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Utility of Terminal Restriction Fragment Analysis on Raw Milk to Evaluate Milk Quality

L. VieBrock[2], H. Guo[1, 2], A. Hamrick[2], R. Jimenez[2], M. Yeung[2]
[1]Shanghai Jiao Tong Univ., Shanghai, China;
[2]Environmental Biotechnology Institute, California Polytechnic State Univ., San Luis Obispo, CA

Contact Info:
Dr. Marie Yeung
California Polytechnic State University
Biological Sciences Department
San Luis Obispo, CA 93407-0401
Phone: (805) 756-2498
Fax: (805) 756-1419
Email: pmyeung@calpoly.edu

Abstract (updated)

The quality of pasteurized milk and other finished dairy products has been previously shown to correlate with raw milk quality. Several conventional culture-dependent methods are available to evaluate raw milk quality yet there is an unacceptable level of variability in the methodology and interpretation of these tests.

There is a critical need to arrive at a consensus on one or more tests that can produce results with the most relevance in assessing and/or predicting the final quality of dairy products. We reasoned that a culture-independent method should be included to understand the entire bacterial community in raw milk. To this end, the objective was to evaluate the utility of Terminal Restriction Fragment (TRF) analysis in assessing raw milk quality.

Raw milk samples were taken from healthy dairy cows and cows shown apparent signs of mastitis (*n*=20). Conventional microbiological methods including standard plate count (SPC), coliform count (CC) and somatic cell count were conducted. DNA extraction method was optimized, which entailed sonication and phenol-chloroform extraction, to isolate DNA from hard-to-lyse endosporeformers that may be present in each of the 200-ml milk samples. Amplified 16S rDNA was labeled at 5' end and digested with DpnII. TRF patterns were analyzed by visual inspection of peaks and comparison of size in base pairs to the GenBank database.

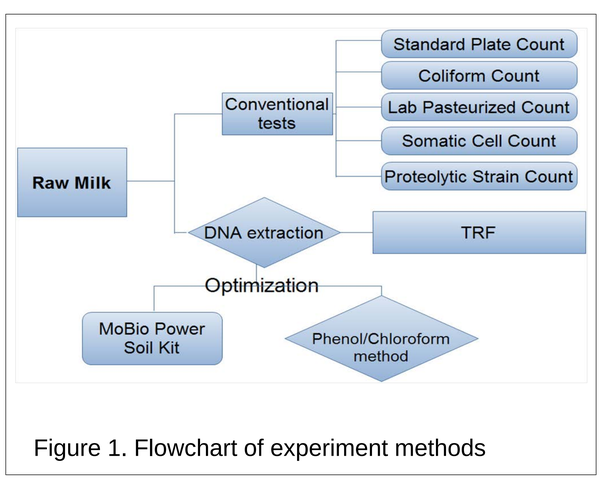

Figure 1. Flowchart of experiment methods

Introduction

Quality of Grade A raw milk is conventionally based on the following tests:

Test	Criteria
Standard Plate Count (SPC)	Not to exceed 1×10^5 cfu/ml
Somatic Cell Count (SCC)	Not to exceed 7.5×10^5 cells/ml
Lab Pasteurized Count (LPC)	Not to exceed 750 cfu/ml
Coliform Count (CC)	Not to exceed 750 cfu/ml

Why is there a need to determine raw milk quality?

•Despite pasteurization of Grade A raw milk, heat resistant organisms and enzymes may adversely affect dairy product quality and shelf life[4]

•Raw milk is not routinely tested directly for heat-resistant endosporeforming microorganisms

•Standard and conventional tests are not sensitive enough to test for specific species responsible for quality defects

Why is there a need to use molecular methods to determine raw milk quality?

•Methods such as Terminal Restriction Fragment (TRF) analysis provide information on presumptive genus and species of bacteria in the samples and therefore the cause of poor quality

•Molecular tools also help evaluate the impact of uncultured bacteria on milk quality

Methods

- Outline shown in Figure 1
- Raw milk samples collected from mastitis and healthy cows at Cal Poly dairy
- Conducted standard and conventional tests to screen for milk quality
- Samples were classified as "good" (*n*=8) and "poor" (*n*=12) according to conventional tests
- Conducted TRF analysis after DNA extraction optimization[7] (Figure 2)

Results

Table 1. TRF peak sizes and presumptive bacterial identification in the raw milk samples.

Peak Size (in base pairs)	Presumptive Genus	Grade A Quality Milk (*n*=8)	Poor Quality Milk (*n*=12)
63 bp	*Bifidobacterium*	75%	50%
167 bp	*Bacillus*	12.50%	8.30%
229 bp	*Lactobacillus*	50%	8.30%
268 bp	*Pseudomonas*	12.50%	67%
272 bp	*Klebsiella, Enterobacter*	50%	75%
304/430bp	*Streptococcus*	62.50%	58.30%
314 bp	*Enterococci*	0%	58.30%
	Uncultured	100%	100%

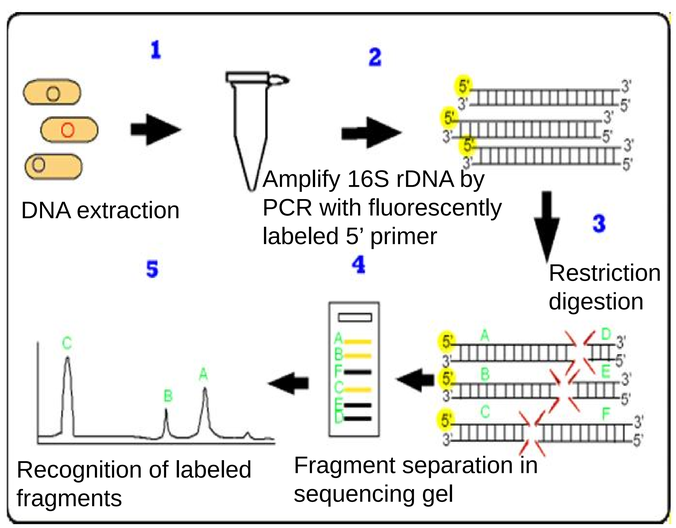

Figure 2.Terminal Restriction Fragment Analysis procedure[5].

Conclusions

- Most raw milk samples below Grade A did not exceed SCC values, indicating microbes are the cause of poor quality
- Good quality samples appeared to contain *Bifidobacterium, Lactobacillus*, and uncultured bacteria
- Only one Grade A sample appeared to contain endosporeformers implying some samples may need less treatment
- Poor quality samples appeared to contain *Klebsiella* and *Enterobacter* that may produce exopolysaccharides leading to the ropiness defect of pasteurized milk[1]
- *Pseudomonas* also appeared to be more prevalent in poor quality milk; they can produce heat stable enzymes[2,3,6,8]
- TRF patterns supported conventional test results in regards to the presence of coliforms
- TRF analysis was able to obtain genus (and species) distribution profiles that are useful in differentiating raw milk having varying microbial qualities

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