

Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (First Year)

Subject: Botany

Course Code: BOG 102

Course Title: Coastal and Mangrove Ecology

Unit: Flora and fauna of Mangroves and Associated Environments

Module Name: Bacteria, fungi and actinomycetes - Collection, preservation and identification techniques

Module No: 18

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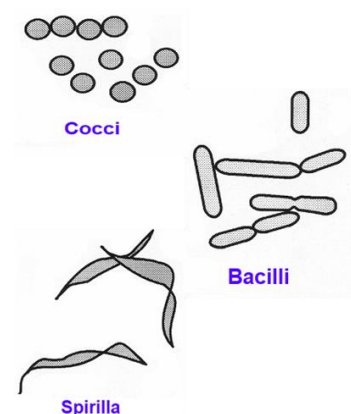
Notes

Flora and fauna of Mangroves and Associated Environments

Module name : **Bacteria, fungi and Actinomycetes - Collection, preservation and identification techniques**

Bacteria

- Bacteria are microscopic unicellular organism
- They are living organism that belongs to the kingdom prokaryotes
- They are extremely tiny thus they cannot be seen individually unless viewed through microscope
- When cultured on agar, bacteria grow as slimy colonies that contain many individual cells



Robinson C. K.
https://commons.wikimedia.org/wiki/File:Bacteria_shapes_01.png

Sampling methods

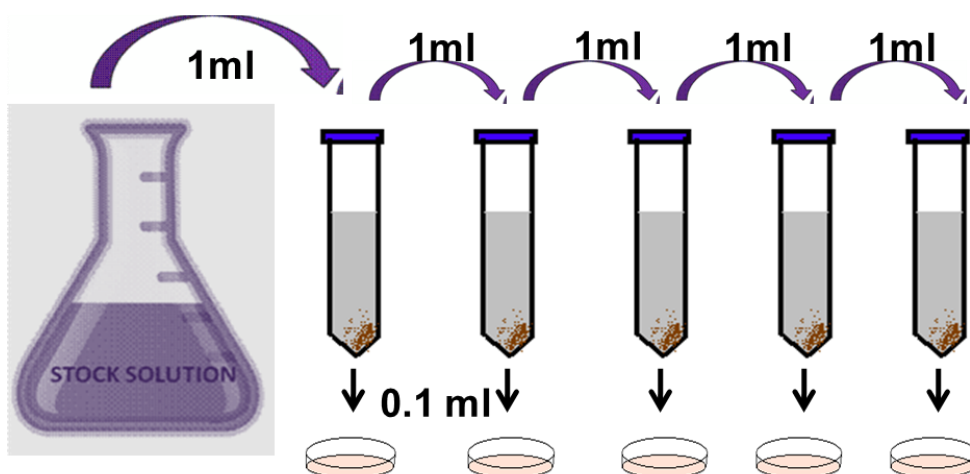
For obtaining bacterial cultures following samples are used

- **Water** - Surface water samples can be hand collected directly from near the shore or from a boat using sterile bottle.
- **Sediment**: Sediment samples near the shore can be collected using a sterile spatula and kept in sterile petri-dishes.

Isolation method

- Samples collected for isolating bacteria should be examined as soon as possible after collection
- If samples are to be transported from distance, it must be maintained at 4° to 10°C
- In laboratory, diluted samples are spread on media in sterile petri-plates to get cultures.
- Water samples are filtered through sterile Whatman No.1 filter paper to remove unwanted debris
- Filtrate is used directly as inoculum after serial dilution or filtered through the sterile membrane filter paper (0.22 μ m).
- The membrane filter paper is used as inoculum source for the isolation of aerobic heterotrophic bacteria.
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Sediment samples are serially diluted in 50% seawater and 0.1 ml of the dilutant to be used for inoculation on sterile agar petri-plates



Identification of bacteria

Gram staining - most widely used method in bacteriology.

- Stain divides bacteria into two large groups.
- Those that retain the primary stain crystal violet throughout the staining procedure, - grampositive.
- Those that are stained the color of counter stain (Safranin) – gramnegative.
- Prepare bacterial on a glass slide using 18-24 hrs old bacterial culture
- Fix smear to the slide by gently warming the slide
- Crystal violet stain is added over the fixed culture. Allowed to stand for 60 seconds.
- Gently rinse with water
- Add about 5 drops of iodine solution on smear and allow to stand for 30 seconds
- Pour off the iodine solution and rinse the slide with running water and Add a few drops of decolorizer
- Rinse it off with water after 5 seconds
- Counter stain with 5 drops of the Safranin solution for 20 seconds, Wash off the Safranin solution
- Examine the finished slide under a microscope.

Preservation of bacteria

- Sequences generated for Identification of Bacteria can be deposited in Gen-Bank.
- Microbial cultures can be maintained for long duration
 - In an active state - on agar medium with frequent sub-culturing
 - Under suspended metabolic condition

– in Cryo-preservation or deep-freeze in -80 temperature, almost permanently.

Fungi

- Fungi are eukaryotic, spore-bearing, achlorophyllous, heterotrophic organisms
- Generally reproduces sexually and asexually
- Usually filamentous, except yeasts which are unicellular
- Marine fungi play a significant role in degradation of litter of mangrove ecosystems
- Lignicolous fungi constitute over 50% of 450 species of obligate marine fungi described so far
- About 150 species are found exclusively on decaying Mangrove wood, aerial roots and seedlings - Manglicolous fungi
- So far 72 species have been described from India

Collection techniques

- The substrates have to be collected and packed in sterile polythene bags
 - Dead and living plant material
 - Soil
 - Humus
 - Animal droppings

Direct examination method: They are directly examined under dissection microscope for the presence of Ascocarps, Basidiocarps, Pycnidia or Conidia. Such fruit bodies are transferred with a needle to a microscopic slide, teased apart in a drop of water to expose the spores and carefully squeezed under a cover glass.

Wood baiting technique - terrestrial wooden logs are immersed in the mangrove waters for certain period then collected and examined for fungal colonization.

Other methods:

- Serial dilution for isolation of soil Fungi
- Particle plating method for isolation of litter Fungi.
- Three step sterilisation method for isolation of Endophytes.

Identification of fungi

- For identification of species, observation of ascospores, basidiospores or conidia usually suffices.
- For allowing development of fruiting bodies, litter samples are incubated in sterile moist chamber / polybags at room temperature.
- Incubated samples can be examined under microscope.

Preservation of fungi

- Same Techniques as described for bacterial preservation can be used.
- Additionally fungal materials can be made into herbarium and deposited in national repositories.

Actinomycetes

- Actinomycetes are unicellular, Gram-positive bacteria that belong to order **Actinomycetales**.
- Members of this group are widely distributed in nature and can be found in a variety of habitats across the world.
- Actinomycetes are unique microorganisms having both characters of bacteria and fungi.

Collection of samples

- The sediments samples have to be collected using a **Peterson grab** from Mangrove sediments.

The samples are air dried for four days at room temperature before using them for isolation

Isolation of Actinomycetes

- Isolation is done by using serial dilution method as described for Bacteria.

Identification of Actinomycetes

Morphological characteristics - 14 day old colonial growth from cultures grown at 28° C shall be examined under Scanning Electron Microscope.

- **Streptomyces**- have discrete aerial mycelium at maturity, forms chains of 3 to many spores.
- **Micromonospora**- well developed, branched, septate mycelium; aerial mycelium found very occasionally.
- **Nocardia**- Rudimentary to extensively branched vegetative hyphae; aerial mycelium at times visible only microscopically.
- **Cultural and physiological characteristics** - Cultures grown on agar plates for 7 and 14 days shall be observed for growth, aerial mycelium, color on both sides and soluble pigments.
- **Preservation of Actinomycetes**: As described in Bacteria and Fungi
