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Short communication

Biosynthesis of hetero-polysaccharides by *Pestalotiopsis microspora* from various monosaccharides as carbon source

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Abstract

Pestalotiopsis microspora was cultured in media containing various monosaccharides as a carbon source. It was found that *P. microspora* metabolizes various monosaccharides and the composition of polysaccharides depends strongly on the monosaccharides used for carbon source. When D-glucose, D-mannose or D-galactose were used as the carbon source, hetero-polysaccharides of similar compositions containing a considerable amount of D-mannose units beside D-glucose units were formed. With D-xylose or *N*-acetyl-D-glucosamine as the carbon source, hetero-polysaccharides containing large amounts of D-mannose and D-galactose units beside D-glucose units were produced. Cultures with L-arabinose as the carbon source produced almost pure glucan, while with L-rhamnose, the yield and the composition of hetero-polysaccharide is the same as that obtained from the culture with D-glucose as the carbon source. The mechanism of biosynthesis of these hetero-polysaccharides is briefly discussed.
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1. Introduction

Biosynthesis of branched β- (1 → 3) glucans by *pestalotiopsis* from media containing ^{13}C-labeled glucoses and their biosynthetic mechanism were reported in our previous paper (Kai et al., 1998). Pathways for cellulose and curdlan have also been reported (Arashida et al., 1993; Kai et al., 1994; Kai et al., 1993). The labeled position in the polysaccharide was determined by ^{13}C-NMR spectroscopy of D-glucitol acetates obtained by hydrolysis of the labeled polysaccharide and then by reduction and acetylation. In the course of these investigations it was noted that in the ^{13}C-NMR spectra some peaks not assignable to D-glucitol acetates were observed. By comparing these peaks with other alditol acetates, they were assigned to D-mannitol acetate. Therefore, in order to elucidate the condition for formation of hetero-polysaccharides, incubation of *pestalotiopsis* in media containing monosaccharides other than D-glucose was attempted.

Ogawa and Tokura (1992) and Shirai et al. (1994) reported that incubation of *Acetobacter xylinum* in *N*-acetyl-D-glucosamine (GlcNAc) as the medium resulted in the formation of hetero-polysaccharides containing 4% of GlcNAc. Formation of polysaccharides differing in their structures depending on the kind of carbon source have not been reported so far, except for the above investigations.

2. Materials and methods

2.1. Culture

Pestalotiopsis microspora (IFO 31056) was used as the microorganism producing branched polysaccharide.

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Table 1
Composition (molar ratio) of hetero-polysaccharides produced by *Pestalotiopsis microspora* from various monosaccharides as the carbon source

Exp. no.	Carbon source	Yield (g/dl)	Method	Glc	Man	Gal
1	D-Glc	0.18	GC	88.9	11.1	0
			NMR	92.2	7.8	0
2	D-Man	0.15	GC	88.1	11.8	0
			NMR	92.4	7.6	
3	D-Gal	0.09	GC	82.2	17.8	0
			NMR	90.7	9.3	0
4	D-Xyl	0.10	GC	58.1	24.9	16.9
			NMR	65.5	22.5	12.0
5	GlcNAc	0.06	GC	51.5	35.2	13.3
			NMR	58.2	34.7	7.1
6	L-Rha	0.08	GC	89.7	10.3	0
			NMR	91.3	8.7	0
7	L-Ara	0.10	GC	99.6	0.4	0
			NMR	100	0	0
8	D-Ara	0.02	GC	88.5	11.5	0
			NMR	92.8	7.2	0

GC: Gas chromatography, NMR: ^{13}C NMR spectroscopy.

The culture was prepared as follows (Kai et al., 1998). An incubated medium (30 ml) containing *P. microspora* was added to a fresh medium (100 ml) containing monosaccharide 3.5 g, peptone 0.05 g, yeast extract 0.05 g, KH_2PO_4 0.1 g and $MgSO_4{\cdot}7H_2O$ 0.05 g. The culture was incubated for 3 days at 28 °C with shaking. The culture was filtered and the concentrated filtrate was precipitated with ethanol. The precipitate was purified by redissolving in distilled water and precipitation with adding ethanol. The precipitate was finally washed with ether and dried under vacuum.

The monosaccharides used for the culture are given in Table 1. They are the main carbon source.

2.2. Alditol acetates

The polysaccharide was hydrolyzed with formic acid, reduced with borohydride and then acetylated to alditol acetates. Monosaccharides were also reduced and acetylated to obtain alditol acetates. The composition of alditol acetates derived from polysaccharides were analyzed by gas chromatography (GC) and NMR.

2.3. GC measurement

GC was performed with a Hewlett Packard Ultra performance series 19091A-102 gas chromatograph equipped with a flame ionization detector and fused silica capillary columns (methyl silicone 12.5 m). The initial temperature was 160 °C and raised to 260 °C at a rate of 2 °C/min and kept at 260 °C. The carrier gas was helium.

2.4. NMR measurement

NMR spectra were measured with a JEOL EX270 spectrometer (270 MHz) at 30 °C in $CDCl_3$ as solvent.

The structure of mannitol acetate and galactitol acetate are symmetrical and they give only three peaks. The assignment of the spectra was performed from 2D-NMR ($^1H-^1H$ and $^1H-^{13}C$ COSY) spectra.

Fig. 1 shows a ^{13}C-NMR spectrum of alditol acetates derived from a polysaccharide cultured in D-xylose. For quantitative determination of mannitol and galactitol acetates, C2 (C5) peaks of mannitol acetate and C1 (C6) peaks of galactitol acetate were used.

3. Results and discussion

Table 1 shows the starting monosaccharides, and the composition of the polysaccharides obtained. It is seen first that the hetero-polysaccharides produced are composed of only D-glucosyl, D-mannosyl and D-galactosyl units, and no other glycosyl units including the monosaccharide units used as the media are found. Determination of composition by GC and NMR measurements are generally in agreement, although determination by GC is inaccurate at low concentration of D-mannosyl units due to overlapping of signals.

It is apparent that if D-mannose or D-galactose are used as the carbon source, polysaccharides with almost the same composition, that is, glucans containing a considerable amount of D-mannosyl units, are biosynthesized. This indicates that rapid interconversion between three

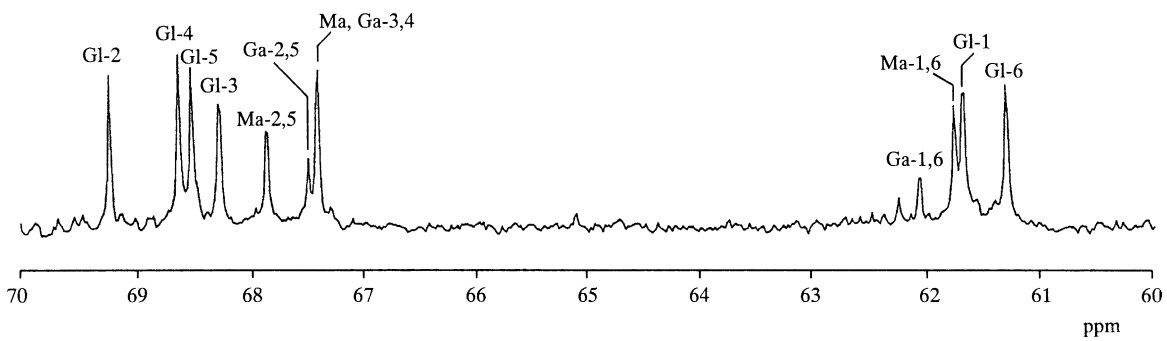

Fig. 1. ^{13}C NMR spectrum of alditol acetates obtained from a hetero-polysaccharide which was produced by *Pestalotiopsis microspora* from D-xylose as the carbon source. Gl: glucitol acetate, Ma: mannitol acetate, Ga: galactitol acetate.

monosaccharides in the microorganism give polysaccharides with the same composition.

Hetero-polysaccharides containing a large amount of D-mannosyl and D-galactosyl units were obtained when D-xylose or *N*-acetyl-D-glucosamine (GlcNAc) are used as the carbon source (Fig. 1). In order to determine the effect of D-xylose or GlcNAc on a medium containing D-glucose or D-mannose, on the composition of polysaccharides produced, D-xylose or GlcNAc was added to the medium up to 50% (50% of D-xylose or GlcNAc and 50% of D-glucose or D-mannose), but only glucans containing D-mannose units were obtained.

It is considered that when D-xylose or GlcNAc is used as carbon source, the enzyme system is perturbed (e.g. polymerization of UDP-Glc is suppressed) and hetero-polysaccharides rich in D-mannosyl and D-galactosyl units may be formed. Addition of D-xylose or GlcNAC to D-glucose or D-mannose media gave no effect, indicating that the perturbation of the enzyme system by D-xylose or GlcNAc in the presence of large amounts of D-glucose or D-mannose did not occur.

When L-rhamnose is used as the carbon source, the yield and the composition of branched polysaccharide are almost the same as those of polysaccharides obtained from the culture with D-glucose. When L-arabinose is used as the carbon source, almost pure glucan was obtained. However, with D-arabinose, the yield was low, while the composition of the polysaccharide was the same as that from D-glucose. *P. microspora* can produce more polysaccharides from lipids and proteins compared with *A. xylinum* or *Agrobacterium* sp. (Kai et al., 1998). It may be considered that the polysaccharide is produced from lipids and proteins and not from D-arabinose.

It is noted that *P. microspora* can metabolize various monosaccharides presumably to D-glucose compared with *A. xylinum* or *Agrobacterium* sp. and the composition of hetero-polysaccharides produced depend strongly on the type of monosaccharide in the media. Since the metabolic process for monosaccharides other than D-glucose is unknown, detailed discussion on the mechanism for the formation of hetero-polysaccharides is difficult at present.

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