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Paper Title **Differentiation Between Viable and Dead Probiotic Microbes Using PCR And DNA-Intercalating Dyes**

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**ABSTRACT**

The determination of cell viability in probiotic products and supplements is of economic, technological and clinical significance. The quality of a probiotic product is evaluated according to the level and viability of probiotic cells it contains. Conventional microbiological methods give an indication of the level of viable cells present in a probiotic product, however, Bifidobacterium species are notoriously difficult to culture. A lack of suitable media to discriminate between the closely related lactic acid bacteria, of which probiotic species form part, led to the increased application of polymerase chain reaction (PCR) methods. PCR is a culture-independent technique but does not discriminate between viable and dead cells. A novel technique using the DNA-intercalating dyes, ethidium monoazide (EMA) or propidium monoazide (PMA), in combination with PCR has been used to distinguish between viable and dead cells.

□ Results indicate that EMA-PCR and PMA-PCR can successfully differentiate between viable and dead *Lactobacillus acidophilus* cells. This method was found to be more sensitive in differentiating between viable and dead Gram negative bacteria as opposed to Gram positive bacteria. Gram positive bacteria require higher concentrations of DNA-intercalating dye to ensure sufficient binding between the DNA and dye, and therefore prevent amplification of the dead bacterial DNA. This technique will allow producers of probiotic products and supplements to not only ensure the presence of the proclaimed bacteria, but also their viability.