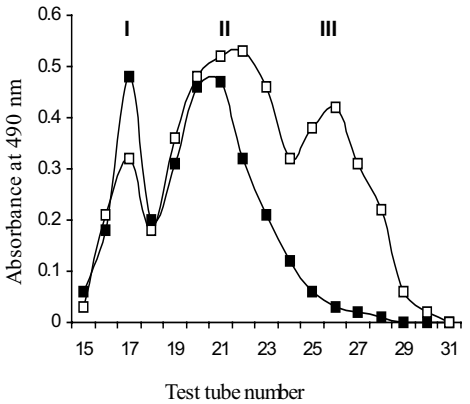

Fig. 1. Fractionation of EPo (–■–) and EPs (–□–) on Superose 12 columns.

Evidently, under stress conditions the yeast produced essentially EP with higher molecular mass.

The neutral sugar composition of EPs, EPo and the main subfractions EPs-I, EPs-II and EPo-II were compared (Table I). As seen, the increased Xyl/Man ratio of 0.62 of the whole EPs resulted from heteromannans of the highest molecular mass subfraction EPs-I, which exhibits a very high Xyl/Man ratio (1.33), and from a lower-branched mannan (Xyl/Man = 0.45) in subfraction EPs-II. In contrast, the Xyl/Man ratio of the main subfraction EPo-II (~ 0.3) resembles that of the whole EPo. The arabinose is present in a relatively high amount in EPs-I (Ara/Man = 0.66). Arabinose was reported only by Schutzbach and Ankel (1969) to be the sugar component of neutral cell wall heteropolysaccharides isolated from the cell walls of

Table I. Analytical characteristics of the crude extracellular polysaccharides produced by *C. laurentii* CCY 17-3-16 under optimal (EPo) and stress (EPs) growth conditions and their main subfractions.

Sample[a]	%[b]	Protein (%)	P (%)	Neutral sugars (mol-%)					Xyl/Man (mol ratio)	OAc
				Ara	Xyl	Man	Glc	Gal		
EPo	100	33.7	7.0	1.6	20.6	60.2	7.8	9.9	0.34	*
EPo-II	57	9.3	3.2	3.5	19.8	65.9	4.8	6.0	0.30	*
EPs	100	13.8	4.8	5.5	27.2	44.3	6.6	15.6	0.62	*
EPs-I	26	nd	nd	10.4	41.1	30.8	6.9	10.7	1.33	+
EPs-II	70	nd	nd	8.6	21.4	47.4	11.4	11.2	0.45	+, *

[a] All samples contained glucuronic acid detected by paper chromatography.
[b] Calculated from the peak area of the gel chromatogram (Fig. 2).
* Acetyl groups were detected by absorption bands at ~ 1730 and 1245 cm^{-1}.
\+ Acetyl groups were detected by chemical shifts at δ 169.9–171.2 (C = O) and cross peaks at δ 1.99–2.17/21.0–2.19 (CH_3).
nd, not determined.

C. laurentii. However, they were not further investigated and probably lost during the applied isolation and purification procedures. The results confirmed that the salt-stress favored the production of heteromannans with a substantially higher amount of xylose residues.

The FT-IR spectra of EPo and EPs and their main subfractions are shown in Fig. 2. The bands at ~ 1650 cm^{-1} (amide I) and ~ 1535 cm^{-1} (amide II) confirmed the presence of protein. The bands at ~ 916 cm^{-1} and 804 cm^{-1} dominating in the anomeric region are attributed to mannose, and the small bands at 874 and 845 cm^{-1} to galactose and α-linked glucose (Mathlouti and Koenig, 1986). The band at 898 cm^{-1} indicates β-linkages of the xylose and glucuronic acid side chains present in GXM polymers (Perry and Webb, 1982; Bartek *et al.,* 2001). The uronic acid shows the carbonyl band at 1730–1720 cm^{-1}, however, it cannot be distinguished from *O*-acetyl groups reported to occur in the EP from *C. laurentii* NRRL Y-1401 (Perry and Webb, 1982; Ross and Taylor, 1981), but not in EP from *C. laurentii* CCY 17-3-5 (Bartek *et al.,* 2001; Kolarová *et al.*, 1997; Matulová *et al.,* 2002).

The mid-infrared region at 1200–800 cm^{-1} has been reported (Stuart, 1997; Kačuráková *et al.*, 1999, 2000) to contain main band maxima which are typical of various structural types in the case of plant polysaccharides and might be, therefore, useful also in characterization of microbial polysaccharides. Table II documents the studied EP samples and the cellular mannan (CM) from *C. utilis* (Kogan *et al.*, 1994). The heteromannans, which were isolated from the *C. laurentii* species (Bartek *et al.,* 2001; Kolarová *et al.*, 1997; Matulová *et al.,* 2002), have the mannose units involved in α-1,3-, α-1,6-, and α-1,2-glycosidic linkages but in different proportions and locations (in the backbone and/or in side chains). The glycosidic ν(C–O–C) stretching vibration was found as distinct bands at 1126–1136 cm^{-1} in CM, EPs and EPo-II, whereas, non-resolved as broad shoulders in the other samples. Also the ring and side group vibrations (C–C), (C–OH), and (C–H) of the samples were seen at 1107–976 cm^{-1} as several distinct band maxima or shoulders. It should be remarked that the main band position is strongly affected by vibrations of the different glycosyl side chains present in plant polysaccharides (Kačuráková *et al.*, 2000). Such effect could be expected also with the various heteromannan components of EP due to the considerable diversity of their side chains. Therefore, it was not possible to detect and distinguish the three known heteromannan types in EPo, EPs and their fractions.

As many NMR data of the carbohydrate components of EP isolated from *C. laurentii* have been published (Perry and Webb, 1982; Bartek *et al.*, 2001; Kolarová *et al.*,1997; Matulová *et al.*, 2002), they were used for assignment of NMR spectra of the fractions EPs-I and EPs-II (Table III). The ^{13}C NMR spectra of these fractions (Fig. 3) together

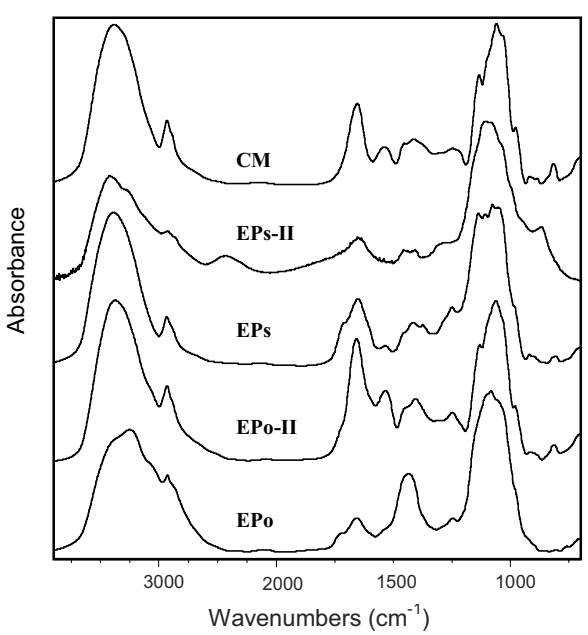

Fig. 2. FT-IR spectra (in KBr) of EPo, EPs, their main subfractions EPo-II and EPs-II, and the cellular mannan (CM) from *C. utilis*.

Table II. Characteristic frequencies (cm^{-1}) of EPo, EPs and their main subfractions, and the cellular mannan (CM) isolated from *C. utilis*[a].

Sample	ν(C–O–C)	Ring and side group vibrations (C–C), (C–OH), (C–H)			
EPo	1156sh; 1130sh	1100vs	1080vs	1047vs,b	985sh
EPo-II	1126s	1103sh	1051vs	1045sh	980m
EPs	1155sh; 1136vs	1107vs	1076vs	1049vs	995sh
EPs-II	1156sh	1103vs	1080vs	1033sh	995sh
CM	1132s	1102sh	1057vs	1028vs	976m

[a] Kogan *et al.*, 1994.
Abbreviations: vs, very strong; s, strong; m, medium; sh, shoulder.

Table III. ^{13}C and ^{1}H NMR chemical shifts (δ) for the subfractions EPs-I and EPs-II.

Chemical shifts (ppm)			Assignments
106.2			C1: *t*-βGal→
104.4–103.8	*A*	4.46/104.5	C1: *t*-βXyl→
	B	4.55/103.8	C1: *t*-βGlcA→
103.1–102.9	*C*	5.16/103.1	C1: *t*-αMan(1→3, →3-αMan(1→
	D	5.06/103.0	C1: *t*-αMan(1→2
101.5	*E*	5.29/101.5	C1: →2-αMan(1→
	F	5.32/101.3	C1: →2-αMan(1→
100.6	*G*	4.91/100.5	C1: →6-αMan(1→
	H	5.40/100.9	C1: →4-αGlc(1→
99.2	*I*	5.11–5.03/99.2–99.1	C1: →2,6-αMan(1→
	J	4.97/89.8	C1: *t*-αGlc→; →6-αGal(1
79.4		79.3	C2: *C*, *D*, *F*; C3: →3-αMan(1→
76.6		76.5	C3: *I*, *J*; C5: *J*
74.7–74.3		74.2	C5: *A*, *B*, *E*
73.8		73.7	C2: *I*
72.1–71.5		72.0–71.4	C2: →3-αMan(1→; C3: *F*, *A*, *t*-αMan(1→3
70.7, 70.3		70.6, 70.1	C4: *I*
69.4		69.3	C2: *t*-αGal(1→; C4: *t*-βGal(1→
68.2–67.4		68–67.2	C4: *A*, *B*, *F*
67.2–66.4		66.9–66.1	C5: *I*
62.0		61.9	C6: *A*, *B*, *C*
61.3		61.2	C6: *t*-Gal(1→

^{13}C NMR chemical shifts were from the spectra of both EPs-I and EPs-II (in D_2O).
$^{1}H/^{13}C$ cross-peaks labelled as *A*–*J* were derived from the $^{1}H/^{13}C$ NMR (HSQC) spectrum of EPs-II.

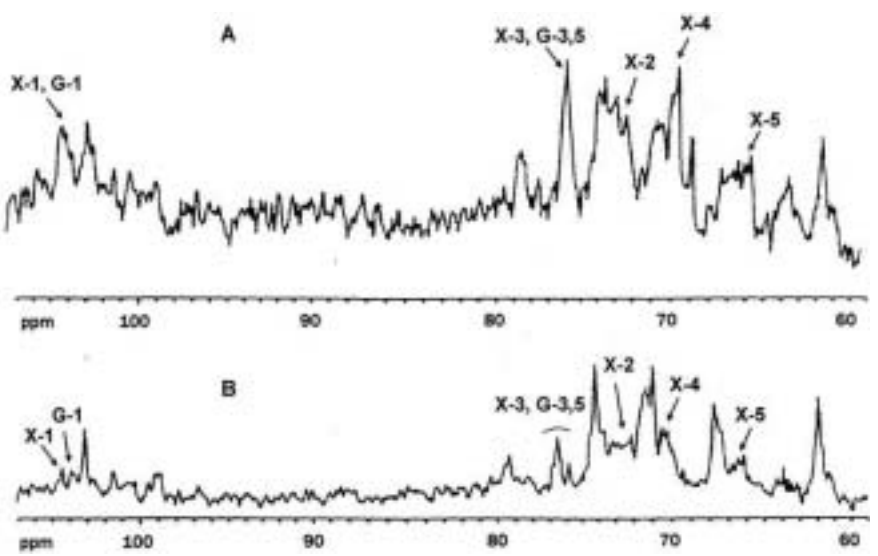

Fig. 3. ^{13}C-NMR spectra in D_2O of subfractions (A) EPs-I and (B) EPs-II. The arrows indicate resonances of the terminal β-D-xylopyranose (X) and β-D-glucuronic acid (G) units.

with the anomeric regions of their ^{1}H NMR spectra (not shown) contain a large number of anomeric bands as well as differences in signal intensities what reflects differences in the proportions of the polysaccharide components and/or changes of the degree of branching of the heteromannan chains. Due to the highly viscous character, particularly of the first fraction (EPs-I) with a molecular mass in the range of ~ 1000 kDa, the spectrum was not well resolved. Small but distinct signals (not shown) at δ 169.9–171.2 (C=O) and δ 21.0–2.19/1.99–2.17 (CH_3) confirmed the presence of acetyl groups in both fractions indicated by FT-IR analysis as well. The signal at δ 178.8 in

EPs-II belongs to C6 of glucuronic acid residues and indicates the presence of the GXM-type. The main anomeric signals were tentatively assigned from the 2D-heterocorrelated (HSQC) spectrum of EPs-II (not shown), which only was measurable, using chemical shift data of other related fungal polysaccharides (Kogan *et al.*, 1994; Kobayashi *et al.*, 1995; Ahrazem *et al.,* 2002) in addition to those reported for EP from *C. laurentii*. The ^{13}C NMR chemical shift (Table III) at $\delta \sim 103.1$ gave two H1/C1 cross peaks, *e.g.* a large (*D*) at δ 5.06/103.0 and a weaker one (*C*) at δ 5.16/103.1 assigned to terminal α-D-mannose residues 2- and 3-linked to other mannose residues. They were seen in the reported spectra of both neutral heteromannans (Bartek *et al.*, 2001; Kolarová *et al.*, 1997; Matulová *et al.*, 2002). However, the last (*C*) can be attributed also to internal 3-linked α-D-mannose constituting the backbone of GXM (Perry and Webb, 1982). The cross peaks at δ 5.29/101.5 (*E*) and 5.32/101.3 (*F*) correspond to internal 2-linked α-D-mannose residues of the side chains of GM and GalGXM and reflect their different chemical environments. The multiple cross peaks at δ 5.11–5.03/99.2–99.1 (*I*) comprise signals of 2,6-linked α-D-mannose units of the backbone as well as of 6- and 3,6-linked mannose units (Kobayashi *et al.*, 1995; Matulová *et al.*, 2002). The presence of non-substituted regions of the 1,6-α-D-mannan backbone is indicated by the cross peak at 4.91/100.5 (*G*) corresponding to 6-linked mannose (Kogan *et al.*, 1994; Ahrazem *et al.*, 2002; Matulová *et al.*, 2002). The cross peak at δ 4.97/89.8 (*J*) can be assigned to terminal α-D-glucose units of GM (Kolarová *et al.*, 1997) as well as to 6-linked α-galactose units found in GalGXM (Matulová *et al.*, 2002). The weak cross peak at δ 5.40/100.9 (*H*) indicates the presence of amylose (McIntyre and Vogel, 1990) detected by the KI/KI_2 test. However, it is difficult to distinguish between the 1,3- and 1,6-α-D-mannans and thus between GXM and both neutral heteromannans GalGXM and GM. Moreover, the indicative chemical shifts of C3 of internal non-branched (in GXM) and 3,6-linked mannose units (in GalGXM), and C2 of 2,6-linked mannose units (in GM) were located all at $\sim \delta$ 79–80.

The cross peaks at δ 4.46/104.5 (*A*) and δ 4.55/103.8 (*B*) in the HSQC spectrum of EPs-II can be assigned according to published data (Bock *et al.*, 1984; Capek *et al.*, 1997) to β-xylose and β-glucuronic acid units, respectively. The broadening and splitting of their anomeric carbon signals (Fig. 3A and B) is due to overlapping and different chemical environments as well. The multiple signals at $\sim \delta$ 104.5, 73.2, 76.5, 70.5, and 66.2 correspond to resonances of C1–C5 of terminal xylose residues in various environments (Kardošová *et al.*, 1998), and dominated in the ^{13}C NMR spectrum of GXM as well (Bartek *et al.*, 2001). Evidently, all mentioned signals showed significant broadening and enlargement in the case of EPs-I (Fig. 3A). The results confirmed the higher proportion of xylose in EPs-I than in EPs-II and indicate that this sugar occurs as single side chains linked to the mannan backbone and/or terminating oligosaccharide side chains of heteromannans produced under stress conditions.

The absence of signals in the region –1.0 to 1.5 ppm in the ^{1}H decoupled ^{31}P NMR spectra of fractions EPs-I and EPs-II (not shown) indicated that the present phosphorus is not linked to the glycan components.

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Abercombie M.-J., Jones J.-K.-N., Lock M.-V., Perry M.-B., and Stoodley R.-J. (1960), The polysaccharides of *Cryptococcus laurentii* NRRL Y-1401. Can. J. Chem. **38**, 1617–1624.

Ahrazem O., Prieto A., Leal J.-A., Jiménez-Barbaero J., and Bernabé M. (2002), Fungal cell-wall mannan isolated from *Geotrichum* spp. and their telemorphs, *Dipodascu*s and *Galactomyxes*. Carbohydr. Res. **337**, 2347–2351.

Ankel H., Ankel E., Schutzbach J.-S., and Garancis J. C. (1970), Mannosyl transfer in *Cryptococcus laurentii*. J. Biol. Chem. **245**, 3945–3955.

Bartek P., Kolarová N., and Capek P. (2001), Isolation and characterization of glycoproteins from the yeast *Cryptococcus laurentii* var. *laurentii*. II. Extracellular glycoproteins. Chem. Papers **55**, 261–268.

Bock K., Pedersen Ch., and Pedersen H. (1984), Carbon-13 nuclear magnetic resonance data for oligosaccharides. Adv. Carbohydr. Chem. Biochem. **125**, 193–225.

Breierová E., Stratilová E., and Šajbidor J. (1996), Production of extracellular polymers by yeast-like genera *Dipodascus* and *Dipodascopsis* under NaCl stress. Folia Microbiol. **41**, 257–263.

Breierová E., Kačuráková M., and Stratilová E. (1997), The effect of higher concentration of NaCl in the cultivation medium on changes in the composition of extracellular yeast glycoproteins. System. Appl. Microbiol. **20**, 348–355.

Cadmus M.-C., Lagoda A.-A., and Anderson R.-F. (1962), The production of a new polysaccharide with *Cryptococcus laurentii* var. *flavescens*. Appl. Microbiol. **10**, 153–156.

Capek P., Matulová M., and Kardošová A. (1997), An acidic heteropolysaccharide from the flowers of *Malva mauritiana* L. J. Carbohydr. Chem. **16**, 1373–1391.

Cohen H. and Feingold D.-S. (1967), Uridine diphosphate D-xylose acceptor xylosyltransferase of *Cryptococcus laurentii*. Biochemistry **6**, 2933–2939.

Dubois M., Gilles K.-A., Hamilton J.-K., Rebers P.-A., and Smith F. (1956), Colorimetric method for determination of sugars. Anal. Chem. **28**, 350–356.

Ebringerová A., Hromádková Z., Machová E., Naran R., and Hříbalová V. (1997), Isolation and characterization of mitogenic pectic polysaccharides from *Cistanche deserticola* Y.C.Ma. Chem. Papers **51**, 289–294.

Foda M.-S.-A., Badr-Eldin S.-M., and Phaff H.-J. (1973), Biochemical investigation on the capsule-amylose relationship in *Cryptococcus laurentii*. Mycologia **65**, 365–372.

Fraser C. G., Jennings H. J., and Moyna P. (1973), Structural features inhibiting the cross-reaction of the acidic polysaccharide from *Tremella mesenterica* with type II anti-pneumococcal serum. Can. J. Biochem. **51**, 225–230.

Golubev V.-I., Sviridov A.-F., and Bab'eva I.-P. (1971), Monosaccharide composition of extracellular and capsular polysaccharides of yeasts. Mikrobiologia **40**, 610–614.

Helms C.-M., Allen P.-Z., and Feingold D.-S. (1969), Comparative immunochemical behavior of acid polysaccharides of *Cryptococcus laurentii, Tremella mesenterica* and of oxidized cellulose with a type II antipneumococcal serum. Immunochem. **6**, 269–277.

Kačuráková M., Wellner N., Ebringerová A., Hromádková Z., Wilson R. H., and Belton P. S. (1999), Characterization of xylan-type polysaccharides and associated cell wall components by FT-IR and FT-Raman spectroscopies. Food Hydrocoll. **13**, 35–41.

Kačuráková M., Capek P., Sasinková V., Wellner N., and Ebringerová A. (2000), FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohydr. Polym. **43**, 195–203.

Kardošová A., Matulová M., and Malovíková A. (1998), (4-*O*-Methyl-γ-D-glucurono)-D-xylan from *Rudbeckia fulgida* var. *sullivantii* (Boynton et Beadle). Carbohydr. Res. **308**, 99–106.

Kobayashi H., Watanabe M., Komiko M., Matsuda K., Ikeda-Hasake T., Suzuki M., Shibata N., Hisamichi K., and Suzuki S. (1995), Assignments of ^{1}H and ^{13}C NMR chemical shifts of a D-mannan composed of α-(1→2), α-(1→6) linkages obtained from *Candida kefyr* IFO 0586 strain. Carbohydr. Res. **267**, 299–306.

Kogan G., Šandula J., and Šimkovicová V. (1994), Glucomannan from *Candida utilis* – structural investigation. Folia Microbiol. **38**, 219–224.

Kolarová N., Matulová M., and Capek P. (1997), Structure of glucomannan-protein from the yeast *Cryptococcus laurentii*. J. Carbohydr. Chem. **16**, 609–623.

Mathlouti M. and Koenig J.-L. (1986), Vibrational spectra of carbohydrates. Adv. Carbohydr. Chem. Biochem. **44**, 7–89.

Matulová M., Kolarová N., and Capek, P. (2002), An extracellular galactoxylomannan-protein from the yeast *Cryptococcus laurentii* var. *laurentii*. J. Carbohydr. Chem. **21**, 521–537.

McIntyre D.-D. and Vogel H.-J. (1990), Two-dimensional nuclear resonance studies of starch products. Starch/Stärke **42**, 287–293.

Perry M.-B. and Webb A.-C. (1982), Structure of the capsular polysaccharide of *Cryptococcus laurentii* NRRL Y-1401. Can. J. Biochem. **60**, 124–130.

Ross A. and Taylor I.-E.-P. (1981), Extracellular glycoprotein from virulent and avirulent *Cryptococcus* species. Infect. Immun. **31**, 911–918.

Schutzbach J.-S. and Ankel H. (1969), Biosynthesis of oligosaccharide components of *Cryptococcus laurentii* cell wall. FEBS Lett. **5**, 145–148.

Sláviková E. and Vadkertiová R. (2000), The occurrence of yeasts in forest soils. J. Basic. Microbiol. **40**, 207–212.

Stuart B. (1997), In: Biological Applications of Infrared Spectroscopy (Ando D. J., ed.). John Wiley & Sons, New York.

Tikhomirová O.-M., Vitovskaya G.-A., and Sinitskaya I.-A. (1998), Cellular polysaccharides of *Cryptococcus laurentii* (Kufferath) Skinner, a producer of extracellular heteroglycan. Mikrobiologia **67**, 73–78.