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Note

NMR-based identification of cell wall galactomannan of *Streptomyces* sp. VKM Ac-2125

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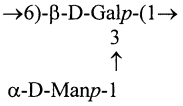
Abstract

The major cell wall polymer of *Streptomyces* sp. VKM Ac-2125, the causative agent of potato scab, is galactomannan with the repeating unit of the following structure:



The polysaccharide with such a structure is found in the bacterial cell wall for the first time. The cell wall also contains small amount of a teichoic acid of the poly(glycerol phosphate) type and 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid.

Keywords: Galactomannan; Teichoic acid; Kdn; Plant pathogenic streptomycete

Cell walls of the majority of species of the genus *Streptomyces* (order Actinomycetales) contain anionic polymers, including teichoic acids, teichuronic acids, and sugar-1-phosphate polymers.[1] Studies of cell walls of pathogenic streptomycetes that are causative agents of potato scab, performed over the last 2 years, have demonstrated that these also contain a polymer of 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (Kdn). Presumably, this polymer is involved in interactions of the pathogen with the potato tuber cells.[2] An acidic polysaccharide containing Kdo-like sugar, belonging to the same family of higher 3-deoxyulosonic acids to which Kdn belongs too, from the plant pathogen *Agrobacterium tumefaciens* has been shown to be involved in the attachment of the microorganism to carrot (host) cells, this being an early step in crown gall tumor formation.[3] Neutral polysaccharides are seldom encountered in cell walls of streptomycetes. Moreover, in those cases where these have been detected as the cell wall components, their structures have not been established.[4] The aim of the present work were structural studies of a neutral galactomannan from the cell wall of a pathogenic *Streptomyces* sp. VKM Ac-2125.

The main acid hydrolysis products of the cell wall containing 0.8% phosphorus were, apart from peptidoglycan components, galactose and mannose and, in addition, small amounts of glycerol mono- and bisphosphates. The composition of the hydrolysate implied the presence of a neutral polysaccharide. This is rather unusual, since cell walls of streptomycetes contain, in the majority of cases, phosphorus-containing polymers, which produce polyol and sugar phosphates upon degradation.

The polysaccharide was isolated from the cell wall by extraction with 10% trichloroacetic acid, the extract was

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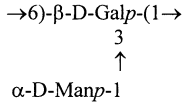
dialysed and freeze-dried. Equimolar amounts of galactose and mannose were detected in the acid hydrolysate of this preparation, glycerol mono- and bisphosphates and glucose being the minor components of the hydrolysate. The absolute D-configurations of mannose and galactose were determined by GLC following their conversion into acetylated (*S*)-octan-2-yl glycosides and comparison with reference standards of octan-2-yl D-glycopyranosides. The ^{13}C NMR spectrum of the neutral polysaccharide contained 12 signals, two of which were present in the anomeric carbon resonance region (δ 104.4 and 97.5). The 'Attached Proton Test' (APT spectrum) revealed two signals for the $-CH_2O-$ group, one of which was observed in the resonance region for the unsubstituted $-CH_2OH$ group of pyranoses (δ 62.25), while the second, in the resonance region of 6-substituted pyranoses (δ 70.5).

In the ^1H NMR spectrum, two signals were observed in the anomeric proton resonance region (δ 5.055, $J_{1,2} < 2$ Hz, and 4.53, $J_{1,2}$ 7.3 Hz). Minor signals (their intensities amounted to approx 5% of those of the major signals) were also present, which belonged to poly(glycerol phosphate) chains and the Kdn residues (H-3_{eq}, δ 2.41, and H-3_{ax}, δ 1.77). The ^1H NMR spectrum of the polysaccharide was assigned using two-dimensional COSY and ROESY experiments. Analysis of the spectra revealed the presence of pyranose residues with α-*manno*- and β-*galacto*-configurations (Table 1). The position of the signal for H-5 of β-Gal was determined from the ROESY spectrum (a correlation peak H-1/H-5), which allowed assignment of the signals for H-6 and H-6′ of this residue from the COSY spectrum.

The assignment of the ^{13}C NMR spectrum was performed using correlations in the HSQC spectrum (Fig. 1). Analysis of the glycosylation effects have shown that the α-Man residue is unsubstituted, while the β-Gal residue is substituted in positions 3 and 6. The structure of the repeating unit of the polymer was established using data from the two-dimensional ROESY and HMBC spectra. The ROESY spectrum contained the correlations between the anomeric proton of β-Gal and H-6 and H-6′ of the same residue and between the anomeric proton of α-Man and H-3 and H-4 of β-Gal.

Correlations through glycosidic bonds were clearly seen in the HMBC spectrum: α-Man H-1/β-Gal C-3 and β-Gal H-1/β-Gal C-6. The experiments altogether prove unambiguously the branched structure of the repeating unit of the polymer:



The characteristic glycosylation effects (Table 2) suggest unequivocally that both sugar residues possess identical absolute configurations,[5,6] which corroborates data from the chemical analysis.[7]

Galactomannan with a different structure has been identified in fungal cell walls,[8] and (1→6)-linked galactan, in the snail *Helix pomatia*.[9] In procaryots, galactomannans have not been found so far. In addition, Kdn has been detected as a cell wall component. Its low percentage precludes conclusion to be made whether this is a constituent of a polymer or of oligomers, which have been detected earlier in cell walls of other pathogenic streptomycetes.[2,10,11] Glycerol mono- and bisphosphates identified in the acid hydrolysate may represent the fragmentation products of poly(glycerol phosphate) chains present in the cell wall in small amount.[12] It is noteworthy that the acid hydrolysates of different batches of mycelium contained variable amounts of glycerol phosphates. In the case where the cell contained 1.6% phosphorus, an alkali-stable glucose-containing glycerol bisphosphate has been identified in its alkaline hydrolysate. The glucose detected in the cell wall may belong to glucosylated poly(glycerol phosphate). Thus, the cell wall of a pathogenic streptomycete *Streptomyces* sp. VKM Ac-2125 comprises galactomannan with a previously unknown structure, as well as poly(glycerol phosphate) chains and 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid.

1. Experimental

Unidentified strain *Streptomyces* sp. VKM Ac-2125, which is phylogenetically close to the type strains *Streptomyces virginae* IFO 3729^T (D85119) and *S.*

Table 1
^1H NMR chemical shifts (δ, *J*/Hz) for the galactomannan from cell wall of *Streptomyces* sp. VKM Ac-2125

Residue	Proton						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
→6)-β-D-Gal*p*-(1→ 3 ↑ α-D-Man*p*-1	**4.53** $J_{1,2}$ 7.3 **5.055** $J_{1,2} < 2$	**3.63** $J_{2,3}$ 9 **4.01** $J_{2,3}$ 3,5	**3.795** $J_{3,4}$ 3 **3.92** $J_{3,4}$ 10	**4.24** $J_{4,5} < 2$ **3.70** $J_{4,5}$10	**3.90** **3.89**	**4.07** **3.885**	**3.93** **3.78**

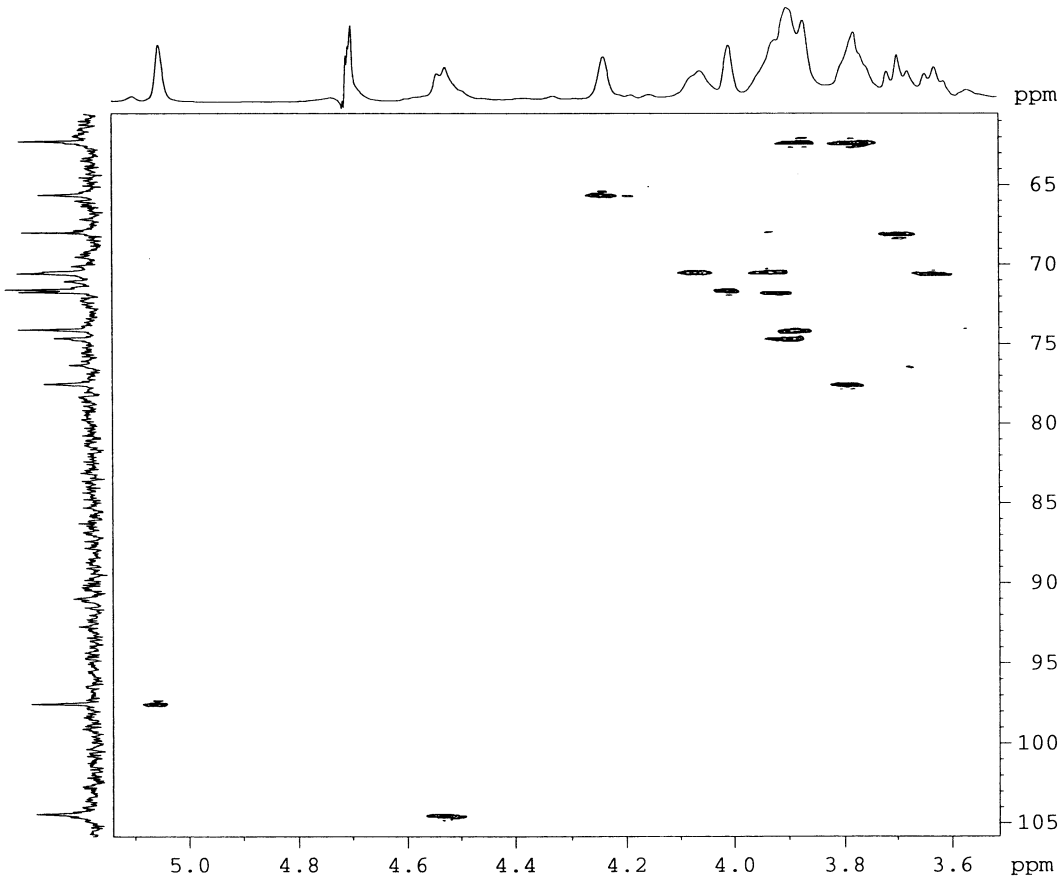


Fig. 1. HSQC-spectrum of the cell wall galactomannan of *Streptomyces* sp. VKM Ac-2125.

subrutilus DSM 40445^T (X80825) (about 98 % 16S rDNA sequence similarity) was grown on a pepton–yeast medium[13] on a shaker at 28 °C and harvested by centrifugation in the middle of the exponential growth phase (24–30 h). The cells were washed with 0.95% NaCl and stored frozen at −20 °C before use. The cell wall and polymers were prepared according to Shashkov and co-workers[10] Descending chromatography and electrophoresis were performed on Filtrak FN-13 paper. Electrophoresis was performed in pyridinium acetate buffer (pH 5.6) to separate phosphate esters (20 V/cm, 3.5 h). Paper chromatography was performed in a pyridine–benzene–butanol–water (3:1:5:3 v/v) solvent system to separate monosaccharides and glycerol. Phosphoric esters were detected with the molybdate reagent; reducing sugars—with aniline hydrogenphthalate; and glycerol and monosaccharides—with 5% $AgNO_3$ in aq ammonia.

Acid and alkali hydrolysis have been described previously.[14] Absolute configuration of Gal and Man was determined by modified method.[7]

NMR spectra were recorded with a DRX-500 (Bruker, Germany) spectrometer for 2–3% solutions in D_2O at 30 °C with acetone (δ_H 2.225, δ_C 31.45) as the internal standard. Pre-saturation of the HDO signal (1 s) was used in the accumulation of the ^1H NMR spectra. Two-

Table 2
^{13}C NMR chemical shifts (δ) for the galactomannan from cell wall of *Streptomyces* sp. VKM Ac-2125

Residue	Carbon					
	C-1	C-2	C-3	C-4	C-5	C-6
→6)-β-D-Gal*p*-(1→ 3 ↑	**104.4**	**70.5**	**77.5** (+3.4) [a]	**65.6** (−4.4)	**74.6**	**70.5**
α-D-Man*p*-1	97.5 (+2.2)	71.5	71.7	67.9	74.06	62.2

[a] The characteristic glycosylation effects for the determination of the absolute configurations of pyranoses[4,5] are given in parentheses.

dimensional spectra were obtained using standard pulse sequences from the Bruker software. A mixing time of 200 ms were used in ROESY experiments. A 60 ms delay was used for the evolution of long-range connectivities in a ^{1}H, ^{13}C HMBC experiments.

Acknowledgements

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