Alginate-degrading bacteriophages as a therapeutic treatment against *Pseudomonas aeruginosa* in cystic fibrosis

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**BACKGROUND**

- *Pseudomonas aeruginosa* can cause up to 20% of nosocomial infections [1].
- *P. aeruginosa* colonizes the lung of cystic fibrosis patients.
- *P. aeruginosa* is difficult to control because its resistance to many antibiotics and disinfectants, mainly due to efflux pumps and biofilm [2].
- Phage therapy has many advantages over antibiotics but needs further research.
- The use of genetically-modified phages is an interesting site-directed treatment.

**EXPECTED RESULTS**

- Reduction in viable cell numbers and total biomass in both early and mature biofilms.
- Significant increase in mice survival in the infected and treated group when compared with non-treated group.
- Cytokine and lactate dehydrogenase levels should be reduced, as well as bacteriophage and viable cell counts, when compared with non-treated group.

**INITIAL HYPOTHESIS AND OBJECTIVES**

- A cocktail composed by *P. aeruginosa* bacteriophages overexpressing an alginate-degrading enzyme could be an effective treatment in cystic fibrosis patients colonized by this bacterium.
- The aim of this project is to design a phage cocktail based in several lytic and non-transductant phages encoding an alginate lyase, and evaluate its safety and effectiveness in vitro and in vivo.

**EXPERIMENTAL DESIGN**

1. The Step-by-step method (SBS) will be used for the selection of infective phages against each resistant strain that could appear. The method ends when last phage-resistant mutant is sensible to the first phage [3].

   - **Bacteriophage**
     - *P. aeruginosa* culture
     - Selective pressure
     - *P. aeruginosa* death
     - Phage1
     - Phage2
     - Phage3
     - Phage1 resistant
     - Phage2 resistant
     - Phage3 resistant

   **Figure 1:** Representation of the SBS method.

2. Bacteriophage Recombining of Electroporated DNA (BRED) will be conducted for gene insertion in the phage genome. The genome and the desired gene are co-electroporated in a strain with a recombination system [4].

   - **Phage genome**
     - DNA purification
     - algl gene
     - Phage1
     - Phage2
     - Phage3

   **Figure 2:** Representation of BRED Technology.

3. **In vitro biofilm and cell viability analysis** will be performed in microtiter plates and continuous flow cells. Total biomass quantification and cell viability will be determined by Syto9 and FDA assays, respectively.

   - **P. aeruginosa** culture (Pa)
   - Phage cocktail (Pc)
   - Syto9 assay
   - FDA assay

   **Figure 3:** Diagram of in vitro analysis of early (up) and mature (down) biofilms.

4. For **in vivo** determination of safety and effectiveness, survival rate of mice will be determined as well as quantification of cytokines, LDH, phages and bacteria in bronchoalveolar fluids.

   - **TEST GROUP**
     - *P. aeruginosa* + phage-cocktail
   - **CONTROL GROUP**
     - *P. aeruginosa* + Phage cocktail

   **Figure 4:** Diagram of in vivo analysis and treatment.

**REFERENCES**