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Mannan and phosphomannan from *Kuraishia capsulata* yeast

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ABSTRACT

Linear mannan and branched phosphomannan were identified as exopolysaccharides produced by *Kuraishia capsulata* yeast. Their structures were determined using nuclear magnetic resonance spectroscopy. The repeating unit of mannan was found to be a trisaccharide →6)-α-Man*p*-(1 → 2)-α-Man*p*-(1 → 2)-α-Man*p*-(1→, while the phosphomannan was shown to be built of β-Man*p*-(1 → 2)-α-Man*p*-(1 disaccharide blocks linked by phosphodiester bonds via C-1 and C-6 of the reducing unit. The production of both polysaccharides was shown to depend on the phosphate concentration in the culture medium. In the absence of phosphate, only mannan was obtained, while an excess of KH_2PO_4 led to the exclusive production of phosphomannan. Chemical depolymerisation of phosphomannan led to the formation of disaccharide β-Man*p*-(1 → 2)-(6-P)-Man*p*, representing the repeating unit of the hydrolysed polysaccharide. The treatment of the disaccharide with alkaline phosphatase resulted in the formation of disaccharide β-Man*p*-(1 → 2)-Man*p*. The latest products can be transformed into glycosyl donors applicable further in the synthesis of oligosaccharides related to *Candida* cell wall polysaccharides.

1. Introduction

Fungal exopolysaccharides (EPSs) are of interest because of their multiple biological activities including antioxidant, immunostimulating, antitumor and antimicrobial ones (Mahapatra & Banerjee, 2013; Osińska-Jaroszuk et al., 2015). The chemical structures of EPSs from different sources vary regarding to monosaccharide composition, types of glycoside linkages, presence or absence of the phosphate components and other characteristics (Mahapatra & Banerjee, 2013; Osińska-Jaroszuk et al., 2015). In particular, EPSs may include mannose, glucose, galactose, xylose, fucose and rhamnose residues as the components of the main chains or branches.

Among fungal polysaccharides, phosphomannans are of special interest due to the presence of the negatively charged phosphate groups in their structures. The extracellular phosphomannan from *Pichia holstii* has been studied in details. It was shown to contain a highly branched phosphomannan core bearing oligosaccharide side chains attached *via* the phosphodiester bonds (Parolis, Parolis, Kenne, Meldal, & Bock, 1998). The phosphomannan core was found to be an effective inhibitor of *in vivo* lymphocyte migration (Weston & Parish, 1991). The oligosaccharide phosphate fraction derived from this polysaccharide was used in the manufacture of the product PI-88, a phosphomannopentaose sulfate possessed of a number of medically significant properties (Ferro, Fewings, Palermo, & Li, 2001; Ferro et al., 2002). Thus, it was reported to reduce tumour growth and metastasis due to heparanase inhibition (Parish, Freeman, Brown, Francis, & Cowden, 1999). Also PI-88 was found to be an effective inhibitor of angiogenesis (Khachigian & Parish 2004).

The production of a phosphomannan by *Kuraishia (Hansenula, Pichia) capsulata* has been observed previously (Slodki, 1963). The major polysaccharide was found to be a linear polymer, containing mannose and phosphate in a molar ratio of 2.5:1 (Slodki, Wickerham, & Cadmus, 1961). Its biosynthesis depends strongly on the phosphate availability (Avigad & Kalina, 1979). The diversity of EPS from this yeast depending on the phosphate concentration in a culture medium was discussed (Slodki, Safranski, Hensley, & Babcock, 1970). The increasing of KH_2PO_4 concentration in a medium led to the accumulation of the phosphate in EPS. In contrast to many yeast species, the cells of *K. capsulata* accumulate not only polyphosphates, but also the extracellular polysaccharide, which contained the major part of inorganic phosphate consumed by the cells (Lichko, Kulakovskaya, & Kulaev, 2013). The biological activities of this phosphomannan have not been studied in details. It was proposed that the major function of this EPS was the phosphorus storage (Lichko et al., 2013). Also the ability of this phosphomannan to reduce *de novo* dental biofilm formation has been reported (Shimotoyodome et al., 2006).

Here we report the study of the phosphomannan and mannan exopolysaccharides produced by *K. capsulata* in different conditions. In

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Table 1
Influence of phosphate concentration in the medium on the production of exopolysaccharides by *K. capsulata*.

Preparation	Phosphate concentration in the medium before incubation, mM	Phosphate concentration in the medium after incubation, mM	Products[a]
1	0	0	**M**
2	14	0.14	**PM** + **M**
3	58	32.6	**PM**

[a] **M** – mannan, **PM** – phosphomannan; their structures are shown on Fig. 1.

addition to the isolation and structural characterisation of the polymers the depolymerisation and de-*O*-phosphorylation of the phosphomannan are described.

2. Experimental procedures

2.1. General methods

The nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance 600 spectrometer for solutions in 99.96% D_2O at 60 °C. 3-(Trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid (TSP) (δ_H 0.0 ppm and δ_C −1.6 ppm) was used as an internal standard for the ^1H and ^{13}C spectra, and 85% H_3PO_4 (δ_P 0.0 ppm) as an external standard for ^{31}P spectra. The two-dimensional (2D) spectra were recorded and treated using the standard methods and packages from Bruker. The ^1H-^1H correlation spectroscopy (COSY) spectra were recorded with a width of 4800 × 4800 Hz, 4 repetition, 512 increments, with presaturation for water signal suppression. The total correlation spectroscopy (TOCSY) spectra were recorded with a width of 4800 × 4800 Hz, 8 repeating, 256 increments. The spin lock time in TOCSY experiments was 100 msec. The rotating frame Overhauser effect spectroscopy (ROESY) spectra were recorded with a width of 4800 × 4800 Hz, 32 repetition, 256 increments, with presaturation for water signal suppression. The mixing time in ROESY experiments was 200 msec. The ^1H-^{13}C heteronuclear single quantum coherence (HSQC) spectra were

Table 2
^1H and ^{13}C NMR data of polysaccharides (**PM**, **M**) and disaccharides (**P-DM**, **DM**) (for structures see Fig. 1).

Residue	C-1 *H-1*	C-2 *H-2*	C-3 *H-3*	C-4 *H-4*	C-5 *H-5*	C-6 *H-6a,6b*
M						
→6)-α-D-Man*p* −(1→ (**A**)	103.5	71.3	72.0	67.4	72.8	66.4
	5.07	*4.09*	*3.86*	*3.88*	*3.82*	*4.08, 3.66*
→2)-α-D-Man*p* −(1 → (**B**)	102.0	79.0	71.3	68.3	74.5	62.3
	5.20	*4.12*	*3.97*	*3.73*	*3.94*	*3.93, 3.77*
→2)-α-D-Man*p* −(1 → (**C**)	99.7	79.0	71.6	68.3	74.0	62.3
	5.08	*4.07*	*3.99*	*3.73*	*3.74*	*3.93, 3.77*
PM						
-(P)-6)-α-D-Man*p*-(1-P- (**D**)	95.3	78.8	70.6	67.7	74.0	65.8
	5.57	*4.24*	*3.98*	*3.88*	*3.94*	*4.20, 4.14*
β-D-Man*p*-(1 → (**E**)	99.9	72.0	74.2	68.2	77.6	62.3
	4.81	*4.11*	*3.70*	*3.62*	*3.45*	*3.93, 3.77*
P-DM(α)						
-(P)-6)-α-D-Man*p*-(1-*O*H (**D**)	93.2	79.0	70.4	68.2	73.2	64.4
	5.28	*4.13*	*3.88*	*3.84*	*3.88*	*4.03, 3.97*
β-D-Man*p*-(1 → (**E**)	99.9	71.9	74.0	68.0	77.5	62.2
	4.77	*4.07*	*3.65*	*3.60*	*3.38*	*3.92, 3.76*
P-DM(β)						
-(P)−6)-β-D-Man*p*-(1-*O*H (**D**[a])	94.9	80.5	73.1	68.1	76.9	64.4
	4.98	*4.17*	*3.65*	3.73	*3.45*	*4.03, 3.97*
β-D-Man*p*-(1 → (**E**[a])	102.1	71.3	74.0	68.0	77.6	62.2
	4.82	*4.20*	*3.65*	*3.60*	*3.38*	*3.92, 3.76*
DM(α)						
α-D-Man*p*-(1-*O*H (**D**)	93.2	79.3	70.9	68.5	73.8	62.0
	5.30	*4.14*	*3.90*	*3.75*	*3.85*	*3.90, 3.80*
β-D-Man*p*-(1 → (**E**)	100.0	72.1	74.1	68.0	77.6	62.2
	4.80	*4.06*	*3.70*	*3.60*	*3.40*	*3.94, 3.76*
DM(β)						
β-D-Man*p*-(1-*O*H (**D**[a])	94.9	80.8	74.1	68.0	77.6	62.2
	5.01	*4.20*	*3.70*	*3.60*	*3.40*	*3.90, 3.80*
β-D-Man*p*-(1 → (**E**[a])	102.2	71.6	73.7	68.4	77.6	62.2
	4.82	*4.20*	*3.70*	*3.60*	*3.40*	*3.94, 3.76*

[a] The signals of β-isomeric disaccharide.

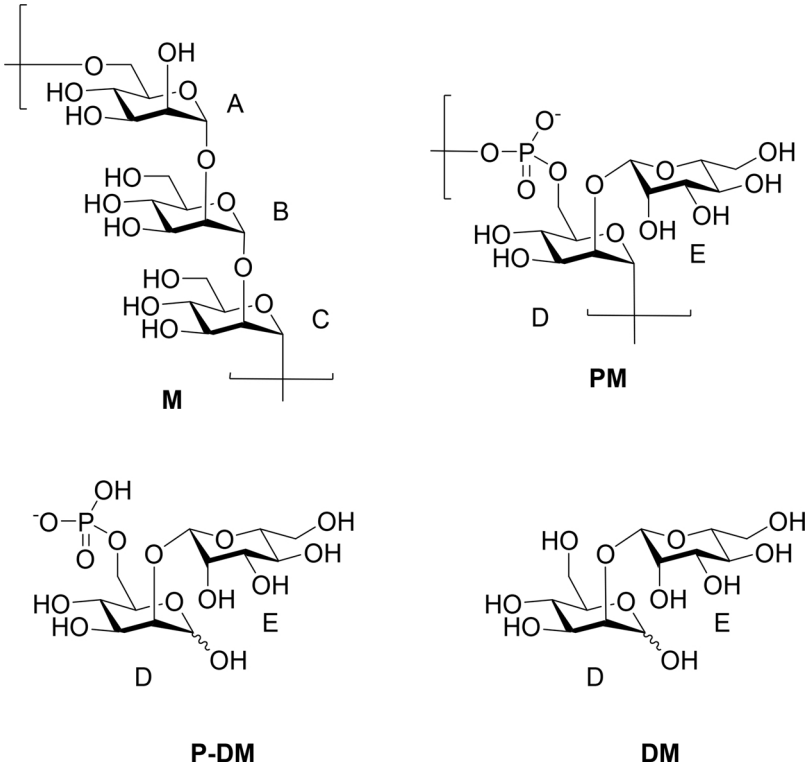

Fig. 1. Structures of the repeating units of mannan **M** and phosphomannan **PM**, and disaccharides **P-DM** and **DM** – the products of **PM** depolymerization and de-O-phosphorylation.