EXPERIMENT 2

MICROSCOPIC ANALYSIS OF MICROORGANISM

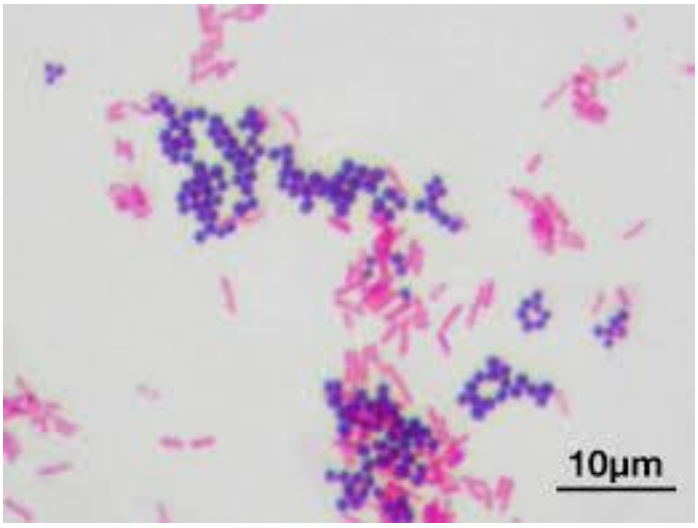

Figure 1: A Gram staining of Staphylococcus aureus (Source: https://en.wikipedia.org/wiki/Gram_staining)

TOPIC OUTCOMES:

Student should be able to

1. To familiarize with the equipment and materials needed to observe microorganism
2. To understand the components, usage and safety procedure of using microscope
3. To understand and differentiate micrscopic characteristic of microorganism

MATERIAL

- Microorganism (s) -Agar plates containing Escherichia coli, Bacillus subtilis and X microbe should be streaked beforehand by each group.
- Wire loop (1 for each group)
- Microscope (will be provided)
- Immersion oil (will be provided)
- Slide glass (10 for each group)
- 50-mL 70% Ethanol (1 Bottle each group)
- Staining reagents (methylene blue, safranin dye, indian dye, crystal violet; will be provided)

METHODOLOGY

Draw a schematic diagram of a working microscope in front of you and label them. Make sure you submit this before starting the experiment.

1) Preparation of bacterial smears OR heat fixation

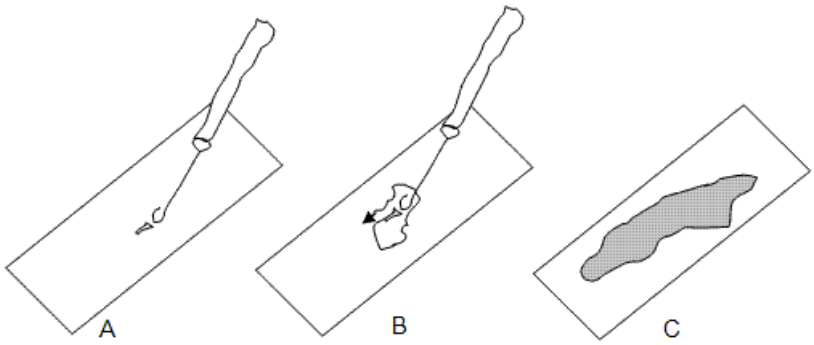

Figure 2: Bacteriological smear: A – transfer of bacteria by bacteriological loop, B – homogenisation of bacteria with water, C – bacteriological smear. (source: http://mmp.vfu.cz/frvs2011/?title=ukoly-biologie-vse&lang=en)

a) Each group should have their own agar plates containing microorganisms needed. The microorganisms should be an overnight culture.
b) Take one slide glass and place one loopful of sterile distilled water and place it on the center of the slide. All should be done ASEPTICALLY.
c) Transfer a small amount of bacterial colonies (preferably one single colony) into the water and spread the mixture into thin layer (see Figure 2).
d) By holding the slide in one end, quickly pass the smear over the flame of Bunsen burner twice to three times.
e) Let it cool and proceed with the microbial staining steps.

CAUTION: TOO MUCH SMEARS WILL CAUSE DIFFICULTIES IN MICROSCOPIC OBSERVATION.

2) Microbial staining

2.1 Simple staining

a) Take out clean slide glasses and place a drop of water (refer to heat fixation steps) to make smears on the slides.
b) After the slides are cooled, add one drop of methylene blue stain and let it sit for one minute.
c) Rinse the slide with distilled water and wipe with tissue paper at the sides of the stained part to get rid of excess water,
d) Examine it under the microscope using oil immersion lens and draw your observation.

2.2 Negative staining

a) Prepare a smear on the slide glasses, but this time do not heat-fixing it, instead let it air dry.
b) Place one drop of indian ink onto the smears.
c) Spread the solution across the slide using the edge of another slide as a spreader by slanting it 45 degree and move to the top slide in one motion to produce smears. You should obtain a thin layer.
d) After the smears are air-dried, observed the shape of the microorganisms under the microscope using high power objective lens (x40) and oil immersion power objective lens (x100).
e) You should observe the morphology of the microorganisms and should be to draw the shapes.

2.3 Gram staining

a) Prepare smears without heat-fixation. Air-dried the slides.
b) Add crystal violet solution to color all cells for one minute.
c) Rinse the slide gently with a slow running tap water or water dispenser.
d) Add one drop of iodine solution and let it sit for one minute before rinsing with water as previously.
e) Add decoloriser solution or 100% Ethanol drop wise until the crystal violet color is merely seen. Do not apply the alcohol too long.
f) If it is gram positive, it will show vivid of blue color retaining the primary color before. If it is gram negative, the smears turn to colorless.
g) Observed the slides, under high power objective lens and oil immersion power objective lens.

QUESTIONS

1. In your report, discuss the reason to prepare new microbial culture beforehand and not to use old microbial cultures (more than 24 hrs).
2. Write a brief description on how to observe fungi under a microscope?