Microbiology, Vol. 69, No. 1, 2000, pp. 48–50. Translated from Mikrobiologiya, Vol. 69, No. 1, 2000, pp. 58–61.

EXPERIMENTAL ARTICLES

Cell Wall Teichoic Acids of *Nocardiopsis prasina* VKM Ac-1880^T

E. M. Tul'skaya*, A. S. Shashkov, L. I. Evtushenko***, and I. B. Naumova***

**Biological Faculty, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia*
***Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii prospekt 47, Moscow, 117913 Russia*
****Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow oblast, 142292 Russia*

Received May 12, 1999

Abstract—The cell wall of *Nocardiopsis prasina* VKM Ac-1880^T was found to contain two structurally different teichoic acids: unsubstituted 3,5-poly(ribitol phosphate) and 1,3-poly(glycerol phosphate) substituted at position 2 by 10% with α-N-acetylglucosamine and by 5% with *O*-acetyl groups. The structure of the polymers was studied by chemical analysis and NMR spectroscopy. The results obtained correlate well with 16S rRNA sequence data and confirm the species-specificity of teichoic acids in the genus *Nocardiopsis*.

Key words: cell wall, teichoic acid, *Nocardiopsis*, taxonomy.

Recent investigations indicate that the genus *Nocardiopsis* belonging to the order *Actinomycetales* consists of at least nine species [1–4].

Of interest is the fact that the structural type of the cell wall teichoic acids of actinomycetes of the genus *Nocardiopsis* can be used for taxonomic purposes as a species-specific criterion.

Thus, the cell walls of all the *Nocardiopsis dassonvillei* subsp. *dassonvillei* strains studied contained a hitherto unknown unique teichoic acid [5]. Teichoic acid of the same structure has also been found in the cell wall of *N. antarctica*, and a similar polymer with a succinyl substituent, in the cell wall of *N. dassonvillei* subsp. *albirubida* [6]. It should be noted that the last two organisms exhibited a high degree of DNA homology with the type strain of *N. dassonvillei* and have been reclassified as members of this species [4].

Similarly, the cell walls of three *N. alba* strains contained structurally identical teichoic acids [7].

The cell wall teichoic acids of some other species of the genus *Nocardiopsis* have also demonstrated species-specificity [8].

In this work, we investigated teichoic acids in the cell wall of the species *N. prasina*, which was not studied earlier.

MATERIALS AND METHODS

The strain *Nocardiopsis prasina* VKM Ac-1880^T was grown in shaken flasks with glucose–yeast extract medium [9] to the mid-exponential growth phase. The mycelium was harvested by centrifugation, washed with 0.95% NaCl, and used to prepare cell walls and to extract teichoic acids. In the latter case, the mycelium was preliminarily defatted and dried with ethanol and acetone.

Cell walls were prepared from ultrasonically disrupted mycelium by differential centrifugation. To prevent a possible contamination with lipoteichoic acid, the crude preparation of cell walls was treated with sodium dodecyl sulfate [10].

Extraction, purification, and hydrolysis of teichoic acids with mineral acids and alkali, as well as the identification of glucosamine, phosphate esters, and glycoside, molecular mass determination, and other analytical procedures were performed as described previously [11, 12]. The amount of oxidized periodate was determined spectrophotometrically [13].

Descending paper chromatography and electrophoresis were carried out on a Filtrak FN-2 paper (Germany), which was preliminarily washed with acetic acid and then with distilled water to the neutral reaction.

Phosphate esters were separated by electrophoresis in pyridine–acetate buffer, pH 5.6 (system A) [12]. Polyols, glycosides, and monosaccharides were separated by descending paper chromatography in a butan-1-ol–pyridine–benzene–water (5 : 3 : 1 : 3) mixture (system B); and amino sugars, in a pyridine–ethyl acetate–acetic acid–water (5 : 5 : 1 : 3) mixture (system C). After separation, phosphorus-containing compounds were detected with the Isherwood reagent; polyols, glycosides, and glucosamine, with 5% $AgNO_3$ in ammonium hydroxide; and glucosamine, with ninhydrin.

The NMR spectra of teichoic acids were recorded as described previously [10].

0026-2617/00/6901-0048$25.00 © 2000 MAIK "Nauka/Interperiodica"

Table 1. Chemical shifts δ (ppm) of the cell wall teichoic acids of *N. prasina* VKM Ac-1880^T

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(d, 5)	78.6(d, 7)	72.3(t, 8)	68.3(d, 5)		
2	–(1Gro3P)–	67.7(6)	70.85(8)	67.7(6)				
	–(1Gro3P)–	66.4*	77.4	65.7*				
	2 ↑							
	DGlcNAcpα1	98.0	55.0	72.5	71.4	73.5	62.1	23.4; 175.6
	–(1Gro3P)–	65.1	73.7	65.1				
	2 | AcO							21.9; 174.8

* Alternative assignment is possible; "d" and "t" stand for "doublet" and "triplet," respectively. Parenthesized are the scalar coupling constants $^2J_{P,C}$ and $^3J_{P,C}$ expressed in Hz.

RESULTS AND DISCUSSION

The acid hydrolysate of the cell wall of *N. prasina* contained 2.25% of acid-stable phosphates, as well as glycerol and ribitol, which might be indicative of the presence of several teichoic acids in the cell wall of this species.

Teichoic acids were extracted from the defatted mycelium with 10% trichloroacetic acid [14]. The subsequent separation and purification of teichoic acids were accomplished by ion-exchange chromatography on a DEAE-Toyopearl 650 M column in a linear NaCl gradient (0–0.5M). Two phosphorus-containing fractions eluted at 0.17 M NaCl (fraction 1) and 0.22 M NaCl (fraction 2) were dialyzed against distilled water, lyophilized, and analyzed for teichoic acids.

Fraction 1. The acid and alkaline hydrolyses of fraction 1 yielded ribitol, ribitol mono- and diphosphate, and inorganic phosphate, indicating the presence of unsubstituted poly(ribitol phosphate) in this fraction. The periodate oxidation of fraction 1 resulted in the formation of equimolar quantities of formaldehyde and phosphorus. In this case, one mole of $NaIO_4$ per one mole of phosphate was consumed. Similar results were obtained in our previous study of fraction 1 of the cell wall teichoic acids of *N. alba* representing unsubstituted 3,5-poly(ribitol phosphate) [7]. Thus, it was reasonable to assume that teichoic acid 1 of *N. prasina* was also unsubstituted 3,5-poly(ribitol phosphate).

Indeed, the NMR spectroscopy of this polymer (Table 1) showed that the ^{13}C-NMR spectrum of fraction 1 was completely identical to that of teichoic acid 1 from *N. alba* [7].

The molecular mass of teichoic acid 1 determined by gel filtration on Sephadex G-50 turned out to be 9.4 kDa, which corresponded to a polymeric chain composed of 41 ribitol phosphate units.

Fraction 2. The acid hydrolysis of fraction 2 yielded glycerol, glycerol mono- and diphosphate, glucosamine, and inorganic phosphate. The alkaline hydrolysis of fraction 2 yielded glycerol, glycerol mono- and diphosphate, and a small amount of glucosamine-containing glycerol phosphodiesters.

The hydrolysis of fraction 2 with 40% aqueous HF yielded glycerol and a glycoside with $R_{Gro} = 0.70$ in solvent system B during descending paper chromatography. The glycoside failed to be stained with ninhydrin or aniline phthalate, yielded equimolar quantities of glycerol and glucosamine during acid hydrolysis (6 M HCl; 100°C; 6 h), and did not produce formaldehyde during periodate oxidation. All this suggested that glucosamine was N-acylated, occurred in the pyranose form, and was linked to the C-2 atom of glycerol by a glycosidic bond.

Based on these data, teichoic acid 2 was assumed to be 1,3-poly(glycerol phosphate) partially substituted at position 2 with N-acylglucosamine.

The ^{13}C-NMR spectrum of this polymer was typical of 1,3-poly(glycerol phosphate) partially substituted with α-N-acetylglucosamine [7] and *O*-acetyl groups [15] (Table 1).

The molecular mass of teichoic acid 2 was estimated to be 6 kDa, which corresponded to a polymeric chain composed of 33 glycerol phosphate units.

A more detailed analysis showed that teichoic acid 2 is 1,3-poly(glycerol phosphate) substituted by 10% with α-N-acetylglucosamine and by 5% with *O*-acetyl groups.

Thus, the cell wall of *N. prasina* VKM Ac-1880^T contains two structurally different teichoic acids, unsubstituted 3,5-poly(ribitol phosphate) and 1,3-poly(glycerol phosphate) substituted by 10% with α-N-acetylglucosamine and by 5% with *O*-acetyl groups. Similar teichoic acids were found earlier in the cell walls of

Table 2. Cell wall teichoic acids of members of the phylogenetic cluster II of the genus *Nocardiopsis*

Teichoic acid	*N. alba* [7]	*N. prasina*	*N. listeri* [8]	*N. lucentensis* [8]
1	+	+	–	–
2	+	+	+	+
3	+	–	+	–

Note: "+" and "–"" stand, respectively, for the presence and absence of a particular teichoic acid in the cell wall of the given species.

three *N. alba* strains, but teichoic acid 2 of these strains had no *O*-acetyl residues. These strains contained also teichoic acid 3, representing 1,5-poly(ribitol phosphate) completely substituted with pyruvic acid [7].

Based on the results of 16S rRNA analysis, Rainey and coworkers [16] assigned *N. prasina* and *N. alba* to the same phylogenetic cluster as *N. listeri* and *N. lucentensis* [4, 16], whose cell walls contain some of the aforementioned teichoic acids (Table 2). Thus, it becomes evident that the structural type of cell wall teichoic acids in the representatives of the genus *Nocardiopsis* is a species-specific characteristic.

ACKNOWLEDGMENTS

This work was supported, in part, by the INTAS grant no. 1571, the Russian Foundation for Basic Research, project no. 98-04-49277, and the Russian State Scientific Program "Universities of Russia."

REFERENCES

1. Grund, E. and Kroppenstedt, R.M., Chemotaxonomy and Numerical Taxonomy of the Genus *Nocardiopsis* Meyer 1976, *Int. J. Syst. Bacteriol.*, 1990, vol. 40, no. 1, pp. 5–11.
2. Labeda, D.P., Testa, R.T., Lechevalier, M.P., and Lechevalier, H.A., *Saccharothrix*: A New Genus of *Actinomycetales* Related to *Nocardiopsis, Int. J. Syst. Bacteriol.*, 1984, vol. 34, no. 2, pp. 426–431.
3. Altai, A.M. and Ruan, J.S., *Nocardiopsis halophilus* sp. nov., a New Halophilic Actinomycete Isolated from Soil, *Int. J. Syst. Bacteriol.*, 1994, vol. 44, no. 2, pp. 474–478.
4. Evtushenko, L.I., Taran, V.V., Akimov, V.N., Kroppenstedt, R.M., Tiedje, J.M., and Stackebrandt, E., *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosei* sp. nov., nom. rev., and Subspecies *Nocardiopsis sassonvillei* subsp. *albirubida* subsp. nov., comb. nov., *Int. J. Syst. Bacteriol.*, 2000, vol. 50 (in press).
5. Tul'skaya, E.M., Streshinskaya, G.M., Naumova, I.B., Shashkov, A.S., and Terekhova, L.P., A New Structural Type of Teichoic Acid and Some Chemotaxonomic Criteria of Two Species, *Nocardiopsis dassonvillei* and *Nocardiopsis antarcticus, Arch. Microbiol.*, 1993, vol. 160, no. 4, pp. 299–305.
6. Shashkov, A.S., Streshinskaya, G.M., Kozlova, Yu.I., Potekhina, N.V., Taran, V.V., Evtushenko, L.I., and Naumova, I.B., The Structure of Cell Wall Teichoic Acid of *Nocardiopsis alborubida, Biokhimiya*, 1997, vol. 62, no. 10, pp. 1127–1131.
7. Tul'skaya, E.M., Shashkov, A.S., Evtushenko, L.I., Taran, V.V., and Naumova, I.B., Novel Cell-Wall Teichoic Acid from *Nocardiopsis albus* subsp. *albus* as a Species-Specific Marker, *Microbiology* (Reading, UK), 1995, vol. 141, no. 8, pp. 1851–1856.
8. Streshinskaya, G.M., Tul'skaya, E.M., Shashkov, A.S., Evtushenko, L.I., Taran, V.V., and Naumova, I.B., Cell Wall Teichoic Acids of *Nocardiopsis listeri, Nocardiopsis lucentensis*, and *Nocardiopsis trehalosei, Biokhimiya*, 1998, vol. 63, no. 2, pp. 230–234.
9. Naumova, I.B., Kuznetsov, V.D., Kudrina, K.S., and Bezzubenkova, A.P., The Occurrence of Teichoic Acids in Streptomycetes, *Arch. Microbiol.*, 1980, vol. 126, no. 1, pp. 71–75.
10. Gnilozub, V.A., Streshinskaya, G.M., Evtushenko, L.I., Naumova, I.B., and Shashkov, A.S., 1,5-Poly(Ribitol Phosphate) with a Tetrasaccharide Substituent in the Cell Wall of *Agromyces fucosus* subsp. *hippuratus, Biokhimiya*, 1994, vol. 59, no. 12, pp. 1892–1899.
11. Naumova, I.B., Yanushkene, N.A., Streshinskaya, G.M., and Shashkov, A.S., Cell Wall Anionic Polymers and Peptidoglycan of *Actinoplanes philippinensis* VKM Ac-647, *Arch. Microbiol.*, 1990, vol. 154, pp. 483–488.
12. Tul'skaya, E.M., Vylegzhanina, K.S., Streshinskaya, G.M., Shashkov, A.S., and Naumova, I.B., 1,3-Poly(Glycerol Phosphate) Chains in the Cell Wall of *Streptomyces rutgersensis* var. *castelarense* VKM Ac-238, *Biochim. Biophys. Acta*, 1991, vol. 1074, pp. 237–242.
13. Dixon, J.S. and Lipkin, D.L., Spectrophotometric Determination of Vicinal Glycols: Application to the Determination of Ribofuranosides, *Anal. Chem.*, 1954, vol. 26, no. 8, pp. 1092–1093.
14. Archibald, A.R., Teichoic Acids, *Methods Carbohydr. Chem.*, 1972, vol. 6, pp. 162–172.
15. Gnilozub, V.A., Streshinskaya, G.M., Evtushenko, L.I., Shashkov, A.S., and Naumova, I.B., Lipoteichoic Acids of Some Species of the Genus *Agromyces, Mikrobiologiya*, 1994, vol. 63, no. 3, pp. 495–502.
16. Rainey, F.A., Ward-Rainey, N., Kroppenstedt, R.M., and Stackebrandt, E., The Genus *Nocardiopsis* Represents a Phylogenetically Coherent Taxon and a Distinct Actinomycete Lineage: Proposal of *Nocardiopsaceae* fam. nov., *Int. J. Syst. Bacteriol.*, 1996, vol. 46, no. 4, pp. 1088–1092.