

Supplemental Material to:

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**Indigenous and acquired modifications
in the aminoglycoside binding sites of
Pseudomonas aeruginosa rRNAs**

2013; 10(8)

<http://dx.doi.org/10.4161/rna.25984>

www.landesbioscience.com/journals/rnabiology/article/25984/

Supplementary figures

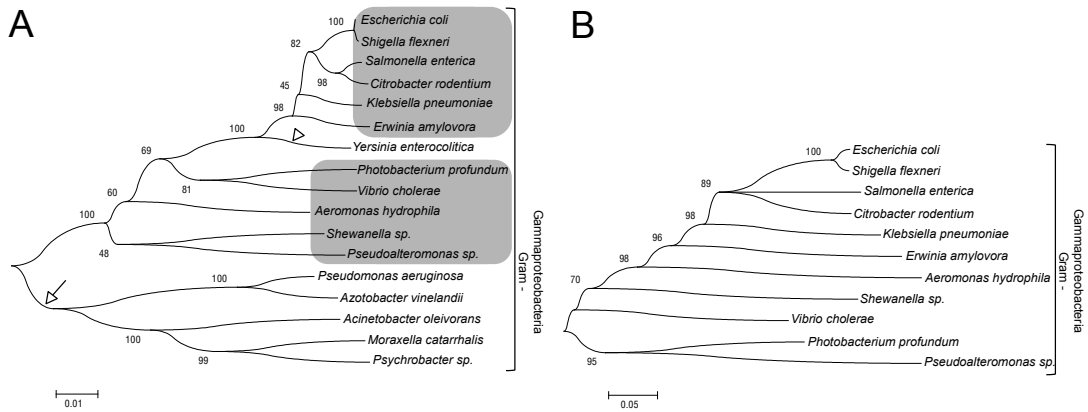


Figure S1. Comparative dendrograms based on bacterial rRNA and *rsmF* sequences. **(A)** Dendrogram based on the 16S rRNA gene sequences from different species. Species carrying an *rsmF* ortholog are shaded in grey. Deletion events are indicated with white symbols. The *rsmF* gene could have been acquired initially by an ancestor common to the bacteria that now have *rsmF*, or alternatively from an ancestor common to a wider range of Gammaproteobacteria followed by loss of *rsmF* in a single deletion event within the Pseudomonadales family (white arrow). The latter scenario is supported by *Yersinia*, enterobacterial genus lacking RsmF, which was presumably lost at the point indicated (white arrowhead). **(B)** Dendrogram based on *rsmF* ortholog sequences. Clustal Omega was used for multiple sequence alignments. Dendrograms were constructed using MEGA software, version 5.05, using the Neighbour-Joining grouping procedure with 10,000 bootstrap replicates. Values at the nodes indicate statistical support for the particular branches, according to the bootstrap test.

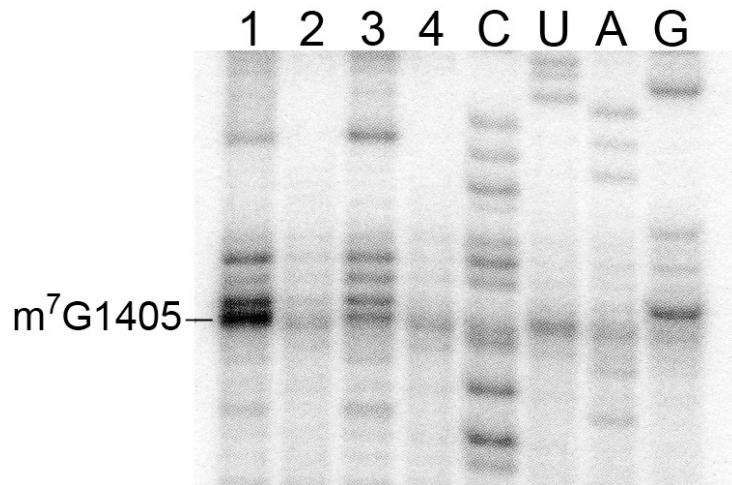


Figure S2. Catalysis of the 16S rRNA m^7G1405 modification by the methyltransferase RmtD. Gel autoradiogram of primer extension through the G1405 region of 16S rRNA. The rRNAs were isolated from *P. aeruginosa* RmtD (lanes 1 and 2) or from *P. aeruginosa* PAO1 (lanes 3 and 4) and were either treated with $NaBH_4$ /aniline (lanes 1 and 3) or left untreated (lanes 2 and 4). In lane 1, rRNA scission stops reverse transcriptase immediately prior to the site of RmtD methylation at G1405. The scission reaction is incomplete, thus the band intensity does not reflect the level of methylation at this nucleotide (see Materials and Methods). Dideoxy sequencing reactions (C, U, A and G) were carried out on PAO1 rRNA.