

Psychrotrophic Spore-Forming Bacteria in Raw and Pasteurized Milk

Background

Preventing **post-pasteurization contamination (PPC)** with Gram-negative psychrotrophic bacteria is essential in ensuring extended shelf-life of pasteurized milk products. When PPC is prevented, the limiting factor of shelf-life becomes the thermotolerant psychrotrophic bacteria that survive pasteurization. Thermotolerant bacteria as a rule are gram-positive organisms. Gram-negative bacteria, as a rule, do not survive pasteurization.



Significant among the thermotolerant psychrotrophs common to milk are endospore (“spore”) forming bacteria. Spores are dormant structures that allow certain bacteria to better withstand stresses that they would not normally survive in the vegetative state (i.e., as actively growing bacteria). When “activated” and/or put into a favorable environment, spores can grow again as vegetative bacteria. The pasteurization process may promote “activation” and outgrowth of spore-forming bacteria.

Spore-Forming Bacteria in Processing

Psychrotrophic strains of spore-forming organisms are widespread in the raw milk supply. They are common contaminants in the farm, 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. ***It is important to understand that it only takes one psychrotrophic spore per milk container to eventually grow and cause spoilage.***

In pasteurized milk, increases in psychrotrophic spore-forming 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 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.

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 for 12 minutes) the milk to “activate” the spores and to kill off the 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 low (<1/ml), a more useful procedure is to heat-shock a larger volume; hold the heated milk at 6-7°C; plating for SPC initially and at 14 days. Growth 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.** A significant increase in counts is indicative of HRSPs in the raw milk and may reflect the spoilage potential of the pasteurized milk. Higher counts at a specific target test day may reflect higher levels of spores in the raw milk and/or the presence of faster growing strains. Little or no increase at 14+ days suggests limited levels in the raw milk.



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 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. 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.

Want more information on [psychrotrophic spore-forming bacteria](#) and the MQIP? Contact Nicole Martin (nicole.martin@cornell.edu) in the Milk Quality Improvement Program or visit our website <https://foodsafety.foodscience.cornell.edu/mqip/>

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