

P-190 - COMBINED TREATMENT WITH BACTERIOPHAGES AND ANTIBIOTICS AS A STRATEGY TO CONTROL BIOFILM ASSOCIATED INFECTIONS

Ergun Akturk¹; Hugo Oliveira¹; Luis D. R. Melo¹; Joana Azeredo¹

1 - CEB-Center of Biological Engineering, LIBRO-Laboratório de Investigação em Biofilmes Rosário Oliveira, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Background

Bacterial biofilms are sessile microbial aggregates with unique community properties, showing a high degree of tolerance/resistance to disinfection by chemicals, antibiotics, and to the human immune system. The opportunistic pathogen *Pseudomonas aeruginosa* is one of the most frequent causes of biofilm-associated infections, causing infections extremely difficult to treat. Currently, bacteriophages (phages) that are specific for pathogenic bacteria are becoming a potential solution for the treat such infections.

Method

In this study, *P.aeruginosa* biofilms were formed and subjected to treatment by *P.aeruginosa* phage vB_PaM_EPA1 (EPA1) alone or in combination with antibiotics (gentamicin, ciprofloxacin, meropenem) of different classes. EPA1 was isolated from the effluent from a wastewater treatment plant. TEM images show that this phage belongs to the *Myoviridae* family. Its genome has a 91,3 kb, a GC content of 49.2% and encodes 178 putative genes, from which 147 have no predicted function. Phage and antibiotics with different multiplicity of infection (MOI) and minimal inhibitory concentration (MIC), respectively, were simultaneously or sequentially (phage suspension was added first then antibiotic were added with a delay of 6 hours) added to 48 hours old-biofilms. After 24-hour treatment, bacterial survival was measured by colony forming unit (CFU) counting method.

Results & Conclusions

Results showed that in individual treatments of phage (at MOI1) and antibiotics (at MIC) generally had significant reductions on the number of viable cells ranging from 0.5 to 3,7 logs. However, when they were sequentially added to the biofilms, a synergistic effect (>8 logs) was detected, namely with phage-gentamicin and phage-ciprofloxacin combinations. In opposition, an antagonistic effect was detected when phage and ciprofloxacin were simultaneously added. Overall, our results show that combination of phages and antibiotics are very effective against *P.aeruginosa* biofilms particularly when they are applied sequentially and this constitutes a good strategy to control biofilm-associated infections.

References & Acknowledgments

- Chaudhry, W. N., Concepción-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., Levin, B. R., ... Gupta, S. (2017). Synergy and Order Effects of Antibiotics and Phages in Killing *Pseudomonas aeruginosa* Biofilms. *Plos One*, *12*(1), e0168615.
- Henry, M., Bobay, L.-M., Chevallereau, A., Sausseureau, E., Ceyssens, P.-J., & Debarbieux, L. (2015). The Search for Therapeutic Bacteriophages Uncovers One New Subfamily and Two New Genera of *Pseudomonas*-Infecting Myoviridae. *PLOS ONE*, *10*(1), e0117163.
- Ives, D. R., Perez-Esteban, P., Kot, W., Bean, J. E., Arnot, T., Hansen, L. H., ... Jenkins, A. T. A. (2016). A novel bacteriophage cocktail reduces and disperses *Pseudomonas aeruginosa* biofilms under static and flow conditions. *Microbial Biotechnology*, *9*(1), 61–74.

Keywords: Bacteriophage, biofilm, antibiotic, treatment