TITLE: EVALUATION OF THE ACTION OF SIGNAL MOLECULES PRODUCED BY *Pseudomonas aeruginosa* IN THE MONO- AND MULTI-SPECIES BIOFILM FORMATION OF *Escherichia coli* AND *Staphylococcus aureus*

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ABSTRACT:
The conditions of the processing environment of food industries favour bacterial adhesion and subsequent biofilms formation on surfaces. Biofilms may become a chronic source of pathogen contamination and are a propitious environment for cell-cell communication, called quorum sensing (QS). The quorum-sensing phenomenon is a process of intra- and inter-species microbial communication and is measured by extracellular chemical signals known as signal molecules or autoinducers (AI). This mechanism allows cells to control many of their functions, including virulence gene expression, exopolysaccharide production, and biofilm formation. The objective of this work was to evaluate the action of signal molecules produced by *Pseudomonas aeruginosa* on mono- and multi-species biofilms formation. The cell-free culture supernatant (CFCS) of *P. aeruginosa* was prepared by activating it in Mueller Hilton broth (MH) for 48h at 28 °C and centrifugation at 14,000 rpm for 15 min. Mono- and multi-species biofilms of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were formed on AISI 304 stainless steel surface, at 25 and 35 °C for 24h. Both MH broth and meat exudate (simulating a common food residue in the meat processing environment) were used as substrates. Parts of these substrates (0%, 30% and 60%) were replaced by the CFCS of *P. aeruginosa*. For biofilm quantification, the violet crystal assay was used and the supernatant was read after discolouring with 95% ethanol in a spectrophotometer at 570 nm. The results demonstrated that, without addition of CFCS of *P. aeruginosa*, the meat exudate substrate favoured the formation of mono- and multi-species biofilms when compared to the MH broth. The substitution of 30% of each substrate by the CFCS of *P. aeruginosa* reduced the density of the mono- and multi-species biofilm formed for both temperatures (25 and 35 °C) and substrates. When 60% of substrate was replaced by the CFCS of *P. aeruginosa* there was no significant reduction in the formation of mono- and multi-species biofilms. In addition, 60% of MH broth at 35 °C favoured mono- and multi-species biofilms formation. Therefore, the signal molecules produced by *P. aeruginosa* influenced the formation of mono- and multi-species biofilms of *E. coli* and *S. aureus* on stainless steel surfaces under the conditions evaluated, demonstrating the great potential of research and application of these molecules as anti-biofilm.

Keywords: biofilm, *quorum sensing*, signal molecules, stainless steel.

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