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Rapid detection and characterization of postpasteurization contaminants in pasteurized fluid milk

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ABSTRACT

Microbial spoilage of pasteurized fluid milk is typically due to either (1) postpasteurization contamination (PPC) with psychrotolerant gram-negative bacteria (predominantly *Pseudomonas*) or (2) growth of psychrotolerant sporeformers (e.g., *Paenibacillus*) that have the ability to survive pasteurization when present as spores in raw milk, and to subsequently grow at refrigeration temperatures. While fluid milk quality has improved over the last several decades, continued reduction of PPC is hampered by the lack of rapid, sensitive, and specific methods that allow for detection of PPC in fluid milk, with fluid milk processors still often using time-consuming methods (e.g., Moseley keeping quality test). The goal of this project was to utilize a set of commercial fluid milk samples that are characterized by a mixture of samples with PPC due to psychrotolerant gram-negative bacteria and samples with presence and growth of psychrotolerant sporeforming bacteria to evaluate different approaches for rapid detection of PPC. Comprehensive microbiological shelf-life characterization of 105 pasteurized fluid milk samples obtained from 20 dairy processing plants showed that 60/105 samples reached bacterial counts >20,000 cfu/mL over the shelf-life due to PPC with gram-negative bacteria. Among these 60 samples with evidence of gram-negative PPC spoilage over the shelf-life, 100% (60/60) showed evidence of contamination with noncoliform, non-*Enterobacteriaceae* (EB) gram-negative bacteria (e.g., *Pseudomonas*), 20% (12/60) showed evidence of contamination with coliforms, and 7% (4/60) showed evidence of contamination with noncoliform EB. Among the remaining 45 samples, 28 showed levels of gram-positive bacteria above 20,000 cfu/mL and the remaining 17 samples did not exceed 20,000 cfu/mL over the shelf-life. Evaluation of the same set of 105 samples using 6 different approaches {all possible combinations of 2 different enrichment protocols (13°C or 21°C for 18 h) and 3 different plating media [crystal violet tetrazolium agar, EB Petrifilm (3M, St. Paul, MN), and Coliform Petrifilm]} showed that enrichment at 21°C for 18 h, followed by plating on crystal violet tetrazolium agar provided for the most sensitive, accelerated detection of samples that reached >20,000 cfu/mL due to PPC with psychrotolerant gram-negatives (70% sensitivity). These results show that tests still required and traditionally used in the dairy industry (e.g., coliform testing) are not suitable for monitoring for PPC. Rather, approaches that allow for detection of all gram-negative bacteria are essential for improved detection of PPC in fluid milk.
Key words: postpasteurization contamination, rapid method, *Pseudomonas*, indicator organism

INTRODUCTION

Although research has shown that fluid milk quality has consistently improved over the last 2 decades (Carey et al., 2005; Martin et al., 2012), postpasteurization contamination (**PPC**) is still a hurdle for some processors. In fact, some studies suggest that ~40 to 50% of conventionally pasteurized fluid milk shows evidence of PPC (Ranieri and Boor, 2009; S. J. Reichler, Cornell University, Ithaca, NY, unpublished). Postpasteurization contamination has previously been associated with rapid bacterial outgrowth (Schröder et al., 1982; Ranieri and Boor, 2009; Martin et al., 2012) and unacceptable sensorial properties (Hayes et al., 2002; Martin et al., 2012), both of which often lead to premature spoilage before the labeled product shelf-life (defined here as the sell-by date provided by the manufacturer). Because premature spoilage is a contributing cause of food loss, which accounts for approximately one-third of the fluid milk processed in the United States, at a value of $6.4 billion (Buzby et al., 2014), reducing PPC is of great importance from a business, consumer, and sustainability perspective.

Postpasteurization contamination can be introduced into the fluid milk continuum at various points, but several research studies indicate that the filling equipment is an area that is particularly susceptible to

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contamination often due to lapses in good manufacturing practices (Eneroth et al., 1998; Ralyea et al., 1998; Gruetmacher and Bradley, 1999). Additionally, although stringent cleaning and sanitation programs reduce the incidence of PPC, some contaminants that exist within resistant biofilms, or in niches that are inaccessible to cleaning and sanitizers, may not be effectively removed from processing equipment and lead to persistent PPC. Methods to detect PPC in fluid milk have primarily relied upon traditional indicator organisms used in the dairy industry. Coliforms have been used, for nearly a century, as indicator organisms in the dairy industry. For example, the US Pasteurized Milk Ordinance specifies a coliform limit of 10 cfu/mL for grade A pasteurized fluid milk by (FDA, 2015). Coliforms are heat labile and are very effectively eliminated by HTST pasteurization. Hence, coliform presence in pasteurized fluid milk can be expected to generally be due to re-introduction of these organisms after the heat step, unless coliforms were present in very high numbers (e.g., $>10^6$ cfu/mL) in raw milk. Detection of coliforms in fluid milk thus is often considered an indication of unhygienic conditions or PPC. Testing for coliforms is also relatively fast, a desirable quality for indicator organisms, with many methods (e.g., Coliform Petrifilm) taking 24 h or less.

Despite the longstanding use of coliforms as indicators of PPC in fluid milk, many studies have identified *Pseudomonas*, a noncoliform, as the primary causative agent of PPC (Ternström et al., 1993; Eneroth et al., 1998; Deeth et al., 2002). Importantly, *Pseudomonas* and other noncoliform gram-negative bacteria are not recovered on coliform media and therefore may go undetected by current indicator tests. Testing methods that allow for the detection of coliforms, *Pseudomonas*, and other gram-negative bacteria (e.g., plating on crystal violet tetrazolium agar, **CVTA**) are not as rapid as coliform testing methods, typically requiring 48 h until results. A variety of methods with varying complexity and time-to-result have hence been developed to detect PPC in fluid milk and other fluid dairy products including impedance measurements (Bossuyt and Waes, 1983), direct epifluorescent filter technique (Griffiths et al., 1984), and bioluminescence assays (Griffiths, 1993). Additionally, several researchers have evaluated various selective enrichment procedures for rapid enumeration of PPC (Byrne et al., 1989). Although some of these methods show high correlation with the shelf-life performance of fluid milk (e.g., r = 0.91 for impedance methods using selective media; White, 1993) and some have rapid time to result (e.g., <20 h for ATP testing following selective enrichment), many have high initial costs for equipment, require the use of numerous chemicals and reagents, or are complex to run. Importantly, almost all of the research that has been conducted on rapid detection of PPC in fluid milk was conducted in the 1980s when milk quality was significantly different and when milk quality issues due to PPC may have been due to different organisms (e.g., more common contamination with coliforms) and may have represented different contamination patterns (e.g., higher levels of initial contamination; Carey et al., 2005). Therefore, the goals of this study were to determine the overall population of bacterial contaminants contributing to PPC in a set of contemporary fluid milk samples and to test the ability of various methodologies, specifically those that do not require specialized or expensive equipment and complex steps, to detect PPC in these samples.

MATERIALS AND METHODS

Sample Collection and Handling

Pasteurized milk samples (n = 105) were collected from 20 fluid milk processing facilities by Milk Quality Improvement Program (**MQIP**; Cornell University, Ithaca, NY) personnel from 2014 to 2015. Processing facilities were all enrolled in the Voluntary Shelf-Life Program, administered by MQIP, and were located in the northeast United States (New York, Maine, Vermont, New Hampshire, and Massachusetts). Facility size ranged from small, on-farm facilities (with processing capacities of approximately 0.5 million kg/yr) to large facilities (approximately 250 million kg/yr). Pasteurized fluid milk samples collected included whole-fat (minimum 3.25% milk fat, n = 35), reduced-fat (2% milk fat, n = 22), low-fat (1% milk fat, n = 24), and nonfat (<0.2% milk fat, n = 24) milk in 12-ounce (355 mL, n = 2), pint (473 mL, n = 1), quart (946 mL, n = 10), half-gallon (1.9 L, n = 91), or gallon (3.8 L, n = 1) containers. None of the processors fortified their milk with nonfat dry milk. All products were pasteurized via HTST (15 facilities; 94 samples) or vat pasteurization (5 facilities; 11 samples) and packaged in glass bottles (n = 9), high-density polyethylene jugs (n = 92), or paperboard cartons (n = 4). After being packed in coolers with ice packs or ice, milk samples were transported to the MQIP laboratory and stored at 4°C until the initial testing, performed within 48 h of sample collection.

Shelf-Life Analysis, Rapid Shelf-Life Screening, and Bacterial Isolation

On initial day of testing, pasteurized milk samples were handled and stored as described previously (Martin et al., 2012) in preparation for microbiological and organoleptic analyses, which were performed on each

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