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EXPERIMENTAL ARTICLES

The Cell Wall Teichoic Acids of Streptomycetes from the "*Streptomyces cyaneus*" Cluster

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Abstract—The cell wall anionic polymers of the 13 species of the "Streptomyces cyaneus" cluster have a similar structure and contain β-glucosylated 1,5-poly(ribitol phosphate) and 1,3-poly(glycerol phosphate). In the degree of glucosylation of the ribitol phosphate units of their teichoic acids, the cluster members can be divided into two groups. The streptomycetes of the first group (*S. afghaniensis*, *S. janthinus*, *S. purpurascens*, *S. roseoviolaceus*, and *S. violatus*) are characterized by a very similar structure of their cell walls, the completely glucosylated 1,5-poly(ribitol phosphate) chains, and a high degree of DNA homology (67–88% according to literature data). The cell wall teichoic acids of the second group (*S. azureus*, *S. bellus*, *S. caelestis*, *S. coeruleorubidus*, *S. curacoi*, and *S. violarus*) differ in the degree of β-glucosylation of their 1,5-poly(ribitol phosphate) chains and have a lower level of DNA homology (54–76% according to literature data). Two streptomycetes of the cluster (*S. cyaneus* and *S. hawaiiensis*) are genetically distant from the other cluster members but have the same composition and structure of the cell wall teichoic acids as the second-group streptomycetes. The data obtained confirm the genetic relatedness of the "*S. cyaneus*" cluster members and suggest that the structure of the cell wall teichoic acids may serve as one of the taxonomic criteria of the species-level status of streptomycetes.

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

The numerical classification of *Streptomyces*, which is the most diverse genus of the order *Actinomycetales*, allowed the species of this genus to be differentiated into 77 clusters [1]. The systematics of species within the genus *Streptomyces* remains unclear [2], which poses the problem of classification of newly isolated streptomycetes and gives rise to numerous synonyms. This problem can be solved by devising new taxonomic approaches and employing chemotaxonomic markers, such as the composition and structure of the cell wall anionic polymers of gram-positive bacteria. There is increasing taxonomic interest in these constituents of bacterial cell walls, which is due to their high structural diversity and important role in bacterial life [3]. Teichoic acids have been successfully used for the taxonomic dissection of the genus *Micrococcus* [4] and for the grouping of representatives of the genus *Arthrobacter* [5]. The study of the particular groups of actinomycetes in the genera *Nocardiopsis* [6], *Streptomyces* [7–9], and *Glycomyces* [10] showed that the structure of their teichoic acids is species-specific. This prompted us to study the cell wall anionic polymers of other clusters of the genus *Streptomyces*.

This work was undertaken to investigate the cell wall anionic polymers of streptomycetes from the "*Streptomyces cyaneus*" cluster with the aim of revealing correlation between the taxonomic position of streptomycetes (confirmed by DNA–DNA homology data [11]) and the structure of their teichoic acids.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. The streptomycetes used in this study are summarized in Table 1. The streptomycetes were grown in a mineral medium supplemented with peptone and glucose [12].

Cell walls. The streptomycete mycelium was grown to the mid-exponential growth phase, harvested by centrifugation, washed, suspended in 0.5% SDS, and disrupted ultrasonically for a total of 12–16 min in 2-min bursts using a UZDN-1 disintegrator (22 kHz). The homogenate was incubated at 100°C for 5 min and then fractionated by differential centrifugation. The fraction of cell walls was washed with distilled water and lyophilized.

Teichoic acids. Teichoic acids were extracted from the lyophilized cell walls by incubating them in 10%

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Table 1. The species of the "*S. cyaneus*" cluster used in this work

Species	Strain code in VKM	Strain code in NRRL
S. afghaniensis	Ac-703	B-5621
S. azureus	Ac-719	B-2655
S. bellus	Ac-573	B-2575
S. caelestis	Ac-1822	2418
S.coeruleorubidus	Ac-576^T	B-2569
S. curacoi	Ac-621	B-2901
S. cyaneus	Ac-1307	B-16305
S. janthinus	Ac-208	B-3365
S. hawaiiensis	Ac-1761	B-1988
S. purpurascens	Ac-755^T	B-12230
S. roseoviolaceus	Ac-1901	B-12177
S. violarus	Ac-528	B-5432
S. violatus	Ac-532	B-2867

TCA at 4°C for 48 h. The extract of teichoic acids was separated from cell debris by centrifugation, dialyzed against distilled water, and lyophilized.

Acid hydrolysis. The preparations of cell walls and teichoic acids were hydrolyzed in 2 M HCl at 100°C for 3 h. The hydrolysate was analyzed for hydrolysis products by electrophoresis and paper chromatography. Alternatively, these preparations were hydrolyzed in 40% HF at 4°C for 24 h. Then HF was removed on a column with Dowex 2×8 in CO_3^{2-} form. The eluate was lyophilized and used for the analysis of glycosides and polyols by paper chromatography.

Alkaline hydrolysis. The preparation of teichoic acids was hydrolyzed in 1 M NaOH at 100°C for 3 h. The hydrolysate was purified on a column with Dowex 50×8 in H^+ form. The eluate was lyophilized and used for the analysis of alkaline hydrolysis products by electrophoresis and paper chromatography.

Enzymatic hydrolysis. The enzymatic hydrolysis of preparations was carried out with β-glucosidase (EC 3.3.1.21) purchased from Sigma, United States [13].

Paper chromatography. Hydrolysis products were analyzed by paper chromatography in two systems. Glucose, polyols, and glycosides were analyzed using system A, which represented a butan-1-ol–pyridine–benzene–water (5 : 3 : 1 : 3, v/v) mixture. Lysine and its derivatives were analyzed using system B, which represented a butan-1-ol–acetic acid–water (4 : 1 : 5, v/v) mixture.

Paper electrophoresis. Polyol phosphates, lysine, and lysine derivatives were analyzed by paper electrophoresis in an acetate–pyridine buffer with pH 5.5–5.6 (system C) run at a voltage difference of 20 V/cm for 3.5–4 h (polyol phosphates) or 1.5–2 h (lysine and its derivatives). Polyol phosphates were visualized with the Isherwood reagent [14]; lysine and lysine amide, with ninhydrin; glucose, with aniline phthalate; polyols, glycosides, and glucose, with 5% $AgNO_3$; amides, with *o*-toluidine [15]; and hydroxamates, with 5% $FeCl_3$ in 0.1 M HCl.

Ammonolysis, hydroxylaminolysis, the analysis of lysine configuration, the Smith decomposition, the structural analysis of ribitol phosphates and glycosylated ribitol, and the analysis of molar proportions between the constituents of teichoic acids were carried out as described earlier [13].

NMR spectroscopy. The ^{13C} NMR spectra of 2–3% solutions of samples in D_2O were recorded at 30°C using a DRX-500 NMR spectrometer (Brucker, Germany) and acetone (31.45 ppm) as the standard.

RESULTS

The structure of the teichoic acid of one of the streptomycetes, *S. azureus*, was studied in our earlier works [13, 16]. The cell walls of the streptomycetes studied in this work (Table 1) contained about 3% phosphorus of teichoic acids, whose structure was investigated by procedures described in detail in the aforementioned paper [13]. The procedures included the acid and alkaline hydrolyses of preparations and the analysis of hydrolysis products by paper chromatography in systems A and B and by paper electrophoresis in system C. In addition, we analyzed the amides and hydroxamates that were produced during the ammonolysis and hydroxylaminolysis of teichoic acids.

Analysis of acid hydrolysates. Among the products of the acid hydrolysis of teichoic acids, we identified glucose, ribitol, anhydroribitol, anhydroribitol monophosphate, inorganic phosphate, lysine, and the mono- and bisphosphates of ribitol and glycerol. The presence of the mono- and bisphosphates of polyols in the acid hydrolysate of teichoic acids indicated that phosphodiester bonds linked neighboring polyol residues (in other words, that the teichoic acids had a poly(polyol phosphate) structure [17]) but not the polyol and sugar residues, as in poly(glycosylpolyol phosphate) [6].

Analysis of alkaline hydrolysates. The presence of phosphate esters in alkaline hydrolysates indicated that phosphodiester bonds linked repeated units. Under the conditions of alkaline hydrolysis, the possible glycosyl substituents of polyol residues are retained. As a result, the hydrolysis of a partially glycosylated poly(polyol phosphate) chain gives rise to the phosphate esters of a glycosylpolyol and polyol. As can be seen from the data presented in Table 2, the cell wall hydrolysates of all of the streptomycetes studied contained the mono- and bisphosphates of glucosylribitol, suggesting that the cell walls of these streptomycetes are composed of glucosylated poly(ribitol phosphate) chains. In addition to glucosylribitol phosphates, the cell wall hydrolysates of *S. azureus*, *S. bellus*, *S. caelestis*, *S. coeruleorubidus*, *S. curacoi*, *S. violarus*, *S. cyaneus*, and *S. hawaiiensis*

Table 2. Products detected in the alkaline hydrolysates of the teichoic acids of streptomycetes from the "*S. cyaneus*" cluster

Species	Major products
First group	
S. afghaniensis *S. janthinus* *S. violatus*	GlcRib-ol*P*, GlcRib-olP_2, GlcRib-ol, Gro*P*(ÒÎ), GroP_2 (traces), Lys
S. roseoviolaceus *S.purpurascens*	GlcRib-ol*P*, GlcRib-olP_2, GlcRib-ol, Gro*P*, GroP_2, Gro, Lys
Second group	
S. caelestis *S. coeruleorubidus*	GlcRib-ol*P*, GlcRib-olP_2, GlcRib-ol, Rib-ol*P* (traces), Gro*P*, GroP_2, Gro, Lys
S. azureus *S. bellus* *S. curacoi* *S. cyaneus* *S. hawaiiensis* *S. violarus*	GlcRib-olP, Rib-ol*P*, Rib-olP_2, GlcRib-ol, Anhrib-ol*P*, Rib-ol, Anhrib-ol, Gro*P*, GroP_2, Gro, P_i, Lys

Note: Rib-ol, ribitol; Anhrib-ol, anhydroribitol; GlcRib-ol, glucosylribitol; Gro, glycerol; Lys, L-lysine; Rib-ol*P*, ribitol monophosphate; Rib-olP_2, ribitol bisphosphate; Anhrib-ol*P*, anhydroribitol monophosphate; GlcRib-ol*P*, glucosylribitol monophosphate; GlcRib-olP_2, glucosylribitol bisphosphate; Gro*P*, glycerol phosphate; GroP_2, glycerol bisphosphate; P_i, inorganic phosphate.

Table 3. The molar proportions between polyols in the cell walls of streptomycetes from the "*S. cyaneus*" cluster

Species	^{1}Rib-ol$_{total}$: ^{2}Gro	^{3}Rib-ol$_{nongl}$: ^{4}Rib-ol$_{gl}$
First group		
S. afghaniensis	1 : traces	1 : 0.0
S. janthinus	1 : traces	1 : 0.0
S. purpurascens	1 : 0.5	1 : 0.0
S. roseoviolaceus	1 : 0.4	1 : 0.0
S. violatus	1 : traces	1 : 0.0
Second group		
S. azureus	1 : 0.15	1 : 0.3
S. bellus	1 : 0.1	1 : 0.2
S. caelestis	1 : 0.3	1 : traces
S. coeruleorubidus	1 : 0.8	1 : traces
S. curacoi	1 : 0.6	1 : 1.5
S. cyaneus	1 : 0.7	1 : 2
S. hawaiiensis	1 : 0.6	1 : 1
S. violarus	1 : 0.3	1 : 1

Note: The proportions between products are calculated from the relative intensities of the NMR signals at 80.0–80.4 ppm (C-4 Rib-ol$_{gl}$), at 71.8–71.9 ppm (C-4 Rib-ol$_{nongl}$), and at 70.8–71.4 ppm (C-2 Gro) (see Table 4). Rib-ol$_{total}$, Gro, Rib-ol$_{gl}$, and Rib-ol$_{nongl}$ stand for the total content of ribitol residues, glycerol residues, glucosylated ribitol residues, and nonglucosylated ribitol residues, respectively.

contained ribitol phosphates, indicating that not all of the ribitol phosphate units of the teichoic acids of these streptomycetes were glucosylated. Estimations by ^{13}C NMR spectroscopy showed that the relative amount of nonglucosylated ribitol phosphate units in the teichoic acids of the aforementioned streptomycetes varied from nearly zero to 67% (Table 3).

It should be noted that the cell wall hydrolysates of the streptomycetes also contained the mono- and bisphosphates of glycerol and lysine.

Analysis of HF hydrolysates. The HF hydrolysates of the cell walls of the streptomycetes contained a glycosylribitol, glycerol, and nonglycosylated ribitol (the latter compound was present only in the cell wall hydrolysates of the eight aforementioned streptomycetes) and did not contain glycosylglycerol. These results confirm the data of alkaline hydrolysis indicating that the poly(glycerol phosphate) chains of the teichoic acids of the streptomycetes are not glycosylated [17].

Determination of the structure of glycosylribitol. One of the HF hydrolysates was fractionated by paper chromatography in system A, and the glycoside was isolated and identified according to the procedure described for the teichoic acid glycoside of *S. azureus* [13].

β-Glucosidase split the glycoside to glucose and ribitol in approximately equimolar amounts, indicating that the glycosidic bond was of a β-configuration and that glucose occurred in pyranose form. The periodate oxidation of the glycoside, followed by the reduction of the reaction products with $NaBH_4$ and their acid hydrolysis (the so-called Smith degradation) gave rise to glycerol (according to the data of paper chromatography in system A). The absence of ethylene glycol in the products of the acid hydrolysis of the reduced sample suggested that the C-1 and C-5 atoms of ribitol were not involved in the glycosidic bond. The blue (but not yellow) color developed in reaction with the Schiff reagent indicated that the C-3 atom of ribitol was not involved either [18]. On the other hand, the formation of formaldehyde during the periodate oxidation of the glycoside in an equimolar amount with glucose indicated that the hydroxyl group was substituted either at the C-2 or at the C-4 atom of ribitol. Consequently, the glycoside at hand is 4(2)-0-β-D-glucopyranosylribitol.

Analysis of the products of hydroxylaminolysis and ammonolysis. These chemical reactions gave rise to hydroxamates and the amides of lysine and acetic acid, suggesting that lysine and acetic acid are bound to the polymer by ester bonds. Circular dichroism analysis showed that lysine had an L-configuration [13].

^{13}C NMR spectroscopy. The study of the structure of the teichoic acids of the streptomycetes by ^{13}C NMR spectroscopy revealed the presence of signals corresponding to the carbon atoms of the 1,5-poly(ribitol

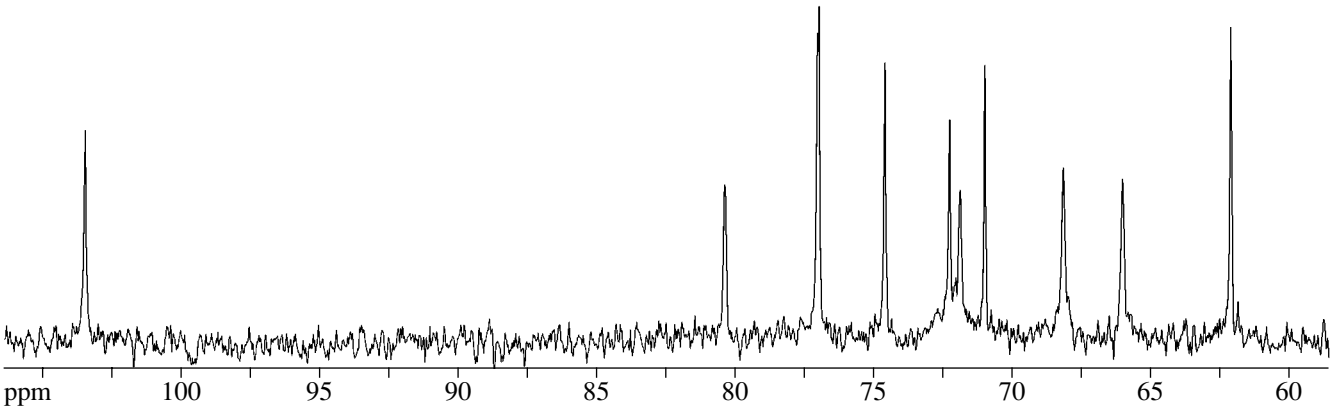

Fig. 1. The ^{13}C NMR spectrum of the teichoic acid of *S. violatus* VKM Ac-532 from the first group of streptomycetes of the "*S. cyaneus*" cluster.

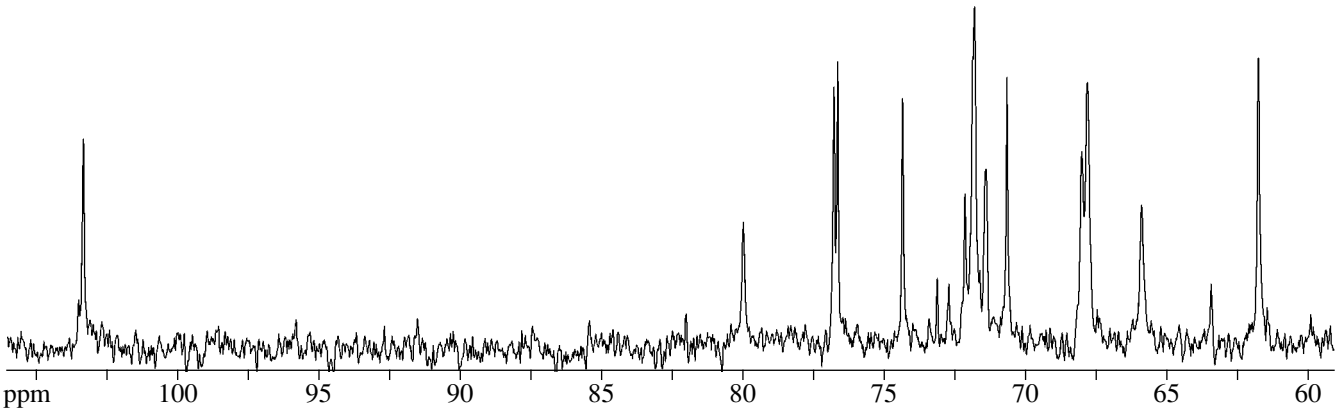

Fig. 2. The ^{13}C NMR spectrum of the teichoic acid of *S. hawaiiensis* VKM Ac-1761 from the second group of streptomycetes of the "*S. cyaneus*" cluster.

phosphate) chain glucosylated with β-*D*-glucopyranose (Fig. 1, Table 4). The NMR spectra of some samples had signals corresponding to nonglycosylated ribitol phosphate units (Fig. 2, Table 4). The NMR spectra of the preparations containing poly(glycerol phosphate) chains contained signals belonging to the C-1 and C-3 (67.7–67.8 ppm), as well as to the C-2 (70.8–71.4 ppm), atoms of the glycerol phosphate units of nonglycosylated 1,3-poly(glycerol phosphate) chains (Table 4).

DISCUSSION

The cell walls of all of the streptomycetes studied (Table 1) were found to contain teichoic acid representing 1,5-poly(ribitol phosphate). The ribitol phosphate units of this teichoic acid were glucosylated with β-D-glucopyranose either at the C-2 or at the C-4 atom. Other monosugars were not detected. On the other hand, the teichoic acids of the streptomycetes contained O-lysyl and O-acetyl groups. Along with poly(ribitol phosphate) chains, the teichoic acids also contained poly(glycerol phosphate) chains.

The cell wall teichoic acids of all of the streptomycetes were qualitatively similar, slightly differing only in the degree of glucosylation of 1,5-poly(ribitol phosphate) chains and the amount of 1,3-poly(glycerol phosphate) chains (Table 3).

According to the structure of their teichoic acids, the streptomycetes of the "*S. cyaneus*" cluster can be divided into two groups. The teichoic acids of the streptomycetes of the first group (*S. afghaniensis*, *S. janthinus*, *S. purpurascens*, *S. roseoviolaceus*, and *S. violatus*) are very similar in structure and composition and are composed of completely glucosylated 1,5-poly(ribitol phosphate) chains (Fig. 1, Tables 2, 3) and 1,3-poly(glycerol phosphate) chains. The amount of the latter chains varies from traces (as in *S. afghaniensis*, *S. janthinus*, and *S. violatus*), detectable only by chemical methods, to sufficiently high levels (as in *S. purpurascens* and *S. roseoviolaceus*), detectable both by chemical methods and by NMR spectroscopy (Tables 2, 3). This group of streptomycetes has a high degree of DNA homology (67–88%) [11] and includes streptomycetes

Table 4. The chemical shifts (δ, ppm) of carbon atoms in the ^{13}C NMR spectra of the teichoic acids of streptomycetes from the "*S. cyaneus*" cluster. The data presented in the table are the average values obtained by the analysis of 12 NMR spectra

Fragment	C-1	C-2	C-3	C-4	C-5	C-6
–C–C–C–C–C–*P*– ↑	68.0–68.1	71.8–71.9	72.1–72.3	80.0–80.4	65.9–66.0	
Glc	103.3–103.4	74.4–74.6	76.8–77.1	70.7–71.0	76.7–77.0	61.7–62.0
–C–C–C–C–C–*P*–	67.9–68.1	71.8–71.9	73.1–73.4	71.8–71.9	67.9–68.1	
–C–C–C–*P*–	67.7–67.8	70.8–71.4	67.7–67.8			
C–C–C–*P*– Terminal	63.4	72.7	67.7–67.8			

of the *S. purpurascens* genospecies, all of which produce red spores.

The teichoic acids of the streptomycetes of the second group (*S. azureus, S. bellus, S. caelestis, S. coeruleorubidus, S. curacoi, S. violarus, S. cyaneus,* and *S. hawaiiensis*) contain both types of anionic polymers and differ in the degree of the β-glucosylation of their 1,5-poly(ribitol phosphate) chains (Table 3). The lowest degree of glucosylation is typical of the 1,5-poly(ribitol phosphate) chains of *S. curacoi* and *S. cyaneus* (in the latter species, the amount of nonglucosylated ribitol phosphate units is twice as large as that of glucosylated ribitol phosphate units). The cell walls of *S. violarus* and *S. hawaiiensis* contain ribitol teichoic acids with equal amounts of the glucosylated and nonglucosylated ribitol phosphate units. In the degree of glucosylation of the ribitol phosphate units of their teichoic acids, the streptomycetes *S. caelestis* and *S. coeruleorubidus* are close to the first-group streptomycetes.

The streptomycetes *S. azureus, S. bellus, S. caelestis, S. coeruleorubidus, S. curacoi,* and *S. violarus* of the second group are characterized by a lower level of DNA homology (58–65%) [11] than are the streptomycetes of the first group (67–88% DNA homology). Two streptomycetes of the "*S. cyaneus*" cluster (*S. cyaneus* and *S. hawaiiensis*) are genetically distant from the other members of the cluster, but they are placed into the second group as having the same composition and structure of their cell wall teichoic acids as the second-group streptomycetes. The streptomycetes of the second group produce either red (*S. violarus*), or blue (*S. azureus, S. bellus, S. caelestis, S. coeruleorubidus, S. curacoi,* and *S. cyaneus*), or yellow (*S. hawaiiensis*) spores.

The data obtained show that the members of the "*S. cyaneus*" cluster are very similar in the composition and structure of their cell wall anionic polymers and confirm the genetic relatedness of the cluster members. It can be suggested that the structure of the cell wall teichoic acids may serve as one of the taxonomic criteria of the species-level status of streptomycetes. The presence of glucosylated ribitol phosphate, lysine, and glycerol phosphates among the products of the alkaline hydrolysis of the cell walls of a streptomycete and the identity of ^{13}C NMR spectra with those presented in Figs. 1 and 2 give sufficient grounds to believe that this streptomycete belongs to the "*S. cyaneus*" cluster.

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REFERENCES

1. Williams, S.T., Goodfellow, M., Alderson, G., Wellington, E.M.H., Sneath, P.H.A., and Sackin, M.J., Numerical Classification of *Streptomyces* and Related Genera, *J. Gen. Microbiol.*, 1983, vol. 129, no. 1-2, pp. 1743–1813.
2. Anderson, A.S. and Wellington, E.M.H., The Taxonomy of *Streptomyces* and Related Genera, *Int. J. Syst. Evol. Microbiol.*, 2001, vol. 51, pp. 797–814.
3. Naumova, I.B. and Shashkov, A.S., The Cell Wall Anionic Polymers of Gram-Positive Bacteria, *Biokhimiya*, 1997, vol. 62, no. 8, pp. 947–982.
4. Stackebrandt, E., Koch, C., Gvozdiak, O., and Schumann, P., Taxonomic Dissection of the Genus *Micrococcus: Kocuria* gen. nov., *Nesterenkovia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend., *Int. J. Syst. Bacteriol.*, 1995, vol. 45, pp. 682–692.
5. Fiedler, F. and Schaffler, M.J., Teichoic Acids in Cell Walls of Strains of the "Nicotinae" Group of *Arthrobacter*: A Chemotaxonomic Marker, *Syst. Appl. Microbiol.*, 1987, vol. 9, no. 1, pp. 16–21.
6. Naumova, I.B., Shashkov, A.S., Tul'skaya, E.M., Streshinskaya, G.M., Kozlova, Yu.I., Potekhina, N.V., Evtushenko, L.I., and Stackebrandt, E., Cell Wall Teichoic Acids: Structural Diversity, Species Specificity in the Genus *Nocardiopsis*, and Chemotaxonomic Perspective, *FEMS Microb. Rev.*, 2001, vol. 25, no. 3, pp. 269–283.
7. Tul'skaya, E.M., Shashkov, A.S., Evtushenko, L.I., Bueva, O.V., and Naumova, I.B., The Structural Identity of the Teichoic Acids of Different Strains of *Streptomyces hygroscopicus*, *Biokhimiya*, 1997, vol. 62, no. 3, pp. 289–293.
8. Kozlova, Yu.I., Streshinskaya, G.M., Shashkov, A.S., Evtushenko, L.I., and Naumova, I.B., The Cell Wall Carbohydrate-containing Anionic Polymers of Two *Strep-*

toverticillum Genospecies, *Biokhimiya*, 1999, vol. 64, no. 6, pp. 805–812.

9. Shashkov, A.S., Kozlova, Yu.I., Streshinskaya, G.M., Kosmachevskaya, L.N., Bueva, O.V., Evtushenko, L.I., and Naumova, I.B., The Carbohydrate-containing Cell-Wall Polymers of Certain Species from the Cluster *"Streptomyces lavendulae," Mikrobiologiya*, 2001, vol. 70, no. 4, pp. 477–486.
10. Potekhina, N.V., Tul'skaya, E.M., Shashkov, A.S., Taran, V.V., Evtushenko, L.I., and Naumova, I.B., Species Specificity of Cell Wall Teichoic Acids in the Actinomycete Genus *Glycomyces*, *Mikrobiologiya*, 1998, vol. 67, no. 3, pp. 414–418.
11. Labeda, D.P. and Lyons, A.J., Deoxyribonucleic Acid Relatedness among Species of the "Streptomyces cyaneus" Cluster, *Syst. Appl. Microbiol.*, 1991, vol. 14, pp. 158–164.
12. Naumova, I.B., Kuznetsov, V.D., Kudrina, K.S., and Bezzubenkova, A.P., The Occurrence of Teichoic Acids in *Streptomyces*, *Arch. Microbiol.*, 1980, vol. 126, no. 1, pp. 71–75.
13. Streshinskaya, G.M., Naumova, I.B., Romanov, V.V., and Shashkov, A.S., The Structure of the Cell Wall Ribitolteichoic Acid of *Streptomyces azureus* RIA 1009, *Bioorg. Khim.*, 1981, vol. 7, no. 9, pp. 1409–1418.
14. Benson, A., *Biokhimicheskie metody analiza rastenii* (The Biochemical Methods of Plant Analysis), Moscow: Izd. Inostr. Lit., 1960, pp. 146–182 (Russian translation).
15. Rendel, F. and Hoppe, W., Uber eine Farbemethode zum Anfarben von Aminosauren, Peptiden und Proteinen auf Papierchromatogrammen und Papierelectropherogrammen, *Chem. Ber.*, 1954, vol. 87, no. 7, pp. 1103–1107.
16. Shashkov, A.S., Streshinskaya, G.M., and Naumova, I.B. The Application of ^{13}C NMR Spectroscopy to the Study of the Ribitolteichoic Acid of *Streptomyces azureus* RIA 1009, *Bioorg. Khim.*, 1979, vol. 5, no. 5, pp. 782–785.
17. Kelemen, M.V. and Baddiley, J., Structure of the Intracellular Glycerol Teichoic Acid from *Lactobacillus casei* ATCC 7469, *Biochem. J.*, 1961, vol. 80, no. 2, pp. 246–254.
18. Armstrong, J.J., Baddiley, J., and Buchanan, J.G., Structure of Teichoic Acid from Cell Walls of *Bacillus subtilis*, *Nature*, 1959, vol. 184, no. 4682, pp. 248–249.