

P-225 - INTERSPECIES INTERACTIONS AND BACTERIOPHAGE CONTROL IN *S. ENTERITIDIS* AND *E. COLI* MIXED BIOFILMS

Catarina Milho¹; Maria Daniela Silva¹; Diana Alves¹; Joana Azeredo¹; Sanna Sillankorva¹

1 - Centre of Biological Engineering, LIBRO – Laboratório de Investigação em Biofilmes Rosário Oliveira, University of Minho, 4710-057 Braga, Portugal

Background

Salmonella Enteritidis and *Escherichia coli* are important foodborne pathogens, commonly related to outbreaks¹. Their ability to form biofilms contributes to their virulence and enables their survival on different food contact surfaces². In mixed biofilms, interspecies interactions can occur, resulting in positive, negative or neutral outcomes to each species^{3,4}. As these microbial communities present great resistance to antimicrobials used in food industries (disinfectants, biocide, antimicrobials), bacteriophages, bacterial viruses, can be regarded as good and safe candidates for biofilm biocontrol⁵. This study aimed at characterizing the interactions established between two *S. Enteritidis* and two *E. coli* strains, previously reported to be strong or weak biofilm producers, respectively, in mixed biofilms. Moreover, the efficacy of a bacteriophage cocktail as a biofilm control agent was assessed.

Method

24h old mono and dual species biofilms, in all possible combinations of weak and strong biofilm producers of *S. Enteritidis* and *E. coli*, were grown under dynamic conditions (120rpm), at 37°C, in 96-well plates. PVP-SE2 and vB_EcoM_CEB1 bacteriophages, which are specific for *S. Enteritidis* and *E. coli*, respectively, were used in the control of these biofilms. For the infection assays, 4, 8 and 24h of treatment were used, and at each time-point, biofilms were disrupted by sonication and the number of viable cells was determined.

Results & Conclusions

Our results showed that the number of viable cells in mono-species biofilms was higher than in dual-species biofilms. Also, there was a tendency for weaker biofilm producers to form stronger biofilms when in the presence of strong biofilm producers, and weaker biofilms when in the presence of weak biofilm producers. Furthermore, the presence of two strong biofilm producers does not benefit any of the species. Regarding the antibiofilm efficacy of the bacteriophage cocktail, the most efficient time of action for both bacteriophages was 8 hours. Overall, higher reductions in bacterial cells numbers were obtained for the *S. Enteritidis* than *E. coli* due to bacteriophage growth characteristics. We suggest that unknown determinants present in *S. Enteritidis* and *E. coli* biofilms formed by strong biofilm producers have a positive influence in the formation of biofilms by weak biofilm producers. Moreover, the bacteriophage cocktail can be used in the control of *S. Enteritidis* and *E. coli* mixed biofilms.

References & Acknowledgments

1. CDC(2012) *Pathogens causing US foodborne illnesses, hospitalizations, and deaths, 2000–2008*.
2. Giaouris *et al.* (2015) *Front Microbiol* **6**,1–26.
3. Esteves *et al.* (2005) *Society* **73**,5198–5203.
4. Wang *et al.* (2014) *J Food Prot* **77**,805–813.
5. Motlagh *et al.* (2016) *World J Microbiol Biotechnol* **32**,1–10.

Financial support is acknowledged to FCT for the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020(POCI-01-0145-FEDER-006684), RECI/BBB-EBI/0179/2012(FCOMP-01-0124- FEDER-027462) and BioTecNorte operation (NORTE-01-0145-FEDER-000004). Catarina Milho is supported by the FCT fellowship SFRH/BD/94434/2013.

Keywords: Biofilms, Bacteriophage, Biocontrol, Foodborne pathogens