How do surface material and hydrodynamics shape horizontal gene transfer in biofilms?

Marta M. Ferreira[1,2], Ana Azevedo[1,2], Rita Teixeira-Santos[1,2], Mette Burmølle[3], Filipe J. Mergulhão[1,2] and **Luciana C. Gomes**[1,2]

[1]*LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*

[2]*ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*

[3]*Section of Microbiology, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark*

Presenter's e-mail address: luciana.gomes@fe.up.pt

Background: Horizontal gene transfer (HGT) in bacterial biofilms is a significant concern because of the dissemination of antibiotic resistance genes, which hinders biofilm control in medical, industrial, and environmental fields. Microorganisms are nearby within biofilms, facilitating the exchange of genetic material. Several mechanisms, including conjugation, enable HGT within biofilms. Conjugation involves the direct transfer of genetic material from one bacterium to another through physical contact and is mediated by a specialised genetic element called conjugative plasmid. This study aimed to gain a comprehensive understanding of the influence of surface properties, hydrodynamics, and contact time on the conjugation process in dual-species biofilms.

Methods: Escherichia coli carrying the conjugative IncP-1 plasmid pKJK5 was used as the donor strain, and biofilm formation assays were performed under two hydrodynamic conditions (0 and 185 rpm of orbital shaking frequency), using three surface materials with distinct physicochemical properties (polyvinyl chloride, PVC; graphene sheet, GS; and glass, G), for 24 and 72 h. Cell quantification and plasmid transfer frequency were assessed using flow cytometry. Confocal laser scanning microscopy (CLSM) was used as a complementary method to evaluate the spatial organisation of biofilms.

Results: When the donor and recipient strains were co-cultured, hydrodynamics affected the total number of biofilm cells at both incubation times. Concerning the transconjugant numbers and transconjugant/donor ratios, both hydrodynamics and incubation times influenced HGT, with no shaking conditions and longer bacterial contact time promoting plasmid transfer events. Additionally, a positive association between 0 rpm, G surface, number of donors and total biofilm cells, and number of transconjugants on dual-species biofilms was demonstrated using linear regression models. In addition to confirming that static conditions increased the number of transconjugants, confocal microscopy suggested that the transconjugants were mainly located in the innermost regions of the biofilm.

Conclusions: Factors such as hydrodynamics and biofilm age can have a huge impact on plasmid transfer and potentially influence the dissemination of antibiotic resistance in microbial communities.

Keywords: Biofilm; Horizontal Gene Transfer; Conjugation; Plasmid; Antibiotic Resistance Genes.

Acknowledgements: This research was funded by LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), and SurfSAFE project funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 952471. A.A. thank FCT for the financial support of a Ph.D. grant (BD.2020.07427). R.T.-S. acknowledges the receipt of a junior researcher fellowship from project PTDC/CTM-COM/4844/2020 (NanoCAT), supported by national funds through the FCT/MCTES (PIDDAC).