Supplemental information for:

GtfA and GtfB are both required for protein *O*-glycosylation in *Lactobacillus plantarum*

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Supplemental Materials and methods

Bacterial strains and growth conditions. *Lactobacillus plantarum* WCFS1 (wild-type) and *tagE* deletion mutants (see Table 1) were cultured in 20 L MRS broth (Merck, Darmstadt, Germany) at 37°C for 20 hours using a Techfors-S bioreactor (Infors, Bottmingen, Switzerland) at a constant pH of 6.8 by titration of 6M NaOH with a built-in peristaltic pump and stirrer at 100 rpm. The cells were collected by centrifugation using an AVANTI J-25 centrifuge (Beckman-Coulter, Brea, USA) at 9000 × g at room temperature and were subsequently washed once with 200 ml of phosphate buffer saline.

Preparation of lipoteichoic acid. Lipoteichoic acid (LTA) of the cells cultured was isolated and purified by butanol extraction, hydrophobic interaction chromatography (HIC), and anion-exchange chromatography as described by Morath *et al*. (1). Briefly, the cells were disrupted by a French press cell (SLM Instruments Inc., Urbana, USA) and supernatants after centrifugation (23000 × g for 30 min at 4°C) were collected by pipetting. Subsequently, the supernatants were extracted with the same volume of butanol for 30 min at room temperature and the water phase was collected and lyophilized after centrifugation at 23000 × g for 30 min at room temperature. The lyophilized fraction was then separated on a Octyl-Sepharose 4 FastFlow column (GE Healthcare, Little Chalfont, UK) with a stepwise elution of 13–31% 1-propanol in 100 mM sodium

acetate buffer (pH 4.7). LTA in the fractions was detected by ashing of organic phosphate (2) as described by Allen R. J. (3). For 2D NMR analysis Δ*tagE5E6*-derived LTA was subsequently purified on a DEAE-Sepharose 4 FastFlow column (GE Healthcare) with a linear gradient elution of 0–1.0 M sodium chloride in sodium acetate buffer by using an AKTA FPLC system (GE Healthcare). LTA in the separated fractions were collected by lyophilisation after dialysis against water.

Preparation of deacylated and dealanylated LTA. To improve NMR spectral resolution for signals from anomeric protons of sugar residues, deacylated and dealanylated LTA (dd-LTA) were prepared as described by Simpson *et al*. (4). Briefly, approximately 10 mg of the isolates of the HIC analyses were dissolved in 250 μl water, mixed with the same volume of 30% ammonium hydroxide, and incubated overnight at room temperature. The mixtures were extracted with the same volume of chloroform–methanol (1:1, v/v) once, followed by two chloroform extractions. dd-LTA in the solution was collected by lyophilisation.

NMR spectroscopic analysis of LTA from *ΔtagE5E6* mutant. LTA from HIC fractions and their dd-LTA were dissolved in 400 μl of D_2O and used for measurement of 1D NMR spectra. Purified LTA from Δ*tagE5E6* was also used for measurement of 2D NMR spectra. The 1D (^{1}H and ^{13}C) and 2D NMR spectra (COSY, H-H correlation spectroscopy; TOCSY, H-H totally correlated spectroscopy; HSQC, H-C hetero-nuclear single quantum coherence; HMBC, H-C hetero-nuclear multiple-bond connectivity) were recorded on an AVANCE III 500 MHz NMR spectrometer (Bruker Daltonics, Bremen, Germany) at 333 K with proton and carbon frequencies of 500.13 MHz and 125.77 MHz. The 2D NMR spectra were acquired with 512 increments of 8 scans for COSY, 512 increments of 16 scans for TOCSY and HSQC, and 1024 increments of 96 scans for HMBC, respectively. Sodium 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (Sigma-Aldrich, Zwijndrecht, The Netherlands) was used as an internal reference for chemical shift (δ_H 0.00 and δ_C 0.00).

Supplemental figures

A.

Protein View: Acm2 isolated from wild type

Sequence coverage of mature Acm2 (753 aa): **75%**
Sequence coverage of Acm2 AST domain (211 aa): **69%**

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1   MKIGMTKKVV TSLLLSTALL PMLSGKADTA SANQKPAAAT KGNSAASAAS
51  QQVTLSAGSQ TETTAAGATD QSVASDGAKT DDQAESTSTT TATTSATSRV
101 TVRAASQAAK ADSTGPQSQS SASEAAKDNA ATSATADSTT SAVDQLDKTA
151 KASAATSQAS HSTTNETAKA SAAASQDSHV TTDQSSVTVT SEVAKSAASS
201 AAPKQATEQA VAAKISPKIE TAVAADAVQS SAMMARSTRA MTSQEIFLSQ
251 IKAGAISGWN KYQVLPSVTA AQAILESGWG QSQLATQGNN LFGIKGSYQG
301 QSIYFPTQEW NGSQYITIQD AFRKYPNWSA SVEDHGAFLV VNPRYSNLIG
351 VTDYRRVASL LQQDGYATAP TYASSLISII EYNKLHEWDQ EALSGQASGG
401 NDNNQVQPDQ DVTPTSGTHK FTKTTTIHNA PDATSAVVGT YNAGETVNYN
451 GKLTVGNATW LRYQSYSGVS RYVMISQTTT NDNNNQATVT PASGSYKFTA
501 KTNIRSAASK TAQVVGTYNA GETVYYNGKI TTGGTTWLRY LSYSGAQHYV
551 AMSGDEVGSV AKPDVVATSG SYRFTKTTAI KSSPATSATT VGSYNAGDTV
601 YYNGKVTTNG QTWLRYMSYS GAQHYVQISG ESTSTNVDKP QVTPQSGSYR
651 FTQTTAIKNT PAGNAPSVGT YSAGDTVYYN AKVTANGQTW LRYLSYSGAQ
701 HYVAISGNAA TGNTTSKPVT NSQGAFRFVT TTNIRTAPST RASVVGEYNP
751 GETVYYNGTV QAEGYTWLRY LSRSGATHYV AKLEG
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B.

Protein View: Acm2 isolated from *ΔtagE5E6*

Sequence coverage of mature Acm2 (753 aa): **52%**
Sequence coverage of Acm2 AST domain (211 aa): **59%**

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1   MKIGMTKKVV TSLLLSTALL PMLSGKADTA SANQKPAAAT KGNSAASAAS
51  QQVTLSAGSQ TETTAAGATD QSVASDGAKT DDQAESTSTT TATTSATSRV
101 TVRAASQAAK ADSTGPQSQS SASEAAKDNA ATSATADSTT SAVDQLDKTA
151 KASAATSQAS HSTTNETAKA SAAASQDSHV TTDQSSVTVT SEVAKSAASS
201 AAPKQATEQA VAAKISPKIE TAVAADAVQS SAMMARSTRA MTSQEIFLSQ
251 IKAGAISGWN KYQVLPSVTA AQAILESGWG QSQLATQGNN LFGIKGSYQG
301 QSIYFPTQEW NGSQYITIQD AFRKYPNWSA SVEDHGAFLV VNPRYSNLIG
351 VTDYRRVASL LQQDGYATAP TYASSLISII EYNKLHEWDQ EALSGQASGG
401 NDNNQVQPDQ DVTPTSGTHK FTKTTTIHNA PDATSAVVGT YNAGETVNYN
451 GKLTVGNATW LRYQSYSGVS RYVMISQTTT NDNNNQATVT PASGSYKFTA
501 KTNIRSAASK TAQVVGTYNA GETVYYNGKI TTGGTTWLRY LSYSGAQHYV
551 AMSGDEVGSV AKPDVVATSG SYRFTKTTAI KSSPATSATT VGSYNAGDTV
601 YYNGKVTTNG QTWLRYMSYS GAQHYVQISG ESTSTNVDKP QVTPQSGSYR
651 FTQTTAIKNT PAGNAPSVGT YSAGDTVYYN AKVTANGQTW LRYLSYSGAQ
701 HYVAISGNAA TGNTTSKPVT NSQGAFRFVT TTNIRTAPST RASVVGEYNP
751 GETVYYNGTV QAEGYTWLRY LSRSGATHYV AKLEG
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FIG. S1 Protein view (Mascot, Matrix Science) and coverage of the LC/MS spectra of Acm2 isolated from (A) *Lactobacillus plantarum* WCFS1 and (B) the *tagE5E6* deletion mutant strain. Detected peptides are shown in red. The predicted signal peptide and the AST domain are presented in bold and underlined, respectively (5).

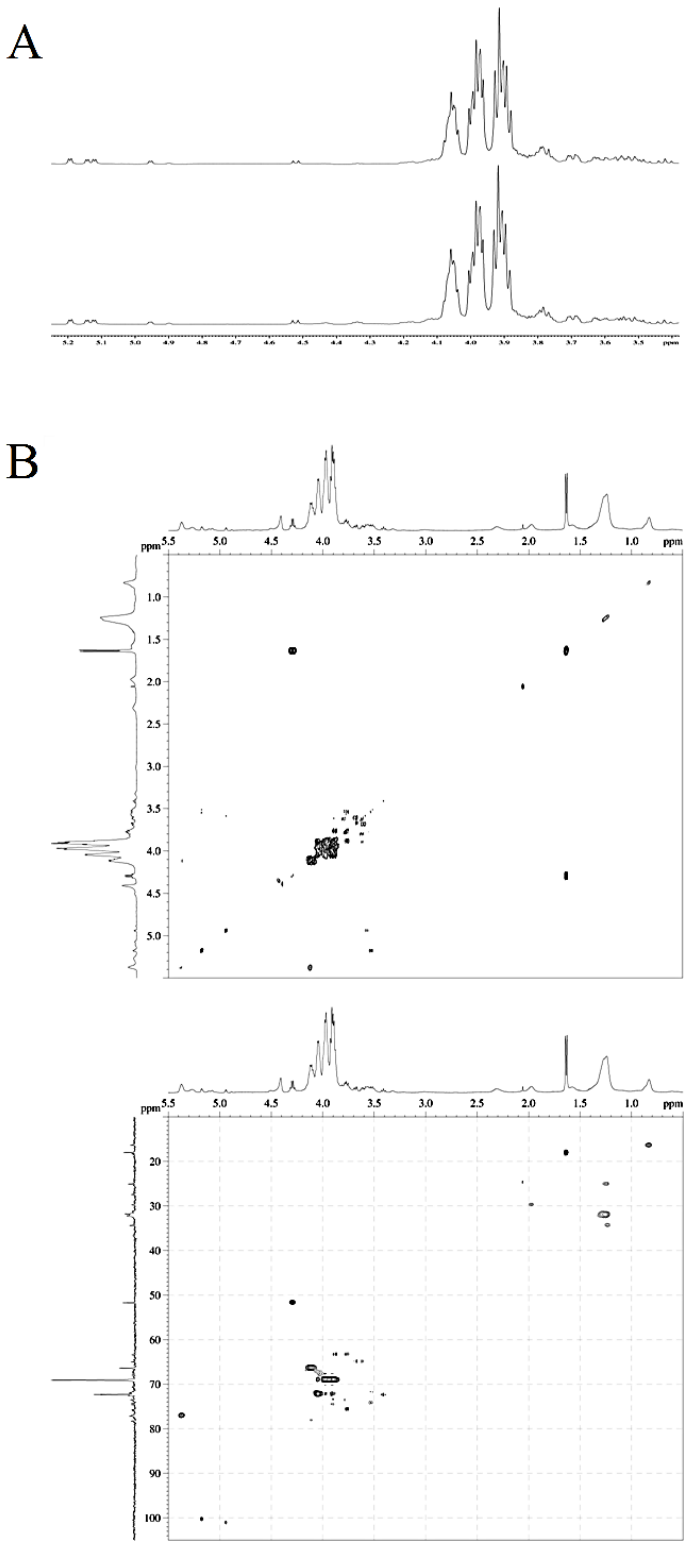

Fig. S2 The nuclear magnetic resonance (NMR) analysis of purified LTA and deacylated dealanylated LTA (dd-LTA) from wild-type and Δ*tagE5E6* mutant. (A) Comparison of ^{1}H NMR spectra of dd-LTA from wild-type (upper panel) and Δ*tagE5E6* (lower panel). (B) COSY (upper panel) and HSQC (lower panel) spectra of purified LTA from *ΔtagE5E6*.

References

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2. **Ames BN.** 1966. Assay of inorganic phosphate, total phosphate and phosphatase. Methods in Enzymology **8:**115-118.
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5. **Rolain T, Bernard E, Beaussart A, Degand H, Courtin P, Egge-Jacobsen W, Bron PA, Morsomme P, Kleerebezem M, Chapot-Chartier M-P, Dufrene YF, Hols P.** 2013. O-glycosylation as a novel control mechanism of peptidoglycan hydrolase activity. Journal of Biological Chemistry.