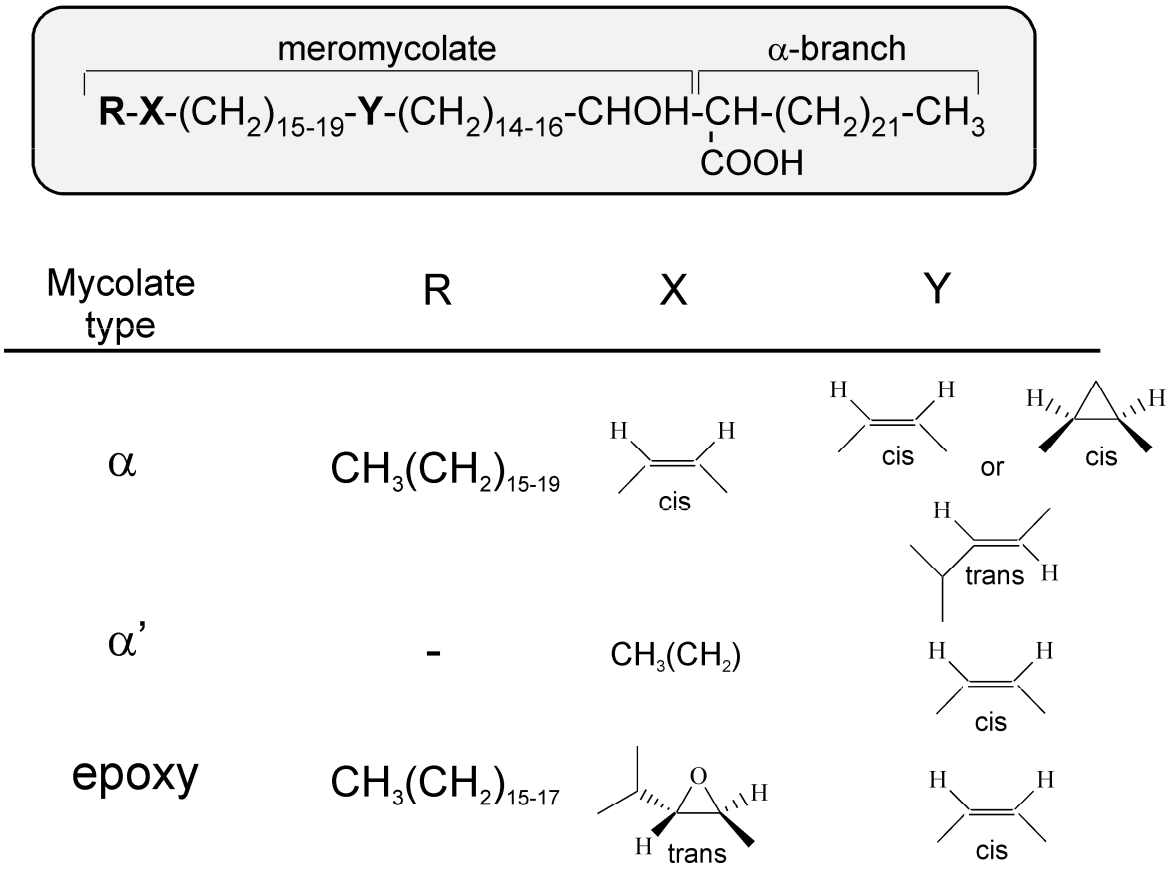
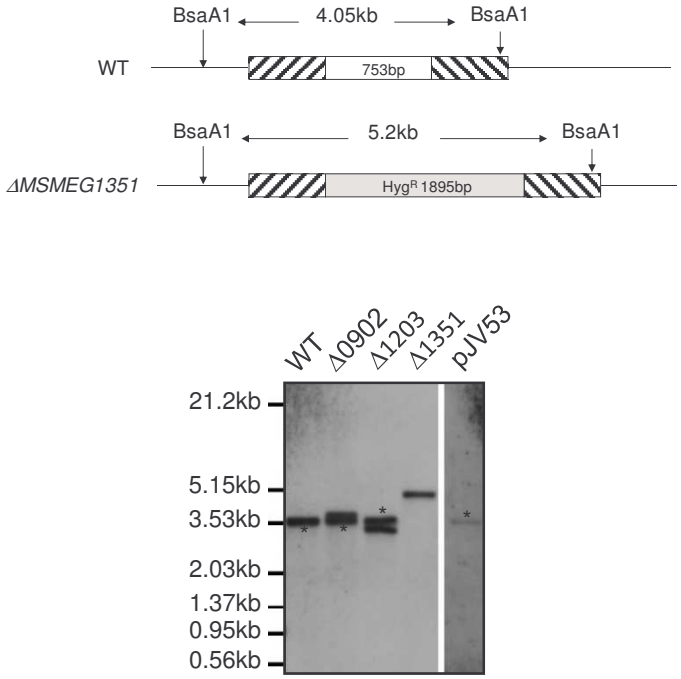


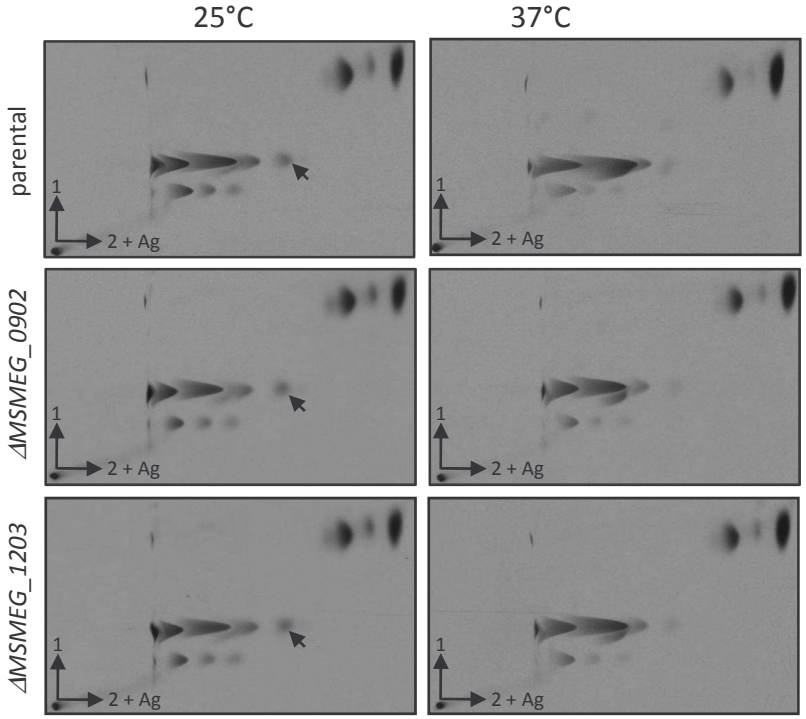
Structures of the major mycolic acids of *M. smegmatis*. X and Y represent the positions of the distal and proximal functional groups relative to the carboxyl group, respectively.

Figure S2



Disruption of *MSMEG_1351* in *Msm* by a hygromycin resistance cassette. BsaA1 restriction site and probe location are indicated. The same strategy was followed for the construction of *ΔMSMEG_0902* and *ΔMSMEG_1203*. The Southern blot of BsaA1-digested genomic DNA in the indicated strains was performed using a hygromycin probe. The band marked with * corresponds to non-specific labeling of a 3611-bp BsaA1-fragment of the pJV53 vector. This band is absent from the *ΔMSMEG_1351* mutant in which pJV53 has been cured. Predicted sizes for *ΔMSMEG_0902*, *ΔMSMEG_1203* and *ΔMSMEG_1351* were 3.85kb, 3.2kb, and 5.2kb, respectively.

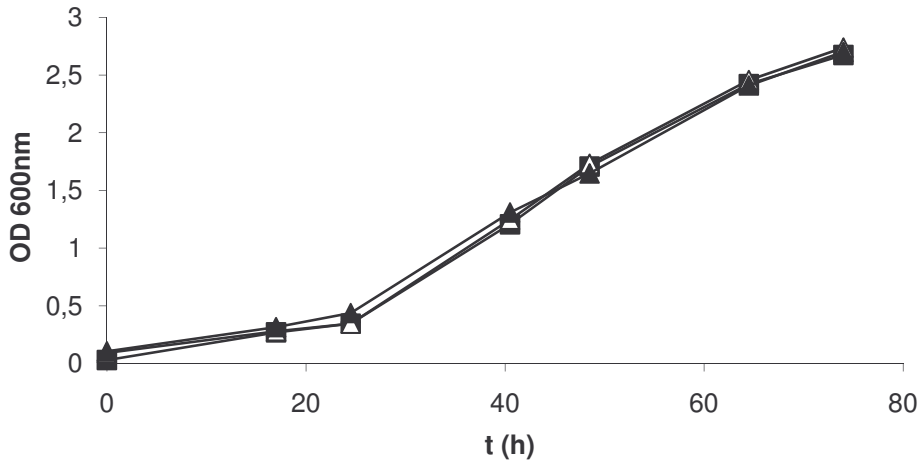
Figure S3



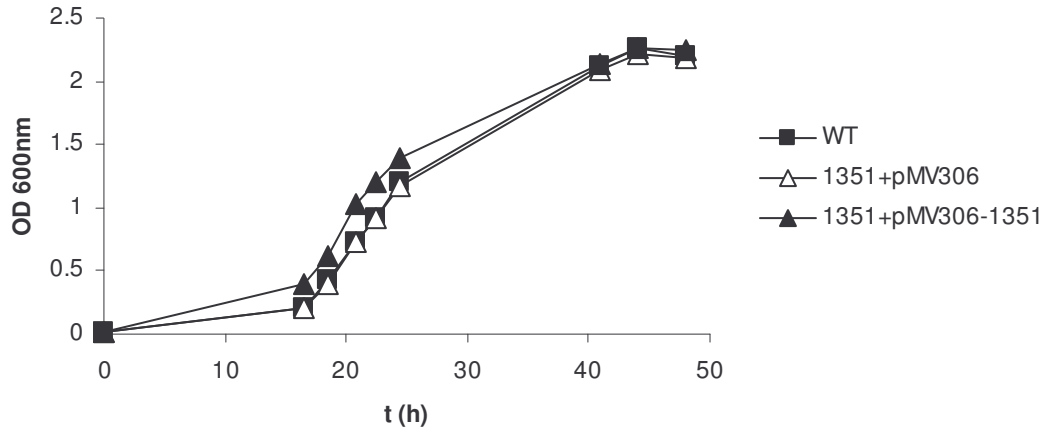
Mycolic acid analysis of *MSMEG_0902* and *MSMEG_1203* disrupted *M. smegmatis* mutants. *MSMEG_0902* and *MSMEG_1203* genes were inactived by introducing a hygromycin resistance cassette, following the same strategy used to inactive *MSMEG_1351*. The parental strain, the *ΔMSMEG_0902* and *ΔMSMEG_1203* mutants were either grown at 25°C or at 37°C and labeled with ^{14}C-acetate. Mycolic acids were then extracted and resolved by 2D argentation-TLC. The arrowhead indicates the position of cyclopropanated α-mycolic acids that accumulate at 25°C, not only in the parental strain but also in the two mutants.

Figure S4

A



B



Growth curves of *M. smegmatis* strains at 25°C (A) or 37°C (B).