

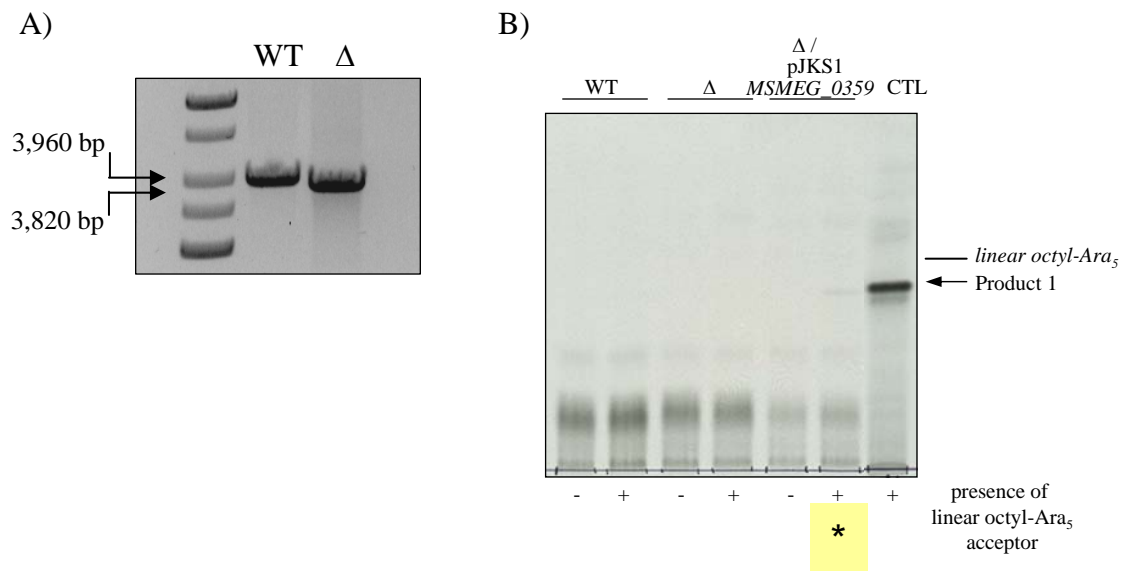
Supplementary Material 1:

A) Allelic replacement at the *aftD* locus of *C. glutamicum*.

Allelic replacement was confirmed by PCR using primers NCgl2757fw and NCgl2757rev (see Materials and Methods). The WT 3,960-bp amplification signal is replaced by a 3,820-bp fragment in the mutant ( $\Delta$ ) due to a 1,340-bp *AgeI* deletion in the *aftD* gene and insertion of a 1,200 bp- kanamycin resistance cassette.

B) Arabinofuranosyltransferase assays using cell-free extracts from WT *C. glutamicum*, *C. glutamicum*  $\Delta$ NCgl2757 and *C. glutamicum*  $\Delta$ NCgl2757 expressing *aftD* gene from pJKS1MSMEG\_0359. The same assays as presented in Fig. 6 were run.

p[<sup>14</sup>C]Rpp and synthetic linear Ara<sub>5</sub> served as the donor and acceptor substrates, respectively. “+” indicates the presence of acceptor substrate in the reaction mixture; “-” indicates that no synthetic acceptor was added.



Supplementary Material 2: GC/MS analysis of cell walls from *M. smegmatis* WT

and the *aftD* single cross-over strain SCO1.

Sugar composition (A) and glycosidic linkage analyses (B) are shown (see Materials and Methods).

