Genome-wide CRISPRi-seq identifies essential genes modulating antibiotic resistance in *Pseudomonas aeruginosa*

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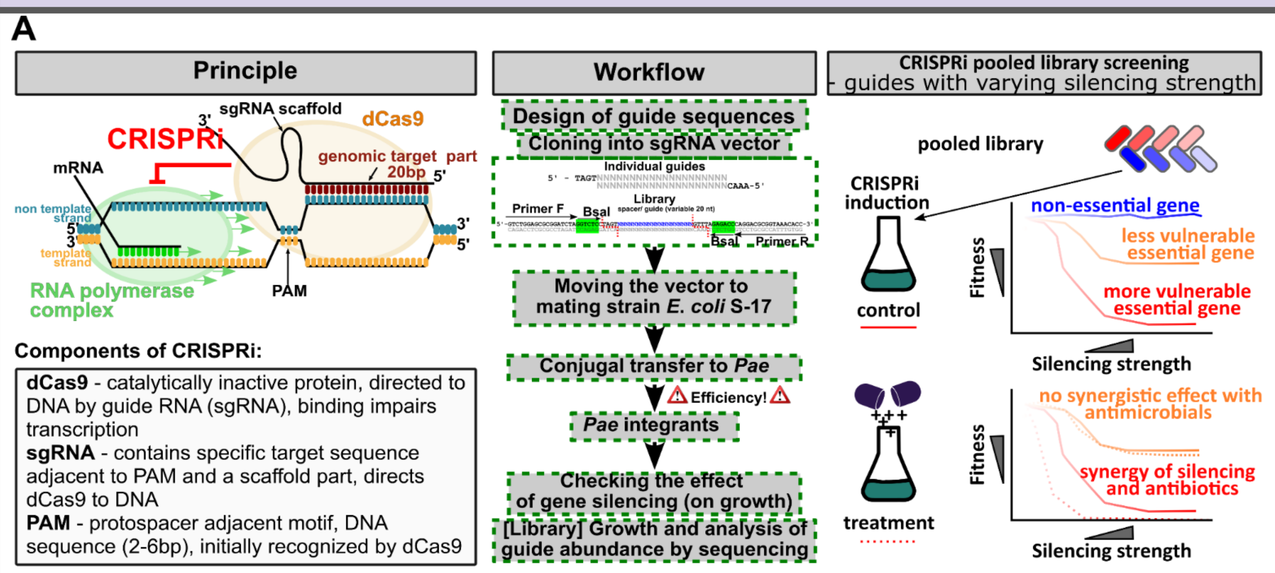
Introduction

Pseudomonas aeruginosa (*Pae*), an ESKAPE group opportunistic pathogen, exhibits intrinsic and acquired resistance mechanisms, limiting effective treatment strategies. We developed CRISPRi system for *Pae* and performed a pooled genome-wide CRISPRi-seq screen under subinhibitory concentrations of antibiotics commonly used to treat *P. aeruginosa* infections, aiming to identify essential genes whose silencing modulates antibiotic resistance. The screen revealed multiple gene-drug interactions and highlighted pathways whose repression altered antimicrobial susceptibility. These essential genes may serve as therapeutic targets or adjuvants to improve antibiotic efficacy while limiting dose-related toxicity.

CRISPRi system developed for *P. aeruginosa*



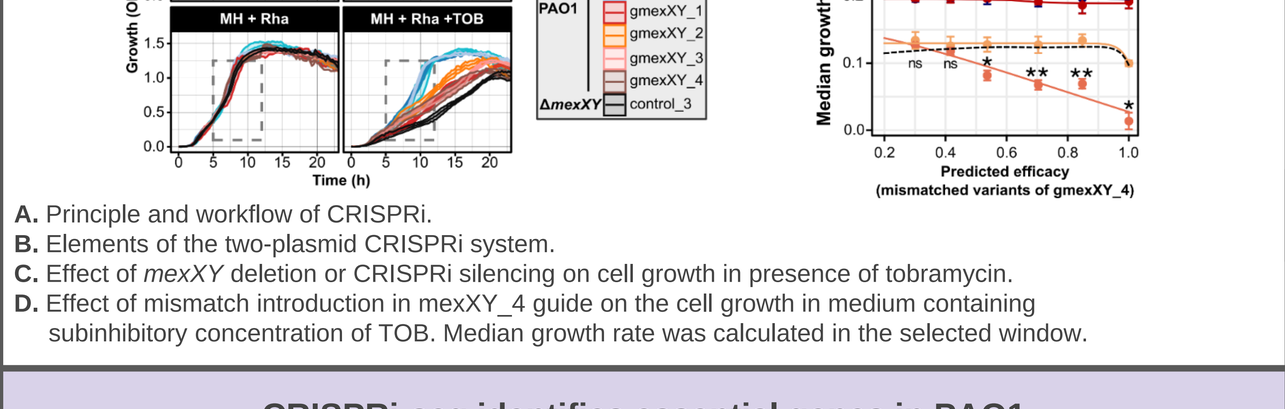
A. Principle and workflow of CRISPRi.
B. Elements of the two-plasmid CRISPRi system.
C. Effect of *mexXY* deletion or CRISPRi silencing on cell growth in presence of tobramycin.
D. Effect of mismatch introduction in mexXY_4 guide on the cell growth in medium containing subinhibitory concentration of TOB. Median growth rate was calculated in the selected window.

CRISPRi-seq identifies essential genes in PAO1

A

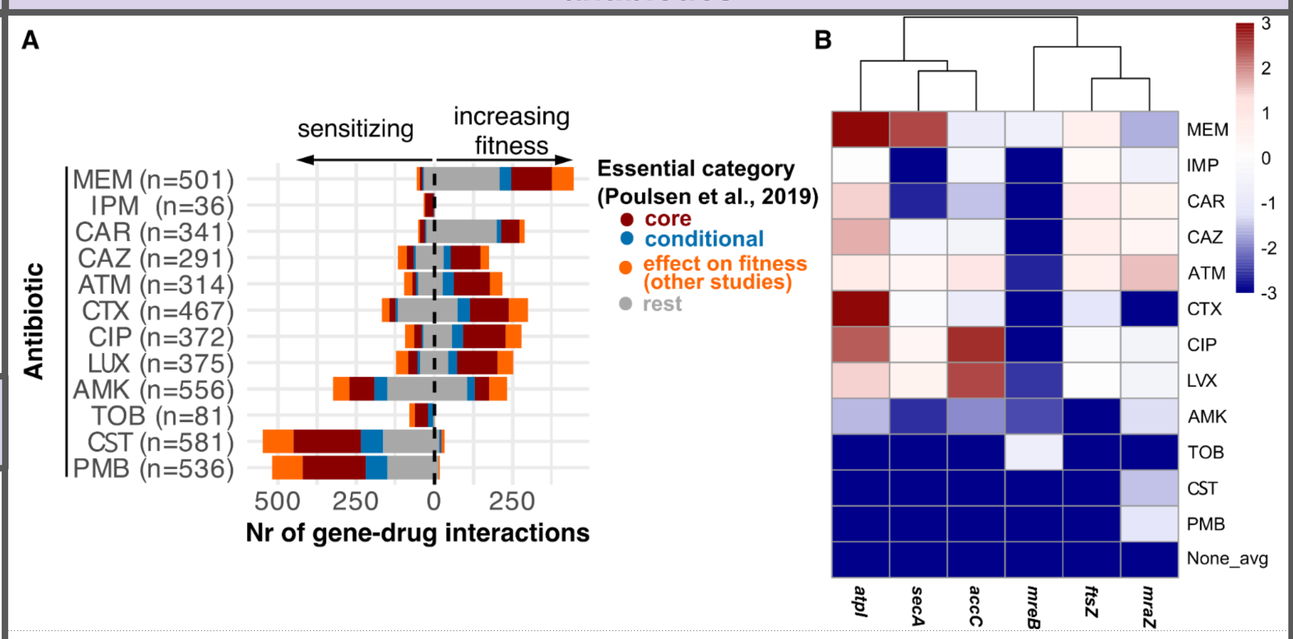
PAO1 Maxi library (~83k guides)

Gene Category	count	guides per gene	mismatched guides
essential (including core)	374	28	15 (for 8)
with effect on fitness	1052	10	8 (for 3)
rest	4287	2	-



A. Composition of CRISPRi PAO1 Maxi library.
B. Experimental workflow for gene involvement analysis using pooled CRISPRi-seq.
C. Number of essential genes identified by CRISPRi in 3 independent experiments, colored according to essential genes categories defined in previous studies.
D. Pathway-level summary of CRISPRi fitness effects in MH and FBS.

CRISPRi-seq identifies genes which silencing modulates tolerance to antibiotics

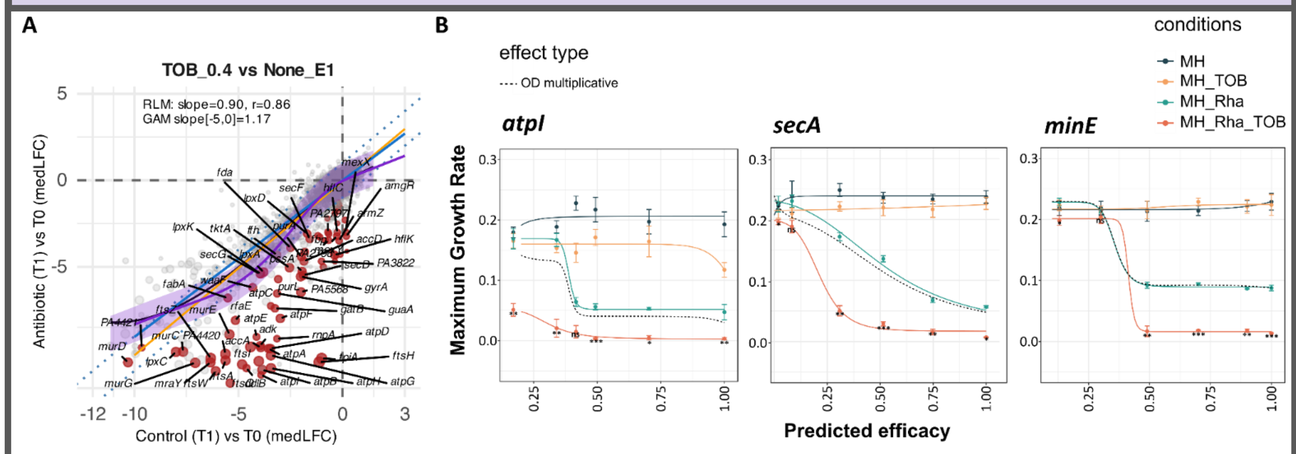


atpI - part of F1-F0 ATP synthase. Inhibition potentiates action of aminoglycosides [PMID: 29906565, 32431678]
secA - essential ATPase that drives the Sec-dependent protein secretion system, facilitating the translocation of preproteins across the inner membrane
accC - biotin carboxylase subunit of acetyl-CoA carboxylase. Catalyzes the ATP-dependent carboxylation of biotin during malonyl-CoA biosynthesis, an essential step in fatty acid synthesis
mreB - actin-like cytoskeletal protein that maintains rod-shaped cell morphology. Coordinates peptidoglycan/cell wall synthesis
ftsZ - forms the bacterial cytokinetic Z-ring, positioned by ***minE***. Coordinates cell division and cell wall synthesis
mraZ - division/cell wall cluster transcriptional repressor

Database browser in preparation

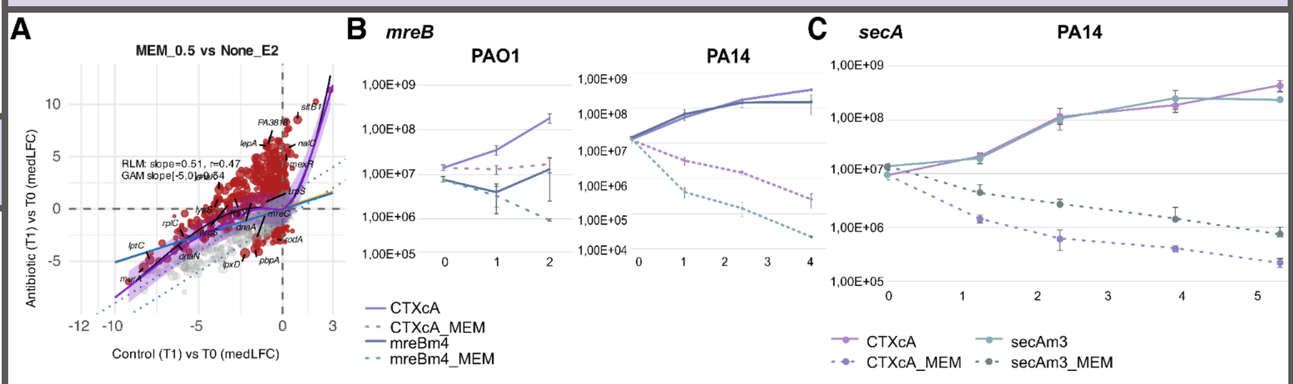
A. Number of identified gene-drug interactions. Bars shows the number of genes which silencing sensitizes or increases fitness in the presence of given antibiotic.
B. Heatmap showing fold change in guide abundance in pooled library of selected genes which silencing modulates antibiotic sensitivity.

Aminoglycosides: tobramycin



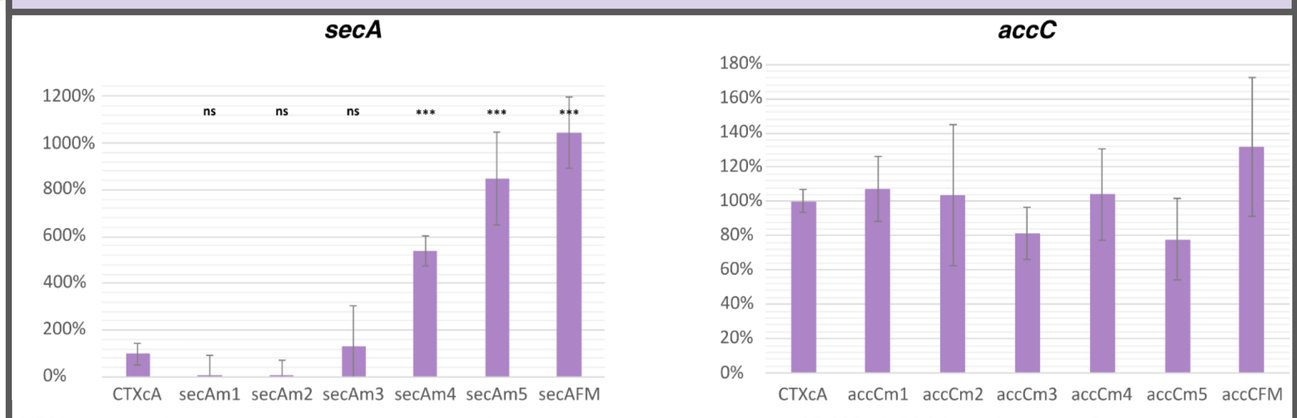
A. Relation between CRISPRi growth defect and tobramycin-specific fitness effect.
B. Relationship between maximum growth rate of PAO1 harbouring guides targeting *secA, atpI* and *minE* and predicted silencing strength.

Carbapenems: meropenem



A. Relation between CRISPRi growth defect and meropenem-specific fitness effect.
B. Meropenem killing assays of PAO1 and PA14 strains harbouring guide targeting *mreB.*
C. Meropenem killing assay in time of PA14 harbouring guide targeting *secA.*

Outer membrane permeability analysis: NPN-mediated fluorescence



NPN-mediated fluorescence measured in relative fluorescence units normalized to OD600 of PAO1 *secA* and *accC* knockdown strains with increasing silencing strength and control (CTXcA), ns - non-significant, * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$.

Conclusions

We preformed a genome-wide screening in *P. aeruginosa* PAO1 towards genetic vulnerabilities of this pathogen using CRISPRi-seq and identified essential genes which inhibition modulates antibiotic resistance. Validation largely confirmed findings from the analysis. In particular, we showed that silencing of *minE, secA* and *atpI* sensitizes *Pae* to tobramycin. We also identified a set of genes, which silencing increased bacterial fitness, particularly in PAO1 treated with meropenem (***secA***). To understand relationship between impact of *secA* inhibition, we additionally tested NPN-mediated fluorescence and proved that inhibition of this gene causes increased permeabilization of outer membrane. Surprisingly, inhibition of ***accC*** didn't show increased permeability in PAO1, suggesting different mechanism behind sensitization to aminoglycosides. Previously shown additional analyses in different media, strains and antibiotics from the same class further support the robustness of the interactions. Taken together, these results contribute to the identification of genes whose inhibition modulates *Pae* susceptibility to antibiotics across diverse conditions.

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