Review

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Structural diversity and biological significance of lipoteichoic acid in Gram-positive bacteria: focusing on beneficial probiotic lactic acid bacteria

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Bacterial cell surface molecules are at the forefront of host-bacterium interactions. Teichoic acids are observed only in Gram-positive bacteria, and they are one of the main cell surface components. Teichoic acids play important physiological roles and contribute to the bacterial interaction with their host. In particular, lipoteichoic acid (LTA) anchored to the cell membrane has attracted attention as a host immunomodulator. Chemical and biological characteristics of LTA from various bacteria have been described. However, most of the information concerns pathogenic bacteria, and information on beneficial bacteria, including probiotic lactic acid bacteria, is insufficient. LTA is structurally diverse. Strain-level structural diversity of LTA is suggested to underpin its immunomodulatory activities. Thus, the structural information on LTA in probiotics, in particular strain-associated diversity, is important for understanding its beneficial roles associated with the modulation of immune response. Continued accumulation of structural information is necessary to elucidate the detailed physiological roles and significance of LTA. In this review article, we summarize the current state of knowledge on LTA structure, in particular the structure of LTA from lactic acid bacteria. We also describe the significance of structural diversity and biological roles of LTA.

Key words: lipoteichoic acid, repeating unit, glycolipid anchor, lactic acid bacteria, probiotics, *Lactobacillus* spp.

OVERVIEW OF TEICHOIC ACIDS

The cell surface of bacteria comprises the cell membrane and cell wall peptidoglycan as its main components. The cell membrane and cell wall play numerous physiologically relevant roles, such as separation of the intra- and extracellular microenvironments, maintenance of homeostasis, and protection against many environmental stresses. Teichoic acids (TAs) are specific polymers on Gram-positive bacterial cell surfaces and are not found in Gram-negative bacterial cells. The word "teichoic" originates from the Greek word teîkhos (τεῖχος), meaning "wall." TAs comprise up to 50% of the cell wall dry weight [1, 2]. Thus, they are believed to play important physiological roles.

Two distinct types of TAs, a wall-teichoic acid (WTA) attached to the cell wall and lipoteichoic acid (LTA) anchored to the cell membrane, have been identified (Fig. 1). WTAs were initially discovered by Armstrong *et al.* in 1958 in cell wall fractions of *Lactobacillus plantarum* (formerly *Lactobacillus arabinosus*), *Bacillus subtilis*, and *Staphylococcus aureus* [3, 4]. LTAs were identified by Kelemen *et al.* in 1961 as structurally similar molecules to WTAs in cell membrane fractions [5]. WTA and LTA backbones are generally anionic polymers consisting of repeating polyol phosphate units that, in rare cases, also contain sugar phosphate. In most LTAs, the backbone is comprised of poly-glycerol phosphate (poly-GroP). By contrast, the WTA backbone varies between bacterial species and strains. Typically, it is comprised of poly-GroP or poly-ribitol phosphate (poly-RboP). In WTA, the backbone consisting of repeating units is covalently linked to C-6 of the cell wall *N*-acetylmuramic acid residue via disaccharide phosphate residues (*N*-acetylmannosaminyl-*N*-acetylglucosamine phosphate, *N*-acetylmannosaminyl-glucosamine phosphate, or glucosyl-*N*-acetylglucosamine phosphate) as linkage units (Fig. 2) [6]. In the case

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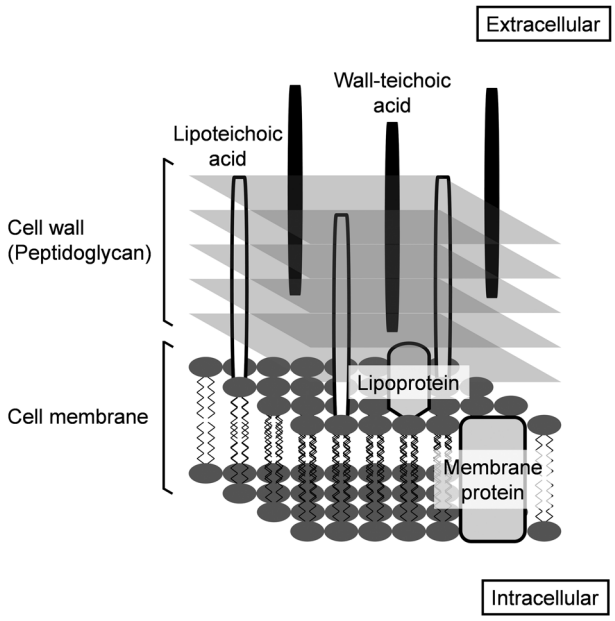


Fig. 1. Overview of the Gram-positive cell surface architecture.
Lipoteichoic acids are anchored to the cell membrane via glycolipids, while the wall-teichoic acids are covalently bound to cell wall peptidoglycan.

of LTA, poly-GroP is covalently linked to hexose residues of cell membrane glycolipids (Fig. 2) [1]. The glycolipids connected to the poly-GroP backbone are called glycolipid anchors. A typical glycolipid anchor is a dihexosyldiacylglycerol (Hex_2DAG) comprised of two hexose residues [typically glucose (Glc), and/or galactose (Gal)] and diacylglycerol.

Gram-positive bacteria generally possess both WTAs and LTAs. However, *Lactobacillus casei* ATCC 334 has only LTAs [2]. LTAs (and related amphipathic macromolecules) have not been found in *Bacillus circulans* AHU 1363, AHU 1365, and AHU 1646, and *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) AHU 1231 and AHU 1385 [7]. Genetically engineered mutants lacking LTA have been constructed in *S. aureus* [8] and *Lactobacillus acidophilus* [9]. These results suggested a dispensable nature of WTA or LTA in Gram-positive bacteria. Alternatively, other macromolecules in the cell surface may fill in for TA functions.

Structural information on WTAs and LTAs from a single strain is available in a few cases, and structural variation of WTAs is greater than that of LTAs [10]. It appears that the physiological roles of LTAs are different from those of WTAs. Polarity constitutes the major difference between these polymers. In contrast to the hydrophilic nature of WTAs, LTAs are amphipathic molecules comprising

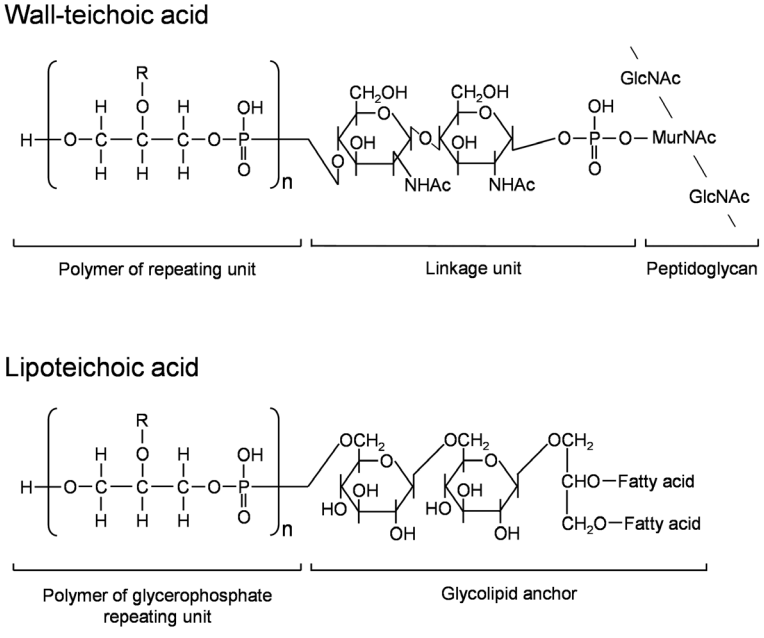


Fig. 2. Typical structures of wall-teichoic and lipoteichoic acids.
In most cases, R is H, D-alanine, and/or hexose. Typical hexose residues include glucose, galactose, and *N*-acetylglucosamine. GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid.

hydrophilic poly-GroP and a hydrophobic glycolipid anchor. In general, the free hydroxyl groups in GroP and RboP repeating units are often substituted by D-alanine (D-Ala), Glc, Gal, and/or *N*-acetylglucosamine (GlcNAc) (Fig. 2). D-Ala is more frequently found as a substituent than other compounds. The structural diversity of TAs is mainly associated with the types of substituents and substitution ratios of the repeating units [7, 11, 12].

Phosphate residues of the repeating units, in addition to those of cell membrane phospholipids, impart a negative charge to the cell surface. On the other hand, D-Ala, which partly substitutes hydroxyl groups in the repeating units, imparts a positive charge. TAs are therefore zwitterionic polymers. Partial and heterogeneous D-Ala substitutions affect the distribution of charge, hydrophobicity, and TA stereostructure. In particular, positive charges of D-Ala residues are involved in the reduction of negative charge of the cell surface. TAs play very important roles in bacterial physiology, e.g., preservation of divalent cations, including Mg^{2+}, for growth [13], maintenance of proton gradient across the cell membrane for energy metabolism [14], and protection against cationic antimicrobial peptides via three-component peptide-sensing systems [15]. WTA is involved in protecting peptidoglycan from bacteriolysis by lysozyme [16] and in the control of lytic enzyme localization during cell division [17]. LTA is also involved in the progression of normal cell division [18]. Cell wall glycopolymers and also LTA, including those derived from lactic acid bacteria, are receptors for bacteriophages [19].

STRUCTURAL DIVERSITY IN LTAS

Structural information is available for LTAs from many bacteria. However, most of the available information is limited to specific bacterial genera/species, including non-opportunistic or opportunistic pathogens: *Bacillus* spp., *Clostridium* spp., *Enterococcus* spp., *Listeria* spp., *Staphylococcus* spp., and *Streptococcus* spp. (Table 1). Little is known about the LTA structure in probiotic and related bacteria (Fig. 3). LTA is a candidate immunomodulatory molecule not only in pathogenic bacteria but also in probiotic and commensal bacteria. Thus, structural information concerning LTA derived from probiotics is important. To the best of our knowledge, structures of both the repeating unit and glycolipid anchor of LTA have been identified in 91 strains from 11 genera/53 species (Tables 1 and 2, Fig. 3). In addition, structural information concerning unspecified strains and/or partial structures of either the repeating unit or the glycolipid anchor have also been reported. Typical LTA structures in most of these bacteria comprise GroP-repeating units as the backbone, with D-Ala, hexose, and/or hexosamine residues as substituents, and a glycolipid Hex_2DAG anchor unit (Fig. 2). In the following sections, the diversity of LTA structures in bacteria other than *Lactobacillus* spp. (Table 1) and *Lactobacillus* strains (Table 2) will be presented.

i) Gram-positive bacteria other than *Lactobacillus* spp.: D-Ala, Glc, Gal, and GlcNAc residues are generally present as C-2 hydroxyl group substituents of the GroP-repeating unit (Fig. 2), but other sugars can also be present, albeit less frequently. Glc oligosaccharides, including di-, tri-, and tetrasaccharides, are found in some *Enterococcus* spp. (formerly *Streptococcus* spp.) [20–29] and *Streptococcus sanguinis* DSM 20567^T [21, 29, 30] and DSM 20068 [30] (Table 1). On the other hand, unique repeating units other than GroP have also been reported. A Gal-Gal-GroP-repeating unit was detected in *Lactococcus garvieae* (formerly *Streptococcus lactis*)

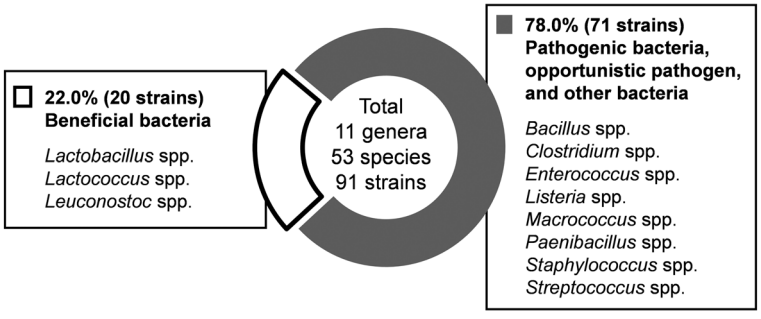


Fig. 3. Bacterial species whose complete lipoteichoic acid structures are known.

Details of lipoteichoic acid of such beneficial lactic acid bacteria as *Lactobacillus* spp., *Lactococcus* spp., and *Leuconostoc* spp. are relatively sparse compared with non-opportunistic and opportunistic pathogens.

Table 1. Structures of lipoteichoic acids from Gram-positive bacteria

Bacterial species (former name)	Strain name	Glycolipid anchor structure	Repeating unit structure (number of units)	Substituent (substitution ratio)	Extraction method	Reference
Bacillus cereus	AHU 1030	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala	Phe/H_2O	[7]
	AHU 1355	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala	Phe/H_2O	[7]
	AHU 1356	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala	Phe/H_2O	[7]
	CH	Glc-Glc-DAG	Gro-P (28)	Ala (41%)	BuOH	[87]
	T	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala	Phe/H_2O	[7]
Bacillus coagulans	AHU 1366	MAG, DAG	Gro-P	Gal (42%)	Phe/H_2O	[11]
	AHU 1634	MAG, DAG	Gro-P	Gal (40%)	Phe/H_2O	[11]
Bacillus clausii	O/C	Glc-Glc-DAG	Gro-P (20)	Ala (3%), GlcNAc (3%)	BuOH	[87]
Bacillus licheniformis	DSM 13^T	Glc-Glc-DAG	Gro-P	Ala (76%), GlcNAc (18%)	Phe/H_2O	[29, 42]
	AHU 1371	Glc-Glc-DAG	Gro-P	Ala, GlcNAc	Phe/H_2O	[11]
	AHU 1372	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala, GlcNAc	Phe/H_2O	[7]
Bacillus megaterium	ATCC 14581^T	DAG	Gro-P	None	Phe/H_2O	[29, 42]
	AHU 1373	MAG, DAG	Gro-P	Gal (5%)	Phe/H_2O	[11]
	AHU 1375	MAG, DAG	Gro-P	Gal (5%)	Phe/H_2O	[11]
Bacillus pumilus	AHU 1650	Glc-Glc-DAG	Gro-P	Ala, GlcNAc	Phe/H_2O	[11]
Bacillus subtilis	AHU 1031	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	None	Phe/H_2O	[7]
	AHU 1035	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala, Glc, GlcNAc	Phe/H_2O	[7]
	AHU 1037	Glc-Glc-DAG	Gro-P	Ala, Glc, GlcNAc	Phe/H_2O	[11]
	AHU 1219	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala	Phe/H_2O	[7]
	AHU 1235	Glc-Glc-DAG	Gro-P	Ala, Glc, GlcNAc	Phe/H_2O	[11]
	AHU 1390	Glc-Glc-DAG	Gro-P	Ala, GlcNAc	Phe/H_2O	[11]
	AHU 1616	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala, Glc, GlcNAc	Phe/H_2O	[7]
	CU1	Glc-Glc-DAG	Gro-P (23)	Ala (17%), GlcNAc (7%)	BuOH	[87]
	DSMZ 1087	Glc-Glc-DAG	Gro-P (22)	Ala (25%),	BuOH	[60]

Bacterial species (former name)	Strain name	Glycolipid anchor structure	Repeating unit structure (number of units)	Substituent (substitution ratio)	Extraction method	Reference
				GlcNAc (25%)		
	W23	Glc-Glc-DAG	Gro-P (24)	Ala (35–68%), GlcNAc (18–37%)	Phe/H_2O	[11, 21, 29, 42]
Clostridium difficile	CM-26	Glc-Glc-Glc-DAG	GlcNAc-GlcNAc(GroA)-P, GlcN-GlcNAc(GroA)-P (<10)	None	Phe/H_2O	[35]
	630	Glc-Glc-Glc-DAG	GlcNAc-GlcNAc(GroA)-P, GlcN-GlcNAc(GroA)-P (<10)	None	Phe/H_2O	[35]
Clostridium innocuum	ATCC 14501^T	GlcN-Glc-DAG	P-Gal-Gro-P-Gal-Gro (8)	GlcN (50%), GlcNAc (25%)	Phe/H_2O	[34]
Enterococcus avium	DSM 20679^T	Glc-Glc-DAG	Gro-P (20–31)	Ala (26%), [Glc, Glc-Glc] (16%)	Phe/H_2O	[21]
Enterococcus casseliflavus	DSM 20680^T	Glc-Glc-DAG	Gro-P (12–25)	Glc-Glc (58%)	Phe/H_2O	[21]
Enterococcus durans	DSM 20633^T	Glc-Glc-DAG	Gro-P (22–39)	Ala (38%), [Glc, Glc-Glc] (38%)	Phe/H_2O	[21]
Enterococcus faecalis (*Streptococcus faecalis*)	DSM 20478^T	Glc-Glc-DAG	Gro-P (19)	Ala (15%), Glc-Glc (40%)	Phe/H_2O	[21]
	DSM 20371 (Kiel 27738)	Glc-Glc-DAG, Glc-Glc (PA) -DAG	Gro-P (12–33)	Ala (28–48%), [Glc or Glc-Glc] (18–47%)	Phe/H_2O	[21, 22, 24, 28, 29, 42]
Enterococcus hirae (*Enterococcus faecalis, Streptococcus faecalis, Streptococcus faecium*)	ATCC 9790^T (NCIB 8191^T)	Glc-Glc-DAG, Glc-Glc (PA) -DAG	Gro-P (8–39)	Ala (0–31%), $(Glc)_{1-4}$ (55–90%)	Phe/H_2O	[20–27, 29]
Enterococcus malodoratus	DSM 20681	Glc-Glc-DAG	Gro-P (18–33)	Ala (37%), [Glc, Glc-Glc] (10%)	Phe/H_2O	[21]
Lactococcus garvieae (*Streptococcus lactis*)	NCDO 2155^T	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gal-Gal-Gro(Gal)-P (9–10)	None	Phe/H_2O	[29, 34]
	Kiel 42172	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gal-Gal-Gro(Gal)-P (6–11)	None	Phe/H_2O or $CHCl_3$/MeOH	[22, 31–33]
	NCFB 2730	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gal-Gal-Gro(Gal)-P (4–12)	None	Phe/H_2O	[27]
Lactococcus lactis subsp. *cremoris*	NCDO 607^T	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gro-P	Ala (58%), Gal (4%)	Phe/H_2O	[29]
Lactococcus lactis subsp. *lactis* (*Streptococcus lactis*)	NCDO 712	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gro-P (18–23)	Ala (21–47%), Gal (28–52%)	Phe/H_2O	[22, 29, 32, 42, 43]
	NCDO 2727	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gro-P	Ala (28%), Gal (66%)	Phe/H_2O	[29, 42]
Lactococcus plantarum	NCDO 1869^T	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gro-P	Ala (45%)	Phe/H_2O	[29, 42]
Leuconostoc citreum (*Leuconostoc mesenteroides*)	DSM 20188^T	Glc-Glc-DAG	Gro-P (20–26)	Ala (0–59%)	Phe/H_2O	[22, 32]
Leuconostoc mesenteroides subsp. *mesenteroides*	DSM 20343^T	Glc-Glc-DAG, Glc-AcylGlc-DAG	Gro-P (31–36)	Ala (29–52%), $(Glc)_{1-4}$ (8–59%)	Phe/H_2O	[24, 29, 42]
Listeria monocytogenes	ATCC 43251	Gal-Glc-DAG, Gal-Glc (PA) -DAG	Gro-P (10–23)	Ala (53–57%), Gal (7–8%)	BuOH	[44]
	NCTC 1383	Gal-Glc-DAG, Gal-Glc (PA) -DAG	Gro-P	Gal	Phe/H_2O	[45]
	NCTC 5214	Gal-Glc-DAG, Gal-Glc (PA) -DAG	Gro-P	Gal	Phe/H_2O	[45]
	NCTC 7973	Gal-Glc-DAG	Gro-P (23)	Ala (31–42%), Gal (21–23%)	Phe/H_2O	[21, 29, 42]
	NCTC 93/65	Gal-Glc-DAG, Gal-Glc (PA) -DAG	Gro-P	Gal	Phe/H_2O	[45]

Bacterial species (former name)	Strain name	Glycolipid anchor structure	Repeating unit structure (number of units)	Substituent (substitution ratio)	Extraction method	Reference
	NCTC F4	Gal-Glc-DAG, Gal-Glc (PA) -DAG	Gro-P	Gal	Phe/H_2O	[45]
Listeria seeligeri	SLCC 3954^T	Gal-Glc-DAG	Gro-P	Ala (34%), Gal (17%)	Phe/H_2O	[29]
Listeria welshimeri	SLCC 5334^T	Gal-Glc-DAG	Gro-P (24)	Ala (56–59%), Gal (36–38%)	Phe/H_2O	[21, 29, 42]
Macrococcus caseolyticus (*Staphylococcus caseolyticus, Micrococcus varians*)	ATCC 29750	Glc-Glc-DAG	Gro-P (37)	None	Phe/H_2O	[22, 29, 42]
Paenibacillus thiaminolyticus (*Bacillus subtilis*)	AHU 1392^T	Glc-Glc-DAG, Glc-Glc-MAG	Gro-P	Ala, Glc, GlcNAc	Phe/H_2O	[7]
Staphylococcus aureus	DSM 20233 (H)	Glc-Glc-DAG	Gro-P (4–48)	Ala (30–81%), GlcNAc (4–21%)	Phe/H_2O or BuOH	[22, 27, 29, 32, 42, 43, 59, 60, 88]
	Hgol$^{-1}\varphi^R$71	Glc-Glc-DAG	Gro-P	Ala (54%)	Phe/H_2O	[29]
Staphylococcus capitis subsp. *capitis*	DSM 20326^T	Glc-Glc-DAG	Gro-P	Ala (25%)	Phe/H_2O	[88]
Staphylococcus carnosus subsp. *carnosus*	DSM 20501^T	Glc-Glc-DAG	Gro-P	Ala (42%)	Phe/H_2O	[88]
Staphylococcus cohnii subsp. *cohnii*	DSM 20260^T	Glc-Glc-DAG	Gro-P (20)	GlcNAc (4%)	Phe/H_2O	[88]
Staphylococcus epidermidis	DSM 20044^T	Glc-Glc-DAG	Gro-P	Ala (47%)	Phe/H_2O	[88]
Staphylococcus haemolyticus	DSM 20263^T	Glc-Glc-DAG	Gro-P	Ala (58%)	Phe/H_2O	[88]
Staphylococcus hominis subsp. *hominis*	DSM 20328^T	Glc-Glc-DAG	Gro-P (29)	Ala (11%), GlcNAc (15%)	Phe/H_2O	[88]
Staphylococcus lentus (*Staphylococcus sciuri*)	DSM 20352^T	Glc-Glc-DAG	Gro-P (29)	Ala (13%)	Phe/H_2O	[88]
Staphylococcus saccharolyticus	DSM 20359^T	Glc-Glc-DAG	Gro-P	Ala (43%)	Phe/H_2O	[88]
Staphylococcus saprophyticus subsp. *saprophyticus*	DSM 20229^T	Glc-Glc-DAG	Gro-P (11)	Ala (14%), GlcNAc (7%)	Phe/H_2O	[88]
Staphylococcus simulans	DSM 20322^T	Glc-Glc-DAG	Gro-P (29)	Ala (17%), GlcNAc (7%)	Phe/H_2O	[88]
Staphylococcus warneri	DSM 20316^T	Glc-Glc-DAG	Gro-P (29)	Ala (15%)	Phe/H_2O	[88]
Staphylococcus xylosus	DSM 20266^T	Glc-Glc-DAG	Gro-P	Ala (31%), GlcNAc (15%)	Phe/H_2O	[29, 42]
Streptococcus agalactiae	0250	Glc-Glc-DAG	Gro-P (34)	Ala (33%)	BuOH	[89]
Streptococcus dysgalactiae	2023	Glc-Glc-DAG	Gro-P (28)	Ala (37%)	BuOH	[89]
Streptococcus mutans	NCTC 10449^T	Glc-Glc-DAG	Gro-P	Ala (57%)	Phe/H_2O	[21, 29, 42]
Streptococcus oralis	Uo5	Glc-DAG	GalNAc(PC)$_2$-Rbo-P-Gal-AATGal (2–5)	Gal (to Gal), Acetyl group (to Gal)	BuOH	[36]
Streptococcus pneumoniae	R6 (ATCC BAA-225)	Glc-DAG or Glc-AATGal-Glc-DAG	GalNAc(PC)-GalNAc(PC)-Rbo-P-Glc-AATGal[*1] or Glc-AATGal-GalNAc(PC)-GalNAc(PC)-Rbo-P[*1] (2–8)	None or GalNAc (to Rbo) (40%)	$CHCl_3$/MeOH or BuOH	[34, 37–40]
	R36A (ATCC 12214)	Glc-DAG	GalNAc(PC)-GalNAc(PC)-Rbo-P-Glc-AATGal	None	$CHCl_3$/MeOH	[37]
Streptococcus pyogenes	D58	Glc-Glc-DAG	Gro-P (25)	None	Phe/H_2O	[32]
	II D298	Glc-Glc-DAG	Gro-P	Ala (47%)	Phe/H_2O	[29]
Streptococcus sanguinis (*Streptococcus sanguis*)	DSM 20567^T	Glc-Glc-DAG	Gro-P (18)	Ala (34–49%), $(Glc)_{1–4}$ (35–46%)	Phe/H_2O	[21, 29, 30, 42]
	DSM 20068	Glc-Glc-DAG	Gro-P	Ala (56%), $(Glc)_{1–4}$ (21%)	Phe/H_2O	[30]
Streptococcus uberis	233	Glc-Glc-DAG	Gro-P (24)	Ala (44%)	BuOH	[89]
Streptococcus sp. (closely related *S. pneumoniae*)	DSM 8747	Gal-DAG	Gro-P (7–17)	Ala	Phe/H_2O	[41]

Bacillus circulans (AHU 1363, AHU 1365, and AHU 1646) and *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) (AHU 1231 and AHU 1385) do not contain LTA [7].
AATGal: 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose; Ala: alanine; DAG: diacylglycerol; Gal: galactose; GalN: galactosamine; GalNAc: *N*-acetylgalactosamine; Glc: glucose; GlcN: glucosamine; GlcNAc: *N*-acetylglucosamine; Gro: glycerol; GroA: glyceric acid; Hex: hexose; MAG: monoacylglycerol; P: phosphate; PA: phosphatidic acid; PC: phosphocholine; Rbo: ribitol; BuOH: butanol; MeOH: methanol; Phe: phenol.
[*1]One repeating unit contains one or two PC residues.

Table 2. Structures of lipoteichoic acids from *Lactobacillus* spp.

Bacterial species (former name)	Strain name	Glycolipid anchor structure	Repeating unit structure (number of units)	Substituent (substitution ratio)	Extraction method	Reference
Lactobacillus brevis	ATCC 8287	Unknown	Gro-P	Ala, Glc, AlaGlc	BuOH	[47]
Lactobacillus casei	BL23	Unknown	Gro-P (42)	Ala (64%)	BuOH	[56]
Lactobacillus delbrueckii subsp. *lactis*	ATCC 15808	Glc-Glc-Glc-DAG, Glc-Glc-AcylGlc-DAG	Gro-P (29–37)	Ala (21–27%), Glc (3%)	Phe/H_2O	[90]
	Ads-5	Glc-Glc-Glc-DAG, Glc-Glc-AcylGlc-DAG	Gro-P (31–36)	Ala (42–49%)	Phe/H_2O	[90]
	LL78	Glc-Glc-Glc-DAG, Glc-Glc-AcylGlc-DAG	Gro-P (30–34)	Ala (25–28%), Glc (26–27%)	Phe/H_2O	[90]
Lactobacillus gasseri	JCM 1131^T	(Gal-Gal-Gal-Glc)^{*1}-DAG, (Gal-Gal-Gal-Glc-Acyl)^{*1,2}-DAG	Gro-P (20–30)	Ala (31%)	BuOH	[49]
Lactobacillus helveticus	DSM 20075^T	Glc-Gal-Glc-DAG, Glc-Gal-AcylGlc-DAG	Gro-P (24)	Ala (57–64%)	Phe/H_2O	[43]
Lactobacillus pentosus (*Lactobacillus plantarum*)	DSM 20314^T	Glc-Gal-Glc-DAG, Glc-Gal-AcylGlc-DAG	Gro-P (22)	None	Phe/H_2O	[32, 42]
Lactobacillus plantarum	JCM 1149^T	Hex-Hex-Hex-DAG, (Hex-Hex-Hex-Acyl)^{*2}-DAG	Gro-P (110)	Ala (42%), Glc (10%)	BuOH	[91]
	L-137	Hex-Hex-Hex-DAG, (Hex-Hex-Hex-Acyl)^{*2}-DAG	Gro-P (96)	Ala (50%), Glc (2%)	BuOH	[91]
	KCTC 10887BP (K8)	Glc-Gal-Glc-DAG, Glc-Gal-AcylGlc-DAG	Gro-P	Ala, Glc, Gal	BuOH	[46, 48]
	NCIMB 8826	Unknown	Gro-P	Ala (42%)	BuOH	[66]
Lactobacillus reuteri	100-23	Unknown	Gro-P (20)	Ala (74–79%), Glc (6%)	BuOH	[92]
Lactobacillus rhamnosus (*Lactobacillus casei*)	DSM 20021^T	Glc-Gal-Glc-DAG, Glc-Gal-AcylGlc-DAG	Gro-P (40)	None	Phe/H_2O	[32, 42, 93]
	GG (ATCC 53103)	Glc-Gal-Glc-DAG	Gro-P (30–78)	Ala (72–74%)	BuOH	[50, 51]
Lactobacillus sakei	KCCM 11175P (K101)	Hex-Hex-DAG, (Hex-Hex-Acyl)^{*2}-DAG	Unknown	Unknown	BuOH	[48]

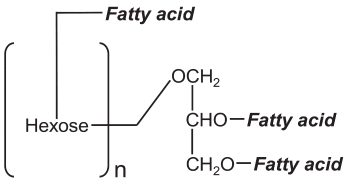
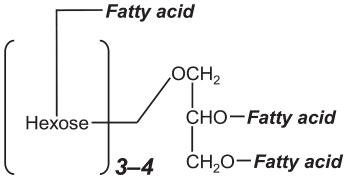
Ala: alanine; AlaGlc: alanyl-glucose; DAG: diacylglycerol; Gal: galactose; Glc: glucose; Gro: glycerol; Hex: hexose; P: phosphate; BuOH: butanol; Phe: phenol.
^{*1}The order of Gal and Glc is unknown.
^{*2}The linkage position of the hexose-bound acyl group is unknown.

[22, 27, 29, 31–34]. Several rare repeating units have been identified in *Clostridium difficile* [35], *Clostridium innocuum* ATCC 14501^T [34], *Streptococcus oralis* Uo5 [36], and *Streptococcus pneumoniae* R6 (ATCC BAA-225) [34, 37–40] and R36A (ATCC 12214) [37] (see Table 1 for detailed structures).

Dihexosyl glycerol is a typical saccharide moiety of the LTA glycolipid anchor (Fig. 2). However, some *Streptococcus* spp. use monohexosyl glycerol [36, 37, 41], and trihexosyl glycerol has been detected in *C. difficile* [35] (Table 1). Glc and Gal are the most commonly found glycolipid anchor residues in LTA in Gram-positive bacteria. In *Bacillus coagulans* AHU 1366 and AHU 1634 [11] and *Bacillus megaterium* ATCC 14581^T [29, 42], AHU 1373, and AHU 1375 [11], GroP polymer directly binds to mono- or diacylglycerol; that is, no hexose residues intervene between the repeating units and the lipid anchor (Table 1). Generally, the glycolipid anchor has two acyl groups. However, dihexosylmonoacylglycerol has been reported in some *Bacillus* spp. and *Paenibacillus thiaminolyticus* (formerly *B. subtilis*) [7]. Other bacteria, such as *Lactococcus* spp. [22, 27, 29, 31–34, 42, 43] and *Leuconostoc mesenteroides* [24, 42], have a third acyl group attached to a hexose residue of the glycolipid anchor (Table 1). This type of glycolipid anchor is termed acyldihexosyldiacylglycerol (AcylHex_2DAG). A glycolipid anchor that contains phosphatidic acid with two acyl groups attached to the hexose residue (i.e., with four acyl groups per LTA molecule) has been reported in *Enterococcus faecalis* DSM 20371 (Kiel 27738) (formerly *Streptococcus faecalis*) [22, 24], *Enterococcus hirae* ATCC 9790^T (NCIB 8191^T) (formerly *E. faecalis*, *S. faecalis*, or *Streptococcus faecium*) [22–25, 27], and many *Listeria monocytogenes* strains [44, 45] (Table 1).

ii) *Lactobacillus* strains: *Lactobacillus* spp. typically comprise beneficial probiotic bacteria. To the best of our knowledge, LTA structures have been reported for 16 strains from 10 species. Information regarding both repeating unit and glycolipid anchor structures is

Table 3. General architecture of lipoteichoic acids from beneficial probiotic lactic acid bacteria

Beneficial probiotic lactic acid bacteria, including *Lactobacillus* spp.	Three acyl groups of the glycolipid anchor
	
Lactobacillus spp.	Tri- or tetrasaccharides in the glycolipid anchor
	

Characteristic structures are indicated by italics. Hexose-linked fatty acids are attached to only one of the hexose residues.

available in 11 out of 16 strains (Table 2).

The *Lactobacillus* spp. LTA repeating unit consists of a typical poly-GroP backbone with D-Ala as a common substituent. In most cases, Glc is also found as another substituent together with D-Ala. In the case of *L. plantarum* KCTC 10887BP (K8), Gal has been detected in addition to D-Ala and Glc [46]. One unusual exception is D-Ala at C-6 of Glc in *Lactobacillus brevis* ATCC 8287 [47] (Table 2). Aminosugars, such as GlcNAc, have not been identified in a substituent of the GroP-repeating unit. It is interesting that all *Lactobacillus* spp., except for *Lactobacillus sakei* KCCM 11175P (K101) [48], do not possess a typical dihexosyl glycerol but, instead, have trihexosyl glycerol as the glycolipid anchor saccharide moiety (Tables 2 and 3). Moreover, we recently found that *Lactobacillus gasseri* JCM 1131^T, an intestinal lactic acid bacterium, has tetrahexosyl glycerol comprising Gal and Glc at a molar ratio of 3:1 [49] (Table 2). This is the first demonstration of a tetrasaccharide in the LTA glycolipid anchor. As *L. gasseri* is often commercially employed as a probiotic, the prevalence of this novel anchor structure in *L. gasseri* and its related species need to be investigated.

All the reported *Lactobacillus* spp. LTA glycolipid anchor structures, except for *Lactobacillus rhamnosus* GG (ATCC 53103) [50, 51], contain not only the typical Hex_xDAG but also $AcylHex_xDAG$, with a hexose-attached third fatty acid residue (Table 2). Interestingly, $AcylHex_2DAG$ is also found in *Lactococcus* spp. and *L. mesenteroides*, as described above (Table 1). Therefore, a glycolipid structure containing three acyl groups may be characteristic for beneficial probiotic lactic acid bacteria, and it might be defined as the lactic acid bacteria-specific LTA (Table 3). However, a glycolipid anchor with three acyl groups has not been found in *L. rhamnosus* GG LTA [50, 51]. A more detailed structural analysis might be required before we can conclude that $AcylHex_xDAG$ is absent from strain GG. It should also be noted that *Lactobacillus* spp. are commonly equipped with glycolipid anchors comprising trisaccharides or tetrasaccharides (for the moment, tetrasaccharides are found only in *L. gasseri* JCM 1131^T) (Table 2). Structural characteristics of *Lactobacillus* spp. glycolipids (Table 3) may potentially affect physiological properties of the cell surface.

The significance of the structural diversity of LTA in Gram-positive bacteria remains unclear. Current knowledge about the structural characteristics of LTA raises interesting questions regarding the relationship between the LTA structural diversity in pathogenic and beneficial bacteria and microbial virulence, pathogenicity, and probiotic functions. However, more data on the structural and biological characteristics of LTA from a broader range of species and strains are required to answer these questions.

FACTORS INFLUENCING LTA STRUCTURE

i) Environmental factors: Environmental stresses imposed by different growth conditions may affect the structural diversity of LTA. For example, a microarray analysis revealed a significant activation of the *Staphylococcus epidermidis* 1457 *dlt* operon, encoding genes for D-Ala substitution of TA, after exposure to human cationic antimicrobial peptide β-defensin 3 [15]. A *Lactococcus lactis* mutant defective in D-Ala substitution (Δ*dltD* mutant) displayed increased susceptibility to a cationic antimicrobial peptide nisin, and to lysozyme in comparison with the parental strain, *L. lactis* MG1363, while a *dltD*-overexpressing mutant showed increased resistance to nisin [52]. Thus, these reports indicate that D-Ala substitution in the repeating unit plays a role in stress resistance. Furthermore, it is thought that the ratio of free hydroxyl groups to D-Ala residues varies not only at species and strain levels but also in response to growth conditions, including pH [53], NaCl concentration [54], and temperature [55]. The number of repeating units and the D-Ala substitution ratio decreased under NaCl-exerted osmotic stress in *L. casei* BL23 LTA [56]. On the other hand, such functions of hexose substituents and the effect of growth conditions on the degree of hexose substitution have not been reported.

ii) Extraction procedures: Hot phenol/water extraction has been used for many years as a typical LTA extraction procedure [57, 58]. A similar hot phenol/water extraction procedure constitutes a conventional extraction procedure for lipopolysaccharides, amphipathic glycolipids of the cell surface of Gram-negative bacteria. The extraction yields an LTA- and nucleic acid-containing water phase, while the phenol phase contains denatured proteins and residual insoluble material. LTAs are further purified by anion exchange chromatography and/or hydrophobic interaction chromatography to eliminate contaminants, such as nucleic acids. Butanol extraction with 1-butanol was proposed in 2001 [59], and this method recently became a common method of LTA extraction. Butanol extraction relies on the polarity of LTA molecules, similar to hot phenol/water extraction, but the principles of butanol extraction are not understood in detail. Whereas careful attention is necessary for handling of phenol because of its toxicity, butanol has the advantage of easy handling. Furthermore, hot phenol may cause partial LTA destruction, such as degradation of the GroP polymer and elimination of the substituents. Morath *et al.* compared chemical structures of *S. aureus* DSM 20233 LTA prepared by phenol or butanol extraction methods [59]. Compared with LTA extracted with butanol, LTA polymer extracted with phenol was shorter, with less than ten GroP-repeating units and a reduced number of D-Ala and GlcNAc substitutions. In particular, the degree of D-Ala substitutions in phenol-extracted LTA was less than half that in butanol-extracted LTA. Thus, butanol extraction can yield a less damaged LTA than the hot phenol/water extraction procedure. Lines of evidence indicate that the extraction procedures clearly affect the results of analysis of the LTA chemical structure. Numerous determinations of LTA chemical structures have been performed with LTA preparations obtained by the conventional hot phenol/water method (Tables 1 and 2). Importantly, commercial LTA preparations have been shown to be inhomogeneous and decomposed. Furthermore, significant amounts of contaminants having immunostimulatory activity are present in the preparations [60]. Thus, the immunomodulation of commercial LTA preparations is quite different compared with that of butanol-extracted LTA. Therefore, comparison of LTA structure between species or strains requires careful consideration, taking into account structural alteration and degradation associated with the different extraction protocols.

LTA-MEDIATED BACTERIA-HOST INTERACTIONS

i) LTA-mediated host adhesion: The interaction between LTA and the host is important, and LTA can act as an adhesion molecule. LTA is involved in the adhesion of *Lactobacillus johnsonii* La1 to human intestinal epithelium Caco-2 cells [61]. Cell-free LTA of *Streptococcus pyogenes* can modulate the attachment of bacterial cells to the host cell surface through cross-linking between a bacterial M protein and host fibronectin [62]. Thus, fibronectin is a candidate host LTA receptor. Recently, Baur *et al.* reported that a nasal epithelial cell scavenger receptor expressed by endothelial cell-I (SREC-I) was a host receptor for *S. aureus*, binding the WTA GroP polymer [63]. They also showed that colonization of the rat nasal cavity by *S. aureus* was inhibited by an anti-SREC-I antibody. Therefore, the GroP polymer, found in both WTA and LTA, might play an important role in bacterial colonization of the host.

ii) Host receptors and LTA immunomodulation: The structural heterogeneity of LTA was suggested to impact host immune response. Host cells recognize LTA via Toll-like receptor 2 (TLR2), a pattern recognition receptor for pathogen-associated molecular patterns that transduces cellular signals to induce proinflammatory cytokines [64–68]. However, the reports concerning immunomodulating activities of LTA via TLR2 have

been contradictory. It has been reported that LTA does not induce TLR2-mediated cytokine production [69], and conflicting reports on LTA cytokine-inducing activity have been published. Suda *et al.* reported that, in conventional *E. hirae* ATCC 9790^T LTA preparations, the cytokine-inducing activity fraction can be separated from LTA by a combination of hydrophobic interaction and anion-exchange chromatography [70]. Hashimoto et al. verified that purified *E. hirae* ATCC 9790^T LTA has no cytokine-inducing activity [20], and the authors concluded that the activity may have been due to the contaminating lipoproteins in such conventional LTA preparations [71, 72]. They confirmed that LTA obtained from a lipoprotein-deficient mutant (Δ*lgt* mutant) had no detectable TLR2 cytokine-inducing activity [69]. The controversy surrounding the cytokine-inducing activity of LTA is still debated [73–75]. Most likely, the interaction between LTA and host cells is very complicated. To clarify it, it is necessary to unify the experimental materials and conditions employed, such as the LTA preparations (LTA from pathogenic or beneficial bacterial or chemically-synthesized LTA), contaminating molecules of the LTA preparations, host immune cells (whole blood, peripheral blood mononuclear cells, dendritic cells, or cell lines originating from macrophages, monocytes, and intestinal epithelial cells), and target molecules for measurement (NF-κB activation and production of IL-1β, IL-8, TNF-α, IL-10, IL-12, and IFN-γ). Several candidates for LTA receptors other than TLR2 have been reported: a lipopolysaccharide-binding protein and CD14, both of which are involved in lipopolysaccharide recognition [21]; a mannose-binding protein [76]; L-ficolin [77]; a type I macrophage scavenger receptor, which is expressed by phagocytes [78]; and paired immunoglobulin-like receptor-B, which is expressed by many immune cells [79]. All these reports suggest that LTA significantly contributes to host immune modulation during bacteria-host interactions. In the future, unambiguous details of the interaction of LTA with human immune response should be understood for application of bacteria as synbiotics.

iii) Structural requirements of LTA for modulation of the host immune response: The relationship between LTA structure and the host immune response has been investigated. The importance of D-Ala substitution of GroP-repeating units for cytokine induction *in vitro* has been reported [51, 80, 81]. Deininger *et al.* evaluated the minimum structural requirements of LTA for cytokine-inducing activity using chemically-synthesized LTA. More than three GroP-repeating units with D-Ala substitutions were required for the induction of proinflammatory cytokines [81]. Different inflammatory responses including proinflammatory cytokine production were induced *in vivo* by D-Ala substitution-deficient mutants (*dlt* operon mutants) as compared with those induced by the parental strain *L. plantarum* NCIMB8826 [66]. Smelt *et al.* constructed an *L. plantarum* WCFS1 Δ*dltX-D* mutant defective in D-Ala substitutions of the GroP-repeating units. Mutant immunomodulatory activities, especially TLR2-dependent NF-κB activation *in vitro* and differentiation of dendritic cells and T-cell populations *in vivo*, were different from those of the parental strain [82]. Acyl groups of the glycolipid anchor are also considered to be important for the immunomodulatory activity of LTA. It was reported that *L. plantarum* KCTC 10887BP LTA, but neither heat-inactivated cells nor peptidoglycan, inhibited Pam2CSK4-induced IL-8 expression and that D-Ala substitutions and lipid moieties of the LTA are required for the agonistic activity [83]. Cytokine-inducing activity was altered by elimination of acyl groups from LTA extracted from *L. rhamnosus* GG [51] and *S. aureus* DSM 20233 [84]. Fatty acid residues (i.e., molecular species, residue number) vary with each LTA molecule. Lines of evidence indicate that LTA is a cytokine-inducing factor of intestinal Gram-positive bacteria, and heterogeneous LTA structures are potentially a key factor in host immunomodulation. On the other hand, it was also reported that D-Ala substitutions of LTA GroP-repeating units [50] and the glycolipid anchor [81] are not important for the induction of cytokines. The cytokine-inducing activity of defined structural elements of LTA has to be clarified. Details of the LTA recognition mechanism by the host will reveal the significance of LTA structural diversity in bacterial-host interactions.

Recently, an LTA-deficient *L. acidophilus* mutant lacking a phosphoglycerol transferase gene (LBA0447) was constructed by a double-crossover gene replacement strategy [9]. The parental *L. acidophilus* NCFM strain and LTA-deficient mutant were examined in a mouse model of dextran sulfate sodium (DSS)-induced colitis [9]. When a viable LTA-deficient *L. acidophilus* mutant was administered orally before the administration of DSS, DSS-induced colitis was significantly suppressed compared with the effect of parental strain administration. Administration of LTA-deficient mutant cells also facilitated the resolution of inflammation of the DSS-induced colitis. Reduced production of proinflammatory cytokines IL-12 and TNF-α and enhanced production of the anti-inflammatory cytokine IL-10 were observed in dendritic cells derived from mice inoculated with the LTA-deficient mutant. It is suggested that the suppression of inflammation in mice inoculated with LTA-deficient

L. acidophilus was caused by an altered induction of cytokines. In addition, regulation of the LTA-deficient mutant-induced IL-10 production was suggested to be mediated by the Erk1/2 mitogen-activated protein kinase signaling pathway [85], and LTA-deficient mutant administration resulted in increased numbers of regulatory dendritic cells and activated regulatory T-cells ($FoxP3^+$ Tregs) [86]. Findings from experiments with the LTA-deficient mutants strongly suggest that LTA affects host immune response. However, the detailed mechanism of host-LTA interaction remains to be elucidated. Noh *et al.* showed that *L. plantarum* KCTC 10887BP LTA inhibited Pam2CSK4-induced IL-8 expression more potently than LTAs from *S. aureus*, *E. faecalis*, and *Streptococcus mutans* [83]. The difference in immunomodulatory effects between *Lactobacillus* spp. LTA and other pathogenic bacterial LTAs is interesting when we consider the structural characteristics of *Lactobacillus* spp. LTA (Tables 2 and 3). Thus, information on the LTA structure might provide a solution to the problem; for example, large numbers of hexoses and acyl groups in the glycolipid anchor and no aminosugar substitution in GroP-repeating units.

CONCLUSION

LTA is regarded as an important cell surface molecule of Gram-positive bacteria, with roles in bacterial physiology and bacterial interaction with the host. Data on the LTA chemical structure, extraction procedures, and LTA immunomodulatory activities are accumulating, and detailed physiological and biological roles of LTA are increasingly understood. On the other hand, numerous questions have been raised. For example, questions about how and why the LTA structural diversity is generated and about the significance of LTA structural diversity for bacterial physiology and host interactions. Full knowledge of LTA chemical structures and biological activities has to be obtained before these questions can be answered.

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REFERENCES

1. Delcour J, Ferain T, Deghorain M, Palumbo E, Hols P. 1999. The biosynthesis and functionality of the cell-wall of lactic acid bacteria. Antonie van Leeuwenhoek 76: 159–184. [Medline] [CrossRef]
2. Kleerebezem M, Hols P, Bernard E, Rolain T, Zhou M, Siezen RJ, Bron PA. 2010. The extracellular biology of the lactobacilli. FEMS Microbiol Rev 34: 199–230. [Medline] [CrossRef]
3. Armstrong JJ, Baddiley J, Buchanan JG, Carss B, Greenberg GR. 1958. 882. Isolation and structure of ribitol phosphate derivatives (teichoic acids) from bacterial cell walls. J Chem Soc 4344–4354. [CrossRef]
4. Armstrong JJ, Baddiley J, Buchanan JG, Carss B. 1958. Nucleotides and the bacterial cell wall. Nature 181: 1692–1693. [Medline] [CrossRef]
5. Kelemen MV, Baddiley J. 1961. Structure of the intracellular glycerol teichoic acid from *Lactobacillus casei* A.T.C.C. 7469. Biochem J 80: 246–254. [Medline] [CrossRef]
6. Araki Y, Ito E. 1989. Linkage units in cell walls of gram-positive bacteria. Crit Rev Microbiol 17: 121–135. [Medline] [CrossRef]
7. Iwasaki H, Shimada A, Yokoyama K, Ito E. 1989. Structure and glycosylation of lipoteichoic acids in *Bacillus* strains. J Bacteriol 171: 424–429. [Medline]
8. Oku Y, Kurokawa K, Matsuo M, Yamada S, Lee BL, Sekimizu K. 2009. Pleiotropic roles of polyglycerolphosphate synthase of lipoteichoic acid in growth of *Staphylococcus aureus* cells. J Bacteriol 191: 141–151. [Medline] [CrossRef]
9. Mohamadzadeh M, Pfeiler EA, Brown JB, Zadeh M, Gramarossa M, Managlia E, Bere P, Sarraj B, Khan MW, Pakanati KC, Ansari MJ, O'Flaherty S, Barrett T, Klaenhammer TR. 2011. Regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. Proc Natl Acad Sci USA 108 Suppl 1: 4623–4630. [Medline] [CrossRef]
10. Tomita S, Furihata K, Tanaka N, Satoh E, Nukada T, Okada S. 2012. Determination of strain-specific wall teichoic acid structures in *Lactobacillus plantarum* reveals diverse α-D-glucosyl substitutions and high structural uniformity of the repeating units. Microbiology 158: 2712–2723. [Medline] [CrossRef]
11. Iwasaki H, Shimada A, Ito E. 1986. Comparative studies of lipoteichoic acids from several *Bacillus* strains. J Bacteriol 167: 508–516. [Medline]
12. Fischer W. 1988. Physiology of lipoteichoic acids in bacteria. Adv Microb Physiol 29: 233–302. [Medline] [CrossRef]
13. Heptinstall S, Archibald AR, Baddiley J. 1970. Teichoic acids and membrane function in bacteria. Nature 225: 519–521. [Medline] [CrossRef]
14. Calamita HG, Ehringer WD, Koch AL, Doyle RJ. 2001. Evidence that the cell wall of *Bacillus subtilis* is protonated during respiration. Proc Natl Acad Sci USA 98: 15260–15263. [Medline] [CrossRef]
15. Li M, Lai Y, Villaruz AE, Cha DJ, Sturdevant DE, Otto M. 2007. Gram-positive three-component antimicrobial peptide-sensing system. Proc Natl Acad

Sci USA 104: 9469–9474. [Medline] [CrossRef]
16. Bera A, Biswas R, Herbert S, Kulauzovic E, Weidenmaier C, Peschel A, Götz F. 2007. Influence of wall teichoic acid on lysozyme resistance in *Staphylococcus aureus*. J Bacteriol 189: 280–283. [Medline] [CrossRef]
17. Yamamoto H, Miyake Y, Hisaoka M, Kurosawa S, Sekiguchi J. 2008. The major and minor wall teichoic acids prevent the sidewall localization of vegetative DL-endopeptidase LytF in *Bacillus subtilis*. Mol Microbiol 70: 297–310. [Medline] [CrossRef]
18. Gründling A, Schneewind O. 2007. Synthesis of glycerol phosphate lipoteichoic acid in *Staphylococcus aureus*. Proc Natl Acad Sci USA 104: 8478–8483. [Medline] [CrossRef]
19. Chapot-Chartier MP. 2014. Interactions of the cell-wall glycopolymers of lactic acid bacteria with their bacteriophages. Front Microbiol 5: 236. [Medline] [CrossRef]
20. Hashimoto M, Yasuoka J, Suda Y, Takada H, Yoshida T, Kotani S, Kusumoto S. 1997. Structural feature of the major but not cytokine-inducing molecular species of lipoteichoic acid. J Biochem 121: 779–786. [Medline] [CrossRef]
21. Hermann C, Spreitzer I, Schröder NW, Morath S, Lehner MD, Fischer W, Schütt C, Schumann RR, Hartung T. 2002. Cytokine induction by purified lipoteichoic acids from various bacterial species—role of LBP, sCD14, CD14 and failure to induce IL-12 and subsequent IFN-γ release. Eur J Immunol 32: 541–551. [Medline] [CrossRef]
22. Fischer W, Rösel P, Koch HU. 1981. Effect of alanine ester substitution and other structural features of lipoteichoic acids on their inhibitory activity against autolysins of *Staphylococcus aureus*. J Bacteriol 146: 467–475. [Medline]
23. Takada H, Kawabata Y, Arakaki R, Kusumoto S, Fukase K, Suda Y, Yoshimura T, Kokeguchi S, Kato K, Komuro T, Tanaka N, Saito M, Yoshida T, Sato M, Kotani S. 1995. Molecular and structural requirements of a lipoteichoic acid from *Enterococcus hirae* ATCC 9790 for cytokine-inducing, antitumor, and antigenic activities. Infect Immun 63: 57–65. [Medline]
24. Leopold K, Fischer W. 1991. Separation of the poly (glycerophosphate) lipoteichoic acids of *Enterococcus faecalis* Kiel 27738, *Enterococcus hirae* ATCC 9790 and *Leuconostoc mesenteroides* DSM 20343 into molecular species by affinity chromatography on concanavalin A. Eur J Biochem 196: 475–482. [Medline] [CrossRef]
25. Toon P, Brown PE, Baddiley J. 1972. The lipid-teichoic acid complex in the cytoplasmic membrane of *Streptococcus faecalis* N.C.I.B. 8191. Biochem J 127: 399–409. [Medline] [CrossRef]
26. Cabacungan E, Pieringer RA. 1985. Evidence for a tetraglucoside substituent on the lipoteichoic acid of *Streptococcus faecium* ATCC 9790. FEMS Microbiol Lett 26: 49–52. [CrossRef]
27. Fischer W. 1993. Molecular analysis of lipid macroamphiphiles by hydrophobic interaction chromatography, exemplified with lipoteichoic acids. Anal Biochem 208: 49–56. [Medline] [CrossRef]
28. Leopold K, Fischer W. 1992. Hydrophobic interaction chromatography fractionates lipoteichoic acid according to the size of the hydrophilic chain: a comparative study with anion-exchange and affinity chromatography for suitability in species analysis. Anal Biochem 201: 350–355. [Medline] [CrossRef]
29. Brade L, Brade H, Fischer W. 1990. A 28 kDa protein of normal mouse serum binds lipopolysaccharides of gram-negative and lipoteichoic acids of gram-positive bacteria. Microb Pathog 9: 355–362. [Medline] [CrossRef]
30. Kochanowski B, Fischer W, Iida-Tanaka N, Ishizuka I. 1993. Isomalto-oligosaccharide-containing lipoteichoic acid of *Streptococcus sanguis*. Basic structure. Eur J Biochem 214: 747–755. [Medline] [CrossRef]
31. Fischer W, Schuster D, Laine RA. 1979. Studies on the relationship between glycerophosphoglycolipids and lipoteichoic acids. IV. Trigalactosylglycerophospho-acylkojibiosydiacyl-glycerol and related compounds from *Streptococcus lactis* Kiel 42172. Biochim Biophys Acta 575: 389–398. [Medline] [CrossRef]
32. Fischer W, Koch HU, Rösel P, Fiedler F, Schmuck L. 1980. Structural requirements of lipoteichoic acid carrier for recognition by the poly(ribitol phosphate) polymerase from *Staphylococcus aureus* H. A study of various lipoteichoic acids, derivatives, and related compounds. J Biol Chem 255: 4550–4556. [Medline]
33. Koch HU, Fischer W. 1978. Acyldiglucosyldiacylglycerol-containing lipoteichoic acid with a poly(3-*O*-galabiosyl-2-*O*-galactosyl-*sn*-glycero-1-phosphate) chain from *Streptococcus lactis* Kiel 42172. Biochemistry 17: 5275–5281. [Medline] [CrossRef]
34. Greenberg JW, Fischer W, Joiner KA. 1996. Influence of lipoteichoic acid structure on recognition by the macrophage scavenger receptor. Infect Immun 64: 3318–3325. [Medline]
35. Reid CW, Vinogradov E, Li J, Jarrell HC, Logan SM, Brisson JR. 2012. Structural characterization of surface glycans from *Clostridium difficile*. Carbohydr Res 354: 65–73. [Medline] [CrossRef]
36. Gisch N, Schwudke D, Thomsen S, Heß N, Hakenbeck R, Denapaite D. 2015. Lipoteichoic acid of *Streptococcus oralis* Uo5: a novel biochemical structure comprising an unusual phosphorylcholine substitution pattern compared to *Streptococcus pneumoniae*. Sci Rep 5: 16718. [Medline] [CrossRef]
37. Seo HS, Cartee RT, Pritchard DG, Nahm MH. 2008. A new model of pneumococcal lipoteichoic acid structure

resolves biochemical, biosynthetic, and serologic inconsistencies of the current model. J Bacteriol 190: 2379–2387. [Medline] [CrossRef]
38. Draing C, Pfitzenmaier M, Zummo S, Mancuso G, Geyer A, Hartung T, von Aulock S. 2006. Comparison of lipoteichoic acid from different serotypes of *Streptococcus pneumoniae*. J Biol Chem 281: 33849–33859. [Medline] [CrossRef]
39. Fischer W, Behr T, Hartmann R, Peter-Katalinić J, Egge H. 1993. Teichoic acid and lipoteichoic acid of *Streptococcus pneumoniae* possess identical chain structures. A reinvestigation of teichoid acid (C polysaccharide). Eur J Biochem 215: 851–857. [Medline] [CrossRef]
40. Behr T, Fischer W, Peter-Katalinić J, Egge H. 1992. The structure of pneumococcal lipoteichoic acid. Improved preparation, chemical and mass spectrometric studies. Eur J Biochem 207: 1063–1075. [Medline] [CrossRef]
41. Roethlisberger P, Iida-Tanaka N, Hollemeyer K, Heinzle E, Ishizuka I, Fischer W. 2000. Unique poly(glycerophosphate) lipoteichoic acid and the glycolipids of a *Streptococcus* sp. closely related to *Streptococcus pneumoniae*. Eur J Biochem 267: 5520–5530. [Medline] [CrossRef]
42. Fischer W, Mannsfeld T, Hagen G. 1990. On the basic structure of poly(glycerophosphate) lipoteichoic acids. Biochem Cell Biol 68: 33–43. [Medline] [CrossRef]
43. Fischer W, Koch HU, Rösel P, Fiedler F. 1980. Alanine ester-containing native lipoteichoic acids do not act as lipoteichoic acid carrier. Isolation, structural and functional characterization. J Biol Chem 255: 4557–4562. [Medline]
44. Dehus O, Pfitzenmaier M, Stuebs G, Fischer N, Schwaeble W, Morath S, Hartung T, Geyer A, Hermann C. 2011. Growth temperature-dependent expression of structural variants of *Listeria monocytogenes* lipoteichoic acid. Immunobiology 216: 24–31. [Medline] [CrossRef]
45. Uchikawa K, Sekikawa I, Azuma I. 1986. Structural studies on lipoteichoic acids from four *Listeria* strains. J Bacteriol 168: 115–122. [Medline]
46. Jang KS, Baik JE, Han SH, Chung DK, Kim BG. 2011. Multi-spectrometric analyses of lipoteichoic acids isolated from *Lactobacillus plantarum*. Biochem Biophys Res Commun 407: 823–830. [Medline] [CrossRef]
47. Sánchez Carballo PM, Vilen H, Palva A, Holst O. 2010. Structural characterization of teichoic acids from *Lactobacillus brevis*. Carbohydr Res 345: 538–542. [Medline] [CrossRef]
48. Jeong JH, Jang S, Jung BJ, Jang KS, Kim BG, Chung DK, Kim H. 2015. Differential immune-stimulatory effects of LTAs from different lactic acid bacteria via MAPK signaling pathway in RAW 264.7 cells. Immunobiology 220: 460–466. [Medline] [CrossRef]
49. Shiraishi T, Yokota S, Morita N, Fukiya S, Tomita S, Tanaka N, Okada S, Yokota A. 2013. Characterization of a *Lactobacillus gasseri* JCM 1131^T lipoteichoic acid with a novel glycolipid anchor structure. Appl Environ Microbiol 79: 3315–3318. [Medline] [CrossRef]
50. Perea Vélez M, Verhoeven TL, Draing C, Von Aulock S, Pfitzenmaier M, Geyer A, Lambrichts I, Grangette C, Pot B, Vanderleyden J, De Keersmaecker SC. 2007. Functional analysis of D-alanylation of lipoteichoic acid in the probiotic strain *Lactobacillus rhamnosus* GG. Appl Environ Microbiol 73: 3595–3604. [Medline] [CrossRef]
51. Claes IJ, Segers ME, Verhoeven TL, Dusselier M, Sels BF, De Keersmaecker SC, Vanderleyden J, Lebeer S. 2012. Lipoteichoic acid is an important microbe-associated molecular pattern of *Lactobacillus rhamnosus* GG. Microb Cell Fact 11: 161. [Medline] [CrossRef]
52. Giaouris E, Briandet R, Meyrand M, Courtin P, Chapot-Chartier MP. 2008. Variations in the degree of D-Alanylation of teichoic acids in *Lactococcus lactis* alter resistance to cationic antimicrobials but have no effect on bacterial surface hydrophobicity and charge. Appl Environ Microbiol 74: 4764–4767. [Medline] [CrossRef]
53. MacArthur AE, Archibald AR. 1984. Effect of culture pH on the D-alanine ester content of lipoteichoic acid in *Staphylococcus aureus*. J Bacteriol 160: 792–793. [Medline]
54. Fischer W, Rösel P. 1980. The alanine ester substitution of lipoteichoic acid (LTA) in *Staphylococcus aureus*. FEBS Lett 119: 224–226. [Medline] [CrossRef]
55. Hurst A, Hughes A, Duckworth M, Baddiley J. 1975. Loss of D-alanine during sublethal heating of *Staphylococcus aureus* S6 and magnesium binding during repair. J Gen Microbiol 89: 277–284. [Medline] [CrossRef]
56. Palomino MM, Allievi MC, Gründling A, Sanchez-Rivas C, Ruzal SM. 2013. Osmotic stress adaptation in *Lactobacillus casei* BL23 leads to structural changes in the cell wall polymer lipoteichoic acid. Microbiology 159: 2416–2426. [Medline] [CrossRef]
57. Coley J, Duckworth M, Baddiley J. 1975. Extraction and purification of lipoteichoic acids from Gram-positive bacteria. Carbohydr Res 40: 41–52. [Medline] [CrossRef]
58. Fischer W, Koch HU, Haas R. 1983. Improved preparation of lipoteichoic acids. Eur J Biochem 133: 523–530. [Medline] [CrossRef]
59. Morath S, Geyer A, Hartung T. 2001. Structure-function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*. J Exp Med 193: 393–397. [Medline] [CrossRef]
60. Morath S, Geyer A, Spreitzer I, Hermann C, Hartung T. 2002. Structural decomposition and heterogeneity of commercial lipoteichoic acid preparations. Infect Immun 70: 938–944. [Medline] [CrossRef]

61. Granato D, Perotti F, Masserey I, Rouvet M, Golliard M, Servin A, Brassart D. 1999. Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. Appl Environ Microbiol 65: 1071–1077. [Medline]
62. Simpson WA, Courtney HS, Ofek I. 1987. Interactions of fibronectin with streptococci: the role of fibronectin as a receptor for *Streptococcus pyogenes*. Rev Infect Dis 9 Suppl 4: S351–S359. [Medline] [CrossRef]
63. Baur S, Rautenberg M, Faulstich M, Grau T, Severin Y, Unger C, Hoffmann WH, Rudel T, Autenrieth IB, Weidenmaier C. 2014. A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Pathog 10: e1004089. [Medline] [CrossRef]
64. Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J Biol Chem 274: 17406–17409. [Medline] [CrossRef]
65. Schröder NW, Morath S, Alexander C, Hamann L, Hartung T, Zähringer U, Göbel UB, Weber JR, Schumann RR. 2003. Lipoteichoic acid (LTA) of *Streptococcus pneumoniae* and *Staphylococcus aureus* activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. J Biol Chem 278: 15587–15594. [Medline] [CrossRef]
66. Grangette C, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J, Pot B, Hartung T, Hols P, Mercenier A. 2005. Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. Proc Natl Acad Sci USA 102: 10321–10326. [Medline] [CrossRef]
67. Park OJ, Han JY, Baik JE, Jeon JH, Kang SS, Yun CH, Oh JW, Seo HS, Han SH. 2013. Lipoteichoic acid of *Enterococcus faecalis* induces the expression of chemokines via TLR2 and PAFR signaling pathways. J Leukoc Biol 94: 1275–1284. [Medline] [CrossRef]
68. Hong SW, Baik JE, Kang SS, Yun CH, Seo DG, Han SH. 2014. Lipoteichoic acid of *Streptococcus mutans* interacts with Toll-like receptor 2 through the lipid moiety for induction of inflammatory mediators in murine macrophages. Mol Immunol 57: 284–291. [Medline] [CrossRef]
69. Hashimoto M, Tawaratsumida K, Kariya H, Kiyohara A, Suda Y, Krikae F, Kirikae T, Götz F. 2006. Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in *Staphylococcus aureus*. J Immunol 177: 3162–3169. [Medline] [CrossRef]
70. Suda Y, Tochio H, Kawano K, Takada H, Yoshida T, Kotani S, Kusumoto S. 1995. Cytokine-inducing glycolipids in the lipoteichoic acid fraction from *Enterococcus hirae* ATCC 9790. FEMS Immunol Med Microbiol 12: 97–112. [Medline] [CrossRef]
71. Hashimoto M, Furuyashiki M, Kaseya R, Fukada Y, Akimaru M, Aoyama K, Okuno T, Tamura T, Kirikae T, Kirikae F, Eiraku N, Morioka H, Fujimoto Y, Fukase K, Takashige K, Moriya Y, Kusumoto S, Suda Y. 2007. Evidence of immunostimulating lipoprotein existing in the natural lipoteichoic acid fraction. Infect Immun 75: 1926–1932. [Medline] [CrossRef]
72. Hashimoto M, Tawaratsumida K, Kariya H, Aoyama K, Tamura T, Suda Y. 2006. Lipoprotein is a predominant Toll-like receptor 2 ligand in *Staphylococcus aureus* cell wall components. Int Immunol 18: 355–362. [Medline] [CrossRef]
73. von Aulock S, Hartung T, Hermann C. 2007. Comment on "Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in *Staphylococcus aureus*". J Immunol 178: 2610, author reply 2610–2611. [Medline] [CrossRef]
74. von Aulock S, Hartung T, Hermann C. 2007. Comment on "Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in *Staphylococcus aureus*". J Immunol 178: 2610–2611, author reply 2610–2611. [Medline] [CrossRef]
75. Bunk S, Sigel S, Metzdorf D, Sharif O, Triantafilou K, Triantafilou M, Hartung T, Knapp S, von Aulock S. 2010. Internalization and coreceptor expression are critical for TLR2-mediated recognition of lipoteichoic acid in human peripheral blood. J Immunol 185: 3708–3717. [Medline] [CrossRef]
76. Polotsky VY, Fischer W, Ezekowitz RA, Joiner KA. 1996. Interactions of human mannose-binding protein with lipoteichoic acids. Infect Immun 64: 380–383. [Medline]
77. Lynch NJ, Roscher S, Hartung T, Morath S, Matsushita M, Maennel DN, Kuraya M, Fujita T, Schwaeble WJ. 2004. L-ficolin specifically binds to lipoteichoic acid, a cell wall constituent of Gram-positive bacteria, and activates the lectin pathway of complement. J Immunol 172: 1198–1202. [Medline] [CrossRef]
78. Dunne DW, Resnick D, Greenberg J, Krieger M, Joiner KA. 1994. The type I macrophage scavenger receptor binds to gram-positive bacteria and recognizes lipoteichoic acid. Proc Natl Acad Sci USA 91: 1863–1867. [Medline] [CrossRef]
79. Nakayama M, Kurokawa K, Nakamura K, Lee BL, Sekimizu K, Kubagawa H, Hiramatsu K, Yagita H, Okumura K, Takai T, Underhill DM, Aderem A, Ogasawara K. 2012. Inhibitory receptor paired Ig-like receptor B is exploited by *Staphylococcus aureus* for virulence. J Immunol 189: 5903–5911. [Medline] [CrossRef]
80. Deininger S, Stadelmaier A, von Aulock S, Morath S, Schmidt RR, Hartung T. 2003. Definition of structural prerequisites for lipoteichoic acid-inducible cytokine induction by synthetic derivatives. J Immunol 170: 4134–4138. [Medline] [CrossRef]

81. Deininger S, Figueroa-Perez I, Sigel S, Stadelmaier A, Schmidt RR, Hartung T, von Aulock S. 2007. Use of synthetic derivatives to determine the minimal active structure of cytokine-inducing lipoteichoic acid. Clin Vaccine Immunol 14: 1629–1633. [Medline] [CrossRef]
82. Smelt MJ, de Haan BJ, Bron PA, van Swam I, Meijerink M, Wells JM, Kleerebezem M, Faas MM, de Vos P. 2013. The impact of *Lactobacillus plantarum* WCFS1 teichoic acid D-alanylation on the generation of effector and regulatory T-cells in healthy mice. PLoS One 8: e63099. [Medline] [CrossRef]
83. Noh SY, Kang SS, Yun CH, Han SH. 2015. Lipoteichoic acid from *Lactobacillus plantarum* inhibits Pam2CSK4-induced IL-8 production in human intestinal epithelial cells. Mol Immunol 64: 183–189. [Medline] [CrossRef]
84. Morath S, Stadelmaier A, Geyer A, Schmidt RR, Hartung T. 2002. Synthetic lipoteichoic acid from *Staphylococcus aureus* is a potent stimulus of cytokine release. J Exp Med 195: 1635–1640. [Medline] [CrossRef]
85. Saber R, Zadeh M, Pakanati KC, Bere P, Klaenhammer T, Mohamadzadeh M. 2011. Lipoteichoic acid-deficient *Lactobacillus acidophilus* regulates downstream signals. Immunotherapy 3: 337–347. [Medline] [CrossRef]
86. Khan MW, Zadeh M, Bere P, Gounaris E, Owen J, Klaenhammer T, Mohamadzadeh M. 2012. Modulating intestinal immune responses by lipoteichoic acid-deficient *Lactobacillus acidophilus*. Immunotherapy 4: 151–161. [Medline] [CrossRef]
87. Villéger R, Saad N, Grenier K, Falourd X, Foucat L, Urdaci MC, Bressollier P, Ouk TS. 2014. Characterization of lipoteichoic acid structures from three probiotic *Bacillus* strains: involvement of D-alanine in their biological activity. Antonie van Leeuwenhoek 106: 693–706. [Medline] [CrossRef]
88. Ruhland GJ, Fiedler F. 1990. Occurrence and structure of lipoteichoic acids in the genus *Staphylococcus*. Arch Microbiol 154: 375–379. [Medline] [CrossRef]
89. Czabańska A, Neiwert O, Lindner B, Leigh J, Holst O, Duda KA. 2012. Structural analysis of the lipoteichoic acids isolated from bovine mastitis *Streptococcus uberis* 233, *Streptococcus dysgalactiae* 2023 and *Streptococcus agalactiae* 0250. Carbohydr Res 361: 200–205. [Medline] [CrossRef]
90. Räisänen L, Draing C, Pfitzenmaier M, Schubert K, Jaakonsaari T, von Aulock S, Hartung T, Alatossava T. 2007. Molecular interaction between lipoteichoic acids and *Lactobacillus delbrueckii* phages depends on D-alanyl and α-glucose substitution of poly(glycerophosphate) backbones. J Bacteriol 189: 4135–4140. [Medline] [CrossRef]
91. Hatano S, Hirose Y, Yamamoto Y, Murosaki S, Yoshikai Y. 2015. Scavenger receptor for lipoteichoic acid is involved in the potent ability of *Lactobacillus plantarum* strain L-137 to stimulate production of interleukin-12p40. Int Immunopharmacol 25: 321–331. [Medline] [CrossRef]
92. Walter J, Loach DM, Alqumber M, Rockel C, Hermann C, Pfitzenmaier M, Tannock GW. 2007. D-alanyl ester depletion of teichoic acids in *Lactobacillus reuteri* 100-23 results in impaired colonization of the mouse gastrointestinal tract. Environ Microbiol 9: 1750–1760. [Medline] [CrossRef]
93. Nakano M, Fischer W. 1978. Trihexosyldiacylglycerol and acyltrihexosyldiacylglycerol as lipid anchors of the lipoteichoic acid of *Lactobacillus casei* DSM 20021. Hoppe Seylers Z Physiol Chem 359: 1–11. [Medline]