Accurate monitoring of living and total bacterial populations in milks for improved microbial management

Zhengyao Xue[1], Mary Kable[1], Jessie Heidenreich [2], Jeremy McLeod[2] and Maria L. Marco[1]

Author Information:

[1]Department of Food Science and Technology, University of California Davis, Davis, California

[2]Hilmar Cheese Company, Hilmar, California

Milk contains a broad diversity of bacteria that enter milks through farm and processing environments. These bacteria are important determinants of the quality and safety of fluid milk and processed dairy products. Currently employed methods for microbial analysis of milk (and other dairy products) typically target only a limited number of bacterial species, and therefore restrict our knowledge on the microbial community dynamics that lead to either high quality or defective dairy products. In order to improve the microbial management of dairy foods, we are developing and applying stream-lined methods for bacterial diversity measurements using culture-independent, high-throughput 16S rRNA gene sequencing. Firstly, we developed protocols for cell collection, DNA extraction, sequencing, and bioinformatic analysis that result in identifying bacterial communities in milk and cheese. Included in these approaches are methods to distinguish between living and total bacteria by the application of propidium-monoazide (PMA) on intact cells. To validate these protocols, we designed a bacterial standard comprising nine strains of dairy-associated bacterial species. DNA was extracted from the strains separately and combined as well as in the presence and absence of milk for sequencing of the 16S rRNA gene V4 regions on two different DNA sequencing platforms. These comparisons revealed significant biases and errors introduced by standard DNA preparation and sequencing methods. These errors could be addressed, in part, by more stringent bioinformatics approaches and sampling and DNA extraction methods that are comparable with automation. Secondly, we are employing these methods to identify raw and pasteurized milks that either result in high quality cheeses or those that are associated with a variety of defects (e.g. slits, soft texture, bitterness, acidic and buttery flavors). We have thus far shown that high-quality and defective cheeses contain distinct microbiomes. Moreover, the bacteria present in cheeses that have a common defect are also variable. These results indicate that different bacterial species may share some conserved enzymatic functions, which can lead to defective products when these bacterial enzymatic properties are disproportionately high. This knowledge combined with the application of our methods to distinguish between living and total bacterial cells in milk pre- and post-pasteurization and during cheese ripening will enable predictive microbial models along dairy processing chains and ultimately result in consistent products with optimal sensory profiles and nutritive benefits.