Cornell CALS College of Agriculture and Life Sciences

Food Science

# Psychrotrophic Spore-Forming Bacteria in Raw and Pasteurized Milk

Preventing post-pasteurization contamination (PPC) with gram-negative psychrotrophic bacteria is essential in ensuring extended shelf-life of pasteurized milk products (e.g., >14 days at 45°F). When PPC is prevented, thermoduric psychrotrophic bacteria that survive pasteurization and grow at refrigerated temperatures become the limiting factor. Thermoduric bacteria as a rule are gram-positive organisms. Gram-negative bacteria as a rule do not survive pasteurization.

Significant among the thermoduric psychrotrophs common to milk are endospore ("spore") forming bacteria, especially certain strains of *Bacillus* and *Paenibacillus*. Spores are dormant structures formed by select groups of bacteria, which allow them to better withstand stresses that they would not normally survive in the vegetative state (i.e., as actively growing bacteria). Spores are more resistant to heat, sanitizers, and other extremes. When "activated" and/or put into a favorable environment, spores can "germinate" and grow again as active, vegetative bacteria. The pasteurization process may promote "activation" and outgrowth of spore-forming bacteria. There is even evidence that raising pasteurization temperatures, well above minimum standards, may result in more rapid outgrowth of some psychrotrophic strains, resulting in a shortened product shelf-life.

Psychrotrophic strains of spore-forming *Bacillus*, *Paenibacillus* and related organisms are widespread in the raw milk supply. They can be common contaminants in the farm environmental, often associated with soil, feed or manure, and may form biofilms within a milking system. Effective pre-milking hygiene procedures and cleaning/sanitation programs are essential to reduce contamination levels in raw milk. Numbers of psychrotrophic spores in raw milk can range from very low to relatively high; counts from 1 spore per liter to several hundred per milliliter (ml) have been reported. It is important to understand that it only takes one psychrotrophic spore per milk container to eventually grow and cause spoilage. Eliminating these contaminants in the raw milk supply would be a significant challenge for the dairy industry, but with proper procedures, they can be reduced.

In pasteurized milk psychrotrophic spore formers do not become apparent in most cases until later in shelf-life; increases in bacteria counts might not be observed until after 10 to 14 days, or even later. This tendency to show up later in shelf-life may be due to low initial numbers, although these organisms may be slow to initiate growth (i.e., longer lag phase) and/or have slower growth rates than other spoilage organism. While producer raw milk can be a primary source of psychrotrophic spore-formers, raw milk transport, storage and handling can be potential sources as well and should not be overlooked. Additionally, poorly cleaned milk processing equipment, both pre- and post-pasteurization can be possible sources of these organisms as they are more likely to withstand the rigors of plant cleaning and sanitization programs. This might happen in

rare situations where a niche in the processing system is created that allows for these organisms to persist.

***Detecting Psychrotrophic Spore-Formers in Raw Milk***. Unfortunately there are no simple tests that can enumerate Heat Resistant Spore-forming Psychrotrophs (HRSP). HRSPs can be detected in raw milk by "heat-shocking" (80°C/12 minutes) the milk to "activate" the spores and to kill off vegetative cells. A psychrotrophic plate count (Standard Plate Count incubated at 7°C for 10 days) can then be used. Since initial numbers may be very low (<1/ml), a more useful procedure to determine the growth potential in pasteurized milk is to heat-shock a larger volume (200 ml), then hold the heated milk at 6-7°C; plating for SPC (32°C for 48 hrs) initially and at 14 days. Growth (increases in SPC) at 6-7°C would likely be due to HRSP. Although this test detects HRSP and may reflect potential growth rates in milk, it does not necessarily predict potential shelf-life; spore-shock treatment differs from pasteurization, thus the microbial response may differ.

**Spore Shock Method for Psychrotrophic Spore Formers:**

1. Measure 200 ml of raw milk into a 300 ml sterile bottle/flask. Avoid touching the bottle lip.

2. Prepare a pilot bottle with a thermometer probe. Cap all bottles/flasks tightly (screw caps preferred).

3. Place bottles/flasks in an 82°C circulating water-bath. Milk level should be at least 4 cm below the water line. Bottles/flasks should be agitated throughout heating (e.g., a water bath with shaking capabilities should be used). When the pilot bottle reaches 79°C, turn down the water bath to 80°C. The complete heating step may take considerable time.

4. Begin timing when the pilot bottle/flask reaches 80°C. Heat for 12 minutes, then immediately cool in an ice bath.

5. Plate the sample for SPC (32°C for 48 hrs) initially and after 14 days at 6-7°C. Longer times may be needed (17-21 days) depending on the growth rate of the organism present and the desired shelf-life of the products. Plating after shorter hold times (e.g., 10 days at 6-7°C) may show increases where levels and/or growth rates are more significant. Generally one target test day (e.g., after 14 days at 6-7°C) should be selected based on individual plant testing goals and schedules.

   **Interpretation**:

   A significant increase is indicative of the presence of HRSP in the raw milk and *may* reflect the spoilage potential of pasteurized milk made from this milk. Higher counts at a specific target test day (e.g., 14 days at 6-7°C) may reflect higher levels in the raw milk and/or the presence of faster growing strains. Little or no increase at 14 plus days suggests limited spore levels in the raw milk.

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