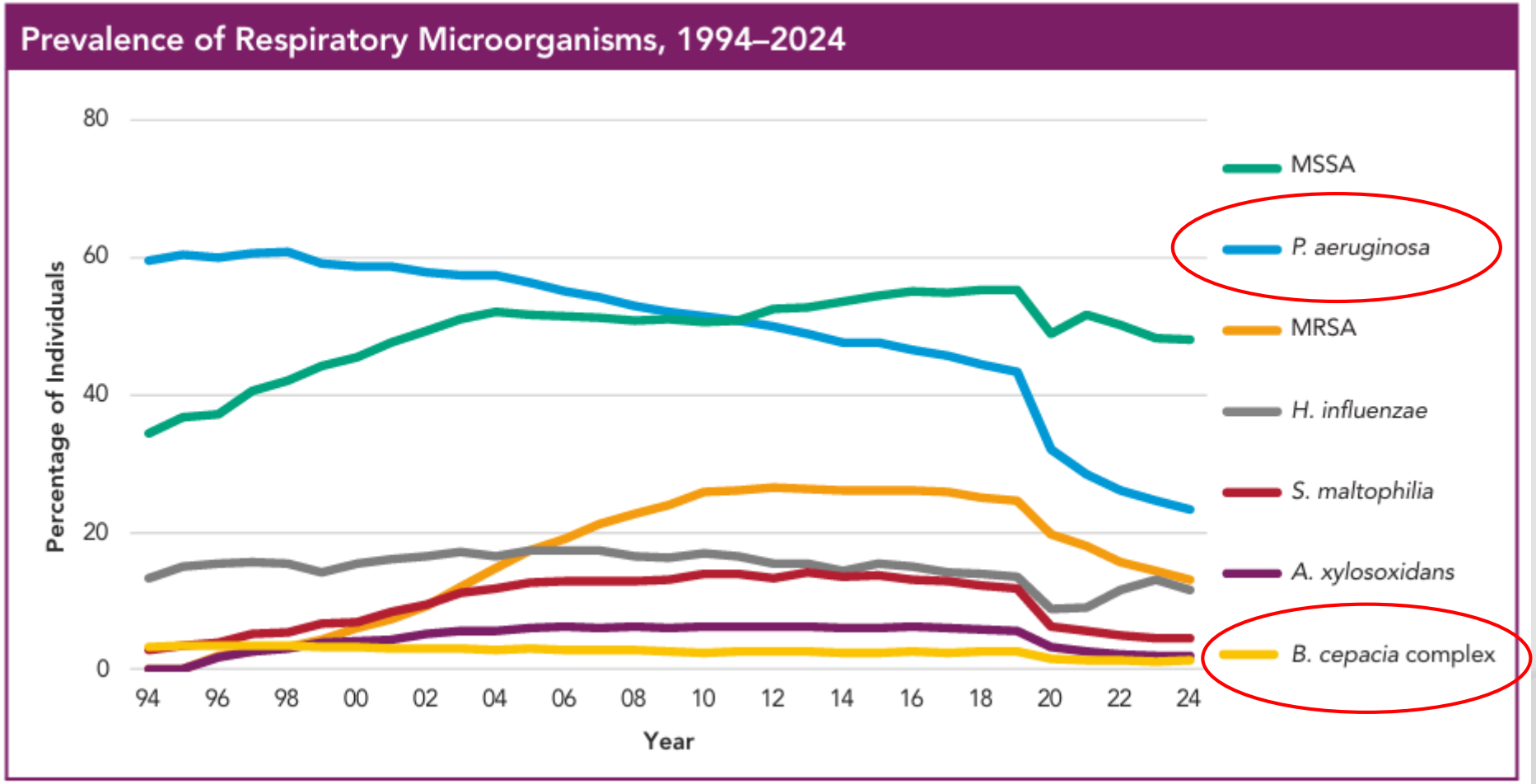
Nuovi approcci terapeutici contro le infezioni da batteri Gram-negativi e -positivi in fibrosi cistica

Silvia Buroni

silvia.buroni@unipv.it

5 Maggio 2026

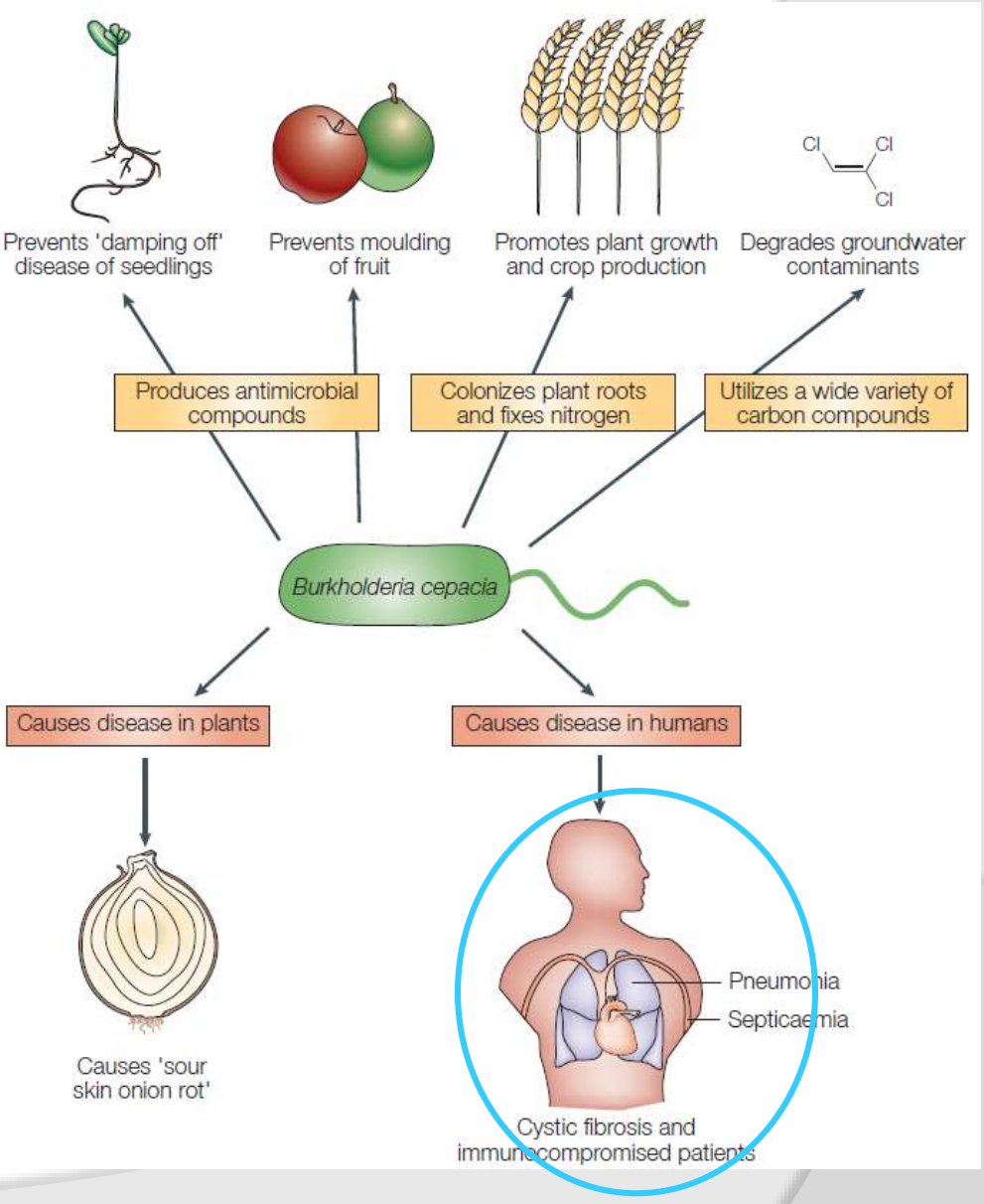
Infections among cystic fibrosis patients



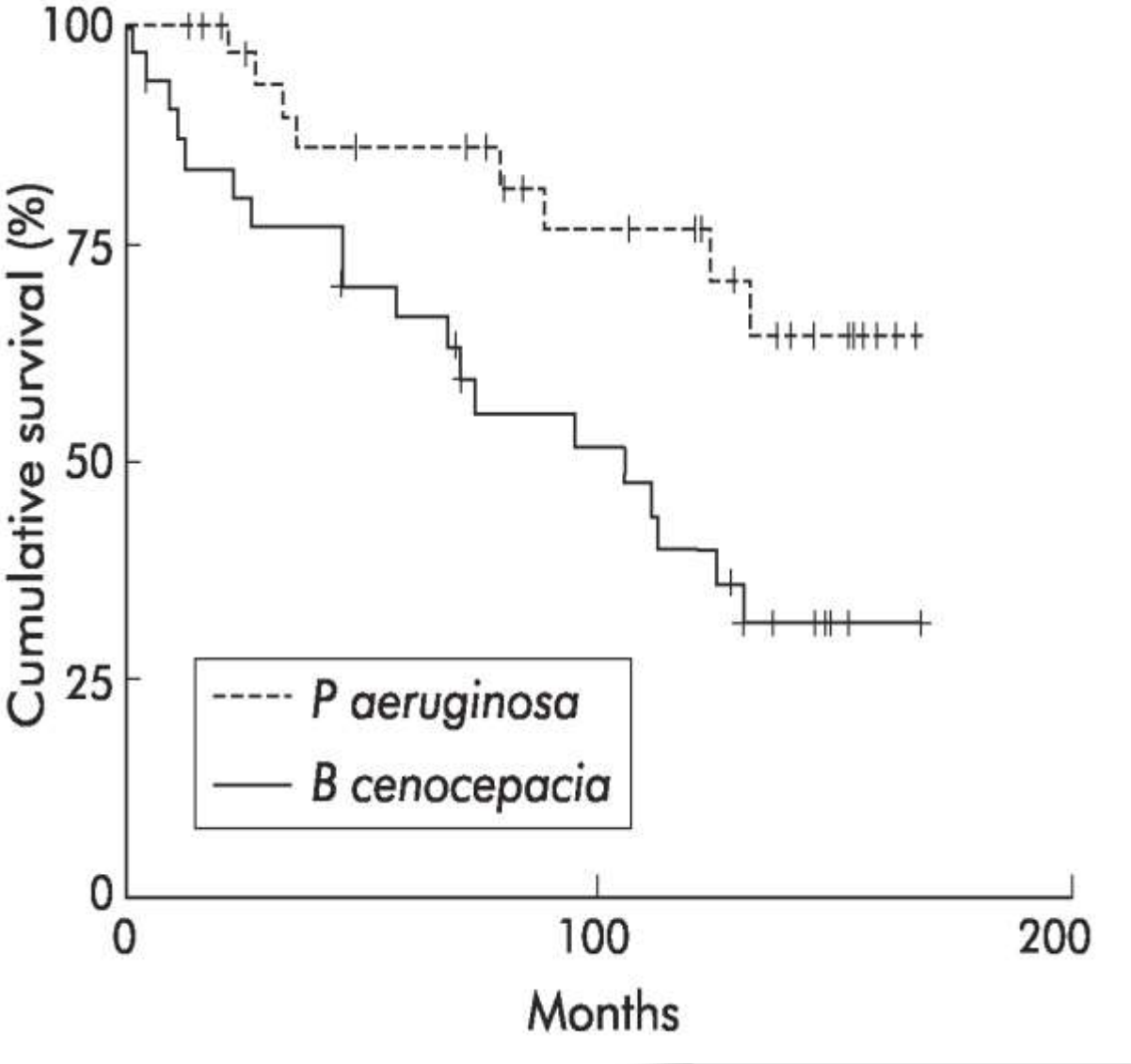
B. cenocepacia: 1-3% patients

Cystic Fibrosis Foundation, Patient Registry 2024 Annual Report

Burkholderia cenocepacia

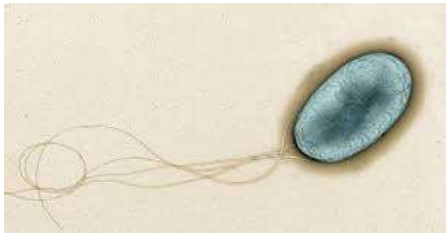


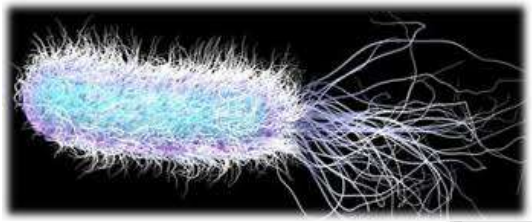
Mahenthiralingam *et al*., 2005. Nature Rev Microbiol 3:144-156



Jones *et al*. (2004), Thorax 59:948-951

Burkholderia vs Pseudomonas





- **Opportunistic** pathogens
- **Ubiquitously distributed** in nature (soil, water, plants and industrial settings)

(i) extraordinary **metabolic versatilities**

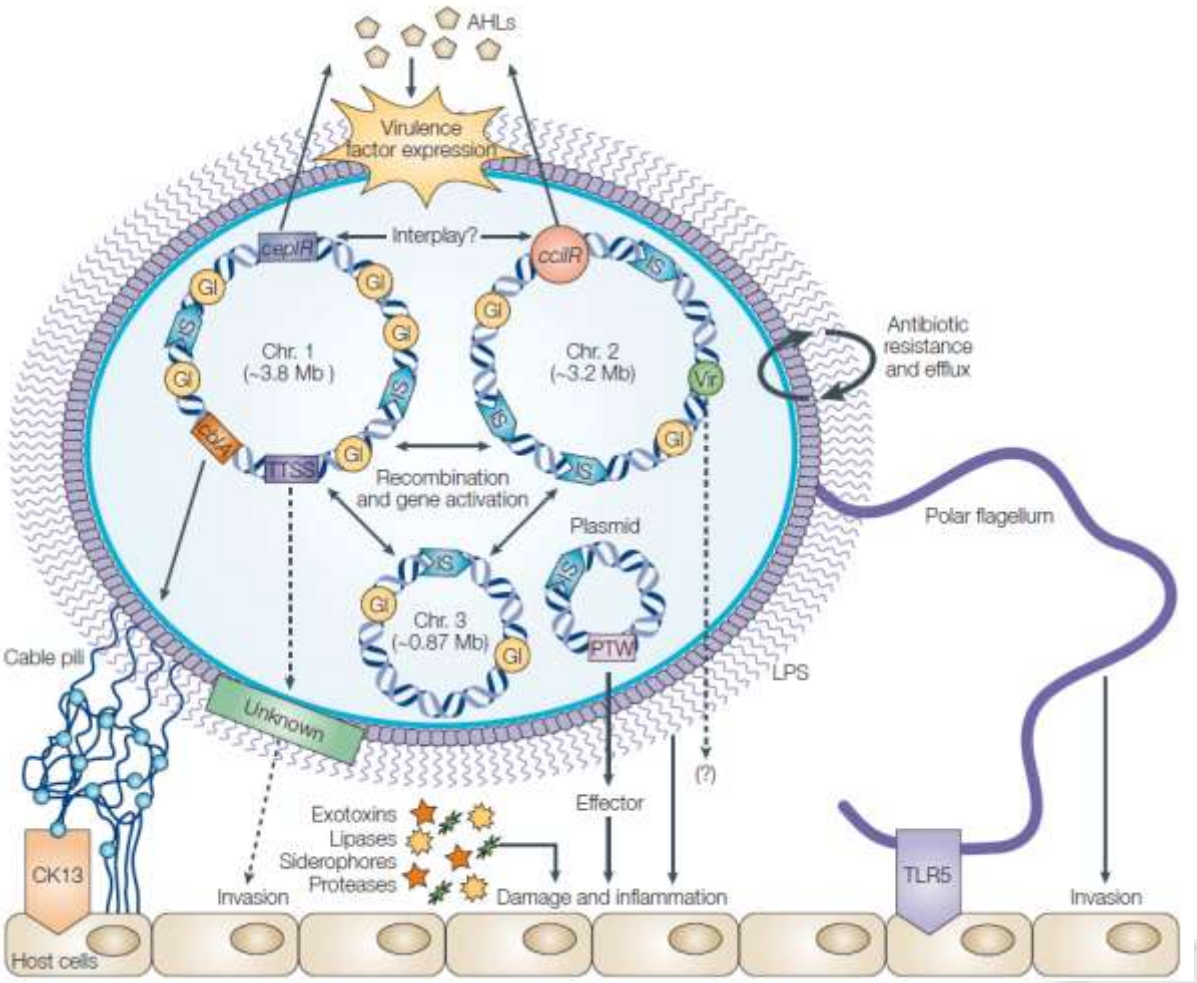
(ii) abilities to **interact with other bacteria** as well as with their eukaryotic hosts via signal molecules

(iii) to adapt their genetic repertoire to the needs of a particular niche by **acquiring genetic material** from other bacteria

- Frequent exchange of genetic material between *P. aeruginosa* an *B. cepacia* → bacteria colonize the same habitats

- *B. cepacia* is capable of perceiving the **QS signals** produced by *P. aeruginosa* but not *vice versa*

B. cenocepacia Genome Structure and principal Virulence Factors



1. Drug resistance;
2. Biofilm formation;
3. EPS synthesis;
4. Cable pili (adherence to mucin and epithelial cells);
5. Flagella (invasion);
6. Production of catalase and SOD (intracellular survival);
6. Secretion of lipases, proteases, hemolysins and siderophores;
7. Quorum sensing signals;
8. Secretion systems.

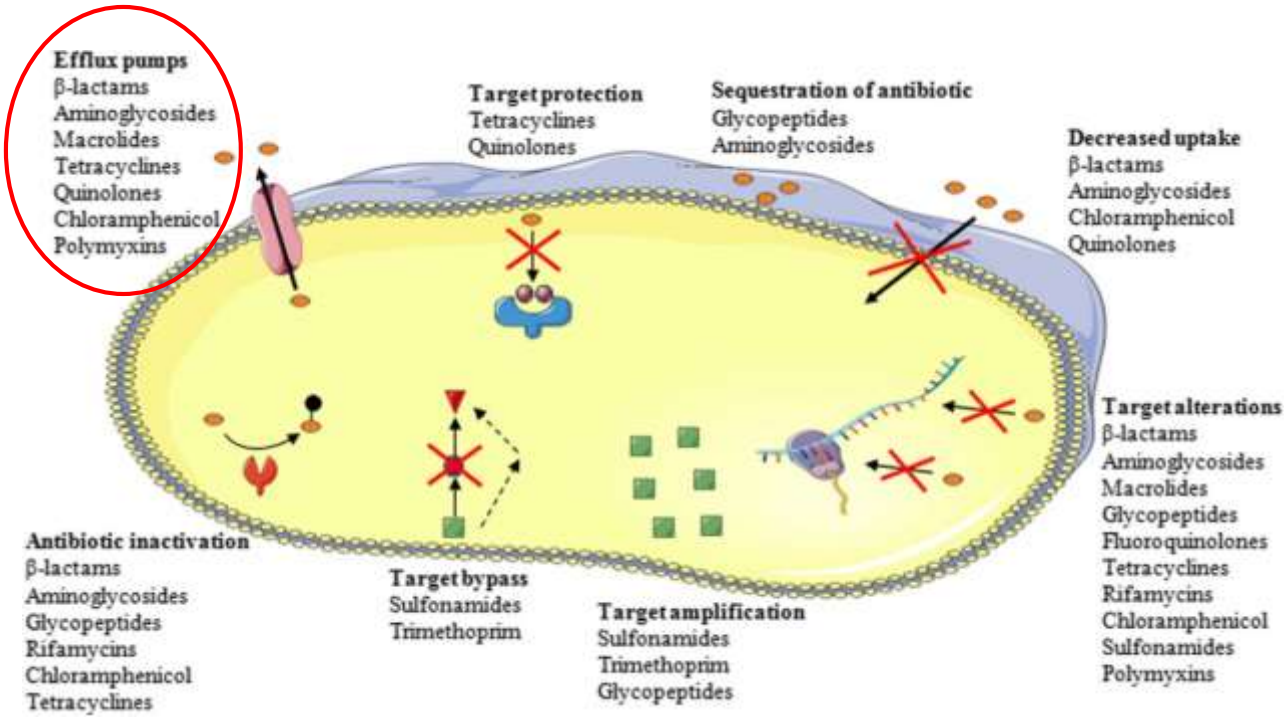
-GI= Genomic Islands

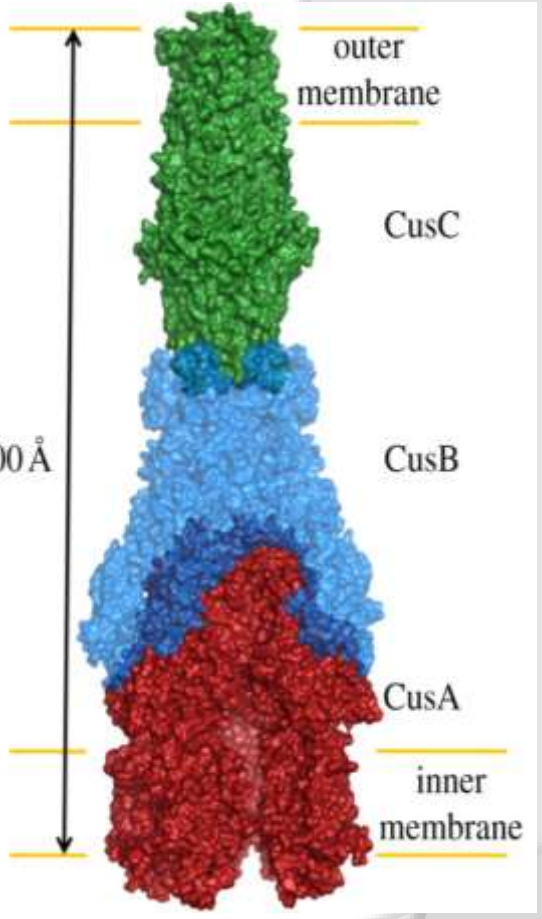
-IS= Insertion Sequence

Mahenthiralingam *et al.*, 2005

Drug Resistance Mechanisms

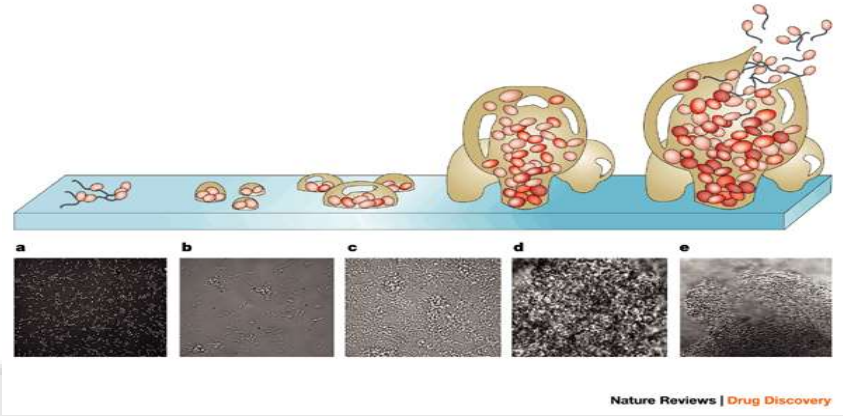
RND Efflux Pumps





Long *et al.*, 2012

Biofilm



Yılmaz and Özcengiz, 2017

Resistance mechanisms in *Burkholderia*

Modification of drug target site

- penicillin binding proteins for β-lactams (ΔPBP3 in *B. pseudomallei*);
- ribosomes for aminoglycosides, macrolides, tetracyclines, and chloramphenicol;
- DNA gyrase for fluoroquinolones (*gyrA* in *B. cepacia*);
- folic acid biosynthesis for trimethoprim and sulphonamides (*dhfr* in *B. cepacia*).

Enzymatic drug inactivation

- Overexpression of β-lactamases;
- Aminoglycoside-inactivating enzymes through N-acetylation
  O-nucleotidylation
  O-phosphorylation.

Reduced membrane permeability

- LPS structure→ aminoglycosides, polymyxins and cationic peptides;
- Porines→ chloramphenicol, trimethoprim, ciprofloxacin or β-lactams;
- bacterial capsule.

Bazzini *et al.* "*Burkholderia:* from genomes to function". Caistee Academic Press

Scoffone *et al.*, 2017. Front Microbiol 8:1592.

No standard eradication protocol

	Medication	Dosing	Frequency	Route
2 weeks	Tobramycin	10 mg/kg	daily	IV
	Ceftazidime	50 mg/kg	3 times daily	IV
	Temocillin	2 g	Twice daily	IV
3 months	Tobramycin	100-300 mg	Twice daily	Nebulised

Kitt *et al*. (2016) BMC Pharmacology and Toxicology 17:14

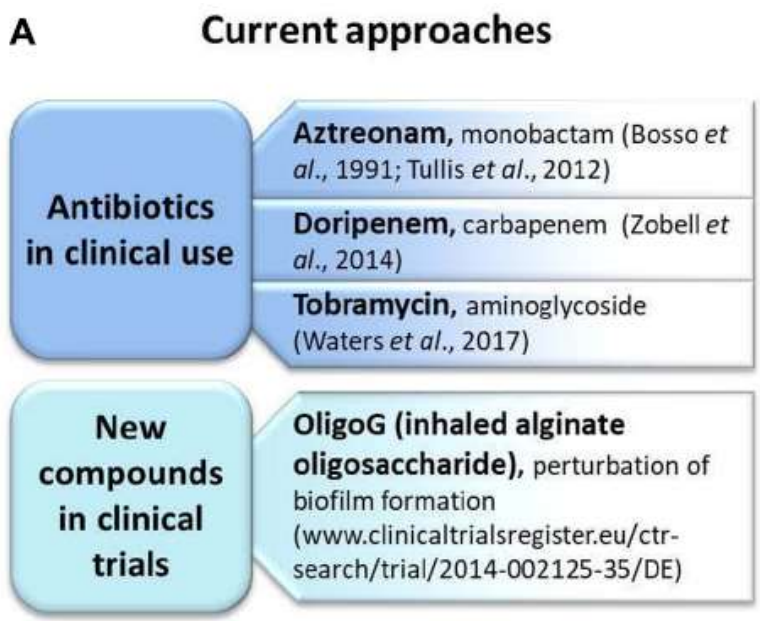
Table 1 *Burkholderia* Eradication Protocol

	Medication	Dosing	Frequency	Route	Bacteria Targeted
Induction Period (21 Days):	Tobramycin	6 mg/kg (per kinetics)	Daily	IV	*PsA, Bcc*
	Ceftazidime	2 g	Every 8 h	IV	*PsA, Bcc*
	Trimethoprim/Sulfamethoxazole	800/160 mg	Twice Daily	Oral	*Bcc*
	Tobramycin inhaled[a]	300 mg	Twice Daily	Nebulized	*PsA, Bcc*
	Azithromycin	250 mg	Daily	Oral	Anti-inflammatory
Consolidation Period (2 months):	Trimethoprim/Sulfamethoxazole	800/160 mg	Twice daily	Oral	*Bcc*
	Tobramycin inhaled[a]	300 mg	Twice daily	Nebulized	*PsA, Bcc*
	Azithromycin	250 mg	Daily	Oral	Anti-inflammatory

[a]Alternative- TIP (tobramycin dry powder for inhalation, 4 caps (28 mg/cap)) every 12 h

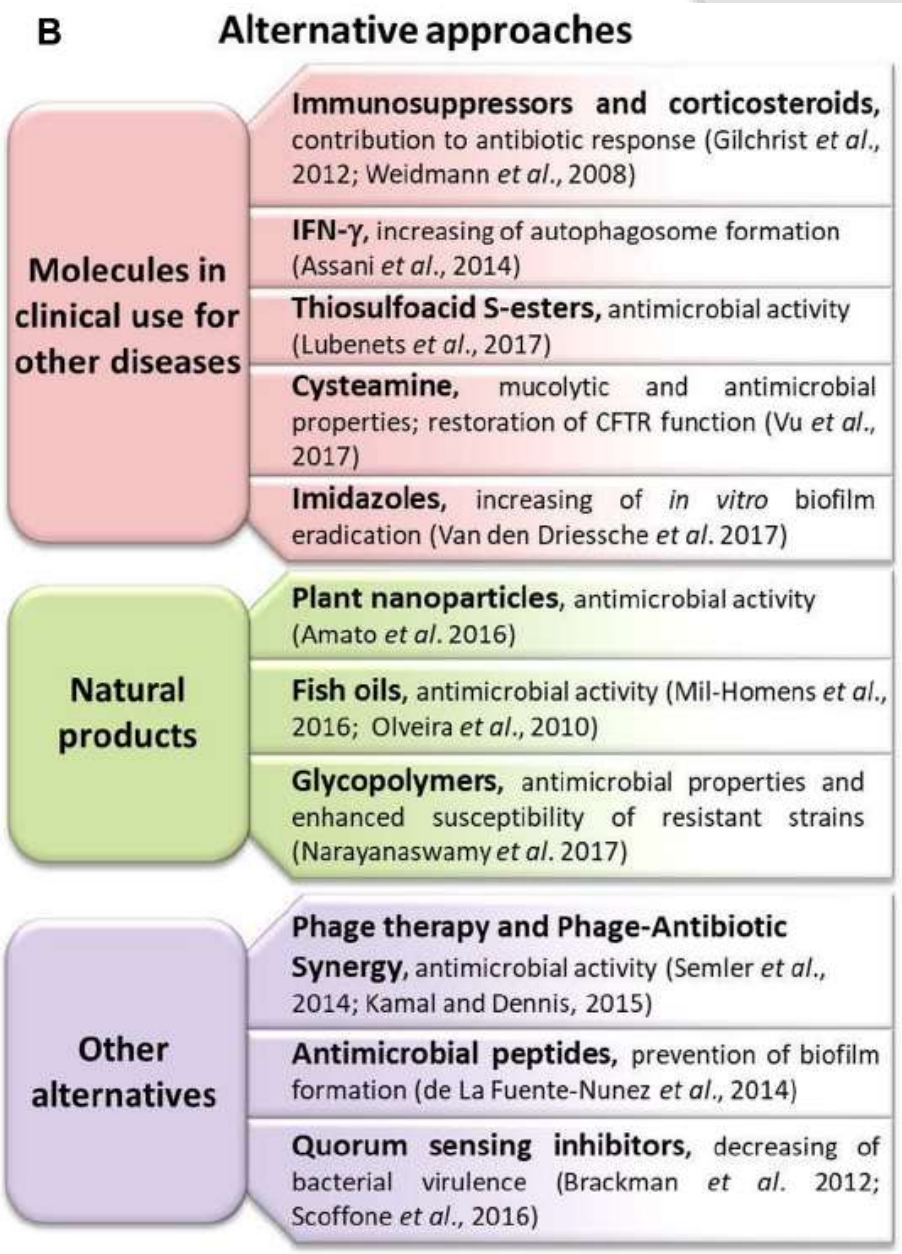
Garcia *et al*. (2018), BMC Pulmonary Medicine 18:35

Only few antibiotics are effective against *B. cenocepacia*

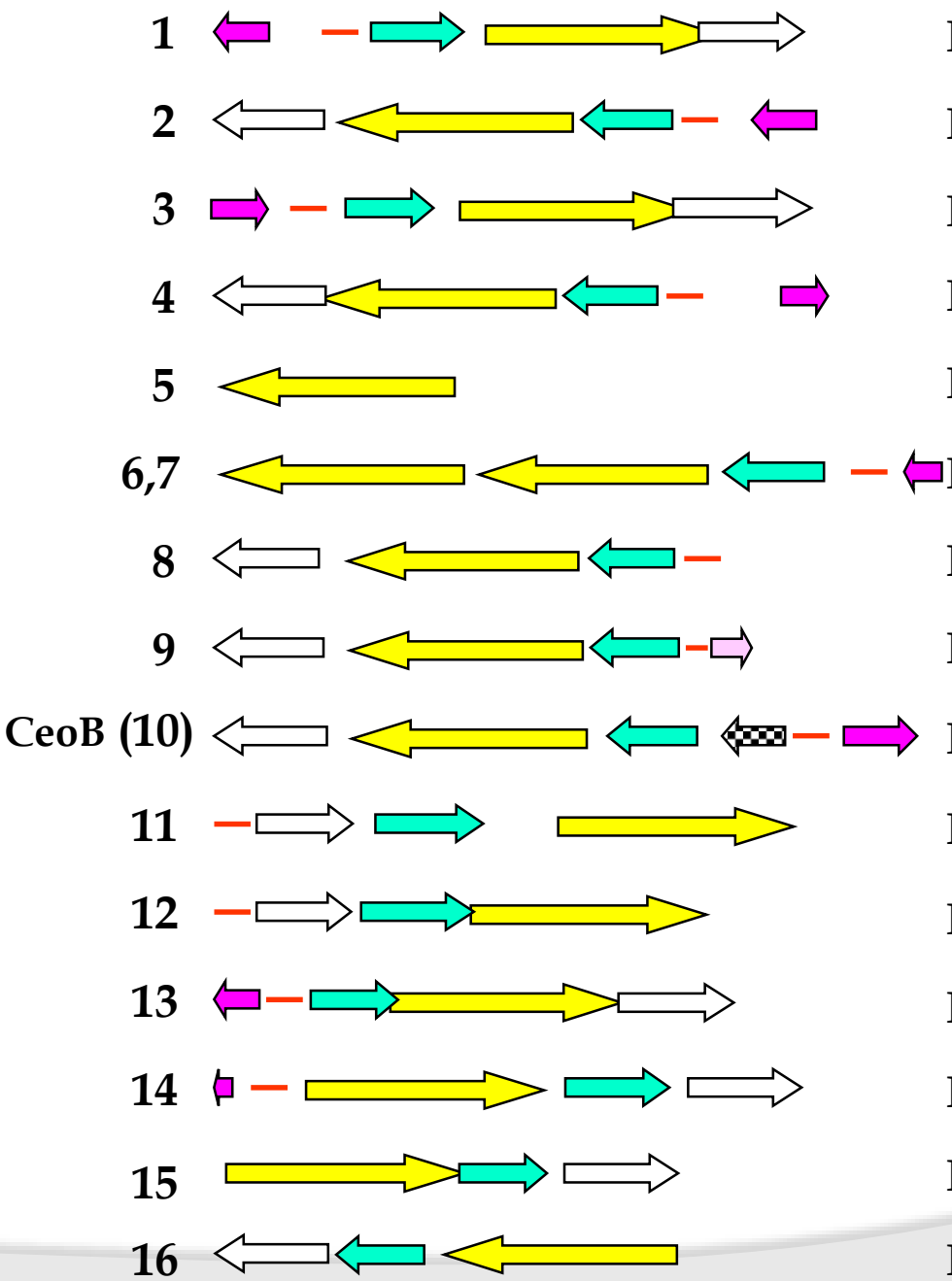


***Burkholderia cenocepacia* Infections in Cystic Fibrosis Patients: Drug Resistance and Therapeutic Approaches**

*Viola C. Scoffone[1], Laurent R. Chiarelli[1], Gabriele Trespidi[1], Massimo Mentasti[2,3], Giovanna Riccardi[1] and Silvia Buroni[1]**



RND efflux pumps in B. cenocepacia



BCAS0591-92-93	55% MexB
BCAS0764-65-66	63% MexB
BCAL1674-75-76	71% MexY
BCAL2820-21-22	64% MexB
BCAL1778	50% MexD
BCAL1079-80-81	
BCAM0925-26-27	56% MexD
BCAM1945-46-47	56% MexF
BCAM2549-50-51	64% MexF
BCAM0710-11-12	70% CzrA
BCAM0433-34-35	
BCAL1811-12-13	
BCAS0582-83-84	
BCAL2134-35-36	
BCAM1419-20-21	

Regulatory gene

Membrane Fusion Protein

Resistance Nodulation Division

Outer Membrane Protein

llpE gene

Promoter region

RND Efflux Pumps in *B. cenocepacia*

BMC Microbiology

BioMed Central

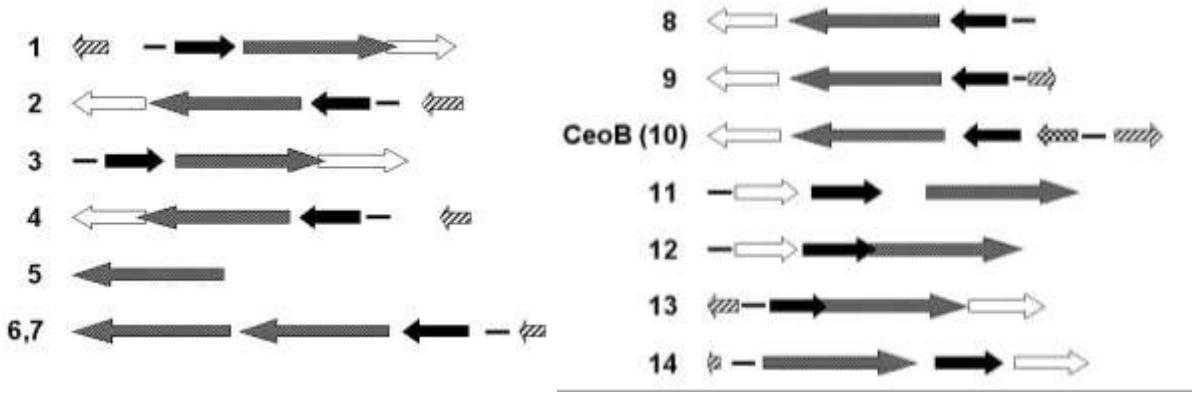
Research article

Open Access

Efflux pump genes of the resistance-nodulation-division family in *Burkholderia cenocepacia* genome

Paola Guglierame[†1], Maria Rosalia Pasca[†1], Edda De Rossi[1], Silvia Buroni[1], Patrizio Arrigo[2], Giulia Manina[1] and Giovanna Riccardi[*1]

→ RND operons identified in J2315



BMC Microbiology

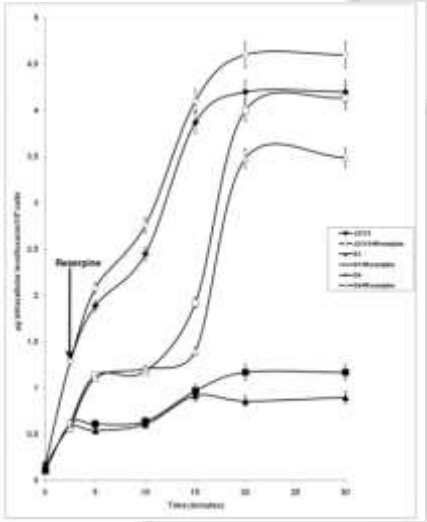
BioMed Central

Research article

Open Access

Assessment of three Resistance-Nodulation-Cell Division drug efflux transporters of *Burkholderia cenocepacia* in intrinsic antibiotic resistance

Silvia Buroni[*1], Maria R Pasca[1], Ronald S Flannagan[2], Silvia Bazzini[1], Anna Milano[1], Iris Bertani[3], Vittorio Venturi[3], Miguel A Valvano[2] and Giovanna Riccardi[1]



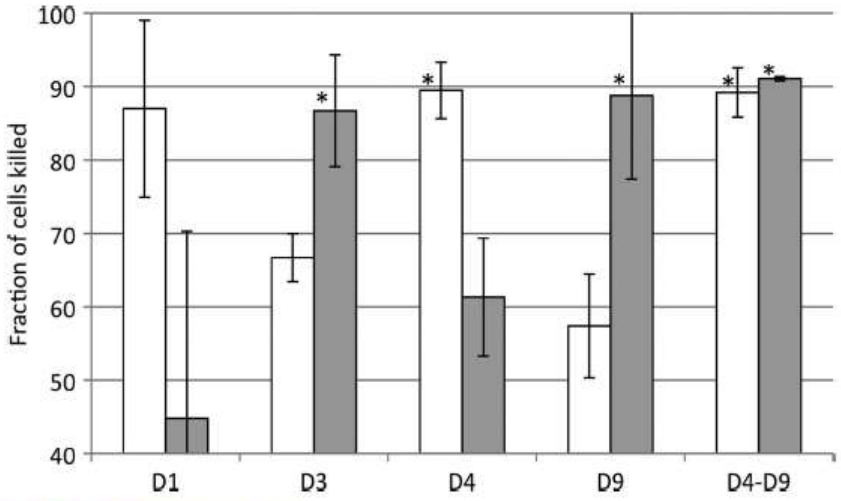
→ RND-4 plays a crucial role in the intrinsic resistance of *B. cenocepacia* to **Azt, Caf, EtBr, Genta, Tobra, Nal Ac, Cip, Levo, Norflo and Sparflo**

→ Transport of **QS** signal molecules

Antimicrobial Agents and Chemotherapy, May 2011, p. 1912–1919
0066-4804/11/$12.00 doi:10.1128/AAC.01571-10
Vol. 55, No. 5

Molecular Mechanisms of Chlorhexidine Tolerance in *Burkholderia cenocepacia* Biofilms

Tom Coenye,[1]* Heleen Van Acker,[1] Elke Peeters,[1] Andrea Sass,[2] Silvia Buroni,[3] Giovanna Riccardi,[3] and Eshwar Mahenthiralingam[2]



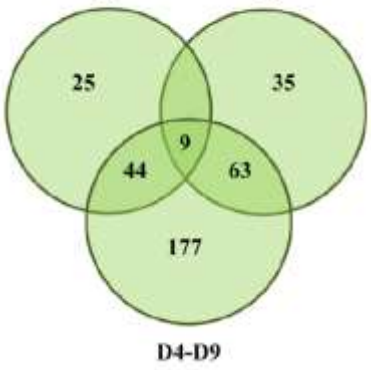
PLoS one

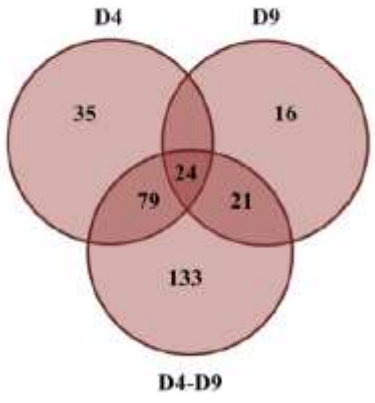
RND-3 and RND-9 → **chlorhexidine** tolerance for biofilm cells
RND-4 → chlorhexidine tolerance for planktonic cells

Deciphering the Role of RND Efflux Transporters in *Burkholderia cenocepacia*

Silvia Bazzini[1☯], Claudia Udine[1☯], Andrea Sass[2☯], Maria Rosalia Pasca[1], Francesca Longo[3], Giovanni Emiliani[4], Marco Fondi[5], Elena Perrin[5], Francesca Decorosi[6], Carlo Viti[6], Luciana Giovannetti[6], Livia Leoni[3], Renato Fani[5], Giovanna Riccardi[1], Eshwar Mahenthiralingam[2], Silvia Buroni[1]*

transcriptome analysis of ΔRND-4 and ΔRND-9 → **wider role than drug resistance**





Motility
Chemotaxis
Biofilm production

AAC
Journals.ASM.org

Differential Roles of RND Efflux Pumps in Antimicrobial Drug Resistance of Sessile and Planktonic *Burkholderia cenocepacia* Cells

Silvia Buroni,[a] Nele Matthijs,[b] Francesca Spadaro,[a] Heleen Van Acker,[b] Viola C. Scoffone,[a] Maria Rosalia Pasca,[a] Giovanna Riccardi,[a] Tom Coenye[b]

16 RND knock-out mutants

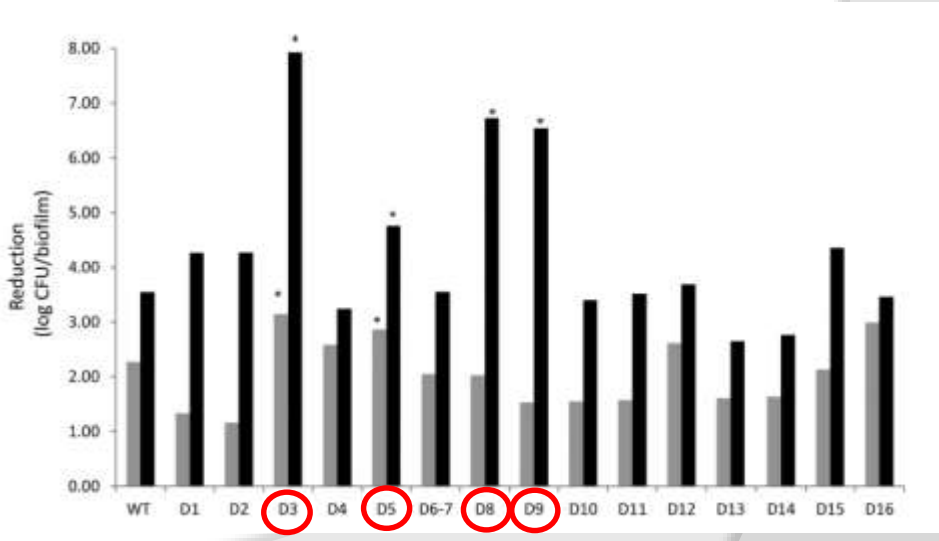
RND-3 and **RND-4** involved in resistance of planktonic cells

RND-3, RND-5, RND-8 and RND-9 involved in resistance of sessile cells

TABLE 2 MICs of the various antibiotics for some of the *B. cenocepacia* strains tested

Strain	MIC (μg/ml) for[a]:				
	CIP	TOB	MIN	MER	CHL
J2315 (WT)	8	256	16	64	32
D3	2	2	—[b]	32	16
D4	2	128	4	64	8
D16	8	256	4	32	16

[a] CIP, ciprofloxacin; TOB, tobramycin; MIN, minocycline; MER, meropenem; CHL, chloramphenicol.
[b] —, no clear minocycline breakpoint was observed for this mutant.



Strategies to fight antibiotic resistance

Essential Proteins

- **Antibacterial compounds for *B. cenocepacia*:**
  C109

Virulence Factors

- **4 antivirulence compounds**
  Diketopiperazines

Vaccines

Adjuvants

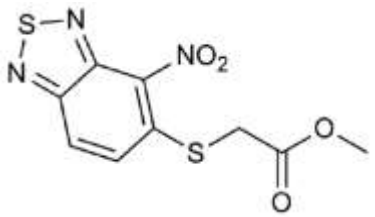
From drug to target: screening of new compounds to find new drugs effective against *Burkholderia*

8000 compounds from AstraZeneca company with unknown chemical structure and mechanism of action. **Resistant**

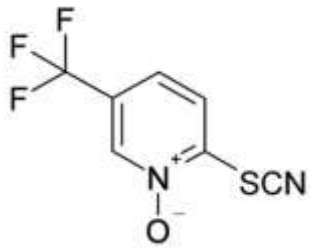
150 antitubercular compounds from Prof. M. Baltas (CNRs, Toulose). **Resistant**

100 new compounds from Dr. Vadim Makarov

One new compound for *B. cenocepacia*
MIC = 8 μg/ml



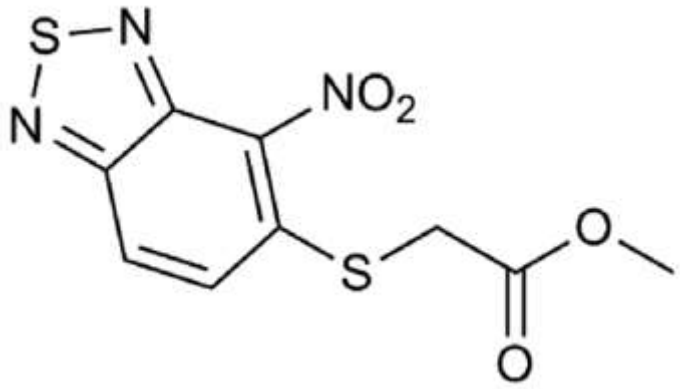
10126109



One new compound for *B. cenocepacia*
MIC = 16 μg/ml

11026103

C109: a benzothiadiazol derivative active against *B. cenocepacia*



It is a **bactericidal** compound

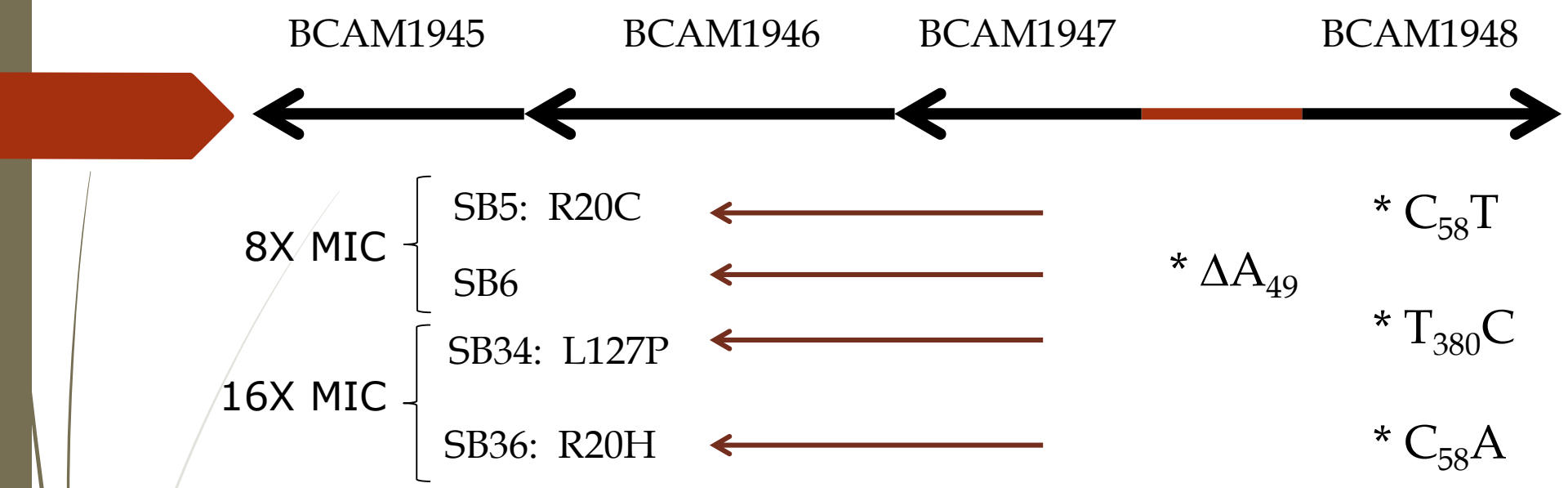
MIC = 8 μg/ml

TC_{50} on human CF cells = 75 μM

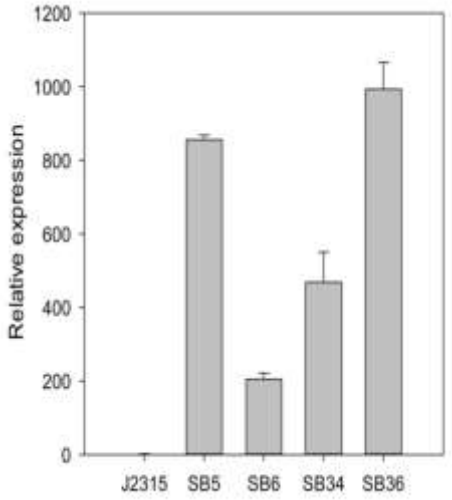
C109 is effective against the *Burkholderia cepacia* complex strains, more than 50 clinical isolates ...

…other Gram negatives and *S. aureus* (including MRSA strains)

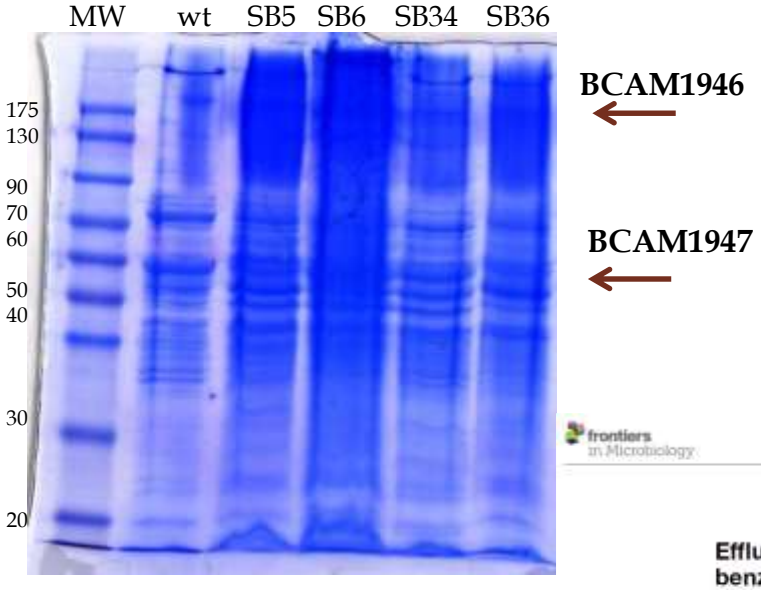
Strain	MIC 10126109 (μg/ml)
E. coli ATCC 25922	**8**
A. baumannii ATCC 19606	**16**
K. pneumoniae ATCC 13883	**64**
P. aeruginosa PAO1	**256**
S. aureus ATCC 25923	**4**



qRT-PCR



Membrane Fraction Extraction



frontiers in Microbiology

Efflux-mediated resistance to a benzothiadiazol derivative effective against *Burkholderia cenocepacia*

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

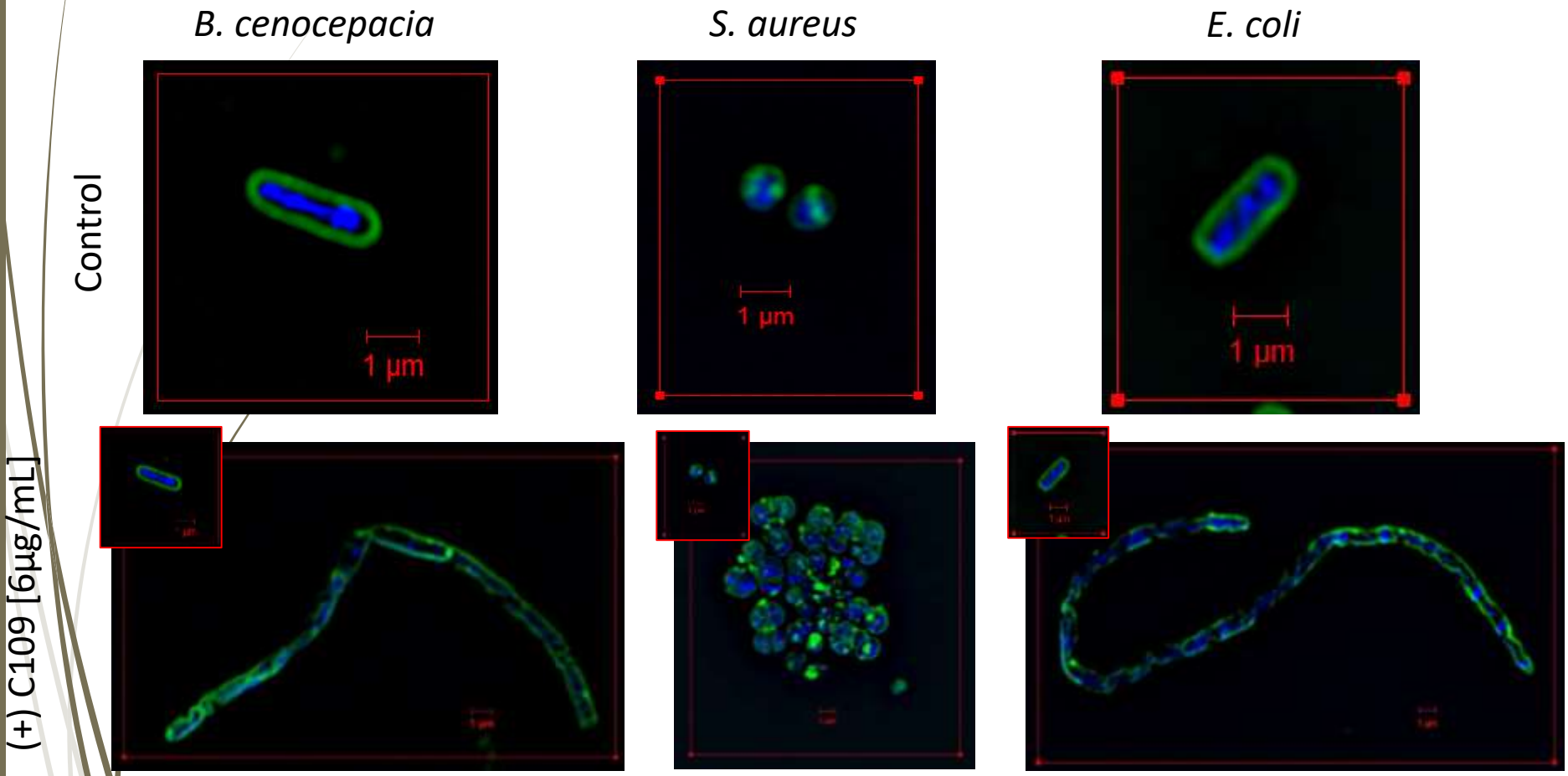
Competitive Fitness of Essential Gene Knockdowns Reveals a Broad-Spectrum Antibacterial Inhibitor of the Cell Division Protein FtsZ

Andrew M. Hogan,[a] Viola C. Scoffone,[b] Vadim Makarov,[c] April S. Gislason,[a] Haben Tesfu,[a] Maria S. Stietz,[a] Ann Karen C. Brassinga,[a] Michael Domaratzki,[d] Xuan Li,[e] Alberto Azzalin,[b,f] Marco Biggiogera,[b] Olga Riabova,[c] Natalia Monakhova,[c] Laurent R. Chiarelli,[b] Giovanna Riccardi,[b] Silvia Buroni,[b] Silvia T. Cardona[a,g]

High-density **transposon mutant library** in *B. cenocepacia* K56-2 by delivering a **transposon element** containing an outward tightly regulated rhamnose-inducible promoter (**P*rhaB***)

Essential genes identified by transposon-based delivery of P*rhaB* throughout the bacterial chromosome followed by screening for **absence or growth without rhamnose**

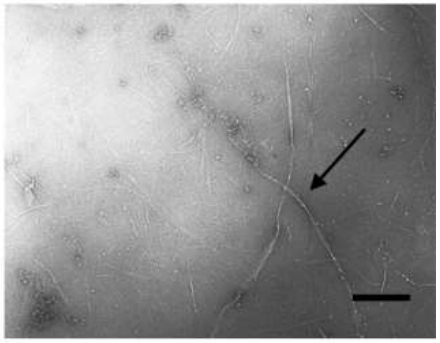
Phenotypic effect of C109 treatment: 3D super resolution microscopy



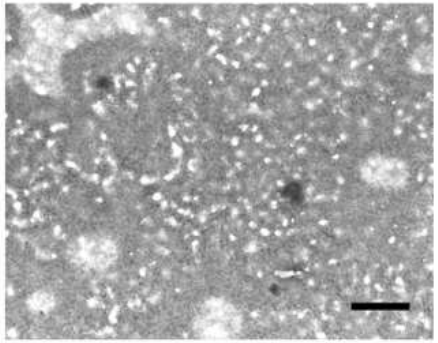
Identification of the cellular target

FtsZ polymers visualized by TEM

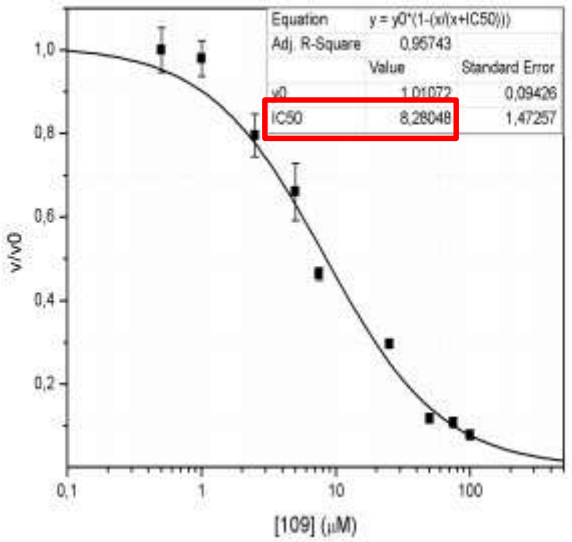
C109 -
GTP +
GDP -



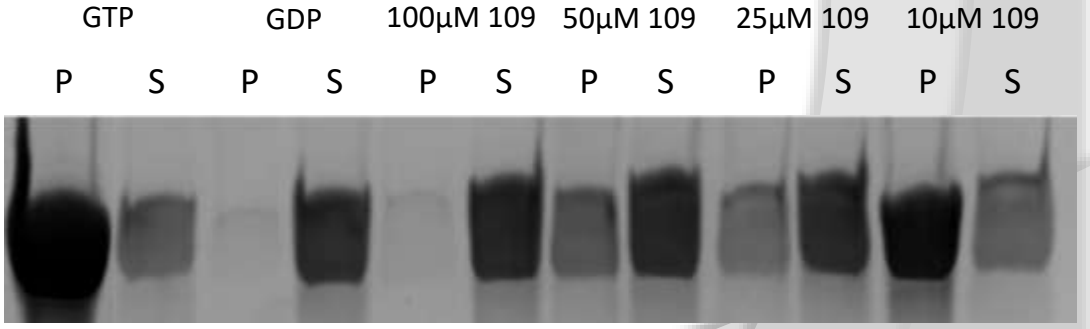
C109 100 µM
GTP +
GDP -



FtsZ GTPase spectrophotometric assay



FtsZ polymerization assay



FtsZ is **C109** cellular target


Antimicrobial Agents and Chemotherapy

MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

Competitive Fitness of Essential Gene Knockdowns Reveals a Broad-Spectrum Antibacterial Inhibitor of the Cell Division Protein FtsZ

Andrew M. Hogan, Viola C. Scoffone, Vadim Makarov, April S. Gislason, Haben Tesfu, Maria S. Stietz, Ann Karen C. Brassinga, Michael Domaratzki, Xuan Li, Alberto Azzalin, Marco Biggiogera, Olga Riabova, Natalia Monakhova, Laurent R. Chiarelli, Giovanna Riccardi, Silvia Buroni, Silvia T. Cardona

P. aeruginosa

A. MICC109+MC207,110

MC207,110 μg/ml

128 64 32 16 8 4 2 0

109 μg/ml 0 0.125 0.25 0.5 1 2 4 8 16 32 64 128

$$\frac{\text{MIC207comb}}{\text{MIC207alone}} + \frac{\text{MICC109comb}}{\text{MICC109alone}} =$$

$$\frac{32}{256} + \frac{1}{256} = 0.125+0.004 = 0.129 < 0.5$$

SYNERGY

The efflux pump inhibitor MC207,110 enhances C109 activity against *P. aeruginosa*

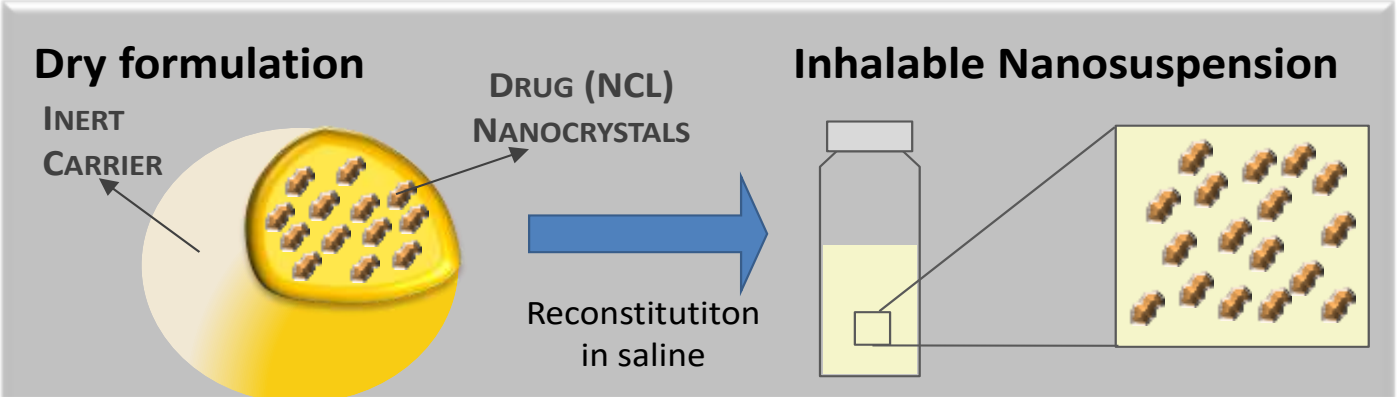
B. FtsZ purification and enzymatic assay

$FtsZ_{PAO}$ has a 53% aa identity with $FtsZ_{J2315}$ ⟹ C109 is effective against the $FtsZ_{PAO}$ purified protein

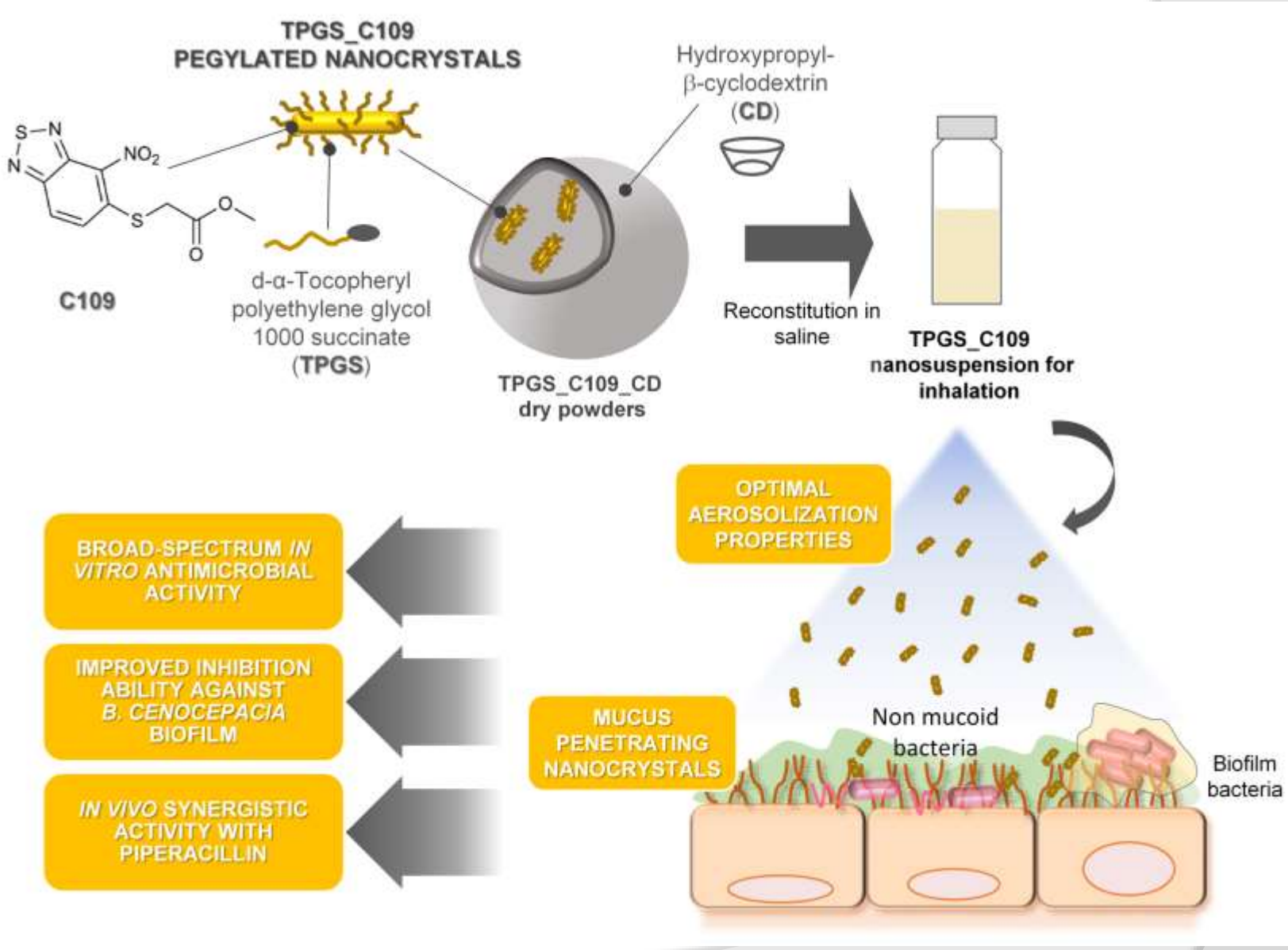
IC50 = 5 μM

FtsZ is C109 cellular target
In *P. aeruginosa* C109 is extruded by efflux pumps

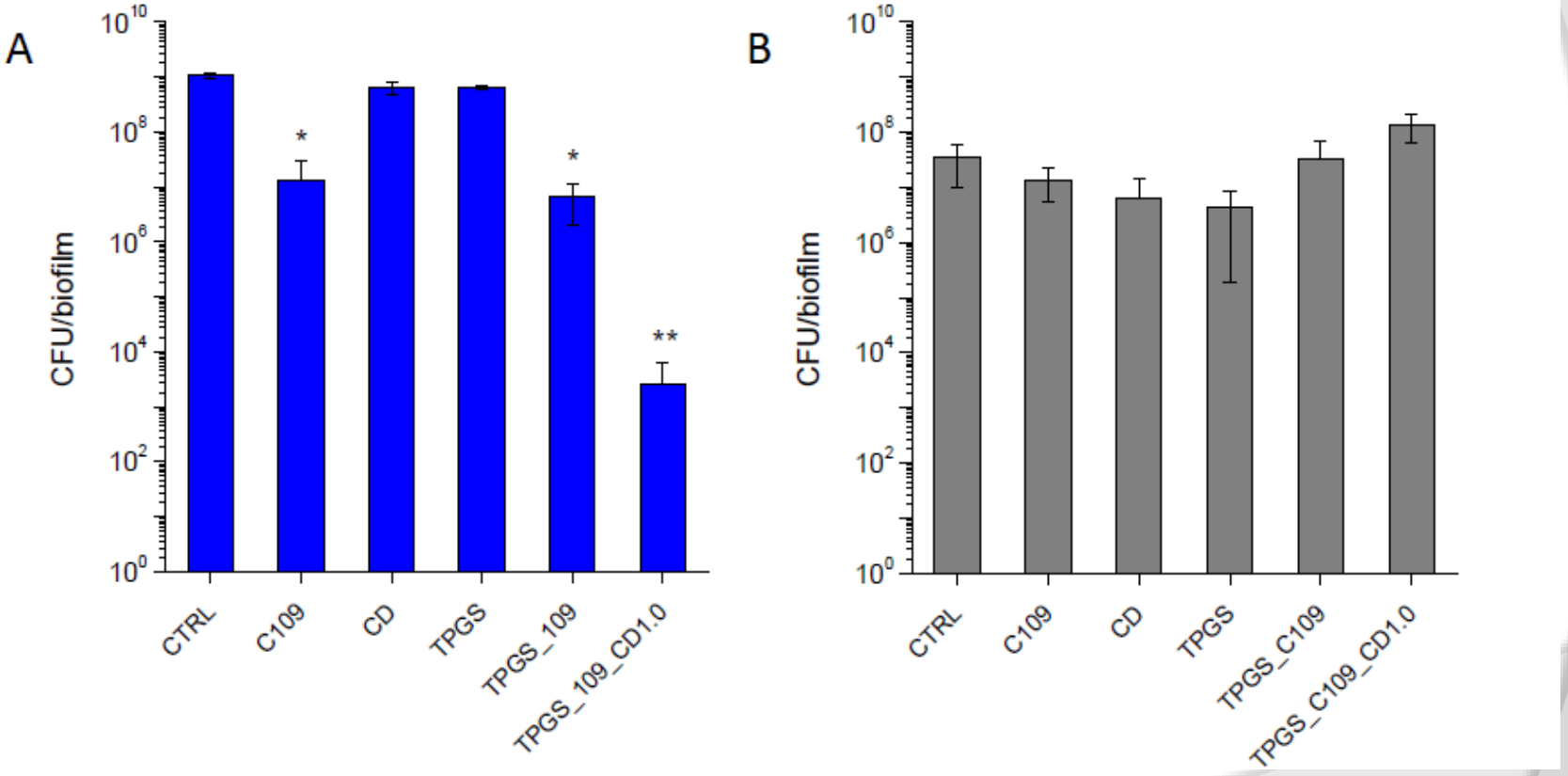
Inhalable formulations of new molecules effective against *Burkholderia cenocepacia*: from *in vitro* to *in vivo* applications



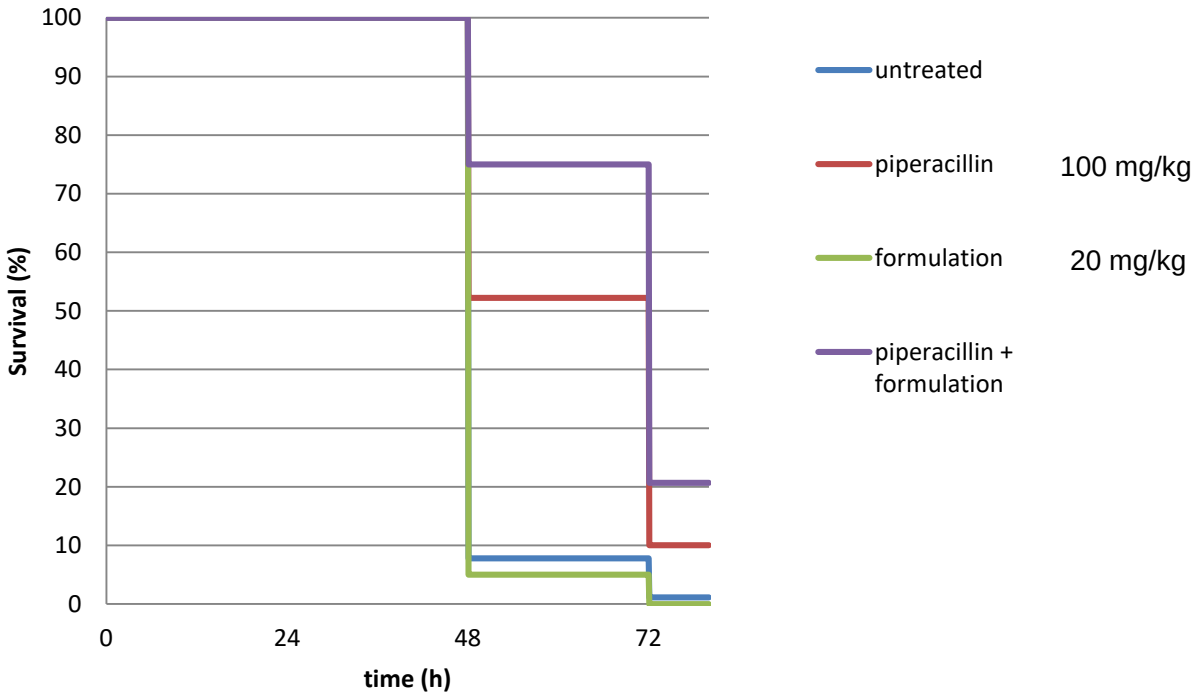
- Biodistribution
- Efficacy in mouse model of *B. cenocepacia* infection


TPGS_C109
PEGYLATED NANOCRYSTALS
Hydroxypropyl-
β-cyclodextrin
(CD)
C109
d-α-Tocopheryl
polyethylene glycol
1000 succinate
(TPGS)
TPGS_C109_CD
dry powders
Reconstitution in
saline
TPGS_C109
nanosuspension for
inhalation
OPTIMAL
AEROSOLIZATION
PROPERTIES
BROAD-SPECTRUM *IN VITRO* ANTIMICROBIAL ACTIVITY
IMPROVED INHIBITION ABILITY AGAINST *B. CENOCEPACIA* BIOFILM
IN VIVO SYNERGISTIC ACTIVITY WITH PIPERACILLIN
MUCUS
PENETRATING
NANOCRYSTALS
Non mucoid
bacteria
Biofilm
bacteria

Inhibition and eradication ability of C109 and of formulations against *B. cenocepacia* J2315 biofilm



Galleria mellonella infection model



ELSEVIER

Nanomedicine: Nanotechnology, Biology, and Medicine
23 (2020) 102113

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

PEGylated mucus-penetrating nanocrystals for lung delivery of a new FtsZ inhibitor against *Burkholderia cenocepacia* infection

Gabriella Costabile, PhD[a], Romina Provenzano, MSci[a], Alberto Azzalin, PhD[b], Viola Camilla Scoffone, PhD[b], Laurent R. Chiarelli, PhD[b], Valeria Rondelli, PhD[c], Isabelle Grillo, PhD[d], Thomas Zinn, PhD[e], Alexander Lepioshkin, MSci[f], Svetlana Savina, MSci[f], Agnese Miro, MSci[a], Fabiana Quaglia, PhD[a], Vadim Makarov, PhD[f], Tom Coenye, PhD[g], Paola Brocca, PhD[c], Giovanna Riccardi, MSci[b], Silvia Buroni, PhD[b,*], Francesca Ungaro, PhD[a,*]

Strategies to fight antibiotic resistance

Essential Proteins

- **Antibacterial compounds for *B. cenocepacia*:**
  C109

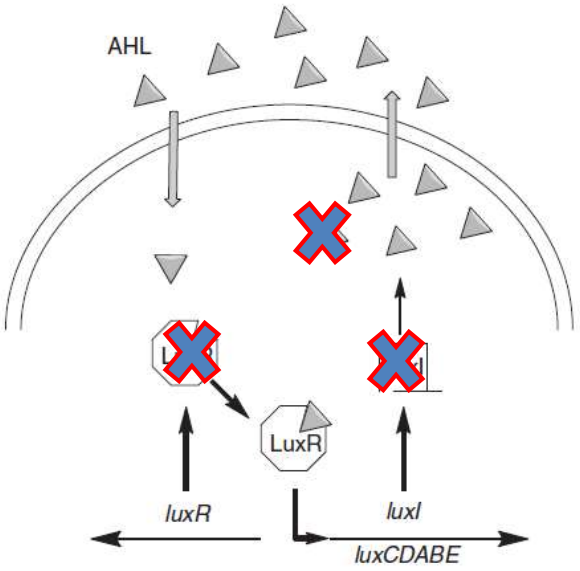
Virulence Factors

- **4 antivirulence compounds**
  Diketopiperazines

Vaccines

Adjuvants

Antimicrobial Compounds targeting Virulence Factors



Jiang and Li, 2013

Quorum Sensing Synthases

CepI (BCAM1870)
CciI (BCAM0239a)
DfsA (BCAM0581)
Ham cluster

1. Bacteria unable to produce virulence factors
2. No direct bacteria killing = development of resistance less probable
3. Anti-virulence drugs could potentially be used in combination with established or novel antimicrobials to improve the current therapy

Targeting *B. cenocepacia* CepI synthase

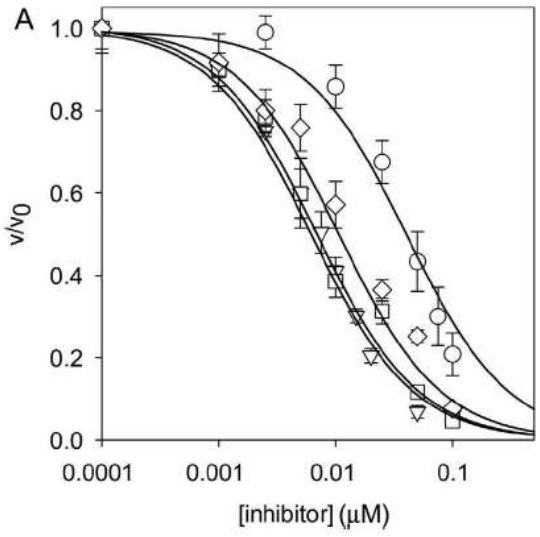
SCIENTIFIC REPORTS

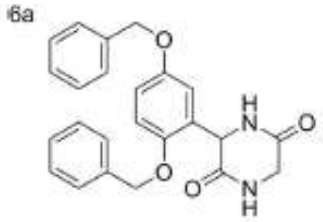
OPEN

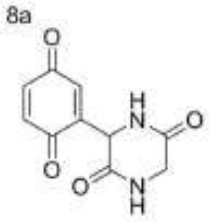
Discovery of new diketopiperazines inhibiting *Burkholderia cenocepacia* quorum sensing *in vitro* and *in vivo*

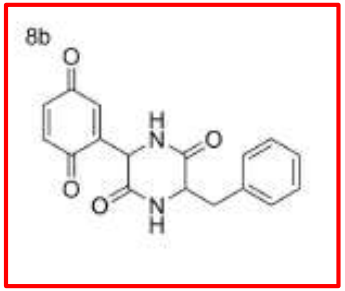
Received: 01 June 2016
Accepted: 08 August 2016
Published: 01 September 2016

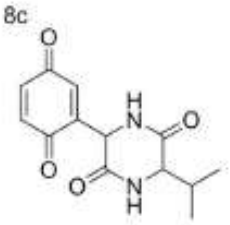
Viola C. Scoffone[1,*], Laurent R. Chiarelli[1,*], Vadim Makarov[2,*], Gilles Brackman[3], Aygun Israyilova[1,4], Alberto Azzalin[5,6], Federico Forneris[1], Olga Riabova[2], Svetlana Savina[2], Tom Coenye[3], Giovanna Riccardi[1] & Silvia Buroni[1]











IC_{50}

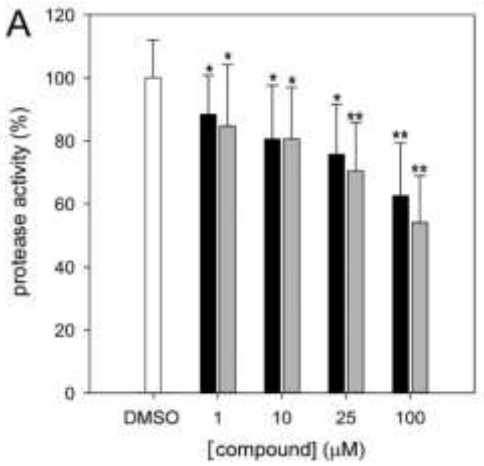
6a ○ 30 μM

8a ◇ 10 μM

8b ▽ 5 μM

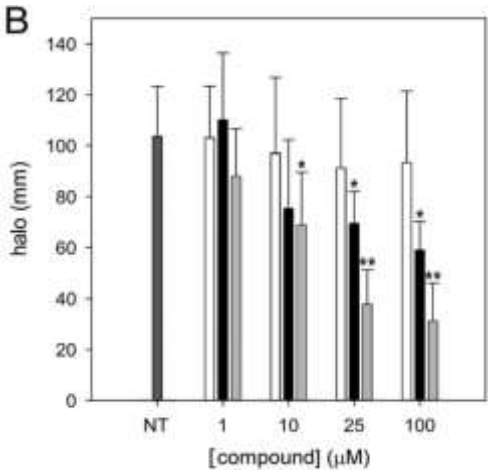
8c □ 6 μM

Protease production



6a (- 16-46%)
8b (- 12-38%)
DMSO

Siderophore production

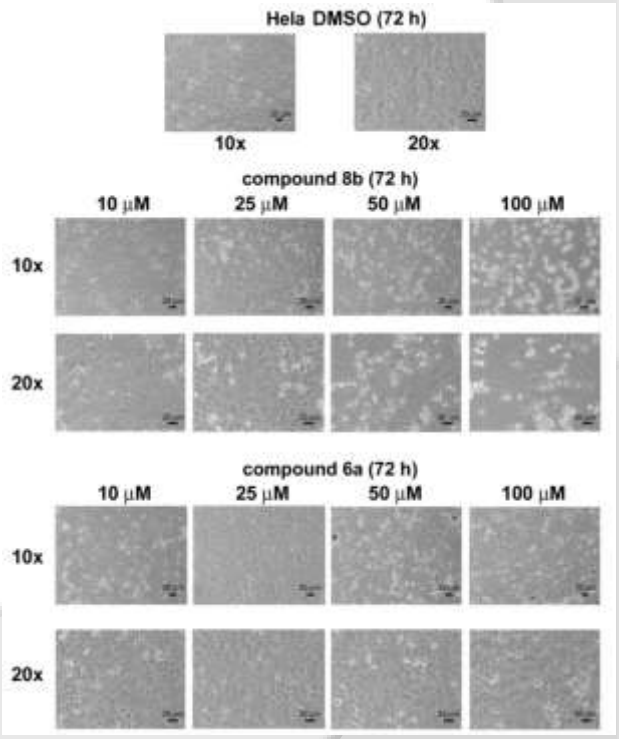


6a (- 13-69%)
8b (- 25-42%)
DMSO

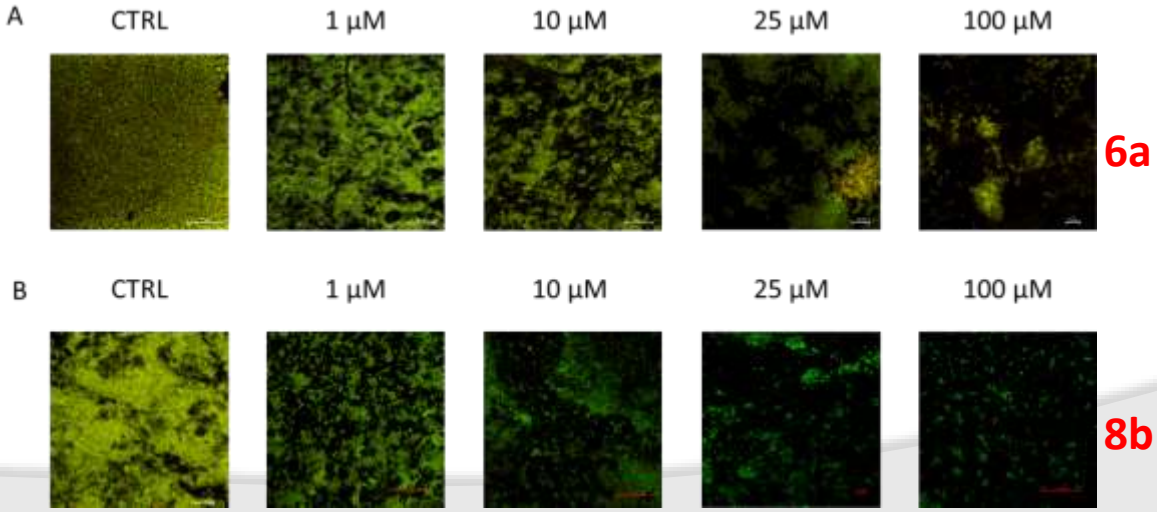
C. elegans survival

Significant increase of survival (30-40%) after 24h and 48h even at low concentrations

No toxicity on HeLa cells



Biofilm formation



Biofilm less structured, with fewer cells and unable to colonize the entire surface

Strategies to fight antibiotic resistance

Essential Proteins

- **Antibacterial compounds for *B. cenocepacia*:**
  C109

Virulence Factors

- **4 antivirulence compounds**
  Diketopiperazines

Vaccines

Adjuvants

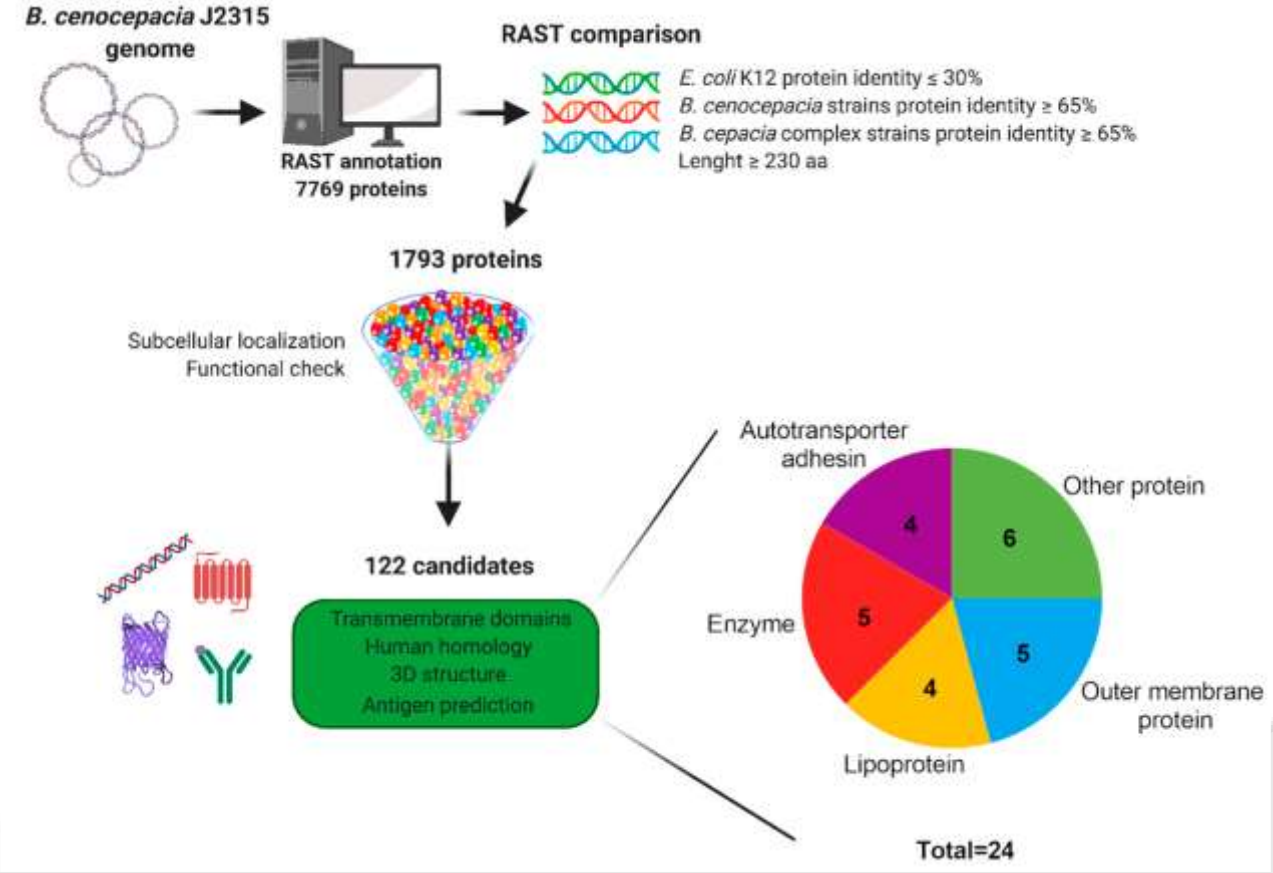
Vaccines for *B. cenocepacia*

vaccines MDPI

Article

Identification by Reverse Vaccinology of Three Virulence Factors in *Burkholderia cenocepacia* That May Represent Ideal Vaccine Antigens

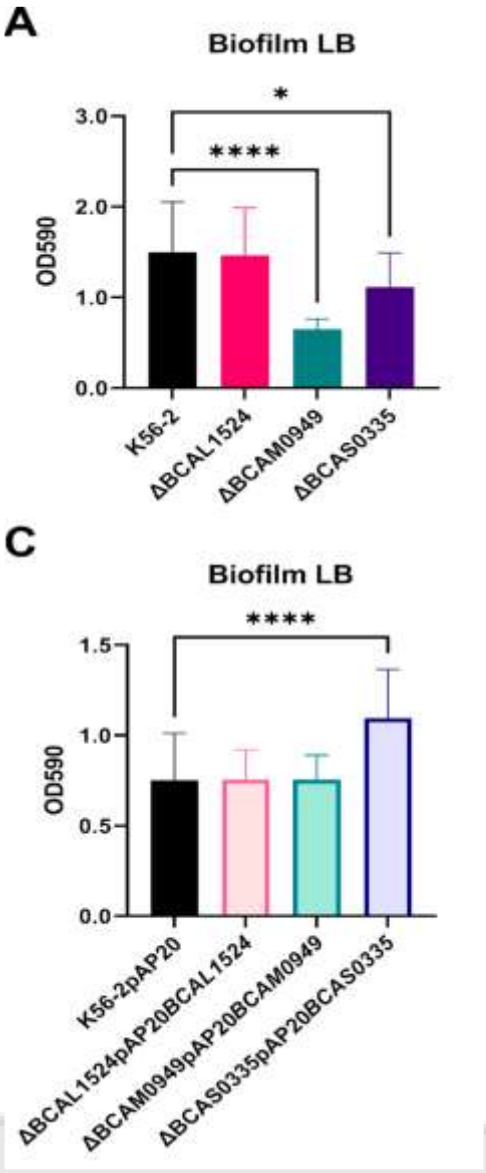
Samuele Irudal [1], Viola Camilla Scoffone [1], Gabriele Trespidi [1], Giulia Barbieri [1], Maura D'Amato [2], Simona Viglio [2], Mariagrazia Pizza [3], Maria Scarselli [4], Giovanna Riccardi [1] and Silvia Buroni [1,*]

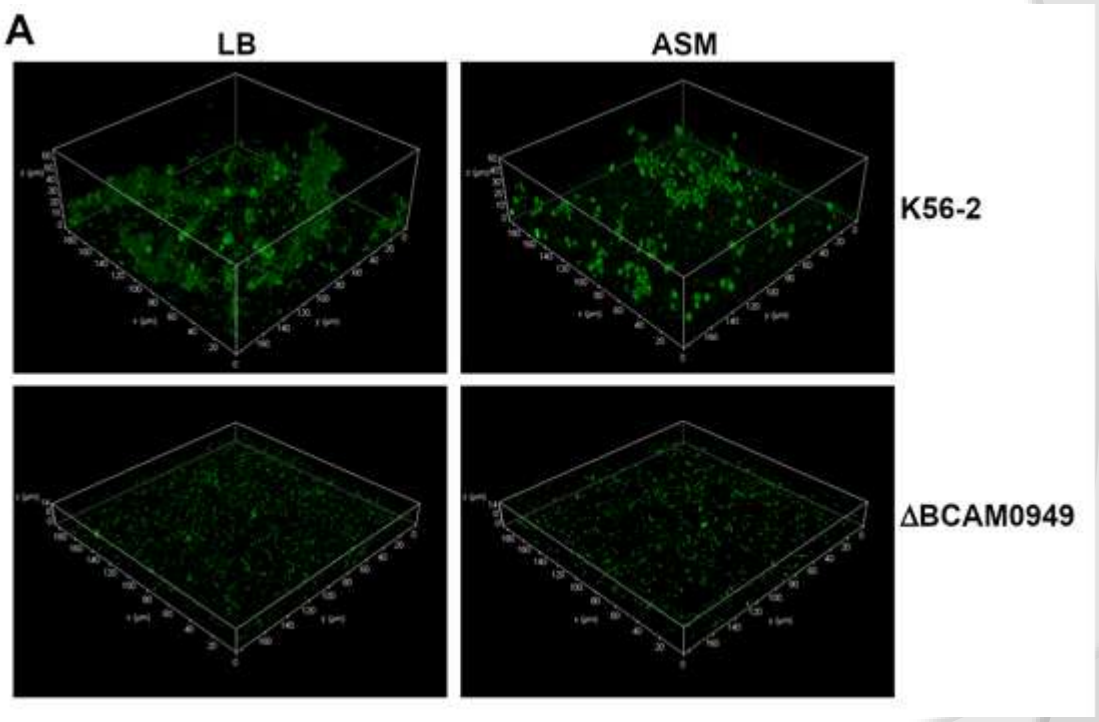


Collage-like protein **BCAL1524**
Lipase LipA **BCAM0949**
Autotransporter adhesin **BCAS0335**

selected for further analysis

Biofilm formation

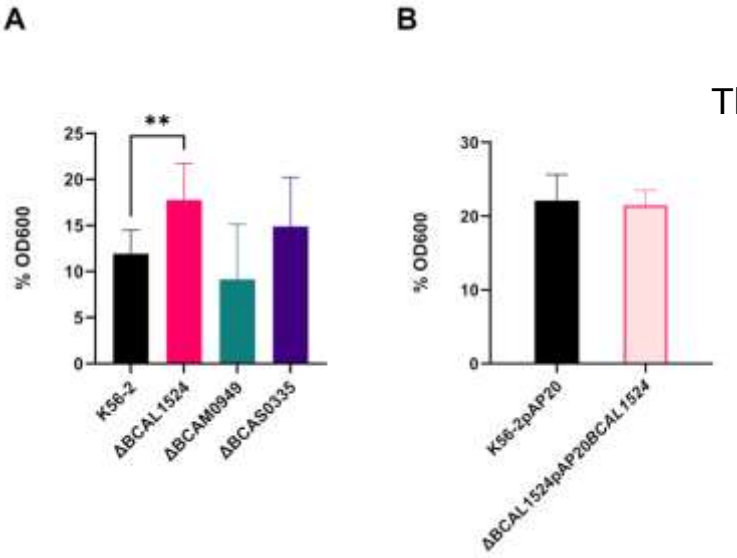




the lipase BCAM0949
and the autotransporter adhesin protein BCAS0335
contribute to biofilm formation

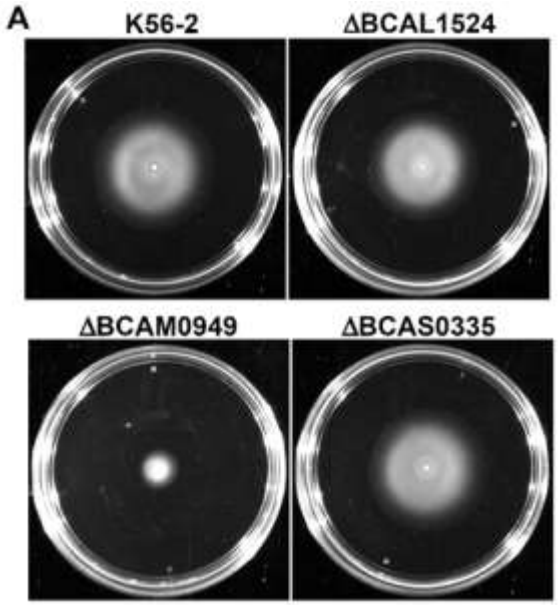
Autoaggregation

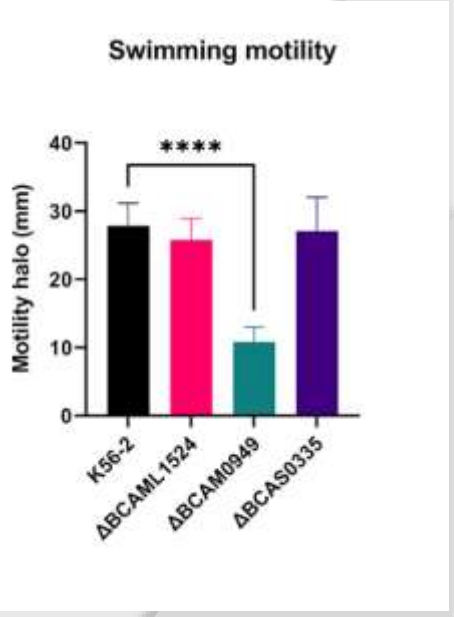
Involved in bacterial colonization and persistence in the host



The Collage-like protein BCAL1524 is involved in autoaggregation

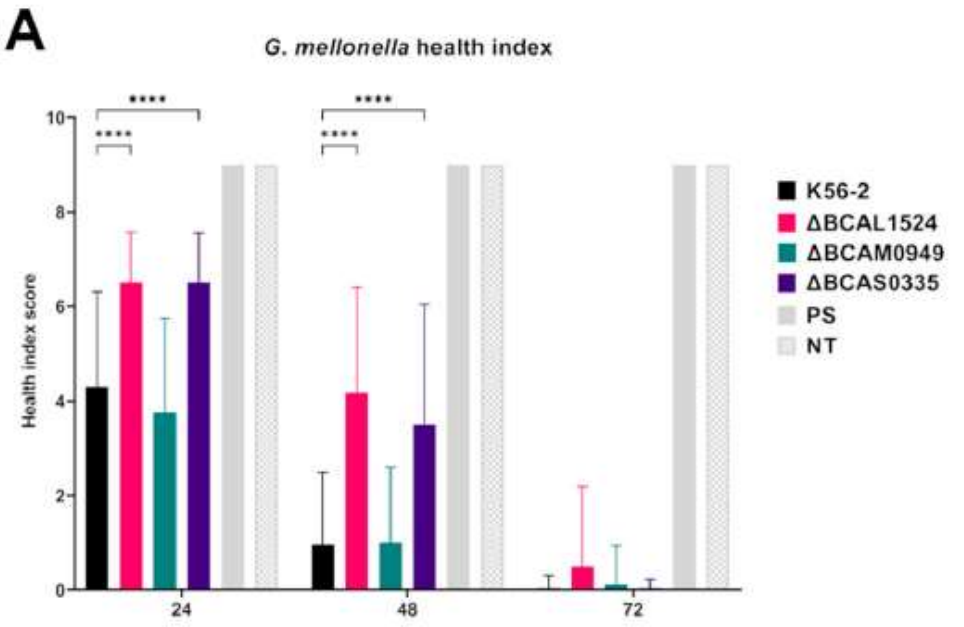
Swimming Motility

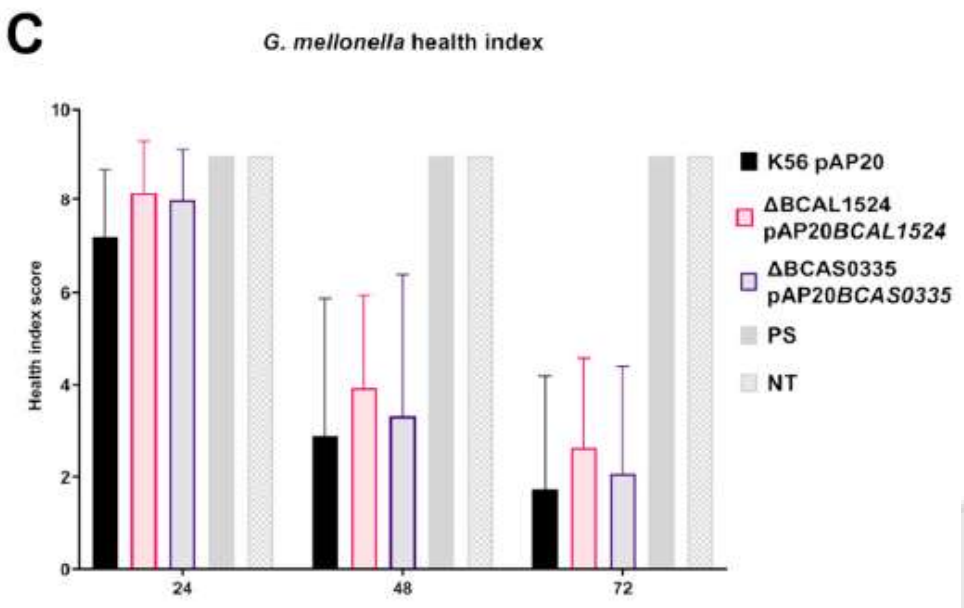


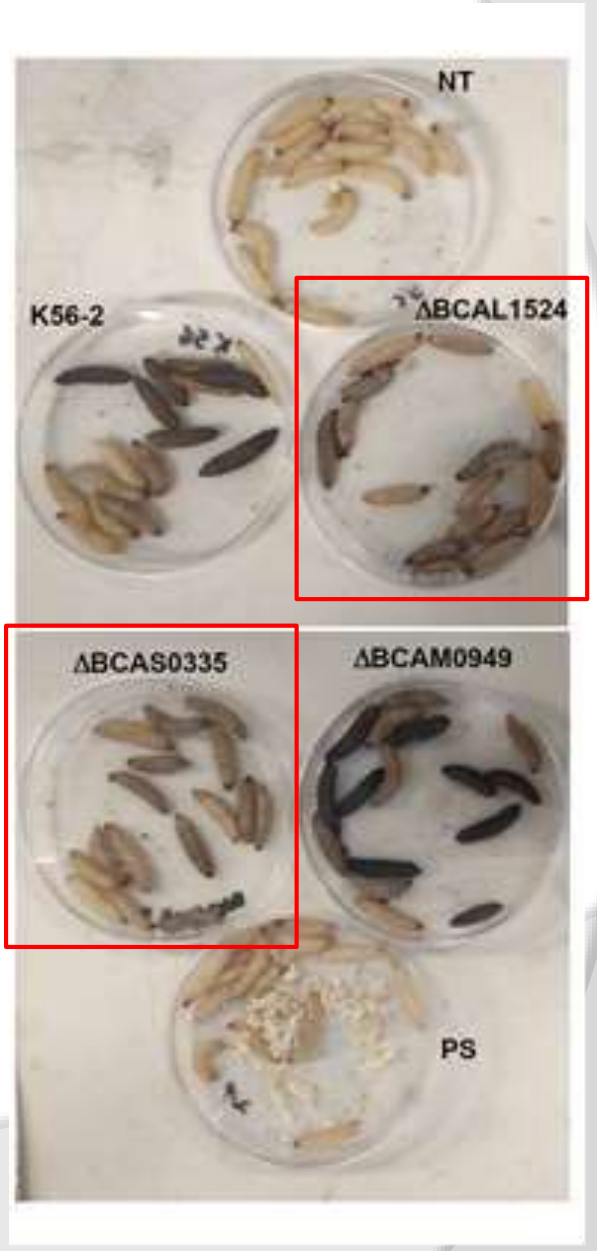


The Lipase LipA BCAM0949 is involved in swimming motility

Virulence in *Galleria mellonella*







Strategies to fight antibiotic resistance

Essential Proteins

- **Antibacterial compounds for *B. cenocepacia*:**
  C109

Virulence Factors

- **4 antivirulence compounds**
  Diketopiperazines

Vaccines

Adjuvants

Characterization of the dispirotripiperazine derivative PDSTP as antibiotic adjuvant and antivirulence compound against *Pseudomonas aeruginosa*

Andrea Bonacorsi[1†], Gabriele Trespidi[1†], Viola C. Scoffone[1], Samuele Irudal[1], Giulia Barbieri[1], Olga Riabova[2], Natalia Monakhova[2], Vadim Makarov[2] and Silvia Buroni[1*]

Front. Microbiol. 15:1357708.
doi: 10.3389/fmicb.2024.1357708

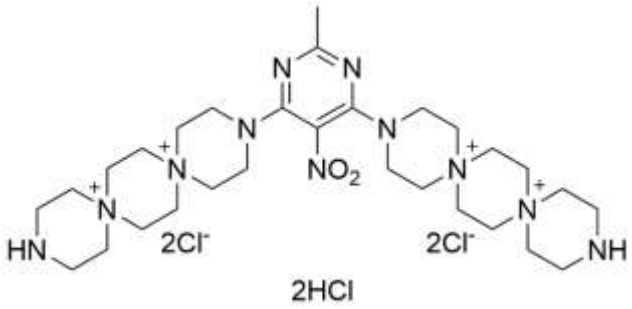
Dispirotripiperazines: broad spectrum antiviral compounds

PDSTP is active against:

- ✓ Herpes simplex virus type-1 and 2
- ✓ Human cytomegalovirus
- ✓ Human immunodeficiency virus
- ✓ Human papilloma virus
- ✓ SARS-CoV-2

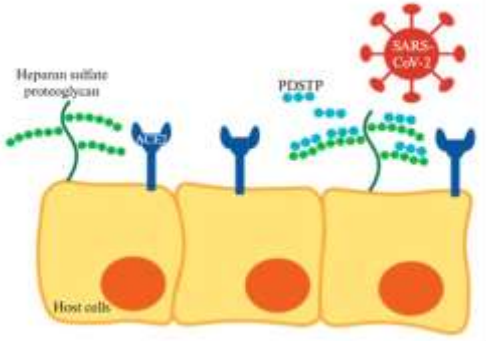
Egorova *et al.*, 2021

PDSTP



Dr. Vadim Makarov

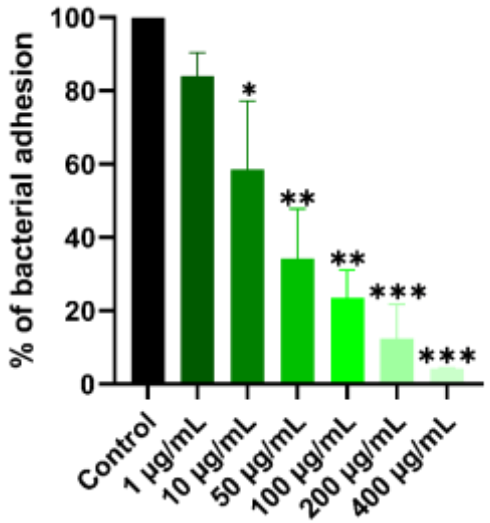
RESEARCH CENTER OF BIOTECHNOLOGY



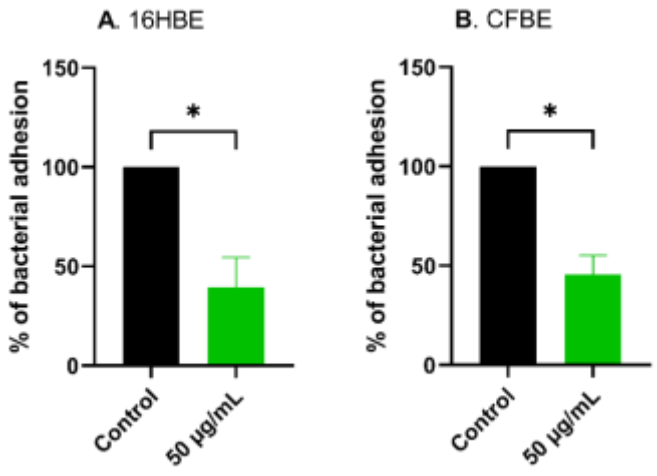
Makarov and Popov, 2022

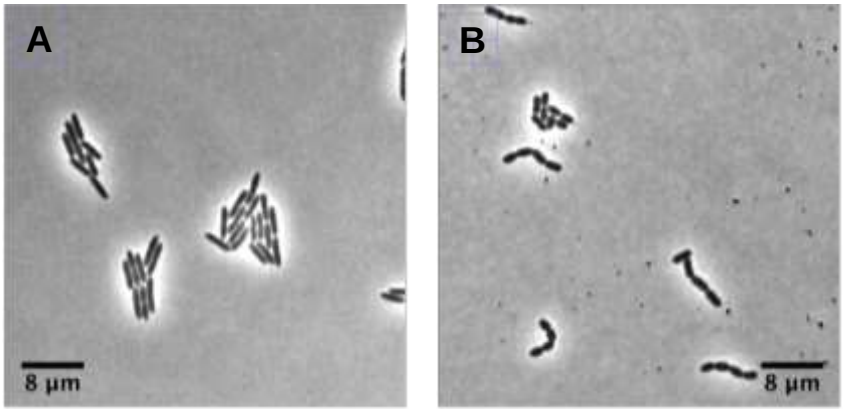
PDSTP impairs *P. aeruginosa* adhesion

PA01 adhesion on A549 monolayers



PA01 adhesion on 16HBE and CFBE monolayers





No treatment **PDSTP 50 µg/mL**

PDSTP enhances efficacy of antibiotics used in clinics

PDSTP-antibiotic combinations tested on PA01 and 9 drug resistant CF clinical isolates

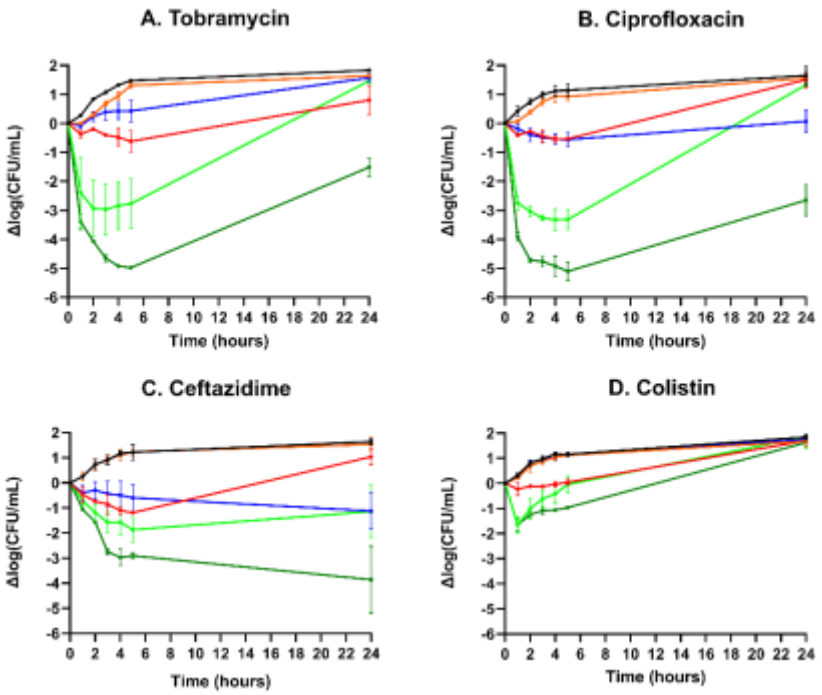
Antibiotic	MIC reduction
Tobramycin	0x to 2x
Ciprofloxacin	2x to 16x
Ceftazidime	4x to **128x**
Meropenem	8x to **32x**
Colistin	16x to **32x**

6/7 resistant strains below the CLSI susceptibility breakpoint

4/4 resistant strains below the CLSI susceptibility breakpoint

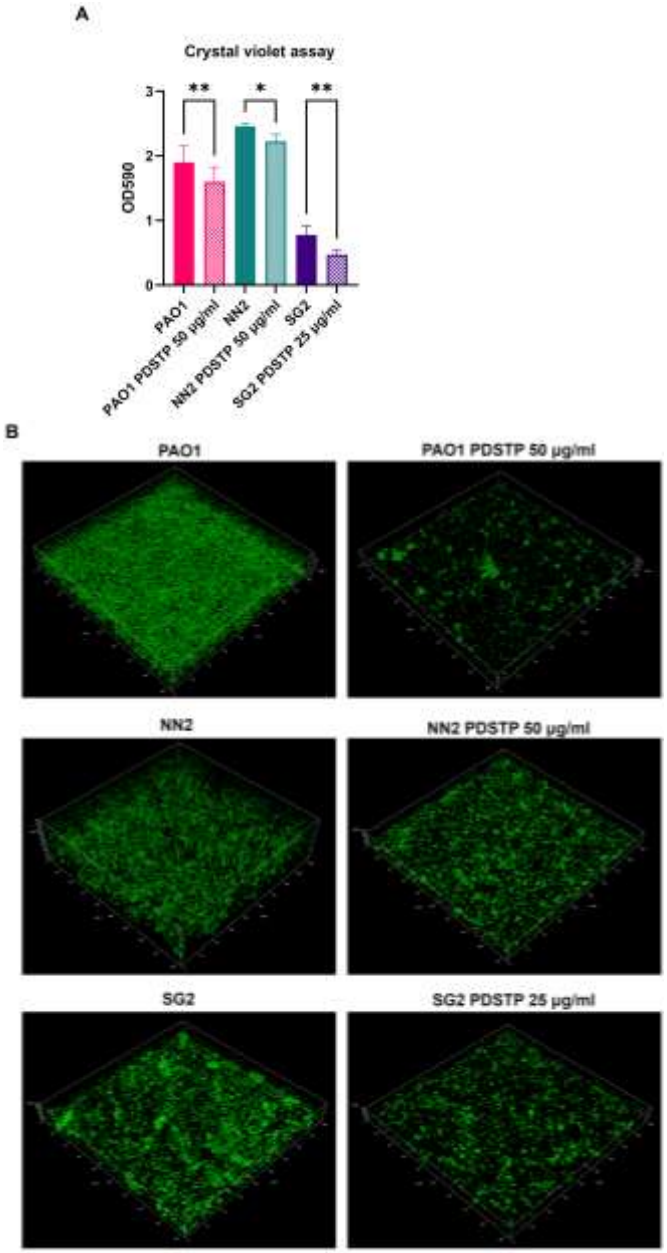
MIC of 2 resistant strains **decreased from 64-128 µg/mL to 4 µg/mL**

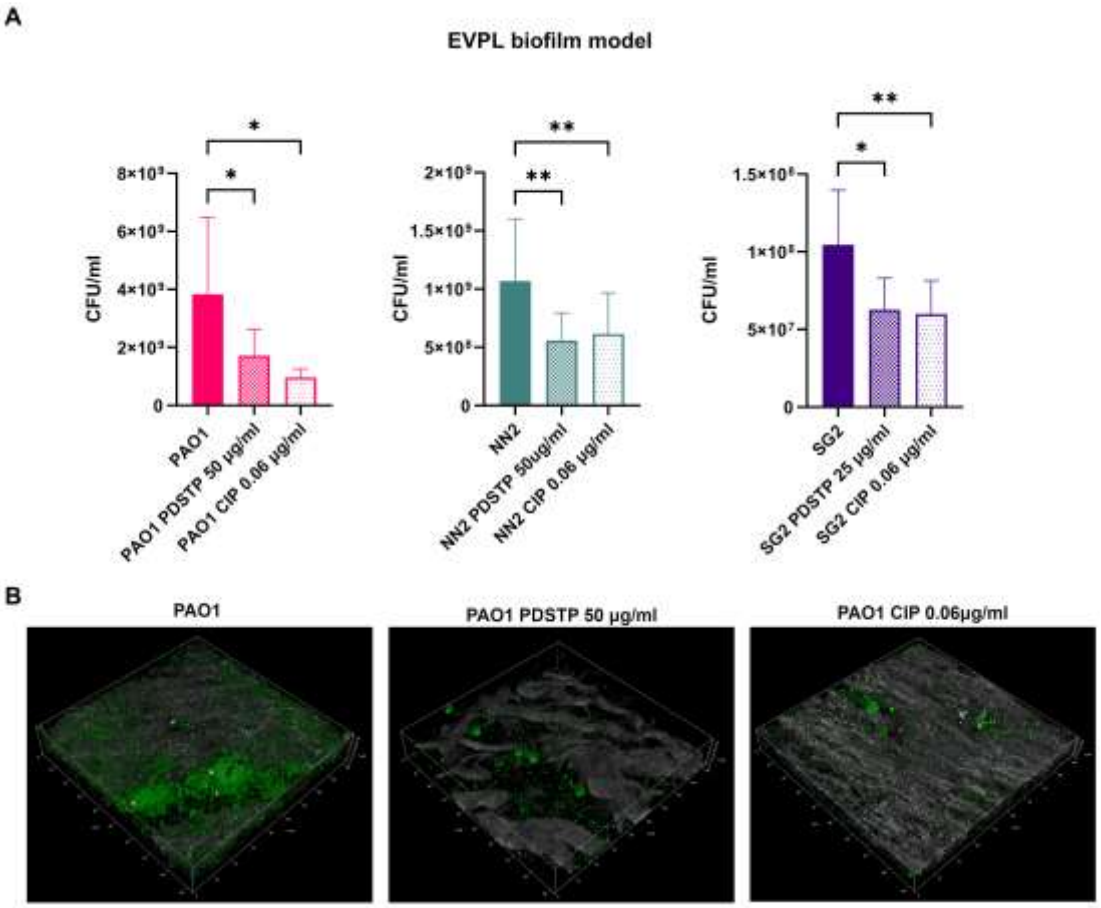
PDSTP-antibiotic combinations tested on PA01 and 9 drug resistant CF clinical isolates



Comparable efficacy of the combinations was observed by time-killing assays on **7/9 clinical isolates**

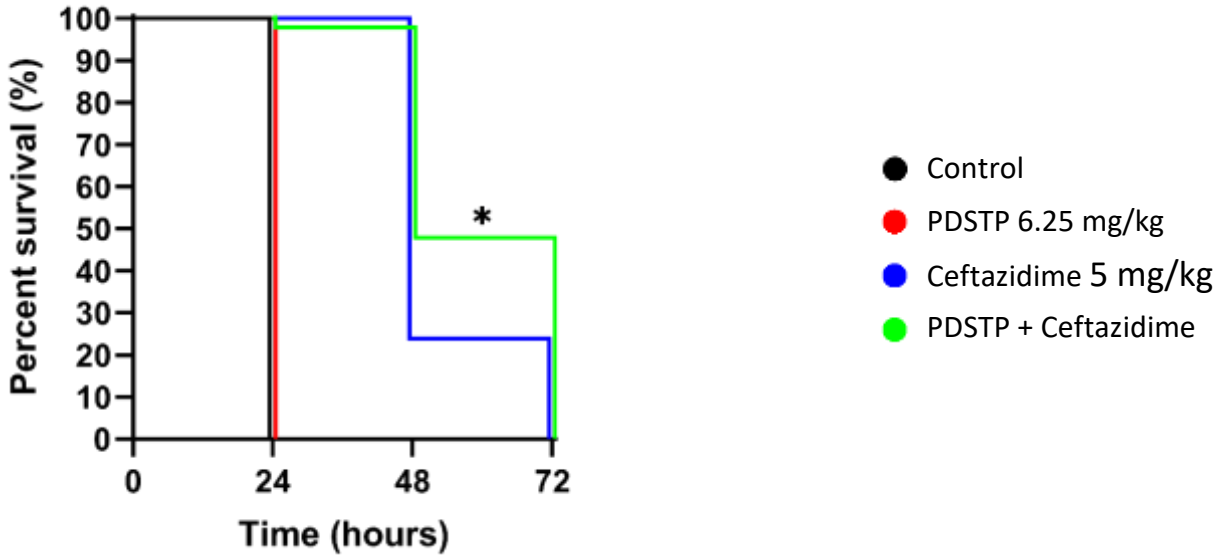
PDSTP inhibits biofilm formation *in vitro* and in an *ex vivo* pig lung model





PDSTP - Ceftazidime combination is effective *in vivo*

***Galleria mellonella* infection model**



Fondazione per la Ricerca sulla Fibrosi Cistica - ETS
italian cystic fibrosis research foundation

FFC#6/2023

Using a Virtual Screening approach to find new drugs against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

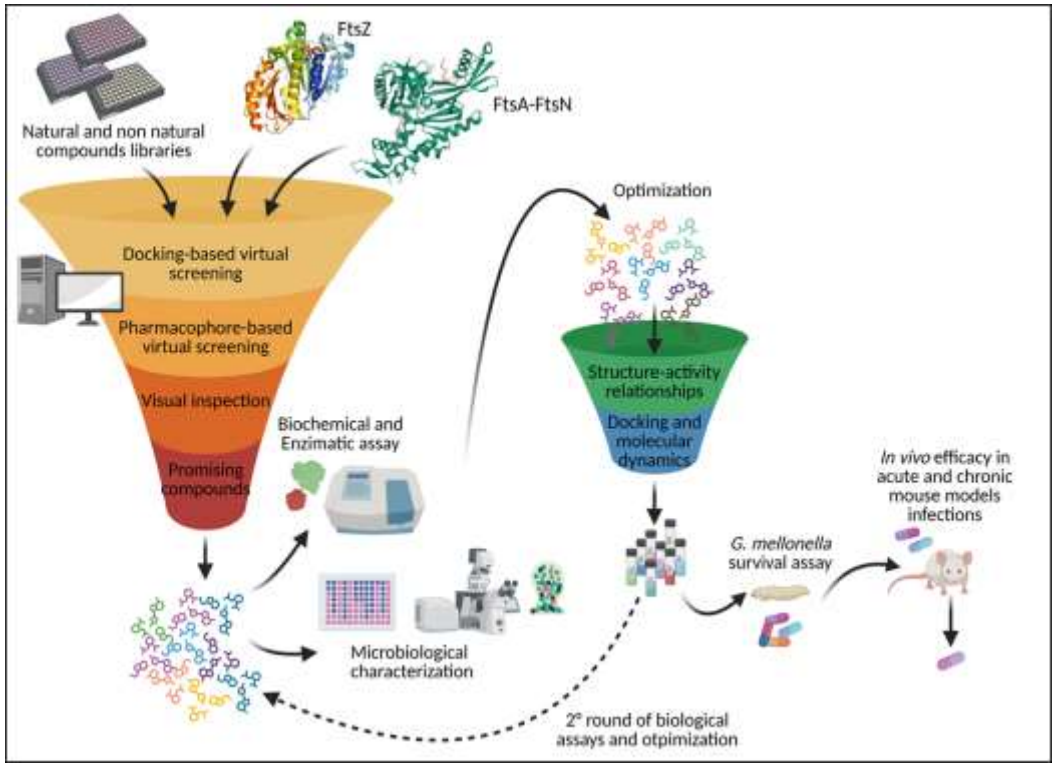
UNIVERSITÀ DI PAVIA
Department of Biology and Biotechnology "Lazzaro Spallanzani"

Silvia Buroni and Antonio Coluccia

SAPIENZA
UNIVERSITÀ DI ROMA
DIPARTIMENTO DI CHIMICA E TECNOLOGIE DEL FARMACO

Workflow and Essential methods

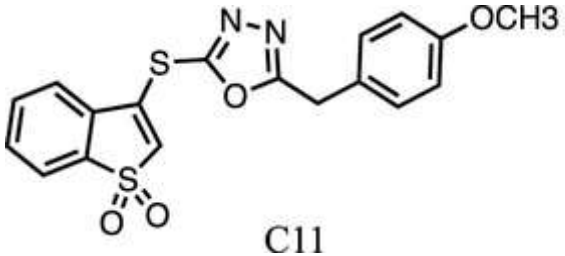
- WP1: **chemistry**
- Task 1.1 Identification of compounds targeting FtsZ or FtsA-FtsN by **VS**.
- Task 1.2 Compound **optimization**.
- Task 1.3 Hit to lead optimization.
- WP2: **compound characterization**
- Task 2.1 Enzymatic **assays**.
- Task 2.2 **MIC** evaluation.
- Task 2.3 Evaluation of the activity of the compounds against **human tubulin**.
- Task 2.4 Evaluation of the **toxicity** of the compounds by MTT.
- WP3: ***in vivo* assays**
- Task 3.1 *In vivo* assays in ***Galleria mellonella***.
- Task 3.2 *In vivo* assays in **mouse models** of infection.



Identification of compounds targeting FtsZ and FtsA-FtsN

S. aureus and *P. aeruginosa* FtsZ

- First protein involved in cell division
- Assembly of “divisome”



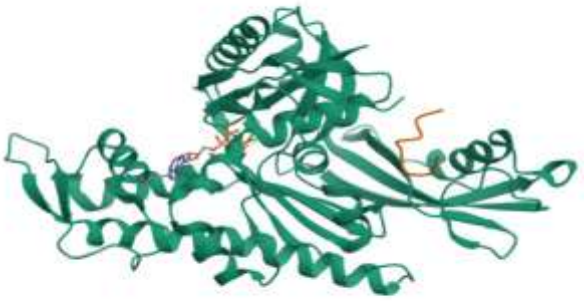
C11

MIC *S. aureus*	2 µg/ml
IC_{50} FtsZSa	47.97 µM
IC_{50} FtsZPa	34 µM

MIC *P. aeruginosa* >128 µg/ml

P. aeruginosa FtsA-FtsN complex

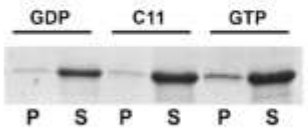
- Vital role for the bacteria
- There are proofs of concept that the impairment of the complex formation has an antibacterial effect
- Important structural information is available



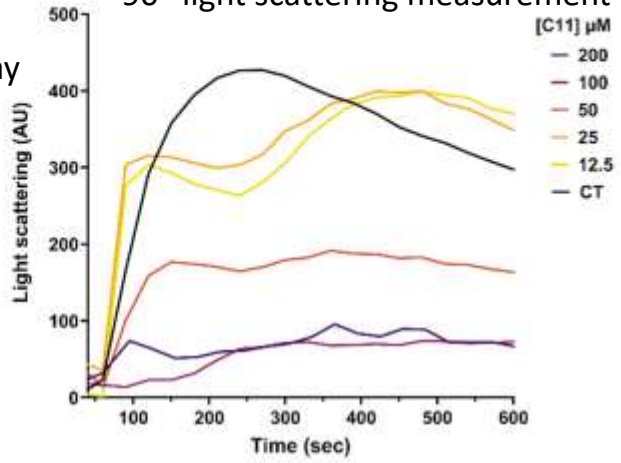
C11 biological characterization

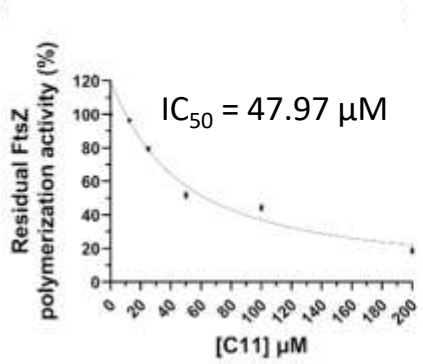
C11 inhibits FtsZ polymerization

In vitro sedimentation assay



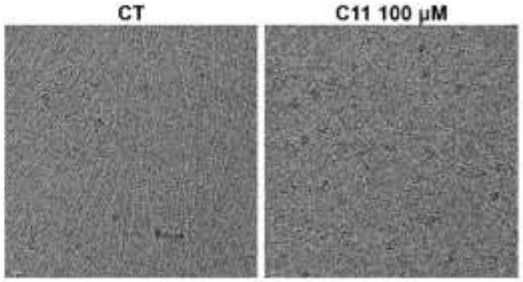
90°-light scattering measurement



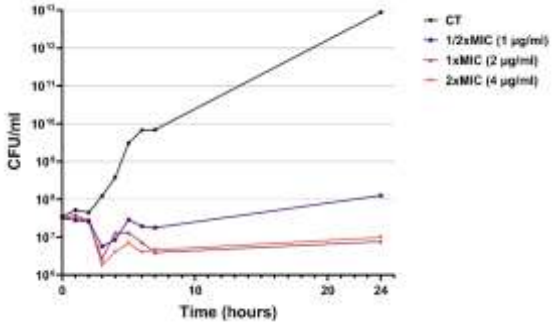


IC_{50} = 47.97 µM

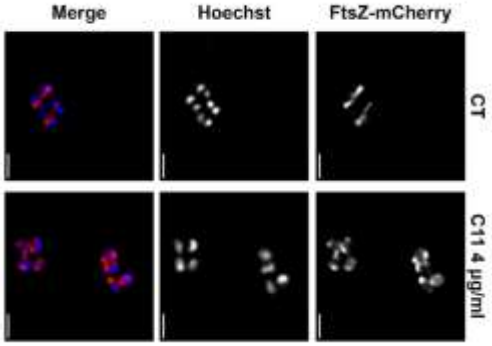
Cryo-electron microscopy



C11 has a bacteriostatic effect also in SCFM3



C11 induces FtsZ mislocalization



C11 has a synergistic/additive effect with other antibiotics

Antibiotics	*S. aureus* ATCC25923 MIC (µg/ml) Single drugs	MIC (µg/ml) combination	FICI[a]
Ceftazidime/C11	16/2	4/0.5	Synergy (0.50)
Erythromycin/C11	0.5/2	0.06/1	Additive (0.62)
Linezolid/C11	2/2	0.5/1	Additive (0.75)
Meropenem/C11	0.25/2	0.03/0.5	Synergy (0.37)
Rifampicin/C11	0.03/2	0.007/0.25	Synergy (0.36)
Tetracycline/C11	0.5/2	0.25/1	Additive (1)

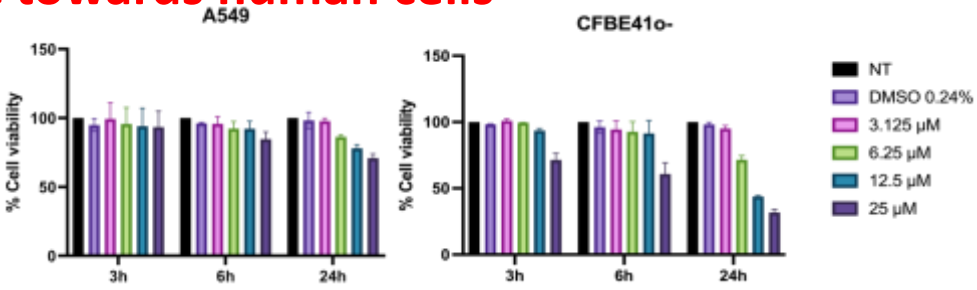
C11 is effective against CF MSSA and MRSA strains but NOT against Gram-negatives

Compound	MIC (µg/ml) *P. aeruginosa* PAO1	*K. pneumoniae* ATCC13883	*A. baumannii* ATCC19606
C11	≥128	≥128	≥128
C11 + PaβN (128 µg/ml)	1	8	nd
C11 + PaβN (64 µg/ml)	16	nd	1

C11 is not toxic towards human cells

At 25 µM C11: - 20% human tubulin polymerization

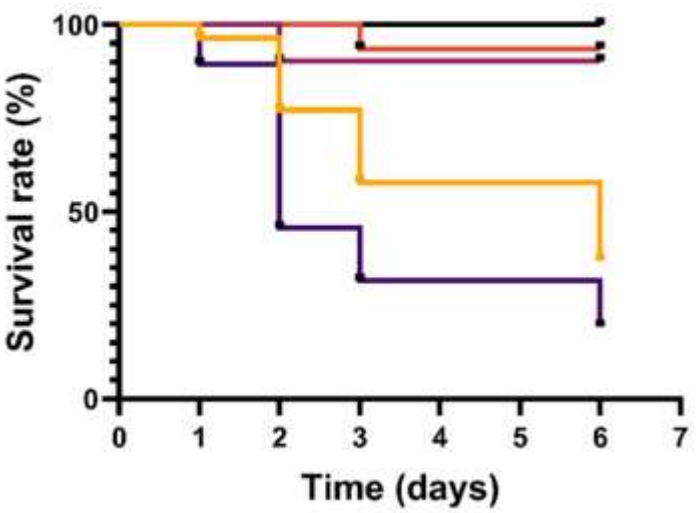
Samples	Vmax (mOD/min)
Positive control (DMSO)	3.78
Paclitaxel (10 µM)*	21.45
11 (5 µM)	3.5
11 (10 µM)	3
11 (25µM)	2.8



At 25 µM the percentage of live cells after 24 h is 90% and 30%

C11 improves the survival of *Galleria mellonella* larvae infected with *S. aureus*

Galleria mellonella infection model: the larvae were infected with *S. aureus* ATCC25923 and treated with 40 mg/kg (4-fold the MIC) of C11 after two hours



77% vs 46% 58% vs 32% 40% vs 20%

- CT
- SA 10^5 + C11 40 mg/kg (4xMIC)
- SA 10^5+ DMSO 8% → 80% of infected larvae died after 6 days
- Saline +C11 40 mg/kg (4xMIC) → C11 is not toxic for *G. mellonella*
- Saline + DMSO 8%

ACS Infectious Diseases

Article

Identification of a New FtsZ Inhibitor by Virtual Screening, Mechanistic Insights, and Structure–Activity Relationship Analyses

Pietro Sciò,* Viola Camilla Scoffone,* Anastasia Parisi, Marianna Bufano, Martina Caneva, Gabriele Trespidi, Samuele Irudal, Giulia Barbieri, Lisa Cariani, Beatrice Silvia Orena, Valeria Daccò, Francesco Imperi, Silvia Buroni, and Antonio Coluccia*

Conclusions

- Using the VS approach targeting FtsZ, compound **C11** was identified.
- C11 biological characterization was completed:

blocks FtsZ **polymerization**;

induces FtsZ **mislocalization;**

retains a good **activity** against *S. aureus,* including CF clinical isolates;

shows a **synergistic** effect in combination with other antibiotics;

is **not toxic**;

has a good ***in vivo*** **activity** in *G. mellonella* infection model.

In vivo activity in a mouse model of acute and chronic infection

Biological and biochemical investigations to understand how they can be optimized to be transposed *in vivo*

Conclusions