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Flow Cytometry as a potential method of measuring bacterial viability in probiotic products: A Review

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1. Introduction.

Bacterial viability currently refers to ability of a cell to grow and reproduce itself under a set of defined environmental conditions (Kennedy et al., 2011). The characteristics of viable cells include the presence and functioning of a range of structural, metabolic, physiological and genetic properties. However, this traditional definition requires that a single" viable" cell must grow and subsequently generate a colony of cells which can be measured optically either in liquid or solid media, thus forming the basis of Koch's principles (Muller & Nebe-von-Caron, 2010). Hence, viability is generally equated with the ability to reproduce and subsequently by cultivability. However, it is clear that not all bacterial cells obey this relationship and indeed, while they may lack the ability to reproduce and grow under certain conditions, they may possess many of the properties of fully functioning viable cells. Hence, we arrive at another classification based on the term "vitality" or the degree to which a cell can perform various aspects of metabolic, physiological and genetic functionality and the extent of structural and morphological integrity (Kramer & Thielman, 2016). In this case, cell vitality is not exclusively related to reproducibility or cultivability and in certain aspects the cell can be described as being in a viable-but-non-culturable state (VBNC) as reviewed by Zhao et al., (2017). A truly "dead" cell may therefore lack the minimal structural integrity and the ability to carry out basic cell functionality such as control and activity of physiology, metabolism and genetic material and possesses neither vitality or viability characteristics (Davis, 2014).

Based on these various states, enumeration of bacterial cells has evolved into two general methodologies; (1) culture dependent and (2) culture independent (Davis, 2014). In the former technique, a liquid sample is plated onto solid media, incubated under defined conditions of time and temperature with data expressed as numbers of colony forming units per gram of original material (cfu/g) (Davey, 2011) . This traditional methodology is still the "gold standard" and is backed by legal status for use in routine enumeration and identification of live/viable microorganisms in food samples (FAO/WHO, (2002); ISO, (2002); Ministero della Salute, (2013); Hill et al., 2014; Sanders et al., (2016). This methodology is a classic example of where viability depends exclusively on the ability of the cell to reproduce with visual inspection of the colony formed from the original single cell. Culture-independent methodologies for bacterial enumeration involve DNA based technologies such as polymerase chain reaction (PCR) or quantitative PCR (qPCR) whereby a specific gene fragment within a DNA sample is amplified and the fluorescent signal directly related to the number of cells in the original sample. This technology is highly useful but may not always distinguish DNA originating from viable or non-viable cells. However, reagents such as ethidium monoazide (EMA) or propidium monoazide (PMA) can be included in the assay to prevent