The *Pseudomonas aeruginosa* DedA protein PA4011 functions as a C55-PP phosphatase via a PAP2-like domain

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The recycling of the lipid carrier undecaprenyl phosphate (C55-P) across the cytoplasmic membrane is a key step of the peptidoglycan biosynthetic pathway. Recent studies have proposed that proteins of the DedA family mediate the flipping of C55-P back to the cytoplasmic side. However, during the recycling process, undecaprenyl pyrophosphate (C55-PP) must first be dephosphorylated.

Pseudomonas aeruginosa has six DedA proteins, one of which, PA4029, has been demonstrated to act as a C55-P flippase. Notably, besides the DedA domain, the PA4011 protein also contains a PAP2-like domain homologous to those present in the *Escherichia coli* phosphatases YbjG, PgpB, and LpxT, which, together with UppP, are involved in C55-PP dephosphorylation. Homologs of UppP and LpxT are also present in *P. aeruginosa*.

By generating double deletion mutants and a triple conditional mutant in PA4011, *uppP* and/or *lpxT*, we confirmed that PA4011 contributes to C55-P(P) recycling by acting as a C55-PP phosphatase. Indeed, deletion of *uppP* in the PA4011 mutant increased its sensitivity to the C55-P synthesis-targeting antibiotic fosmidomycin and caused growth arrest at 25°C, a condition that strongly reduces *P. aeruginosa lpxT* expression. Accordingly, growth was completely inhibited upon LpxT depletion in ΔPA4011 Δ*uppP* cells. Moreover, expression of a PA4011 variant mutated in a conserved catalytic residue and of PAP2-like domain alone demonstrated that PA4011 activity relies on its phosphatase domain. Notably, deletion of PA4011 and/or *uppP* also reduces the emergence of colistin resistance, likely due to increased LpxT-mediated transfer of phosphate from C55-PP to lipid A, which hampers its aminoarabinosylation. Finally, although inactive in C55-PP dephosphorylation, both the DedA domain alone and the catalytically-inactive mutant of PA4011 appear to have C55-P flipping activity, as their expression restored fosmidomycin resistance in the ΔPA4029 mutant.