

**Fig. S4** 2D-TLC lipid analysis and cell wall permeability test of  $\Delta accD6$  mutant versus wild-type *M. smegmatis*. **(A)** Total apolar and polar lipids were resolved using 2D-TLC and the solvent systems according to Besra *et al.*, 1998 (4) (the letters in the upper left corner denote the utilized solvent). Samples were labeled with [2-<sup>14</sup>C]acetate and equal counts of total radioactivity (50,000 cpm) were examined. Abbreviations: TG, triacylglycerols; MAT, multiacylated trehalose; GMM, glycerol monomycolate; DAT, diacylated trehalose; TDM, trehalose dimycolate; PIM; phosphatidylinositol mannosides. **(B)** The rate of [<sup>3</sup>H]rifampin uptake by  $\Delta accD6$  mutant versus wild-type *M. smegmatis*. The analysis was performed at 37°C (closed symbols), and passive adsorption of [<sup>3</sup>H]rifampin to the cell wall (background) was estimated by performing the experiment at 0°C (open symbols).