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Assessing Probiotic Viability in Mixed Species Yoghurt Using a Novel Propidium Monoazide-Quantitative PCR Method

Tlaleo Azael Marole, Thulani Sibanda, Elna Maria Buys

University of Pretoria, Pretoria, South Africa

Abstract

Introduction: Viability is a prerequisite for any therapeutic benefits associated with the ingestion of probiotic bacteria. Current culture-based techniques are inadequate for the enumeration of probiotics in mixed-species food products. In particular, the inability to selectively enumerate *Bifidobacterium* spp. and *Lactobacillus delbrueckii* subsp. *bulgaricus* in yoghurt containing other LAB species such as *Lacticaseibacillus rhamnosus* is a major limitation. Hence, this study focuses on the development of a novel propidium monoazide (PMA) - quantitative PCR (qPCR) method for the selective enumeration of *Streptococcus thermophilus*, *L. bulgaricus*, *L. rhamnosus* and *Bifidobacterium* spp. in mixed species probiotic yoghurt. At present, no PMA-qPCR Rapid viability technique for the enumeration of multi probiotic species has been published. This would enable inline viability verification during processing.

Methodology: The elongation factor Tu (*tuf*) gene-specific primers for *L. bulgaricus*, *Bifidobacterium* spp. and *L. rhamnosus* were designed and tested for specificity in silico using primer 3 plus and the NCBI BLAST database, while *S. thermophilus*-specific primers were obtained from literature. The specificity of these primers and the amplification conditions were confirmed experimentally against non-target LAB species using melting curve analysis and gel electrophoresis. PMA treatment followed by qPCR was used to selectively amplify DNA from viable cells. Viability was determined by use of standard curves. The optimised qPCR protocol was validated in single and mixed species yoghurt samples against standardised plate counts of each organism.

Results and discussion: The assay of the target probiotic and yoghurt cultures with the designed primers, generated PCR products with expected sizes and melt peaks. Selective quantification of viable cells by propidium monoazide (PMAxx ™) treatment at 100 µM, effectively inhibited and removed >99% of DNA from dead control cells.

The PCR efficiency, linear dynamic range and limit of quantification for each probiotic species will be presented. The experimental validation of viability in yoghurt against standardised plate count will also be presented.

Conclusions: The results of this study show the reliability, improved lead time, and high selectivity of PMA-qPCR over culture-dependent method for quantification of *L. rhamnosus*, *Bifidobacterium* spp. and starter cultures in mixed species yoghurt.