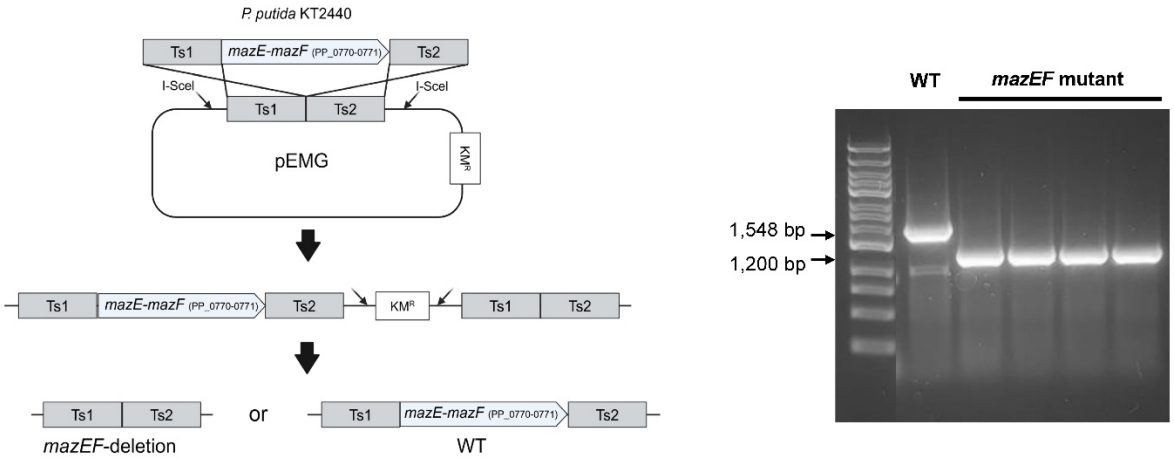


Fig. S1. Process for markerless deletion of the *mazEF* gene from the chromosome of *P. putida.* Schematic representation of the markerless *mazEF* gene deletion procedure in *P. putida* using homologous recombination, following the method described by Martínez-García and de Lorenzo (2011). The diagram illustrates the two-step homologous recombination mediated by plasmids pEMG and pSW-I containing the I-SceI restriction enzyme system. Plasmid pEMG facilitates initial recombination events, whereas plasmid pSW-I introduces the I-SceI endonuclease to induce double-strand breaks, promoting markerless gene deletion (upper panel). PCR confirmation of the *mazEF* gene deletion mutant. PCR was performed using the forward primer Ts1 and reverse primer Ts2. The mutant strain produces an amplified DNA fragment of 1,200 bp, whereas the wild-type (WT) strain produces a fragment of 1,548 bp (lower panel).

References

Martínez-García E, de Lorenzo V. 2011. Engineering multiple genomic deletions in Gram-negative bacteria: Analysis of the multi-resistant antibiotic profile of *Pseudomonas putida* KT2440. *Environ Microbiol*. 13: 2702–2716.