

New tools for drug susceptibility testing against non-tuberculous mycobacteria

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PhD candidate

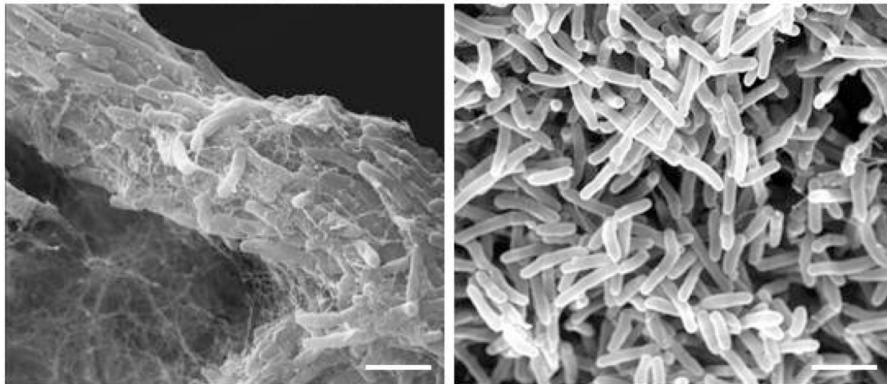
i3S – Instituto de Investigação e Inovação em Saúde, University of Porto (Host Targets of Infection group)

Supervised by Tânia Silva and Maria Salomé Gomes

I Introduction

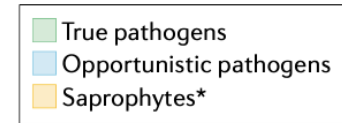
Mycobacterium abscessus (Mab)

- Most **significant** mycobacterial isolates associated with pulmonary infections, particularly in cystic fibrosis patients
- Most **pathogenic** rapidly growing mycobacteria
- Resistant to high chlorine levels, disinfectants, high temperatures and can form **biofilms**
- Suggested to be involved in **person-to-person transmission**
- Associated with intrinsic and acquired **resistance** to most antimycobacterial agents



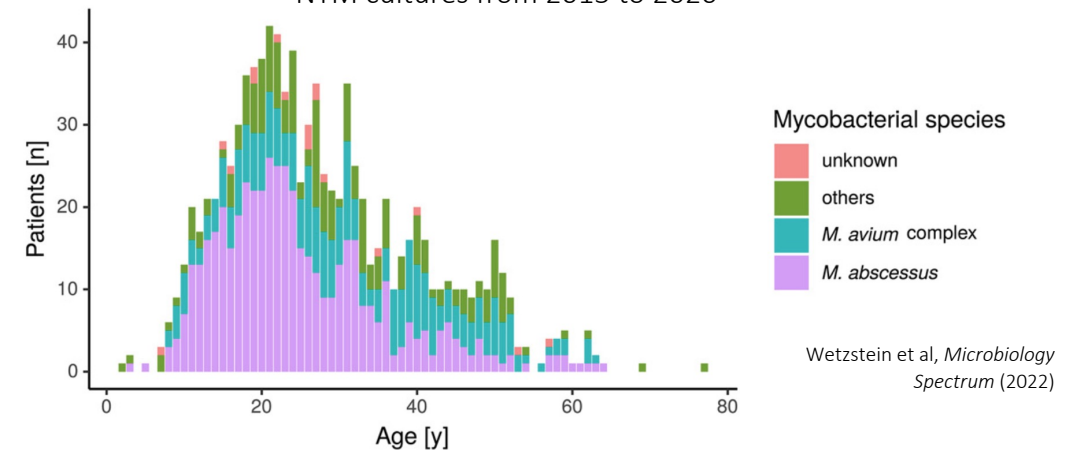
Sánchez-Chardi et al, *Microscopy and Microanalysis* (2012)

Non-tuberculous mycobacteria		
Rapidly growing mycobacteria	Slowly growing mycobacteria	
<ul style="list-style-type: none"> M. chelonae-abscessus complex <u>M. abscessus subsp. abscessus</u> M. abscessus subsp. bolletii M. abscessus subsp. massiliense M. chelonae M. fortuitum 	<ul style="list-style-type: none"> M. marinum M. ulcerans 	<ul style="list-style-type: none"> M. tuberculosis complex M. leprae
<ul style="list-style-type: none"> M. smegmatis M. vaccae 	<ul style="list-style-type: none"> M. avium complex <ul style="list-style-type: none"> M. avium M. intracellulare M. chimaera M. haemophilum M. xenopi M. kansasii M. simiae 	
	<ul style="list-style-type: none"> M. terrae complex M. goodii 	



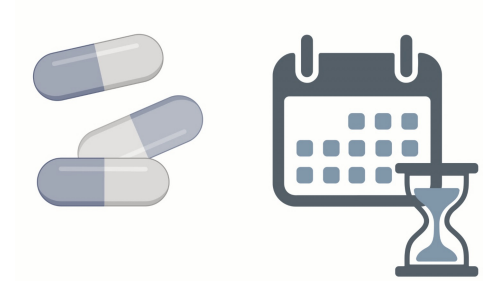
Johansen M. et al. *Nat Rev Microbiol* (2020)

Species and age distribution of CF-patients with positive NTM cultures from 2015 to 2020



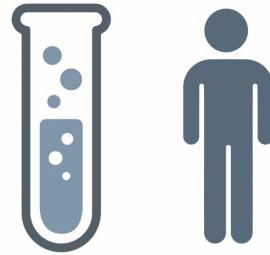
Wetzstein et al, *Microbiology Spectrum* (2022)

II Objective



Very long multi-drug therapy

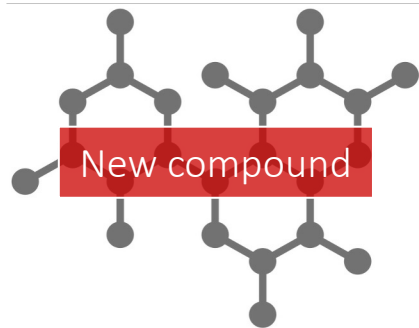
Mab infection



In vitro vs in vivo discrepancy

Methods for drug susceptibility testing are sub-optimal

- High costs in terms of time, work and consumables
- Do not adequately mimic in vivo conditions



Antimicrobial peptides
Ionic liquids
Algae extracts

...

Complex in vitro models

High-throughput screening

New tools to combat antibiotic resistance
and improve treatment outcomes

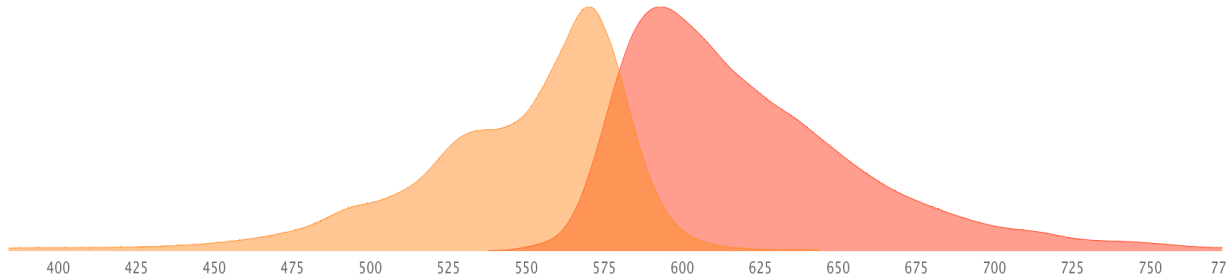
Find molecules with the better
potential to be clinically effective

III *M. abscessus* double-reporter strains

Fluorescence

mScarlet

$\lambda_{ex} = 569 \text{ nm}$ $\lambda_{em} = 594 \text{ nm}$

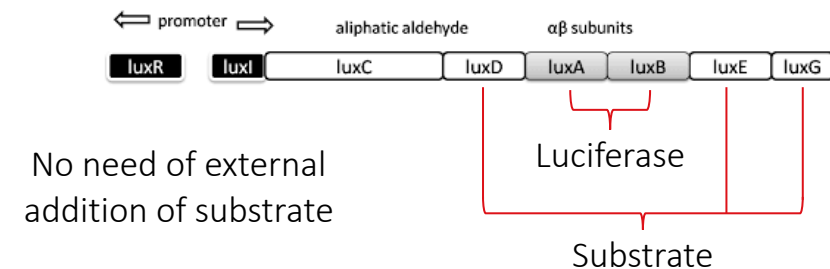
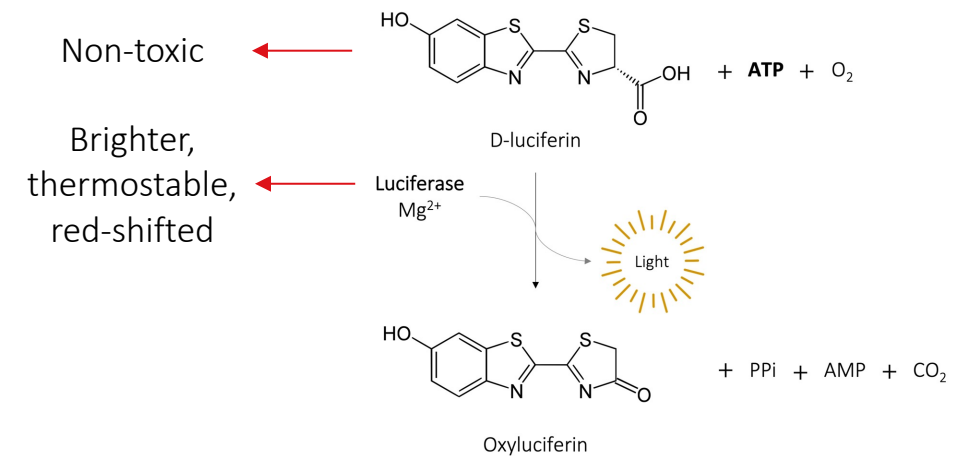


Adapted from: fpbase.org/protein/mscarlet/

- Synthetic gene based on red fluorescent proteins (corals)
- Monomeric protein with high “quantum yield” (emits strong fluorescence, even at low concentrations)

Luminescence

Firefly luciferase or lux operon



IV Growth curves



Mab FF_mScarlet

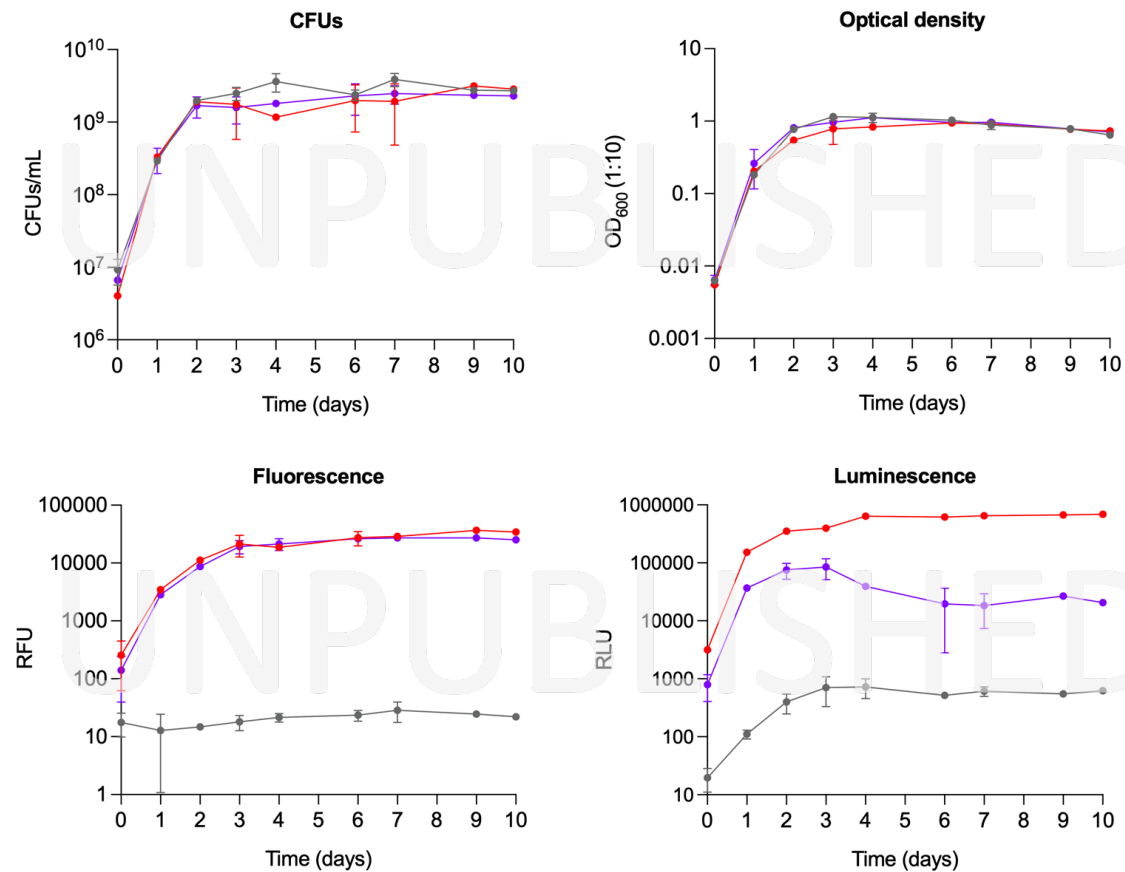


Mab operon_mScarlet

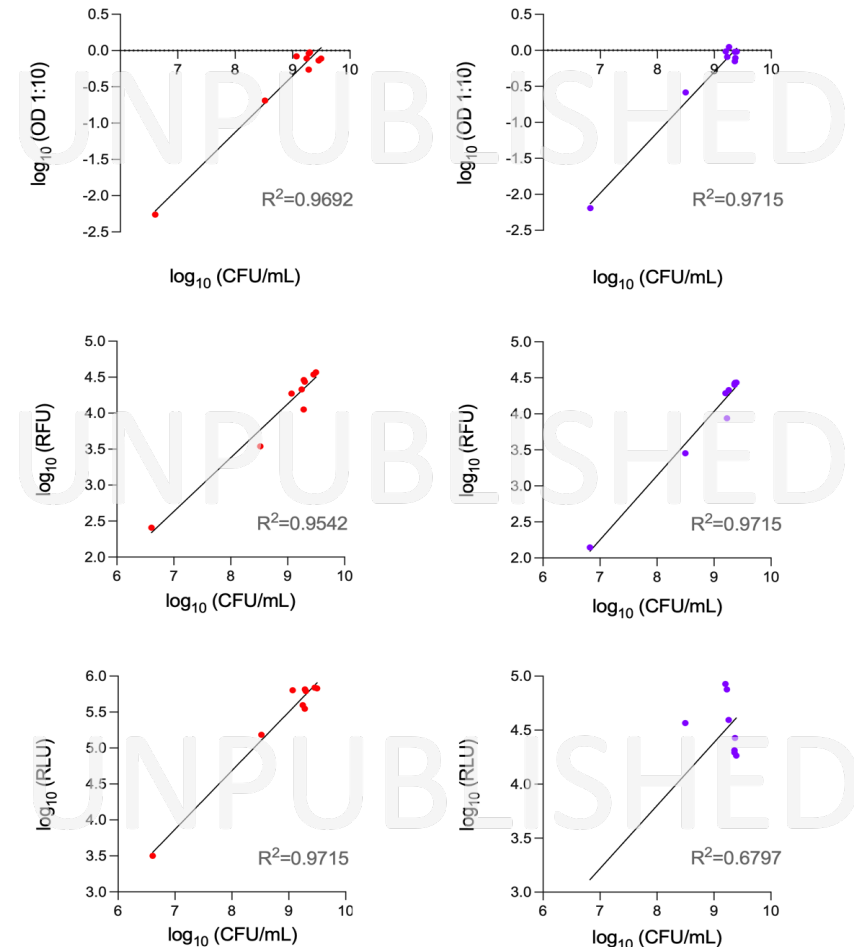


Mab WT

The double-reporter Mab strains grow similarly to the non-transformed strain



The fluorescence and luminescence correlate with CFU counting





Mab FF_mScarlet

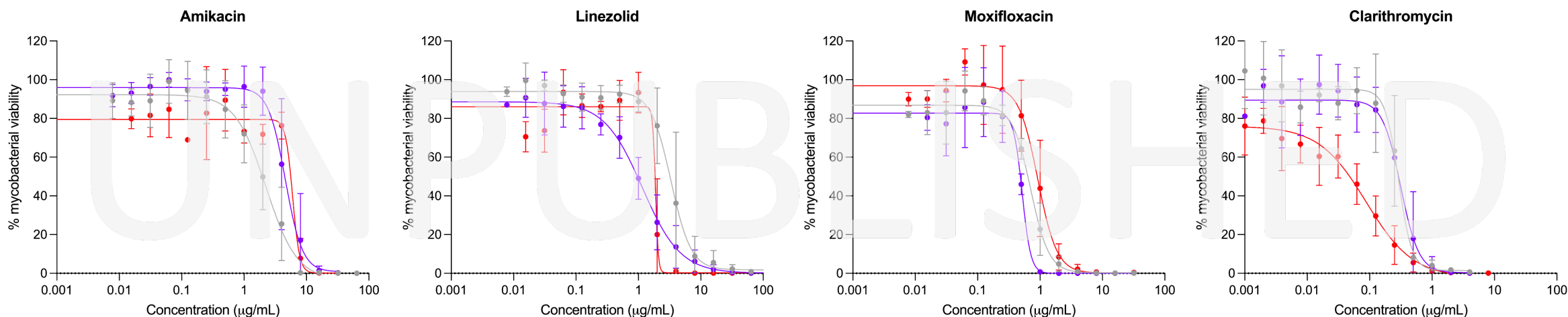


Mab operon_mScarlet



Mab WT

The double-reporter Mab strains have a similar antibiotic susceptibility profile to the non-transformed strain

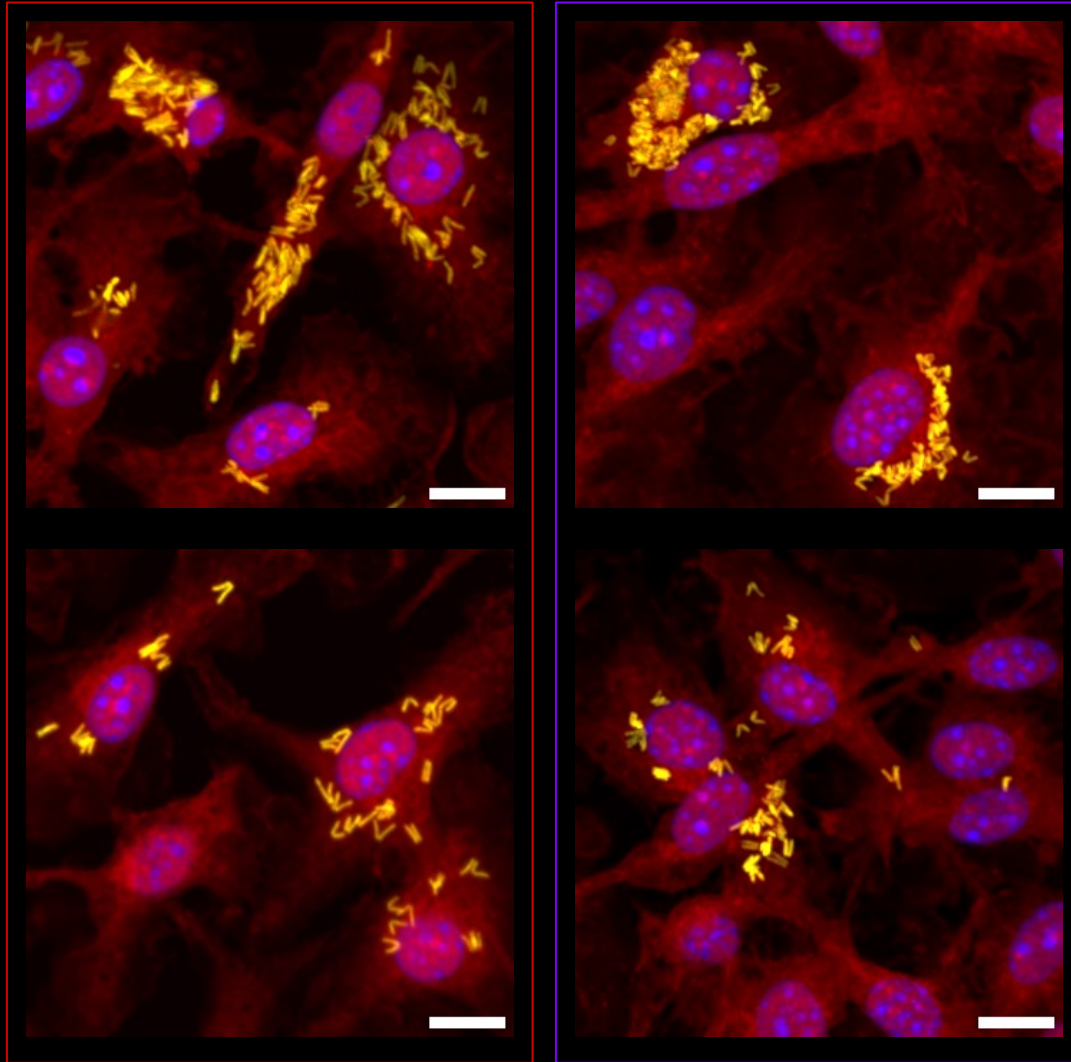
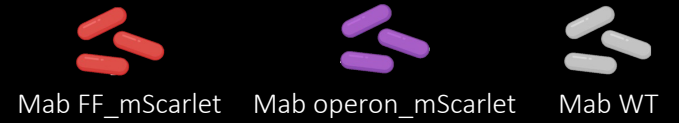


Antibiotic	Mab FF_mScarlet		Mab operon_mScarlet		Mab WT	
	MIC interval (µg/mL)	IC ₉₉ (µg/mL)	MIC interval (µg/mL)	IC ₉₉ (µg/mL)	MIC interval (µg/mL)	IC ₉₉ (µg/mL)
Amikacin	[8,32]	12.96	[8,32]	10.71	[8,16]	31.42
Linezolid	[4,32]	28.67	[2,8]	2.41	[4,32]	n.c.
Moxifloxacin	[2,8]	3.64	[2,4]	1.04	[2,8]	4.91
Clarithromycin	[0.5,1]	1.36	[0.5,1]	1.58	[0.5,1]	n.c.

The IC₉₉ by luminescence (reporter strains) correlates better with the MIC than the IC₉₉ by resorufin fluorescence (WT)

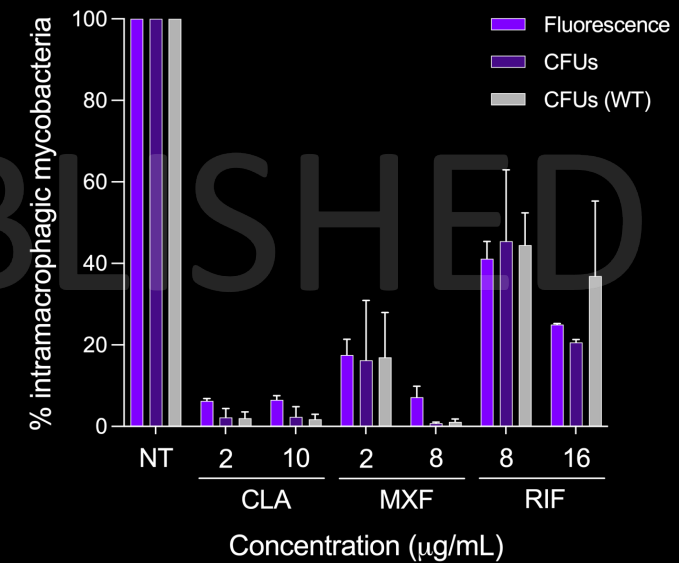
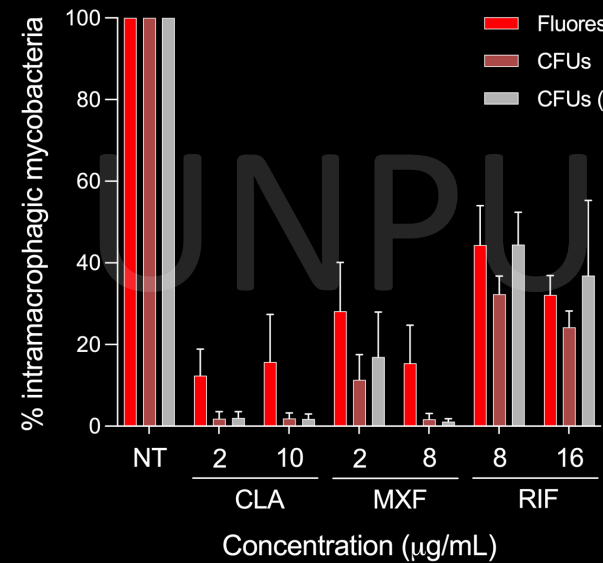
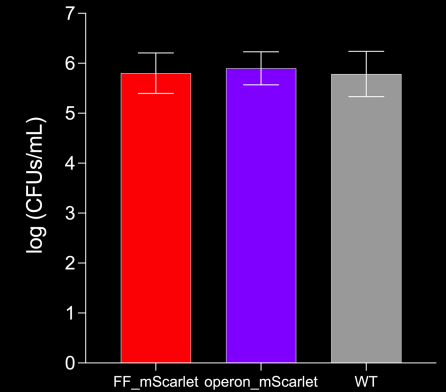
Z prime factor > 0.5 supports the use of the assay for high-throughput drug screening

VI Infection of macrophages

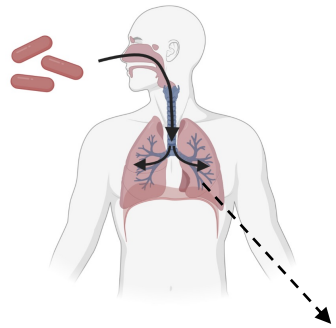


Mab – yellow Macrophages – red Nuclei – blue Confocal 63x Scale: 10 μ m

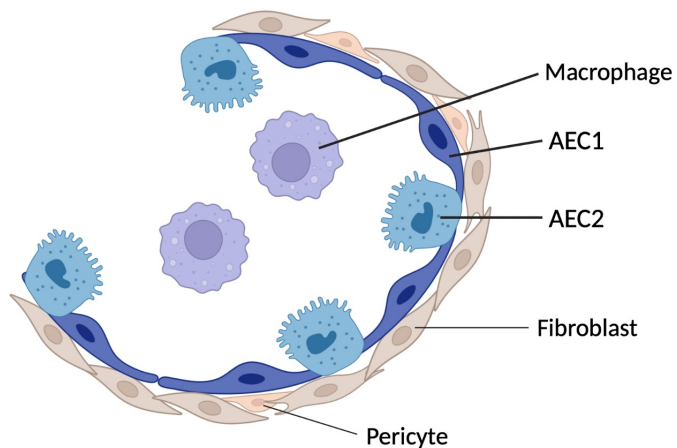
The double-reporter Mab strains can infect mammalian host cells and the intracellular bacterial load can be assessed by fluorescence



VII Infection of lung organoid-derived cells

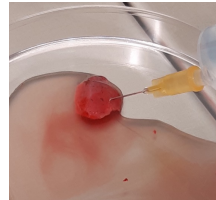


Human alveolus



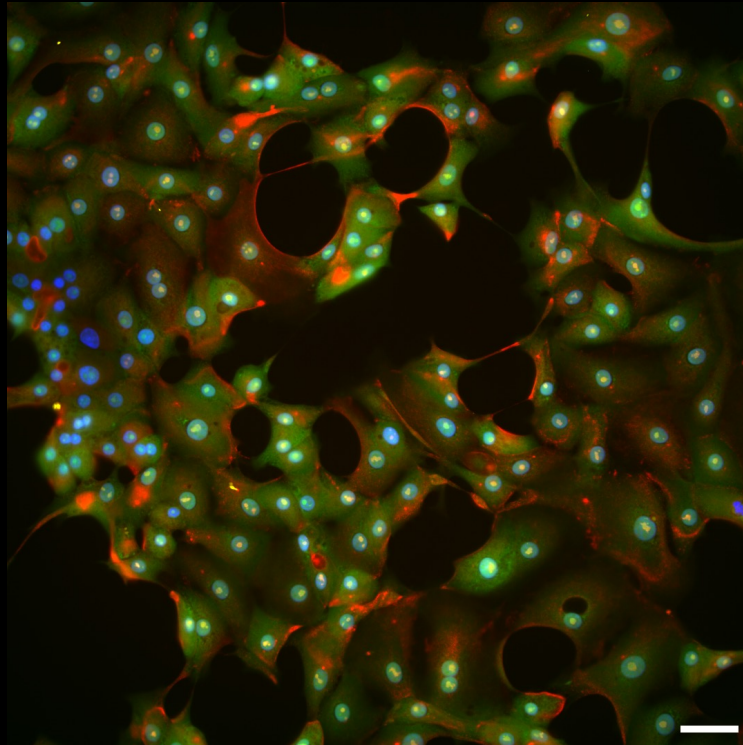
AEC1 - alveolar epithelial type I cell
AEC2 - alveolar epithelial type II cell

Lung biopsies from CHUSJ



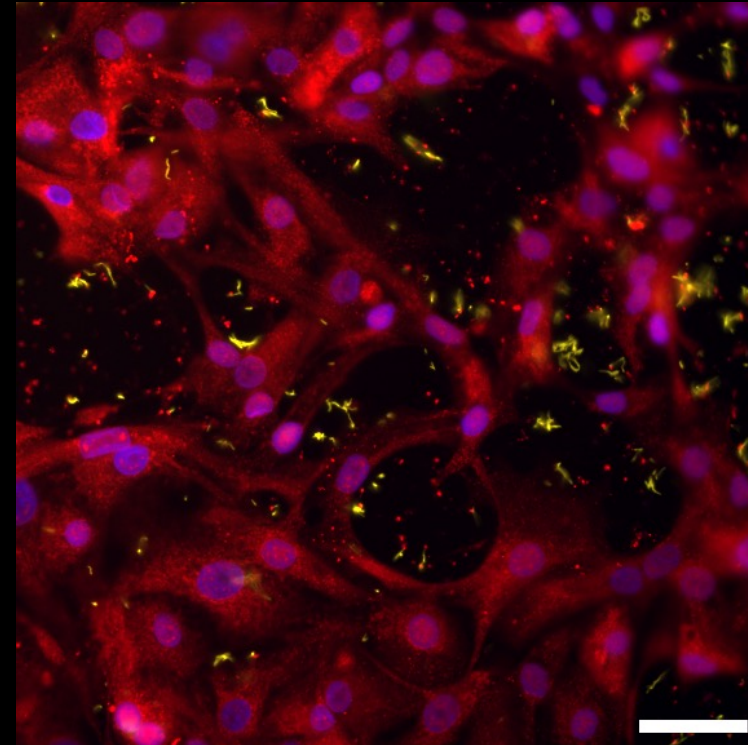
VII Infection of lung organoid-derived cells

Mab infects around 20% of the alveolar cells



Mab FF_mScarlet – yellow
GPRC5a (AEC1) – red
Cell mask - green
Nuclei – blue

Scale: 50 μ m



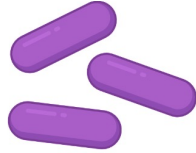
Mab FF_mScarlet – yellow
Pro-SPC (AEC2) – red
Nuclei – blue

Scale: 50 μ m

VIII Conclusions



Mab FF_mScarlet



Mab operon_mScarlet

The new double-reporter Mab strains are useful tools for drug discovery

- ✓ Luminescence can be used for determining the drugs' activity with high sensitivity
- ✓ Fluorescence is a reliable measure of intracellular bacterial load
- ✓ Maintain the characteristics of the non-transformed strain

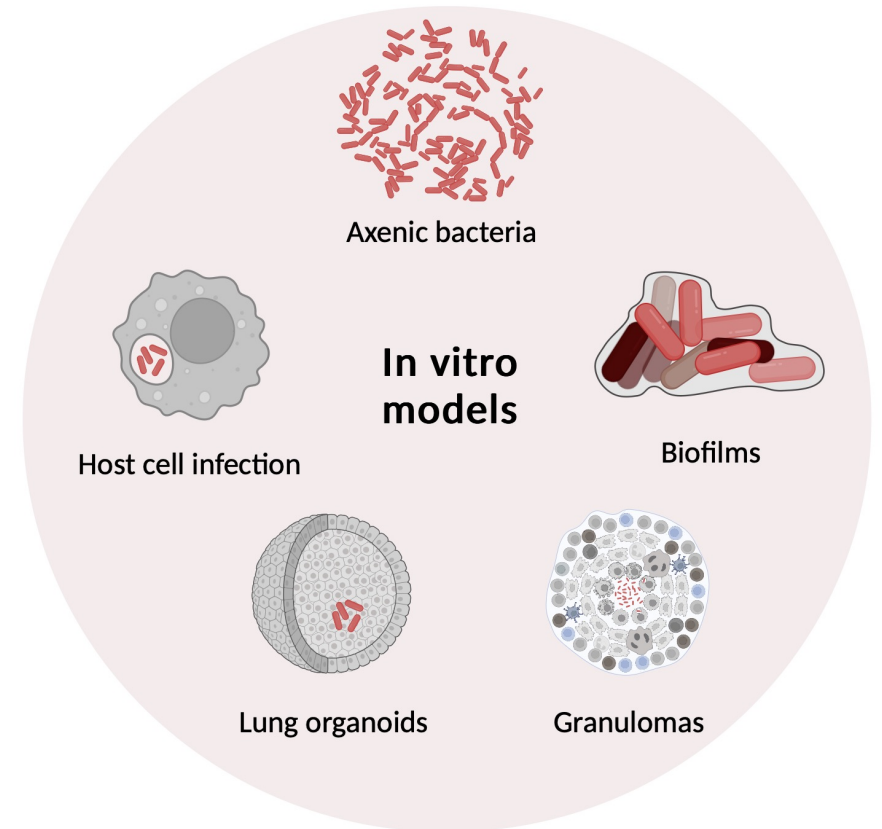


Image created with BioRender

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