Advances in Fish Microbiology and Pathology
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Microorganisms

- Microorganisms is the existence of every minute living organisms or they are living features that can be seen with the aid of microscope, most of them are normally single-celled while some may exist in multicellular forms.

- These microorganism, though minute and microscopic, are a very powerful group of creatures.
- They can make healthy animals sick, destroy plantations, decay food (e.g fish) and cause world-wide epidemic.
- Some of them however, are very useful in that they can be used for the manufacture of drugs capable of destroying harmful microorganisms while a few can convert certain fruit juices into wine, vinegar and alcohol(Ramalingam,1987).

- Microorganisms are found everywhere except in a vaccum that is, they are ubiquitous; on and inside living organisms.
- These organisms vary in sizes from certain algae just large enough to be seen by the naked eye to viruses which are too small to be seen by normal microscope but can be discerned by an electron microscope.

- Microorganisms are chief agents of spoilage of food especially fish.
- These microorganisms responsible for food spoilage especially fish include bacteria, fungal and viruses.

Bacteria

- These are unicellular microscopic organisms smaller in size than mould and yeasts
- different from one another in size and shape,
- widely distributed in the environment-air,
- water and soil,
- on the surface of all living features,
- on the moist lining of the mouth, nostril and throat and
- inside intestinal tract of almost all animals.

- Their natural habitat is the soil.
- They are varied requirement for growth.
- Some bacteria are thermophilic; they grow best at a temperature range of 45^0C – 55^0C.
- Others are mesophilic which grow best at room temperature (200- 300C) and
- pyschrophilic which grow best at refrigeration temperature between 4^0C – 10^0C (Shewan,1977).

- Moreso, some bacteria will grow in the presence of oxygen (aerobic)
- while other grow in the absence of oxygen (anaerobic).
- Therefore, bacteria are classified based on temperature on oxygen preference.
- Bacteria do not generally grow at low pH especially below 3.5.

- Bacteria exist in four typical shapes:
- Spherical in shape (cocci),
- cylindrical or rod shaped (bacilli),
- long coiled thread or spiral shape (spirillae) and
- comma shaped (vibrio or filamentous (Ramalingam, 1987).

- They possess cell wall which sometimes may be surrounded by capsule or slime layer.
- Some bacteria are motile (capable of movement) through the use of flagella.
- They normally produce asexually by a process of binary fission.
- Bacteria spore with a very resistance bodies are produced for protection in unfavourable environment.

Fungi

- These are microscopic plant, most of which are multicellular
- although yeast and some aquatic species are unicellular.
- Fungi living in a variety of habitat aquatic ranges to moist situation on land however,
- some of them are able to withstand drier/dried condition e.g *Aspergillus sp*, Penicillum spp).
- Fungi by nature are heterotrophic organisms that is they require an organic source of carbon for energy and depend upon this source for their life.

- Some fungi can be found feeding on non-living organic matter that is, dead or decaying organic matter called saprophytes (this is important for food spoilage).
- Many other fungi obtain their organic nutrient from the host and live as parasites.
- Some are also plants and animal pathogens they cause diseases (Young et al., 1984).
- The two main group of fungi important in food spoilage are mould and yeast which is made up of single cell.

Laboratory Investigation of Microorganisms Associated Fish Production

- 2 grams of a particular size/quality of sample will be aseptically taken and homogenized in 18mls of sterile distilled water using sterile mortar and pestle under hood.
- These will be taken as the original stock culture of the feed sample.
- The procedure will be repeated to get the original stock culture for other feed samples.

Laboratory Analysis

- **Preparation of Glass-Wares and working areas**
- Materials such as glass wares and media will be sterilized before use.
- The glass wares including test tubes, pipette's, petri dishes, bijou bottles will be washed with detergent, rinsed thoroughly with clean tap water and allowed to air dry before sterilizing in the hot air oven at 160^0C of an hour.
- The media will be sterilized and bench working areas swabbed with cotton wool soaked in ethanol to sterilize before any microbiological analysis was carried out to avoid contamination.

Microbiological Analysis

- Samples from each category will be taken to the laboratory for microbiological analysis.
- 2ml of a particular section will be aseptically taken and homogenized in 20mls of sterile distilled water.
- These will be taken as the original stock culture of each category.
- The procedure will be repeated to get the original stock culture for other categories.

Preparation of the serial dilution of each fish sample stock culture

- Each of the original stock culture will be serially diluted as follows:
- 1ml of the original stock solution will be aseptically poured into 9ml sterile distilled water and mixed thoroughly to give 10^{-1} dilution of original stock culture. 1ml of dilution 10^{-1} of the original stock culture will be aseptically poured into another tube of 9ml sterile distilled water to give 10ml of 10^{-2} dilution of the original stock culture.
- Repeating the above procedure dilutions of 10^{-3}, 10^{-4}, and 10^{-5} will be obtained.

Viable Bacterial count on Nutrient Agar

- The pour plate technique will be employed.
- 0.5ml of dilution 10^{-1} of the stock culture will be introduced into each of three sterilized Nutrient agar plates.
- Sterile molten nutrient agar at about 45^0C will be added and then mixed thoroughly and allowed to set undisturbed.
- The set agar plates will be incubated at 37^0C for 24 hours. This procedure will be repeated using dilutions 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.
- Finally, the number of colonies per plate will be counted and recorded.

Isolation of Microorganisms from the stock culture

- A loopful each of the stock culture will be inoculated into a sterile Nutrient Agar and a sterile Potato Dextrose Agar plates.
- The Nutrient Agar plate will be incubated at 37^0C for 24 hours while the Potato Dextrose Agar plate will be incubated at 25^0C for 3-7 days for microbial growth.

Purification of micro-organisms

- Purification of bacteria: Characteristics colonies from original culture on Nutrient agar plates will be picked by sterile platinum wire loop and streaked to isolate on sterile Nutrient agar, MacConkey agar and Blood agar plates for purity.
- Distinct colonies will be gram stained to know the gram reaction and recorded.
- These isolates will be transferred to their appropriate agar slants, labeled and incubated at 37^0C for 24 hours for growth after which they will be kept in the refrigerator at 4^0C for identification.

Purification of mould

- Characteristic colonies of moulds will be picked with a sterilized wire loop
- and inoculated into a freshly prepared Potato Dextrose Agar incubated at 25^0C for 3-7 days for purification.
- Pure mould isolates will be observed for colonial characteristics and kept on Potato Dextrose agar slants for identification.

IDENTIFICATION

- Staining: All the isolates will be transferred from the slants into appropriate agar plates
- and incubated appropriately and used for identification.
- Identification of bacteria: each pure bacterium isolated will undergo the following tests.

Gram’s staining

- A very small drop of distilled water will be placed on a clean slide.
- The inoculating wire loop will be sterilized by flaming it until it is red hot in the blue flame of a Bunsen burner.
- The loop will be allowed to cool and a small portion of distilled water on the slide and spread into a thin smear along the slide.

- The smear will be allowed to air dry and heat fixed briefly over flame.
- The smear will be stained with 1% crystal violet for 60 seconds
- and washed with distilled water
- this will be stained with Lugols iodine for 60 seconds and washed with distilled water.
- The slide will be decolourised rapidly with 75% alcohol and washed off immediately with distilled water.
- The slide will finally be flooded with counter stain-Safranine for 30 seconds and washed off with distilled water and air dried.
- This will then be observed under the microscope with the aid of oil immersion lens (100 objective lens).

- Capsule staining: Grown micro-organisms will be picked with sterile needle and will be mixed with a drop of sterile distilled water to make a smear which will be allowed to air dry.
- The dry smear will be fixed by passing over Bunsen flame.
- The smear will then be covered with crystal violet stain.

- Heat will be applied gently with the steam just began to rise, and then left to stain for 1 minute.
- The stain will be washed off with copper-sulphate solution.
- The back of the slide will be wiped clean and placed on a draining rack for the smear to air dry.
- The smear will then be observed microscopically under x 100 objective lens to look for capsules which appeared pale blue with bacteria cells stained dark purple.

Spore staining

- A drop of distilled water will be placed on a clean slide and a small loopful of organisms will be added, aseptically using sterile wire loop and mixed then spread out into a thin smear.
- It will then be covered with 2 drops of Ziel-Nelson Carbol Fuschin solution for 5 minutes after which it will be decolourised with 95% alcohol for 2 minutes and
- counter stained with Noeflers methylene blue for 1 minute.
- Thereafter, it will be washed and air dried.
- The spores appeared red and the bacteria bodies (vegetative form) appeared blue.

Mould Staining (Methylene-Blue Staining)

- A drop of methylene blue stain will be put on a clean slide and with the aid of an inoculating needle, a small portion of mycelium will be removed from the mould culture and placed on the drop of methylene blue.
- The mycelium will be spread very well on the slide with the aid of two mounted needles.
- Gently, a cover slip will be lowered on it, excess liquid wipe off by putting the slide between two filter papers and applying a gentle pressure around the cover slip.

Biochemical Tests

- Catalase Test
- Coagulase Test
- Citrate Utilization Test
- Urease test
- Nitrate Reduction Test
- Indole Reaction
- Oxidase reaction
- Hydrogen Sulphide Production
- Gelatin Hydrolysis
- Voges Proskauer
- Sugar utilization

FURTHER READING LIST

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