# DISSECTING THE ROLE OF THE SMALL RNA ERSA IN *PSEUDOMONAS AERUGINOSA* MOTILITY AND BIOFILM REGULATION

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**Background**

ErsA is a novel *Pseudomonas aeruginosa* small RNA responsive to infection-relevant host stimuli. Its transcription is under the control of the alternative sigma factor $\sigma^{22}$ (AlgT/U), implicated in bacterial virulence. In strain PAO1, ErsA exerts a direct negative post-transcriptional regulation in an incoherent feed-forward loop with $\sigma^{22}$ on the bi-functional enzyme AlgC, which is involved in the biosynthesis of sugar precursors for alginate and polysaccharide exoproducts.

**Objectives**

We aim at the characterization of novel target genes regulated by ErsA to expand the knowledge about the ErsA-based regulatory network.

**Methods**

We employed different approaches: i) bioinformatics analysis, ii) *in vitro* electrophoretic mobility shift assays, iii) *in vivo* translational fusions based on the *gfp* reporter gene, iv) RNA-seq, and v) biofilm and motility assays on mutant strains.

**Conclusions**

Our results comprise:

i) *In silico* analyses identified new putative target genes mainly involved in biofilm formation and exopolysaccharides production.

ii) Among these targets, we specifically validated the interaction between ErsA and *amrZ* mRNA, both *in vitro* and *in vivo*

iii) The RNA-seq analysis showed that some of the genes whose expression is positively controlled by ErsA are important for biofilm development (i.e. genes belonging to the *pel* operon, *algD*).

iv) In line with the other results, the ErsA deletion mutant shows a hyper-motile phenotype compared to the wildtype and develops a thin and flat biofilm.

Overall, our results suggest that the small RNA ErsA might represent a relevant post-transcriptional regulator in biofilm development at different levels, likely interacting with different mRNA targets.