Genomic Identification and Laboratory Characterization of a Putative Transcriptional Repressor of Virulence in *Pseudomonas aeruginosa* UCBPP-PA14

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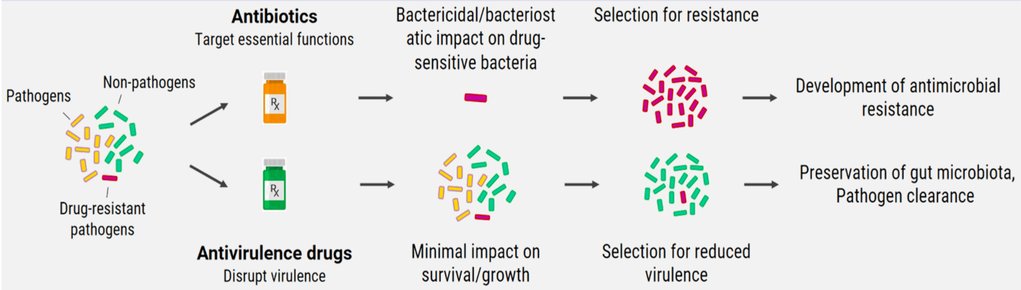
Antivirulence as an alternative approach to antibiotics[1]



Pathogen-associated genes (PAGs) as antivirulence drug targets

Genes found exclusively in bacterial pathogens, but not in related non-pathogens[2]

- Likely associated with virulence-related functions
- Likely not essential for bacterial growth and survival
- Likely more pathogen-specific drug targets

Pseudomonas aeruginosa – a WHO priority pathogen[3,4]

- Gram-negative, ecologically diverse, opportunistic plant and animal pathogen
- Common cause of morbidity and mortality in immunocompromised patients
- Broad range of intrinsic and extrinsic AMR Mechanisms

Hypothesis:

Novel antivirulence drug targets can be discovered via comparative genomic analyses to identify genes solely present in pathogens and potentially under positive selection.

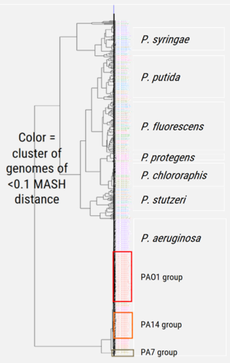
Genus-specific approach of detecting PAGs: *Pseudomonas*

Full genome dataset
606 NCBI RefSeq *Pseudomonas* genomes

PAG analysis 1
- based on one-way BLAST
- ID PAGs by finding genes with BLAST hits only in pathogen genomes

(Dataset too large for OrthoFinder analysis)

Dataset reduction
MASH distance cut-off of 0.1



Reduced genome dataset
206 representative genome (1 gene chosen per cluster)

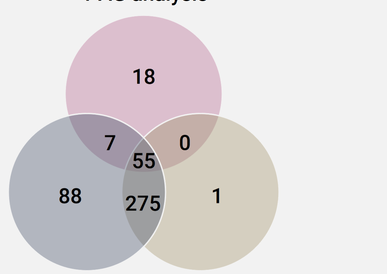
PAG analysis 2

OrthoFinder analysis 3
- Based on RBB normalized by gene length
- ID PAGs by finding orthogroups in which genes are only from pathogen genomes

Three methods of PAG identification to increase accuracy

55 *Pseudomonas aeruginosa* specific PAGs were identified and prioritized, 3 with evidence of positive selection in *Pseudomonas aeruginosa PA14*

A



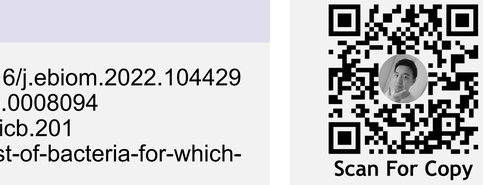
B

RefSeq protein accession	Protein name	Protein length (aa)
WP_003139757.1	Pentapeptide repeat-containing protein	215
WP_003141470.1	DUF3218 domain-containing protein	221
WP_003095466.1	Hypothetical protein	248
WP_003116317.1	Class I SAM-dependent methyltransferase	253
WP_003111331.1	Hypothetical protein	128
WP_003140882.1	Hypothetical protein	212
WP_003118883.1	Hypothetical protein	384
WP_025297936.1	BlaI/MecI/CopY family transcriptional regulator	132
WP_016254216.1	M56 family metallopeptidase	331
WP_003134054.1	Hypothetical protein	137
WP_003089506.1	Hypothetical protein	177
WP_003139692.1	Hypothetical protein	486
WP_071535563.1	Amino acid permease	55
WP_003096896.1	Hypothetical protein	78
WP_071534232.1	Response regulator GacA	105
WP_003140763.1	Hypothetical protein	94
WP_011347386.1	Type I toxin-antitoxin system ptaRNA1 family toxin	76

M56 family metallopeptidase (WP_016254216.1; PA14_RS12695)

- Predicted to be cytoplasmic membrane-localized
- Located on a genomic island which is potentially an integrative and conjugative element (UCBPP-PA14).
- A pathogen associated probable BlaI/MecI/CopY family transcriptional regulator (PA14_RS12700) is located directly upstream.
- BlaI/MecI/CopY family of genes are associated with virulence functions in *Staphylcoccus aureus.*

UCBPP-PA14 integrative plasmid (43kb)

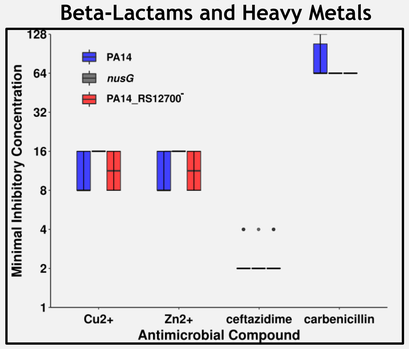


A) A total of 55 PAGs were identified by all 3 methods in *P. aeruginosa*.
B) Of the 55 PAGs, 17 were found in more than 3 genomes in the reduced dataset, 3 of which showed positive selection in *P. aeruginosa* PA14.

PA14_RS12700 knockout does not affect antimicrobial sensitivity and biofilm formation, but is associated with hyper-virulence phenotype

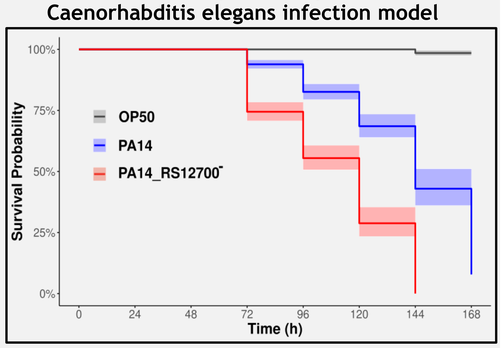
A

Minimum Inhibitory Concentration of Beta-Lactams and Heavy Metals



B

Survival probability of Caenorhabditis elegans infection model



A) The PA14_RS12700 transcriptional repressor knockout strain showed no difference in susceptibility to ß-lactam antibiotics or heavy metals.
B) PA14_RS12700 displays a hyper-virulent phenotype in a *C. elegans* infection model (Gehan-Breslow-Wilcoxon test; $p < 0.0001$)

SUMMARY & FUTURE DIRECTIONS

- Using genomics, we identified and prioritized 17 PAGs in the *P. aeruginosa* PA14 genome that are conserved in *Pseudomonas* pathogens but not in *Pseudomonas* non-pathogens.
- Two genes of interest, PA14_RS12700 and PA14_RS12695, have sequence similarities with Bla/Cop/Mec sensor-regulator systems for β-lactam and heavy metal resistance in Gram positives.
- In *P. aeruginosa* PA14, loss of the transcriptional repressor, PA14_RS12700, results in increased virulence, but no difference in resistance observed.
- **Computationally predicted PAGs warrant further study including possible development as targets for novel antivirulence therapeutics.** However, there is a need to understand their mechanisms of action.
- Future plan: Characterize an overexpression strain of the BlaR-like component, PA14_RS12695.

SFU

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