The 3rd International Electronic Conference on Antibiotics
Rise of Antibiotic Resistance: Mechanisms Involved and Solutions to Tackle it

Exploring Aldehydes as PQS System Targeting Agents to Combat Pseudomonas aeruginosa Biofilm-Associated Infections

Miguel Leitão, PhD student in Chemical and Biological Engineering (3rd cycle of studies)

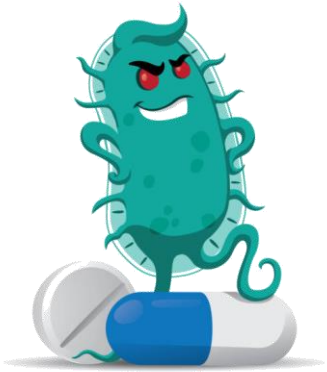
Supervisor: Anabela Borges (PhD)
Co-supervisors: Manuel Simões (PhD) and Fernanda Borges (PhD)

1–15 Dec 2023

U.PORTO FEUP FACULDADE DE ENGENHARIA UNIVERSIDADE DO PORTO
ALiCE
Lepabe Laboratory for Process Engineering, Environment, Biotechnology and Energy
Biofilm Engineering Lab

BIOFILMS: AN EMERGENT PROBLEM

Medicine | Agriculture | Industry | Environment

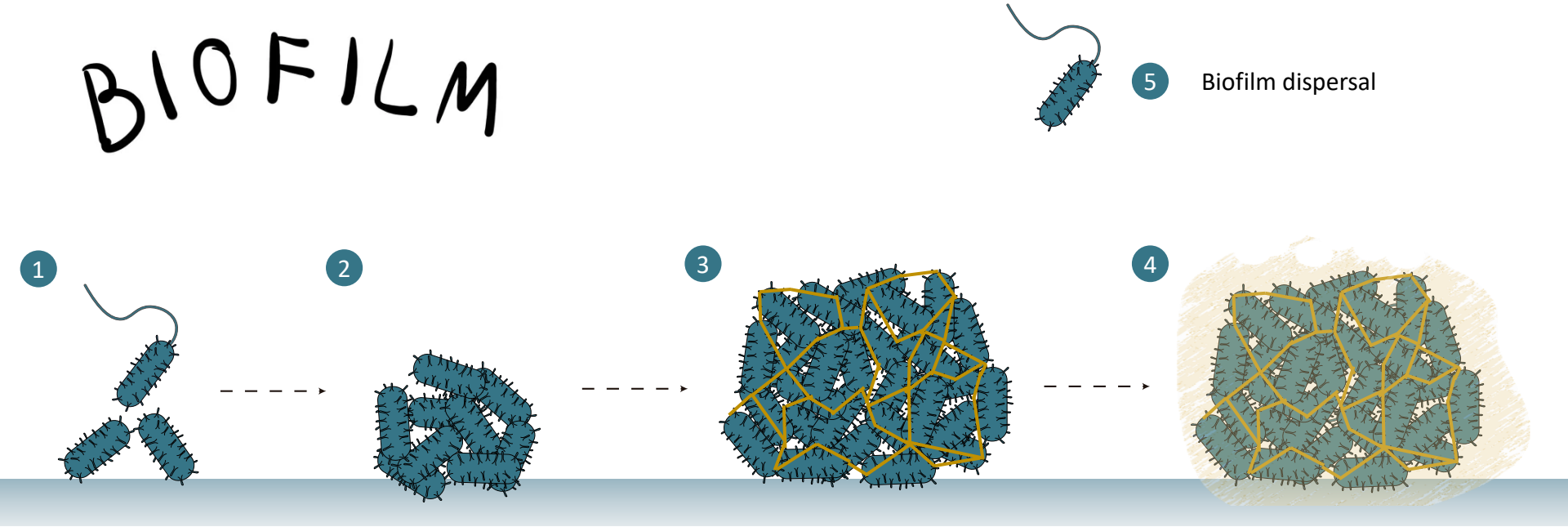


- Responsible for 80% of bacterial infections in humans;
- Their structure and physiology make bacteria 10 to 1000 times more resistant to antibacterial agents;
- Recalcitrance to antibacterial agents and host immunity.

No drug or treatment is completely effective for the treatment of biofilm-related infections

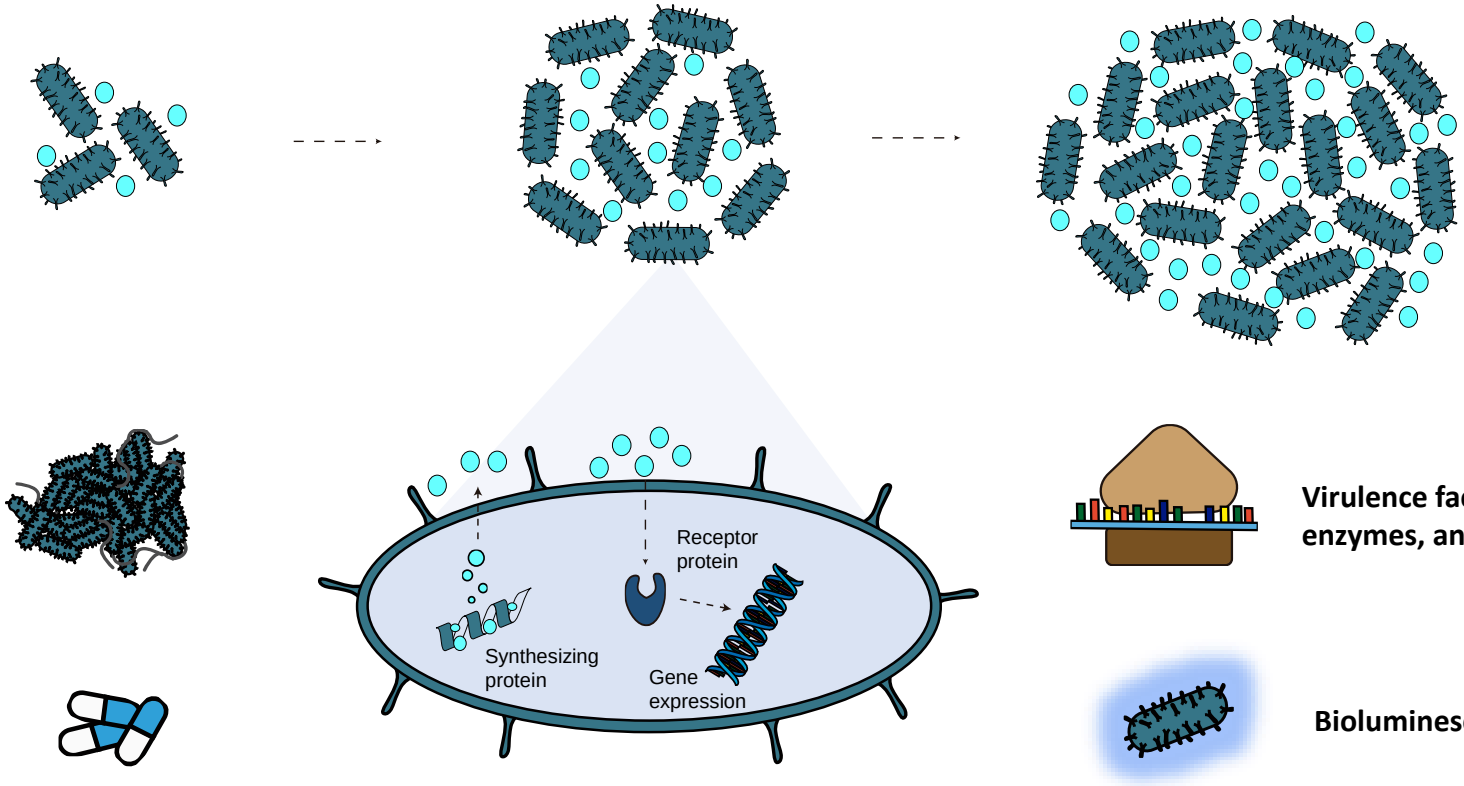
Borges *et al*. 2016. "New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms". Molecules; Gonçalves *et al.* 2023. "The action of phytochemicals in biofilm control." Natural product reports.

BIOFILM FORMATION



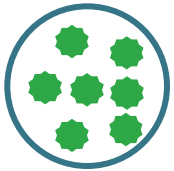
Borges *et al*. 2015. "Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents." Current medicinal chemistry.

QUORUM SENSING



Gonçalves et al. 2023. "The action of phytochemicals in biofilm control." Natural product reports; Borges *et al*. 2017. “Furvina inhibits the 3-oxo-C12-HSL-based quorum sensing system of *pseudomonas aeruginosa* and QS-dependent phenotypes” Biofouling.

NATURAL PRODUCTS: PHYTOCHEMICALS



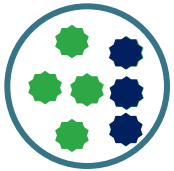
Phytochemicals

- Source of chemical diversity
- Chemical stable
- Multiple targets
- Eco-friendly (“green”)
- Natural and sustainable source

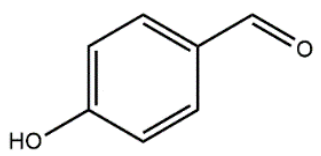
Simões *et al.* 2009. “Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms” Natural product reports; Borges *et al*. 2016. "New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms." Molecules.

NATURAL PRODUCTS: PHYTOCHEMICALS

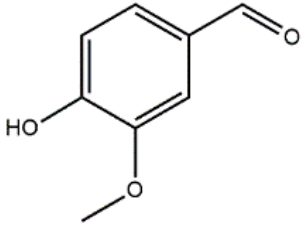
Aldehydes



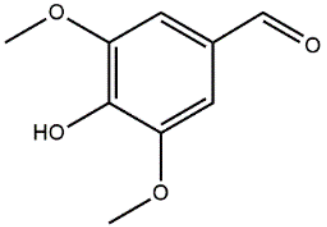
Phytochemicals



***P*-hydroxybenzaldehyde**



Vanillin



Syringaldehyde

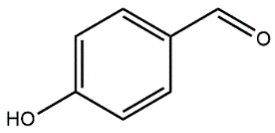
Simões *et al.* 2009. “Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms” Natural product reports; Borges *et al*. 2016. "New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms.” Molecules.

AIM

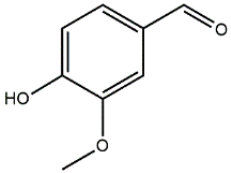
Development of new aldehyde-antibiotic combinations to control *Pseudomonas aeruginosa* biofilms by interfering with pseudomonas quinolone signal system

Assessment of the potential of aldehydes as PQS inhibitors

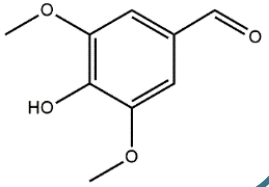
P-hydroxybenzaldehyde



Vanillin



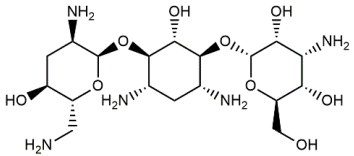
Syringaldehyde

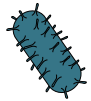


P. aeruginosa
ATCC 10145

MIC > 1000 µg mL^{-1}

Tobramycin



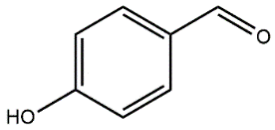


P. aeruginosa
ATCC 10145

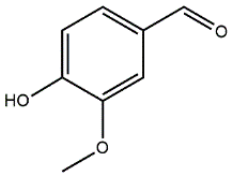
MIC = 8 µg mL^{-1}

Assessment of the potential of aldehydes as PQS inhibitors

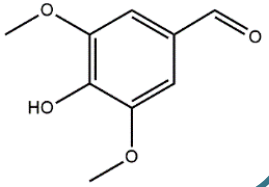
P-hydroxybenzaldehyde



Vanillin



Syringaldehyde

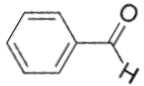


QS Assays

P. aeruginosa PAO1L (WT)

P. aeruginosa PAO1L-CTX

MIC > 1000 µg mL^{-1}

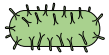


Aldehyde

P. aeruginosa PAO1L (WT)

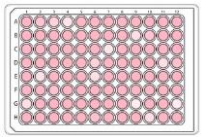
Incubated

Filter

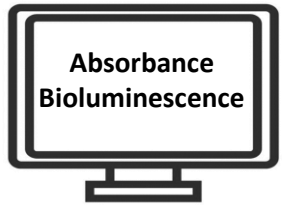


P. aeruginosa PAO1L-CTX

Incubated



4h



Assessment of the potential of aldehydes as PQS inhibitors

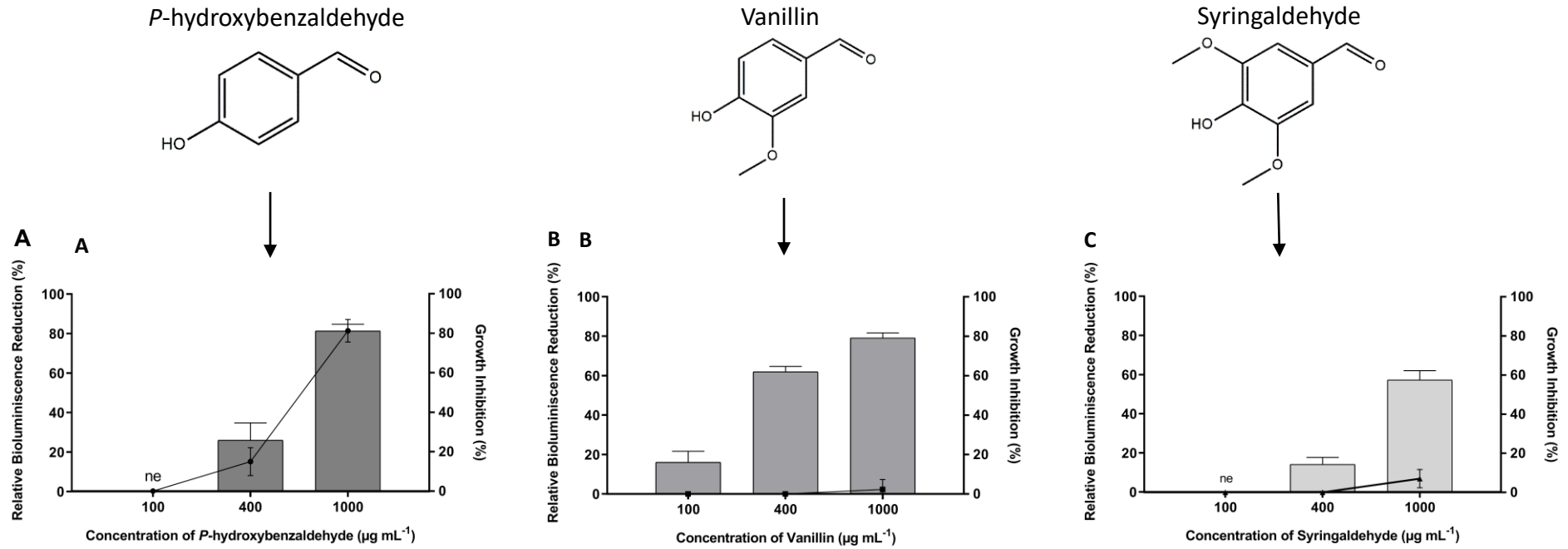
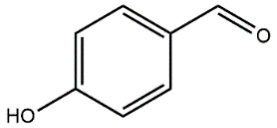


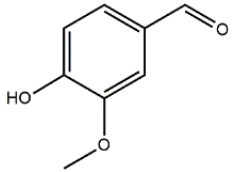
Figure 1. Effect of increasing concentrations of *P*-hydrorxybenzaldehyde (A), Vanillin (B), and Syringaldehyde (C) (100, 400, and 1000 µg mL^{-1}) on the *P. aeruginosa* pqs system (primary y-axis; bars) and on growth inhibition (secondary y-axis; dashed line), ne = no effect. Values are the means ± standard deviations of three independent experiments.

Assessment of the potential of aldehydes as PQS inhibitors

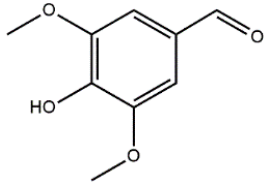
P-hydroxybenzaldehyde



Vanillin



Syringaldehyde



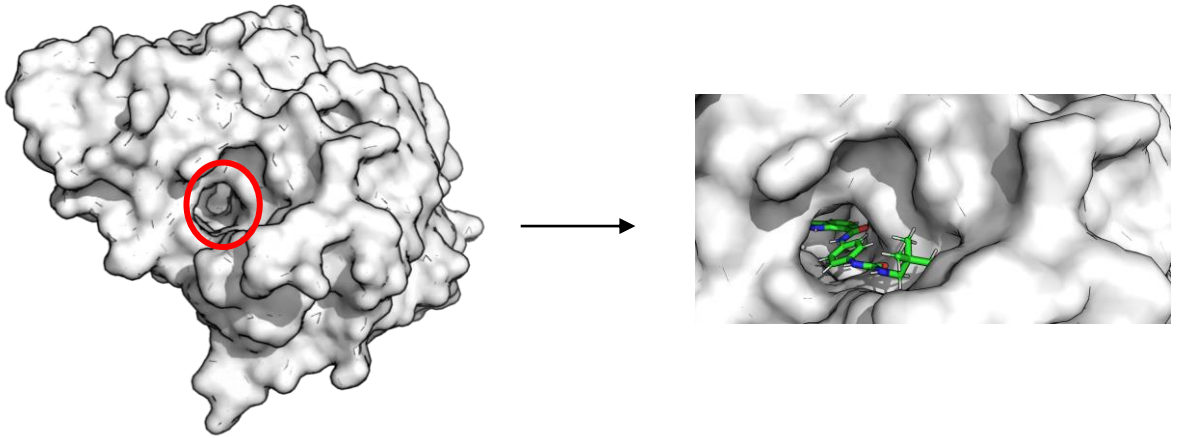
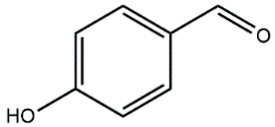


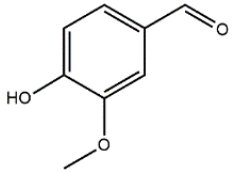
Figure 2. PqsE (Protein 7tza obtained from protein data bank) and simulations in the PqsE to show binding mode of reference ligand.

Assessment of the potential of aldehydes as PQS inhibitors

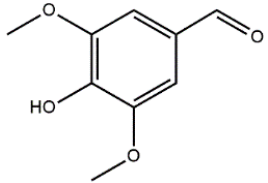
P-hydroxybenzaldehyde



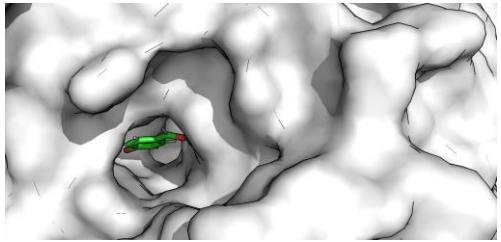
Vanillin



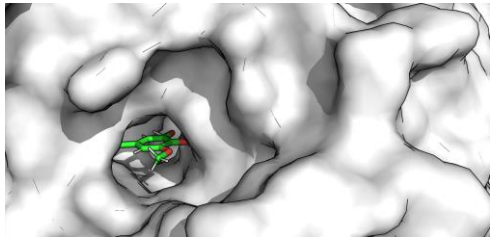
Syringaldehyde



A



B



C

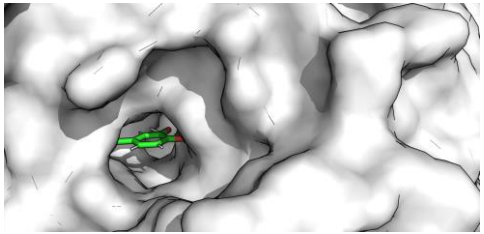
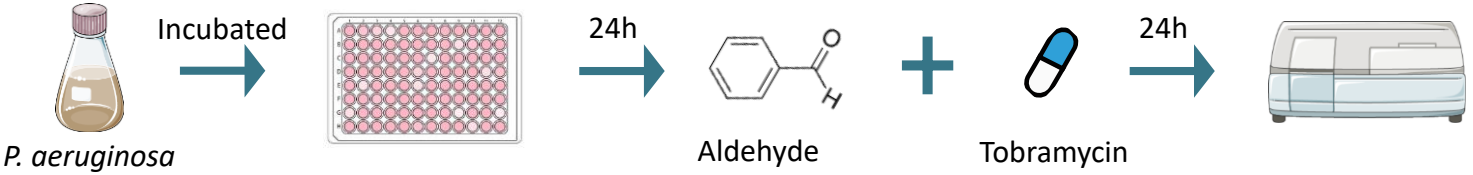


Figure 3. Simulations in the PqsE to show binding mode of *P*-hydrorxybenzaldehyde (A), Vanillin (B), and Syringaldehyde (C).

Depending on the concentration, vanillin and syringaldehyde were able to inhibit the PQS system of *P. aeruginosa*

Biofilm Control Evaluation



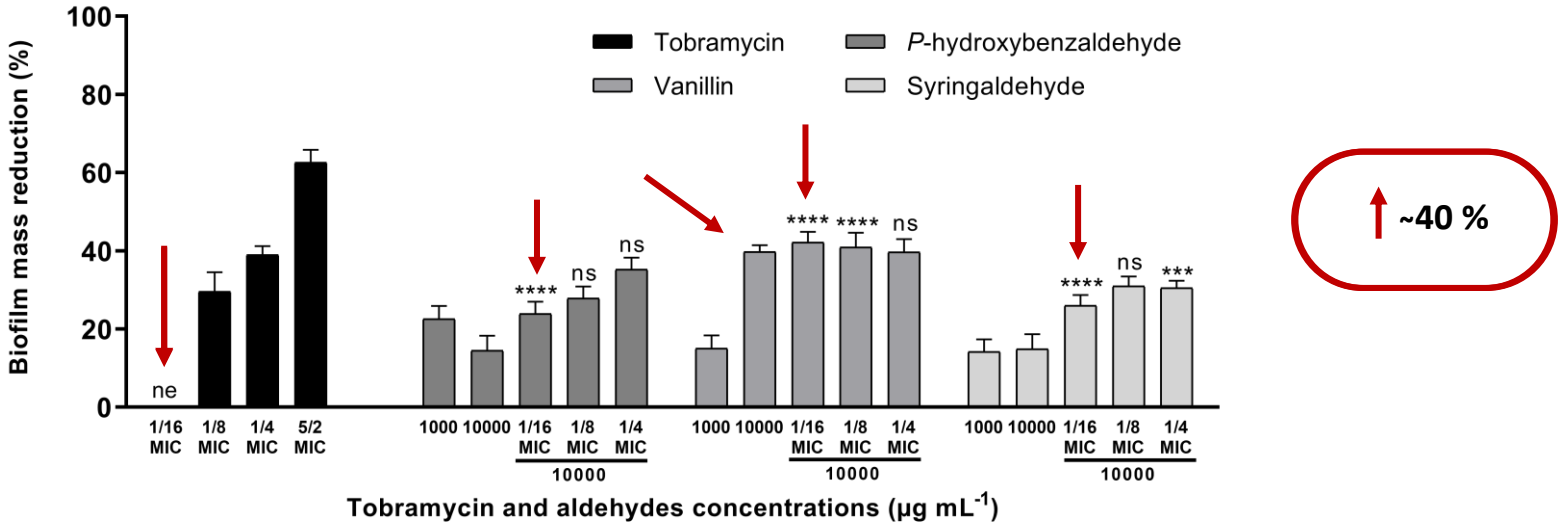
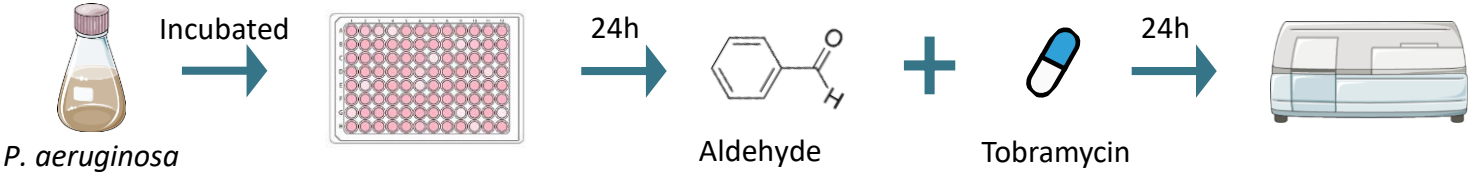
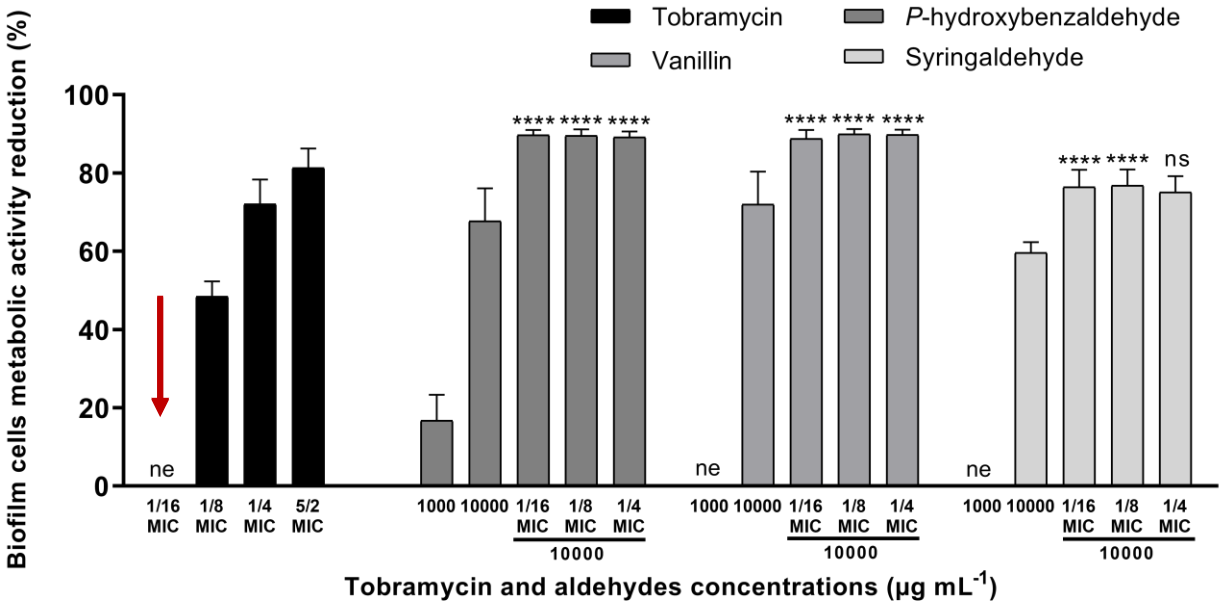


Figure 4. Effect of antibiotic tobramycin, aldehydes, and their combination (tobramycin + aldehyde) against pre-established 24 h old *P. aeruginosa* biofilms, in terms of biomass removal. Bars with '*' are statistically different from tobramycin alone for a confidence level greater than 95 % ($p < 0.05$), ne= no effect, ns = non-significant. Values are the means ± SDs of three independent experiments.

Biofilm Control Evaluation

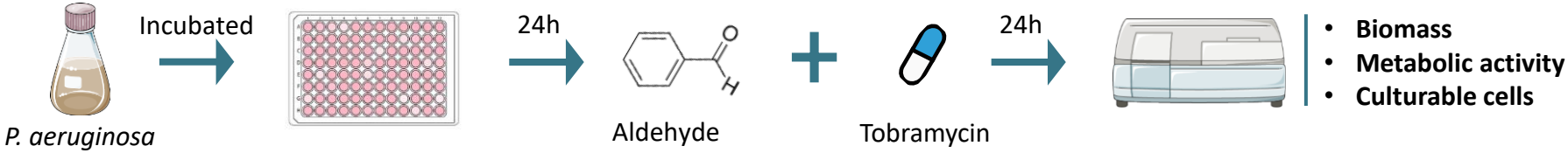




~90 %

Figure 5. Effect of antibiotic tobramycin, aldehydes, and their combination (tobramycin + aldehyde) against pre-established 24 h old *P. aeruginosa* biofilms, in terms of metabolic activity. Bars with '*' are statistically different from tobramycin alone for a confidence level greater than 95 % ($p < 0.05$), ne= no effect, ns = non-significant. Values are the means ± SDs of three independent experiments.

Biofilm Control Evaluation



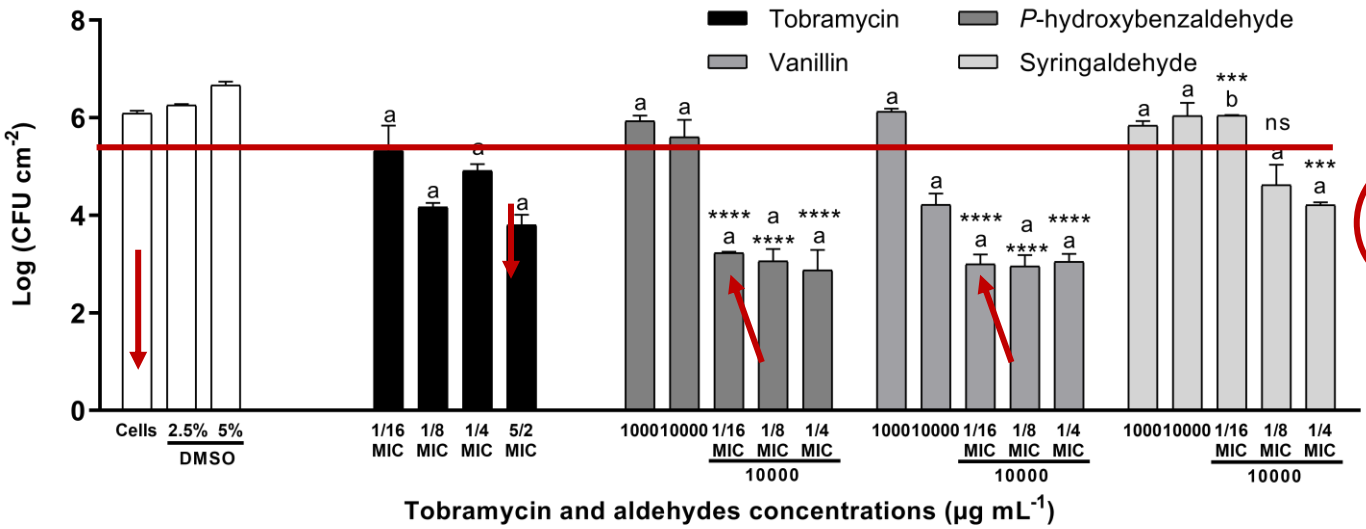
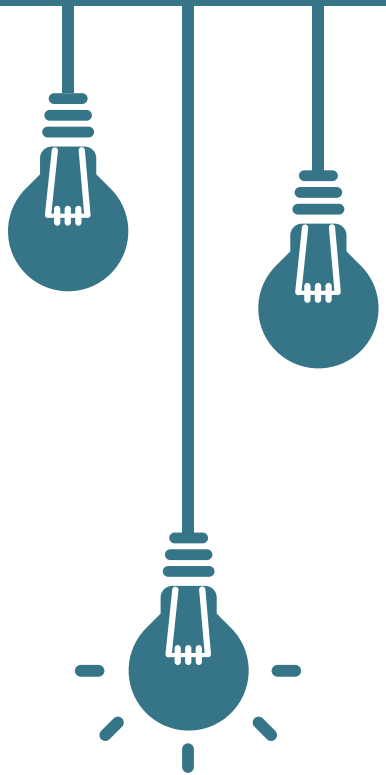


Figure 6. Effect of antibiotic tobramycin, aldehydes, and their combination (tobramycin + aldehyde) against pre-established 24 h old *P. aeruginosa* biofilms, in terms of biofilm culturable cells (log CFU per cm^2) . Bars with '*' are statistically different from tobramycin alone for a confidence level greater than 95 % ($p < 0.05$), ne= no effect, ns = non-significant. Bars with lowercase letters are statistically different from the control (biofilms without treatment and exposed to DMSO at 5 % or 2.5 %) for a confidence level greater than 95 % ($p < 0.05$, where a = **** and b = ns). Values are the means ± SDs of three independent experiments.

CONCLUSIONS



1. Aldehydes inhibit the PQS system depending on the concentration used;
2. Molecular docking studies support the potential of aldehydes as potent QS inhibitors;
3. The combined use of aldehydes with tobramycin increases its efficacy;
4. The concentration of the antibiotic is reduced.

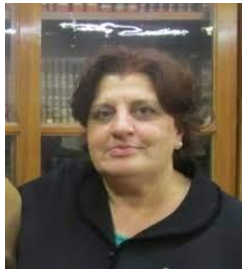
Aldehydes are promising as QS inhibitors and enhancers of antibiotic antibiofilm activity against *P. aeruginosa*

ACKNOWLEDGEMENTS

- This work was supported by: LA/P/0045/2020 (ALiCE) and UIDP/00511/2020 (LEPABE) funded by national funds through FCT/MCTES (PIDDAC);
- Miguel thanks the FCT, for the financial support of her PhD fellowship (2021.07145.BD);
- Anabela Borges thanks FCT, for the Scientific Employment Stimulus—Individual Call—[CEECIND/00823/2021];
- Biofilm engineering Lab (BEL);

Anabela Borges
(Junior Researcher - LEPABE/FEUP)

Manuel Simões
(Associate Professor with Habilitation - LEPABE/FEUP)



Fernanda Borges
(Associate Professor with Habilitation – CIQ(UP)/FCUP)

Thank you for your attention!