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Physiological and transcriptional response of *P. aeruginosa* PAO1 cells lacking six major RND pumps

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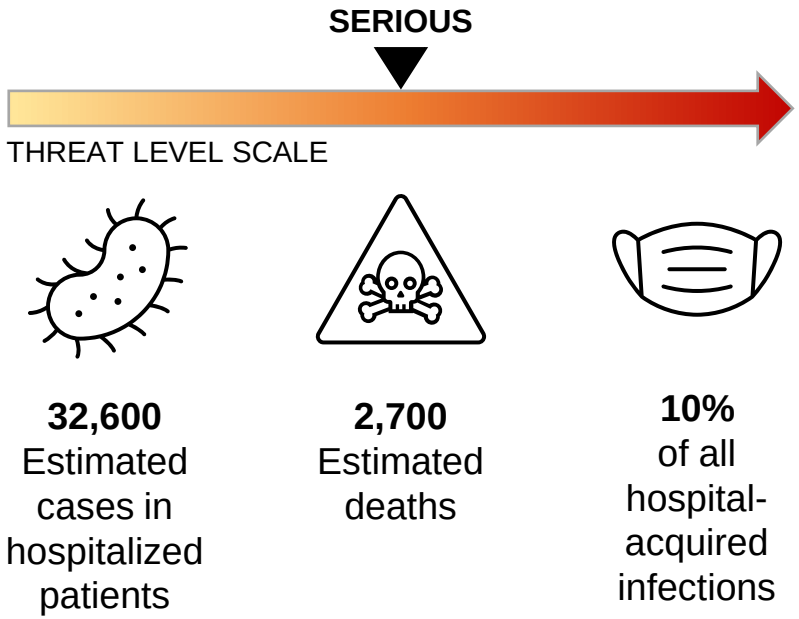
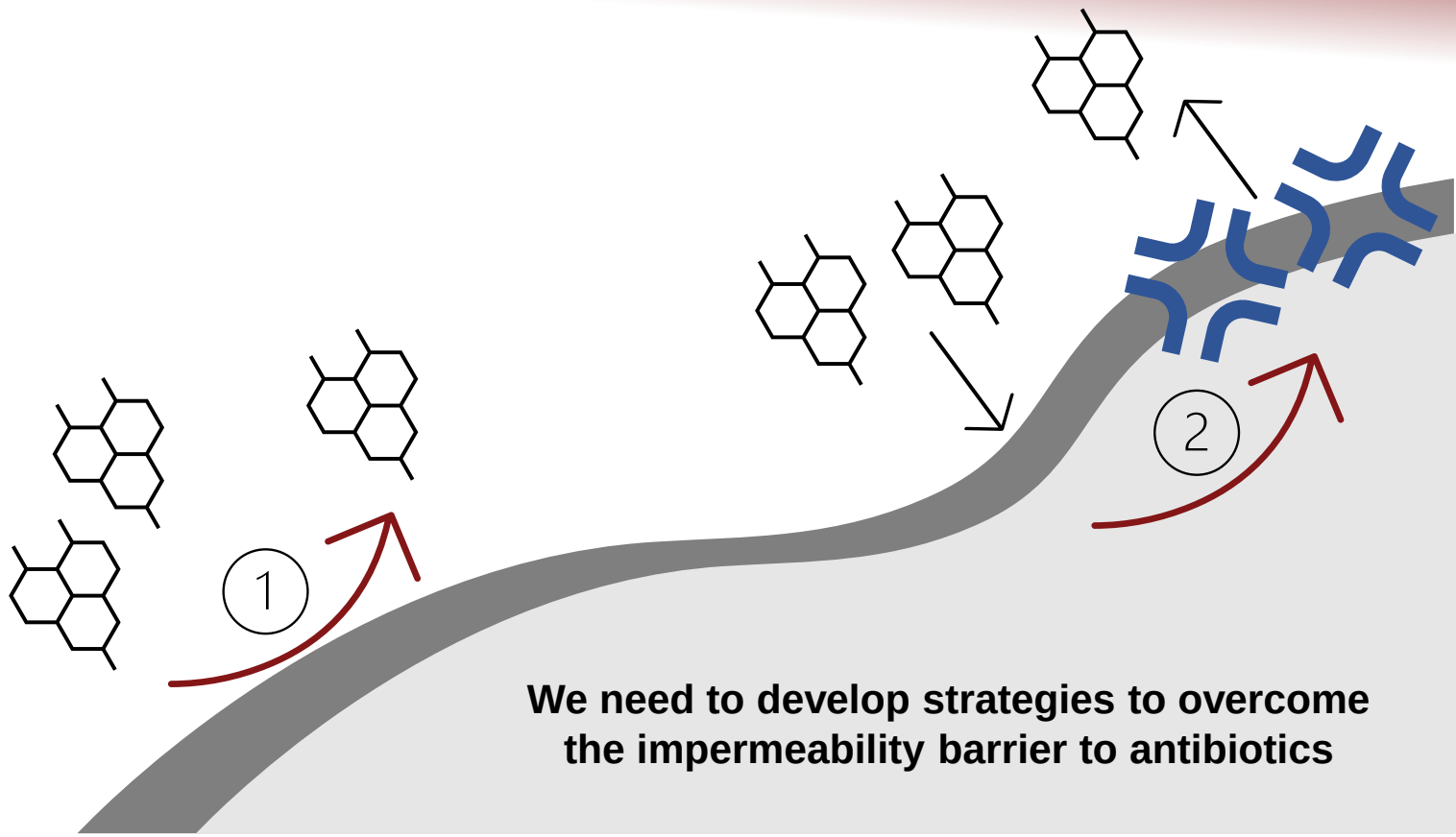
Multidrug-resistant *Pseudomonas aeruginosa*





The development of new antibiotics for *P. aeruginosa* infections is difficult due to outer membrane permeability (1) and Resistance-Nodulation-Division superfamily (RND) transporters (2) protecting the cell.

There are **twelve** RND efflux pumps in *P. aeruginosa*, of which **eleven** are involved in multidrug efflux: MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY, MexJK, MexGHI-OpmD, MexVW, MexPQ-OpmE, MexMN, MuxABC-OpmB, and TriABC.

CDC's Antibiotic Resistance Threats in the United States, 2019; Aloush V. et al. (2005) Antimicrobial Agents and Chemotherapy 50: 43-48

Aim of the study

The aim of the study was to analyze how *P. aeruginosa* changes its physiology due to the lack of six best described RND efflux pumps.

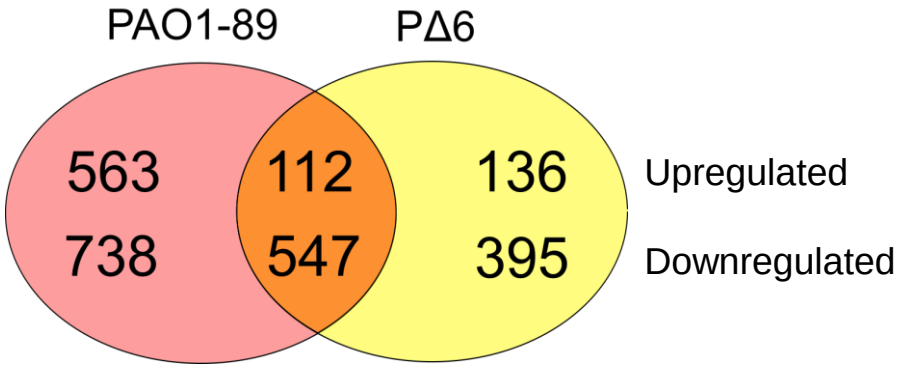
We compared the transcriptomes (RNAseq) and secreted metabolites (MS/MS) of the exponentially growing and stationary PΔ6 and its parent PAO1 cells and identified a specific adaptation response triggered by the lack of efflux.

PAO1 – Pseudomonas aeruginosa wild type
PΔ6 – PAO1 Δ*mexAB-oprM* Δ*mexCD-oprJ* Δ*mexEF-oprN* Δ*mexJKL* Δ*mexXY* Δ*triABC*

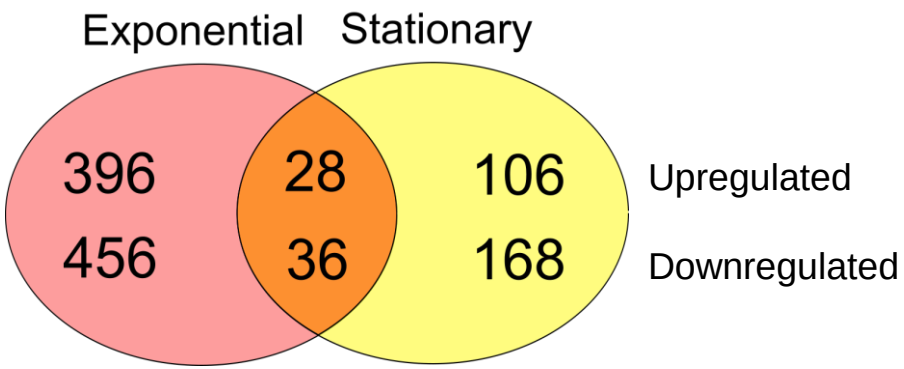
This research is currently being published and is undergoing review in Antimicrobial Agents and Chemotherapy

Expression pattern analysis in PAO1 and PΔ6 strains

A Stationary/Exponential

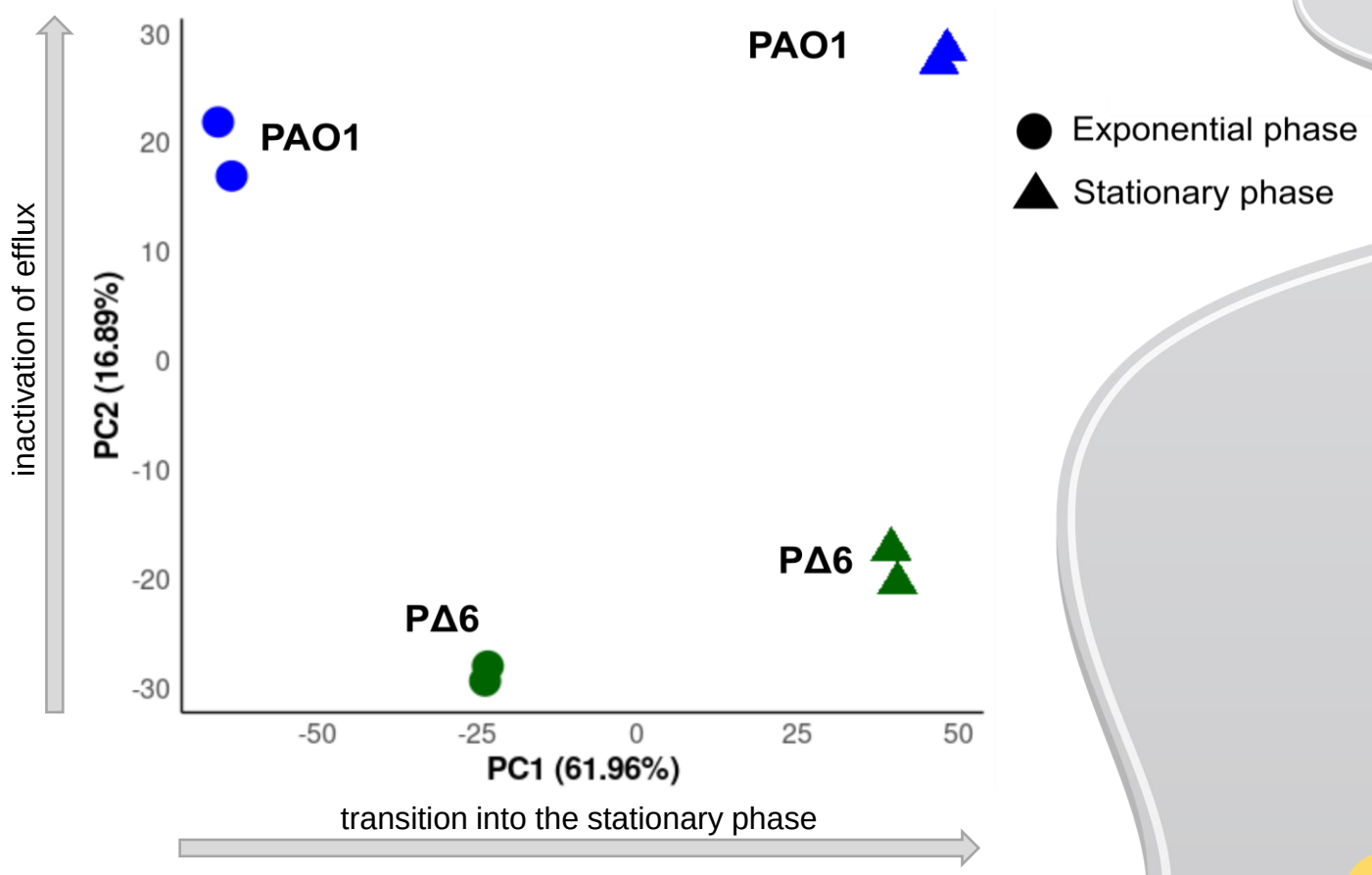


B PΔ6/PAO1-89



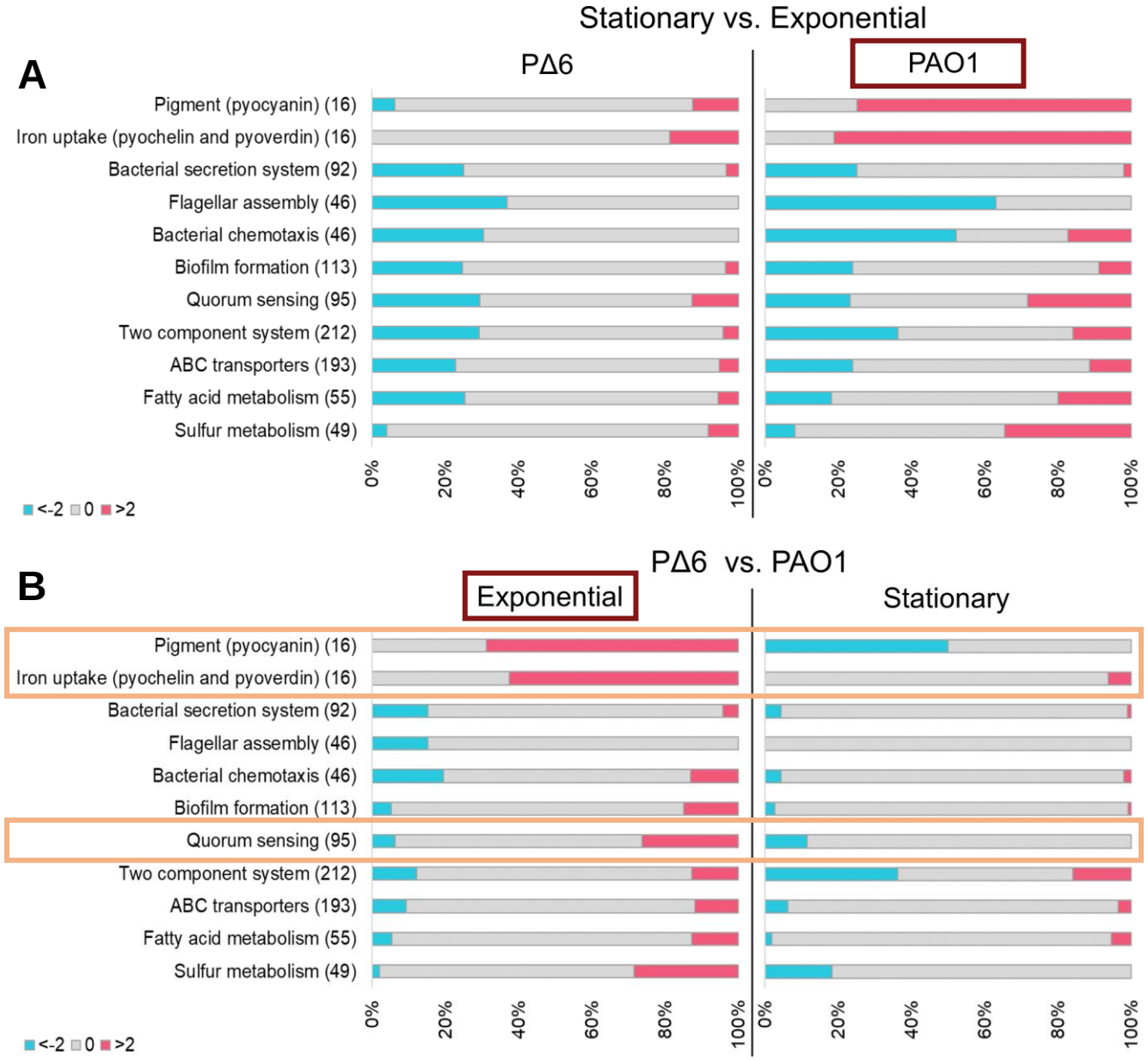
(A) Expression of many genes is affected when PAO1 and PΔ6 cells stop growing.
(B) Significant number of genes affected by the growth phase was strain-specific.

PCA log2 RPKMM
Exponential and stationary phase



Loss of active efflux generates a specific transcriptomic response

Pathways affected in the stationary phase of PAO1 and PΔ6 cells



Annotation of pathways that are affected by
(A) the transition of PAO1 and PΔ6 cell cultures into the stationary phase;
(B) the loss of efflux.

Significantly downregulated

Significantly upregulated

Loss of efflux pumps triggers the changes in the expression of quorum sensing signals and other genes associated with the transition into a stationary phase

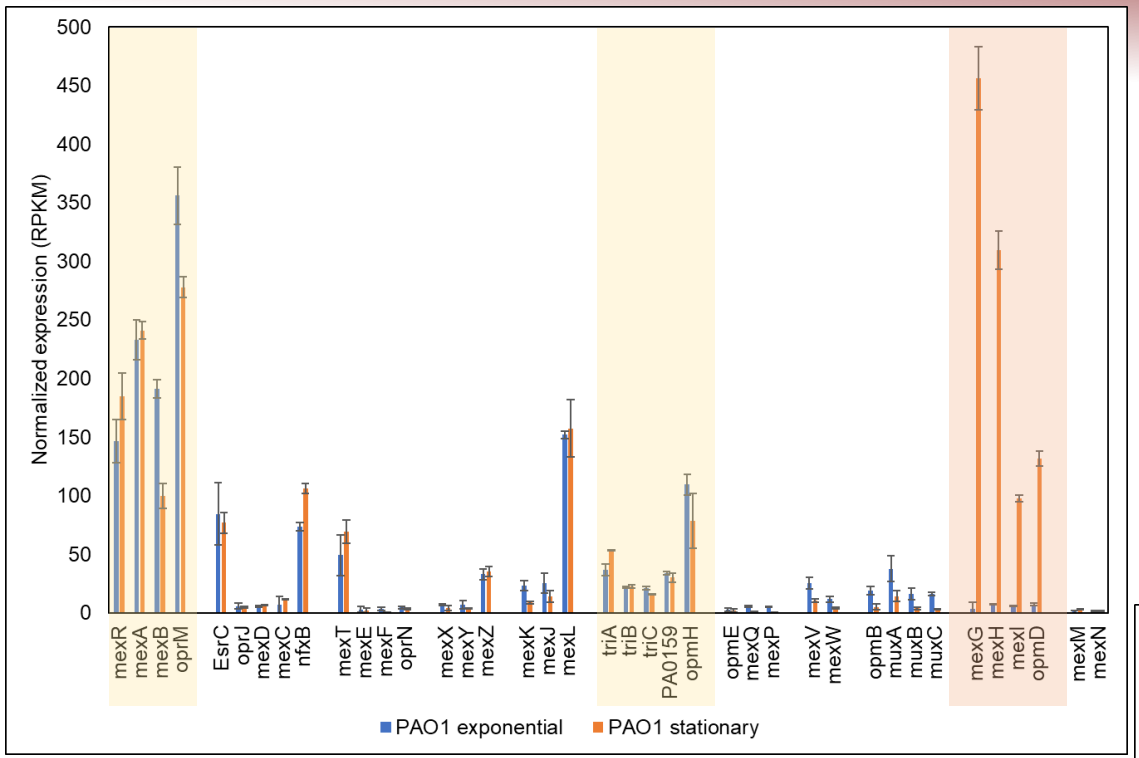
Quorum sensing, pyocyanin pyochelin, pyoverdine biosynthesis – related genes were significantly increased in the stationary PAO1 cells and in the exponential PΔ6 cells.

Pseudomonal quorum signaling molecules (PQS) chelates ferric iron, which may explain higher expression of pyochelin operons in exponential PΔ6 strain, as well as rusty red color of the cell pellet.

PΔ6

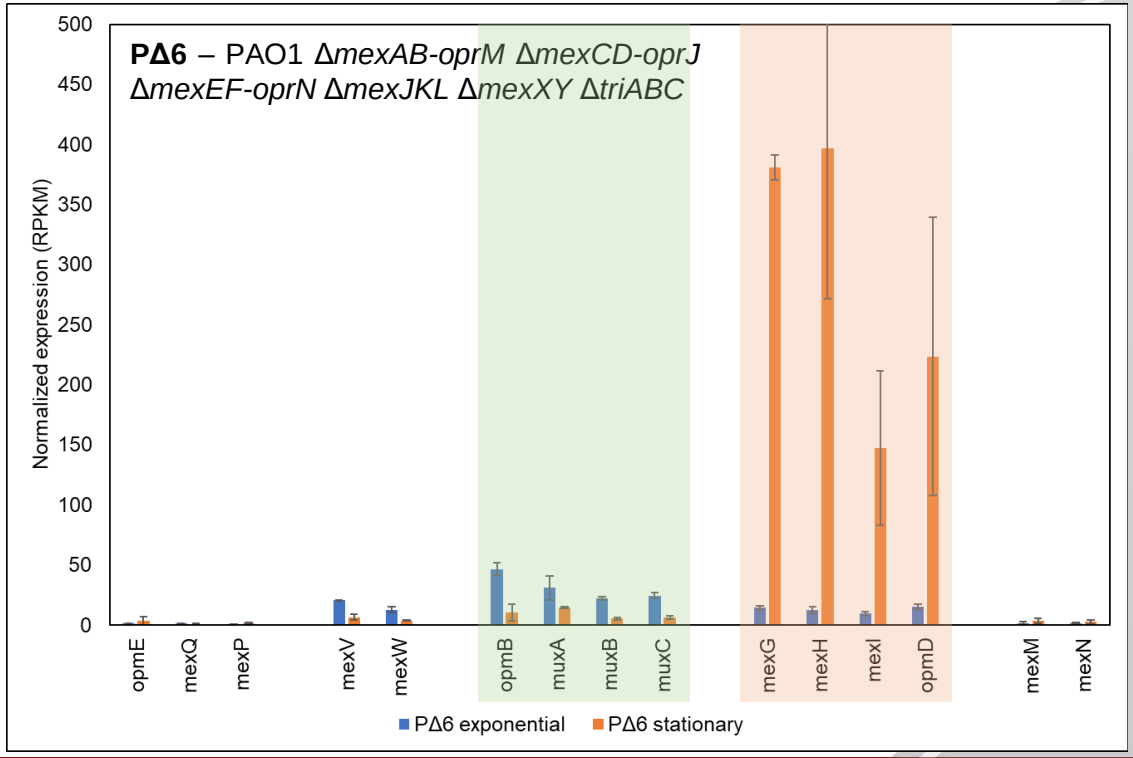
Expression of efflux transporters

Expression of efflux transporters is affected in both exponential and stationary PΔ6 cells. These transporters likely function in the adaptation of PΔ6 cells to the changes in cell physiology as a result of the lack of efflux



MuxABC-OpmB – aminocoumarin-specific transporter. Gene knock-out studies showed 16-32 times higher susceptibility to novobiocin than the parental strains.

Strains	MIC [μg/mL]
PAO1	512
PΔ6	16-32
PΔ6 Δ*mexVW*	32
PΔ6 Δ*mexVW* Δ*muxABC-OpmB*	2-1



Summary

RND-type efflux transporters play a significant role in the physiology of *Pseudomonas aeruginosa*. The inactivation of six best characterized RND efflux pumps led to a specific transcriptomic response associated with the transition into a stationary phase, i.e.:

- changes in transport across the cell envelope (expression of efflux transporter MuxABC-OpmB),
- cell to cell communication (increased amounts of PQS molecules),
- environmental responses (higher expression of pyochelin and increased concentration of Fe^{3+}),

which protect the cell of efflux-deficient *Pseudomonas aeruginosa*.

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