Supplementary Material

A method for total lipid analysis by thin-layer chromatography-flame ionization detection (TLC-FID) in *Rhodococcus* and *Williamsia* species

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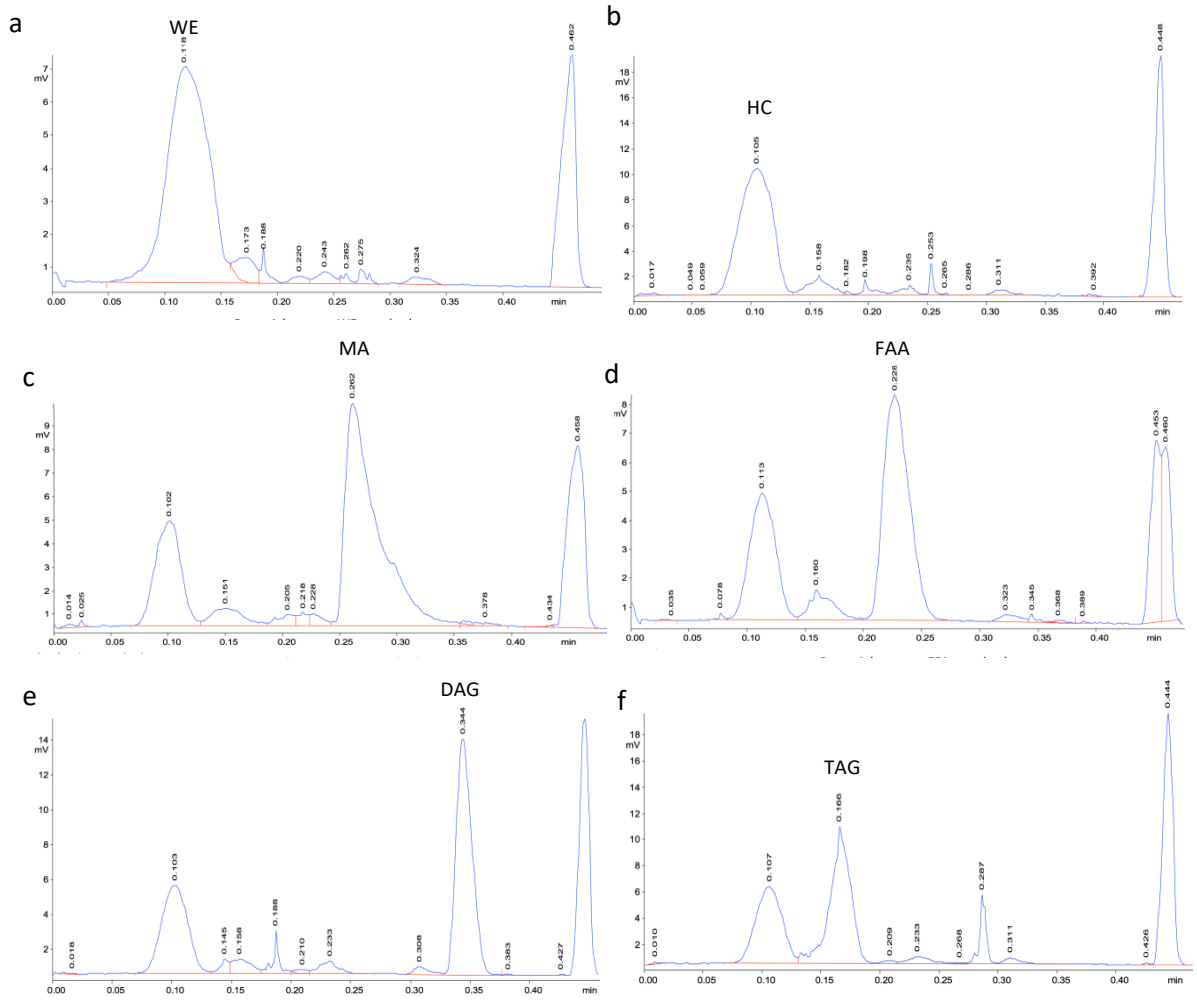

Figure S1. TLC-FID chromatograms of bacterial extract (*Williamsia* sp. 1138) mixed with different standards to demonstrate co-elution of unknown and standard compounds. a) WE, b) HC, c) MA, d) FFA, e) 1,2-DAG and f) TAG. Panels a) and b) show that the retention time of the non-polar lipid peak coincided with the WE and HC standards, respectively. Abbreviations are shown in Figure 2. Panel d) shows a split peak in the region of the PL peak, likely due to monoacylglycerols in the free fatty acid standard A peak near the retention time pf PL Panel c) shows the tailing peak for the mycobacterial free MA standard and a minor merged peak on the side of this region which we suggest corresponds to free MA in strain 1138, noting that

the MA chain length is considerably shorter in the *Williamsia* and *Rhodococcus* strains compared to the standard.

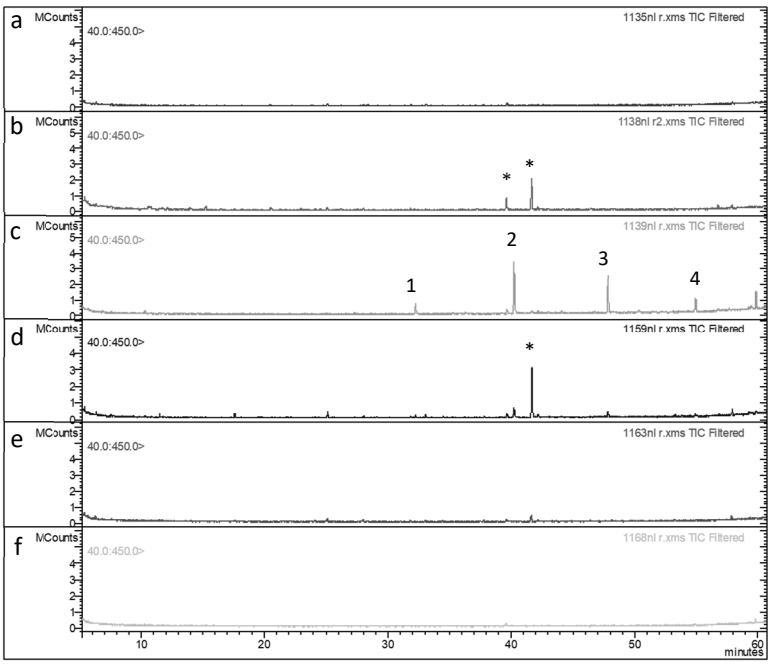

Figure S2. Full scan GC chromatograms of neutral lipid extracts of a) *Williamsia* ap. 1135, b) *Williamsia* sp. 1138, c) *Rhodococcus qingshengii* strain 1139, d) *Rhodococcus erythropolis* strain 1159 (1. Octadecanal, 2. Docosanal, 3. Tetracosanal and 4. Hexacosanal), e) *Rhodococcus* sp. 1163, f) *Rhodococcus* sp. 1168. * = artefact. The absence of non-saponifiable (HC) or saponifiable (alcohol) neutral lipids indicated the absence of WE complexes in the bacterial extracts for these strains although other species of *Rhodococcus* are reported to synthesise WE compounds.