J. Dairy Sci. 100:1–12
https://doi.org/10.3168/jds.2016-12408
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Strains of the *Lactobacillus casei* group show diverse abilities for the production of flavor compounds in 2 model systems

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ABSTRACT

Cheese flavor development is directly connected with the metabolic activity of microorganisms used during its manufacture, and the selection of metabolically diverse strains represents a potential tool for the production of cheese with novel and distinct flavor characteristics. Strains of *Lactobacillus* have been proven to promote the development of important cheese flavor compounds. As cheese production and ripening are long-lasting and expensive, model systems have been developed with the purpose of rapidly screening lactic acid bacteria for their flavor potential. The biodiversity of 10 strains of the *Lactobacillus casei* group was evaluated in 2 model systems and their volatile profiles were determined by gas chromatography-mass spectrometry. In model system 1, which represented a mixture of free AA, inoculated cells did not grow. In total, 66 compounds considered as flavor contributors were successfully identified, most of which were aldehydes, acids, and alcohols produced via AA metabolism by selected strains. Three strains (DPC2071, DPC3990, and DPC4206) had the most diverse metabolic capacities in model system 1. In model system 2, which was based on processed cheese curd, inoculated cells increased in numbers over incubation time. A total of 47 compounds were identified, and they originated not only from proteolysis, but also from glycolytic and lipolytic processes. Tested strains produced ketones, acids, and esters. Although strains produced different abundances of volatiles, diversity was less evident in model system 2, and only one strain (DPC4206) was distinguished from the others. Strains identified as the most dissimilar in both of the model systems could be more useful for cheese flavor diversification.

Received December 6, 2016.
Accepted April 22, 2017.
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Key words: *Lactobacillus*, flavor, biodiversity, model system

INTRODUCTION

Formation of cheese flavor is a complex process, which results mainly from the metabolic activities of microorganisms present during cheese manufacture (Marilley and Casey, 2004; Smit et al., 2005). Lactic acid bacteria are the most commonly found bacteria in dairy products and their metabolic diversity represents a potential tool for flavor diversification and improvement (Smit et al., 2005). Nonstarter lactic acid bacteria (**NSLAB**) that originate from the cheese-making environment dominate the cheese microbiota during ripening (Vaughan et al., 2001). The metabolic activity of NSLAB during ripening leads to the production of compounds contributing to the flavor characteristics of cheese (Fitzsimons et al., 2001; Banks and Williams, 2004), and this effect has been shown to be highly strain specific (Randazzo et al., 2007; Bouton et al., 2009; Pogačić et al., 2016).

The mesophilic lactobacilli dominate the NSLAB flora of cheese, as seen in a broad survey of NSLAB diversity, where 18 species of mesophilic lactobacilli were detected in 38 cheese varieties with *Lactobacillus paracasei* and *Lactobacillus plantarum* as the most prevalent species. These are considered as very adaptable to the cheese environment, and along with *Lactobacillus casei*, *Lactobacillus curvatus*, and *Lactobacillus rhamnosus*, represent the core species of the nonstarter microbiota (Gobbetti et al., 2015). Adjunct cultures are essentially selected strains of NSLAB that are added to cheese milk with the purpose of controlling the indigenous NSLAB population, and thus, directing the development of desired cheese flavor compounds (Milesi et al., 2010; Singh and Singh, 2014). Strains of the *Lactobacillus casei* group (*L. casei*, *L. paracasei*, and *L. rhamnosus*) have been successfully used as adjuncts, solely

or in combination with other lactobacilli in Cheddar cheese manufacture (Crow et al., 2001; Swearingen et al., 2001; Coolbear et al., 2008).

Ideally, the evaluation of the flavor-forming ability of new strains should be performed in cheese-making trials, but this is only practical as a final step as such trials are expensive, laborious, and time consuming (Milesi et al., 2007). To a certain extent, model systems mimic some aspects of the cheese ripening environment and enable rapid assessment of the development of the cheese microbiota and the resultant biochemical processes. Several types of cheese models have been developed based on miniature cheese production (Di Cagno et al., 2006; Milesi et al., 2008; Cavanagh et al., 2014), cheese slurry (Smit et al., 1995), or processed curd (Pogačić et al., 2015; Velez et al., 2015). In addition, synthetic systems that consist of solutions of a similar content to cheese could be used as model systems, such as those based on AA-rich media (Engels and Visser, 1996; Kieronczyk et al., 2001; van de Bunt et al., 2014). Besides these, cheese serum extracts (Peralta et al., 2014), freeze-drying of cheese and extraction with water (Budinich et al., 2011), or lysate of cells (Sgarbi et al., 2013) were also successfully used as cheese models. A model based on miniature cheeses made from as little as 1.7 mL of milk enabled screening of flavor-forming capacities of microorganisms (Bachmann et al., 2009). In most cheese or curd-based model systems, inoculated cells increased in numbers, whereas in synthetic medium model systems, inoculated cells were not growing (Kieronczyk et al., 2001; van de Bunt et al., 2014). Additionally, cell-free extracts have been used as a source of enzymes to investigate the flavor-forming capacity of *Lactococcus lactis* (Engels and Visser, 1996).

The aim of this study was to evaluate the diversity between strains of the *L. casei* group based on determination of their volatile profiles generated in 2 model systems: a model consisting of a mixture of free AA and a processed curd model. Afterward, the strain diversity was mapped using a chemometric approach, which showed different abilities of strains for volatile production in the 2 model systems used.

MATERIALS AND METHODS

Bacterial Strains

Ten strains of the *Lactobacillus casei* group of dairy origin were used in this study (DPC1116, DPC2068, DPC2071, DPC3990, DPC4026, DPC4206, DPC4536, DPC5408, DPC6753, and DPC6800). Strains used in this study were previously confirmed (by 16S rRNA PCR) to belong to species *L. casei* or *L. paracasei* and were selected based on genomic profiles (pulsed-field gel electrophoresis) and biochemical characterization (activities of proteolytic cascade enzymes) of a set of 310 isolates obtained from the DPC Culture Collection held at the Teagasc Food Research Centre, Moorepark, Cork, Ireland (Stefanovic et al., 2017). Strains were kept frozen at −80°C in de Man, Rogosa, Sharp broth (MRS, Oxoid, UK) with 20% (vol/vol) of glycerol, and before the experiment they were grown on MRS agar plates at 30°C in aerobic conditions.

Model System 1: Resting Cells in Medium Containing Free Amino Acids

Model system 1 (**MS1**) consisted of a suspension of nongrowing cells in a concentrated (35% (wt/vol) AA-rich medium Bacto Tryptone [BD, Franklin Lakes, NJ; containing a minimal level of total carbohydrates (4.3 mg/g)] supplemented with 12 g/L of NaCl. This medium was chosen based on the composition of a similar model described by van de Bunt et al. (2014). Medium for MS1 was prepared from the same batch of Bacto Tryptone, and after addition of NaCl, it was autoclaved (121°C, 15 min). Cell manipulation was performed as described by van de Bunt et al. (2014), with some modifications. Briefly, strains were pre-incubated for 18 h at 30°C in MRS broth, re-inoculated (1% vol/vol) in 500 mL of MRS broth, and incubated for 24 h at 30°C. Cells were centrifuged (4,000 × *g*, 10 min, 4°C), washed twice with 0.1 mol/L phosphate buffer pH 6, and finally resuspended in 5 mL of the same buffer containing 15% glycerol and kept at −80°C until required. Thawed cell suspensions (1 mL) were added in 9 mL of the prepared AA-rich medium including 10 μL of a vitamin and microelement solution, which contained 2 mg of biotin, 4.8 mg of Ca-pantothenate, 8 mg of thiamine, 8 mg of $FeSO_4$, 1.6 mg of $MgSO_4$, and 8 mg of $MnSO_4$ dissolved in 4 mL of deionized water and filter sterilized (Filtropur S syringe filter, 0.45 μm pore size, Sarstedt, Wexford, Ireland). Inoculated samples were incubated for 24 h at 30°C. For cell enumeration, samples of 100 μL were taken before and after incubation of the inoculated model system (at time = 0 h and time = 24 h) and serially diluted before plating on MRS agar followed by incubation at 30°C for 72 h. After incubation, pH values of the samples were also measured. Samples were kept at −80°C until volatile analysis was performed. The control consisted of an un-inoculated model system. Both the test strains and the un-inoculated control were evaluated in triplicate.

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