Characterization of wall teichoic acid degradation by the bacteriophage φ29 appendage protein GP12 using synthetic substrate analogs

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# Supplementary Information

**Table S1. Strains, plasmids and oligonucleotides used in this study**

| Strains | Description | Source |
|---|---|---|
| *E. coli* BL21-CodonPlus (DE3)-RIL | *E. coli* protein overexpression strain ($F^-$ *ompT hsdS* ($r_Bm_b^-$) *dcm*$^+$ $Tet^r$ *gal endA* Hte [*argU ileY leuW* $Cam^r$]) | Agilent |
| **Plasmids** | | |
| pET28b-GP12 | Expression plasmid for N-terminal hexahistidine-GP12 | Ref. 27 |
| pΔN88GP12 | Expression plasmid for N-terminal hexahistidine-ΔN88GP12 | This study |
| **Oligonucleotides** | | |
| ΔN88GP12-Forward | CTAGTAGCCATGGGCCACCATCATCATCATCATGATGAAAACC TGTATTTTCAGGGCGACCTCGTTATCCAAGTTATC | |
| GP12-Reverse | CTAGTAGTTAGTTCAAGAATAGTTGTTTTGGAGACGATTGAGA CCGTTTCTAGTAAGCTTCAAAGGAAAAAGGATACGGTG | |

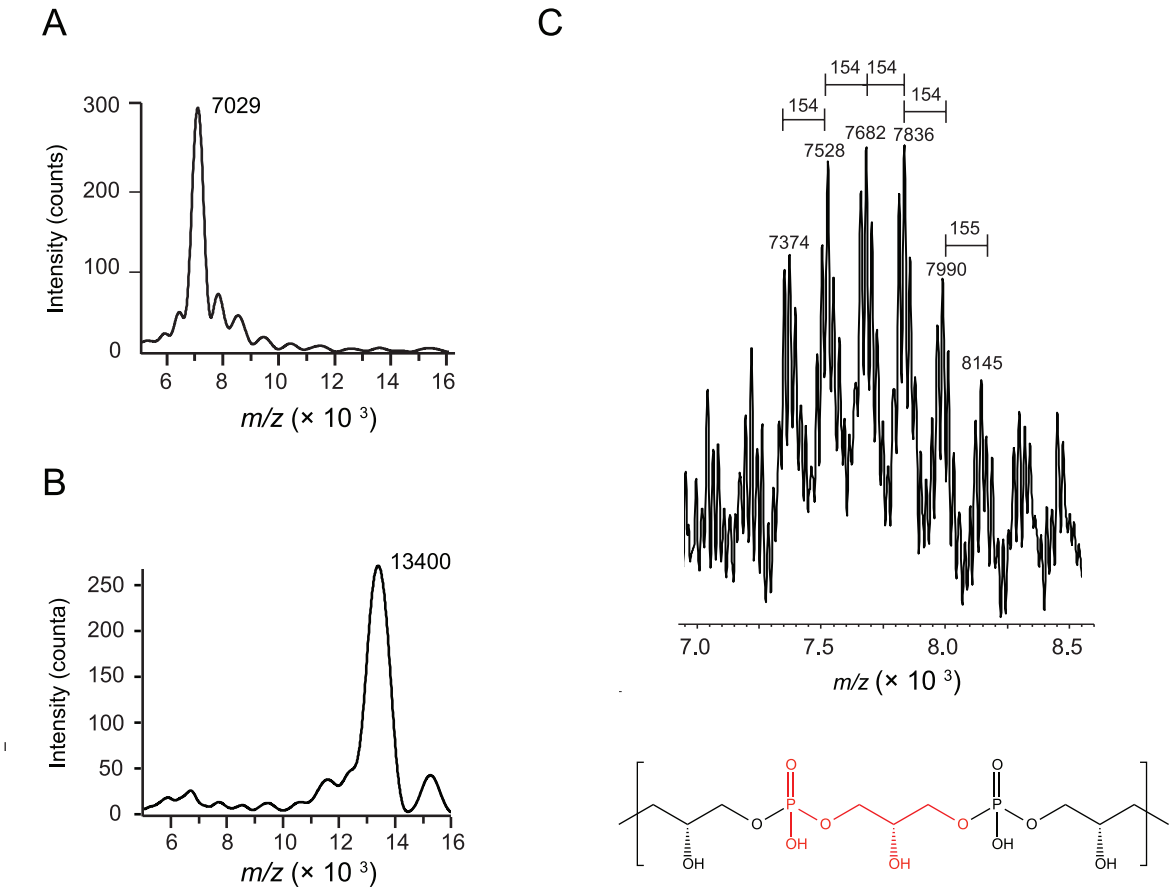


**Figure S1. MALDI-TOF mass spectrometry of WTA analogs**. Synthetic reactions for WTA analogs were performed as described in the main text. Samples were prepared for analysis by mixing a 5 μL aliquot of the reaction mixture with an equal volume of a saturated matrix solution (2,5-dihydroxybenzoic acid in 50% acetonitrile and 0.1% trifluoroacetic acid). 1 μL of this mixture was spotted onto a stainless steel MALDI plate and spectra were acquired in positive ion linear mode, using a Bruker UltrafleXtreme mass spectrometer, calibrated with external standards. Spectra are shown for *A*, the non-glycosylated analog, *m/z* 7029: M + 4 $Na^+$; M corresponds to the mass of a polymer containing 40 GroP units and *B*, the glycosylated analog, *m/z* 13400 corresponds to addition of 39 glucose molecules to the non-glycosylated analog. *C*, MALDI-MS analysis of a larger scale (200 μM) synthetic reaction for the lipid φ.n (n = 43) analog shows the products generated differ by the mass of the repeating glycerol phosphate polymer unit, colored red in the structure below the spectrum.

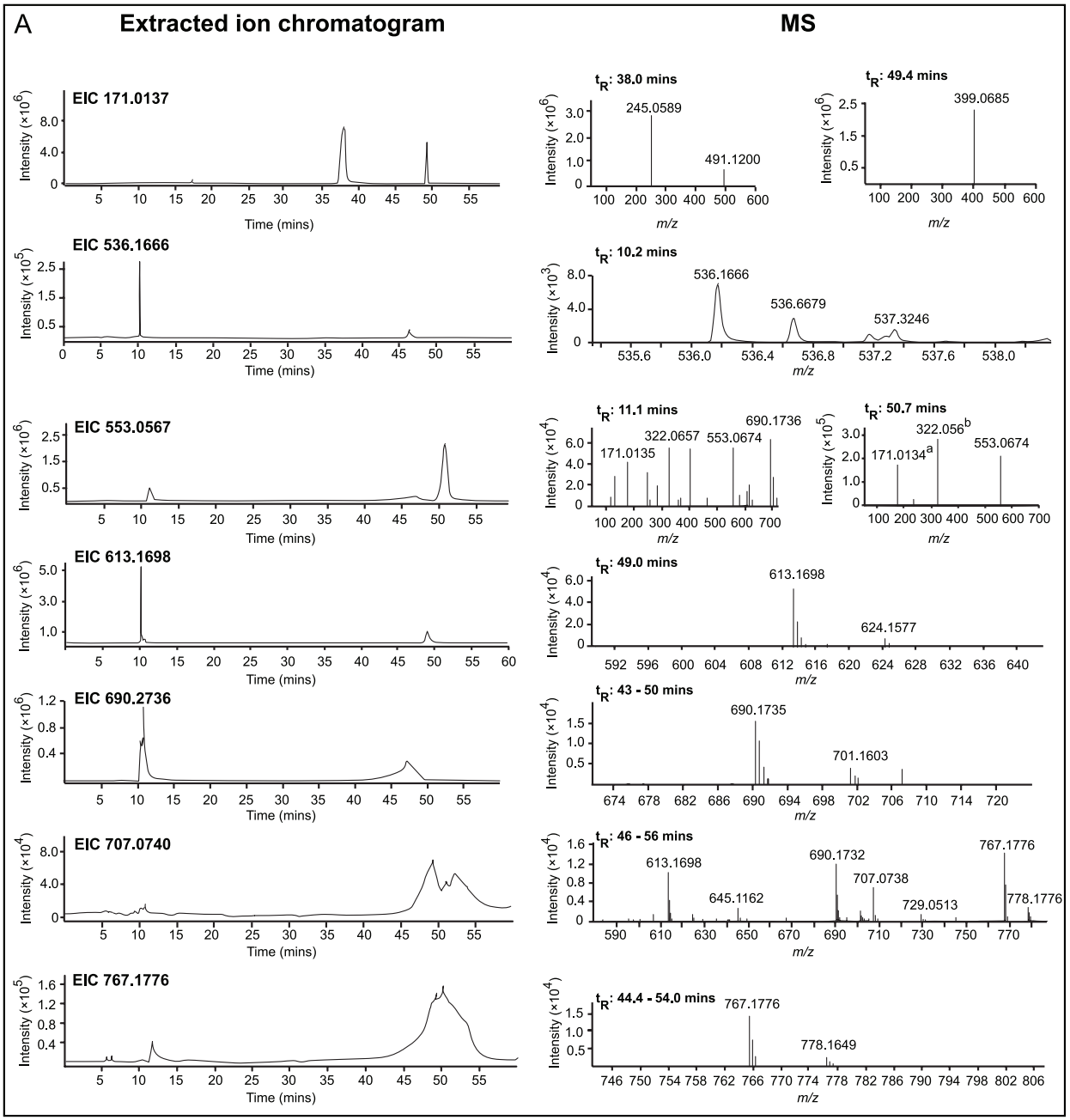


**Figure S2. Negative ion LC-MS analysis of WTA degradation products.** Extracted ion chromatograms and mass spectra (mass assignments are given in Table 3 in the main text) are shown for products from reactions of GP12 with *A*, lipid φ.40 analog (a, glycerol phosphate; b, cytidine monophosphate) and *B,* glycosylated lipid φ.40 analog (c, uridine monophosphate).