Disarming *Pseudomonas aeruginosa*

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Background

Genetically-modified strains of *Escherichia coli* have been largely used as hosts for the production of recombinant proteins but with some limitations.

As an alternative, *Pseudomonas aeruginosa* has an extraordinary repertoire of catabolic genes and secretion pathways making it attractive for biotechnological purposes, but its pathogenicity has prevented any applications so far.

Our objective was to genetically modify strain PAO1 to reduce its capacity to produce virulence factors and to become resistant to antibiotics.

Methods

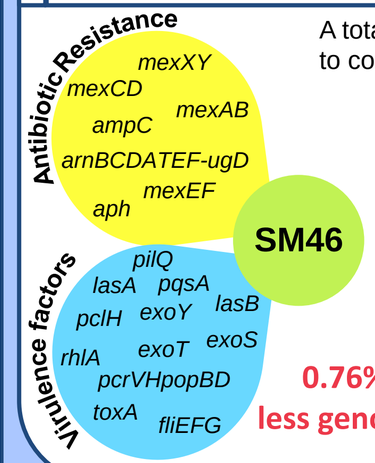
Gene inactivation experiments by homologous recombination were performed to decrease the capacity of strain PAO1 to produce virulence factors and develop antibiotic resistance.

Virulence factor production was evaluated *in vitro* using different specific growth media.

Cytotoxicity was evaluated on J774 A.1 murin macrophages by measuring the release of LDH enzyme into the supernatant.

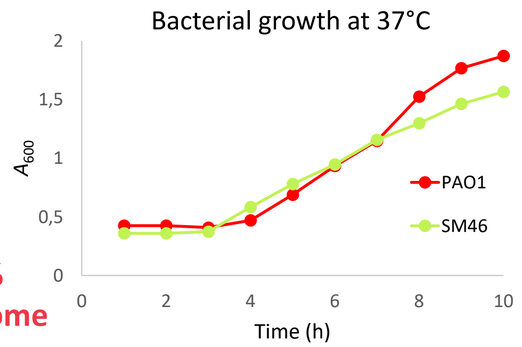
Minimal Inhibitory Concentrations (MICs) of selected antibiotics were determined using the micro-dilution technique as recommended by EUCAST.

1 Construction of SM46 by gene deletion

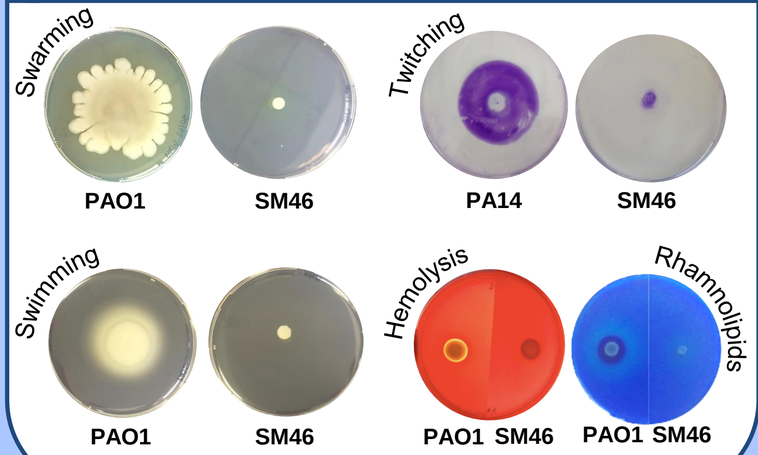


A total of 37 genes have been deleted from strain PAO1 to construct mutant SM46, with no impact on fitness.

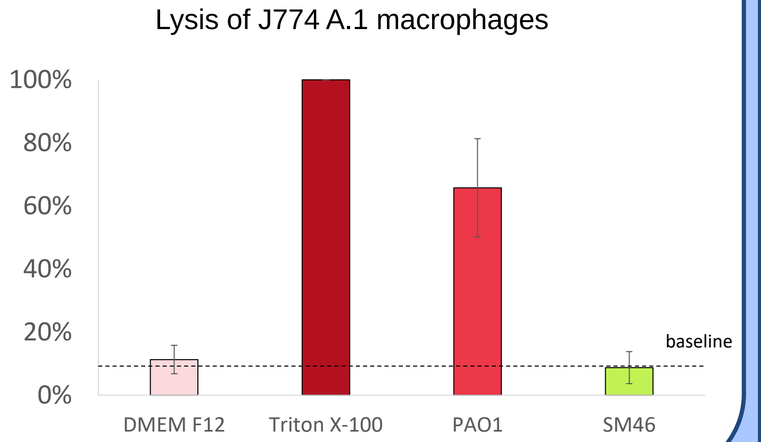
0.76%
less genome



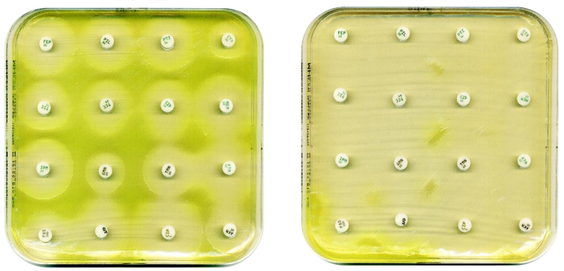
2 Inactivating Virulence Factors



3 Reduction of Cytotoxicity



4 Inactivating Antibiotic Resistance Mechanisms



Compared to PAO1, mutant SM46 is 4- to 256-fold more susceptible to antibiotics.

Strains	Minimal Inhibitory Concentration (MIC) (µg/mL)											
	ATM	TIC	CAZ	FEP	CTX	IPM	CIP	NOV	AKN	TMN	KMN	TET
PAO1	4	32	2	2	32	1	0.125	512	8	2	128	32
SM46	0.125	0.25	0.5	0.25	0.125	0.25	0.03	32	1	0.5	1	0.5

ATM, aztreonam; TIC, ticarcillin; CAZ, ceftazidime; FEP, cefepime; CTX, cefotaxime; IPM, imipenem; CIP, ciprofloxacin; NOV, novobiocin; AKN, amikacin;TMN, tobramycin; KMN, kanamycin, TET, tetracyclin

Conclusion

Most of the virulence and resistance traits typically found in *P. aeruginosa* have been abolished in SM46.

SM46 is a good model to assess specific pathogenic determinants of clinical strains.

SM46 will be engineered to produce recombinant proteins.

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