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Use of propidium monoazide for selective profiling of viable microbial cells during Gouda cheese ripening

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ABSTRACT

DNA based microbial community profiling of food samples is confounded by the presence of DNA derived from membrane compromised (dead or injured) cells. Selective amplification of DNA from viable (intact) fraction of the community by propidium monoazide (PMA) treatment could circumvent this problem. Gouda cheese manufacturing is a proper model to evaluate the use of PMA for selective detection of intact cells since large fraction of membrane compromised cells emerges as a background in the cheese matrix during ripening. In this study, the effect of PMA on cheese community profiles was evaluated throughout manufacturing and ripening using quantitative PCR (qPCR). PMA effectively inhibited the amplification of DNA derived from membrane compromised cells and enhanced the analysis of the intact fraction residing in the cheese samples. Furthermore, a two-step protocol, which involves whole genome amplification (WGA) to enrich the DNA not modified with PMA and subsequent sequencing, was developed for the selective metagenome sequencing of viable fraction in the Gouda cheese microbial community. The metagenome profile of PMA treated cheese sample reflected the viable community profile at that time point in the cheese manufacturing.

1. Introduction

Profiling tools to reveal the composition of microbial communities, such as denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene profiling, 16S rRNA targeted quantitative PCR approaches (qPCR) and metagenomics deduce information from total DNA pool of the community. However, these community profiles commonly fail to reflect the presence of subpopulations with different viability state (Ben-Amor et al., 2005). This is due to the persistence of DNA in the sample matrix although the cells might have (long) lost their integrity (Masters et al., 1994; Novitsky, 1986). A recent study compared the microbial community profiles of human faecal sample fractions that were sorted based on the bacterial RNA concentration (Peris-Bondia et al., 2011). The profiles of the active fraction in all samples were different from the whole community profiles. A similar study by Ben-Amor et al. (2005) reported that up to 60% of faecal microbiota may be membrane compromised. The dominant DGGE bands that were separately obtained from viable, dead, and injured cells involved phylogenetic diversity differences.

Viability dyes are increasingly being used to overcome the indicated limitations of DNA-based community profiling methods, and discriminate the intact (viable) and membrane compromised (dead and injured) cells in microbial communities (Elizaquivel et al., 2014). Propidium monoazide (PMA) is a modified version of propidium iodide (PI), a fluorescent dye that is routinely used for staining membrane compromised cells. The iodide group of PI was replaced with an azide group that covalently links to DNA (and other organic moieties) upon photo activation and inhibits DNA amplification by PCR (Nocker et al., 2006). The dye is membrane impermeable, modifying only the DNA freely present in the sample matrix or in the membrane compromised cells, but not of the intact cells. Therefore, pretreatment of the sample with PMA prior to DNA extraction and subsequent PCR allows the selective amplification of the DNA derived from intact cells in the community (Nocker et al., 2007).

PMA is a promising tool to study the viable community dynamics in food fermentations. The community in food fermentations varies due to succession and differential loss of viability among the microbial cells. For example, Gouda cheese manufacturing employs mixed-strain starter cultures, including closely related blends of *Lactococcus lactis* and *Leuconostoc mesenteroides* strains. The composition of starter culture is dynamic and succession of strains is observed under the cheese

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manufacturing regime (Erkus et al., 2013). The loss of viability occurs after the brining step and the relative abundances of citrate fermenting *L. lactis* ssp. *lactis* and *Ln. mesenteroides* strains increase in the cheese ripening period (Erkus et al., 2013). These strains were determined to be more salt and acid tolerant compared to the other strains in the community (data not shown). This is probably due to their functional citrate utilizing pathways contributing to the pH homeostasis in the cell (Sanchez et al., 2008). Furthermore, the balance between intact and membrane-compromised (dead and injured) cells is crucial for the final flavour formation and texture in cheese (Crow et al., 1995). The membrane-compromised cells are considered essential for the release of intracellular peptidases into the cheese matrix, converting the casein derived peptides into amino acids (Chapot-Chartier et al., 1994). On the other hand, the intact cells synthesize the catabolic enzymes for the formation of key aroma compounds from degradation of amino acids, carbohydrates and/or lipids (Smit et al., 2005). Due to this complexity, understanding the aroma formation in cheese requires a multidiciplanary approach combining the data from viability selective profiling of the starter culture community and meta-omics studies such as metabolomics and metatranscriptomics.

In this study, the use of PMA was evaluated for the selective profiling of viable cells in the starter community during Gouda cheese manufacturing. The study was organized in three sections (Fig. 1). In the first section, PMA treatment procedure was optimized on purified *L. lactis* DNA, and on sub-populations of *L. lactis* cell cultures with different viability state (intact, injured, and dead). In the second section, community dynamics of

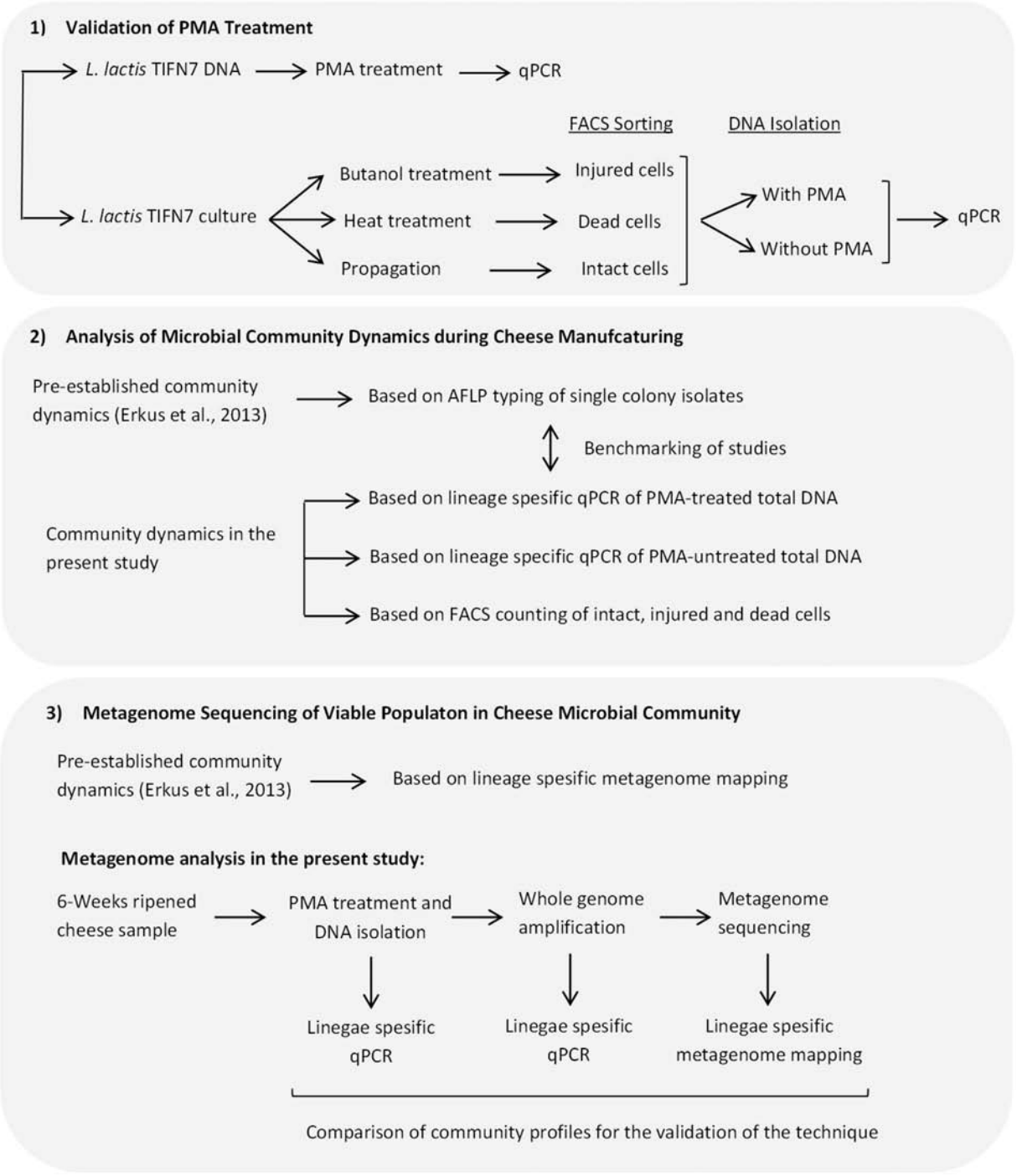

Fig. 1. The flow diagram of the experimental design presented in this study.