ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 12, pp. 1368-1374. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © N. V. Potekhina, G. M. Streshinskaya, Yu. I. Kozlova, E. B. Kudryashova, S. N. Senchenkova, A. S. Shashkov, L. N. Anan'ina, 2009, published in Biokhimiya, 2009, Vol. 74, No. 12, pp. 1678-1685.

Heterogeneous Set of Cell Wall Teichoic Acids in Strains of *Bacillus subtilis* VKM B-760 and VKM B-764

N. V. Potekhina[1]*, G. M. Streshinskaya[1], Yu. I. Kozlova[1], E. B. Kudryashova[2], S. N. Senchenkova[3], A. S. Shashkov[3], and L. N. Anan'ina[4]

[1]*Faculty of Biology, Lomonosov Moscow State University, 119899 Moscow, Russia; fax: (495) 939-4309; E-mail: potekchina@hotbox.ru*
[2]*All-Russian Collection of Microorganisms, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia; fax: (495) 923-3602*
[3]*Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky pr. 47, 119992 Moscow, Russia; fax: (499) 135-5328*
[4]*Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, ul. Goleva 13, 614081 Perm', Russia; fax: (342) 244-6711*

Received July 11, 2008
Revision received October 20, 2008

Abstract—Cell walls of *Bacillus subtilis* VKM B-760 and VKM B-764 are characterized by heterogeneous composition of teichoic acids. Polymer I with structure -6)-β-*D*-Gal*p*-(1→1)-*sn*-Gro-(3-P-, polymer II with structure -6)-α-*D*-Glc*p*-(1→1)-*sn*-Gro-(3-P-, and a small amount of unsubstituted 1,3-poly(glycerol phosphate) were detected in strain VKM B-760. Strain VKM B-764 contains an analogous set of teichoic acids, but a characteristic feature of polymer II is the presence of disubstituted glycerol residue with α-glucopyranose localization in the integral chain at C-1 hydroxyl and β-glucopyranose as a side branch at C-2 hydroxyl (polymer III): -6)-α-*D*-Glc*p*-(1→1)-[β-*D*-Glc*p*-(1→2)]-*sn*-Gro-(3-P-. The structures of polymer I in bacilli and polymer III in Gram-positive bacteria are described for the first time. Teichoic acids were studied by chemical methods and on the basis of combined analysis of one-dimensional ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra, homonuclear two-dimensional ^{1}H/^{1}H COSY, TOCSY, and ROESY, and heteronuclear two-dimensional ^{1}H/^{13}C gHSQC- and HMQC-TOCSY experiments. Simultaneous presence of several different structure teichoic acids in the bacillus cell walls as well as chemotaxonomical perspectives of the application of these polymers as species-specific markers for members of the *Bacillus* genus is discussed.

DOI: 10.1134/S0006297909120116

Key words: *Bacillus subtilis*, cell wall, teichoic acids, NMR spectroscopy

Bacillus subtilis is a member of a large family of Gram-positive bacteria – *Bacillaceae*. According to the present classification, the *B. subtilis* species includes several subspecies. Structures and composition of teichoic acids of several strains described in the literature as well as our preliminary data [1] are indicative of species heterogeneity and necessity for further investigations using modern methods of gene taxonomy.

This work is the continuation of investigations of variability of anionic bacillus polymers [1, 2] and chemotaxonomical perspectives of the application of their structural peculiarity as a species-specific marker for members of the *Bacillus* genus. The paper deals with descriptions of teichoic acid structures for cell walls of two *B. subtilis* strains, VKM B-760 and VKM B-764, that basically differ from polymers of the type strain *B. subtilis* subsp. *subtilis* VKM B-501^{T} [2].

MATERIALS AND METHODS

Bacillus subtilis strains VKM B-760 and VKM B-764, obtained earlier by the All-Russian Collection of Microorganisms (VKM) as non-valid "*B. subtilis* subsp. *aterrimus*" and "*B. subtilis* subsp. *niger*", respectively, were grown under aerobic conditions at 28°C to exponential growth phase as described previously [2].

Cell walls were obtained from sonicated biomass by differential centrifugation, repeatedly washed with water, and lyophilized [3]. Teichoic acids were extracted from

* To whom correspondence should be addressed.

cell walls with 10% trichloroacetic acid (TCA) (1 : 10 w/v, three times, 24 h each at 4°C). Extracts were combined and dialyzed, the undialyzed residue was lyophilized, and preparations B-760 and B-764 were obtained. Conditions of acid hydrolysis and other analytical methods were described previously [4]. Forms of phosphorus were determined according to the earlier described method [3].

Products of acid degradation of cell wall preparations and teichoic acids were analyzed by high-voltage electrophoresis and paper chromatography by comparison with control samples. Electrophoresis was carried out in pyridine–acetate buffer (A), pH 5.6 (20 V/cm, 4 h). The following solvent systems were used for descending paper chromatography: pyridine–benzene–butan-1-ol–H_2O (3 : 1 : 5 : 3 v/v) (B) for separation of glycerol and monosaccharides and butan-1-ol–AcOH–H_2O (5 : 1 : 4 v/v) (C) for separation of hydroxamates. Electrophoresis and paper chromatography were carried out on Filtrak FN-3 chromatographic paper (Germany).

To obtain alanine hydroxamates, 0.1 ml freshly prepared hydroxylamine solution was added to 10-30 mg teichoic acid dissolved in 1 ml H_2O [5]. The solution was alkalized with 1 M NaOH to pH 7.6 and left for 1 h at 37°C, then vacuum evaporated to dryness, and hydroxamates were extracted by aqueous ethanol (96% ethanol–H_2O, 4 : 1 v/v) 5-6 times. Ethanol extracts were dried and analyzed by electrophoresis in buffer A and paper chromatography in system C by comparison with a standard sample of alanine hydroxamate.

To obtain glycosides, the teichoic acid preparation was hydrolyzed with 48% HF (4°C, 16 h) with subsequent lyophilization using a trap with NaOH. Hydrolysis products were separated on a column (90 × 1.5 cm) with TSK gel HW-40S (Toyopearl, Japan), elution being carried out in 1% acetic acid and using a differential refractometer (Knauer, Germany). The resulting fractions were analyzed by NMR spectroscopy.

NMR spectra were registered on a Bruker DRX-500 spectrometer (Germany) for solutions in D_2O at 30°C. Internal TSP (δ_H 0.0) and acetone (δ_C 31.45) were used as standards for chemical shift readings. Two-dimensional spectra were registered and processed using standard Bruker techniques. The spin lock time in TOCSY and HMQC-TOCSY experiments was 200 and 150 msec, respectively. Mixing time in ROESY experiments was 200 msec. The HMBC experiment was optimized for $J_{H,C}$ constant of 8 Hz.

Base sequence for the 16S rRNA gene fragment comprising approximately 500 bp and including variable regions V1-V3 was determined using a CEQ Dye Terminator Cycle Sequencing kit on a MegaBASE 1000 automatic sequencer (JSC GE Healthcare, USA).

Preliminary phylogenetic analysis of the 16S rRNA gene fragment base sequence was carried out using the BLAST software package of National Center of Biotechnological Information of USA (http://www.ncbi.nlm.nih.gov). Then the 16S rRNA base sequences of studied strains were compared with those of typical strains of closely related species using the CLUSTAL W program [6].

RESULTS AND DISCUSSION

Cell walls of *B. subtilis* strains VKM B-760 and VKM B-764 contained 2.8 and 2.1% organic phosphorus, respectively, and acid hydrolysis with 2 M HCl for 3 h at 100°C gave identical products: glycerol mono- and diphosphates, phosphoric esters (m_{groP} 0.45 and 0.61), inorganic phosphate, galactose, glucose, and glycerol, which suggested the possible presence of teichoic acids.

Teichoic acids were extracted from the cell wall using fractional extraction with 10% TCA, and the resulting preparations were analyzed. Products of their acid degradation were identical to each other and to those obtained previously upon cell wall hydrolysis, which made it possible to combine them and then to work with overall preparations (preparations B-760 and B-764).

Electrophoretic study of the latter showed the presence of two fractions stained by Isherwood reagent for organic phosphorus. Fraction 1 was prevalent, had mobility in electric field m_{groP} 0.90, and after acid degradation it formed glycerol monophosphate, inorganic phosphate, phosphoric esters with m_{groP} 0.45 and 0.61, as well as glucose, galactose, and glycerol, which could be indicative of the presence of teichoic acids of poly(glycosyl glycerol phosphate) nature. Polymers of similar structure do not form glycerol diphosphate upon acid hydrolysis [7]. Further studies by NMR spectroscopy showed that the teichoic acid preparations contain two polymers with glucose and galactose in each integral chain. Such polymers have similar charge and are not separated in an electric field.

Fraction 2 with mobility m_{groP} 1.3 was minor, formed only glycerol mono-and diphosphates upon acid hydrolysis, and corresponded to unsubstituted poly(glycerol phosphate) [8].

To detect in teichoic acids the O-ester-bound alanine residues, preparations B-760 and B-764 were treated by hydroxylamine solution. Hydroxylaminolysis products were separated in an electric field in buffer A and identified by paper chromatography in system C by comparison with a standard sample. Alanine hydroxamate was revealed in trace amounts and was not detected by NMR spectroscopy, which can be explained both by the O-ester bond lability and by the method of polymer isolation [4].

Final structure of the cell wall polymers in the studied bacilli was established by NMR spectroscopy.

The ^{13}C-NMR spectrum of B-760 preparation contained two signals of unequal intensity at δ_C 104.8 and 99.9 in the resonance region of the sugar anomeric carbon atoms. In the resonance region of carbon atoms bound to

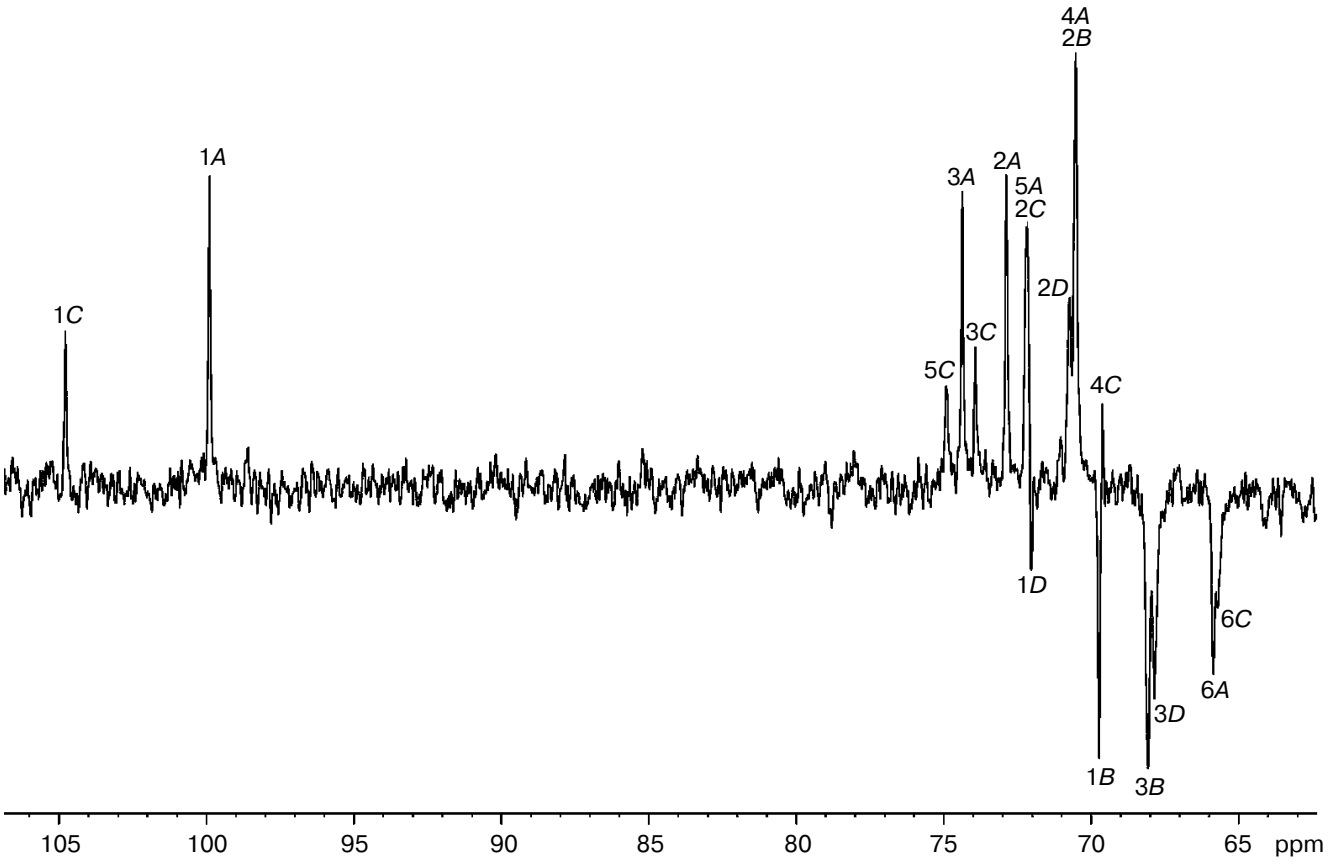

Fig. 1. ^{13}C-APT spectrum of teichoic acids from the *B. subtilis* VKM B-760 cell wall. Arabic numerals correspond to numbers of carbon atoms in residues designated by Latin letters according to the table.

one oxygen atom, the APT spectrum (Fig. 1) identified signals of substituted oxymethyl groups at δ_C 69.7, 68.1, and 65.8 (intense peaks) and at δ_C 72.0, 67.8, and 65.7 (less intense peaks). One broad signal with peak at δ_P +0.9 was detected in the ^{31}P-NMR spectrum. In the ^{1}H-NMR spectrum, a doublet at δ_H 4.93 (*J* 4 Hz) and another less intense doublet at δ_H 4.43 (*J* 8 Hz) were present in the proton resonance region at anomeric carbon atoms. The remaining signals were localized in the δ_H 4.2-3.5 region.

Two equal intensity signals δ_C 103.8 and 99.9 and the lower intensity signal at δ_C 104.8 were present in the resonance region of anomeric carbon atoms in the ^{13}C-NMR spectrum of the B-764 preparation. The APT spectrum (Fig. 2) contained seven signals of substituted oxymethyl groups at δ_C 72.0-65.9 and an intense signal of unsubstituted hydroxymethyl group at δ_C 62.3. Two broad partially overlapping signals with peaks at δ_P +0.5 and +0.7 were detected in the ^{31}P-NMR spectrum. Two equally intense doublets were present in the proton resonance region at anomeric carbon atoms in the ^{1}H-NMR spectrum: a doublet at δ_H 4.94 (*J* 4 Hz) and 4.64 (*J* 8 Hz) and a less intense doublet at δ_H 4.43 (*J* 8 Hz). The remaining signals were localized in the δ_H 4.2-3.3 region.

^{1}H-NMR spectra were decoded using two-dimensional ^{1}H/^{1}H COSY, TOCSY, and ROESY experiments. Analysis of two-dimensional spectra of the B-760 preparation showed that the more intense doublet belongs to an α-glucopyranose residue (α-*D*-Glc*p*, *A* residue; table) while the less intense one belonged to β-galactopyranose residue (β-*D*-Gal*p*, *C* residue). The spectra also revealed glycerol residue signals (two series of signals with higher and lower intensity). Signals in the one-dimensional ^{13}C-NMR spectrum of the B-760 preparation were subtracted upon analysis of heteronuclear ^{1}H/^{13}C two-dimensional spectra of gHSQC- and HMQC-TOCSY (table). The weak-field displacement of sugar C-1 and C-6 hydroxyls and glycerol C-1 and C-3 hydroxyls signals compared to the same signals in the spectrum of the corresponding unsubstituted sugars and glycerol was indicative of sugar substitution at C-1 and C-6 hydroxyls and glycerol substitution at C-1 and C-3 hydroxyls. Displacements of sugar C-6 and glycerol C-3 signals were typical of effects of substitution by phosphoric acid residues [9], whereas changes in C-1 chemical shifts in glycerol residues correlated with effects of glycosylation by α-pyranose (+7.3 ppm, glycerol residues with more intense signals) or by β-pyranose (+9.6 ppm, glycerol residues with less intense signals).

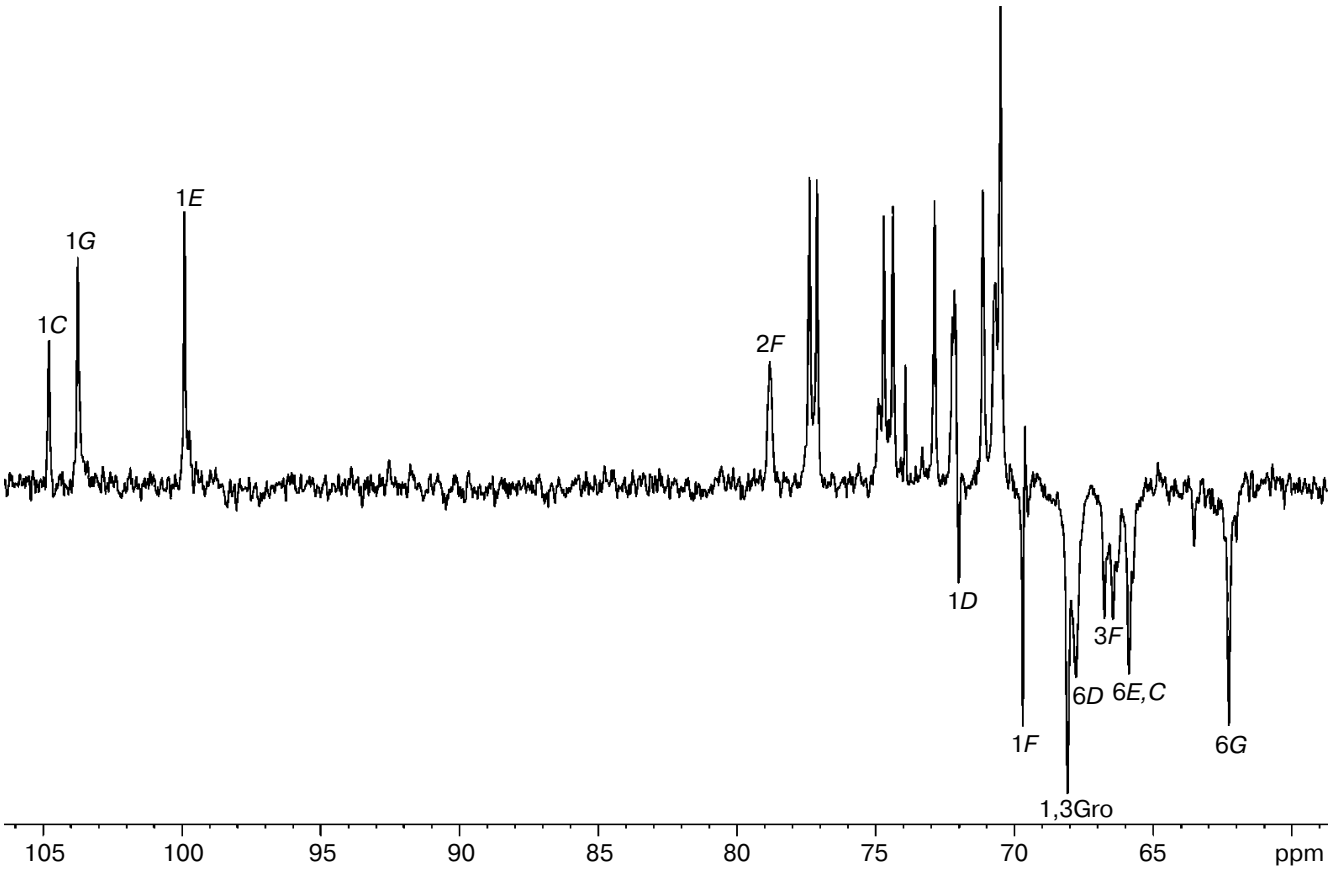

Fig. 2. ^{13}C-APT spectrum of teichoic acids from the *B. subtilis* VKM B-764 cell wall. Designations as in Fig. 1.

Thus, the spectral data showed that preparation B-760 contains a mixture of two poly(glycosyl glycerol phosphate) polymers with repeated units: -6)-α-*D*-Glc*p*-(1→1)-*sn*-Gro-(3-P- (the prevalent component polymer I) and -6)-β-*D*-Gal*p*-(1→1)-*sn*-Gro-(3-P- (the minor component polymer II).

Absolute configuration of glycerol residues were attributed on the basis of existing concepts of teichoic acid biosynthesis, according to which residues of *sn*-glycerol-3-phosphate are incorporated into the polymer molecule [10]. Some investigations of glycosylation enzymes point to *D*-hexose incorporation into the teichoic acid molecule [11-13], which is supported by our data on determination of the monosaccharide absolute configuration in analogous polymers [14].

In addition to the abovementioned polymers of poly(glycosyl glycerol phosphate) nature, the ^{13}C-NMR spectrum of the B-760 preparation showed the presence of a small amount of 1,3-poly(glycerol phosphate) chains. Treatment of this preparation by 48% hydrofluoric acid (HF) resulted in complete dephosphorylation of the polymers and formation of two glycosides (table) whose structure correlated with that of the initial polymers.

Similar spectral and chemical investigations of the B-764 preparation showed that it contained three polymers, two of which were identified by NMR spectra as 1,3-poly(glycerol phosphate) with characteristic signals in the ^{13}C-NMR spectrum at δ_C 67.8 (C-1,3) and 70.7 (C-2) [9] and as teichoic acid II identical to that in B-760 (table). According to the analysis of the homonuclear two-dimensional spectra, the third polymer (polymer III) contained two glucopyranose residues with different anomeric configuration along with one glycerol and one phosphoric acid residue. Analysis of ^{1}H/^{13}C gHSQC and HMQC-TOCSY spectra of the preparation showed that the glycerol residue is glycosylated at positions 1 and 2 and carries a phosphate group in position 3, the α-glucopyranose residue has a phosphate group in position 6, while the β-glucopyranose residue is terminal. Finally, the structure of the repeated unit of the polymer was detected upon analysis of the two-dimensional ^{1}H/^{13}C gHMBC spectrum (Fig. 3), where correlation peaks pointing to α-glucopyranose localization at C-1 and β-glucopyranose at C-2 hydroxyl of glycerol residue were found:

-6)-α-*D*-Glc*p*-(1→1)-*sn*-Gro-(3-P- (polymer III)
2)
↑
β-*D*-Glc*p*-(1

Chemical shifts in ^{1}H- and ^{13}C-NMR spectra of teichoic acids of *B. subtilis* VKM B-760 and *B. subtilis* VKM B-764 cell walls

Residue		Chemical shifts (acetone δ_C 31.45, TSP δ_H 0.0)					
		C-1 *H-1*; *1′*	C-2 *H-2*	C-3 *H-3*; *3′*	C-4 *H-4*	C-5 *H-5*	C-6 *H-6*; *6′*
Polymer I							
-6)-α-*D*-Glc*p*-(1→	(***A***)	99.9 *4.93*	72.9 *3.58*	74.4 *3.76*	70.5 *3.53*	72.2 *3.79*	65.8 *4.15*; *4.04*
→1)-*sn*-Gro-(3-P-	(***B***)	69.7 *3.83*; *3.63*	70.5 *4.10*	68.1 *4.01*; *3.98*			
Glycoside I							
α-*D*-Glc*p*-(1→	(***A′***)	99.7 *4.93*	72.9 *3.57*	74.5 *3.74*	71.0 *3.42*	73.3 *3.68*	62.0 *3.87*; *3.77*
→1)-*sn*-Gro	(***B′***)	70.0 *3.78*; *3.59*	71.9 *3.96*	63.9 *3.70*; *3.66*			
Polymer II							
-6)-β-*D*-Gal*p*-(1→	(***C***)	104.8 *4.43*	72.2 *3.56*	73.9 *3.67*	69.6 *3.99*	74.9 *3.85*	65.7 *4.11*; *4.05*
→1)-*sn*-Gro-(3-P-	(***D***)	72.0 *4.01*; *3.72*	70.8 *4.03*	67.8 *3.99*; *3.91*			
Glycoside II							
β-*D*-Gal*p*-(1→	(***C′***)	104.8 *4.42*	72.3 *3.56*	74.1 *3.67*	70.1 *3.93*	76.6 *3.71*	62.5 *3.79*; *3.77*
→1)-*sn*-Gro	(***D′***)	72.4 *4.01*; *3.69*	72.2 *3.95*	63.8 *3.71*; *3.63*			
Polymer III							
-6)-α-*D*-Glc*p*-(1→	(***E***)	99.9 *4.94*	72.9 *3.58*	74.4 *3.75*	70.5 *3.53*	72.2 *3.79*	66.1 *4.14*; *4.04*
→1)-*sn*-Gro-(3-P- 2) ↑	(***F***)	69.8 *3.83*; *3.62*	78.7 *4.22*	66.5 *4.13*; *4.06*			
β-*D*-Glc*p*-(1	(***G***)	103.7 *4.64*	74.7 *3.33*	77.2 *3.52*	71.2 *3.40*	77.4 *3.47*	62.3 *3.91*; *3.75*
Glycoside III							
α-*D*-Glc*p*-(1→	(***E′***)	99.7 *4.91*	72.9 *3.55*	74.5 *3.72*	71.1 *3.40*	73.3 *3.66*	62.0 *3.85*; *3.76*
→1)-*sn*-Gro 2) ↑	(***F′***)	70.0 *3.75*; *3.56*	82.4 *3.90*	62.9 *3.73*; *3.71*			
β-*D*-Glc*p*-(1	(***G***)	103.4 *4.59*	74.7 *3.31*	77.1 *3.49*	71.1 *3.39*	77.3 *3.45*	62.2 *3.90*; *3.70*

The investigations showed that cell walls of the studied bacillus strains VKM B-760 and VKM B-764 contain a heterogeneous set of teichoic acids: a low amount of 1,3-poly(glycerol phosphate) and two polymers of poly(glycosyl glycerol phosphate) nature with α-glucopyranose and β-galactopyranose in the integral chain, in which a phosphodiester bond joins the hydroxyl at C-3 atom of glycerol and hydroxyl at C-6 of a glycosyl residue. The characteristic feature of the VKM B-764 cell wall is the presence in poly(glycosyl glycerol phosphate) of the β-glucopyranose side residue at the C-2 hydroxyl of glycerol. In the VKM B-760 cell wall, polymer I prevailed quantitatively, while polymer III was prevalent in the cell wall of VKM B-764 (table). A small amount of O-ester-bound alanine residues was found in teichoic acid preparations from the studied bacilli.

It was shown previously that the heterogeneous set of teichoic acids is a characteristic feature of actinomycetes, some members of which contained in their cell walls up to four structurally different polymers [15, 16]. As for bacilli that are up to now less studied, simultaneous presence of several polymers was described in three microorganisms: *Lactobacillus plantarum* NIRD C106 [17], *Bacillus coagulans* AHU 1631 [18, 19], and *Bacillus licheniformis* [20]. In some cases it was possible to separate the mixture of teichoic acids to components with a regular repeated unit by chromatography, electrophoresis, or by immunological methods, which excluded the supposition concerning the polymer heterogeneity as such. We believe by analogy that a mixture of polymers rather than a polymer with heterogeneous chains was studied.

It should be noted that several structurally different poly(glycosyl glycerol phosphates) were found in cell walls of the studied and abovementioned strains. The unsubstituted or poorly substituted 1,3-poly(glycerol phosphate), often incorporated in heterogeneous set of

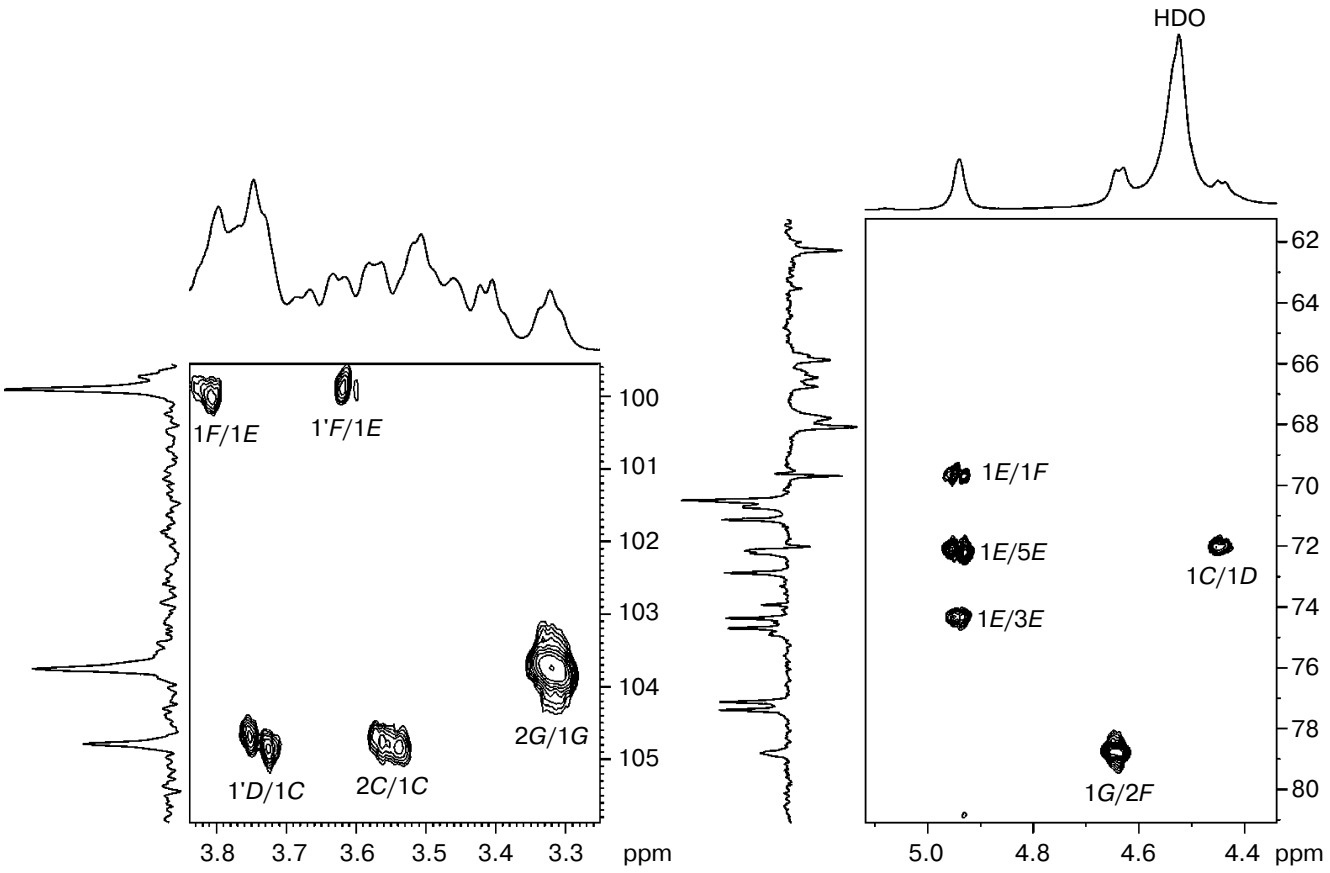

Fig. 3. Fragments of the $^{1}H/^{13}C$ gHMBC spectrum of teichoic acids from *B. subtilis* VKM B-764 cell wall. Parts of one-dimensional ^{1}H- and ^{13}C-APT spectra are shown at the top and to the left, respectively. Figures in front of oblique stroke relate to proton numbers, while figures after this stroke relate to numbers of carbon atoms in residues designated by Latin letters according to the table.

polymers of actinomycete cell walls, was also found among bacillar teichoic acids in the VKM B-760 and VKM B-764 strains.

Teichoic acids of poly(glycosyl glycerol phosphate) nature are widespread among Gram-positive bacteria and are described in representatives of genera – *Bacilli, Streptomyces, Actinoplanes, Nocardioides, Agromyces*, etc. [9]. Structural variations are associated with the phosphodiester bond localization on the glycosyl substituent (C-3, C-4, and C-6) and the glycosyl residue on polyol (C-1 and C-2 in the case of glycerol) as well as with the amount and nature of carbohydrate components in the repetitive unit and with the glycoside bond configurations.

Poly(galactosyl-(β 1→1)-glycerol phosphates) with the phosphodiester bond localization at C-6 hydroxyl of β-galactopyranose, appearing in the actinomycete cell walls [9], were not found earlier in cell walls of bacilli. On the contrary, similar polymers but with α-glucopyranose already appeared in cell walls of the streptomycete *S. kanamyceticus* RIA 690 [7] and the bacillus *B. stearothermophilus* B 65 [21].

Teichoic acid III of the VKM B-764 strain with disubstituted glycerol as the integral chain monomer is of interest. Such structural peculiarity was described within the hydrophilic part of lipoteichoic acids of *Lactococcus garvieae* NCFB 2730, *Streptococcus lactis* Kiel 42172, *Clostridium innocuum* [22], teichoic acids of the cell wall of *Actinomadura viridis* VKM Ac-1315^{T} [23], and two bacillus strains—*B. coagulans* AHU 1638 and AHU 1631 [18]. However, disubstituted glycerol with the integral chain α-glucose localization at C-1 hydroxyl and the side branch β-glucose localization at C-2 hydroxyl was found for the first time within Gram-positive bacterial cell surface layers polymers.

The cell wall teichoic acids of the studied bacilli are close to each other in structure and composition, but teichoic acid II, whose integral chain is identical in the two strains, differs in VKM B-764 by the presence of disubstituted glycerol residue. This peculiarity separates strains VKM B-760 and VKM B-764. The studied bacilli also essentially differ from the typical strain *B. subtilis* subsp. *subtilis* VKM B-501^{T} [2] that contains in its cell wall structurally different polymers.

Our data correlate with results of phylogenetic analysis of the 16S rRNA gene fragments. Strain VKM B-764 is characterized by a high level of its base sequence

similarity (100%) with those of *Bacillus subtilis* subsp. *subtilis* DSM10^T and *Bacillus tequilensis* 10b^T, and the level of similarity between strain VKM B-760 and *Bacillus licheniformis* DSM13^T and *Bacillus sonorensis* NRRL B-23154^T is 99.5%. It is necessary to note that the similarity of the analyzed 16S rRNA gene fragment for some species of *Bacillus sensu stricto* group is in the range of 99-100%, which suggests the necessity of additional investigations for more exact definition of species affiliation of VKM B-764 and VKM B-760 strains. Taking into attention structural distinctions of the cell wall polymers and their genetic similarity with different valid bacillus species, it can be supposed that the strains are members of different species.

Thus, our investigations show the heterogeneity of *B. subtilis* strains and the necessity for further revision of the species. Owing to repeatedly discussed species specificity of teichoic acids [15, 24-28], the improvement or confirmation of species affiliation of VKM B-760 and VKM B-764 strains is the subject of further investigations using modern methods of gene taxonomy.

This work was supported by the Russian Foundation for Basic Research (grant 07-04-01054).

REFERENCES

1. Potekhina, N. V., Streshinskaya, G. M., Tul'skaya, E. M., Kozlova, Yu. I., Kudryashova, E. B., and Shashkov, A. S. (2008) *Abstr. IV Congr. Russ. Association of Biochemists and Molecular Biologists* [in Russian], Novosibirsk, p. 883.
2. Shashkov, A. S., Potekhina, N. V., and Kudryashova, E. B. (2009) *Biochemistry (Moscow)*, **74**, 543-548.
3. Streshinskaya, G. M., Naumova, I. B., and Panina, L. I. (1979) *Mikrobiologyia*, **48**, 814-819.
4. Streshinskaya, G. M., Naumova, I. B., Romanov, V. V., and Shashkov, A. S. (1981) *Bioorg. Khim.*, **7**, 1409-1418.
5. Naumova, I. B., and Dmitrieva, N. F. (1974) *Biokhimiya*, **39**, 201-209.
6. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.*, **22**, 4673-4680.
7. Naumova, I. B., Shashkov, A. S., and Stroganova, M. P. (1978) *Bioorg. Khim.*, **4**, 1529-1537.
8. Naumova, I. B., Digimbay, K., Potekhina, N. V., Shashkov, A. S., Terekhova, L. P., and Preobrazhenskaya, T. P. (1986) *Bioorg. Khim.*, **12**, 670-678.
9. Naumova, I. B., and Shashkov, A. S. (1997) *Biochemistry (Moscow)*, **62**, 809-840.
10. Neuhaus, F. C., and Baddiley, J. (2003) *Microbiol. Mol. Biol. Rev.*, **67**, 686-723.
11. Maino, V. C., and Young, F. E. (1974) *J. Biol. Chem.*, **249**, 5169-5175.
12. Shimada, A., and Ito, E. (1986) *J. Biochem.*, **100**, 1507-1521.
13. Shibaev, V. N. (1982) *Uspekhi Biol. Khim.*, **23**, 61-101.
14. Streshinskaya, G. M., Shashkov, A. S., Senchenkova, S. N., Bueva, O. V., Stupar, O. S., and Evtushenko, L. I. (2007) *Carbohydr. Res.*, **342**, 659-664.
15. 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. (2001) *FEMS Microbiol. Rev.*, **25**, 269-283.
16. Shashkov, A. S., Streshinskaya, G. M., Evtushenko, L. I., and Naumova, I. B. (2001) *Carbohydr. Res.*, **336**, 237-242.
17. Archibald, J. J., and Coapes, H. E. (1971) *Biochem. J.*, **224**, 449-460.
18. Kojima, N., Uchikawa, K., Araki, Y., and Ito, E. (1985) *J. Biochem*. (Tokyo), **97**, 1085-1092.
19. Kojima, N., Uchikawa, K., Araki, Y., and Ito, E. (1986) *Eur. J. Biochem.*, **155**, 521-526.
20. Schipper, D. (1995) *Carbohydr. Res.*, **279**, 75-82.
21. De Boer, W. R., Wouters, J. T. M., Anderson, A. J., and Archibald, A. R. (1978) *Eur. J. Biochem.*, **85**, 433-436.
22. Fischer, W. (1994) *New Compr. Biochem.*, **27**, 199-215.
23. Shashkov, A. S., Potekhina, N. V., Naumova, I. B., Evtushenko, L. I., and Widmalm, G. (1999) *Eur. J. Biochem.*, **262**, 688-695.
24. Shashkov, A. S., Kozlova, Yu. I., Streshinskaya, G. M., Kosmachevskaya, L. N., Bueva, O. V., Evtushenko, L. I., and Naumova, I. B. (2001) *Mikrobiologiya*, **70**, 477-486.
25. Streshinskaya, G. M., Kozlova, Yu. I., Shashkov, A. S., Evtushenko, L. I., and Naumova, I. B. (2003) *Mikrobiologiya*, **72**, 510-515.
26. Potekhina, N. V., Tul'skaya, E. M., Shashkov, A. S., Taran, V. V., Evtushenko, L. I., and Naumova, I. B. (1998) *Mikrobiologiya*, **67**, 399-403.
27. Shashkov, A. S., Streshinskaya, G. M., Senchenkova, S. N., Kozlova, Yu. I., Alferova, I. V., Terekhova, L. P., and Evtushenko, L. I. (2006) *Carbohydr. Res.*, **341**, 796-802.
28. Kozlova, Yu. I., Streshinskaya, G. M., Shashkov, A. S., Evtushenko, L. I., and Naumova, I. B. (1999) *Biochemistry (Moscow)*, **64**, 671-677.