

## BIOFILM FORMATION BY *Listeria monocytogenes* STRAINS EXPOSED TO COLD STRESS

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**Introduction:** *Listeria monocytogenes* is a foodborne psychrotrophic pathogen that causes listeriosis in humans, with a mortality rate of approximately 20%. *L. monocytogenes* can form biofilms; however, this is usually evaluated under optimal temperature conditions for this bacterium. It has been reported that some strains of *L. monocytogenes* could improve their adhesion and ability to form biofilms if exposed to refrigeration and freezing, which poses a risk of contamination in the food industry.<sup>1,2</sup> **Objective:** The objective of this work was to evaluate the biofilm formation capacity of *L. monocytogenes* strains exposed to cold stress. **Material and Methods:** Cheese cubes were inoculated with 100  $\mu$ L of a bacterial culture ( $10^8$  CFU/g) of an *L. monocytogenes* strain isolated from cheese (*Lm5*) and stored at 8°, 4°C and -20°C for 30 days. On days 2, 7 and 30, dilutions (1:10) in peptone water were prepared from the inoculated cubes and plated on Oxford agar. Biofilm formation was evaluated at 24 h using the crystal violet assay and classified according to their optical density (OD) as absent (OD<0.120), weak (OD 0.121-0.480), moderate (OD 0.481-0.720) and strong (OD>0.721).<sup>3</sup> The results were analyzed using ANOVA (P<0.05 statistically significant) and the differences between means with Tukey's Test, in GraphPad Prism 8.0.1. **Results:** The strain not exposed to cold stress formed a moderate biofilm (OD 0.526); the biofilms of day 2, 7 and 30 at 8°C showed significant differences, obtaining a strong (OD 0.999), moderate (OD 0.503) and weak (OD 0.468) biofilm, respectively, indicating a decreasing capacity. No differences were found at 4°C and, after 30 days of storage, the isolates retained the initial capacity of moderate biofilm formation (OD 0.512). The strain at -20°C after 30 days of storage, biofilm formation increased to a strong biofilm (OD 0.969). In the comparison between temperatures, there was a significant difference from day 30 to 8°C and -20°C, obtaining the strongest biofilm formation of the strains maintained at -20°C. **Conclusion:** *L. monocytogenes* retained its ability to form biofilms even after exposure to cold stress. Exposed for 30 days to freezing stress, the bacteria improved its biofilm-forming capacity.

### References

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