IV. MICROBIOLOGY

1. Introduction to micro-organisms

'Micro-organisms are small organisms that can only be observed through a microscope. Many of these organisms consist of a single cell. They can be found everywhere in the environment. Some have the ability to take up nutrients and metabolise them into a large number of end products. Micro-organisms often have the ability to react to changes in their environment and some have been known to adapt to new environments.

Many micro-organisms are beneficial to humans. Some are involved in the production of fermented foods such as bread, cheese, wine, beer and sauerkraut. Others are used by industry in the production of products such as enzymes, antibiotics and glycerol. Additional microbial functions such as degradation of organic matter and enrichment of soils also benefit mankind. Pathogenic micro-organisms, however, have the potential of causing food-borne illnesses.

Pathogenic micro-organisms can contaminate fruits and vegetables through contact with the soil and dust, as well as through poor production and handling practices such as the application of untreated manure during production, the use of contaminated irrigation water or unsanitary handling practices.

Micro-organisms can also cause fresh fruit and vegetable to decay, thus reducing their shelf-life and marketability, resulting in heavy losses to farmers'[1].

2. Setting up a basic microbiology laboratory

Infrastructural requirements

In order to set up a basic microbiology laboratory, the following will be required:

- A small room with washable surfaces (walls, floor and ceiling);
- One or two tables with washable surfaces;
- A shelf or two and a cupboard (or storage cabinet); and
- A basin with running tap water or a portable bowl.

The room must be washed and disinfected prior to commencing microbiological work. A commercial disinfectant such as Dettol or household bleach can be used as a disinfectant.

Purpose

The purpose here is to demonstrate that a microbiology laboratory can be set up with minimum equipment requirements and at minimal cost.

Equipment and supplies

Commercial equipment can be purchased from a company that supplies laboratory equipment. An 'alternative' level of equipment could also be sourced from a hardware store (see below).

[1] University of Maryland, 2002.

Materials

Commercial	***Alternative***
Autoclave	Pressure cooker
Analytical top pan balance	Digital kitchen balance, battery operated
Hot plate/magnetic stirrer	Gas stove/electrical hot plate
Water bath	Large cooking pot

Instruments

Commercial	***Alternative***
Bunsen burner with LP[2] gas/ethanol burner	Candle
Inoculation holder with loop	Wooden rod and thin wire
Tweezers	Fine pair of pliers
Spatula	Spoon
Scalpel with blade	Sharp knife
Weighing boats	Foil/plastic lids
70 per cent ethanol	Surgical spirit/methylated spirit
Spreading rod – plastic (sterile)	Spreading rod made of wire
Sterile swabs	Cotton ear buds
Distilled water	Bottled water/untreated, clear water, boiled

Other

Thermometer
Scissors
Foil
Masking tape
Permanent marking pen
Matches and/or gas lighter or cigarette lighter
Oven gloves

Description of items of equipment and supplies

a. Equipment

- An autoclave (pressure cooker) is used to sterilise items or culture media for microbiological use
- A balance is used to weigh out chemicals or culture media according to a prescribed recipe, or to weigh fruit or vegetables to be studied
- A cooking pot is used to heat water on the gas stove/hot plate to create a water bath
- A thermometer is used to measure the temperature of the water and the settings of the stove/hot plate, in order to control the temperature of the water bath. A water bath, kept at 50°C, is used to keep agar molten until it can be poured

b. Instruments

- A Bunsen burner/ethanol burner/candle is used during aseptic work to create a sterile environment and to sterilise instruments prior to use

[2] Liquefied petroleum gas (LPG).

- An inoculation holder with loop is used to transfer bacterial cultures
- Tweezers are used to transfer fungal cultures and to pick up and transfer plant material
- A spatula/spoon, as well as the weighing boats/foil, are used to weigh out chemicals
- A scalpel/knife is used to aseptically cut, either fungal cultures or fruit pieces
- A spreading rod is used to spread a sample over the surface of an agar plate
- Swabs are used to collect samples from surfaces
- Ethanol (70 per cent)/surgical/methylated spirit is used to disinfect working surfaces, as well as instruments before use
- Distilled water is essential in the preparation of culture media, as chlorine in treated water will inhibit the growth of microbes; a water purifier would be ideal

c. Chemicals and culture media

Chemicals and culture media are commercially available from companies that supply laboratory chemicals. Some chemicals can be substituted by home-grown vegetables. Potatoes can, for example, serve as a substitute for potato dextrose agar.

d. Glass ware and Plastic ware

These can also be obtained from companies that supply laboratory ware. A simple laboratory will require a few Erlenmeyer flasks (conical) or screw-cap bottles (Schott bottles) ranging from 250 to 1000ml, a few beakers, ranging from 100 to 1000ml, some test tubes and test tube racks, disposable sterile Petri dishes (90mm in diameter), and measuring cylinders 250, 500 and 1000ml.

3. Demonstrating the omnipresence of micro-organisms

Introduction

Micro-organisms can be found in every habitat on earth. They are found in the air, sea, rivers and soil, on plants and in fish and humans. They can even be found in extreme habitats such as Antarctica and in hot springs.

Purpose

The purpose of this task is to demonstrate that micro-organisms are everywhere.

Materials

- Contact plates with nutrient agar
- Petri dishes with nutrient agar

Procedure

This task can be carried out as a group exercise, with different groups investigating different surfaces.

Surface sampling

- Choose a surface for investigation
- Label the base of the contact plate with your name and the identity of the surface you have chosen to investigate
- Remove the lid of the contact plate and press it for 30 seconds against the selected surface
- Incubate the plate for 24-48 hours at room temperature

Air sampling

- Identify a sampling point
- Label the base of the contact plate with your name and the location of the sampling point you have chosen to investigate
- Place an open nutrient agar plate at the selected sampling point and leave it open at this point for 30-60 minutes
- Note the time for which the plate was exposed
- Replace the lid
- Incubate the plate for 24-48 hours at room temperature

Results

Surface/sampling point investigated	**Total number of colonies**	**Number of fungal colonies**	**Number of bacterial colonies**

Questions and discussion issues

- Which sampling point had the least number of colonies?
- Which sampling point had the largest number of colonies?
- Can you explain the difference?