	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*	

Title: Bacterial Cell Extraction from Fluid Milk			
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Bacterial Cell Extraction from Fluid Milk

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this experiment is to purify bacterial cells from other milk components, namely protein and butterfat. This is necessary for downstream applications such as microscopy and DNA or RNA extraction.

1.2 Scope

This SOP applies to the Milk Quality Improvement Program Laboratory and the Food Safety Lab.

1.3 Definitions

Tween-20: also called polysorbate 20, a nonionic surfactant. Its stability and relative nontoxicity allow it to be used as a detergent and emulsifier in a number of domestic, scientific, and pharmacological applications. In this protocol, it emulsifies the butterfat from the milk, allowing for its removal from the bacterial cell pellet through washes, without adversely affecting bacterial membrane integrity or viability.

1.4 Safety

Centrifuges must be carefully balanced to avoid damaging the instrument and causing catastrophic failures.

SECTION 2 MATERIALS

- Milk containing bacteria at high levels (≥ 5 log CFU/mL). This milk can be raw, pasteurized, creamline, or homogenized, and any fat level.
    - Coagulated milk will not work for this SOP. The coagulated protein partitions with the pellet during centrifugation and will prevent downstream use of the extracted bacterial cells for microscopy or molecular biology.
- Phosphate Buffered Saline (PBS) with 0.1% volume/volume Tween-20
    - To prepare, pipette 100 µL of Tween-20 into a sterile 99 mL bottle of pre-prepared PBS (Weber Scientific). You will need a wide-bore ("blunt") pipette tip to pipette the Tween-20. These tips are available in the sterile supply cabinet in room 350 and are typically used in the lab to pipette glycerol. Shake the PBS bottle to mix. This solution can be stored at room temperature for up to 6 months.
- Sterile PBS
- Sterile 2 mL microcentrifuge tubes – stored in the sterile supply cabinet in room 350
- Sterile cotton swabs – stored in the sterile supply cabinet in room 350
- P1000 micropipette and sterile filter tips
- Ice

- Microwave oven
- Benchtop vortex mixer with horizontal microtube holder attachment (Scientific Industries Item # SI-H524)
- Microcentrifuge

SECTION 3 PROCEDURES

1. Pipette 2 mL of each milk sample into a 2 mL microcentrifuge tube. Place tubes in a microcentrifuge tube rack.
2. Microwave the samples in the rack for a few seconds (5-8, assuming the milk is cold) to warm the milk. The target temperature for the milk is approximately 40°C, which should feel just warm to the touch through the wall of the tube.
    a. This step ensures that the butterfat is melted, which is necessary for the subsequent step to be successful.
3. Place samples, cap side out, into the horizontal microtube holder on the vortex mixer. Turn the vortex mixer speed to maximum and turn the vortex mixer on. Shake samples for 10 minutes.
    a. This shaking detaches the bacteria that typically partition into the butterfat upon centrifugation (including many sporeformers) and forces them into the aqueous phase of the milk.
4. Immediately transfer samples to the microcentrifuge and centrifuge at 5,000×*g* for 5 minutes.
    a. This separates the milk into 3 phases – a pellet containing mostly bacterial cells and any precipitated protein (bottom layer), skim milk (middle layer), and butterfat (top layer).
5. Place microcentrifuge tubes on ice and chill for 10 minutes.
    a. This solidifies the butterfat layer, allowing for it to be easily removed.
6. Use sterile cotton swabs to remove the butterfat layer from the top of the tube. Remove as much as possible, but it doesn't need to be perfect because the rest of the butterfat will be removed in subsequent steps. Discard the swabs.
    a. The butterfat layer, now depleted of bacterial cells, can be removed and discarded.
7. Pipette off and discard the supernatant (skim milk), preserving the pellet at the bottom of the tube.
8. Use sterile cotton swabs to remove any remaining butterfat clinging to the walls of the tube.
9. Resuspend the pellets in 2 mL of PBS with 0.1% Tween-20 by breaking up pellet with the pipette tip, pipetting up and down to further break up the pellet, and then vortexing.
    a. The Tween-20 is a surfactant, and will dissolve any remaining butterfat in the tube, allowing for it to be removed after subsequent centrifugation and washing steps.
10. Centrifuge the tubes at 5,000×*g* for 5 minutes.
11. Pipette off and discard the supernatant, preserving the pellet at the bottom of the tube.
12. Repeat steps 9-11 one more time, for a total of 2 washes. A third wash can optionally be performed.
13. Resuspend the cell pellet in 20 µL of PBS (without Tween-20).

At this point, the cell suspension contains mostly bacterial cells, with some residual precipitated protein. It may be used for microscopy or for molecular biology applications at this point. Store the suspensions at 4°C. Stability will depend on the type(s) of bacteria present in the sample.

SECTION 4 TROUBLESHOOTING

- The final cell pellet should be very small, barely more than a small white patch at the bottom of the microcentrifuge tube. If the pellet is larger than this, there may be precipitated protein contaminating the pellet. This will likely interfere with visualizing the cells using microscopy and may adversely affect molecular biology applications. For molecular biology applications, precipitated protein could be digested using proteinase K, but this will not work for microscopy as it will destroy the bacterial cells as well.

SECTION 5 REFERENCES

Brewster, J. D. and M. Paul. 2016. Short communication: Improved method for centrifugal recovery of bacteria from raw milk applied to sensitive real-time quantitative PCR detection of *Salmonella* spp. J. Dairy Sci. 99(5):3375-3379. https://doi.org/10.3168/jds.2015-9655.

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	2020-12-08	sjr267	Original SOP
Version 2			
Version 3			