Visualization of unstained bacterial cells using phase-contrast microscopy

FILE NAME: 7.30-Visualization of unstained bacterial cells using phase-contrast microscopy.docx

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this experiment is to visualize bacterial cell structures microscopically using phase-contrast microscopy, which does not require staining or other treatments. Bacterial endospores are visible using phase-contrast microscopy without performing an endospore stain.

1.2 Scope

This SOP applies to the Milk Quality Improvement Program Laboratory.

1.3 Definitions

Phase-contrast microscopy: an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image, allowing for the visualization of unstained biological structures. For more information, visit https://en.wikipedia.org/wiki/Phase-contrast_microscopy.

Bacterial endospore: a dormant, tough, and non-reproductive structure produced by some bacteria in the phylum Firmicutes. Endospores are refractive and appear bright against dark bacterial cells in phase-contrast microscopy.

1.4 Safety

Caution must be taken while melting the agarose solution in the microwave. PPE must be worn (lab coat, gloves, safety glasses), and a silicon mitt must be used when touching the hot bottle. The cap of the bottle must be loosened whenever it is being heated. The microwave should never be left unattended. Only tempered borosilicate glass (e.g., Pyrex, Kimax) should be used in the microwave, and the microwave should only ever be used to reheat very small volumes of liquid, such as those used in this experiment.

If you need to reheat microbiological media, do not use the microwave. See SOP 5.2.5 on the Food Safety Wiki for detailed guidelines on how to safely reheat larger volumes of media.

SECTION 2 MATERIALS

- **A bacterial culture**:
  - A 1 mL aliquot of each culture grown in liquid media should be centrifuged (10,000×*g*, 1 min), and then have the supernatant discarded and the pellet resuspended in approximately 1/10 the original volume of phosphate buffered saline (PBS).
  - Resuspend cultures grown on solid media in 100 µL of PBS and vortex well to mix. The suspension should be quite heavy.
  - Bacteria from milk samples can be prepared according to SOP 7.29 and used as-is for this procedure.
- 0.7% agarose solution
  - Add 50 mL of dH_2O to a 100 mL Pyrex media bottle. Add 0.35 g agarose and swirl to mix. Boil in the microwave (approximately 30 sec) to fully dissolve the agarose.
  - Solution can be stored at room temperature for up to 6 months.
  - Solution must be used while agar is molten. Reheat in the microwave for 20-30 sec until boiling immediately before use.
- Glass microscope slides
- Glass cover slips
- Microscope immersion oil

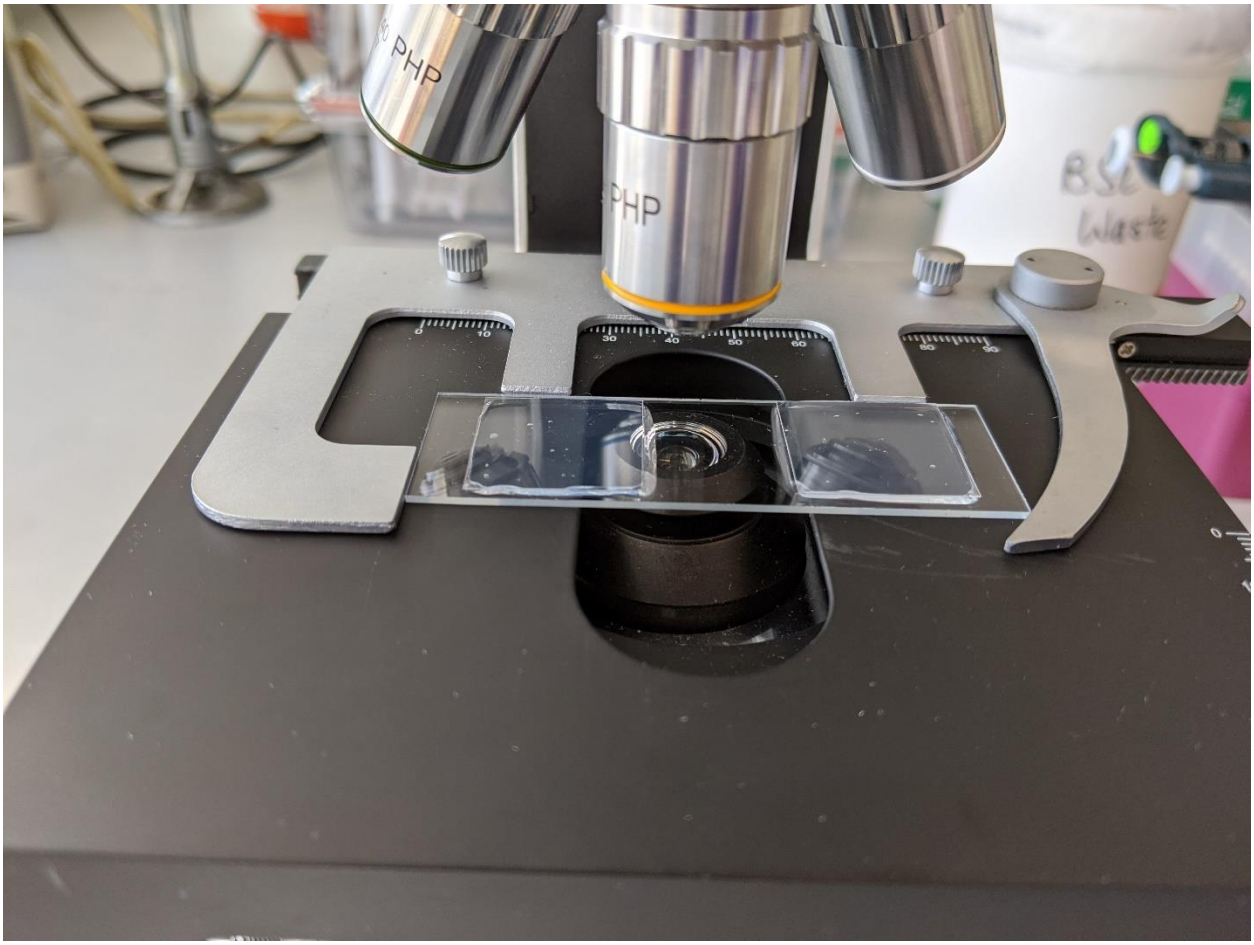
- Phase-contrast microscope with 100× oil immersion objective lens
- Micropipettes and tips (P1000 and either P20 or P10)

SECTION 3 PROCEDURES

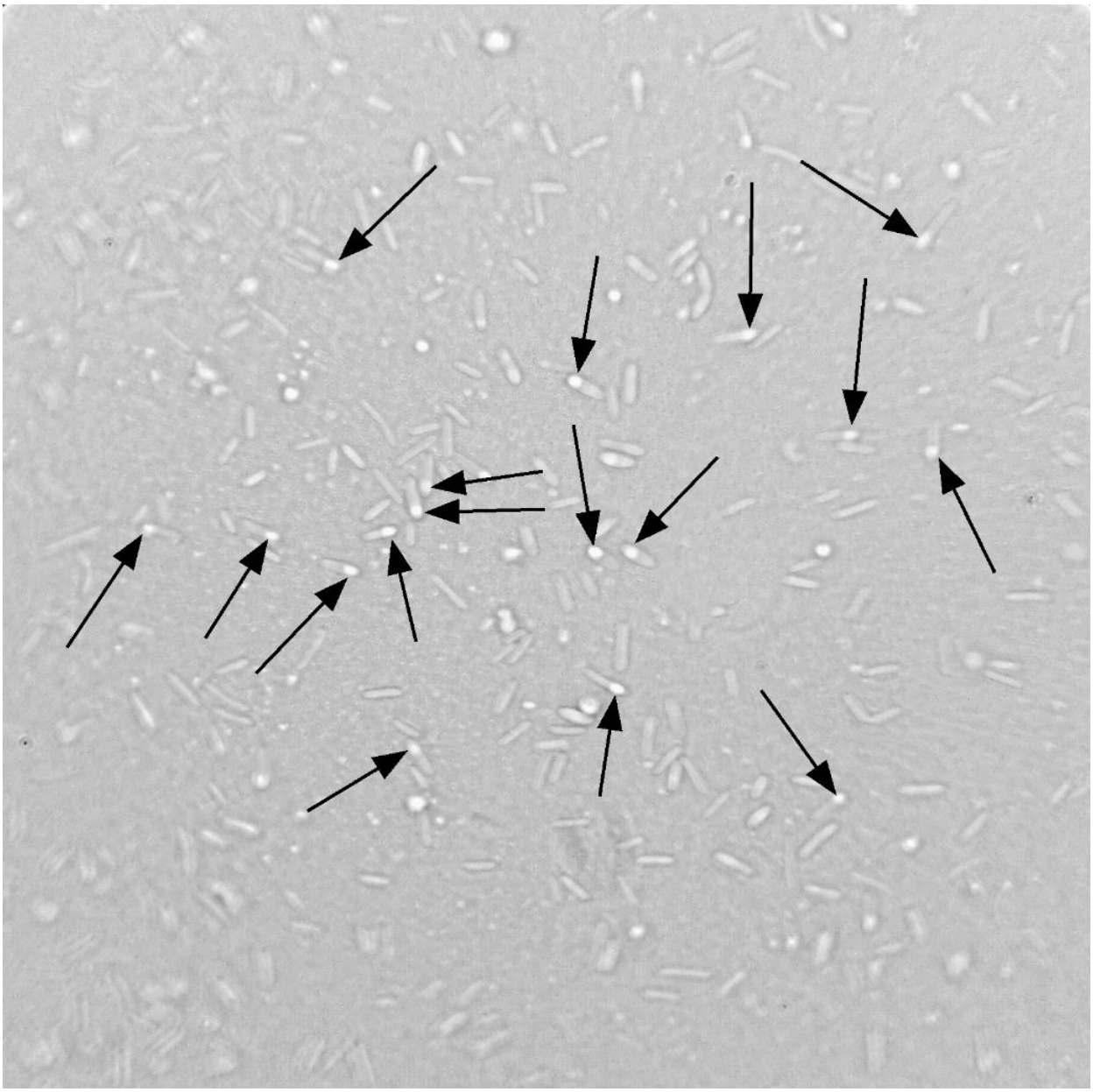
1. Pipette 500 µL of molten 0.7% agarose solution onto a clean microscope slide. Aim for an even, thin layer that touches both the top and bottom of the slide and is at least twice as wide as the cover slip.
2. Allow the agarose layer to cool and set for 2 min undisturbed before proceeding.
    a. The agarose pad immobilizes the bacteria, allowing them to be visualized more easily.
3. Pipette 5-7 µL of prepared bacterial culture in a straight, horizontal line across the center of the agarose pad, approximately the width of the cover slip.
4. Carefully place a cover slip over the deposited bacterial culture, being careful not to trap any air bubbles. Press gently on the cover slip and move it around in a small circular motion to distribute the culture evenly under the surface and dislodge any trapped air.
    a. This compresses the bacterial culture into a single, immobilized layer which can be focused on and viewed easily.
5. View the prepared and mounted bacterial culture on the phase-contrast microscope using the 100× oil immersion objective for a total magnification of 1,000×.
    a. It is easiest to first focus the microphone on the edge of the cover slip before adjusting the view to the bacterial culture.

Interpretation note: Vegetative bacterial cells in phase-contrast microscopy typically appear as dark structures against a lighter background. Spores are phase-bright, meaning they appear as lighter structures when compared to the darker vegetative cells.

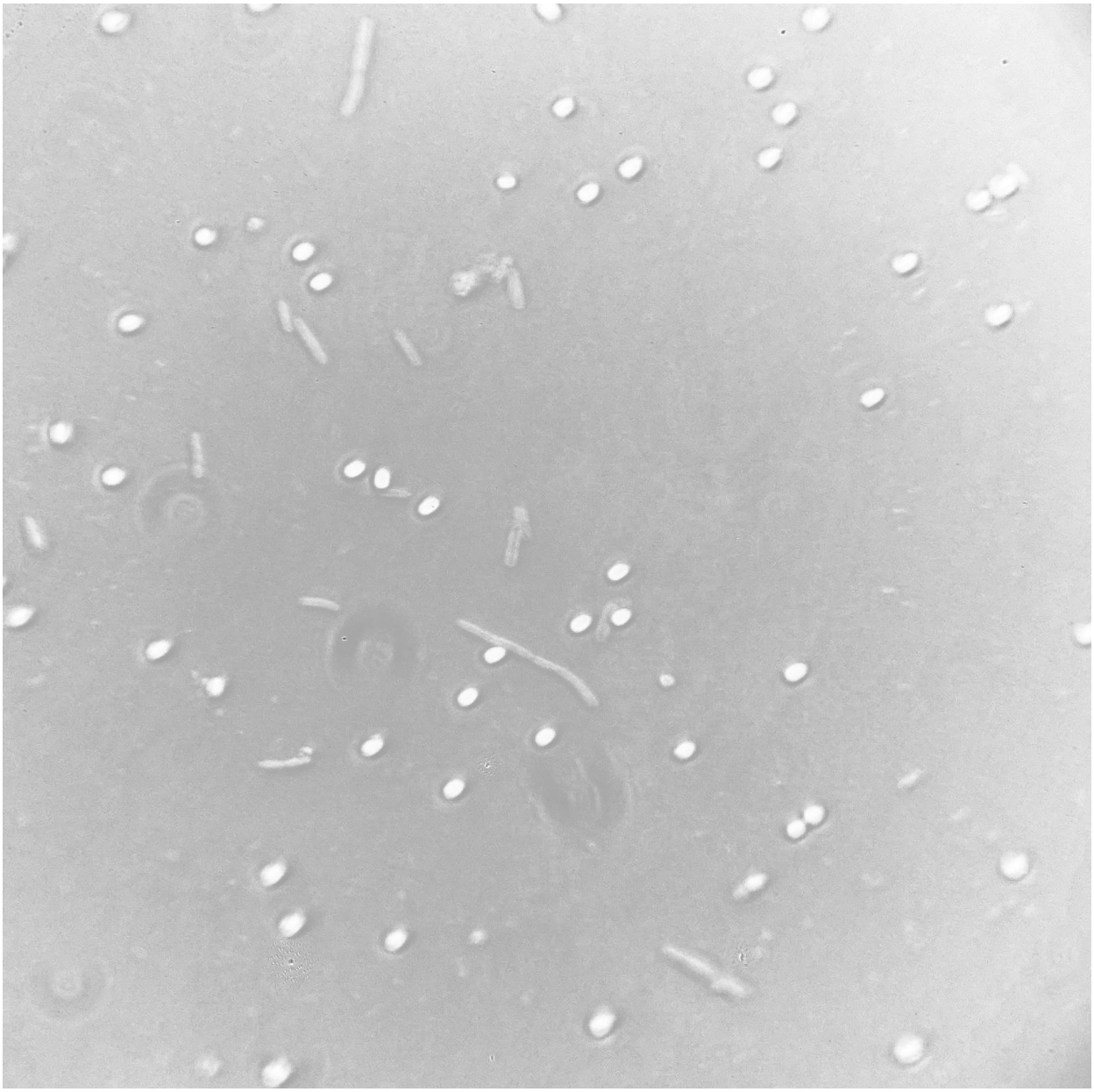
Examples:



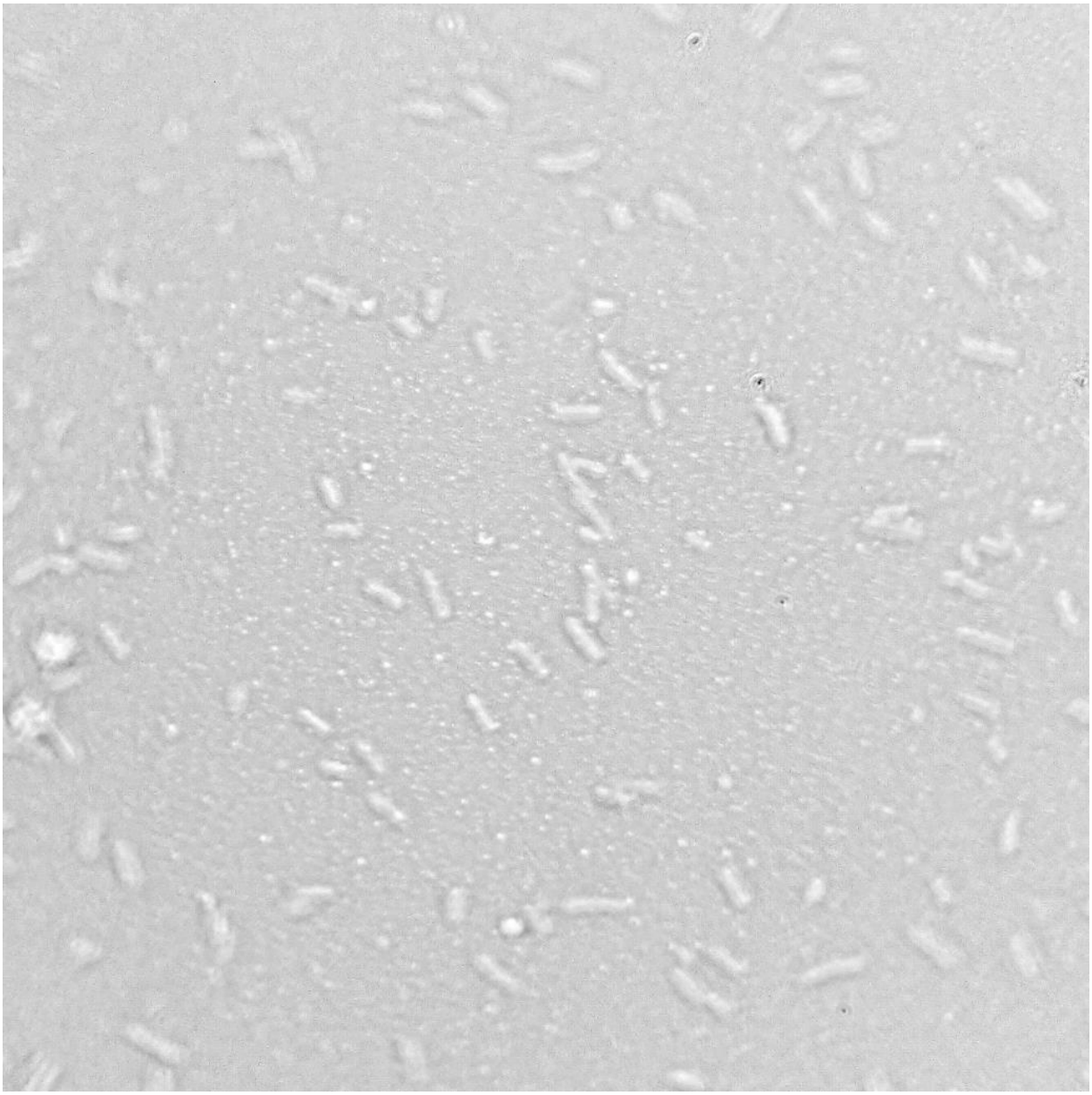
A prepared slide mount containing 2 cultures, ready to view using the phase-contrast microscope.



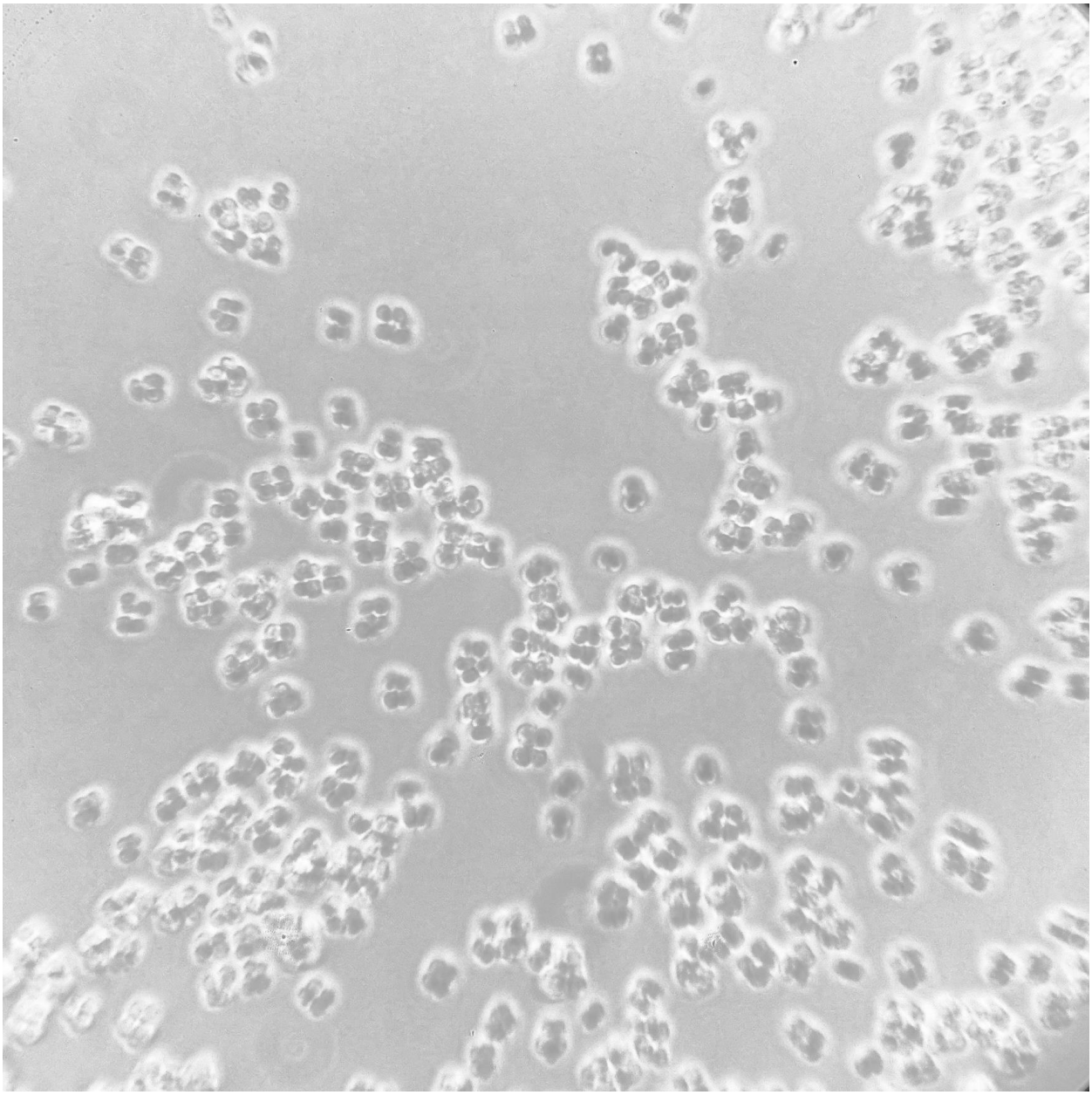
FSL H8-0237, *Paenibacillus odorifer*, extracted from 2% UHT milk after 35 days of incubation at 6°C. Terminal endospores appear as bright structures within darker cells, and are pointed out by the arrows. 1,000× magnification. Photo has been enhanced using GIMP and ImageJ.



FSL J3-0120, *Paenibacillus peoriae* s.l. culture containing a mix of vegetative cells (dark) and naked endospores (bright) viewed after 4 days of shaking incubation at 32°C in 2×SG broth. 1,000× magnification. Photo has been enhanced using ImageJ.



FSL H7-0689, *Paenibacillus amylolyticus*, extracted from 2% UHT milk after 35 days of incubation at 6°C. Vegetative cells only, no endospores present. 1,000× magnification. Photo has been enhanced using GIMP and ImageJ.



Unknown culture contaminant, likely *Staphylococcus*, viewed after 4 days of shaking incubation at 32°C in 2×SG broth. 1,000× magnification. Photo has been enhanced using ImageJ

SECTION 4 TROUBLESHOOTING

- Too many or too few cells on the mount can be remedied by adjusting the concentration of the prepared culture by either adding additional PBS or by centrifuging and resuspending in a smaller volume of PBS.
- Milk samples contaminated with large amounts of precipitated protein will obscure bacterial cells, making them difficult to view. See SOP 7.30 for more information.

SECTION 5 REFERENCES

Edwards, A. N. and S. M. McBride. 2016. Isolating and purifying *Clostridium difficile* spores. Pages 117-128 in *Clostridium difficile*: Methods and protocols. A. P. Roberts and P. Mullany, eds. Springer New York, New York, NY. https://doi.org/10.1007/978-1-4939-6361-4_9.

SECTION 6 METHOD VERSION & CHANGES

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