Expt. No. 5

Date 04/11/2021

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CAPSULE x STAINING

Aim: To prepare an India ink wet mount for microscopic examination of Cryptococcus.

REQUIRED MATERIALS:

1. Inoculating Loop
2. Saline
3. Microscope slide
4. Blood agar plate
5. India Ink
6. Plastic transfer pipette
7. Coverslip
8. Toothpick
9. Standard bright field light microscope.

PROCEDURE :-

- A loop was pre-flamed to ensure that it is sterile.
- Using this sterile loop, loop full of saline was placed onto a microscope slide.
- Then the loop was resterilized.
- A small amount of growth from a well-isolated colony on a blood agar plate was taken.
- A light suspension was made by emulsifying the yeast in the saline.
- The suspension should be barely visible to the naked eyes as cloudiness.
- If it is too densed, it will be difficult to examine.
- The loop was resterilized.
- An equal volume of India Ink was added to yeast suspension using a drop from plastic transfer pipette.

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10) This was mixed with a toothpick antill the suspension become homogenous.
11) The toothpick was discarded into the disinfectant.
12) A coverslip was placed on the slide and it was gently push down using a toothpick.
13) Then, the stained wet mount was examined by standard bright field light microscopy.
14) It is started with 10 times objective and ensured that the condenser was raised.
15) The focus in low power was moved to 40 times objective.

OBSERVATION

The presence of capsule excluded the particles of India ink and a clear appearance around each cell was observed.

CONCLUSION

From this experiment, we have successfully performed capsule staining.

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ENDOSPORE STAINING

Aim:- To perform endospore staining.

REQUIRED MATERIALS

1. Prepared smear.
2. Paper towel.
3. Bunsen Burner.
4. Metal or Aluminium Cup
5. Malachite green stain.
6. Slide
7. Safranin.

Theory :-

The endospore stain is useful for detecting endospore forming bacteria. Endospores are inclusions inside of cells that are very resistant to heat, chemicals and radiation. Under favourable conditions spores can germinate to form vegetative cells, because a thick coat covers the endospore, it does not take up stain easily, and it is necessary to heat the smear in the presence of some to force it into the endospore. After cooling the slides are exposed to safranin in a second counter stain which stains vegetative cells.

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PROCEDURE :-

1. A smear was first prepared as outlined in smear preparation.
2. A paper towelling was cut to a dimension larger than the smear, but smaller than the edge of the slide. This allows a larger amount of stain to be placed on the smear and prevents the stain from drying out.
3. During endospore stain a water bath was set up with a bunsen burner and simple metal or aluminium cup as a heating platform.
4. It is important to keep water bath gets to a high temperature so that steam should be raised before adding malachite green stain.
5. After the boiling of water bath a large amount of malachite green was added to the slide and the slide was stained for 5 minutes. It is important to watch the slide carefully during the staining to prevent the malachite green from completely evaporating.
6. It is also neccessary to add extra stain during the 5 minutes boiling period.
7. After 5 minutes, slide was allowed to cool and malachite green had to be removed.
8. A wash with water removed any excess malachite green.
9. Then safranin was used as a counter stain at room temperature to stain vegetative cells.
10. Finally, the slide was rinsed with water and blotted dry using a soft paper towel. (or blotting paper)

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OBSERVATION

The endospore forming bacteria Bacillus cereus was observed under microscope. Long chains of pink cells were found with green coloured endospores.

CONCLUSION :-

From this experiment, we have successfully distinguished the endospores from vegetative cells using endospores staining process.