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Lactobacillus demonstrate thiol-independent metabolism of methylglyoxal: Implications toward browning prevention in Parmesan cheese

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ABSTRACT

Endogenous production of α-dicarbonyls by lactic acid bacteria can influence the quality and consistency of fermented foods and beverages. Methylglyoxal (MG) in Parmesan cheese can contribute toward undesired browning during low temperature ripening and storage conditions, leading to the economic depreciation of affected cheeses. We demonstrate the effects of exogenously added MG on browning and volatile formation using a Parmesan cheese extract (PCE). To determine the influence of *Lactobacillus* on α-dicarbonyls, strains were screened for their ability to modulate concentrations of MG, glyoxal, and diacetyl in PCE. It was found that a major metabolic pathway of MG in *Lactobacillus* is a thiol-independent reduction, whereby MG is partially or fully reduced to acetol and 1,2-propanediol, respectively. The majority of lactobacilli grown in PCE accumulated the intermediate acetol, whereas *Lactobacillus brevis* 367 formed exclusively 1,2-propanediol and *Lactobacillus fermentum* 14931 formed both metabolites. In addition, we determined the inherent tolerance to bacteriostatic concentrations of MG among lactobacilli grown in rich media. It was found that *L. brevis* 367 reduces MG exclusively to 1,2-propanediol, which correlates to both its ability to significantly decrease MG concentrations in PCE, as well as its significantly higher tolerance to MG, in comparison to other lactobacilli screened. These findings have broader implications toward lactobacilli as a viable solution for reducing MG-mediated browning of Parmesan cheese.
Key words: *Lactobacillus*, α-dicarbonyl, methylglyoxal, Parmesan cheese browning

INTRODUCTION

The complexity of browning reactions arises partly from a series of parallel and sequential reactions stemming from the glycation of peptides in the Maillard reaction. Oxoaldehydes are reactive intermediates of the Maillard reaction that augment and redirect glycation, adding to this complexity. The carbonyl carbon of α-oxoaldehydes such as glyoxal and methylglyoxal (**MG**) are highly electrophilic due to their oxidative state and vicinal position to a second carbonyl group. This instability enhances elimination, condensation, and other degradation mechanisms that can form stable end-stage adducts called advanced glycation end products (**AGE**) as well as brown, nitrogenous, polymeric substances responsible for browning known as melanoidins (Nemet et al., 2006, Ramasamy et al., 2006). Because of the reactivity of these α-oxoaldehydes, controlling the formation of AGE and melanoidins during processing, cooking, and prolonged storage of food and beverages has remained a difficult undertaking.

The development of brown pigmentation and concomitant alteration in flavor profiles during the aging process of cheese is an enduring issue, causing economic detriment to the industry (Yann et al., 2005). Brown pigmentation may materialize during the latter ripening and storage periods of Parmesan cheese and is atypical of Maillard reactions because it occurs in the relative absence of reducing sugars and at low temperature (Divine et al., 2012). The microbial production of MG has been implicated as a major contributor toward browning defects during low-temperature storage, leading to the economic depreciation of affected cheeses (Divine and Rankin, 2013). The major source of MG in biological systems is from the degradation of the glycolytic intermediate, dihydroxyacetonephosphate (**DHAP**), but less is known about MG metabolism in lactic acid bacteria (**LAB**) with high effect on fermented food systems.

The browning defect is more prominently observed in cheeses that have undergone direct salting of the curd before pressing compared with the more time-intensive

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brine salting of already pressed cheese (McDonald, 1992). A directly salted cheese has a uniform dispersion of salt throughout the wheel, whereas a brined cheese has a gradient concentration of salt, with less diffusion toward the center. Therefore, it is likely that MG formation is linked to the discrepancy in ripening microbiota associated with the 2 manufacturing styles. Currently, natural solutions to prevent or decrease browning do not exist outside of using chemical reducing agents and bleaching applications (Divine and Rankin, 2013). As a result, there is great interest within the industry to develop a microbial solution for reducing MG-mediated browning that is amendable to the standard of identity of Parmesan cheese.

In many long-ripened cheeses, nonstarter lactic acid bacteria (**NSLAB**) previously existing in low levels in the milk or processing environment begin to thrive during later stages of ripening. Nonstarter lactic acid bacteria, predominated by facultative and obligate heterofermentative lactobacilli, have a significant role in cheese flavor development. Their effect on cheese flavor development can be either positive or negative; therefore, industry expends significant effort to control the NSLAB microbiota of cheese. One approach used by industry to control the NSLAB microbiota is to select NSLAB with desired properties and to add these cultures (referred to as culture adjuncts) to cheese at relatively high numbers (e.g., 1×10^5 cfu/mL; Broadbent et al., 2011). If a NSLAB capable of reducing the level of MG in cheese could be identified, it may have potential as a culture adjunct for the control of MG-mediated browning in cheese.

The present study demonstrates the effects of exogenously added MG on the color and volatile profile of Parmesan cheese extract (**PCE**). We assess the ability of different strains of *Lactobacillus* to modulate glyoxal, MG, and diacetyl after prolonged single-strain fermentation in PCE. Furthermore, we identify a major metabolic pathway of MG in *Lactobacillus* growing in PCE and examine strain-specific tolerance to MG.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Lactobacillus selected for this study are listed in Table 1. Stock cultures were maintained at −80°C in de Man, Rogosa, and Sharpe (**MRS**) medium (BD Biosciences, Sparks, MD) containing 15% (vol/vol) glycerol. All media containing MG was made daily. Working cultures in PCE were prepared from frozen stocks by a single transfer in MRS followed by 2 successive transfers in PCE. When screening strains for MG tolerance, working cultures of *Lactobacillus* were prepared from frozen stocks by 2 successive transfers in MRS. All sub-cultures and working cultures of *Lactobacillus* were propagated at 37°C, without shaking, for 16 to 18 h.

Parmesan Cheese Extract

Parmesan cheese extract was obtained from a 10-mo-old Parmesan cheese and processed by the Western Dairy Center at Utah State University. A Parmesan cheese block was cut into 5.08-cm cubes and processed through a Commitrol grinder (Urschel, Chesterton, IN) using a 0.317-cm cutting head. The ground cheese was vacuum sealed and cooled at 4°C for 24 h. A minimum of 54.4 kg of cheese was combined with 265 L of deionized water in a stainless-steel, steam-heated, jacketed vat with a total capacity of 321 L. The cheese and water mixture was continuously stirred and heated from 15.5 to 48.8°C over 40 min and held at 48.8°C for 20 min. Cheese solids were removed from the cheese-water extract by processing through a cheesecloth. The extract (~265 L) was processed through 2 spiral-wound UF membranes with a MW cutoff of 5,000 kDa while using an operating flow of 18.9 L/min, operating temperature of 48.8°C, and operating pressure of 100 to 1,000 kPa. The permeate was collected and recycled once with the concentrated retentate through the UF system before a final reverse osmosis filtration treatment. The reverse osmosis filtration system used the aforementioned conditions albeit at an operating pressure of 3,000 to 6,000 kPa. The extract was packaged and stored at −20°C. Prior to use as a bacterial growth media, PCE was thawed, filter sterilized (0.2 μm filter, Thermo Scientific Bottle-top Nalgene), and stored at 4°C.

Identification of Volatiles Formed from Exogenous Addition of MG to PCE by Solid-Phase Microextraction GC-MS

Increasing concentrations of MG were added to PCE and incubated at 65°C for 24 h to accelerate reactivity. Samples were visualized in cuvettes placed against a light source. A browning index (defined as the optical density difference measured at 420 and 500 nm) was used to define positive discoloration. In parallel, volatile profiles of PCE containing either 0, 1, 10, 50, or 100 m*M* MG were determined using solid-phase microextraction (**SPME**) and GC-MS. Ten-milliliter samples were prepared in 16 × 60 mm screw-cap glass vials with rubber septums (Fisher Scientific, Pittsburgh, PA) and heated at 65°C for 24 h to accelerate reactivity. Volatiles were trapped by exposing an 85-μm polydimethylsiloxane/Carboxen SPME fiber (Supelco, Bellefonte, PA) to the head space for 20 min at 40°C. The initial temperature of the front inlet was 220°C at a flow of 33.9 mL/

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