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**EXPERIMENTAL ARTICLES**

# The Carbohydrate-containing Cell-Wall Polymers of Certain Species from the Cluster "*Streptomyces lavendulae*"

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**Abstract**—The type strains of the species of the cluster "*Streptomyces lavendulae*" with a low level of DNA–DNA relatedness were found to contain different cell-wall carbohydrate polymers, whereas the species of this cluster with a level of DNA–DNA relatedness of about 60% contain similar or identical carbohydrate polymers. The type strains *Streptomyces katrae* VKM Ac-1220$^T$ and *S. polychromogenes* VKM Ac-1207$^T$ synthesize mannan with different amounts of α-1,2- and α-1,3-substituted mannopyranose units and a small number of 1,3-poly(glycerol-phosphate) chains. The cell walls of *S. lavendulocolor* VKM Ac-215$^T$ and *Streptomyces* sp. VKM Ac-2117 were found to contain a hitherto unknown teichuronic acid, whose repeating unit is a disaccharide consisting of diaminomannuronic acid and *N*-acetylgalactosamine: ⟶ 4)-β-D-Man*p*NAc3NAcA-(1 ⟶ 3)-α-D-Gal*p*NAc-(1 ⟶. In addition, the cell walls of these two streptomycetes contain β-glucosylated 1,5-poly(ribitol phosphate). The cell walls of *S. virginiae* VKM Ac-1218$^T$ and *S. flavotricini* VKM Ac-1277$^T$ contain the same poly(glucosyl-glycerolphosphate). The results presented in this paper are in accordance with the DNA–DNA relatedness data and indicate a taxonomic significance of the structure of the cell-wall polysaccharides for the delineation of phenetically related *Streptomyces* species.

*Key words*: *Streptomyces*, cell wall, mannan, teichoic acid, taxonomy, NMR spectroscopy.

*Abbreviations*: Rib-ol, ribitol; Rib-ol*P*, ribitol monophosphate; Rib-ol$P^2$, ribitol diphosphate; Gro, glycerol; Gro*P*, glycerol phosphate; Gro$P^2$, glycerol diphosphate; Man*p*NAc3NAcA, 2,3-diacetamido-2,3-dideoxy-β-mannuronic acid; Gal*p*NAc, 2-acetamido-2-deoxy-α-galactopyranose; APT, attached proton test; COSY, correlated spectroscopy; HMQC, heteronuclear multiquantum correlation; ROESY, rotating frame Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; HMBC, heteronuclear multibond correlation; HSQC, $^1$H-detected single-quantum correlation.

The genus *Streptomyces* of the order *Actinomycetales* presently includes about 500 validated species, many of which are useful in biotechnology. Most of the species of this genus were identified based on their cultural and morphological characteristics and the ability to utilize sugars as carbon sources. In many cases, mere phenotypic characterization is not sufficient to reliably identify newly isolated streptomycetes. In view of this, novel approaches to the taxonomy of streptomycetes were proposed, which are based on the cultural, morphological, and physiological characterization of species [1–3]. The numerical analysis of *Streptomyces* species allowed taxonomists to reduce the number of nominal species and to classify them into 80 arbitrary groups (clusters) [1, 3]. The analysis of the DNA–DNA homology of the species within particular clusters showed that some of them are heterogeneous and formed the basis for the reclassification of some streptomycetes in accordance with the modern definition of bacterial species, whose main criterion is the level of DNA–DNA relatedness of about 70% [4]. Nevertheless, the problem of the phenotypic characterization of species that would allow for their reliable delineation remains challenging.

Our earlier studies showed that the structure of the anionic carbohydrate-containing cell-wall polymers of *Nocardiopsis*, *Glycomyces*, and *Agromyces* is species-specific, while the NMR spectra and some structural features of these polymers can be used for the identification of representatives of these genera at a species level [5–9]. We also revealed differences in the structure of the anionic cell-wall polymers of *Streptomyces*

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species [10] and demonstrated the presence of identical polymers in the representatives of one streptomycete genospecies [7, 11].

This work is a continuation of the study of the diverse carbohydrate-containing cell-wall polymers of actinomycetes and the taxonomic significance of such polymers for the delineation of close species. Specifically, the present work is aimed at studying the structure of the cell-wall polymers of species from the phenetic cluster *"Streptomyces lavendulae,"* some of which are closely related and others that are genetically different [3, 12].

## MATERIALS AND METHODS

**Strains.** *Streptomyces katrae* VKM Ac-1220$^T$, *Streptomyces polychromogenes* VKM Ac-1207$^T$, *Streptomyces lavendulocolor* VKM Ac-215$^T$, *Streptomyces* sp. VKM Ac-2117, and *Streptomyces virginiae* VKM Ac-1218$^T$ were obtained from the All-Russia Collection of Microorganisms (VKM).

**Cultivation conditions.** Streptomycetes were grown at 28°C in flasks with 100 ml of peptone–yeast extract medium [10] on a shaker. The growth medium was inoculated with 1-day-old cultures grown under the same conditions. Cells for the preparation of cell walls were harvested in the logarithmic phase (17–24 h of growth), washed with distilled water, and stored at –20°C until required.

**The cultural and morphological characteristics** of streptomycetes were studied as described earlier [13].

**Cell walls** were prepared by disintegrating the streptomycete mycelium in 1% SDS using an ultrasonic disintegrator. The homogenate was fractionated by differential centrifugation [14]. The fraction of cell walls was lyophilized.

**Polymers** were extracted from the cell walls and defatted mycelium with 10% trichloroacetic acid (TCA). The polymers extracted from the mycelium were purified by ion-exchange chromatography on DEAE-Toyopearl 650 M [15].

**Acid hydrolysis.** To identify phosphate esters, polyols, aminosugars, and monosaccharides, the preparations of the cell walls, polysaccharides, or glycosides were hydrolyzed in 2 M HCl at 100°C for 3 h. To prepare glycosides, polymers were hydrolyzed in 40% HF at 4°C for 24 h. The hydrolysate was treated with Dowex 2 × 4 ion-exchange resin in $CO_3^{2-}$ form, lyophilized, and then chromatographed.

**Electrophoresis and chromatography.** Phosphate esters and aminosugars were separated by electrophoresis on Filtrak FN-13 paper in pyridine–acetate buffer (pH 5.6) at an electric field strength of 20 V/cm for 3–4 h. Monosaccharides, glycosides, aminosugars, and polyols were separated by descending chromatography on the same paper in a pyridine–benzene–butanol-1-ol–water (3 : 1 : 5 : 3, v/v) system.

**Spot visualization.** Separated substances were visualized by spraying the developed paper with the Isherwood reagent (phosphate esters), 5% $AgNO_3$ (polyols), ninhydrin (aminosugars), or aniline phthalate (monosugars). The quantitative analytical methods used were described earlier [16].

**The NMR spectra** of 2–3% solutions of preparations in $D_2O$ were recorded at 30°C using a Bruker DRX-500 device (Germany) with acetone as the standard (2.225-ppm peak in $^1$H NMR spectra and 31.45-ppm peak in $^{13}$C NMR spectra). Two-dimensional NMR spectra were recorded according to manufacturer's instructions.

## RESULTS

***Streptomyces katrae* VKM Ac-1220$^T$ and *Streptomyces polychromogenes* VKM Ac-1207$^T$.** The cell walls of *S. katrae* VKM Ac-1220$^T$and *S. polychromogenes* VKM Ac-1207$^T$were found to contain identical sets of monosaccharides (Table 1) and small amounts of organic phosphorus (less than 1%), whereas the streptomycete cell walls frequently contain up to 60% of phosphorus-containing polymers [17]. The extracts of the cell walls of these two streptomycetes with 10% TCA contained mainly mannose, as well as galactose, glucose, glycerol monophosphate, and glycerol diphosphate. The detection of glycerol mono- and diphosphates suggested the presence of a poly(glycerophosphate) chain in the cell walls of the streptomycetes [17]. To verify this suggestion, the streptomycete polymers were fractionated by ion-exchange chromatography and the phosphorus-containing fractions were collected, dialyzed, lyophilized, and analyzed by NMR spectroscopy. Analysis of the $^{13}$C NMR spectra of these preparations showed the presence of the 70.55-ppm signal of the C-2 atom and the 67.5-ppm signal of the C-1 and C-3 atoms of glycerol [17], indicating that the cell walls of *S. katrae* and *S. polychromogenes* contain some amount of teichoic acid with the 1,3-poly(glycerophosphate) chain.

The detection of a great amount of mannose suggests the presence of mannan in the cell walls of the two streptomycetes. Analysis of the one-dimensional $^{13}$C and $^1$H NMR spectra and the two-dimensional COSY, TOCSY, ROESY, and HSQC spectra of carbohydrate polymers confirmed the presence of mannan in the cell walls. The polysaccharide preparation from the cell wall of *S. katrae* exhibited the 101.8- and 103.4-ppm signals of the α-1,2- and α-1,3-substituted mannopyranose units; the 104.4-, 71.8-, 74.0-, 69.9-, 76.2-, and 62.25-ppm signals of the C-1, C-2, C-3, C-4, C-5, and C-6 atoms of β-galactosyl residues; and the 99.5-, 79.85-, and 74,8-ppm signals of the C-1, C-2, and C-3

**Table 1.** Chemical composition of the cell walls of streptomycetes

| Species | Strain | Monosaccharides[1, 3] | Aminosugars[1] | Polyols[1] | Phosphate esters[1] |
|---|---|---|---|---|---|
| *S. katrae* | VKM Ac-1220$^T$ | Mann, Gal, Glc | GlcNAc[2] | Gro | Gro*P*, Gro$P_2$ |
| *S. polychromogenes* | VKM Ac-1207$^T$ | Mann, Gal, Glc | GlcNAc[2] | Gro | Gro*P*, Gro$P_2$ |
| *S. lavendulocolor* | VKM Ac-215$^T$ | Glc | GlcNAc[2] GalNAc | Rib-ol, Gro | Rib-ol*P*, Rib-ol$P_2$ , GroP, Gro$P_2$ |
| *Streptomyces* sp. | VKM Ac-2117 | Glc | GlcNAc[2] GalNAc | Rib-ol, Gro | Rib-ol*P*, Rib-ol$P_2$, Gro*P*, Gro$P_2$ |
| *S. virginiae* | VKM Ac-1218$^T$ | Glc, Gal | GlcNAc[2] | Gro | Gro*P*, Gro$P_2$ |
| *S. flavotricini*[4] | VKM Ac-1277$^T$ | Glc, Gal | GlcNAc[2] | Gro | Gro*P*, Gro$P_2$ |

[1] Products of the cell wall hydrolysis with 2 N HCl at 100°C for 3 h.
[2] Components of peptidoglycan.
[3] In the order of decreasing content.
[4] Data from Kozlova *et al.* [20].

**Table 2.** Chemical shifts in the $^{13}$C NMR spectrum of teichuronic acid

| Residue | Chemical shift δ, ppm | | | | | |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
| →4)-β-D-Man*p*NAc3NAcA-(1→ (A) | 100.95 | 53.3* | 54.8* | 71.7 | 79.3 | 175.1 |
| →3)-α-D-Gal*p*NAc-(1→ (B) | 98.4 | 49.05* | 79.1 | 68.2 | 71.7 | 62.1 |

* The chemical shifts of $\underline{C}H_3CON$ are 23.4, 23.3, and 23.2 ppm and those of $CH_3\underline{C}ON$ are 175.6 and 176.1 ppm.

**Table 3.** Chemical shifts in the $^1$H NMR spectrum of teichuronic acid

| Residue | Chemical shift δ, ppm | | | | | |
|---|---|---|---|---|---|---|
| | H-1 | H-2 | H-3 | H-4 | H-5 | H-6,6' |
| →4)-β-D-Man*p*NAc3NAcA-(1→ (A) | 5.02 | 4.25* | 4.28* | 4.89 | 3.95 | |
| →3)-α-D-Gal*p*NAc-(1→ (B) | 5.10 | 4.20* | 3.94 | 4.20 | 3.93 | 3.70 |

* The chemical shifts of $CH_3CON$ are 2.02, 1.93, and 1.87 ppm.

atoms of the 2-substituted β-mannosyl residues glycosidically linked to the C-3 atom of mannose. The polysaccharide preparation from the cell wall of *S. polychromogenes* also contained mannan, which differed from that of *S. katrae* in that it had a lower amount of 1,3-linked α-mannopyranosyl residues.

It was difficult to perform a comprehensive analysis of mannan because of its heterogeneity. In view of this, the glucose detected in small amounts in the cell walls of both species may be a component of a minor cell-wall polysaccharide other than mannan.

***Streptomyces lavendulocolor* VKM Ac-215$^T$.** The cell wall of *S. lavendulocolor* VKM Ac-215$^T$ was found to contain 0.24% of the teichoic acid phosphorus, as well as galactosamine, glucose, ribitol, anhydroribitol, glycerol, an unidentified ninhydrin-reactive compound, and trace amounts of the ribitol and glycerol phosphate esters (Table 1). The extract of the cell wall of this streptomycete with 10% TCA exhibited the presence of the same substances that were detected in the cell wall. The detection of only trace amounts of phosphate esters suggested that the content of teichoic acids in the cell wall of *S. lavendulocolor* VKM Ac-215$^T$ was low. Attempts to separate polymers by ion-exchange chromatography were not successful, since all the polymers were eluted by 0.23 M NaCl. The elution of the polymers by such a high concentration of NaCl suggests that they are acidic in nature.

The $^{13}$C NMR spectrum of the cell-wall polymers (Fig. 1) exhibited the two major signals of the anomeric carbon atoms of sugars at 100.95 and 98.4 ppm. Three signals were within the region of the resonance of carbon atoms bound to nitrogen atoms (49 to 55 ppm), and one signal (62.1 ppm) corresponded to an unsubstituted oxymethyl group. Three other intense signals were

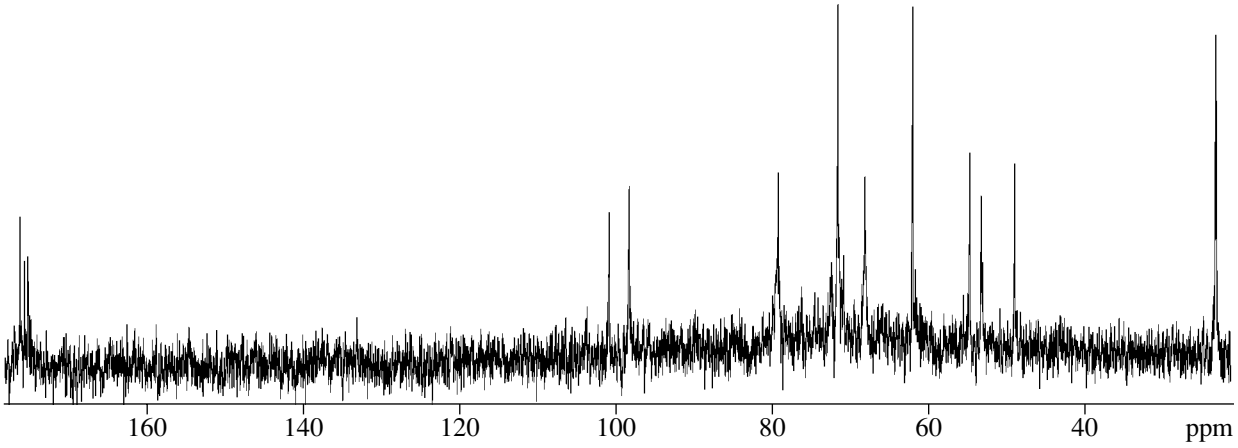


**Fig. 1.** The $^{13}C$ NMR spectrum of anionic polymers from the cell wall of *S. lavendulocolor* VKM Ac-215$^T$.

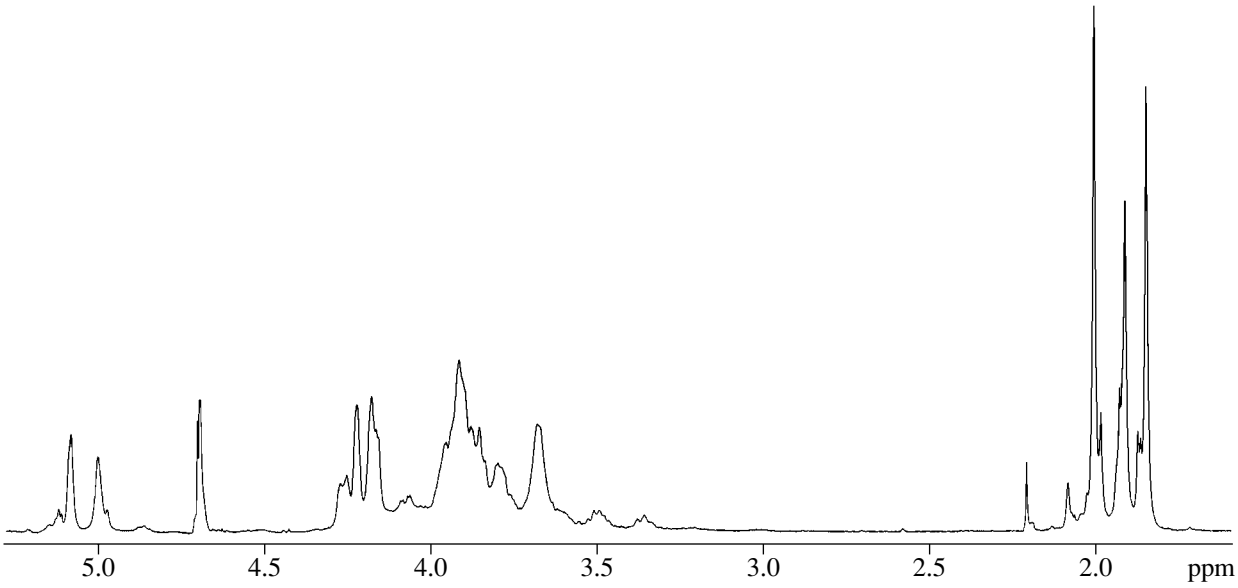


**Fig. 2.** The $^1H$ NMR spectrum of anionic polymers from the cell wall of *S. lavendulocolor* VKM Ac-215$^T$.

attributed to $CH_3CON$ groups; this corresponded to the estimated number of carbon atoms bound to nitrogen and suggested the presence of three *N*-acetylamide groups in the repeating polysaccharide unit. In the low-field region (175–176 ppm), there were four signals of the CO groups (taking into account the double-intensity signal at 176.1 ppm). Several weak and broad signals in the region 62–72 ppm probably belonged to teichoic acids.

The $^1H$ NMR spectrum (Fig. 2) had two signals of anomeric carbon atoms at 5.02 and 5.10 ppm, three-proton singlets of the $CH_3CO$ groups in the region 1.87–2.02 ppm, several intense signals in the region 3.7–4.3 ppm, and weak signals within 3.3–4.2 ppm corresponding to teichoic acids.

Analysis of the COSY, TOCSY, HMQC (Fig. 3), and HMQC-TOCSY (Tables 2 and 3) spectra of the cell-wall carbohydrates showed that they contained a repeating unit composed of two residues, 2,3-diacetamido-2,3-dideoxy-β-mannuronic acid (residue A) and 2-acetamido-2-deoxy-α-galactopyranose (residue B). The two-dimensional ROESY spectrum had two correlation peaks of protons, H-1(A)/H-3(B) and H-1(B)/H-4(A), corresponding to the carbohydrate sequence ⟶ 4)-β-D-Man*p*NAc3NAcA-(1 ⟶ 3)-α-D-Gal*p*NAc-(1 ⟶. The low-field chemical shifts of the C-4 atom of residue A and the C-3 atom of residue B (Table 2) were in accordance with this sequence.

Thus, the major cell-wall polymer of *S. lavendulocolor* is teichuronic acid with the repeating disaccha-

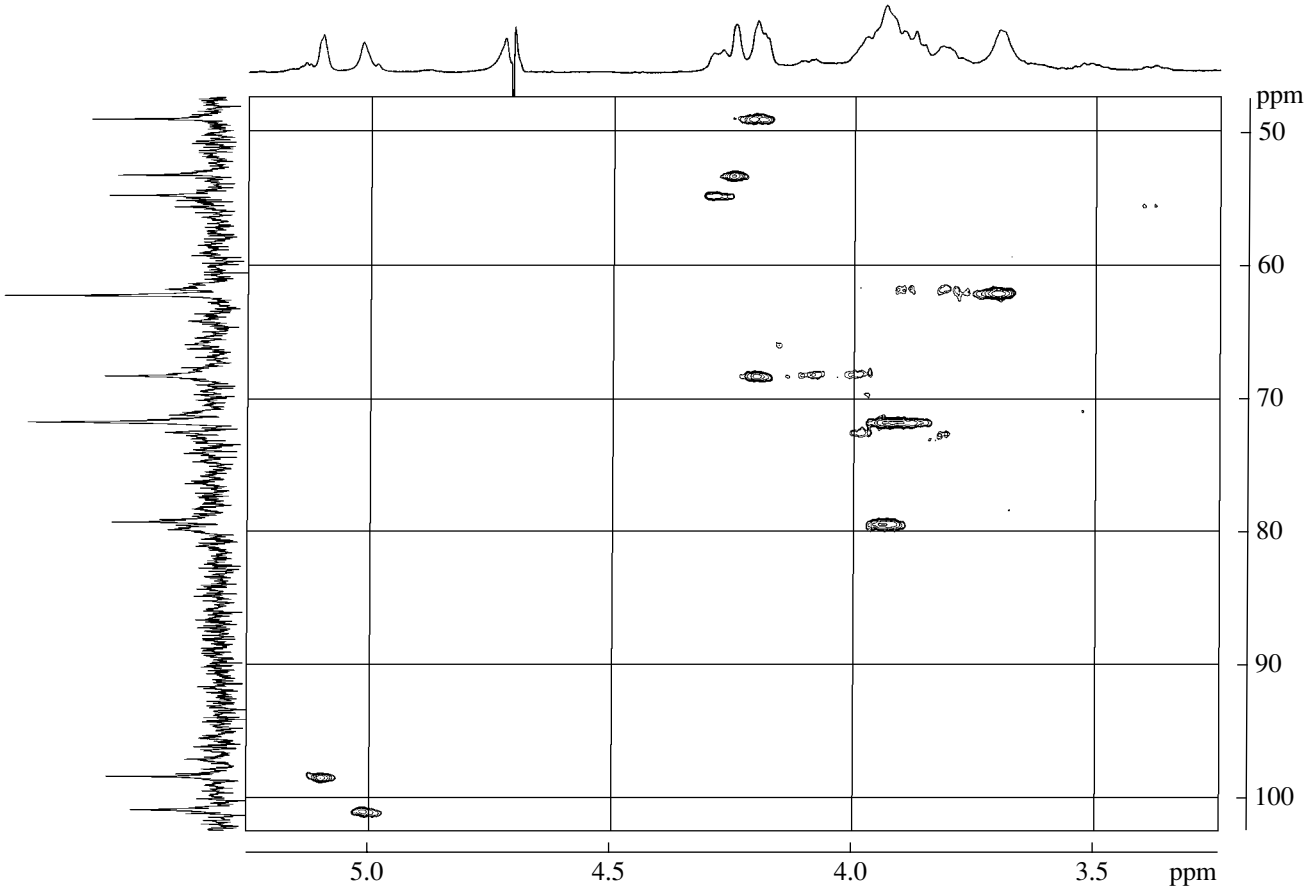


**Fig. 3.** The HMQC spectrum of anionic polymers from the cell wall of *S. lavendulocolor* VKM Ac-215$^T$.

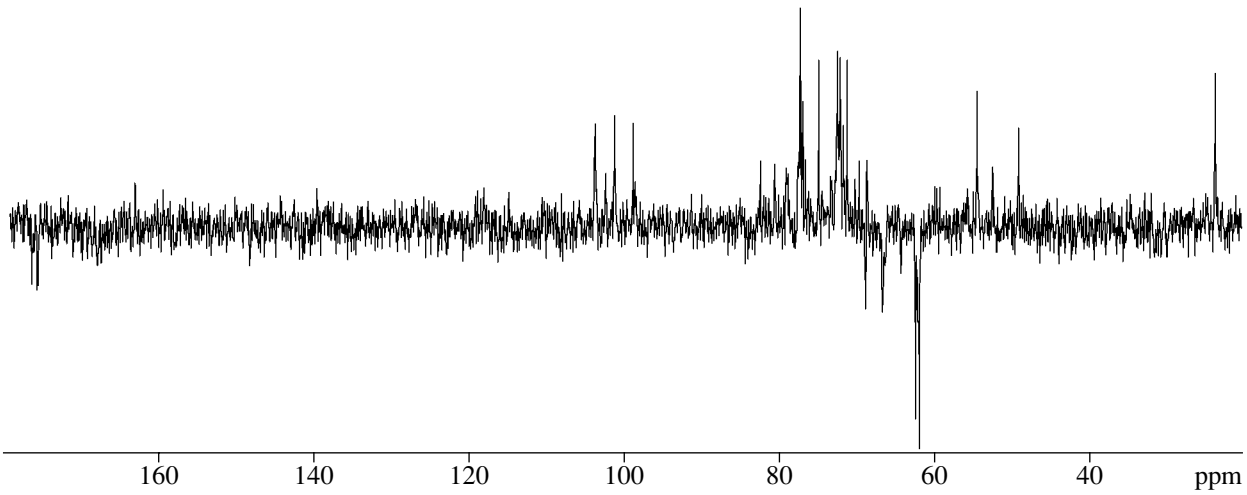


**Fig. 4.** The APT spectrum of anionic polymers from the cell wall of *Streptomyces* sp. VKM Ac-2117. The signals of the carbon atoms of methine are below the baseline and those of quaternary carbon atoms are above the baseline.

ride unit composed of diaminomannuronic acid and galactosamine. The detection of ribitol phosphates and glycerol phosphates suggests the presence of, respectively, ribitol-containing and glycerol-containing teichoic acids in the cell wall of *S. lavendulocolor.* Alternatively, glycerol phosphate esters may result from the hydrolysis of oligomeric units composed of a peptidoglycan and anionic polymers [17].

**Table 4.** Chemical shifts in the $^{13}C$ NMR spectrum of ribitolteichoic acid

| Residue | Chemical shift δ, ppm | | | | | |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
| -1)-Rib-ol-(5-*P*- | 68.1 | 72.1 | 73.1 | 72.1 | 68.1 | |
| -1)-Rib-ol-(5-*P*- (C) | 68.1 | 71.8 | 72.1 | 80.4 | 65.9 | |
| 4 | | | | | | |
| ↑ | | | | | | |
| β-D-Glc*p*-1 (D) | 103.6 | 74.6 | 77.1 | 70.9 | 77.0 | 61.9 |

**Table 5.** Chemical shifts in the $^{1}H$ NMR spectrum of ribitolteichoic acid

| Residue | Chemical shift δ, ppm | | | | | | |
|---|---|---|---|---|---|---|---|
| | H-1 | H-1' | H-2 | H-3 | H-4 | H-5 | H-5' |
| -1)-Rib-ol-(5-*P*- (C) | 4.13 | 4.01 | 3.86 | 3.97–4.20 | | 4.18 | 4.02 |
| 4 | | | | | | | |
| ↑ | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | H-6' |
| β-D-Glc*p*-1 (D) | 4.60 | 3.26 | 3.46 | 3.30–3.45 | | 3.85 | 3.67 |

***Streptomyces* sp. VKM Ac-2117.** The strain produces smooth spores contained in spiral sporophores. The gray aerial mycelium develops well on mineral agar 1. On oat and glycerol–nitrate agars, the aerial mycelium is white-grayish and spotty. On organic agar 2, the aerial mycelium is scarce. The substrate mycelium grown on mineral media is either colorless or yellowish and that grown on organic agar 2 is brown [13]. In its cultural and morphological characteristics, *Streptomyces* sp. VKM Ac-2117 differs from the other species of the cluster *"Streptomyces lavendulae"* and is closest to the species *Streptomyces diastatochromogenes* [13].

The cell-wall monosaccharides and polyols of *Streptomyces* sp. VKM Ac-2117 are identical to those of *S. lavendulocolor* VKM Ac-215$^{T}$ (Table 1), although the content of ribitol phosphate esters is somewhat higher in the former streptomycete.

Analysis of the NMR spectra of the cell-wall preparations of these two streptomycetes showed that they contain identical teichuronic acids. The relatively high content of the ribitol-containing teichoic acid in the cell wall of *Streptomyces* sp. VKM Ac-2117 allowed us to establish its structure. For this purpose, in addition to the two-dimensional NMR spectra described above, we recorded the APT [18] spectrum (Fig. 4) and the HMBC spectrum (Fig. 5) of the cell-wall polymers of this streptomycete. As follows from the correlation peak H-1(B)/C-4(A) with the shifts 5.10/71.7 ppm (Fig. 5), *N*-acetylgalactosamine (residue B) is bound to the C-4 atom of diaminomannuronic acid (residue A). In turn, as follows from the correlation peak H-1(A)/C-3(B) with the shifts 5.02/79.1 ppm, residue A is bound to the C-3 atom of residue B. The anomeric proton of the β-glucopyranosyl residue C gives the correlation peak H-1(C)/C-4(D) with the shifts 4.60/80.4 ppm (Tables 4 and 5). Based on these results and taking into account the structure of the analogous teichoic acid of *Streptomyces chrysomallus* [19], the structure of the ribitol-containing teichoic acid of *Streptomyces* sp. VKM Ac-2117 was determined as 1,5-poly(ribitol phosphate) partially substituted by β-glucopyranose.

***Streptomyces virginiae* VKM Ac-2118$^{T}$.** The cell wall of *S. virginiae* VKM Ac-1218$^{T}$ contained 1.42% of the teichoic acid phosphorus, as well as glucose, galactose, glycerol monophosphate, and inorganic phosphate (Table 1). The teichoic acid extracted from the defatted mycelium with 10% TCA was found to contain glucose, glycerol, glycerol monophosphate, and inorganic phosphate. The absence of galactose among the products of the acid hydrolysis of teichoic acid suggests that this monosugar may be a component of another cell-wall polymer. Among the products of the teichoic acid hydrolysis with 40% HF, we detected 1-*O*-glucopyranosylglycerol and analyzed it according to the scheme used during the study of the teichoic acid glycoside from the cell wall of *S. flavotricini* [20].

The presence of this glycoside among the products of the teichoic acid hydrolysis by 40% HF and the absence of glycerol diphosphate among the products of the hydrolysis of the cell wall by HCl suggest that either the polymer is 2,3-poly(glycerolphosphate), in which the OH-1 group of glyceryl residue is substituted by a glycosyl group, or the polymer is poly(glycosylglycerolphosphate), in which repeating units are linked

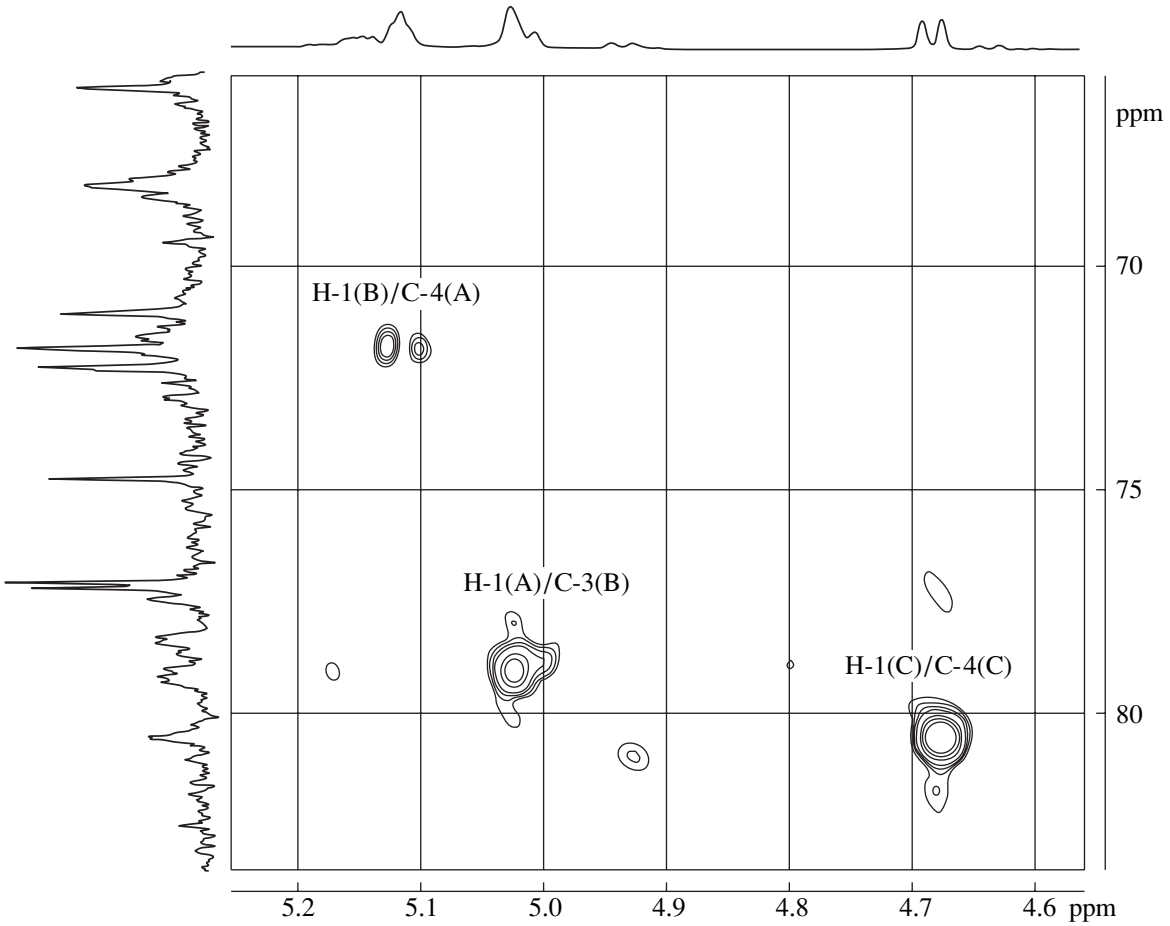


**Fig. 5.** A part of the HMBC spectrum of anionic polymers from the cell wall of *Streptomyces* sp. VKM Ac-2117. Residues A, B, C, and D are diaminomannuronic acid, *N*-acetylglucosamine, glucose, and ribitol, respectively.

by phosphodiester bonds with the involvement of the OH-3 group of glycerol and one of the hydroxyl groups of the sugar moiety [17]. Analysis of the $^{13}C$ and $^{1}H$ NMR spectra showed that the polymer represents poly(glycosyl-glycerolphosphate) whose phosphodiester bonds are formed by the OH-3 group of glycerol and the OH-4 group of β-D-glucose. Therefore, the teichoic acid of *S. virginiae* VKM Ac-1218$^{T}$ is completely identical to the teichoic acid of *S. flavotricini* [20].

## DISCUSSION

The investigation of the chemical composition of the cell walls of four streptomycetes from the cluster *"Streptomyces lavendulae"* [1] and *Streptomyces* sp. VKM Ac-2117 showed that the cell walls of ***S. katrae*** and ***S. polychromogenes*** are very similar and have a chemical composition atypical of other streptomycetes. Along with peptidoglycan, the major cell-wall polymer of these two streptomycetes is mannan with α-1,2- and α-1,3-glycosidic linkages. Unlike the mannan of *S. katrae*, that of *S. polychromogenes* contains predominantly α-1,3-glycosidic bonds. Because of the mannan heterogeneity, it remains unclear whether or not galactose is a component of mannan or of another cell-wall polysaccharide. In addition to mannan, the cell walls of the two streptomycetes contain a small amount of 1,3-poly(glycerolphosphate) chains. The chemical similarity of the cell walls of *S. katrae* and *S. polychromogenes* is in accordance with the phenotypic similarity of these streptomycetes [1] and the high level (59%) of their DNA–DNA relatedness [12].

The major anionic polymer of the cell wall of ***S. lavendulocolor*** is teichuronic acid with the disaccharide ⟶ 4)-β-D-Man*p*NAc3NAcA-(1 ⟶ 3)-α-D-Gal*p*-NAc-(1 ⟶ as the repeating unit. Earlier, teichuronic acid was detected in the cell walls of species of the genera *Bacillus, Micrococcus, Staphylococcus, Streptococcus,* and the four genera of the order *Actinomycetales*: *Actinoplanes, Catellatospora, Corynebacterium,* and *Propionibacterium* [17] but not in the cell walls of streptomycetes. In addition to teichuronic acid, the cell wall of *S. lavendulocolor* contains a small amount of ribitol phosphate–containing teichoic acid. It should be noted that this streptomycete is very distant from the

other species of the cluster "*Streptomyces lavendulae*" in the degree of DNA–DNA relatedness (8–32%) [12].

The cell wall of ***Streptomyces* sp. VKM Ac-2117** contains the same teichuronic acid and 1,5-poly(ribitol phosphate) partially substituted by β-glucopyranose at the C-4(2) atoms. Such ribitol-containing teichoic acids with the β-glucopyranose and α-*N*-acetylglucosamine substituents are widely spread among actinomycetes [10, 17].

Thus, the cell walls of *S. lavendulocolor* and *Streptomyces* sp. VKM Ac-2117 contain two anionic polymers: teichuronic acid, which prevails, and 1,5-poly(ribitol phosphate). The presence of several anionic polymers in the cell wall of one actinomycete has already been reported [16]. The identical chemical composition of the cell walls of the two streptomycetes suggests their close taxonomic relatedness. This suggestion requires experimental confirmation.

The cell wall of ***S. virginiae*** contains poly(glycosylglycerophosphate), which is completely identical to that from the cell wall of *S. flavotricini* [20]. The similarity of the chemical composition of the cell walls of these streptomycetes agrees with their phenotypic similarity [1] and the high level (59%) of their DNA–DNA relatedness [12].

Thus, the results of the analysis of the chemical composition of the cell walls of streptomycetes agree with the DNA–DNA homology data [12] and the phagotyping evidence [21], indicating a taxonomic significance of such an analysis in the delineation of phenetically close streptomycetes. A comparative study of the cell-wall polymers, phenotypic characteristics, and the phylogenetic relations between actinomycetes may essentially contribute to the refinement of their taxonomic status and to the emendation of bacterial classification in general.

## ACKNOWLEDGMENTS

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