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Gram staining technique

Aim, principles, theory

(a) to introduce the most common bacterial staining technique

b) to introduce the use of the classical taxonomic division of bacteria into Gram-negative and Gram-positive bacteria - morphological analysis

The bacterial cell wall is an important surface structure of the prokaryotic cell. The wall ensures **the shape of the bacteria** and allows them to survive in **a hypotonic environment**.

According to the Gram staining technique, the cell wall structure distinguishes Gram-positive (G+), Gram-negative (G-), bacteria without cell wall and bacteria with damaged cell wall. Bacterial cell walls contain **peptidoglycan (murein)**, which belongs to polymers. Gram staining, one of the most widely used techniques for staining bacteria in microbiology. The technique is named after the Danish bacteriologist Hans Christian Gram. The method is designed to distinguish bacterial species into two main groups according to the chemical and physical properties of their cell walls.

The words VLAK or VLAS are used as an aid for remembering the successive dyes and steps; the individual letters indicate the dye solutions used - V (crystal violet), L (Lugol's solution), A (alcohol - ethanol or acetone), K (carbolfuchsin) or S (safranin).

We start by applying the first dye, crystal violet, to a fixed sample of the bacterial culture. After rinsing, a Lugol's solution of iodine is then added to act as a mordant. The sample is then treated with acetone/ethanol, which acts as a decolouriser. The role of acetone/ethanol is very important as it determines the final colour of the bacteria. Gram-positive bacteria have a relatively thick layer of peptidoglycan and therefore the crystal violet remains in the cell wall, resulting in a blue-purple appearance. In contrast, Gram-negative bacteria have a thin peptidoglycan layer and the crystal violet is washed out upon exposure to ethanol. Therefore, an additional dye, safranin or carbolfuchsin, must be used subsequently, turning the gram-negative bacteria pink or red.

Gram staining can be affected by the physiological state of the cells, the age of the cell culture and the composition of the culture medium. Therefore, in certain cases, we can speak of so-called Gram-lability. Freshly grown bacteria are used for staining. Cells can lose their gram-positivity, e.g. due to mechanical damage, UV radiation, exposure to acids, alkalis or solvents.

The actual workflow was innovated on the basis of practical experience from laboratory exercises in previous years, in particular by shortening the staining time.

Material and equipment

Bacterial cultures of various bacterial species from the group evaluated as BSL 1, commonly found in the environment - Bacillus subtilis, Micrococcus luteus, lactobacilli, Streptococcus thermophilus, etc., optical microscope, preparation kit, slides, immersion oil, pipettes, distilled water, crystal violet solution, Lugol's solution, safranin, ethanol, beakers, work gloves.

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Procedure

a) Apply the bacterial culture to a drop of sterile distilled water on a clean slide to form a bacterial suspension. Let it dry well!!!

b) Perform fixation of the slide, i.e., pass the slide through the flame of a blowtorch 3 times in succession. After cooling, perform staining.

c) Stain the preparation for 20-50 seconds with crystal violet solution (blue colour).

d) Overcoat for 20-50 seconds with Lugol's solution.

e) Rehydrate with ethanol or acetone for 10-15 seconds max., the purple solution flows off. (Only gram-negative bacteria are decoloured, gram-positive bacteria remain blue!).

f) Rinse thoroughly with distilled water.

g) Stain for 30-60 seconds with carbolfuchsin or safranin solution. (We stain gram-negative bacteria red).

h) Dry with filter paper and observe with an immersion objective.

i) Determine from the staining of the slide whether the culture submitted contains G+ or G- bacteria.

Results

In the protocol, we carefully describe the time of staining in individual steps for each preparation, the time of rinsing the preparations and the result of staining.

Describe any time and other differences that occurred during the actual work.

Discussion and conclusion

To find out what results other students in the group encountered, conduct a discussion.