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Influence of temperature on biofilm formation by *Listeria innocua*

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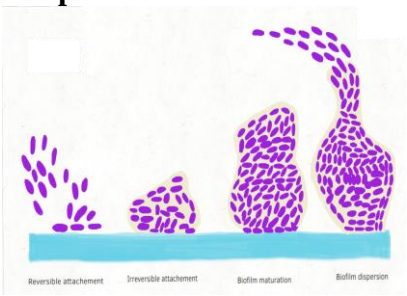
Graphical Abstract



Introduction

The genus *Listeria* is constituted by several species; however, *Listeria monocytogenes* is the most well-known species due to its impact as common foodborne pathogen and also is the leading cause for major food recalls. In recent years, several others Listeria species had been identified worldwide due to the availability of molecular tools for rapid characterization of putative *Listeria* isolates, such as *L. innocua, L. seeligeri and L. welshimeri* (2). Nowadays, several studies had been identifying *Listeria monocytogenes* with co-infection with *Listeria innocua*, on food plants surfaces, it is really worrying because *L. innocua* difficult the detection of *L. monocytogenes* due to its accelerated growth rate. Our previous results identified several *Listeria* sp. on fresh cheese from several farm markets, where *L. innocua* was also identified among the contaminated samples. The origin of the contamination of these processed foods is due to the persistence of Listeria in the environment.

L. monocytogenes has the ability to colonize surfaces in food processing plants and it is really difficult to eliminate because of its ability to form biofilms and survive at different temperatures(1). On the other hand, *Listeria innocua* biofilms have been detected on food processing plants surfaces although it usually coexists with *Listeria monocytogenes*, it is really important to mention that it was detected on surfaces at different temperatures including freezer areas. Little is still known about *L. innocua* and *L. monocytogenes* interactions and mixed biofilm formation. Therefore, it is important to understand the dynamic of biofilm formation of *L. innocua* to further analyze mixed biofilm with *L. monocytogenes.* Therefore, our main goal was to evaluate *L. innocua* ability on biofilm formation under a variability of temperatures, more exactly, at 4, 22 and 37°C.

Materials and Methods

The isolate of *L. innocua* was obtained from artisanal soft cheese used in this study. The biofilm assays were realized on a glass surface placed in a 6-well plate and growth in static conditions. Briefly, an initial inoculation of 1 – 2

Log 8 CFU/ml, through a standard growth curve, was prepared in each well and then incubated for 48h at different temperatures (4, 22 and 37°C). The medium was changed at 24 hours for sterile TBS and each assay had negative controls (coverslip with sterile medium). Each biofilm assay and samples were done in duplicate. The results were analyzed by crystal violet measurement (OD 630 nm) and CFU counting. This CFU counting allowed to evaluate the biofilm viability by culturing in TSA. Finally, each biofilm growth was also evaluated through microscopy analysis.

Results and Discussion

L. innocua demonstrated the ability to form biofilms in all temperatures (4, 22 and 37°C). The biofilm production index was higher at 37°C and gradually decreased to 4°C. Furthermore, biofilm viability showed the same pattern for 37°C (log 9), 22°C (log 8) and 4°C (log 7). Meanwhile, microscopy analysis confirmed biofilm formation in all temperatures, where at 4°C *L. innocua* showed less surface coverage in contrast to 37°C where the same strain was able to cover the entire glass surface.

Conclusions

To conclude, *Listeria innocua* had undoubtedly demonstrated its ability to form biofilms in a large range of temperatures, showing a great adaptation to the thermal conditions and comparable to *L. monocytogenes* ability to form biofilm, as previously described by other studies. To our best knowledge, this is the first study in Ecuador to evaluate *L. innocua* biofilms (3)

References

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