Supplemental Material

Characterization of lipoteichoic acid from *Lactobacillus gasseri* JCM 1131^{T}: A novel glycolipid anchor structure

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Bacterial strain and growth conditions

Lactobacillus gasseri JCM 1131^{T} was obtained from the Japan Collection of Microorganisms, RIKEN BioResource Center (Tsukuba, Japan). The cells were cultured at 37°C under anaerobic conditions in 0.5 × Difco Lactobacilli MRS Broth (Becton Dickinson and Co., Franklin Lakes, NJ), which was supplemented with 200 mM 2-morpholinoethanesulfonic acid and adjusted to pH 6.5 with NaOH. The bacterial cells were grown to an optical density at 660 nm of 0.6 (log phase).

Purification of lipoteichoic acid (LTA)

The bacterial cells were collected by centrifugation (10,000 × *g*, 8 min, 20°C). The cells were disrupted by a French pressure cell (Ohtake Works, Tokyo, Japan), and the residues were removed by centrifugation (10,000 × *g*, 15 min, 4°C). The supernatant was stirred with an equal volume of n-butanol for 30 min at room temperature, and then centrifuged (10,000 × *g*, 10 min, 20°C). The lower aqueous layer was lyophilized, and then dissolved with 15% (v/v) n-propanol in 100 mM sodium acetate buffer (pH 4.7). After centrifugation (10,000 × *g*, 10 min, 4°C), the supernatant was applied to an Octyl Sepharose 4 Fast Flow column (GE Healthcare UK Ltd., Little Chalfont, UK). Bound material was eluted in a stepwise manner with 15, 25, 35, and 45% (v/v) n-propanol in 100 mM sodium acetate buffer (pH 4.7). LTA was eluted in 35% (v/v) n-propanol-containing buffer. The combined LTA fractions were concentrated, dialyzed against water, and lyophilized.

Analysis of the repeating unit structure of LTA by nuclear magnetic resonance (NMR) spectroscopy

The LTA preparation was dissolved in 99.96% D_2O (Cambridge Isotope Laboratories, Inc., Andover, MA) and analyzed using ^{1}H and ^{13}C distortionless enhancement by polarization transfer (DEPT)-135 NMR spectroscopy with a Bruker AMX-500 spectrometer at 500 MHz for ^{1}H NMR and 126 MHz for ^{13}C DEPT-135 NMR. Two-dimensional homonuclear correlation spectroscopy (COSY) (^{1}H-^{1}H) and two-dimensional heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) (^{1}H-^{13}C) were carried out. The chemical shifts were given with 3-(trimethylsilyl)propionic-2, 2, 3, 3-D_4 acid as an external standard (δ_H and δ_C 0.00).

Preparation of the glycolipid anchor and carbohydrate portion of the glycolipid anchor

The glycolipid anchor fraction of LTA was prepared by treatment with 98% (v/v) acetic acid at 100°C for 3 h. After the removal of acetic acid, the product was partitioned with chloroform/water (1:1, v/v). The organic layer was used as the glycolipid anchor fraction. The carbohydrate portion of the glycolipid anchor was obtained via deacylation by treatment with 20% (w/v) ammonia at room temperature for 12 h. After the removal of ammonia, the product was partitioned with chloroform/methanol/water (2:1:3, v/v/v). The aqueous layer was recovered.

The glycolipid anchor structure of LTA as determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

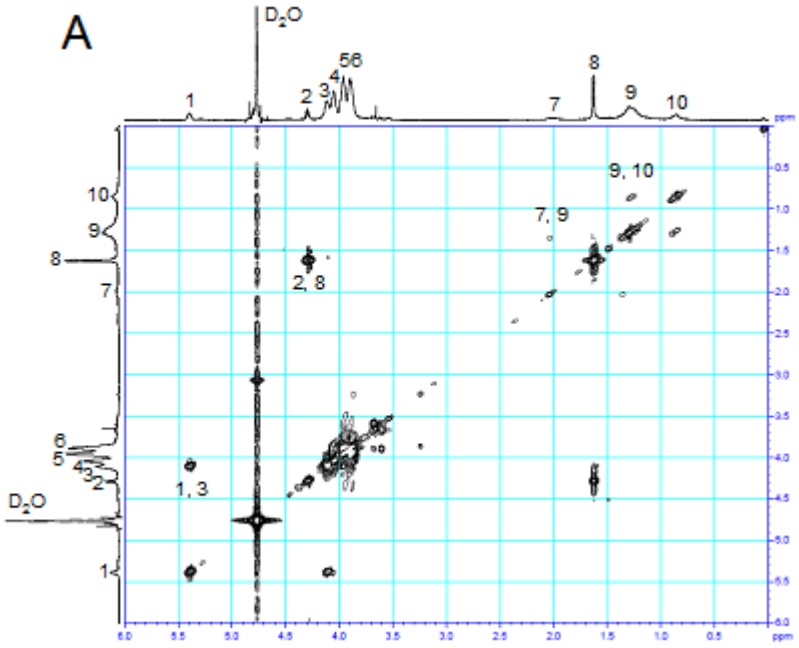
The glycolipid anchor fraction dissolved in chloroform/methanol (2:1, v/v) at a concentration of 1 μg/μl was mixed with an equal amount of matrix [10 mg/ml of 2,5-dihydroxybenzoic acid (DHBA) in water/methanol (7:3, v/v) containing 0.1% (w/v) trifluoroacetic acid (TFA)] on a plate of MTP 384 target ground steel TF (Bruker Daltonics Inc., Billerica, MA). The carbohydrate fraction (0.1 μg/μl in water) was mixed with an equal amount of matrix [saturated DHBA dissolved in 50% (v/v) acetonitrile] on the plate. After co-crystallization, MALDI-TOF mass spectra were acquired in the positive ion and reflectron modes, and the molecular mass was determined using Autoflex II TOF/TOF with the flexControl software (Bruker Daltonics Inc.).

Chemical composition of the glycolipid anchor by gas chromatography (GC)

The carbohydrate portion of the glycolipid prepared above was dissolved in 50 mM ammonium bicarbonate buffer, and further purified by gel-filtration chromatography on a Superdex Peptide HR 10/30 column (GE Healthcare UK Ltd.). Elution of the carbohydrate was monitored by the phenol-sulfuric acid method (1), and the major sugar fractions were pooled and lyophilized. The oligosaccharide was hydrolyzed with 1 M HCl at 100°C for 3 h. After the removal of HCl, mannitol was added as an internal standard. The hydrolysate was peracetylated with pyridine and acetic anhydride for 20 h at room temperature. The products

were analyzed by GC using GC-1700 (Shimadzu Corp., Kyoto, Japan) with 50 m × 0.22 mm (internal diameter) of a BPX70 capillary column (SGE Analytical Science Pty. Ltd., Ringwood, Australia) at 170°C for 6 min, 170-260°C (20°C/min), and maintained at 260°C for 25 min.

Fatty acid composition was determined as described previously (2). The LTA preparation was hydrolyzed with 1 M NaOH for 3 h at 100°C, and then neutralized with HCl. The resulting material was extracted with chloroform. After the addition of heneicosanoic acid as an internal standard, the materials were treated with 10% (v/v) acetyl chloride in methanol for 3 h at 100°C, and then extracted with n-hexane. The methyl esters of the fatty acids were analyzed by GC using a GC-1700 with 25 m × 0.22 mm (internal diameter) of a BPX70 capillary column (SGE Analytical Science Pty. Ltd.) at 160-260°C (10°C/min), and maintained at 260°C for 8 min. The peaks of the fatty acids were identified using FAME Quantitative & Qualitative Mixtures (No. 1021-58110; GL Sciences, Inc., Tokyo, Japan) as standards.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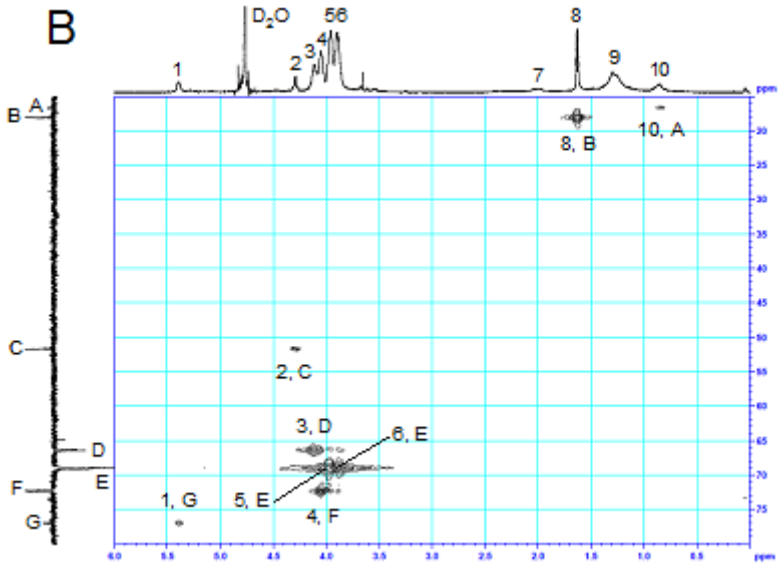
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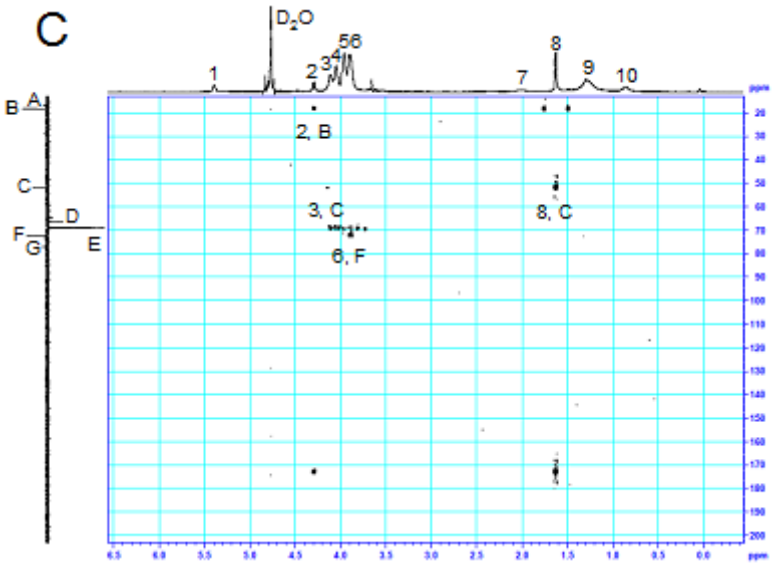

FIG S1 NMR spectra for purified *L. gasseri* JCM 1131^T LTA. Two-dimensional COSY (A), HMQC (B) and HMBC (C) spectra are shown. 1, AlaGro H-2; 2, Ala H-2; 3, AlaGro H-1 and -3; 4, Gro H-2; 5, Gro H-1 or -3; 6, Gro H-3 or -1; 7, protons linked to carbons next to a double-bonded carbon in an unsaturated fatty acid; 8, Ala H-3; 9, protons of CH_2 in fatty acids; 10, protons of CH_3 in fatty acids; A, carbons of CH_3 in fatty acids; B, Ala C-3; C, Ala C-2; D, AlaGro C-1 or -3; E, Gro C-1 or -3; F, Gro C-2; and G, AlaGro C-2.

References

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2. **Morita N, Nishida T, Tanaka M, Yano Y, Okuyama H.** 2005. Enhancement of polyunsaturated fatty acid production by cerulenin treatment in polyunsaturated fatty acid-producing bacteria. Biotechnol. Lett. **27**:389-393.