Supporting Information

Bansal-Mutalik and Nikaido 10.1073/pnas.1112572108

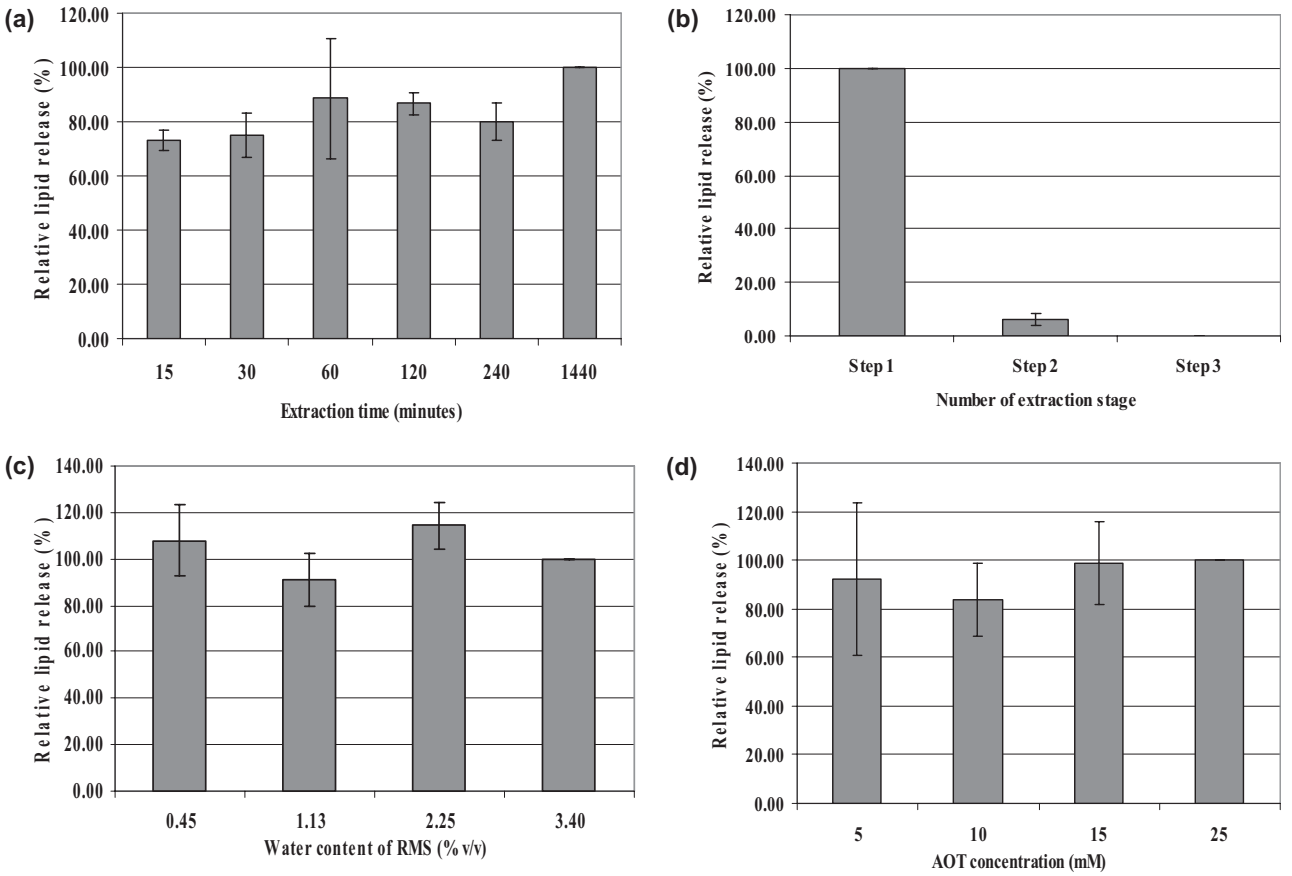

Fig. S1. Optimization of RMS extraction for maximum lipid release from *C. glutamicum*. Error bars represent SDs. (*A*) Effect of time: Separate aliquots of wet cells corresponding to similar dry weights were extracted with 25mM AOT in heptane (containing 0.45% (vol/vol) water) for varying times; Values expressed as % lipid release relative to that in maximum time. (*B*) Effect of number of extraction cycles: An aliquot of wet cell of known dry weight was subjected to multiple extractions of 15 min each under conditions similar to *A*; Values are expressed as % lipid release relative to that in first extraction cycle. (*C*) Effect of % (vol/vol) of water in the RMS: Separate aliquots of wet cells corresponding to similar dry weights were extracted with RMS of 25mM AOT in heptane containing varying amounts of water, for 15 min each; Values expressed as % lipid release relative to that with maximum water content. (*D*) Effect of AOT concentration. Separate aliquots of wet cells corresponding to similar dry weights were extracted with varying concentrations of AOT in heptane (containing water at molar concentrations 10 times higher than that of AOT) for 15 min each. Values are expressed as % lipid release relative to that with maximum AOT concentration.

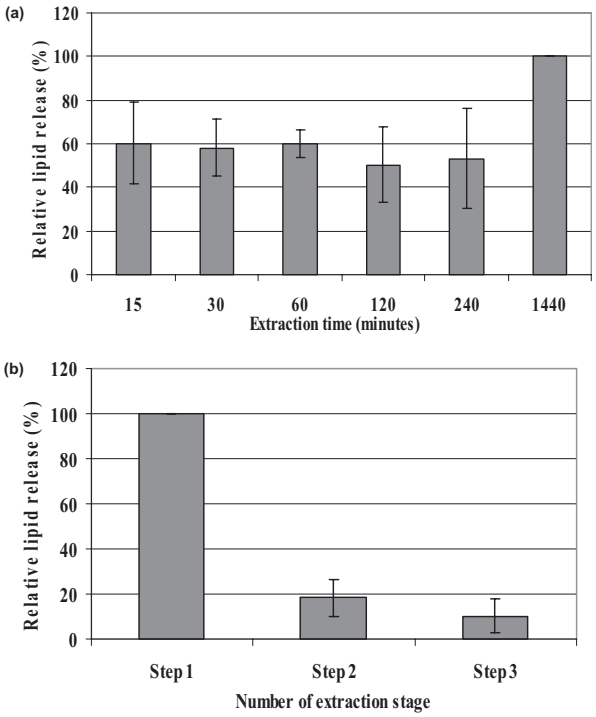

Fig. S2. Optimization of CMW extraction for maximum lipid release from *C. glutamicum*. Error bars represent SDs. (*A*) Effect of time. Separate aliquots of wet cells corresponding to similar dry weights were extracted with CMW for varying times; Values expressed as % lipid release relative to that in maximum time. (*B*) Effect of number of extraction cycles. An aliquot of wet cells of known dry weight was subjected to multiple extractions of 15 min each. Values are expressed as % lipid release relative to that in first extraction cycle.

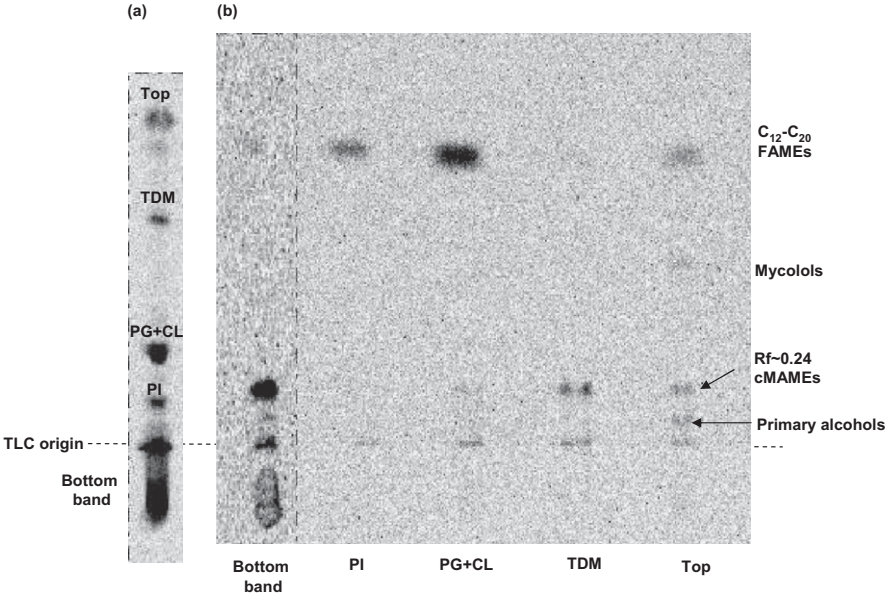

Fig. S3. (*A*) TLC profile of CMW extract of *C. glutamicum* using CMW (30:8:1) as the developing solvent. Individual lipid bands from this TLC profile were subjected to transmethylation using 15% aqueous tetrabutyl ammonium hydroxide. (*B*) This was followed by TLC of the products with petroleum-ether:diethyl ether (85:15). FAME, fatty acid methyl ester; cMAME, corynomycolic acid methyl ester.

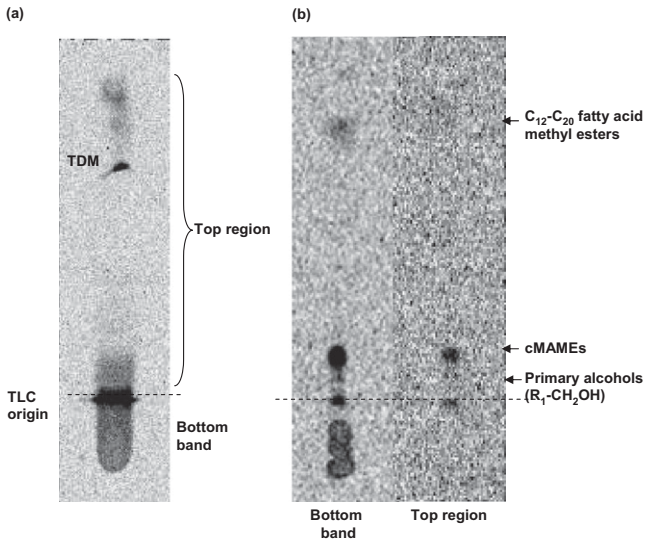

Fig. S4. (*A*) TLC profile of RMS extract of *C. glutamicum* using CMW (30:8:1) as the developing solvent. Individual lipid bands from this TLC plate were subjected to transmethylation using 15% aqueous tetrabutyl ammonium hydroxide. (*B*) This was followed by TLC of the products with petroleum-ether:diethyl ether (85:15). cMAME, corynomycolic acid methyl ester.

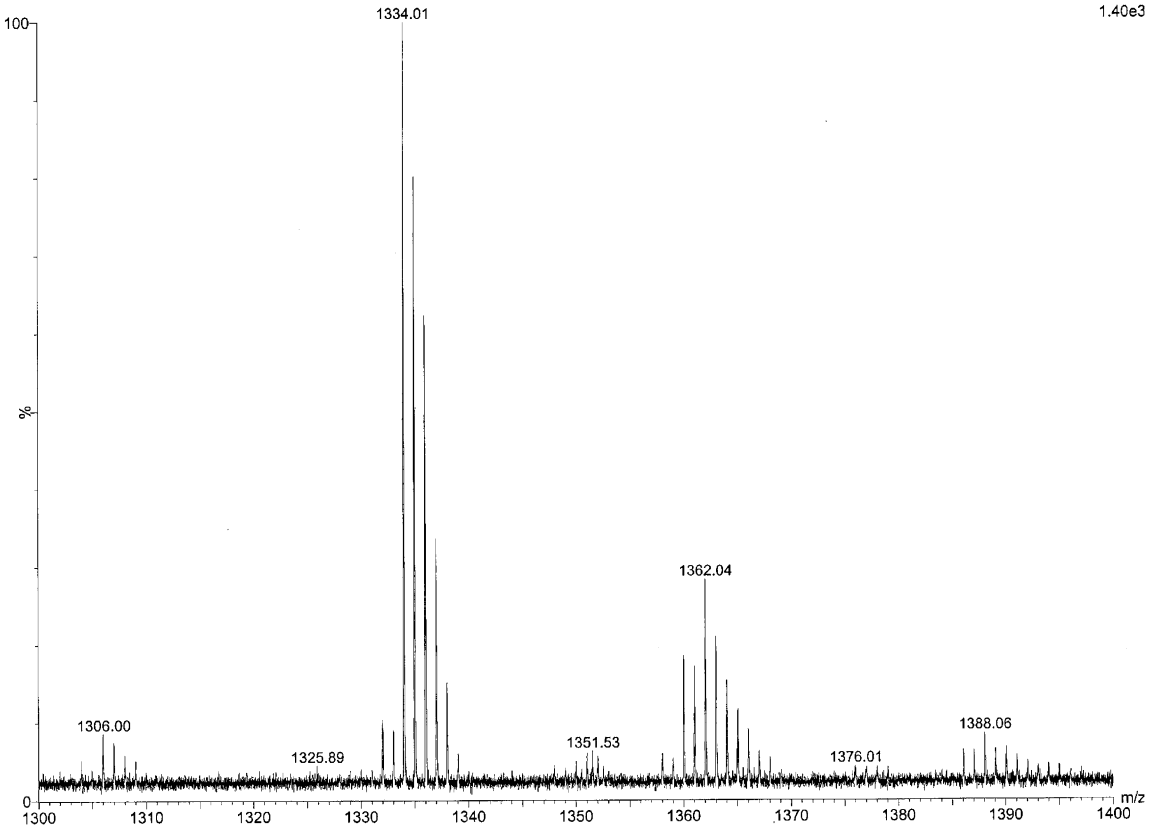

Fig. S5. ESI-MS profile of the major glycolipid band seen in the CMW and RM extract, identified as TDM (isolated from the TLC plate of CMW extracts of intact cells).

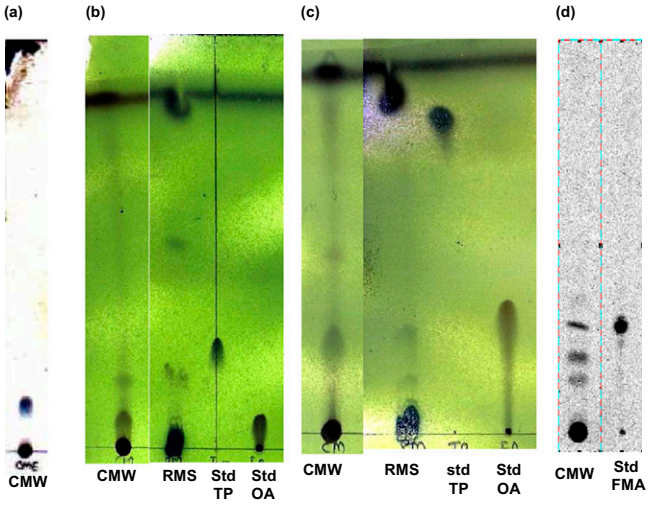

Fig. S6. Identification of lipids in the TLC profiles of CMW and RMS extracts using various 1D-TLC developing systems and lipid standards. (*A*) TLC with chloroform:methanol (9:1) for identification of the OM-specific glycolipid of modest polarity, visualized by anthrone reagent. (*B–D*) TLC for identification of triacylglycerols and FFAs with petroleum ether:diethyl ether (9:1) (*B*), petroleum ether:diethyl ether (7:3) (*C*), and hexane-diethyl ether-acetic acid (70:30:1) (*D*). Visualization was done for all lipid groups by spraying with phosphomolybdotungstate reagent (*B* and *C*) or by phosporimaging of radioactive lipids (*D*). Samples: CMW, CMW extract; RMS, RMS extract. Standards: std TP, tripalmitin (Sigma-Aldrich); std OA, oleic acid (Sigma-Aldrich); std FMA, radiolabeled mycolic acid isolated from *Mycobacterium smegmatis* mc^2 155.

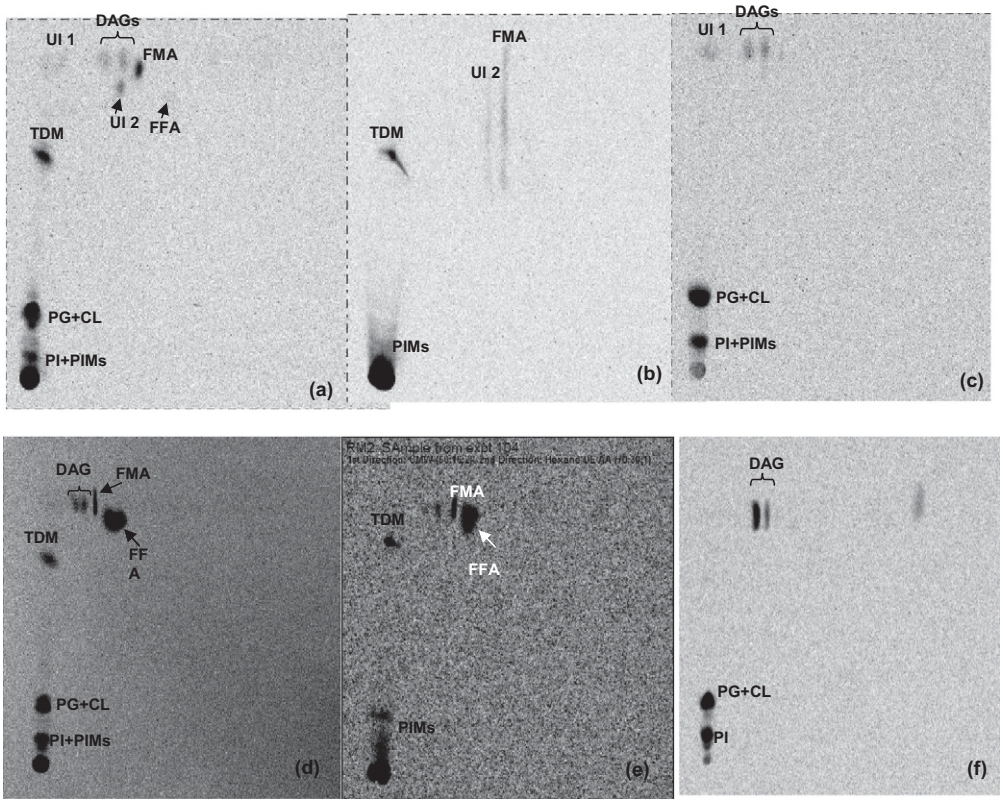

Fig. S7. 2D-TLC profiles of lipids from *C. glutamicum* using CMW (30:8:1) in the first direction and hexane-diethyl ether-acetic acid (70:30:1) in the second direction, followed by phosphorimaging. (*A–C*) TLC profiles of lipids from fresh cells. (*D–F*) TLC profiles of lipids from cells that were kept frozen for more than 1 mo. (*A* and *D*) CMW extract of whole cells. (*B* and *E*) RMS extract of whole cells. (*C* and *F*) CMW extract of cells pretreated with RMS. PG, phosphatidylglycerol; CL, cardiolopidin; DAGs, diacyl gycerols; FMA, free (coryno)mycolic acids; UI, unidentified lipid.

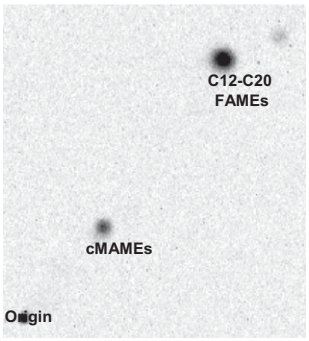

Fig. S8. Fatty acid methyl esters obtained after alkaline hydrolysis and transmethylation of delipidated (CMW-extracted) cells. Fatty acid esters were converted into methyl esters by phase-transfer catalysis as described in *Experimental Procedures*. 2D-TLC was performed with petroleum ether-acetone (95:5) (three times) in the first direction and with toluene-acetone (97:3) in the second direction. cMAME, corynomycolic acid methyl ester; FAME, fatty acid methyl ester.

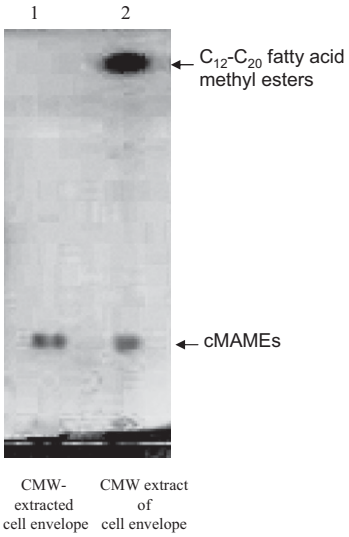

Fig. S9. TLC profiles of the fatty acid methyl esters from cell envelope obtained by alkaline hydrolysis with 15% aqueous tetrabutyl ammonium hydroxide followed by transmethylation. The samples were the cell wall after CMW extraction, intended to remove the IM lipids as well as the extractable lipids of OM (lane 1) and the CMW extract of the cell envelope (lane 2). TLC was done with petroleum-ether:diethyl ether (85:15). cMAME, corynomycolic acid methyl ester. GC-MS quantitation of the methyl esters of C12-20 fatty acids found that the sample in lane 1 contained only 1% of the amount in the sample in lane 2.

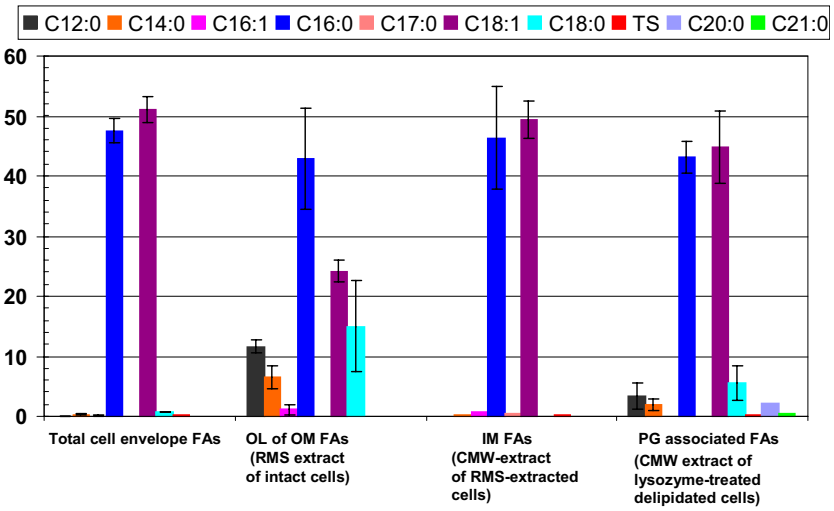

Fig. S10. Relative abundance of individual fatty acids in various fractions. The figure shows the distribution, from left, in the total cell envelope, the outer leaflet (OL) of OM (RMS extract of cells), IM (CMW extract of cells preextracted with RMS), and peptidoglycan-embedded fraction (CMW reextract of lysozyme-treated delipidated cells). Fatty acids were converted into methyl esters as described in *Experimental Procedures*, and then separated and quantitated using GC-MS. Note that mycolic acid esters are not eluted in the GC system used. TS, tuberculostearic acid. Error bars represent SDs.