EXPERIMENT 3 CULTURING AND IDENTIFICATION OF MICROORGANISMS

Structure

3.1 INTRODUCTION

Microorganisms are ubiquitous. They are found in soil, air, water, food, sewage and body surfaces. In short, every area of our environment is replete with them. When grown on a variety of media, microorganisms will exhibit differences in the microscopic appearance of their growth. These differences, called cultural characteristics, are used as basis for separating microorganisms into taxonomic groups. The cultural characteristics for all non-microorganisms are contained in Bergy's Manual of Systemic Bacteriology with their morphological characteristics.

Objectives

After studying and performing this experiment, you should be able to:

- differentiate microorganisms into bacteria, yeasts and moulds; and
- know the different forms / shapes of microorganisms.

3.2 EXPERIMENT

3.2.1 Principle

The microorganisms can be divided into bacteria, yeasts and moulds on basis of the difference in their morphological, cultural and physiological characteristics.

Bacteria

Among the major characteristics of bacterial cells are their size, shape, structure and arrangement. These characteristics constitute the morphology of the cell. Bacteria are very small, most being approximately 0.5 to 1.0 micrometers in diameter. They are unicellular, have cell wall and cytoplasm but the nucleous is not well developed. The shape of a bacterium is governed by its rigid cell wall. Typical bacterial cells are spherical (cocci), straight rods (bacilli) or rods that are helically covered (spirilla).

Different patterns for arrangement for identification purposes are monococci, diplococci, streptococci, tetrads, staphylococci and sarcinae (Figure 3.1). Cocci generally reproduce by binary fission. Rod shaped bacteria may be sporulating type like *Bacillus* species and *Clostridium* species which produce endospores or they are non-sporulating like *Lactobacillus* species (Figure 3.2). Bacteria may be both motile (having flagella) or non-motile (no flagella).

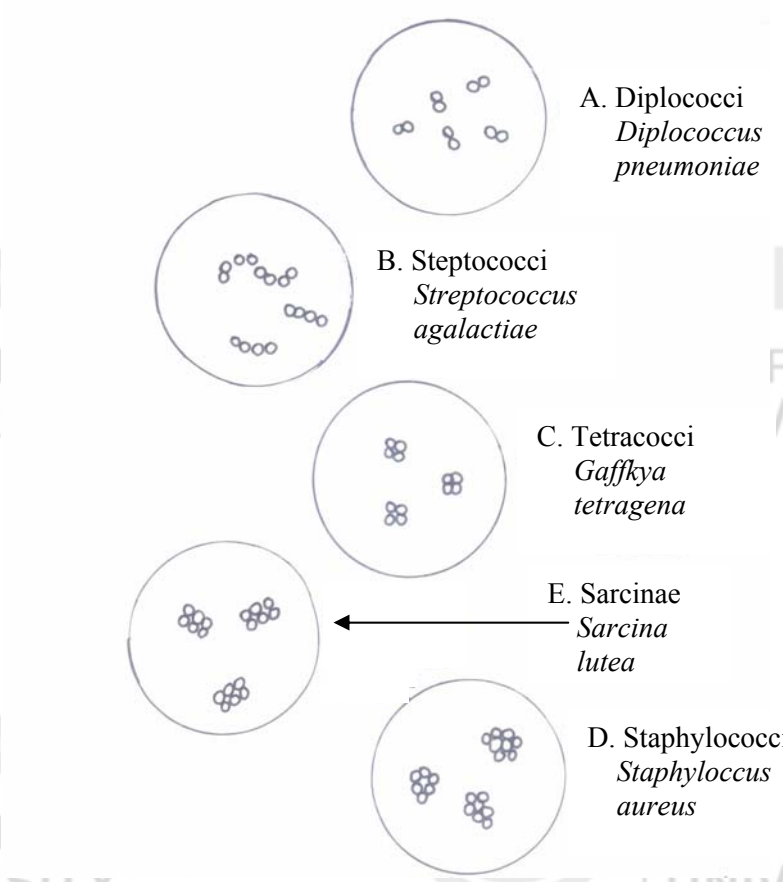


Figure 3.1: Characteristic arrangements of cocci

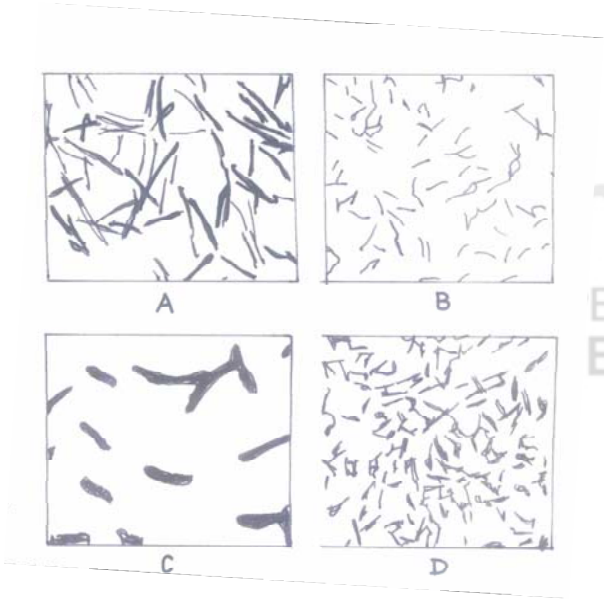


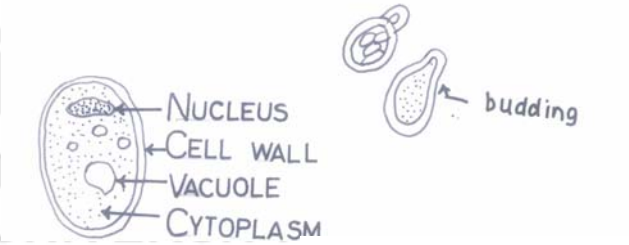
Figure 3.2: Types of rod-shaped bacteria. A) *Clostridium sporogenes*; B) *Pseudomonas sp.*; C) *Bacillus megaterium*; and D) *Salmonella typhi*

Fungi

Fungi is a group of eukaryotic organisms. They comprise of yeasts and moulds. Whereas moulds are filamentous and multicellular, yeasts are unicellular.

Yeasts

In general yeast cells are larger than most bacteria. Yeasts vary considerably in size ranging from 1-5 micrometer in width and from 5-30 micrometer in length. They are commonly egg-shaped, but some are elongated and some spherical. Yeasts lack flagella and other means of locomotion (Figure 3.3).



Saccharomyces sp. (a common bread yeast)

Figure 3.3: Yeast cell

Moulds

The thallus of moulds consist essentially of two parts: the mycelium and the spores. The mycelium is a complex of several filaments called hyphae. Filaments are made up of cells arranged end to end, branched and intertwined. Cells are like cells of higher plants in that they have visible nuclei, cell wall of varying thickness and cytoplasm. Mycelia in some fungi are divided into individual cells separated by cross walls and each cell containing a nucleus (Figure 3.4 and 3.5).

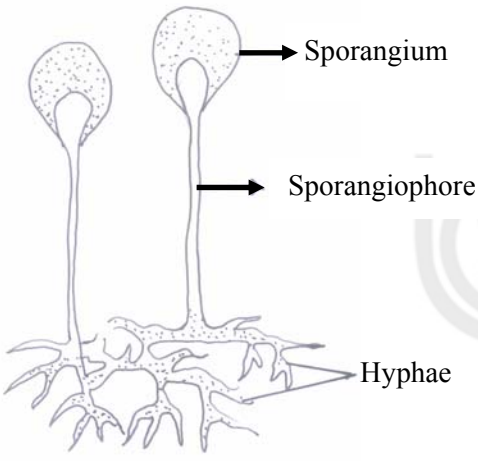


Figure 3.4: *Mucor* sp.

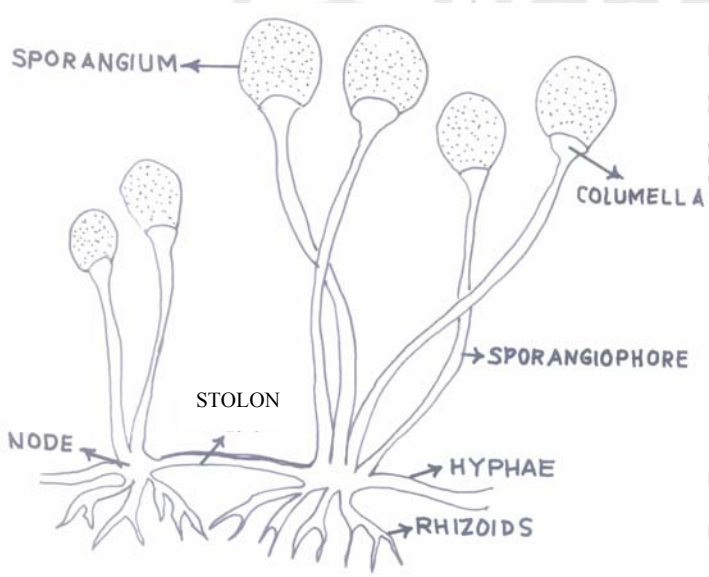


Figure 3.5: *Rhizopus* sp.

3.2.2 Requirements (Equipment/ Machinery/ Instrument and Chemicals/ Material)

- Compound microscope
- Bunsen burner
- Immersion oil
- Glass slides
- Inoculating needle
- Cover slips
- Tissue paper
- Microbial culture
- Distilled water

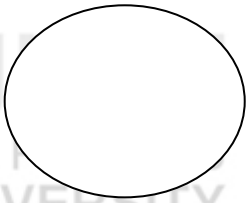
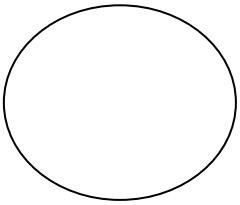
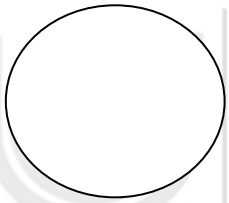
3.2.3 Procedure

1. Prepare the required media (broth or agar) for culturing the microorganisms
2. Place a small amount of media into test tubes, plug and sterilize them in an autoclave.
3. In case of solid media tubes, cool them in an incline position (slants)
4. When the medium is cold and solid, inoculate the surface of the medium using pre-sterilized needle. Move the needle gently on the agar surface in a snakelike motion from the butt to the top. In case of broth tubes, inoculate in the liquid media
5. Incubate both culture tubes at 30°C for few days.
6. In case of solid media, scoop out the mass of surface growth in which organism grows and put on clean, dry slide. From liquid broth, place a drop of culture on slide.
7. Observe under microscope.

3.2.4 Observations

In the chart provided:

1. Draw several cells from a typical microscopic field as viewed under each magnification.
2. Give the total magnification for each objective.
3. Observe spores or conidia and their arrangement.

	Bacteria	Yeast	Mould
Drawing of a representative field			
Magnification	----------	----------	----------

3.2.5 Results

Different types of spoilage have been encountered caused by various microorganisms. The type of microorganism proliferating depends on the composition of the material. The different spoilage microorganisms include bacteria, yeasts and moulds that can be observed and identified under a microscope by studying the morphological characteristics. These organisms vary in size, shape, colour, growth habit and mode of reproduction.

3.3 PRECAUTIONS

1. Use clean glass slides for smear preparation.
2. Thick, dense smears should be avoided.
3. Sterilize the inoculating needle before inoculation to avoid contamination.
4. The agar tubes should be properly sterilized.
5. Do not place the cotton plugs on ground during experiment.
6. Carefully view the characteristics of the microorganisms so as to differentiate them correctly.