



MICROSCOPIC EXAMINATION OF BACTERIA ON AGAR COATED SLIDES

Dieter Claus

Chemnitzerstr. 3, 37085 Göttingen, Germany.

Fax : 0049 551 55791 ; E-mail : clausgoettingen@flynet.de

The morphological examination of bacterial isolates is important for their characterization. Since bacteria in aqueous suspensions have optical properties similar to those of water they are difficult to observe by normal bright-field microscopy. Therefore, one has to apply methods by which the visibility of cells is increased. A widely use method to achieve this is to stain bacteria by certain dyes which react with cytoplasmic elements of the cells. However, cells in fixed and stained preparations may suffer some degree of distortion. Therefore, stained preparations may not give a true impression of cellular shape and size.

For the microscopical examination of living bacterial cells wet preparations should be studied using phase contrast microscopy. Through this a more accurate impression of cell morphology can be obtained than from the stained preparations. However, for obtaining good results simple wet mount preparations are not recommended as the thickness of the film between slide and cover glass is much higher than the width of a bacterial cell. This results in the focusing of only few cells and, in addition, cells might show movements which makes difficult to determine the exact shape of the cells.

Therefore, the use of agar coated slides is recommended. This technique helps to immobilize bacterial cells between the coverslip and the agar layer of the slide. Cells get slightly pressed to the cover glass as the water of the cell suspension gets absorbed by the agar layer. In this way the "depth" of the liquid layer (as it appears in simple wet mount preparations) is eliminated and all cells can be seen in a single plane.

For the preparation of photomicrographs (for documentation of bacterial cell morphology), the use of agar coated slides is highly recommended.

Following precautions are recommended while using agar coated

slides :

1. To standardize all procedures and to define the cultural conditions clearly as morphology of cells may be affected by the medium used and by the growth conditions.

2. To grow the cultures at the lower limit of their optimal temperature range or below, but never near the maximum growth temperature. At the latter temperature cell shape and size may become atypical.

3. To examine cultures immediately after growth becomes just visible since bacterial cells are in their most typical state in young cultures. Cultures grown on solid media are often more heterogenous in shape than cells from liquid media. This may be due to different supply conditions of nutrients and oxygen in different places within a colony grown on agar.

4. To shake liquid cultures which are strictly aerobic or cultures which tend to form a sediment. However, shaking may disturb the natural arrangement of cells by breaking up chains, clumps or other fragile associations. So it would be wise to study also material from unshaken cultures or from colonies.

With some bacteria the shape of cells is markedly influenced by the composition of medium. In order not to neglect any typical morphological changes arising during the growth cycle, it is also important to examine cultures of different age up to several days.

5. To use only purified agar brands (such as Oxoid Agar No.1, Difco Bacto Agar, and others) since normal agar is often heavily contaminated with bacteria or other particles which may interfere with the observations.

6. To obtain good preparations the thickness of the agar film should be less than 1mm, otherwise the image contrast will be strongly reduced. Agar coated slides should be prepared on a leveled surface in order to have all cells

-at least those within a single field or vision - in focus. For this the use of a levelling table is recommended.

7. To use only a small drop of the bacterial suspension so that the liquid can be absorbed by the agar layer.

Preparation of agar coated slides

1. Prepare 2% water agar, distribute the agar in 5ml amounts into screw-

cap test tubes. Sterilize the tubes (with caps loosened) for 20 min at 121°C. Tighten the screw caps

2. Put a slide in a 90 mm Petri dish, placed on a level surface.
3. Place a tube containing 5ml of a 2% water agar in a boiling water bath or in a microwave oven (if a Bunsen flame is used directly to liquefy the agar, heating must be done very carefully).
4. After the agar is liquefied pour the whole amount (while still very hot) into the Petri dish and on the surface of the slide. By slightly moving the Petri dish to and fro the agar will get evenly distributed to cover the bottom of the dish and the slide. Allow the agar to set, this takes few minutes.
5. Cut out the agar coated slide in the Petri dish from the surrounding agar layer by using a knife or a spatula.
6. Clean the lower surface of the slide from agar residues.

Procedure of Examination

1. Using an inoculation loop (inner diameter about 1mm) transfer one drop of a distinctly turbid bacterial suspension onto the agar layer of the slide. Liquid medium used for growing the particular organism or 0.1% peptone water should be used for preparation of such suspension.
2. Prepare a 1:10 diluted suspension using the same medium. Transfer one drop of this suspension also on the agar slide.
3. Take a cover glass with tweezers, touch the agar surface with one edge of the glass about 5mm apart from the drop. Carefully lower the cover glass onto the agar surface. Take care not to include air bubbles. Cover each drop separately.
4. Wait for a few minutes. The preparation is then ready for microscopic examination.
5. Place the slide on the microscopic stage. Observe first with a dry phase contrast objective (e.g. 40 x). If cells are in focus, the oil immersion objective should be applied for more detailed observations.

OBSERVATIONS :

Cells within a single microscopic field of view should be in focus. If not then either the coating of the slide was not done on a leveled surface or the agar used for coating was not sufficiently hot to cover the bottom of the whole plate or the drop of the bacterial suspension was not sufficiently small. Repeat the procedure to get good results.

After observation discard the slide in a container for sterilization.

Published by : UNESCO / WFCC-Education Committee 1998