DUAL SPECIES BIOFILMS AND THEIR CONTROL WITH BACTERIOPHAGES

S. Sillankorva[1,2], P. Neubauer[3], J. Azeredo[1]

[1] IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Braga, Portugal; [2] Bioprocess Engineering Laboratory, Department of Process and Environmental Engineering and Biocenter Oulu, University of Oulu, Finland; [3]Department of Biotechnology, Technische Universität Berlin, Berlin, Germany

AIMS

Although several strategies are daily adopted to prevent biofilm formation and their removal, biofilms do persist in a wide range of industrial surfaces. This is largely due to biofilm tolerance toward antimicrobial agents and therefore possible alternative controlling agents are being investigated. Bacteriophages (phages) are bacterial viruses which, today, are seen as good controlling agents due to their specificity, efficacy against biocide resistant bacteria, innocuousness to the environment and are able to self-reproduce as long as their host bacterium are present. Also, the production of phages is fast, simple and relatively inexpensive. Despite the outcomes of several studies with phages which shown that they are capable of controlling their host population grown in monospecies biofilms, there are limited studies of phage interaction with their target bacterium present within dual species biofilms. Here we characterize dual species bacterial biofilms formed by Gram-negative (*Pseudomonas fluorescens)* and Gram-positive bacteria (*Staphylococcus lentus)*, and their infection with phages.

METHODS

Biofilms were formed on stainless steel slides immersed on microplates for 24 and 72 hours and under the influence of different conditions: with/without shaking and with/without medium renewal every 12 hours. After, the formed dual species biofilms were exposed to phages using two strategies: a phage cocktail for each species present or a single phage (phiIBB-PF7A) for the less predominant bacterium (*P. fluorescens*) of the biofilm consortia. The viable cells on biofilms and the amount of phages were enumerated after 2 and 4 hours of biofilm exposure to phages.

RESULTS

Infection with the phage cocktail was very effective and the biofilms were well removed from the substratum. Additionally, the phage cocktail also controlled the bacteria which had been released from the biofilms to the planktonic phase. Regardless of the low amounts of *P. fluorescens* observed in dual species biofilms, this study evidenced that phage phiIBB-PF7A easily reached its' target host and the encounter was not influenced by the presence of a non-susceptible bacterium (S. *lentus*). After 4 hours of phiIBB-PF7A application to the biofilm consortium, the number of *P. fluorescens* cells on the biofilms was highly reduced and there was an increase of the quantity of planktonic cells from 2 to 4 hours, most likely due to the release of *S. lentus*, the non-susceptible host, from the partially damaged dual species biofilms.

CONCLUSIONS

This study evidences that phages are well capable of reducing and controlling the target hosts present in dual species biofilms, however phage treatment depends of the phage application strategy employed.