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REVIEW

Phosphate-Containing Cell Wall Polymers of Bacilli*

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Abstract—Anionic phosphate-containing cell wall polymers of bacilli are represented by teichoic acids and poly(glycosyl 1-phosphates). Different locations of phosphodiester bonds in the main chain of teichoic acids as well as the nature and combination of the constituent structural elements underlie their structural diversity. Currently, the structures of teichoic acids of bacilli can be classified into three types, viz. poly(polyol phosphates) with glycerol or ribitol as the polyol; poly(glycosylpolyol phosphates), mainly glycerol-containing polymers; and poly(acylglycosylglycerol phosphate), in which the components are covalently linked through glycosidic, phosphodiester, and amide bonds. In addition to teichoic acids, poly(glycosyl 1-phosphates) with mono- and disaccharide residues in the repeating units have been detected in cell walls of several *Bacillus subtilis* and *Bacillus pumilus* strains. The known structures of teichoic acids and poly(glycosyl 1-phosphates) of *B. subtilis*, *B. atrophaeus*, *B. licheniformis*, *B. pumilus*, *B. stearothermophilus*, *B. coagulans*, *B. cereus* as well as oligomers that link the polymers to peptidoglycan are surveyed. The reported data on the structures of phosphate-containing polymers of different strains of *B. subtilis* suggest heterogeneity of the species and may be of interest for the taxonomy of bacilli to allow differentiation of closely related organisms according to the "structures and composition of cell wall polymers" criterion.

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Bacilli, the representatives of the genus *Bacillus* (family Bacillaceae, order Bacillales), are Gram-positive endospore-forming rod-shaped microorganisms. They are widespread in diverse habitats and environmental niches (soil, aquatic ecosystems, foodstuffs) and manifest considerable pathogenic variability. Many strains are able to produce valuable substances, such as enzymes, antibiotics, surfactants, organic acids, insecticide peptides, and can be used in their biotechnological manufacturing. Since spores of bacilli withstand different physical factors, such as pressure, temperature, humidity, etc., these bacteria are used as test organisms in assaying sterilization methods. *Bacillus subtilis* 168 is a popular model for studies of biology, biochemistry, and genetics of Gram-positive bacteria (like *E. coli* for Gram-negative bacteria). Comprehensive knowledge of the *B. subtilis* genome enables its wide use in gene engineering. Considerable information has also been accumulated to date on the structure of cell walls of bacilli, in particular, on the nature and structure of the constituent polymers.

Cell walls of bacilli contain A1γ-type peptidoglycan [1] and, in addition, high content of secondary polymers attributed, according to a recent classification [2], to teichoic acids, teichuronic acids, and "non-classical" polysaccharides.

Teichoic acids are anionic polymers comprising residues of a polyol and orthophosphoric acid as indispensable components. Often, they also contain monosaccharide residues. This is the most abundant and the

Abbreviations: GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Gro, glycerol; GroA, glyceric acid; ManNAc, N-acetylmannosamine; *P*, phosphate; Qui4N, 4-amino-4,6-dideoxyglucose (4-amino-4-deoxyquinovose); Ribol, ribitol.

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best-studied class of cell wall components of bacilli. Teichoic acids are typical of representatives of a *B. subtilis* group [3, 4], including *B. subtilis* [1, 5], *B. atrophaeus* [5], *B. licheniformis* [1], *B. pumilus* [6, 7], and detected in some other species of the *Bacillus* genus, namely, *B. coagulans* (several strains) [8, 9] and *B. cereus* AHU 1030 [10].

Teichuronic acids are far less common components of cell walls of bacilli; structures of polymers of several strains have been established to date [1]. Their constituents are uronic acids, which impart anionic properties to the polymers, and usually amino sugars. Teichuronic acids are either the only polymers of the cell wall of bacilli or are encountered together with teichoic acids [11, 12]. In certain cases, their presence depends on the composition of the culturing medium: phosphate deficiency in the course of growth can trigger production of teichuronic acids instead of teichoic acids. This phenomenon was first documented in experiments with bacilli [13]. It was shown that the change in the phosphate content in the medium could direct the biosynthesis towards either type of acidic polymers [1].

The group of "non-classical" polysaccharides, i.e. those different from teichoic and teichuronic acids, includes linear and branched neutral and acidic (due to the presence of pyruvic acid residues) polymers usually containing N-acetylmannosamine and N-acetylglucosamine residues [2, 14, 15]. "Non-classical" polysaccharides are specific cell wall components of representatives of the *B. cereus* group [14, 15]. These polymers have been detected also in cell walls of *B. sphaericus* CCM 2177 and several strains of the genera *Paenibacillus* and *Aneurinibacillus* [2].

In the present review, attention is mainly focused on phosphate-containing cell wall polymers of the representatives of the genus *Bacillus*, which include poly(glycosyl 1-phosphates) in addition to teichoic acids.

TEICHOIC ACIDS

The notions on teichoic acids, their structures, functioning, and biological properties have extended significantly over the past decades; however, the anionic nature of these compounds due to the presence therein of phosphoric acid residues is still regarded as the principal chemical feature that determines their functioning in the cell. Teichoic acids together with peptidoglycan are responsible for the maintenance of the cell shape; they are involved in construction of the electrolyte matrix, which forms the negatively charged network of cell surface layers; they are related to the cation exchange and regulation of autolysin activity; possess immunological and adhesive properties; take part in bacteriophage reception, microbial co-aggregation, and specify the pathogenicity of bacteria [16-19].

Teichoic acids are indispensable cell wall components of the majority of the bacilli studied and can account to 60% of its weight [1]. These polymers are covalently attached to peptidoglycan through a linking oligomer the structure of which is similar to that of *B. subtilis* W23, (GroP)$_2$–ManNAc-(β1→4)-GlcNAc [20] (Table 1). Studies by Japanese scientists of the 1980s [8, 10, 20-23] revealed that the structure of the linking oligomer is rather conserved; the oligomers may only differ in the number of the glycerol phosphate residues or in the presence of glucose in place of N-acetylmannosamine (Table 1). On the contrary, teichoic acids demonstrate structural variability. Probably, these polymers underwent considerable modifications during evolution, like O-antigens of lipopolysaccharides of Gram-negative bacteria [21].

The first teichoic acids of bacilli were studied in the late 1950s/early 1960s, and the elucidation of their structures made recourse mainly to chemical and enzymatic methods. The polymers were isolated from cell walls of *B. subtilis* 168 and W23, the classical objects of research, and

Table 1. Structures of the linkage oligomers binding the phosphate-containing polymers and the cell wall peptidoglycan in representatives of the genus *Bacillus*

Strains	Linkage oligomer*	Reference
B. subtilis W23, AHU 1390	(Gro*P*)$_2$–ManNAc(β1→4)GlcNAc	[20]
B. pumilus AHU 1650	**(Gro*P*)$_7$–ManNAc(β1→4)GlcNAc	[23]
B. subtilis AHU 1035, AHU 1037, AHU 1392, AHU 1235	(Gro*P*)$_x$–ManNAc(β1→4)GlcNAc	[21]
B. licheniformis AHU 1371	ManNAc(β1→4)GlcNAc	[21]
B. cereus AHU 1030	ManNAc(β1→4)GlcNAc	[10]
B. pumilus AHU 1650	ManNAc(β1→4)GlcNAc	[23]
B. coagulans AHU 1366	(Gro*P*)$_2$–Glc(β1→4)GlcNAc	[22]
B. coagulans AHU 1631, AHU 1634, AHU 1638	Glc(β1→4)GlcNAc	[8]

* The structures of the linkage oligomers are reproduced as in the original publications.
** The linkage oligomer for the poly(glycosyl 1-phosphate).

the type strain of the species, *B. subtilis* NCTC 3610. Investigations into the structure of teichoic acids gained new impetus in the 1970s-1980s owing to the advent of non-destructive analytical methods, such as NMR spectroscopy. Teichoic acids of the microorganisms *B. stearothermophilus* B65 and *B. subtilis* var. *niger* WM were the first whose structures were established using NMR spectroscopy [24, 25]. Later it was shown that this method allows structural studies of teichoic acids in polymer mixtures without isolation of individual fractions [26]. Thus the presence of three different chains of teichoic acids was observed in a strain of *B. licheniformis*, and their structures were established [26].

The structures of a considerable part of teichoic acids of bacilli (AHU collection strains, Table 2) have been proposed by Japanese scientists based on studies by chemical and enzymatic degradations. Occasionally, NMR spectroscopy was applied to characterize glycosides formed as dephosphorylation products of the teichoic acids. Therefore, many structures of teichoic acids are referred to as "the most likely structures" [8-10, 20-23]. Besides that, in certain studies by Japanese and some other authors, the ring size and absolute configuration of the monosaccharides have not been determined.

Over nearly 50 years that have elapsed since the first teichoic acids of bacilli were described, it was shown that these polymers are structurally variable in terms of both position of the phosphodiester bond in the main chain of the polymer and the nature of constituents. The known structures of the teichoic acids of bacilli may be classified into three main types, viz. poly(polyol phosphates), poly(glycosylpolyol phosphates), and poly(acylglycosylpolyol phosphates). In turn, each type may further be subdivided into subtypes and variants depending on the nature of the polyol (for bacilli, glycerol and ribitol) and the sugar component (for bacilli, galactose, glucose, their amino derivatives, and 4-amino-4-deoxyquinovose), configurations of the glycoside linkages (α, β), and position of the glycosyl residues (O1 and O2 of glycerol or O4 of ribitol) (for more comprehensive information on teichoic acids see reviews [17, 27, 28]).

Investigations using modern techniques, such as HPLC, NMR spectroscopy, and mass spectrometry, into cell wall polymers of some representatives of the *B. subtilis* group that have not hitherto been studied, revealed minor polymers with unique structures and novel polyols, monosaccharides, and acyl residues as structural elements that have not earlier been encountered (unpublished results).

Here we present the literature data on the structures of teichoic acids (according to the classification mentioned above) and poly(glycosyl 1-phosphates) of representatives of the genus *Bacillus* (Table 2).

Poly(polyol phosphates). Teichoic acids with poly(polyol phosphate) structures are represented by glycerol- and ribitol-containing polymers comprising from 40 to 60 repeating units [10, 29, 30]. Poly(glycerol phosphates) with 1,3- and 2,3-phosphodiester bonds were found in the cell walls of bacilli. All teichoic acids of bacilli contain ester-linked D-alanine residues. Recent NMR studies of these polymers suggest that the amino acid residues can be partially N-acetylated (unpublished results) (the role of O-linked D-alanine in bacterial cell functioning has been reviewed [16, 17]).

1,3-Poly(glycerol phosphates). The phosphodiester bond connects the hydroxyl groups at C1 and C3 of the adjacent glycerol residues in the polymer with the glycosyl and/or D-alanine substituents attached at the hydroxyl group at C2 of glycerol (PDB 1,3-type; Table 2). Nonglycosylated 1,3-poly(glycerol phosphates) can dominate among cell wall polymers, as, e.g. in *B. licheniformis* (strain is not specified) [26] and *B. pumilus* AHU 1650 [23]; however, they are often minor components and are present in the cell wall together with other polymers [37].

The most common teichoic acids of bacilli are those bearing monosaccharide residues attached by α- or β-glycosidic linkages. 1,3-Poly(glycerol phosphate) with an α-glucopyranose substituents was isolated from *B. subtilis* NCTC 3610 (=VKM B-501^T). Its structure was established by chemical methods [31] and confirmed later by NMR spectroscopy [32]. Teichoic acids with a similar structure were identified in *B. subtilis* 168 [29], *B. subtilis* AHU 1392 [21], and *B. cereus* AHU 1030 [10]. At the same time, *B. subtilis* AHU 1235 [21], *B. atrophaeus* VKM B-723, VKM B-763 (=VKM B-911), and *B. subtilis* VKM B-520 [5] contain a cell wall 1,3-poly(glycerol phosphate) bearing β-glucopyranose residues.

Biosynthesis of teichoic acids is known to involve glycosylation by specific glycosyl transferases [33]. In certain strains, e.g. in *B. subtilis* 168 and W23, it was shown that the glycosylating enzymes occupy positions close to those of enzymes of teichoic acid biosynthesis, and the stereochemistry of the glycosidic bond (α or β) is strain-specific [19].

Teichoic acids from two strains of *B. pumilus* contain N-acetyl-α-glucosamine and N-acetyl-α-galactosamine as glycosyl substituents. It was supposed that these polymers are localized in the cell walls. Their structures were inferred from analysis of derived degradation products. Most likely they are as follows: 1,3-poly(glycerol phosphate) bearing N-acetyl-α-glucosamine in *B. pumilus* Sh17 [6], and 1,3-poly(glycerol phosphate) bearing N-acetyl-α-glucosamine and N-acetyl-α-galactosamine in *B. pumilus* Sh18 [7].

2,3-Poly(glycerol phosphates). In these polymers, the phosphodiester bond connects the hydroxyl groups at C2 and C3 of the adjacent glycerol residues. The glycosyl substituents and/or D-alanine residues are linked to the hydroxyl group at C1 of glycerol. A teichoic acid with the 2,3-poly(glycerol phosphate) backbone substituted nonstoichiometrically with β-D-glucopyranose was isolated from the cell wall of *B. subtilis* var. *niger* WM [24, 25].

Table 2. Structures of cell wall teichoic acids and poly(glycosyl 1-phosphates) of representatives of the genus *Bacillus*

Type of PDB	Repeating unit, the presence of *O*-linked D-alanine	Strains	Reference
1	2	3	4
	Poly(polyol phosphates)		
1,3	-1)-sn-Gro-(3-*P*-	*B. licheniformis**, *B. pumilus* AHU 1650	[26] [23]
1,3	α-D-Glc*p*-(1 ↓ 2) -1)-sn-Gro-(3-*P*- D-Ala	*B. subtilis* ssp. *subtilis* VKM B-501^T, *B. subtilis* 168, *B. subtilis* AHU 1392, *B. cereus* AHU 1030	[32] [29] [21] [10]
1,3	β-D-Glc*p*-(1 ↓ 2) -1)-sn-Gro-(3-*P*- D-Ala	*B. subtilis* AHU 1235, *B. atrophaeus* VKM B-723, VKM B-763 (= B-911), *B. subtilis* VKM B-520	[21] [5] [5]
1,3	*α-GlcNAc-(1 ↓ 2) -1)-Gro-(3-*P*-	*B. pumilus* Sh17, Sh18	[6, 7]
1,3	*α-GalNAc-(1 ↓ 2) -1)-Gro-(3-*P*	*B. pumilus* Sh18	[7]
2,3	β-D-Glc*p*-(1 ↓ 1) -2)-sn-Gro-(3-*P*-	*B. subtilis* var. *niger* WM	[24, 25]
1,5	-1)-Rib-ol-(5-*P*-	*B. pumilus* Sh18	[7]
1,5	β-D-Glc*p*-(1 ↓ 2) -1)-Rib-ol-(5-*P*- D-Ala	*B. subtilis* W23, S31, *B. subtilis* CMM 234 (R-1), *B. licheniformis* CMM 454 (1-1G-2), *B. subtilis* VKM B-761	[29] [34] [34] [5]
1,5	α-D-Glc*p*-(1 ↓ 4) -1)-Rib-ol-(5-*P*- D-Ala	*B. subtilis* VKM B-722, VKM B-922	[5]
1,5	β-D-Glc*p*NAc-(1 ↓ 4) -1)-Rib-ol-(5-*P*- D-Ala	*B. subtilis* VKM B-762	[35]
	Poly(glycosylpolyol phosphates)		
3,6	-6)-β-D-Gal*p*-(1→1)-sn-Gro-(3-*P*- D-Ala	*B. licheniformis* ATCC 9945, *B. subtilis* VKM B-760, VKM B-764	[36] [37]
3,6	*-6)-α-Gal-(1→2)-Gro-(3-*P*-	*B. licheniformis* AHU 1371, *B. coagulans* AHU 1366, AHU 1638	[22] [8]
3,6	*α-Glc-(1 ↓ 1) -6)-α-Gal-(1→2)-Gro-(3-*P*-	*B. coagulans* AHU 1638	[8]

Table 2. (Contd.)

1	2	3	4
3,6	*β-Glc-(1 ↓ 1) -6)-α-Gal-(1→2)-Gro-(3-*P*-	*B. coagulans* AHU 1631, AHU 1634	[8]
3,6	-6)-α-D-Glc*p*-(1→1)-sn-Gro-(3-*P*- D-Ala	*B. stearothermophilus* B65, *B. subtilis* VKM B-760, VKM B-764	[25] [37]
3,6	β-D-Glc*p*-(1 ↓ 2) -6)-α-D-Glc*p*-(1→1)-sn-Gro-(3-*P*- D-Ala	*B. subtilis* VKM B-764	[37]
3,6	-6)-β-D-Glc*p*-(1→1)-sn-Gro-(3-*P*-	*B. licheniformis* ATCC 9945	[36]
3,6	*-6)-β-Glc-(1→6)-β-Gal-(1→6)-α-Glc-(1→1)-Gro-(3-*P*- 3) ↑ β-Glc-(1	*B. coagulans* AHU 1631	[9]
3,3	-3)-α-D-Gal*p*-(1→1)-sn-Gro-(3-*P*-	*B. licheniformis**	[26]
3,3	β-D-Glc*p*-(1 ↓ 2) -3)-α-D-Gal*p*-(1→1)-sn-Gro-(3-*P*-	*B. licheniformis**	[26]
3,3	*α-Glc-(1 ↓ 6) -3)-α-Gal-(1→1)-Gro-(3-*P*-	*B. subtilis* AHU 1035, AHU 1037	[21]
3,3	*-3)-β-GlcNAc-(1→4)-Rib-ol-(1-*P*-	*B. pumilus* Sh18	[7]
	Poly(acylglycosylpolyol phosphate)		
–	-2)-D-GroA-(1→4)-β-D-Qui*p*4N-(1→1)-sn-Gro-(3-*P*-	*B. subtlis* VKM B-762	[35]
	Poly(glycosyl 1-phosphates)		
–	-6)-β-D-Glc*p*-(1→3)-α-D-Gal*p*NAc-(1-*P*-	*B. subtilis* 168, *B. subtilis* ssp. *subtilis* VKM B-501^T	[32] [38]
–	(OAc)$_{0,6}$ | 6) -4)-β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*-(1-*P*- 3) | (OAc)$_{0,4}$	*B. subtilis* VKM B-761	[5]
–	*-6)-α-ManNAc-(1-*P*-	*B. pumilus* Sh17	[6]
–	*-4)-GlcNAc-(1-*P*-	*B. pumilus* AHU 1650	[23]

Note: 1,3, 2,3 and 1,5, the phosphodiester bond connects the hydroxyl groups at C1 and C3, C2 and C3 of glycerol and C1 and C5 of ribitol, respectively; 3,6 and 3,3, the phosphodiester bond connects the hydroxyl groups at C3 of glycerol and C6 or C3 of a monosaccharide residue, respectively. PDB, phosphodiester bond.

* The structures of the polymers and the strain names are reproduced as in the original publications.

1,5-Poly(ribitol phosphates). In these polymers, adjacent ribitol residues are connected by the phosphodiester bond between the hydroxyl groups at C1 and C5. Carbohydrate substituents and D-alanine residues are usually attached at position 4 of ribitol (PDB 1,5-type; Table 2).

A non-glycosylated 1,5-ribitol teichoic acid was identified in the cell wall of *B. pumilus* Sh18 [7]. Cell wall ribitol teichoic acids of *B. subtilis* W23 and S31 contain β-glucopyranose and D-alanine residues [29]. Later, NMR spectroscopic evidence was presented for the existence of analogous teichoic acid in two strains of Caribbean marine bacilli (Collection of marine microorganisms of the Pacific Institute of Bioorganic Chemistry, Far-East Branch of the Russian Academy of Sciences), viz. *B. subtilis* CMM 234 (R-1) and *B. licheniformis* CMM 454 (1-1G-2) (no indication of the presence of D-alanine is given) [34] as well as in *B. subtilis* VKM B-761 [5].

α-Glucopyranose-containing ribitol teichoic acids were found in cell walls of *B. subtilis* VKM B-722 and VKM B-922 [5], and a ribitol teichoic acid with N-acetyl-β-glucosamine was identified in *B. subtilis* VKM B-762 [35]. Both structures were established by NMR spectroscopy.

Poly(glycosylpolyol phosphates). The teichoic acids with the poly(glycosylpolyol phosphate) structures contain a glycosyl component in the main chain together with a polyol, chiefly glycerol. The phosphodiester bond connects the hydroxyl groups at C3 of glycerol and C3 or C6 of the sugar moiety. In the main chain, the latter is glycosidically linked to the hydroxyl group at C1 or C2 of glycerol. The majority of studied poly(glycosylpolyol phosphates) belong to the polymers with 6-phosphorylated monosaccharide (Sug) residues with the general formula -6)-Sug-(1→1/2)-sn-Gro-(3-*P*- (PDB 3,6-type; Table 2). The monosaccharide constituents of the main chain of poly(glycosylpolyol phosphates) are represented by α- and β-galactopyranose and α- and β-glucopyranose.

3,6-Linked polymers. This subtype of teichoic acids with β-galactose and β-glucose in the main chain was first described in cell walls of *B. licheniformis* ATCC 9945 in 1966 [36]. Later, polymers comprising α-galactopyranose and α-glucopyranose were detected (PDB 3,6-type; Table 2).

Cell wall teichoic acids of *B. subtilis* VKM B-760 and B-764 as well as of *B. licheniformis* ATCC 9945 contain in the main chain β-galactopyranose glycosidically linked to the hydroxyl group at C1 of glycerol [37]. The main chains of teichoic acids of *B. licheniformis* ATCC 9945 [36] and *B. stearothermophilus* B65 [25] have the same structural pattern with β-glucopyranose and α-glucopyranose, respectively, instead of β-galactopyranose. Later, a similar teichoic acid was isolated from the cell walls of *B. subtilis* VKM B-760 and B-764 [37].

Teichoic acids with α-galactopyranose glycosidically linked to the hydroxyl group at C2 of glycerol in the main chain (Table 2) were detected in cell walls of *B. licheniformis* AHU 1371, *B. coagulans* AHU 1366 [22], and *B. coagulans* AHU 1638 [8]. Yet another teichoic acid was identified in *B. coagulans* AHU 1638 [8], which contained a disubstituted glycerol residue bearing α-galactopyranose at the hydroxyl groups at C2 as the main chain constituent and α-glucose at C1 as the side-chain substituent. A similar structure with β-glucose as the side-chain substituent was established for teichoic acids of *B. coagulans* AHU 1631 and AHU 1634 [8].

The structure of yet another cell wall teichoic acid can be regarded as a variation of the polymer from the above-mentioned strain of *B. subtilis* VKM B-764. The polymer repeating unit comprises disubstituted glycerol with α-glucopyranose as the main chain monomer at the hydroxyl group at C1 and β-glucopyranose as the side-chain at the hydroxyl group at C2. This structure was established by NMR spectroscopy [37].

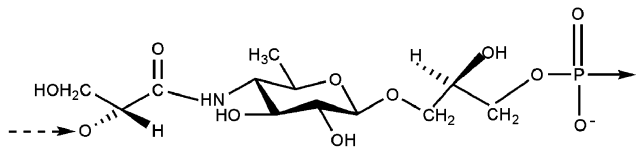
An unusual polymer was isolated from the cell wall of *B. coagulans* AHU 1631 as a minor teichoic acid [9]. The carbohydrate component of the main chain of the poly(glycosylpolyol phosphate) is a linear -6)-β-Glc-(1→6)-β-Gal-(1→6)-α-Glc-(1→ trisaccharide. The majority of the repeating units bear a branching β-glucose residue at position 3 of the terminal β-glucose. The most likely structure of a teichoic acid from the strain AHU 1631 is shown in Table 2. Presumably, the polymer is directly attached to the muramic acid residue of the peptidoglycan by the phosphodiester bond without involvement of any linkage oligomer [9]. This teichoic acid is a unique poly(glycosylpolyol phosphate) with a tetrasaccharide repeating unit; however, it should be noted that the polymer structure has not been confirmed by NMR spectroscopic data.

3,3-Linked polymers. Several poly(glycosylpolyol phosphate) teichoic acids where the sugar residue bears the phosphate group at O3 and glycosylates the glycerol residue at O1 are known in bacilli (PDB 3,3-type; Table 2). The main chain of this subtype of teichoic acids is given here as an example: -3)-α-Gal-(1→1)-sn-Gro-(3-*P*-. This teichoic acid was identified as a component of a polymer mixture of *B. licheniformis* cell wall (strain is not specified) [26]. Structural variations of this group of polymers include the presence and the nature of lateral glycosyl substituents on the main-chain galactose residue, viz. β-glucopyranose in *B. licheniformis* [26] and α-glucose in *B. subtilis* AHU 1035 and AHU 1037.

A single example of a poly(glycosylpolyol phosphate) teichoic acid with ribitol in the main chain is reported in *B. pumilus* Sh18 [7]. This teichoic acid is a minor cell wall component, and its structure has not been finally elucidated.

Poly(acylglycosylpolyol phosphate). Expanding the subject of investigations aimed at studying the distribution of the anionic polymers in representatives of *B. subtilis* (unpublished data) resulted in discovery of a

poly(acylglycosylpolyol phosphate) teichoic acid with a novel type of linkages between the monomeric units in the main chain in the cell wall of *B. subtilis* VKM B-762. A characteristic feature of this type of a polymer is that the phosphodiester bond connects the hydroxyl group at C3 of glycerol with the hydroxyl group at C2 of glyceric acid, which is the N-acyl substituent of the 4-amino-4,6-dideoxy-β-glucose residues of the main chain [35]. Thus, the novel unique natural polymer is built with involvement of three types of covalent bonds, viz. phosphodiester, glycosidic, and amide bonds (figure).



Repeating unit of the teichoic acid of *B. subtilis* VKM B-762

POLY(GLYCOSYL 1-PHOSPHATES)

Poly(glycosyl 1-phosphates) were discovered in the early 1970s. In their composition, they are akin to teichoic acids and for years bore the same name. The repeating units in poly(glycosyl 1-phosphates), mono- or oligosaccharide residues, are linked with a phosphodiester bond between the anomeric hydroxyl group of one monomer and the hydroxyl group at C3, C4, or C6 of the other. Like teichoic acids, poly(glycosyl 1-phosphates) are localized in the cell wall and are covalently bound to the peptidoglycan through a linkage oligomer (Table 1) [17, 27]. Currently, poly(glycosyl 1-phosphates) have been identified in micrococci, staphylococci, streptococci, actinomycetes [27], and certain strains of bacilli [5, 6, 23, 32, 38]. The polymers are linear or branched and their repeating units comprise from one to eight monosaccharide residues [27]. In bacilli, poly(glycosyl 1-phosphates) with mono- and disaccharide repeating units were found (Table 2).

The phosphodiester bond in this class of polymers is highly labile and their extraction from the cell walls is usually performed under milder conditions [39] as compared to those employed for the isolation of teichoic acids.

It should be noted that poly(glycosyl 1-phosphates) have not been taken into consideration in the classification of the secondary bacterial polymers (teichoic acids, teichuronic acids, and "non-classical" polysaccharides) suggested in 2005 [2], presumably, due to small number of the established structures. Nevertheless, this class of polymers, together with teichoic acids, corresponds to the first group of "classical" polysaccharides.

The first poly(glycosyl 1-phosphate) of bacilli was discovered in 1973 in the cell wall of *B. subtilis* 168 [38]. The polymer contained a disaccharide built of 1→3-linked β-D-glucopyranose and N-acetyl-α-D-galactosamine with the phosphodiester bond between the hydroxyl groups at C6 of β-glucopyranose and C1 of the amino sugar: -6)-β-D-Glc*p*-(1→3)-α-D-Gal*p*NAc-(1-*P*-. An analogous polymer has recently been isolated from the cell wall of *B. subtilis* ssp. *subtilis* VKM B-501^T (=*B. subtilis* NCTC 3610), and its structure has been established by NMR spectroscopy [32]. Structurally identical phosphate-containing polymers, teichoic acids and poly(glycosyl 1-phosphates), are present in the cell walls of both strains, which are referred to as the subspecies *B. subtilis* ssp. *subtilis* according to the modern classification.

A polymer with a different disaccharide structure, viz. -β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*-(1-*P*-, was isolated from the cell wall of *B. subtilis* VKM B-761 [5]. Its characteristic feature is the presence of two *O*-acetyl groups at O3 and O6 of N-acetyl-β-D-glucosamine (Table 2). Earlier, *O*-acetyl substituents have been found only in teichoic acids of actinomycetes [27].

Poly(glycosyl 1-phosphates) with monosaccharide repeating units were identified in two strains of *B. pumilus*. A polymer with N-acetylmannosamine residues linked by a phosphodiester bond between the hydroxyl groups at C1 and C6 was found in *B. pumilus* Sh17. The polymer that was supposed to be localized in the cell wall manifested the properties of a group antigen [6]. A cell wall polymer with N-acetylglucosamine residues linked by a phosphodiester bond between the hydroxyl groups at C1 and C4 was identified in *B. pumilus* AHU 1650 [23].

Three types of teichoic acids as well as poly(glycosyl 1-phosphates) with mono- and disaccharide repeating units were revealed in cell walls of several strains of bacteria of the genus *Bacillus*. The teichoic acids are mainly represented by glycerol-containing polymers bearing *O*-D-alanine residues as the acyl substituents and α- and β-linked glucose or galactose. In their composition, the teichoic acids of bacilli are akin to those of actinomycetes, though they are far less structurally different, which is related probably to the lesser number of the microorganisms investigated.

A characteristic feature of the teichoic acids of bacilli is the presence of the D-alanine residues, which are believed to play a significant role in the manifesting of the biological and functional activities of these polymers [16]. The D-alanine residues can change the surface charge of Gram-positive bacteria, control the ligand binding to cations and cell autolysins, and maintain homeostasis and control the electromechanical properties of the cell wall [16]. It is the degree of substitution with D-alanine that is the mechanism involved in the cell adaptation to the environmental changes, e.g. increase in temperature and salt concentration. The D-alanine residues seem to be

related to manifestation of some other properties of the cell, such as involvement in adhesion, the ability to interact with different positively charged molecules, as well as virulence and pathogenicity of bacteria [16, 17].

Many strains of bacilli, like actinomycetes, contain a heterogeneous set of cell wall phosphate-containing polymers, either teichoic acids with different structures or teichoic acids and poly(glycosyl 1-phosphates) (Table 3). Unsubstituted poly(glycerol phosphate) was observed in certain strains of bacilli as a minor cell wall teichoic acid [37] or a predominant polymer [23, 26]. No unsubstituted poly(glycerol phosphate) was found in the strains from the Japanese collection AHU; this may be partly rationalized as being due to the fact that it was glycosides, i.e. the degradation products of the polymers, rather than the intact teichoic acid chains, that were studied. At the same time, in strains *B. subtilis* AHU 1035 and 1037 [21], the amount of glycerol phosphate in a mixture of the degradation products of the carbohydrate-containing fractions was higher than that required for structure of the poly(glycosylglycerol phosphate) teichoic acid identified in these microorganisms.

In view of our data on the heterogeneous set of cell wall teichoic acids of bacilli, one can assume that glycerol phosphate is a repeating unit of yet another polymer, viz. poly(glycerol phosphate) bearing no glycosyl substituents.

Investigation into the structures of teichoic acids has been performed so far for an inconsiderable number of species of bacilli: *B. subtilis*, *B. atrophaeus*, *B. licheniformis*, *B. pumilus*, *B. coagulans*, and one strain of *B. cereus*. As follows from the present review, one cannot also ignore the fact that different strains of the same species, e.g. *B. subtilis*, contain teichoic acids with different structures, which may suggest the inhomogeneity of the species and different taxonomic statuses of the investigated strains.

Over years, the possibility of employing teichoic acids and other cell wall glycopolymers in the taxonomy

Table 3. Strains of bacilli containing several cell wall polymers

Strains	Phosphate-containing cell wall polymers*
B. subtilis VKM B-760, VKM B-764	TA1: poly(glucosyl(1→1)glycerol) TA2: poly(galactosyl(1→1)glycerol) TA3: 1,3-poly(glycerol phosphate)
*B. licheniformis***	TA1: 1,3-poly(glycerol phosphate) TA2: poly(galactosyl(1→1)glycerol phosphate) TA3: poly(galactosyl(1→1)glycerol phosphate) with β-D-Glc*p*
B. licheniformis ATCC 9945	TA1: poly(glucosyl(1→1)glycerol phosphate) TA2: poly(galactosyl(1→1)glycerol phosphate)
B. subtilis VKM B-762	TA1: 1,5-poly(ribitol phosphate) TA2: poly(acylglycosylglycerol phosphate)
B. pumilus Sh18	TA1: 1,3-poly(glycerol phosphate) TA2: 1,5-poly(ribitol phosphate) TA3: poly(glycosylribitol phosphate)
B. coagulans AHU 1631	TA1: poly(glycosylglycerol phosphate) with β-Glc TA2: poly(galactosyl(1→2)glycerol phosphate)
B. coagulans AHU 1638	TA1: poly(galactosyl(1→2)glycerol phosphate) TA2: poly(galactosyl(1→2)glycerol phosphate) with α-Glc*p*
B. subtilis ssp. *subtilis* VKM B-501^T, *B. subtilis* 168	TA: 1,3-poly(glycerol phosphate) Poly(glycosyl 1-phosphate)
B. subtilis VKM B-761	TA: 1,5-poly(ribitol phosphate) Poly(glycosyl 1-phosphate)
B. pumilus AHU 1650	TA: 1,3-poly(glycerol phosphate) Poly(glycosyl 1-phosphate)
B. pumilus Sh17	TA: 1,3-poly(glycerol phosphate) Poly(glycosyl 1-phosphate)

Note: TA, teichoic acid.

* The polymers structures are listed in Table 2.

** The structures of the polymers and the strain names are reproduced as in the original publications.

of Gram-positive bacteria has been disputed. Initially, teichoic acids were supposed to be used to classify lactobacilli [40] and staphylococci [41], while the absence of teichoic acids from micrococci is an important chemotaxonomic characteristic that distinguishes them from staphylococci [42]. Currently, the characteristics "the presence/absence of cell wall teichoic acids" enters a compulsory list of markers used in the description of certain genera of Gram-positive cocci [43]; it is also recommended for the notation of strains of a new genus *Macrococcus* [44] and new species of staphylococci [45].

It has earlier been observed that certain representatives of some species of the genus *Bacillus* contain no cell wall teichoic acids. Thus no phosphate-containing polymers were found in 14 of 17 strains of *B. megaterium* and *B. cereus* studied [46]. Later it was shown that strains of *B. anthracis*, *B. cereus*, and *B. thuringiensis*, belonging to the group *B. cereus* are also devoid of teichoic acids and other phosphate-containing polymers; their cell walls were reported to contain unique "non-classical" polysaccharides. Studies of the genome of separate representatives of this group revealed the absence of the genes encoding the enzymes of teichoic acid biosynthesis [47].

Strains of the group *B. cereus* manifest diverse pathogenic properties. Thus *B. thuringiensis* is an insect pathogen; soil *B. cereus* in rare cases is a causative agent of non-lethal poisoning in humans or, occasionally, of a severe pneumonia; *B. anthracis*, the causative agent of anthrax, is extremely dangerous. On the contrary, microorganisms of the group *B. subtilis* are, for the most part, non-pathogenic. They can produce various useful substances, such as enzymes, surfactants, antibiotics and probiotics, which make them especially attractive for biotechnology [4, 48]. Certain strains of the group *B. subtilis* contain "classical" cell wall polysaccharides, viz. teichoic acids and poly(glycosyl 1-phosphates); the enzymes of their biosynthesis are well studied with *B. subtilis* 168 and W23 as examples [19, 49]. Thus, strains of two groups, *B. cereus* and *B. subtilis*, that form phylogenetically different clusters differ also in the composition and nature of the respective cell wall glycopolymers. This corroborates data inferred from studies of the genomes of these bacilli [47].

The characteristics "the presence/absence of cell wall teichoic acids" as well as the nature of cell wall glycopolymers can be employed for the differentiation of the bacilli belonging to two groups, whereas the set and the structures of the glycopolymers may be of interest for the taxonomy of closely related strains within each group. Thus the structures of "non-classical" secondary glycopolymers are considered as species-specific characteristics of the strains of *B. cereus* and *B. anthracis* [14]. One cannot rule out that "classical" polymers of the representatives of the *B. subtilis* group, the taxonomy of which is impeded by the high level of similarity of sequences of the 16S rRNA gene fragments and similar physiological biochemical characteristics [3, 4], can serve as markers for the subdivision of the strains of bacilli at the species/subspecies level.

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