Novel cell-wall teichoic acid from *Nocardiopsis albus* subsp. *albus* as a species-specific marker

Elena M. Tul'skaya,¹ Alexander S. Shashkov,² Ludmila I. Evtushenko,³ Victoriya V. Taran³ and Irina B. Naumova¹

Author for correspondence: I. B. Naumova. Fax: +7 095 939 43 09.

¹ Department of Biology, Moscow State University, Moscow 119899, Russia

² N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

³ Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, Russia

Cell walls of the three *Nocardiopsis albus* subsp. *albus* strains, DSM 43377^T, 43378 and 43120 contain structurally identical teichoic acids. The cell wall of each strain has three distinct types of teichoic acids: (1) unsubstituted 3,5-poly(ribitol phosphate), (2) 1,3-poly(glycerol phosphate) partially substituted at C-2 with α -N-acetylglucosamine residues, and (3) 1,5-poly(ribitol phosphate) with each ribitol unit carrying a 2,4-pyruvate ketal group. Types 1 and 3 are reported in prokaryotes for the first time. The structure of the teichoic acids was elucidated by chemical analysis and NMR-spectroscopic methods. Structural identity of the teichoic acids from the three strains belonging to the same species may demonstrate the species-specificity of these polymers.

Keywords: *Nocardiopsis*, cell walls, teichoic acids, 3,5-poly(ribitol phosphate), pyruvic acid, NMR spectroscopy

INTRODUCTION

The genus *Nocardiopsis* belonging to the order *Actinomycetales* was segregated from the genus *Actinomadura* based on morphological features and cell wall composition (Meyer, 1976). Later, the heterogeneity of this genus was demonstrated (Grund & Kroppenstedt, 1990), and some *Nocardiopsis* species were transferred into the genus *Saccharothrix* proposed by Labeda *et al.* (1984).

The genus *Nocardiopsis* comprises seven species including *N. dassonvillei* and *N. albus* subsp. *albus* (Altai & Ruan, 1994). Earlier, we studied the cell-wall teichoic acid from 15 strains of *N. dassonvillei* and showed that all the strains contained the same polymers of unique structure, hitherto never found in bacterial cell walls (Tul'skaya *et al.*, 1993; Akimov *et al.*, 1994).

The same teichoic acids were also found in *N. antarcticus* and *N. albus* subsp. *prasina* which show a high degree of DNA relatedness with the type strain *N. dassonvillei* and have been reclassified as members of that species (Akimov *et al.*, 1994).

In this work we have investigated the structure of the cell-wall teichoic acids from three strains of *N. albus* subsp. *albus* and obtained further evidence of species-specificity of these polymers.

Some preliminary information on the composition of the *N. albus* subsp. *albus* DSM 43120 cell wall was reported previously (Tul'skaya *et al.*, 1995).

METHODS

Organisms. *N. albus* subsp. *albus* DSM 43377^T, DSM 43378 and DSM 43120 were cultivated as described by Naumova *et al.* (1980).

Analytical procedures. Cell walls were prepared according to Gnizozub *et al.* (1994). Extraction and purification of teichoic acids, structural determination, molecular mass determination and other analytical procedures have been described previously (Naumova *et al.*, 1990; Tul'skaya *et al.*, 1991). Pyruvic acid was quantified according to Telegina (1980). The amount of periodate consumed during oxidation was quantified according to Dixon & Lipkin (1954).

Ribitol content of the cell wall was assayed after hydrolysis of the wall with 40% (v/v) aqueous HF at 20 °C for 24 h; glycerol was quantified after hydrolysis with 2 M HCl at 100 °C for 5 h and subsequent treatment of the hydrolysate with phosphomonoesterase (EC 3.1.3.1, Sigma). Polyols were resolved by paper chromatography in system B and quantified by measuring the amount of formaldehyde formed after periodate oxidation (Hanahan & Olley, 1958).

Chromatography and electrophoresis. These were carried out on Filtrak FN-13 paper. Electrophoresis was run in the pyridine/acetate buffer A of Tul'skaya *et al.* (1991). The following solvent systems were used in descending paper chromatography: (B) butan-1-ol/pyridine/benzene/water (5:3:1:3, by vol.) to separate polyols, glycosides and

Abbreviations: Gro, glycerol; Rbo, ribitol.

aminosugars; (C) pyridine/ethyl acetate/acetic acid/water (5:5:1:3, by vol.) to separate aminosugars; (D) propan-1-ol/2 M NH_4OH (7:3, v/v) and (E) amyl alcohol/5 M formic acid (1:1, v/v) to identify pyruvic acid.

To detect the compounds, the following spray reagents were used: the reagent of Isherwood for phosphorus-containing compounds, 5% (w/v) AgNO_3 in aqueous NH_3 for polyols, glycosides and glucosamine; ninhydrin for glucosamine; aniline xylose (Hais & Macek, 1958) to identify pyruvic acid.

NMR. ^{13}C and ^1H NMR spectra of the teichoic acids solutions were recorded under the conditions described previously (Gnilozub *et al.*, 1994). The ^1H -NMR and ^{13}C -NMR spectra were recorded with a Bruker WM-250 and a AM-300 respectively, at 60 °C with acetone as internal standard ($\delta_{\text{H}} = 2.225$ p.p.m., $\delta_{\text{C}} = 31.45$ p.p.m.). Two-dimensional ^1H nuclear Overhauser effects (nOe) in rotating frames (Shashkov *et al.*, 1994) were obtained using a standard Bruker program from the software package for the Aspect 3000 computers for ROESY. The HMQC spectrum (Bax & Subramanian, 1986) was taken with the AM-300 spectrometer without suppression proton-carbon coupling constants and using a standard Bruker program.

RESULTS AND DISCUSSION

Results of the studies on the teichoic acids from three strains are described. Except for the quantitative differences discussed below, the results were identical with all strains.

Teichoic acid was obtained by extraction of the cell walls with 10% trichloroacetic acid. To prevent possible contamination with lipoteichoic acid, cell walls were treated with SDS before extraction. Acid hydrolysates of the preparation contained glycerol, ribitol, phosphorus, glucosamine, and pyruvic acid. This implied the possible presence of several teichoic acids. This conclusion was substantiated by the results of the electrophoretic analysis of the preparation in buffer A. Three phosphorus-containing zones were detected.

Efforts to use preparative electrophoresis to completely resolve the zones and to accumulate amounts sufficient for further analysis failed. Therefore, in the subsequent work, the preparation isolated from the crude fat-free mycelium by extraction with 10% trichloroacetic acid (Archibald, 1972) was used throughout. Teichoic acids were separated and purified by ion-exchange chromatography on a DEAE-Toyopearl 650 M column in a linear NaCl gradient (0–0.5 M). Three phosphorus-containing fractions were eluted: fraction 1 at 0.16–0.19 M NaCl; fraction 2 at 0.22 M NaCl; and fraction 3 at 0.27–0.30 M NaCl. Each fraction was pooled, dialysed against deionized water, lyophilized, and analysed separately.

Fraction 1

Qualitative analysis of this fraction revealed the presence of ribitol and phosphorus. Acid hydrolysis yielded ribitol mono- and diphosphates. The same esters were detected as the products of the alkaline hydrolysis of the polymer.

The results of acid and alkaline degradations suggested that the polymer was an unsubstituted poly(ribitol phos-

phate). In all poly(ribitol phosphates) from the bacterial cell walls described hitherto, ribitol units are joined by phosphodiester bonds involving C-1 and C-5 hydroxy groups (1,5-type). On periodate oxidation of polymers of this type, formaldehyde is formed only from the terminal ribitol residue (Armstrong *et al.*, 1961). However, periodate oxidation of the polymer resulted in the formation of formaldehyde in an amount equimolar to phosphorus content. This was interpreted as evidence of a different position of the phosphodiester bond.

In the course of polymer biosynthesis, D-ribitol-5-phosphate moieties originating from the nucleotide precursor are transferred to a growing polymer chain (Baddiley, 1972). Therefore, formation of the phosphodiester bond should necessarily involve the ribitol C-5 hydroxy group. Assuming that the other hydroxy group forming the phosphodiester bond is located at C-4 or C-3, one should expect formation of 1 mole of formaldehyde for each ribityl residue making up the polymer chain.

One mole of NaIO_4 per mole phosphate was consumed during oxidation of the polymer. The only explanation of this result is that the hydroxy group at C-3 of ribitol is involved in phosphodiester bond formation.

The polymer structure was independently examined by NMR spectroscopy. The ^{13}C -NMR spectrum (Fig. 1, Table 1) contained five main signals and several minor signals derived from the contaminating fraction 2 (see below). Some of the main signals had the characteristic splitting at phosphorus atoms. The presence of five signals was evidence of the asymmetrical substitution of ribitol. The APT spectrum (Patt & Shoolery, 1982) showed that the signals at 68.3 p.p.m. and 64.0 p.p.m. could be assigned to CH_2 groups, and the other three to CH groups. The downfield position of signals at 68.3 p.p.m. (CH_2O) and 78.6 p.p.m. (CHO) indicated that they belonged to the carbon atoms substituted with phosphate groups. The signal from one of the CH_2 groups shifted upfield (64.0 p.p.m.) had no characteristic splitting due to the phosphorus atom which ruled out the 2,5 type of phosphodiester linkage. However, consistent with the 3,5 type of linkage, all the signals from the CH groups were split. If the phosphodiester bond had linked the OH groups at C-4 and C-5, the signal from C-2 would not have been split. Pseudo-triplet splitting of the signal from C-4 due to interaction with two phosphorus atoms provided additional indication of the ribitol residue being substituted at positions 3 and 5.

Thus, teichoic acid 1 is an unsubstituted 3,5-poly(ribitol phosphate). The molecular mass of the teichoic acid was determined by Sephadex G-50 gel filtration chromatography using reference teichoic acids and found to be about 5600 Da. This suggests a chain length of about 26–27 ribitol phosphate units (Fig. 2).

Fraction 2

Acid hydrolysis of fraction 2 yielded glycerol mono- and diphosphates, glucosamine, glycerol and inorganic phosphate. Alkaline hydrolysis of the polymer yielded glycerol

Fig. 1. ^{13}C -NMR spectrum of teichoic acid 1 from the cell wall of *N. albus* subsp. *albus* DSM 43120. Expanded signals are shown above. The spectrum contains minor signals arising from contamination with teichoic acid 2.

Table 1. Chemical shifts of carbons in the ^{13}C spectra of cell-wall teichoic acid from *N. albus* subsp. *albus*

The coupling constants $^2J_{\text{P,C}}$ and $^3J_{\text{P,C}}$ (in Hz) are shown in parentheses.

Teichoic acid	Fragment of teichoic acid	C-1	C-2	C-3	C-4	C-5	C-6	CH_3CO
1	-(3Rbo5P)-	64.0	72.5 (5)	78.6 (7)	72.3 (8)	68.3 (5)		
2	-(1Gro3P)-	67.7 (6)	70.85 (8)	67.7 (6)				
	-(1Gro3P)- 2 ↑	66.4*	77.4	65.7*				
	D-GlcNAc α 1	98.0	55.0	72.5	71.4	73.5	62.1	23.4; 175.6
3	-(1Rbo5P)- ↗ ↖ 2 4 ↖ ↗ Pyr	65.45 ($\approx$ 5)	76.6 (8)	61.2	76.6 (8)	65.45 ($\approx$ 5)		
		176.6	102.1	25.8				

* Arbitrary assignment.

mono- and diphosphates, glycerol and a small amount of glucosamine-containing glycerol phosphodiester. Hydrolysis of the polymer with 40% aqueous HF yielded glycerol and a glycoside. The latter was accumulated by preparative paper chromatography in system B and subjected to analysis. It displayed an R_{Gro} of 0.70, was not stained with ninhydrin or aniline phthalate, yielded equimolar amounts of glycerol and glucosamine on acid hydrolysis (6 M HCl, 100 °C, 6 h), and no formaldehyde upon periodate oxidation. These results showed that the glucosamine was in the pyranose form, was linked via a glycoside bond to the secondary hydroxyl of glycerol, and had an *N*-acyl substituent. On the basis of these data the polymer under investigation was inferred to be a 1,3-poly(glycerol phosphate) partially substituted at C-2 with *N*-acetylglucosamine. The molar ratio of the constituents P-Gro-GlcN in the polymer was 1.0:1.06:0.08 (1:1:0.1).

The ^{13}C NMR spectra were typical of the 1,3-poly(glycerol phosphate) partially substituted with α -*N*-acetylglucosamine (Table 1) (Naumova *et al.*, 1982).

The molecular mass of the polymer, estimated in the same way as that of polymer 1 was 7600 Da. Based on the molar content of the polymer constituents, this suggested a chain length of 40–41 glycerol phosphate units (Fig. 2).

Thus, teichoic acid 2 is a 1,3-poly(glycerol phosphate) with about 10% of glycerol phosphate units being substituted with α -*N*-acetylglucosamine residues.

Fraction 3

Fraction 3 polymer was eluted from the column at high NaCl concentration (0.3 M) suggesting a higher negative charge as compared to the fraction 1 and 2 polymers.

Fig. 2. Chromatography of cell-wall teichoic acids from *N. albus* subsp. *albus* on a Sephadex G-50 column (990 mm $\times$ 9.5 mm; fraction volume 2 ml). $\circ$, Teichoic acid 1; $\triangle$, teichoic acid 2; $\square$, teichoic acid 3. Molecular mass standards ($\bullet$): 1', teichoic acid from *Streptomyces antibioticus* (7000 Da) (Shashkov *et al.*, 1979); 2', teichoic acid from *S. violaceus* (4000 Da) (Naumova *et al.*, 1969); 3', teichoic acid from *S. azureus* (3300 Da) (Streshinskaya *et al.*, 1981); 4', glycerol monophosphate.

Electrophoretic behaviour of the polymer was in agreement with this conclusion (E_{GROF} 1.96). Acid hydrolysis of the polymer yielded ribitol mono- and diphosphates, ribitol, inorganic phosphate and pyruvic acid. The latter was identified by comparison with an authentic sample using paper chromatography in systems D and E. Glycoside substituents were not detected. Alkaline hydrolysis and periodate oxidation did not degrade the polymer.

Table 2. Data from ^1H NMR spectra of teichoic acid 3

Proton	Chemical shift (δ , p.p.m.)	Coupling constant (J , Hz)
H-1a, H-5a	4.23	$J_{1a,1b}$ 12.5
H-1b, H-5b	4.17	$J_{1b,2}$ 4.5
H-2, H-4	3.88	$J_{1a,2}$ 2.5
H-3	3.81	$J_{2,3}$ 9.5
CH_3	1.54	

Ribitol mono- and diphosphate formation on acid hydrolysis suggested that the teichoic acid was poly(ribitol phosphate). The resistance of the polymer to alkaline hydrolysis indicated the absence of free hydroxyl groups in the vicinity of phosphodiester groups (Kelemen & Baddiley, 1961), while resistance to periodate oxidation was indicative of the absence of non-substituted glycol groups in the polymer. It was conceivable that ribitol residues were substituted with pyruvic acid bound via an alkali-stable ketal bond (Thurrow *et al.*, 1975). The complete removal of pyruvate was achieved within 3 h on acid hydrolysis (2 M HCl, 100 $^\circ\text{C}$). The polymer released only 10% of its pyruvic acid under conditions (2 M HCl, 4 h, 100 $^\circ\text{C}$) employed previously (Thurrow *et al.*, 1975) to completely hydrolyse the 4,6-ketal of its pyruvic acid and galactose.

The stability of the bond formed by pyruvate with ribitol prevented removal of the substituent without hydrolysis of the polymer chain. This, in turn, prevented determination of the localization of both the phosphodiester link and pyruvate.

The polymer was analysed by NMR spectroscopy. The ^{13}C NMR spectrum contained six main signals, two of

Fig. 3. HMQC spectrum of teichoic acid 3 from the cell wall of *N. albus* subsp. *albus* DSM 43120. One-dimensional ^{13}C -NMR spectrum of teichoic acid 3 is shown on the left. Signals from CH_3 at 25.8 p.p.m., from quaternary carbon atom at 102.1 p.p.m., and from $\text{C}=\text{O}$ at 176.6 p.p.m. are not shown. The spectrum contains minor signals arising from contamination with teichoic acid 2 (imp. of 2).

Table 3. Phosphorus, polyols and pyruvic acid contents and chain ratios of teichoic acids in the cell wall of *N. albus* subsp. *albus*

Strain	Phosphorus	Ribitol	Glycerol	Pyruvic acid	Chain ratio		
	μmol (100 mg cell wall) ⁻¹				1	2	3
DSM 43377 ^T	114.9	50.7	60.2	32.4	4	8	10
DSM 43378	100.3	58.8	37.8	30.6	6	5	10
DSM 43120	99.6	66.2	29.6	31.4	8	4	10

which were of double integral intensity and were either broadened or split due to interaction with phosphorus atoms (Fig. 3). Three signals at 25.8 p.p.m. (CH_3), 102.1 p.p.m. (quaternary carbon atom), and 176.6 p.p.m. (CO) were unequivocally identified as belonging to a pyruvic acid residue in a six-membered ring (Garegg *et al.*, 1980). Hence, the other three signals, including the two of double intensity mentioned above belonged to the five carbon atoms of the ribitol residue. This was evidence of symmetrical substitution of ribitol residues with pyruvate. Broadening of the signal at 65.45 p.p.m. (CH_2 group, APT-spectrum) and splitting of the signal at 76.6 p.p.m. were indicative of a 1,5-poly(ribitol phosphate) chain.

Additional evidence of the polymer structure was furnished by the ^1H NMR spectrum. The polymer proton spectrum contained the singlet of the CH_3 group of pyruvate at 1.54 p.p.m. (Table 2) and three series of peaks with the integral intensity ratio 1:2:4. These signals were assigned using homonuclear double resonance. The magnitudes of the coupling constants were typical of an axial orientation of all the protons in the six-membered ring.

As shown in Table 1, two-dimensional homonuclear $^1\text{H}/^{13}\text{C}$ spectrum (HMQC) (Bax & Subramanian, 1986) (Fig. 3) confirmed the assignment of the signals in the carbon spectrum. The downfield position of the signal from the CH_3 group of pyruvate was unequivocal evidence of an equatorial orientation in the six-membered ring (Garegg *et al.*, 1980). Therefore, the repeating unit of the polymer has the structure depicted in Fig. 3.

The molecular mass of the polymer estimated in the same way as that of polymers 1 and 2 was about 5400 Da corresponding to a chain length of about 18 repeating units (Fig. 2).

To assess the amounts of these polymers in the cell walls of the three strains under study, glycerol and ribitol were quantified (Table 3). In a preliminary experiment, treatment of the cell wall with 40% aqueous HF (24 h, 20 °C) was shown to result in a complete cleavage of the ketal bond. Based on the content of ribitol and pyruvic acid in the cell walls and taking into account the complete substitution of polymer 3 with pyruvate, the proportions of polymers 1 and 3 were estimated. Knowing a polymer chain length, one can calculate the ratio of the teichoic

acid chains in the cell walls. Cell walls of the strains differed quantitatively in the content of the three teichoic acids (Table 3).

Thus, the cell wall of *Nocardiosis albus* subsp. *albus* contains three structurally distinct teichoic acids. 3,5-poly(ribitol phosphate) and pyruvylated 1,5-poly(ribitol phosphate) are reported in prokaryotes for the first time. Pyruvic acid was reported as a component of mannitol teichoic acid isolated from *Brevibacterium iodinum* NCTC 9742 cell wall (Anderton & Wilkinson, 1980). 1,3-Poly(glycerol phosphate) with α -N-acetylglucosaminyl substituents was previously described in the cell wall of a *Streptomyces* species (Naumova *et al.*, 1982).

The bacterial cell wall is known to be structurally heterogeneous (Graham & Beveridge, 1994). This heterogeneity may stem from the structural variability of cell wall teichoic acids. Being non-uniformly localized in the cell wall, these teichoic acids would perform different physiological functions, in particular furnishing both autolysin-sensitive and -resistant areas and making up domains with different anionic properties.

The fact that each of the three strains of *N. albus* subsp. *albus* examined has an identical set of cell wall teichoic acids taken together with the recent observation on the structure of the teichoic acids from *N. dassonvillei* strains (Tul'skaya *et al.*, 1993; Akimov *et al.*, 1994) may serve as an indication of species-specificity of these polymers in the genus *Nocardiosis*.

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REFERENCES

- Akimov, V. N., Taran, V. V., Evtushenko, L. I., Naumova, I. B. & Kalakoutskii, L. V. (1994). Grouping of *Nocardiosis* strains on the basis of DNA relatedness. In *The 9th International Symposium on the Biology of Actinomycetes (Theses)*, P 3-34, p. 242. July 10-15, Moscow, Russia.

- Altai, A. M. & Ruan, J. S. (1994).** *Nocardiopsis halophilus* sp. nov., a new halophilic actinomycete isolated from soil. *Int J Syst Bacteriol* **44**, 474–478.
- Anderton, W. J. & Wilkinson, S. G. (1980).** Evidence for the presence of a new class of teichoic acid in the cell wall of bacterium NCTC 9742. *J Gen Microbiol* **118**, 343–351.
- Archibald, A. R. (1972).** Teichoic acids. *Methods Carbohydr Chem* **6**, 162–172.
- Armstrong, J. J., Baddiley, J. & Buchanan, J. G. (1961).** Further studies on the teichoic acid from *Bacillus subtilis* walls. *Biochem J* **80**, 254–261.
- Baddiley, J. (1972).** Teichoic acids in cell walls and membranes of bacteria. *Essays Biochem* **8**, 35–77.
- Bax, A. & Subramanian, S. (1986).** Sensitivity-enhanced two-dimensional heteronuclear shift correlation NMR spectroscopy. *J Magnet Reson* **67**, 565–569.
- Dixon, J. S. & Lipkin, D. L. (1954).** Spectrophotometric determination of vicinal glycols. Application to the determination of ribofuranosides. *Anal Chem* **26**, 1092–1093.
- Garegg, P. J., Jansson, P. E., Lindberg, B., Lindh, F., Lönngren, J., Kvarnstrom, I. & Nimmich, W. (1980).** Configuration of the acetal carbon atom of pyruvic acid acetals in some bacterial polysaccharides. *Carbohydr Res* **78**, 127–132.
- Gnilozub, V. A., Streshinskaya, G. M., Evtushenko, L. I., Naumova, I. B. & Shashkov, A. S. (1994).** The 1,5-poly(ribitol phosphate) with tetrasaccharide substituents in the cell wall of *Agromyces fucosus* subsp. *hippuratus*. *Biokhimiya* **59**, 1892–1899.
- Graham, L. L. & Beveridge, T. J. (1994).** Structural differentiation of the *Bacillus subtilis* 168 cell wall. *J Bacteriol* **176**, 1413–1421.
- Grund, E. & Kroppenstedt, R. M. (1990).** Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5–11.
- Hais, I. M. & Macek, K. (1958).** *Handbuch der Papierchromatographie*, p. 739. Jena: Gustav Fischer Verlag.
- Hanahan, D. J. & Olley, J. N. (1958).** Chemical nature of monophosphoinositides. *J Biol Chem* **231**, 813–828.
- Kelemen, M. V. & Baddiley, J. (1961).** Structure of the intracellular glycerol teichoic acid from *Lactobacillus casei* ATCC 7469. *Biochem J* **80**, 246–254.
- Labeda, D. P., Testa, R. T., Lechevalier, M. P. & Lechevalier, H. A. (1984).** *Saccharothrix*: a new genus of *Actinomycetales* related to *Nocardiopsis*. *Int J Syst Bacteriol* **34**, 426–431.
- Meyer, J. (1976).** *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.
- Naumova, I. B., Rogozina, S. V. & Zaretskaya, M. Sh. (1969).** On the structure of the cell wall teichoic acid from *Actinomyces violaceus*. *Dok Akad Nauk* **188**, 710–712.
- Naumova, I. B., Kuznetsov, V. D., Kudrina, K. S. & Bezzubenkova, A. P. (1980).** The occurrence of teichoic acids in *Streptomyces*. *Arch Microbiol* **126**, 71–75.
- Naumova, I. B., Shashkov, A. S., Skoblilova, N. K., Agre, N. S. & Romanov, V. V. (1982).** Lysylteichoic acid of the cell wall of *Streptomyces roseoflavus* var. *roseofungini* 1128. *Bioorg Khim* **8**, 848–856.
- Naumova, I. B., Yanushkene, N. A., Streshinskaya, G. M. & Shashkov, A. S. (1990).** Cell wall anionic polymers and peptidoglycan of *Actinoplanes philippinensis* VKM Ac-647. *Arch Microbiol* **154**, 483–488.
- Patt, S. I. & Shoolery, I. N. (1982).** Attached proton test for carbon-13 NMR. *J Magnet Reson* **46**, 535–539.
- Shashkov, A. S., Zaretskaya, M. Sh., Yarotsky, S. V., Naumova, I. B., Chizhov, O. S. & Shabarova, Z. A. (1979).** On the structure of the teichoic acid from the cell wall of *Streptomyces antibioticus* 39. *Eur J Biochem* **102**, 477–481.
- Shashkov, A. S., Kochanowski, H., Kozlova, Yu. I., Streshinskaya, G. M., Naumova, I. B., Terekhova, L. P. & Galatenko, O. A. (1994).** Teichuronic acid of the cell wall of *Actinoplanes brasiliensis*. *Biochim Biophys Acta* **1201**, 333–338.
- Streshinskaya, G. M., Naumova, I. B., Romanov, V. V. & Shashkov, A. S. (1981).** Structure of ribitol teichoic acid from the cell wall of *Streptomyces azureus* RIA 1009. *Bioorg Khim* **7**, 1410–1418.
- Telegina, T. A. (1980).** Quantity determination of pyruvic acid. In *Biochemical Methods*, pp. 154–157. Moscow: Nauka.
- Thurrow, H., Choy, Y. M., Frank, N. & Niemann, H. (1975).** The structure of *Klebsiella* serotype 11 capsular polysaccharide. *Carbohydr Res* **41**, 241–255.
- Tul'skaya, E. M., Vylegzhanina, K. S., Streshinskaya, G. M., Shashkov, A. S. & Naumova, I. B. (1991).** 1,3-Poly(glycerol phosphate) chains in the cell wall of *Streptomyces rutgersensis* var. *castelarensis* VKM Ac-238. *Biochim Biophys Acta* **1074**, 237–242.
- Tul'skaya, E. M., Streshinskaya, G. M., Naumova, I. B., Shashkov, A. S. & Terekhova, L. P. (1993).** A new structural type of teichoic acid and some chemotaxonomic criteria of two species *Nocardiopsis dassonvillei* and *Nocardiopsis antarcticus*. *Arch Microbiol* **160**, 299–305.
- Tul'skaya, E. M., Shashkov, A. S., Evtushenko, L. I., Taran, V. V. & Naumova, I. B. (1995).** The cell wall teichoic acids of *Nocardiopsis albus* subsp. *albus* DSM 43120. *Biokhimiya* **60**, 179–186.

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