

Novel therapeutic approaches against *Pseudomonas aeruginosa*



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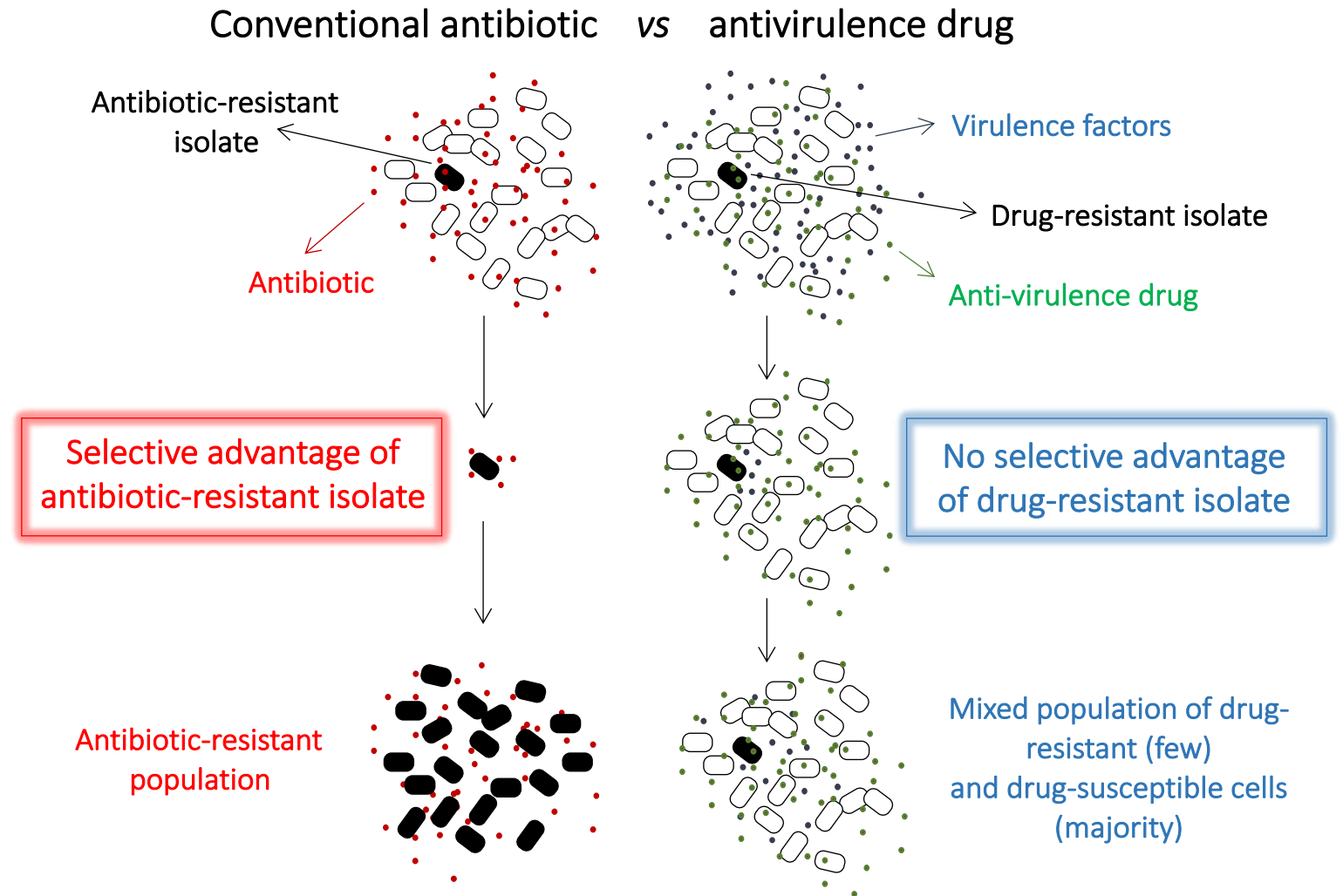
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The strategy

Antivirulence drugs inhibit the expression or activity of virulence factors without affecting bacterial growth.

Antivirulence drugs are expected to:

- ✓ prevent/inhibit the establishment of the infection
- ✓ reduce the damage caused by pathogens
- ✓ have less adverse effects on the host microbiota
- ✓ impose a weaker selective pressure for the emergence of drug resistance



NATURE REVIEWS | MICROBIOLOGY 300 | APRIL 2014 | VOLUME 12

Targeting virulence: can we make evolution-proof drugs?

Richard C. Allen, Roman Popat, Stephen P. Diggle and Sam P. Brown

The Sociomicrobiology of Antivirulence Drug Resistance: a Proof of Concept

Brett Mellbye and Martin Schuster

Department of Microbiology and Molecular and Cellular Biology Program, Oregon State University, Corvallis, Oregon, USA

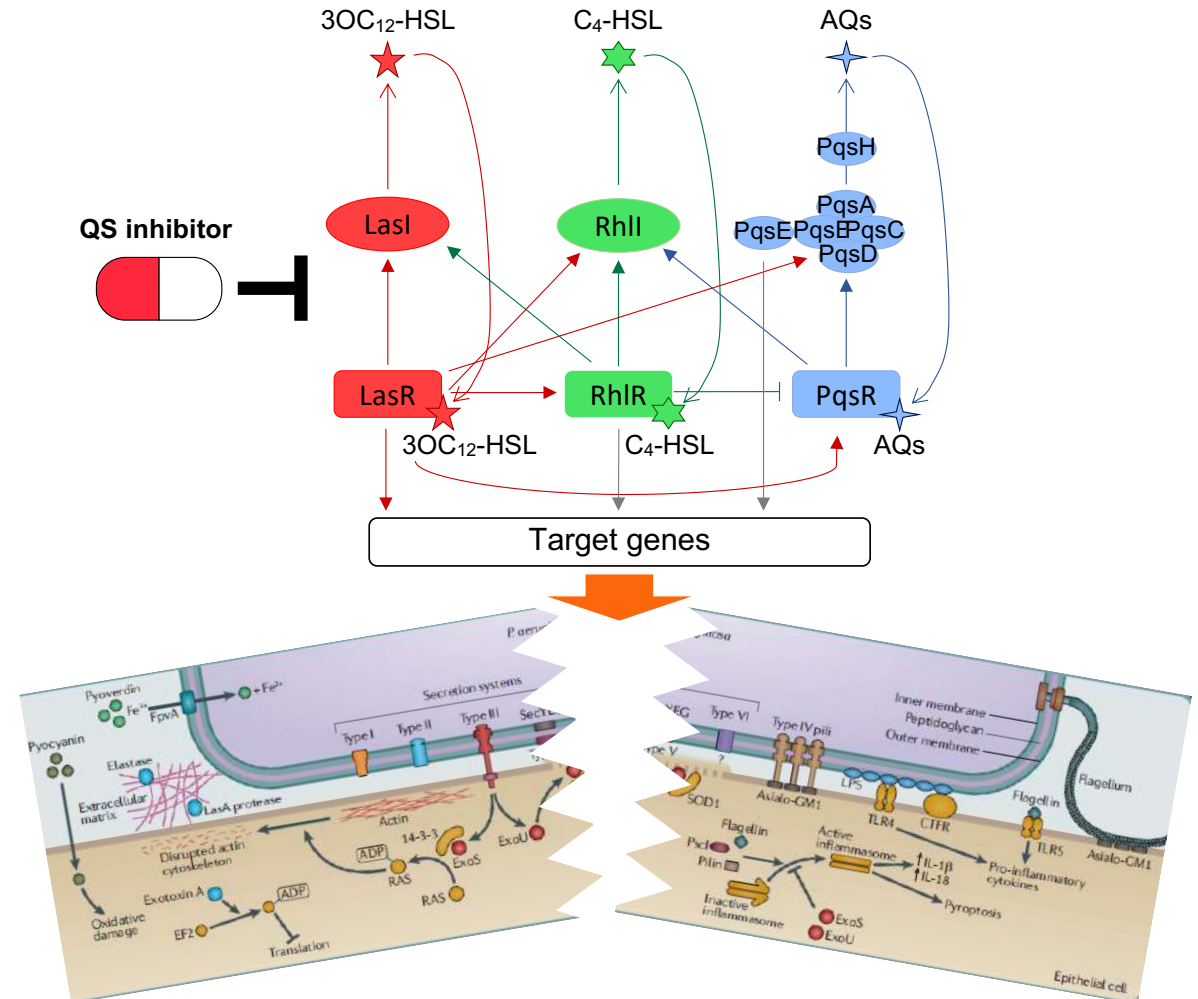


The organism and the target

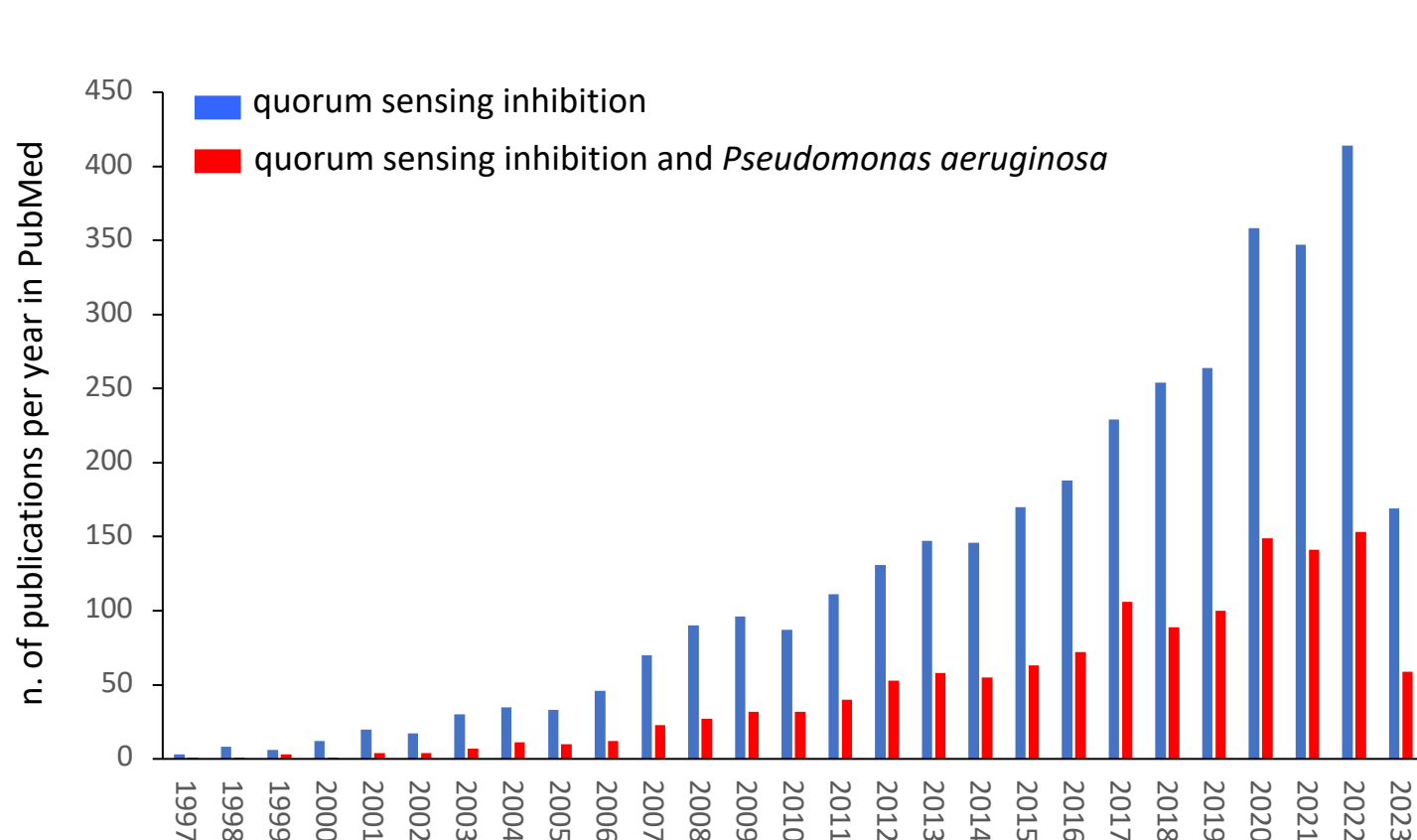
Pseudomonas aeruginosa

- Opportunistic human pathogen
- Top ranked in both intensive care unit (ICU) and non-ICU hospital-acquired infections
- Main cause of mortality in cystic fibrosis (CF) patients and one of the main causative agents of chronic wounds
- High antibiotic resistance, also as a consequence of biofilm formation
- Included by the WHO in the priority list of pathogens for which new antimicrobial therapies are urgently needed (Priority 1: Critical)

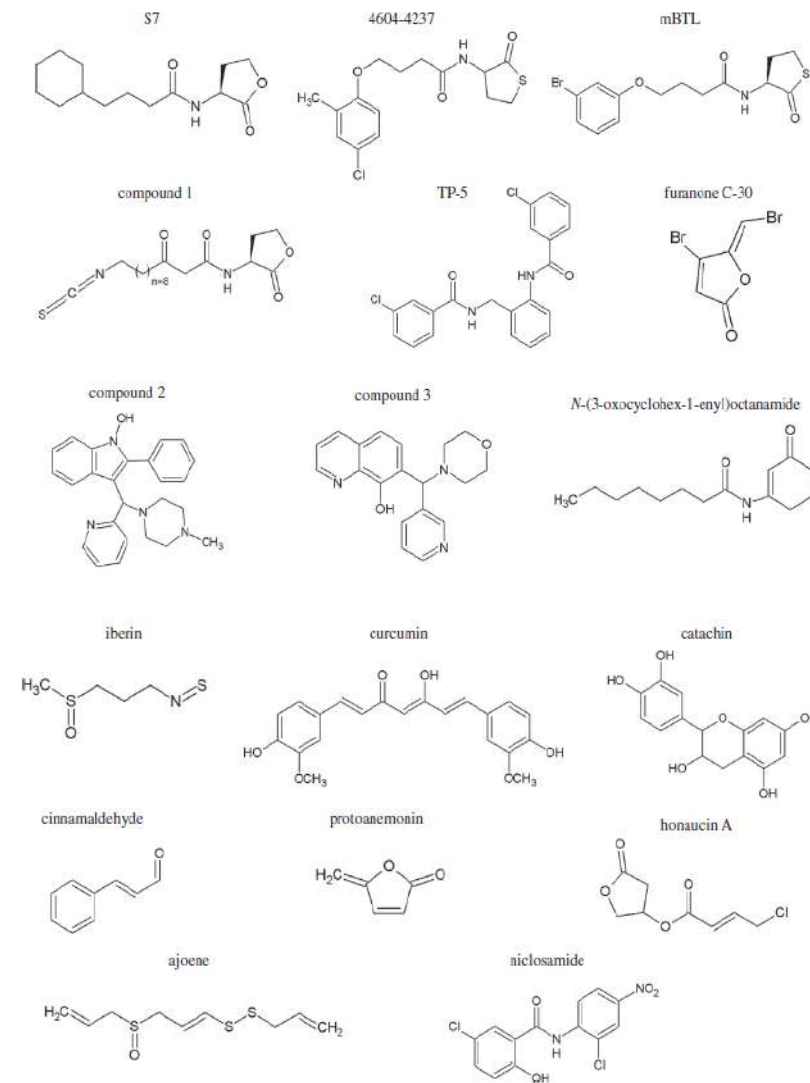
quorum sensing (QS)



Many antivirulence drugs targeting QS with poor pharmacological properties



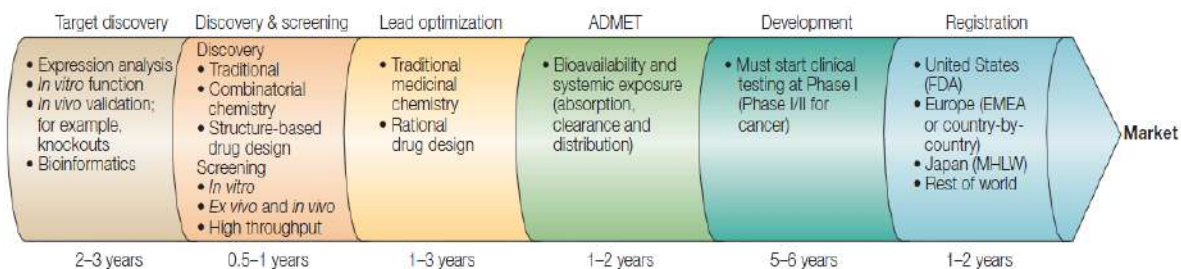
Unfortunately, most QS inhibitors have unfavourable pharmacological properties



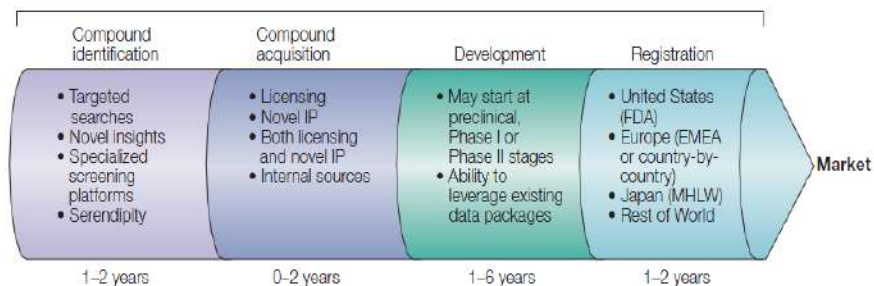
The approach

Drug repurposing is based on the use of “old” drugs already approved for use in humans to treat different diseases.

As compared to *de novo* drug discovery, the drug repurposing approach has a higher probability of yielding bioavailable and safe hit compounds.



De novo drug development



Drug repurposing

Ashburn and Thor (2004) *Nat Rev Drug Discov* 3:673-683.

Emerging Topics in Life Sciences (2017)
DOI: 10.1042/EBC20170018

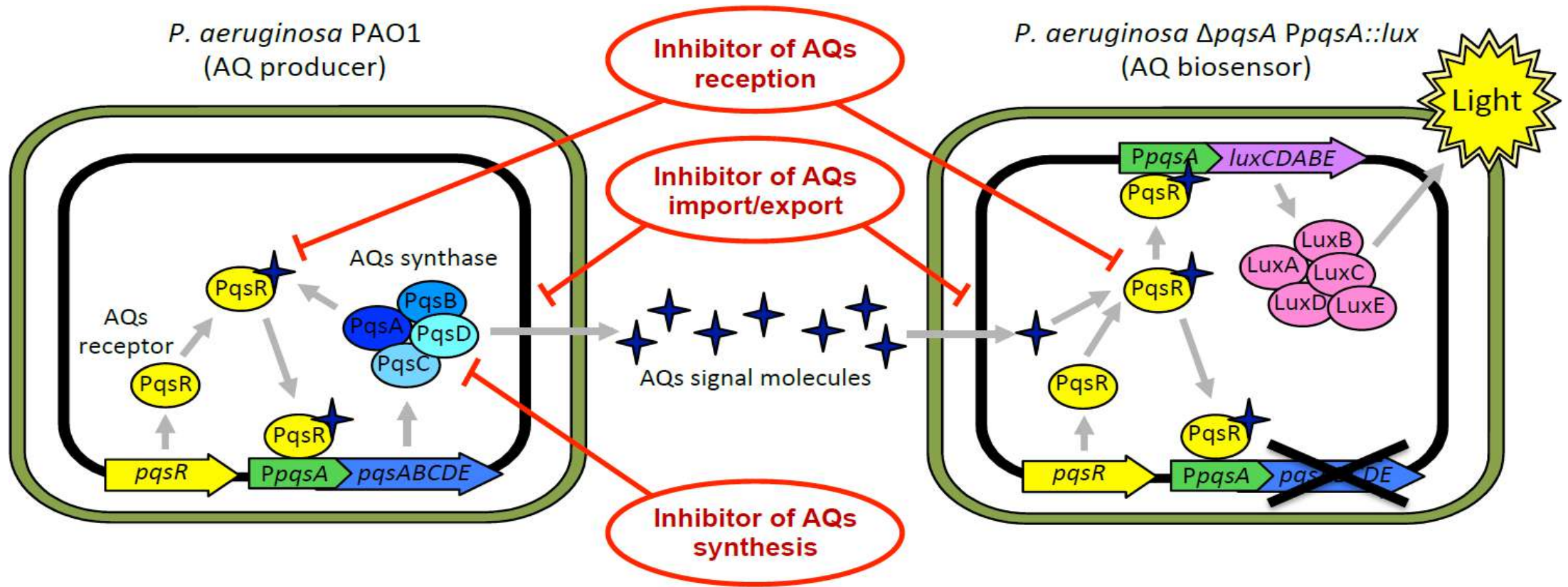


Review Article

Drug repurposing for antivirulence therapy against opportunistic bacterial pathogens

Giordano Rampioni¹, Paolo Visca¹, Livia Leoni¹ and Francesco Imperi²

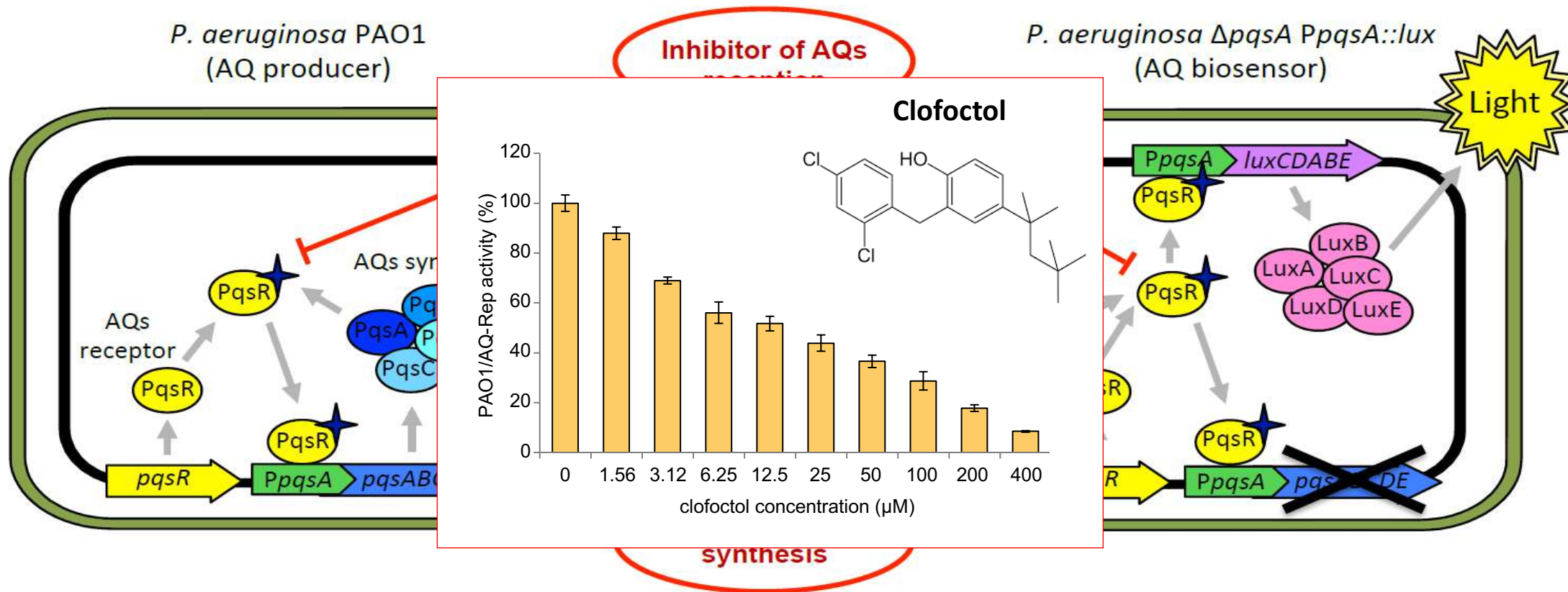
The screening system



PHARMAKON Library: 1600 FDA-approved drugs



The inhibitor

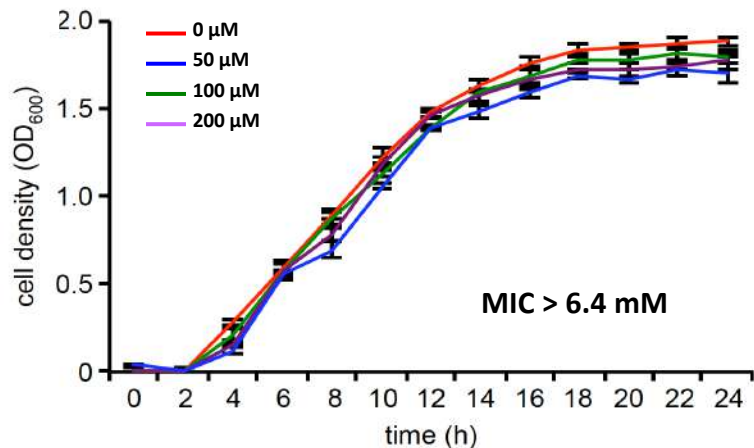


PHARMAKON Library: 1600 FDA-approved drugs

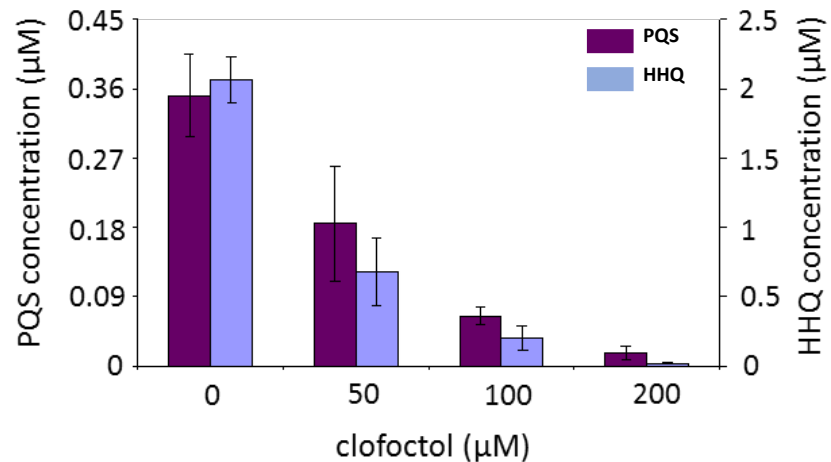


Clofoctol inhibits QS-dependent virulence traits

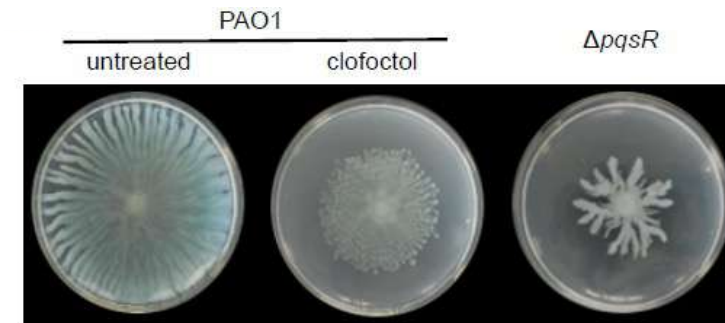
Growth curves



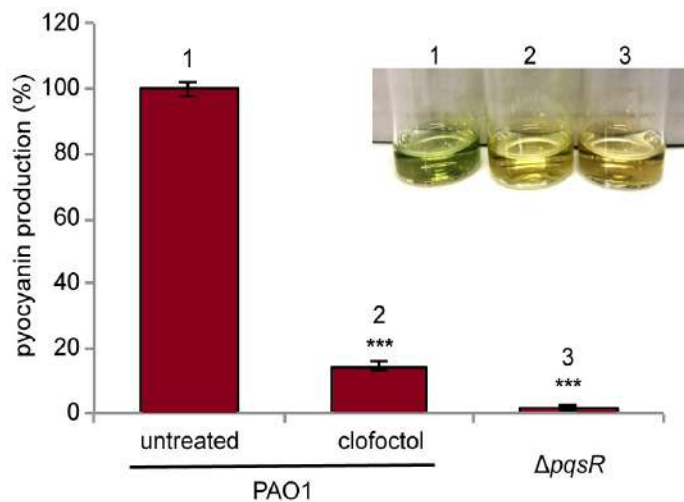
QS signal molecules



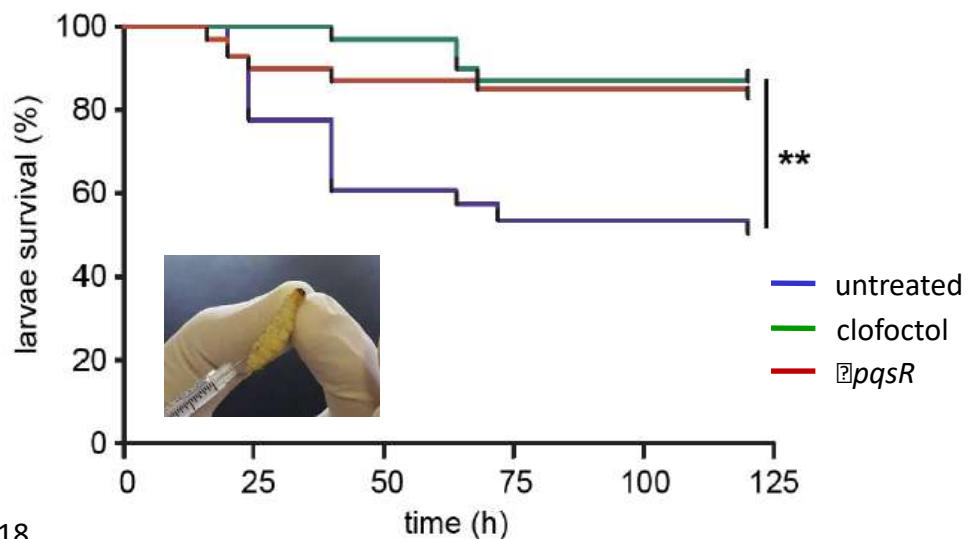
Swarming motility



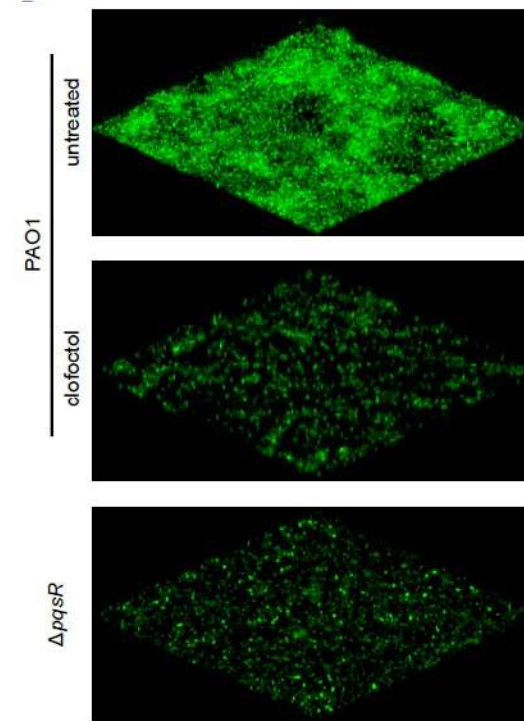
Pyocyanin production



G. mellonella killing assay



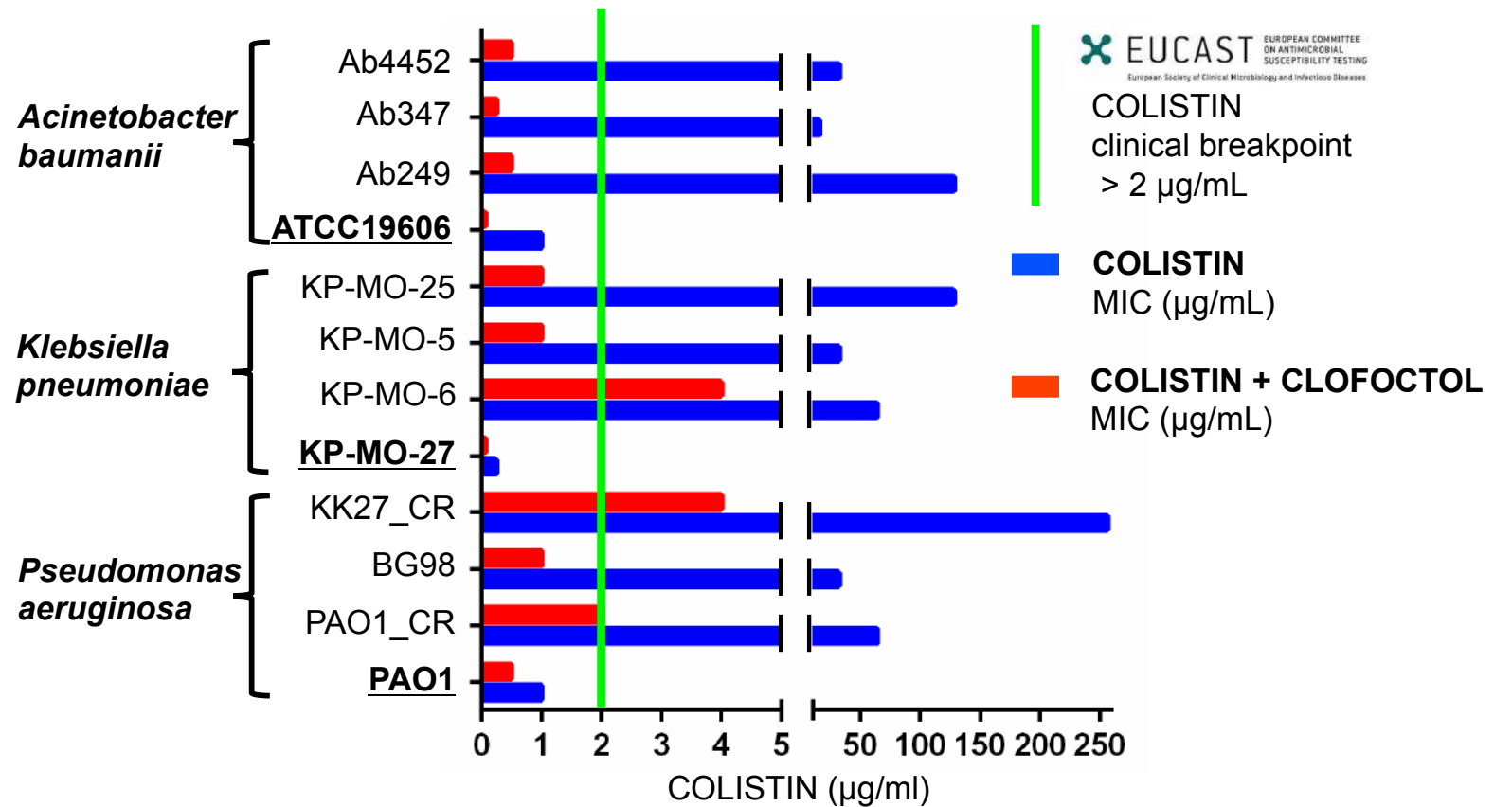
Biofilm formation



Clofoctol restores colistin sensitivity in colistin-resistant Gram-negative pathogens

Clofoctol (also known as octofene) is an antibiotic active against Gram-positive pathogens, commonly prescribed to treat pulmonary lung infections in infants due to its low toxicity.

Clofoctol is a resistance breaker active against different Gram-negative colistin-resistant pathogens.



We are currently investigating the mechanism of action of clofoctol as a colistin-resistance breaker.

Identification, characterization and optimization of other antivirulence drugs

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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

Check for updates

Identification of FDA-Approved Drugs as Antivirulence Agents Targeting the *pqs* Quorum-Sensing System of *Pseudomonas aeruginosa*

Francesca D'Angelo,^a Valerio Baldelli,^a Nigel Halliday,^b Paolo Pantalone,^b Fabio Politicelli,^{a,c} Ersilia Fiscarelli,^d Paul Williams,^b Paolo Visca,^a Livia Leoni,^e and Giordano Rampioni^a*

OPEN ACCESS Freely available online

PLOS PATHOGENS

Structural Basis for Native Agonist and Synthetic Inhibitor Recognition by the *Pseudomonas aeruginosa* Quorum Sensing Regulator PqsR (MvfR)

Aravindan Ilangoan^{1,2,3a}, Matthew Fletcher^{1,2}, Giordano Rampioni^{1,2b}, Christian Pustelný^{1,2c}, Kendra Rumbaugh⁴, Stephan Heeb^{1,2}, Miguel Cámara^{1,2}, Alex Truman^{1,2}, Siri Ram Chhabra^{1,2}, Jonas Emsley^{1,2}, Paul Williams^{1,2a}

¹ Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, United Kingdom, ² School of Molecular Medical Sciences, University of Nottingham, University Park, Nottingham, United Kingdom, ³ School of Pharmacy, University of Nottingham, University Park, Nottingham, United Kingdom, ⁴ Department of Surgery, University of Texas, Lubbock, Texas, United States of America

VIROLENCE

2020, VOL. 11, NO. 1, 652-668
<https://doi.org/10.1080/21505594.2020.1779568>

Taylor & Francis

RESEARCH PAPER

OPEN ACCESS

Identification of FDA-approved antivirulence drugs targeting the *Pseudomonas aeruginosa* quorum sensing effector protein PqsE

Valerio Baldelli^a, Francesca D'Angelo^{a*}, Viola Pavoncello^{a*}, Ersilia Vita Fiscarelli^b, Paolo Visca^a, Giordano Rampioni^a, and Livia Leoni^a

frontiers in Microbiology

ORIGINAL RESEARCH

published: 10 October 2019
doi: 10.3389/fmicb.2019.02355

In silico Selection and Experimental Validation of FDA-Approved Drugs as Anti-quorum Sensing Agents

Marta Mellini¹, Elena Di Muzio¹, Francesca D'Angelo^{1*}, Valerio Baldelli¹, Serena Ferrillo¹, Paolo Visca¹, Livia Leoni¹, Fabio Politicelli^{1,2*} and Giordano Rampioni^{1*}

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OBSERVATION

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Synergistic Activity of Colistin in Combination with Clofocetol against Colistin Resistant Gram-Negative Pathogens

Diletta Collalto,^a Alessandra Fortuna,^a Paolo Visca,^{a,b} Francesco Imperi,^{a,b} Giordano Rampioni,^{a,b} and Livia Leoni^a*

AAC Journals/ASM.org

New Life for an Old Drug: the Anthelmintic Drug Niclosamide Inhibits *Pseudomonas aeruginosa* Quorum Sensing

Francesco Imperi,^a Francesco Massai,^b Cejcoje Ramachandran Pillai,^b Francesca Longo,^b Elisabetta Zennaro,^b Giordano Rampioni,^a Paolo Visca,^a Livia Leoni^a*

Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, Rome, Italy^a; Department of Biology, University Roma Tre, Rome, Italy^b

molecular pharmaceuticals

Article

pubs.acs.org/moleculapharmaceutics

Toward Repositioning Niclosamide for Antivirulence Therapy of *Pseudomonas aeruginosa* Lung Infections: Development of Inhalable Formulations through Nanosuspension Technology

Gabriella Costabile,¹ Ivana d'Angelo,² Giordano Rampioni,³ Roslen Bondi,³ Barbara Pompili,¹ Fiorentina Ascenzioni,¹ Emma Mitidieri,⁷ Roberta d'Emmanuele di Villa Bianca,⁷ Raffaella Sorrentino,⁷ Agnese Miro,⁷ Fabiana Quaglia,⁷ Francesco Imperi,¹ Livia Leoni,³ and Francesca Ungaro^{8,†}

frontiers in Microbiology

ORIGINAL RESEARCH

published: 25 April 2022
doi: 10.3389/fmicb.2022.840291

In vitro Activity of Antivirulence Drugs Targeting the *las* or *pqs* Quorum Sensing Against Cystic Fibrosis *Pseudomonas aeruginosa* Isolates

Diletta Collalto¹, Giulia Giallonardi^{2*}, Alessandra Fortuna¹, Carlo Meneghini¹, Ersilia Fiscarelli¹, Paolo Visca^{1,4}, Francesco Imperi^{1,4}, Giordano Rampioni^{1,4} and Livia Leoni^{1*}

Antivirulence agents targeting QS

Antibiofilm agents targeting c-di-GMP

Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Novel genetic tools to tackle c-di-GMP-dependent signalling in *Pseudomonas aeruginosa*

S. Vishnu Pawar¹, M. Messina¹, S. Rinaldo², F. Cutruzzolà², V. Kaever³, G. Rampioni¹ and L. Leoni¹

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Synthesis of Triazole-Linked Analogues of c-di-GMP and Their Interactions with Diguanylate Cyclase

Silvia Ferricola,^{†,‡} Ilaria Torquati,^{†,‡} Alessandro Paiardini,[§] Giorgio Giardina,[†] Giordano Rampioni,^{||} Marco Messina,^{||} Livia Leoni,^{||} Fabio Del Bello,[‡] Riccardo Petrelli,[‡] Serena Rinaldo,^{*,†} Loredana Cappellacci,^{†,‡} and Francesca Cutruzzolà^{†,‡}

AMERICAN SOCIETY FOR MICROBIOLOGY Journal of Bacteriology

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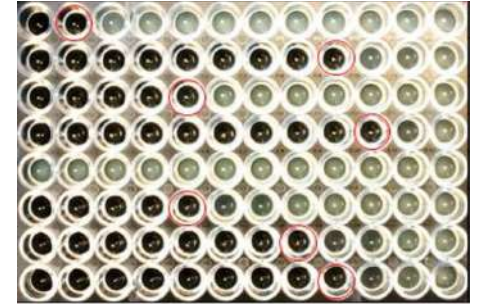
In Silico Discovery and *In Vitro* Validation of Catechol-Containing Sulfonohydrazide Compounds as Potent Inhibitors of the Diguanylate Cyclase PleD

Silvia Ferricola,^a Alessandro Paiardini,^b Giorgio Giardina,^a Giordano Rampioni,^c Livia Leoni,^c Francesca Cutruzzolà,^a and Serena Rinaldo^a*

Skills and expertise possibly useful to EUROSTOP

- ✓ **Antibiotic susceptibility assays**
- ✓ Selection and characterization of antibiotic-resistant mutants
- ✓ Generation of mutants by random transposon mutagenesis
- ✓ Generation of site-specific mutants
- ✓ Characterization of the mutants *via* transcriptomics and phenotypic analysis
- ✓ Protein purification and binding assays to DNA
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- ✓ Collection of > 100 *P. aeruginosa* cystic fibrosis isolates
- ✓ *Galleria mellonella* and lettuce leaf infection models
- ✓ Cellular infection models with lung epithelial cells

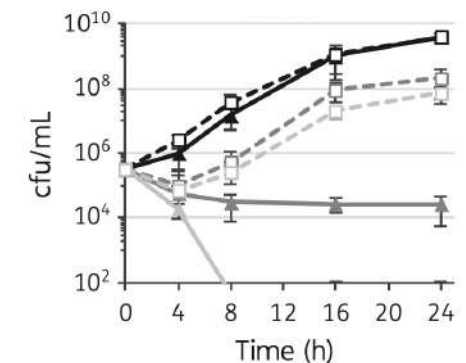
MIC assay



Kirby-Bauer assay

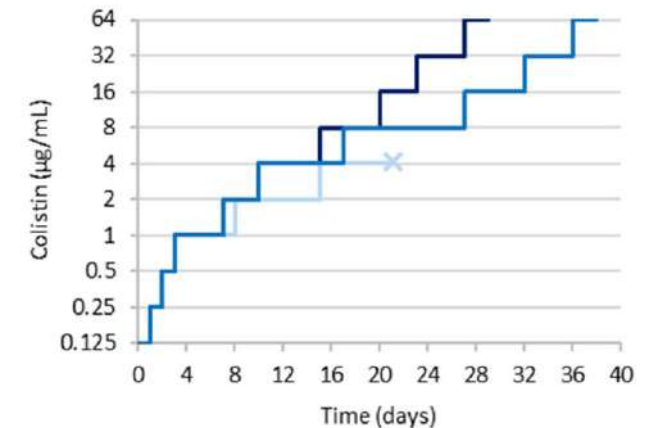
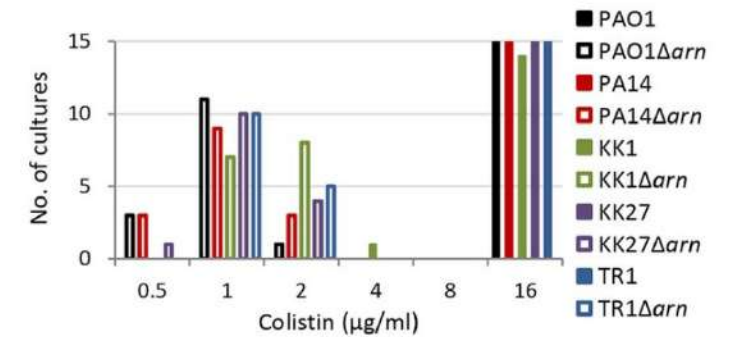
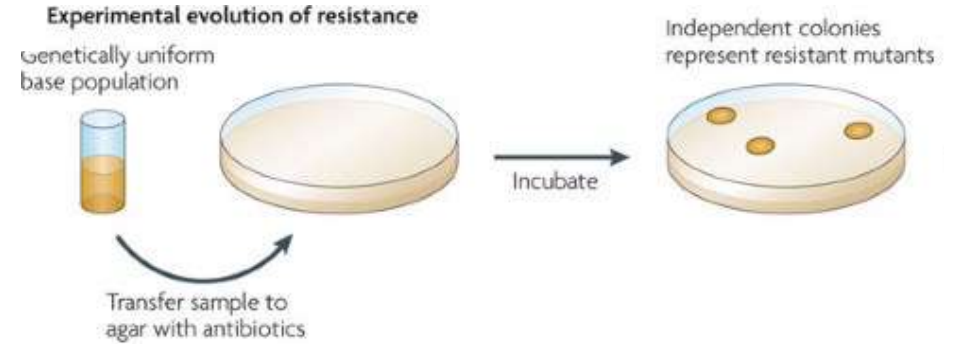


Time-kill assay



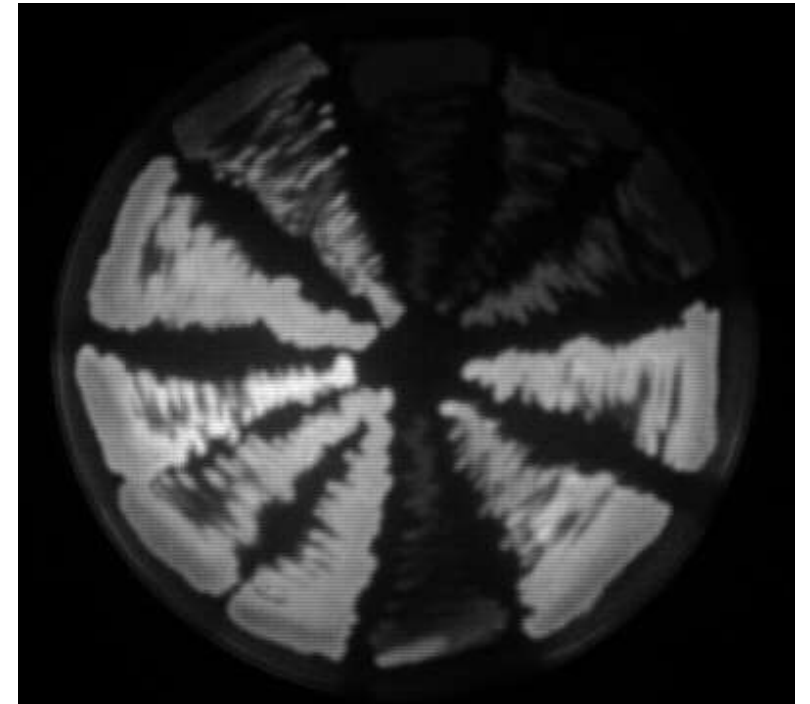
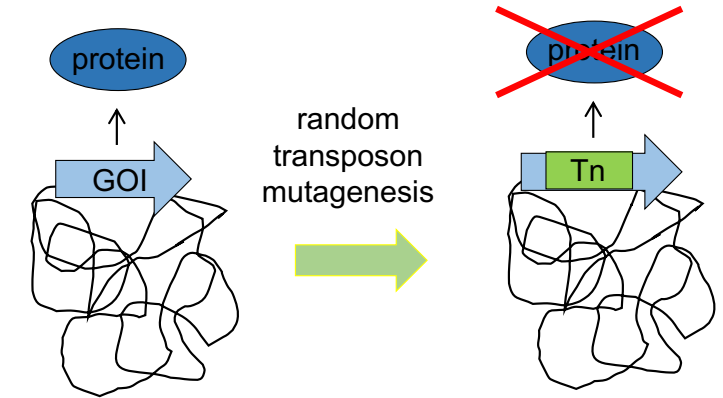
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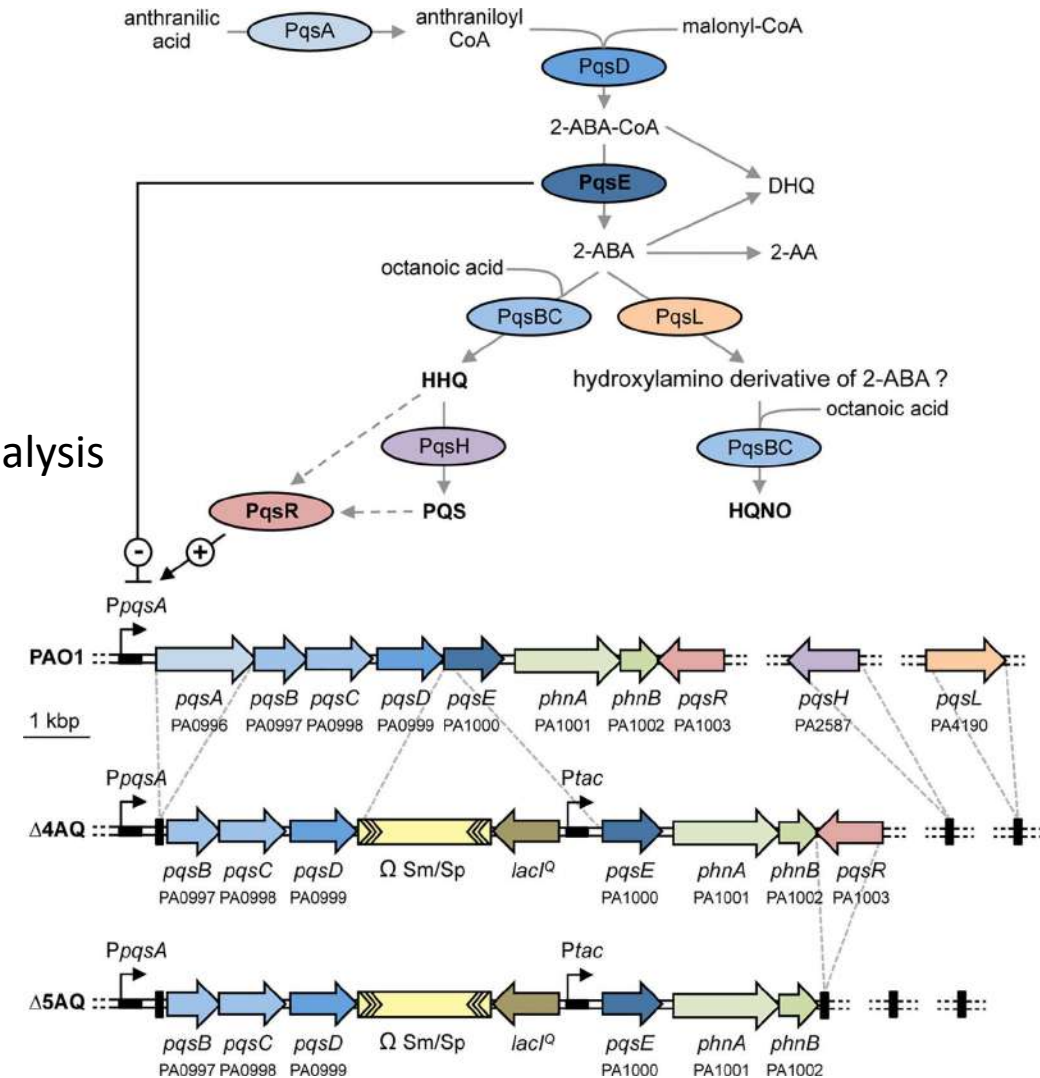
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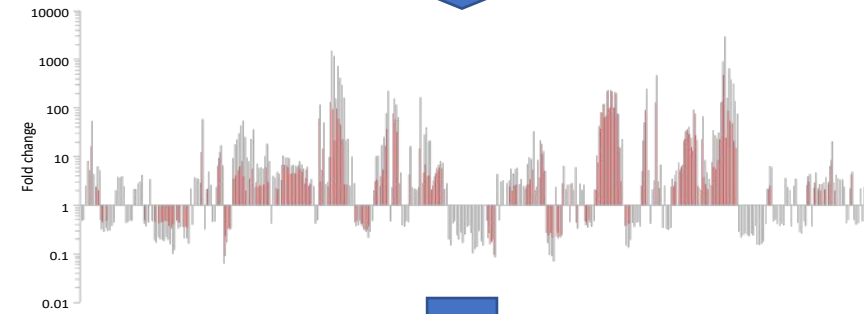
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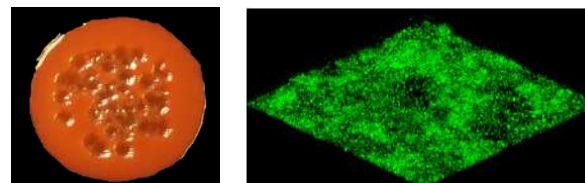
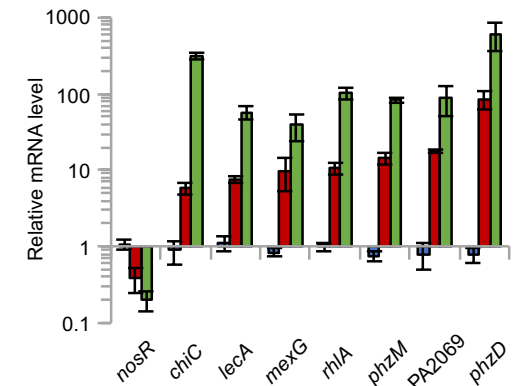


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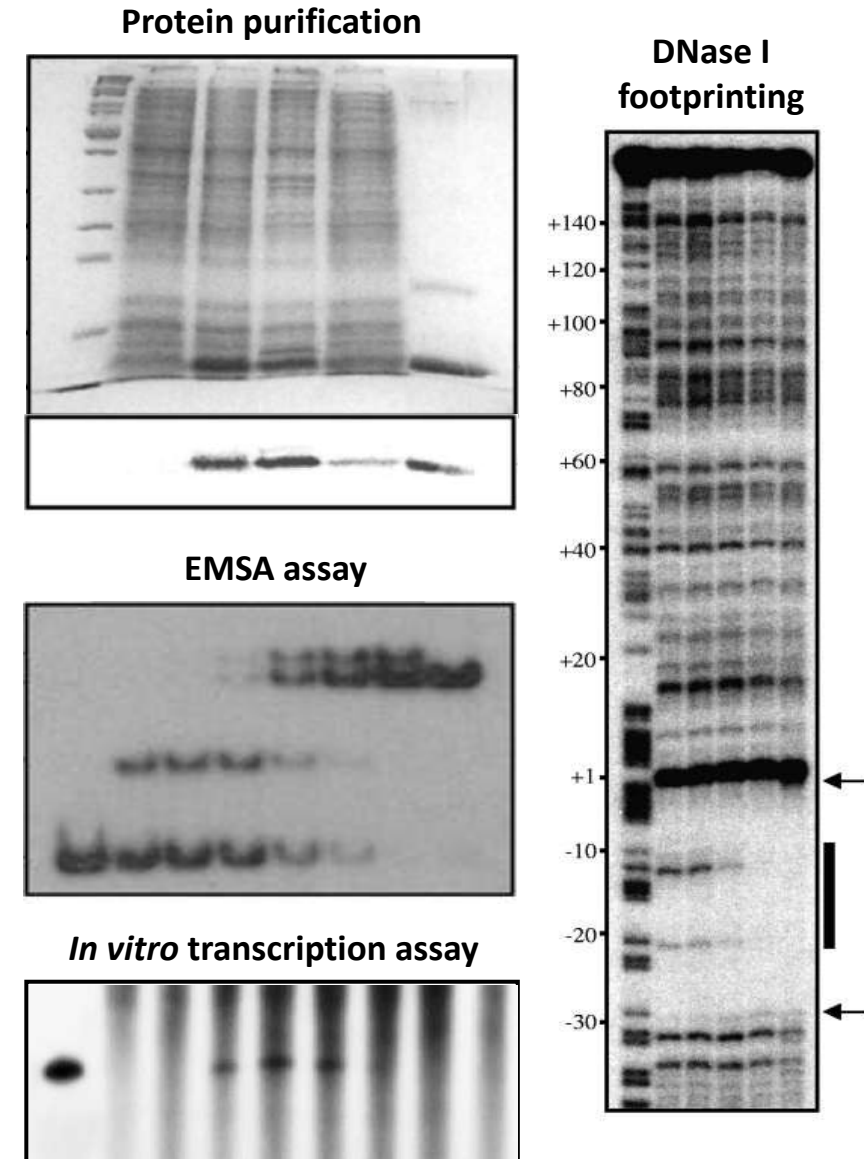


RT-qPCR



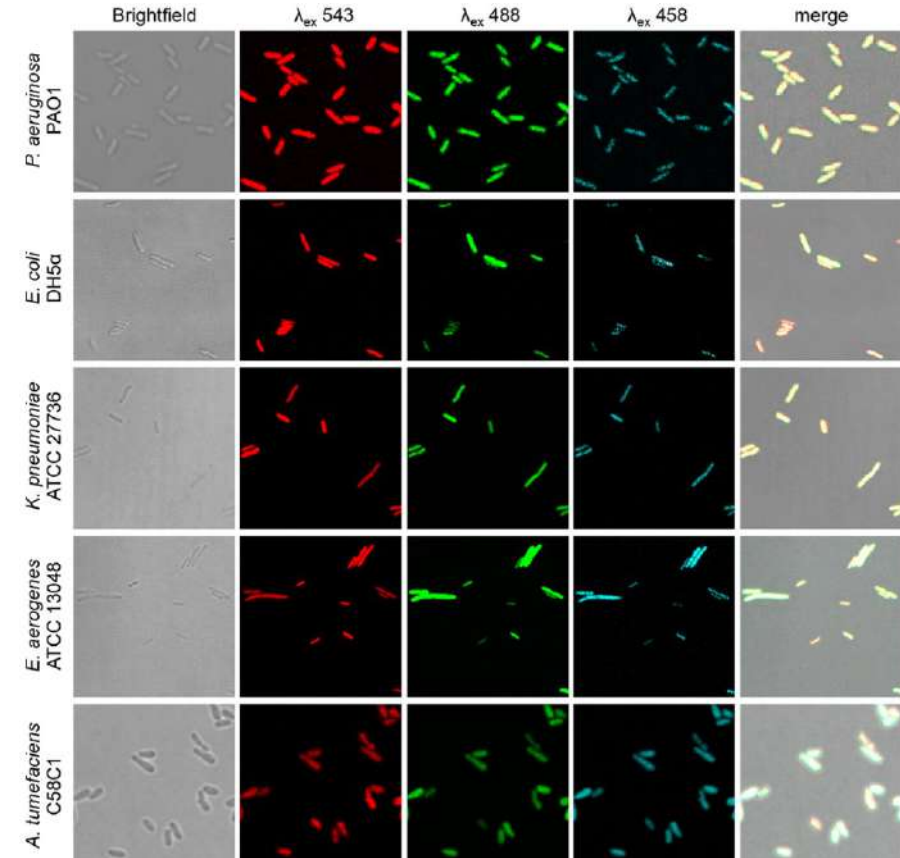
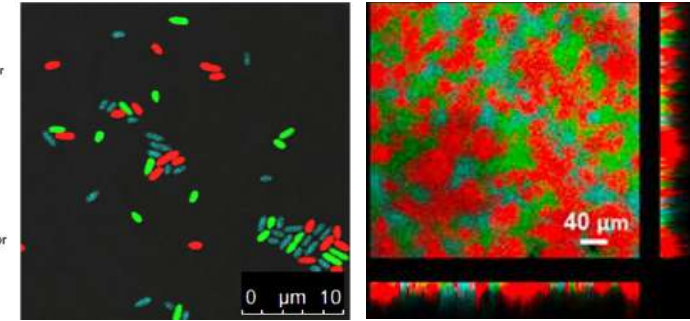
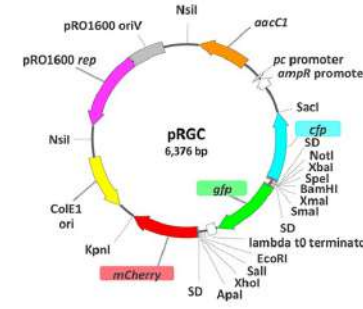
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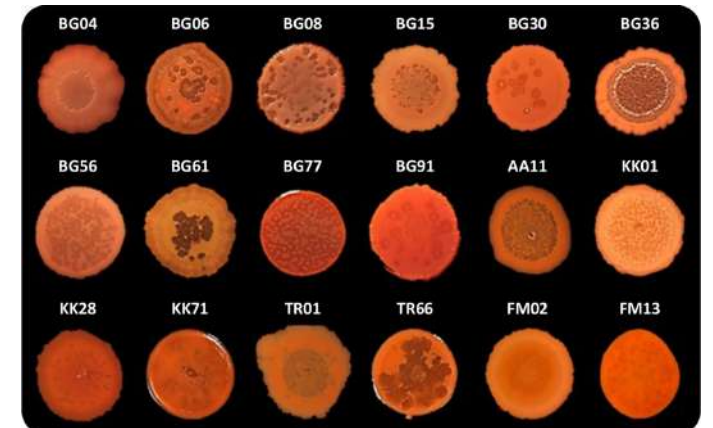
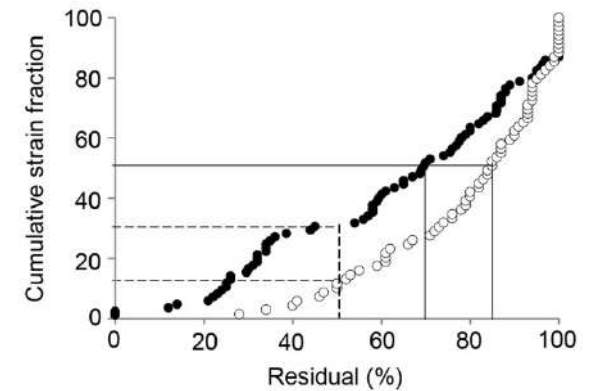
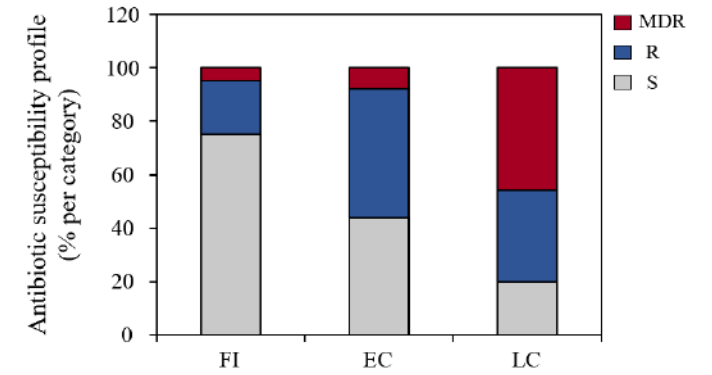
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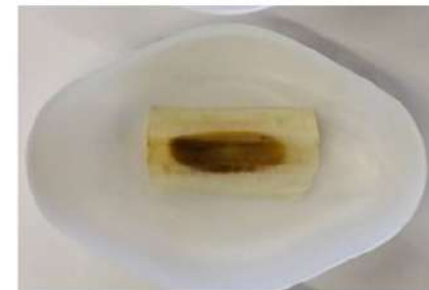
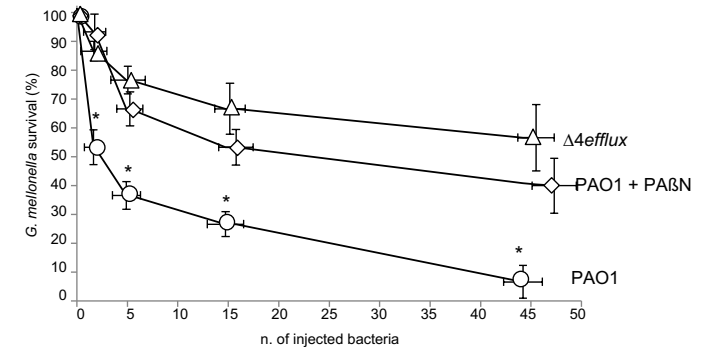
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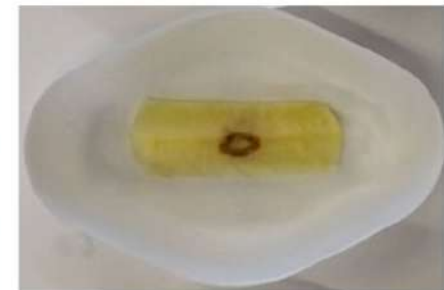


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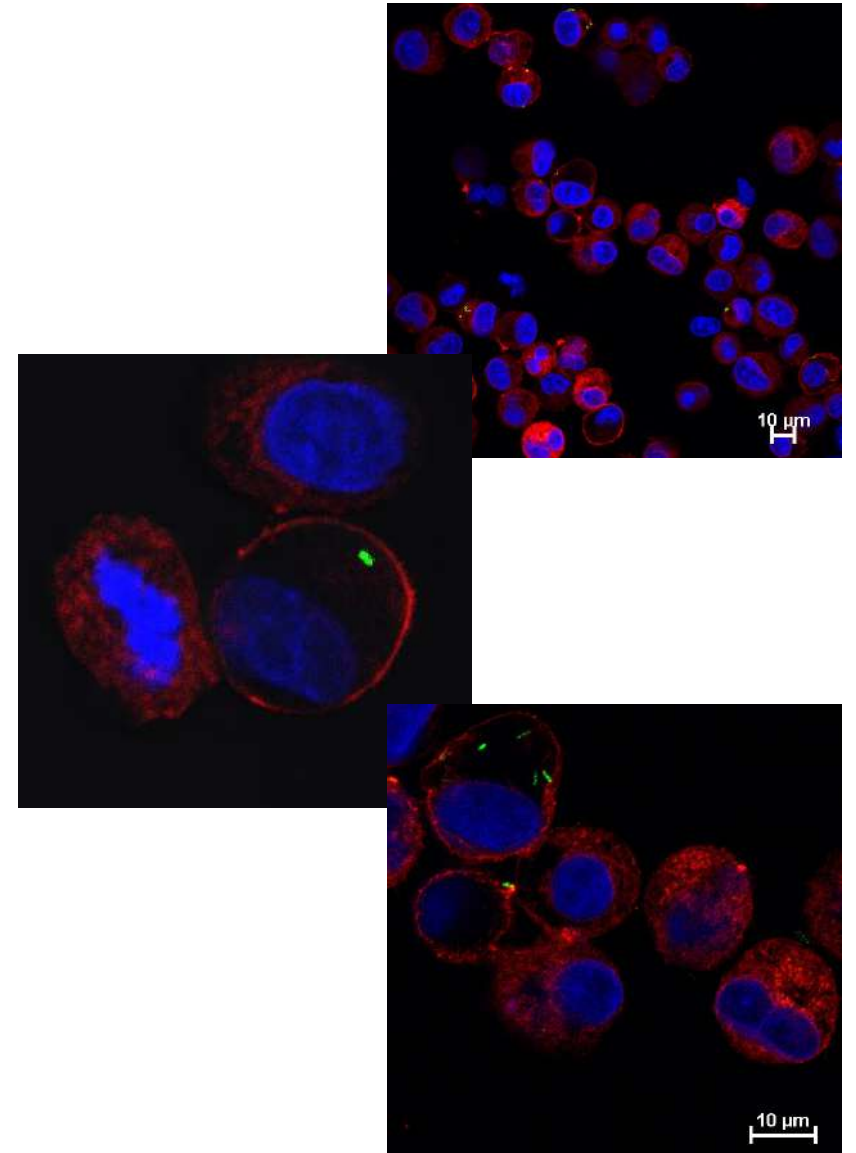
wild type(pME6032)



$\Delta dksA1$ (pME6032)

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Livia Leoni

Paolo Visca

Francesco Imperi

Fabio Polticelli

Elisabetta Zennaro

Emanuela Frangipani

Marta Mellini

Morgana Letizia

Alessandra Fortuna

Marco Messina

Valerio Baldelli

Francesca D'Angelo

Francesca Longo

Roslen Bondi

Marilena Falcone

Marco Grasso

Alessandro Zennaro



Vittorio Venturi

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**Oregon State
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Martin Schuster



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Funding



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Fibrosi Cistica - Onlus**
italian cystic fibrosis research foundation



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