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Short Communication

An improved method of preparing wet mounts for photomicrographs of microorganisms

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Summary

A method is described to prepare agar-coated dry slides for wet mounts of living microorganisms that need to be photographed by bright field, phase-contrast or epifluorescence microscopy.

Key words: *Microscopy – Photomicrograph – Wet mounts*

Introduction

Anyone who has prepared micrographs of bacteria with the light microscope has experienced the problem of trying to obtain uniformly sharp pictures of the cells. The difficulties with wet mounts are caused by the following circumstances.

(1) Cellular motility, streaming and Brownian movement of the cells.

(2) Depth of focus for cells not incidentally in horizontal position.

(3) Number of cells in focus and dependency of the thickness of the wet mount.

The problems are the more pronounced the smaller the objects are and the higher the power of the objective to be used (see Fig. 1, left).

The troubles may be overcome if slides, which are coated with a layer of dry water agar, are used for micrographs rather than using cut out wet agar blocks [2].

Preparation of slides

1. Good quality powdered agar is repeatedly washed with large volumes of distilled water to remove dust and small particles as well as soluble substances which form crystals if the molten agar is dried again. For washing, the agar suspension is magnetically stirred for several minutes and then allowed to settle. The supernatant

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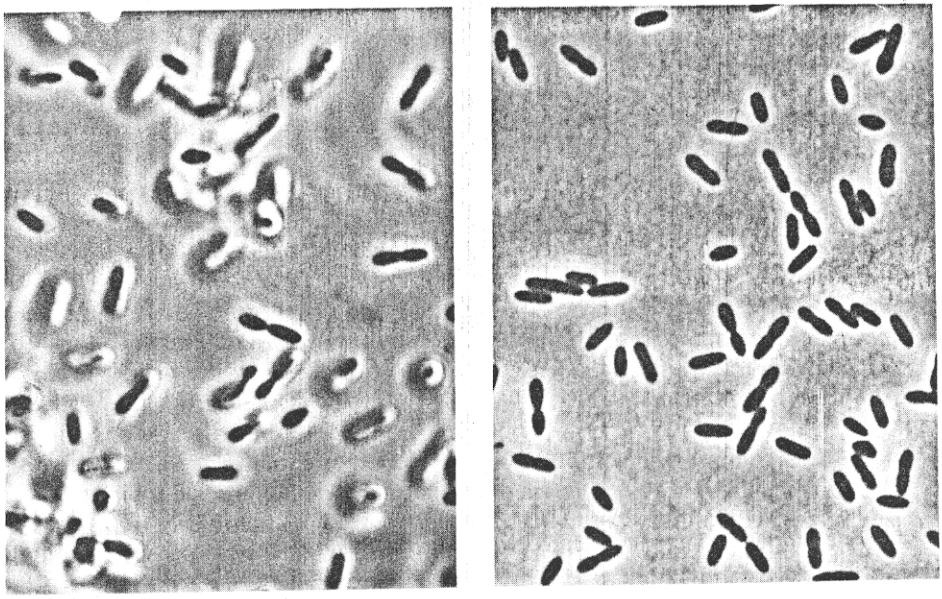

Fig. 1. Phase contrast micrographs of *Acetobacterium woodii*. Left, ordinary wet mount on plain glass slide. Right, wet mount on an agar-coated slide as explained in the text. Magnification, 2000×.

is decanted and replaced by fresh distilled water. After the last washing, the agar is suspended in an amount of distilled water to yield a final agar concentration of about 2% w/v. This agar suspension is autoclaved for 20 min at 120 °C.

2. Clean microscope slides to be coated with an agar layer are placed at some distance from each other on an even horizontal table. The autoclaved water agar is kept molten in a boiling water bath while samples are removed with a 5 ml graduated pipette. 2 ml of water agar are distributed over the surface of each slide by moving the tip of the pipette in a zigzag line from one end to the other. Care must be taken not to let the agar run off the slide. The slides should be allowed to dry overnight, and care must be taken to keep dust off the slides. The dried agar slides should be stored dust free in a slide box.

3. When photomicrographs of bacteria in liquid medium or a suspension need to be taken, the cell density is first checked by the usual wet mount. If the number of cells per field is too low, the cell suspension should be concentrated by centrifugation. From the cell suspension with the proper cell density, three doplets in amounts of about 0.02, 0.022 and 0.025 ml are placed with a graduated 0.1 or 0.2 ml pipette on the dry agar surface of a slide. Each droplet is immediately covered with a clean cover slip of 18 × 18 mm size. In order to prevent evaporation of the liquid, all four sides of each cover slip are covered with a molten mixture of paraffin plus paraffin oil (one part paraffin plus three parts paraffin oil). The sealing is most readily performed

with a metal spatula, the flat front edge of which is about as wide as the cover slip.

In the meantime the agar gradually swells up and soaks up most of the liquid under the cover slip. The three mounts on the slide are now inspected with a high dry objective. Only a mount in which regions of attached cells, as well as regions of motile or still floating cells, are detected is useful for taking photographs. If all cells under a cover slip are attached, the amount of cell suspension was insufficient to prevent the strong squeezing of the cells against the cover slip by the swelling agar. As a result, not only the morphology of the cells is distorted but also the phase contrast is drastically reduced. However, if a wet mount with areas of still actively or passively moving cells is chosen, the best positions for photographs are found in areas of attached cells that are close to regions of still moving cells. In those areas all cells are motionless and gently held in place by the swollen agar without being squeezed. As a result, all cells are horizontally oriented in one plane and thus all are in focus. Now, a drop of immersion oil is placed on the cover slip and the oil immersion objective (100 × magnification) is used for photomicrographs (see Fig. 1).

4. If the autofluorescence of, e.g., methanogenic bacteria, is to be documented using UV radiation (300–450 nm) and epifluorescence microscopy [1], sudan black [3] should be added to the agar to reduce the contrast-decreasing background fluorescence of the agar (see Fig. 2). The sudan black agar is prepared as follows: 25 ml of sudan black solution (1 or 2 mg sudan black in 100 ml ethanol) are mixed with 25 ml of distilled water. The mixture is heated to about 60 °C and added to 50 ml molten water agar which is prepared as described under **1**, using, however, 4%

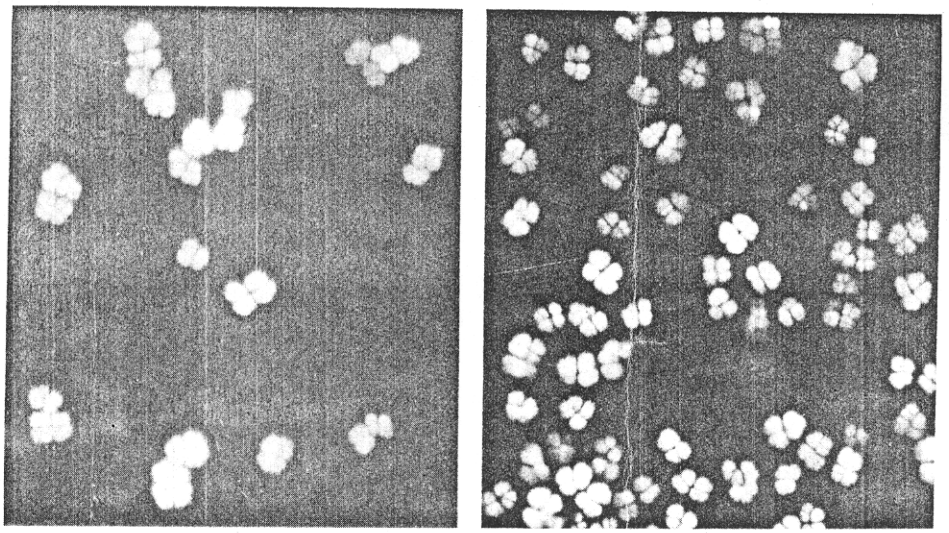

Fig. 2. Autofluorescence of *Methanosarcina barkeri* strain fusaro as revealed by epifluorescence microscopy using wet mounts on agar-coated slides. Left, the background fluorescence of plain water agar results in a poor contrast. Right, with sudan black agar better contrast is obtained. Magnification, 625 ×.

agar. After boiling and thorough mixing, the sudan black agar is used in the same way as the plain water agar to prepare agar-coated slides.

At first glance, the method appears complicated, however, after practice it becomes obvious that the greatly improved quality of the photomicrographs is a sufficient reward for the effort.

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