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ENZYME DIRECTED EVOLUTION BY EP-PCR FOR THE TREATMENT AGAINST *PSEUDOMONAS AERUGINOSA* INFECTIONS IN CYSTIC FIBROSIS

Lluïsa Mas Fons
Bachelor's Degree in Biotechnology

Research proposal

BACKGROUND

Cystic fibrosis (CF) affects more than 80.000 people around the world. ***Pseudomonas aeruginosa*** (PA) is the most prevalent and common bacteria causing infections in CF patients[1] and it is difficult to eradicate due to the formation of **biofilms** that enhance **antimicrobial tolerance**[2].

A large number of **enzymes** with **biofilm disruptive** activity have been identified[3]. Despite that, most of them present low stability and not enough disruptive capacity to be applied as a treatment. Thus, **directed evolution** is an interesting approach to obtain efficient **therapeutic enzymes** to treat PA infections in CF patients.

HYPOTESIS

The use of **error-prone PCR** in the development of mutants could allow the obtention of efficient **therapeutic enzymes** for the treatment of infections of ***P. aeruginosa*** in **cystic fibrosis** patients.

OBJECTIVES

- Selection of the 4 candidates with more effect in PA biofilm disruption.
- Generation of enzymatic mutants with enhanced stability, specificity and biofilm disruption activity.
- Assessment of mutant efficiency in *Cftr*^{tm1Unc} Tg(FABPCFTR)1Jaw/J mice.

EXPERIMENTAL DESIGN

1. SELECTION OF ENZYMATIC CANDIDATES. Enzyme candidates will be incubated with PAO1 biofilms. Biofilm disruption will be assessed by Laser Confocal Scanning Microscopy (LCSM). Elastin production will be quantified by Elastin Congo-Red (ECR) assay. Pyocyanin production will be quantified by spectrophotometry. The 4 candidates with higher efficiency will be selected.

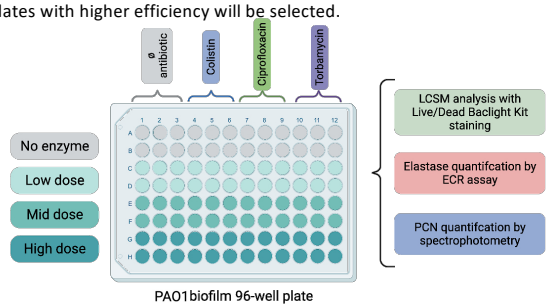

Figure 1. Representation of the enzymatic selection.

2. MUTANT GENERATION. Mutants will be generated by Error-Prone PCR using the DNA polymerase Mutanzyme II. PCR products will be cloned in a pET-28 vector, previously digested. The construction will be transferred by electroporation to *Escherichia coli* BL21 (DE3) strain its expression.

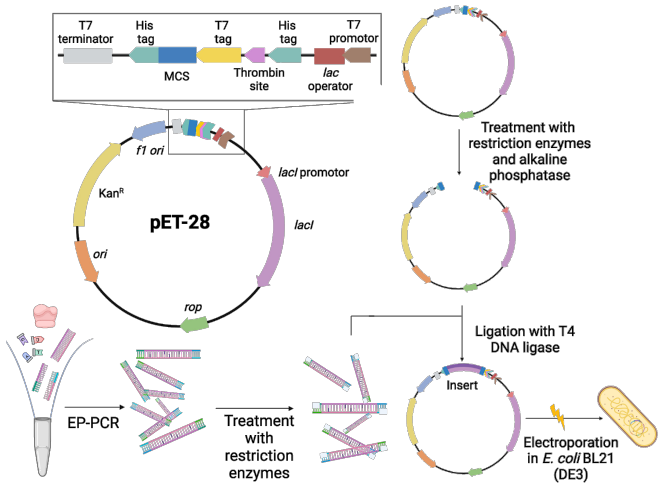

Figure 2. Representation of the process of mutant generation.

3. MUTANT SCREENING. Transformants will be selected in LB agar plates supplemented with kanamycin. Mutants will be transferred in 96-well plates with LB supplemented with kanamycin, where protein production will be induced by adding IPTG. Cell lysis will be performed by sonication, and the cell lysate will be added to 96 well-plates with PAO1 biofilms. Biofilm disruption will be analysed by LCSM.

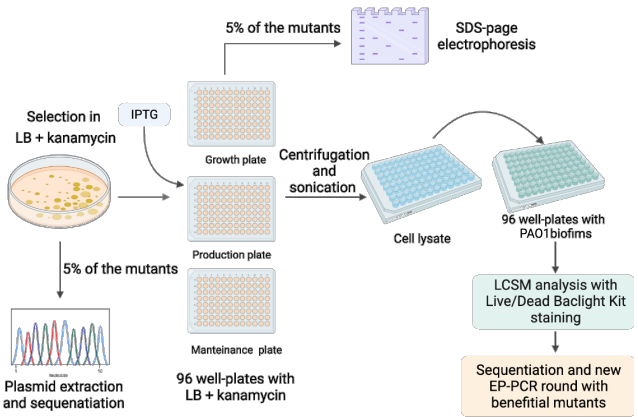

Figure 3. Diagram of the screening process.

4. PURIFICATION, SELECTION ANALYSIS AND *IN VIVO* STUDIES. Selected mutants will be recombinantly produced in *E. col* BL21 (DE3) and purified by Ni-NTA affinity chromatography. PCN quantification, LCSM analysis and ECR assay will be repeated. PAO1 infected *Cftr*^{tm1Unc} Tg(FABPCFTR)1Jaw/J mice will be exposed to purified enzymes. Bronchoalveolar lavage (BAL), bacterial quantification (BQ) and lung histopathologic (LH) studies will be performed.

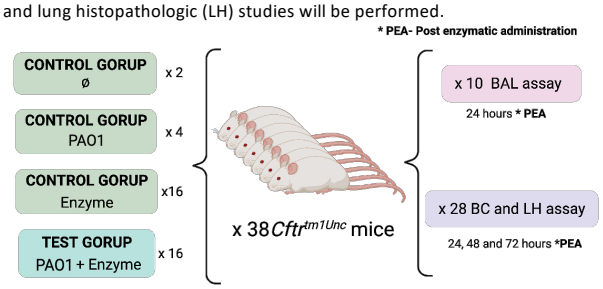

Figure 4 . Diagram of *in vivo* studies.

EXPECTED RESULTS AND PERSPECTIVES

- Enzymes with higher stability and enhanced antibiofilm activity.
- Reduction on the pyocyanin and elastase production ⟶ less virulence.
- Biofilms with higher sensibility to antibiotics.
- Reduction of *Pseudomonas aeruginosa* infection in *Cftr*^{tm1Unc} Tg (FABPCFTR) 1Jaw /J mice test group.
- Future use as therapeutic enzymes and/or coatings in medical equipment.

DISSEMINATION PLAN

- **Publications** in scientific journals focused on microbiology, nosocomial infections and molecular biology.
- Results presentation in national and international **conferences** such as the annual congress of the *Sociedad Española de la Fibrosis Cística*.

REFERENCES

1. Gbian, D. L. & Omri, A. Current and novel therapeutic strategies for the management of cystic fibrosis. *Expert Opin. Drug Deliv.* 00, 1–18 (2021).
2. Sousa, A. M. & Pereira, M. O. Pseudomonas Aeruginosa diversification during infection development in cystic fibrosis Lungs-A review. *Pathogens* 3, 680–703 (2014).
3. Thi, M. T. T., Wibowo, D. & Rehm, B. H. A. Pseudomonas aeruginosa biofilms. *Int. J. Mol. Sci.* 21, 1–25 (2020).
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