

Integration of PMA-qPCR and Flow Cytometry for Detecting *Listeria monocytogenes* Under Environmental Stress and Biocide Exposure

黃婉萍 (5139)

2025/09/24

Outline

- I. Introduction
- II. Sublethal injury and viable but nonculturable (VBNC) under acidic conditions and disinfectants
- III. Formation and recovery of VBNC state under different temperature combined with low nutrients and high NaCl concentration
- IV. Estimation by flow cytometry of survival treated with tetracycline, with or without prior exposure to several biocides
- V. Conclusion

Abstract

Listeria monocytogenes is a major foodborne pathogen capable of surviving harsh environmental and chemical stresses by entering dormancy states such as sublethal injury, persistence, and the viable but nonculturable (VBNC) state. This report aims to identify environmental stresses and biocides that induce VBNC formation, and to evaluate advanced detection methods, namely PMA-qPCR and flow cytometry, in overcoming the limitations of conventional culture-based approaches. Evidence indicates that factors such as temperature, high NaCl concentrations, low pH, and nutrient limitation drive VBNC induction, with food isolates showing greater tolerance than clinical strains, and recovery occurring more effectively in nutrient-rich environments during a “recovery window.” Disinfectants also play a critical role in dormancy and resistance, with peracetic acid (PAA) shown to induce persistence and VBNC states. Notably, pre-exposure to PAA increased *L. monocytogenes* survival under tetracycline (TE) treatment, suggesting a cross-protection mechanism that may reduce antibiotic effectiveness. To avoid underestimating microbial risk, accurate and sensitive detection is essential. PMA-qPCR provides precise quantification of VBNC cells, while flow cytometry using SYTO 9 and propidium iodide (PI) enables rapid discrimination of live and dead cells. The combination of both methods offers a powerful, accurate, and rapid approach for detecting VBNC *L. monocytogenes* and assessing their public health risks.

References

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