Assessment of tolerance development in *L. monocytogenes* – *E. coli* dual – species biofilms to Pronase and Benzalkonium chloride treatments

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1. BACKGROUND

*L. monocytogenes* is considered one of the main food-borne pathogens in food industry presenting an increasing incidence tendency over the last years [1]. Its ability to form biofilms associated with other bacteria developing higher tolerance to antimicrobial treatments [2] is nowadays one of the main issues regarding food safety.

2. OBJECTIVE

To assess the capacity of a *L. monocytogenes* and *E. coli* MIXED-SPECIES BIOFILM isolated from food industry [3] grown on a stainless steel (SS) to develop TOLERANCE to SUBLETHAL CONCENTRATIONS of a PRONASE (PRN) and BENZALKONIUM CHLORIDE (BAC) sequential treatment.

3. EXPERIMENTAL DESIGN

3.1. BIOFILM SETUP

Adjust inocula to 10^3-10^4 CFU/ml 1:1 mixed in TSB + 2.5 g/l D-glu + 0.6 g/l YE

1 ml culture in each well

Incubation: 25ºC

4. RESULTS

4.1. INCREASING CONCENTRATIONS

**Before** treatment

- **EXPOSED BIOFILMS** (void bars) presented LOWER CELL COUNT compared with control (filled bars).

- **LOWER OCCUPIED AREA VALUES** at 72 h before treatment in EXPOSED SAMPLES (void bars). However, NO DIFFERENCES were appreciated AFTER TREATMENT.

**After** treatment

- After treatments NO SIGNIFICANT DIFFERENCES were observed in the cell count compared with samples before treatment, neither in exposed samples (void bars) nor in controls (filled bars).

- *L. monocytogenes* COUNTS WERE AFFECTED by the previous sublethal exposure.

**CONTROL**

24 h 48 h 72 h (BT) 72 h (AT)

**EXPOSED**

- **EXPOSED SAMPLES PRESENTED AN ALTERED STRUCTURE** if compared with control biofilms.

- Differences were more evident when samples before treatments (BF) and after treatments (AF) are compared where MATRIX STRUCTURE was LOST due to the proteolytic action of the pronase.

5. CONCLUSIONS

1. NO TOLERANCE DEVELOPMENT WAS DETECTED in *L. monocytogenes* – *E. coli* mixed-species biofilm to the PRN – BAC treatment applied in the experimental conditions used. 2. PRN – BAC TREATMENTS WERE MORE EFFECTIVE in YOUNG BIOFILMS, indicating that MATURATION of the STRUCTURE PLAYS AN ESSENTIAL ROLE in antimicrobial treatments.

3. Considering the effects on individual species, *L. monocytogenes* APPEARED TO BE MORE SENSITIVE to the treatment than *E. coli*. Viable cell numbers remained almost unaltered in all approaches followed.

4. The application of a PRN – BAC sublethal treatments, DRAMATICALLY AFFECTS THE MATRIX STRUCTURAL FEATURES of the biofilm, especially in prolonged exposures. These changes may be one of the major causes of the higher sensitivity to BAC.

6. REFERENCES


7. ACKNOWLEDGMENTS

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