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Heterogeneity of the Rhamnomannans from One Strain of the Human Pathogen *Sporothrix schenckii* Determined by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The synthesis of different rhamnomannans in a strain of *Sporothrix schenckii* (1099.12) was shown by use of ^{13}C nuclear magnetic resonance spectroscopy. Fractionation of a polysaccharide preparation from cells grown at 25°C provided a neutral monorhamnosyl rhamnomannan and an acidic rhamnomannan containing 4-*O*-substituted glucuronic acid units and also (1 → 2)-linked dirhamnosyl side chains.

Previous studies (7) have shown that one strain of *Sporothrix schenckii* (1099.12), a dimorphic human pathogen, synthesized at 25°C a polysaccharide which was similar to that formed by several other well-characterized *S. schenckii* strains. On the basis of ^{13}C and proton nuclear magnetic resonance (NMR) spectroscopy, such a polysaccharide differed from the rhamnomannans synthesized by *Ceratocystis stenoceras*, a morphologically related ascomycete, in a few aspects of its fine structure (8). *S. schenckii* 1099.12 was originally described as a pathogenic mutant of *C. stenoceras* on the basis of ecological and morphological studies (3). The deoxyribonucleic acid of strain 1099.12 associated extensively with that from a typical strain of *S. schenckii* but not with that from the presumed wild-type strain of *C. stenoceras* (1099.11). Accordingly, strain 1099.12 was reclassified as *S. schenckii* (6). These results did not favor the hypothesis that *C. stenoceras* is the perfect form of *S. schenckii* (4). More strains must be examined, however, before a definite conclusion can be drawn.

A more detailed study using *S. schenckii* 1099.12 was carried out with the aim of determining the correlation of morphological differentiation and the synthesis of specific polysaccharides (5). Yeast-like forms synthesized monorhamnosyl rhamnomannans, whereas mycelium cultures formed rhamnomannans with (1 → 2)-linked dirhamnosyl side chains (5, 7). Minor amounts of a galactomannan were also detected in an unsporulated mycelium culture of strain 1099.12, although a similar polysaccharide was not observed in another *S. schenckii* strain (1099.18) studied simultaneously. Variations in the ^{13}C NMR spectra of rhamnomannans from cells and supernatant fluid of strain 1099.12 cultures in several media were observed, but they were attributed to the different proportions of different morphological cell types in each culture. A careful analysis of several NMR spectra of strain 1099.12 polysaccharides showed, however, that the variations observed could be a reflection of fluctuations of the fungal population of this strain. Additional evidence for the heterogeneity of the rhamnomannans of *S. schenckii* 1099.12 is now given by use of a fractionation procedure and ^{13}C NMR spectroscopy.

Since many signals of the ^{13}C NMR spectra of *S. schenckii* and *C. stenoceras* polysaccharides have been assigned to known structures (see Table 1), the simple inspection of such spectra suffices for an immediate interpretation of most of the structural variations. Polysaccharides and spectra were obtained as previously described (5, 7, 8). Methylation analysis was carried out as in Travassos et al. (7). An exo-cellular polysaccharide from *S. schenckii* 1099.12 grown in yeast nitrogen base medium (7) at 25°C gave a ^{13}C NMR spectrum (Fig. 1A) indicating the presence of a dirhamnosyl rhamnomannan in which a (1 → 6)-linked α-D-mannopyranose main chain is mainly substituted on the 3 positions by α-L-rhamnopyranosyl-(1 → 2)-L-rhamnopyranose residues and are responsible for the signals at δ_c 103.7, 96.9, and 80.4 (assignments in Table 1). A smaller proportion of single-unit (1 → 3)-linked α-L-rhamnopyranose side chains is also evident from the C-1 signal at δ_c 98.2. This

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TABLE 1. *Assignments of signals in ^{13}C NMR spectra of rhamnomannans from S. schenckii and C. stenoceras*

Signal, $\delta_c \pm 0.2$ (ppm)	Assignment	Reference
105.5	C-1 of β-D-glucopyranosyluronic acid units	1
103.7	C-1 of α-L-rhamnopyranose nonreducing end unit of *O*-α-L-Rha*p*-(1 → 2)-*O*-α-L-Rha*p*-(1 → 3)-D-Man*p*	5
102.3	C-1 of 4-*O*-substituted α-D-mannopyranose units	1
	C-1 of L-rhamnopyranose nonreducing end unit of *O*-L-Rha*p*-(1 → 4)-*O*-β-D-Glu*p*A-(1 → 2)-α-L-Rha*p*-	1
101.1	C-1 of 3,6-di-*O*-substituted α-D-mannopyranose units	8
100.3	C-1 of 2,4-di-*O*-substituted α-D-mannopyranose units	8
98.2	C-1 of α-L-rhamnopyranose nonreducing end unit of *O*-α-L-Rha*p*-(1 → 3)-D-Man*p*	8
97.2	C-1 of 2-*O*-substituted α-L-rhamnopyranose units of -β-D-Glu*p*A-(1 → 2)-α-L-Rha*p*-(1 → 3)-D-Man*p*	1
96.8	C-1 of 2-*O*-substituted α-L-rhamnopyranose units of α-L-Rha*p*-(1 → 2)-α-L-Rha*p*	8
81.4	C-2 of 2-*O*-substituted α-L-rhamnopyranose units of -β-D-Glu*p*A-(1 → 2)-α-L-Rha*p*-	1
80.3	C-2 of 2-*O*-substituted α-L-rhamnopyranose units of α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-	
	C-4 of 4-*O*-substituted β-D-glucopyranosyluronic acid units	1
62.8	C-6 of unsubstituted α-D-mannopyranose units	8
18.4	C of methyl group of α-L-rhamnopyranose units	5

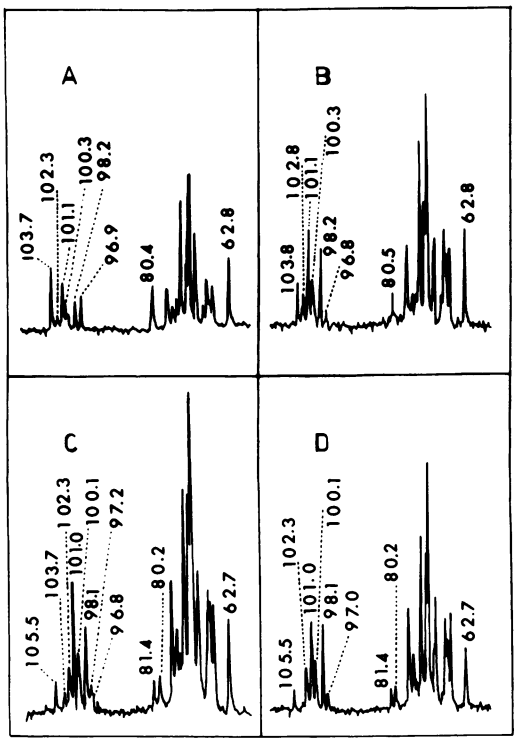

FIG. 1. *Partial ^{13}C NMR spectra of (A) exo-cellular polysaccharide from S. schenckii 1099.12 grown in yeast nitrogen base medium at 25°C; (B) cellular rhamnomannan from a mycelial culture of strain 1099.12 in yeast nitrogen base medium at 37°C; (C) rhamnomannan of S. schenckii 1099.12 grown in Sab medium at 25°C; (D) C. stenoceras-like S. schenckii 1099.12 acidic rhamnomannan.*

polysaccharide also contains a few 4-*O* and 2,4-di-*O*-substituted α-D-mannopyranose units (δ_c 102.2 and 100.3). The nature of the dirhamnosyl side chains was determined previously by isolation and characterization of the trisaccharide *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 3)-D-mannopyranose, which arose by partial acetolysis of an *S. schenckii* 1099.12 rhamnomannan obtained under similar conditions (2). A rhamnomannan obtained from cells of a predominantly mycelial culture of strain 1099.12 in yeast nitrogen base medium at 37°C gave a similar spectrum (Fig. 1B), which showed, however, an increased proportion of the monorhamnosyl substitution in the α-D-mannopyranose main chain although signals denoting the presence of dirhamnosyl side chains were also clearly evident. Upon growing strain 1099.12 in liquid Sabouraud (Sab) medium at 25°C, the cellular polysaccharides were usually similar to those obtained from cultures in other media (5). In one instance, however, a polysaccharide from a Sab culture gave the spectrum depicted in Fig. 1C. In addition to the usual signals arising from *S. schenckii* rhamnomannans obtained at 25°C, peaks at δ_c 105.5, 97.2, and 81.4 indicated the occurrence of a new structural feature. The latter signals are characteristic of spectra of *C. stenoceras* polysaccharides (1, 8). Detailed studies on a *C. stenoceras* polysaccharide showed that these signals corresponded to the presence of 4-*O*-substituted β-D-glucopyranosyluronic acid units in the sequence L-Rha*p*-(1 → 4)-β-D-Glu*p*A-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-D-Man*p* (1). This side chain replaced the dirhamnosyl side chain of the *S. schenckii* polysaccharides, which

was not found in the *C. stenoceras* acidic rhamnomannans.

To characterize better the heterogeneity of the polysaccharide preparation from *S. schenckii* 1099.12 grown in Sab at 25°C, the preparation was fractionated on a diethylaminoethyl-cellulose column equilibrated with acetic acid (5%). Elution with 5% aqueous acetic acid gave a typical monorhamnosyl-containing rhamnomannan (Fig. 2A) with an unusually high proportion of 4-*O*- and 2,4-di-*O*-substituted α-D-mannopyranose units as indicated by the C-1 signals at δ_c 102.3 and 100.0 and by the C-6 signal at δ_c 62.6. The eluate also contained traces of galactose. On subsequent elution with 5% aqueous formic acid, a second polysaccharide was obtained (Fig. 2B) which would be a typical *C. stenoceras* rhamnomannan were it not for the presence in the spectrum of signals at δ_c 103.5 and 96.7, which suggested the presence of di-rhamnosyl side chains. Methylation analysis of both eluates showed the absence of 3,4-di-*O*-methyl-1,2,5,-tri-*O*-acetyl rhamnitol in the acetic acid fraction and a proportion of 20% of this derivative (corresponding to 60% of side chains with 2-*O*-substituted α-L-rhamnopyranose units) in the formic acid eluate. This result suggests that *S. schenckii* 1099.12 formed at 25°C a monorhamnosyl rhamnomannan along with an acidic rhamnomannan that also contained di-rhamnosyl side chains.

To study the possible heterogeneity of strain 1099.12, yeast cultures obtained in brain heart infusion at 37°C were plated on solid Sab, and several clones were picked up and subcultured in liquid Sab at 25°C. The polysaccharides extracted from the cells of most clones gave spectra similar to that for the rhamnomannan of the original culture (Fig. 1C). Cells from one clone synthesized, however, an acidic rhamnomannan which gave a ^{13}C NMR spectrum identical to that of *C. stenoceras* rhamnomannan (Fig. 1D) lacking signals at δ_c 103.5 and 96.7. This result suggests that there may be at least two fungal variants in *S. schenckii* 1099.12 or that this strain is particularly unstable. Noteworthy is the fact that the deoxyribonucleic acid hybridization studies (6) were made with deoxyribonucleic acid extracted from *S. schenckii* 1099.12 grown in Sab medium at 25°C, a growth condition which according to the present data stimulates the selection of cells synthesizing acidic rhamnomannans similar but in most instances not identical to those of *C. stenoceras*.

The presence of glucuronic acid residues in *S. schenckii* 1099.12 rhamnomannans suggested in the present communication by inspection of ^{13}C NMR spectra is probably a more general structural feature of *S. schenckii* polysaccharides, thus not restricted to this particular strain. ^{13}C NMR spectra of rhamnomannans from a few other strains (8) support this assertion.

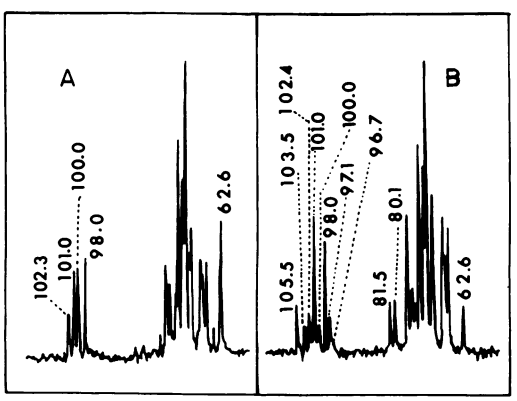

FIG. 2. *Partial ^{13}C NMR spectra of (A) diethylaminoethyl-cellulose neutral fraction consisting of a monorhamnosyl rhamnomannan; (B) acidic rhamnomannan from diethylaminoethyl-cellulose fractionation of a cellular polysaccharide from S. schenckii 1099.12.*

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