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Comparative analysis of two membrane-based methods for the differentiation of viable and dead cells

Inhalt

Listeria monocytogenes (Lm) is a foodborne pathogen with high hospitalization and mortality rates. Common route of infection is ingestion of contaminated food. Its persistence in food-processing environments can be facilitated by the induction of the viable but non-culturable (VBNC) state. VBNC cells have lost their ability to grow on culture media, but maintain their metabolic activity.

Bacterial load in food-processing environments is monitored using culture-based analysis of surface samples. With VBNC cells present, viable cell numbers may be underestimated.

In this study, two culture-independent methods, namely LIVE/DEAD™BacLight™ staining with fluorescence microscopy and viability real-time PCR using propidium monoazide (PMA-qPCR), were compared for their power to discriminate between viable and dead Lm and their ability to detect VBNC cells after long- and short-term induction.

We were able to show that both methods are reliable tools for differentiating viable and dead Lm. The dual staining of BacLight™ detected viable cells at ratios up to 1:1000 within mixtures of dead cells. PMA-qPCR detected viable cells at ratios up to 1:10,000. As PMA did not completely inhibit amplification of DNA from dead cells, viable cell numbers were overestimated.

Both methods detected VBNC cells after long- and short-term induction. While BacLight™ staining and PMA-qPCR detected similar cell numbers after short-term induction, long-term induced VBNC cells were underestimated by PMA-qPCR.

Keywords

Listeria monocytogenes, VBNC, LIVE/DEAD BacLight, PMA-qPCR

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Yes, I am a Junior Scientist.

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