EDO UNIVERSITY IYAMHO

Department of Microbiology

MCB 212 General Microbiology

Instructor 1: *Mr. A. C. Okafor,* email: arthur.okafor@edouniversity.edu.ng
Lectures: Wednesday, 2.00pm – 4.00 pm, NLT3, phone: (+234) 8053248678
Office hours: Wednesday, 2.30 to 3.30 pm,
Instructor 2: *Miss Ozolua O. Phebean,* email: ozolua.phebean@edouniversity.edu.ng**;** Central Laboratory, Muhamadu Buhari Faculty of Science.

Description: This course is intended to give the students a thorough knowledge of the role of microorganisms in food, soil. Also to train them on proper identification of selected microbial groups: Bacteria, Fungi, Viruses, Protozoa and Algae. To help them know some of the important tools and techniques used in Microbiology. This course will also give students a thorough knowledge of the principles of sterilization and disinfection.
Prerequisites: Students should be able to understand the role of microorganisms in food and soil, know how microbial growth and spoilage occur, know what factors that influence the nature of spoilage microorganisms, understand the basic approaches to food preservation, know some soil microorganisms, and their functions in the soil. They should be able to understand the growth of microorganisms including the effect of environmental factors on growth, survival, inhibition and death of microorganism. Also, they should be able to understand the co-existence between microorganisms and their host, how to properly identify selected microorganisms from specimens, know the economic importance of selected microorganisms

Assignments: We expect to have 8 individual homework assignments throughout the course in addition to a Mid-Term Test and a Final Exam. Home works are due at the beginning of the class on the due date. Home works are organized and structured as preparation for the midterm and final exam, and are meant to be a studying material for both exams.

Grading: We will assign 10% of this class grade to home works, 10% for the seminar presentations, 10% for the mid-term test and 70% for the final exam. The final exam is comprehensive.

Textbook: The recommended textbooks for this class are as stated:

Title: *Microbiology*
Authors: Michael J. Pelczar, Jr., E. C. S. and Noel R. Krieg
Publishers: Tata McGraw-Hill, 5th edition
ISBN: 10:0-07-462320-6
Year: 1993.

Title: *Essential Microbiology*
Authors: Stuart Hogg
Publisher: John Wiley & Sons Ltd
ISBN: ISBN 0 471 49753 3
Year: 2005

Title: *Prescott's Microbiology*
Authors: Joanne,
Publisher: Mc Graw Hill Education, 9th Edition (2013),
ISBN: 1-558600-698-X
Year: 2013

Title: *Microbiology*
Author: Lansing M. Prescott, John P. Harley, Donald A. Klein,
Publisher: Morgan-Kaufmann Publishers
ISBN: 1-558600-320-4

Title: *A Textbook of Basic and Applied Microbiology*
Authors: K. R. Aneja, Pranay Jain, Raman Aneja
Publishers: New Age
ISBN: 978-81-224-2367-9
Year: 2008.

Title: *A Textbook of Fungi, Bacteria and Viruses*
Authors: H. C. Dube
Publishers: Vikas Publishing House, 3rd edition
ISBN: 81-88826-38-7
Year: 2014.

Lectures: Below is a description of the contents.

Role of microorganisms in food and soil

Food often provides an ideal environment for microbial survival and growth. Microbial growth in food involves successional changes, with intrinsic (food related) and extrinsic (environmental) factors interacting with the microbial communities. Wines, beers and other alcoholic products are also produced through microbial activities. On the other hand, food can also serve as vehicles for disease transmission, and the detection and control of pathogens and food spoilage microorganisms are important parts of food microbiology. Microbial growth in foods can result in either preservation or spoilage depending on the microorganisms involved and the food storage conditions. Contamination by disease-causing can occur at any point in the food-handling sequence.

Microbial growth in foods

Foods are excellent environment for the growth of microorganisms. Microbial growth is controlled by:

1. **Intrinsic factors:** These are food-related factors that influence microbial growth. The intrinsic factors include pH, moisture content, water activity or availability, oxidation-reduction potential, physical structure of the food, available nutrients and the possible presence of natural antimicrobial agents.
2. **Extrinsic factors:** Extrinsic or environmental factors include temperature, relative humidity, gases (CO2 and CO) and the types and numbers of microorganisms present in food.

Microbial growth and food spoilage

Microbial growth in foods can lead to visible changes, including a variety of colours caused by spoilage microorganisms. Meat and dairy products with their high nutritional value and the presence of easily usable carbohydrates, fats and proteins provide ideal environment for microbial spoilage. Proteolysis and putrefaction are typical results of microbial spoilage of such high protein materials. Unpasteurized milk undergoes a predictable four step succession during spoilage: acid production by *Lactococcus lactis*. *Lactis* is followed by additional acid production associated with the growth of more tolerant organisms such ass Lactobacillus. At this point, yeasts and molds become dominant and degrade the accumulated lactic acid, and the acidity gradually decreased. Eventually, protein-digesting bacteria become active, resulting in a putrid odour and bitter flavor. The milk, originally opaque, can eventually become clear.

In comparison with meat and dairy products, most fruits and vegetables have a much lower protein and fat content and undergo a different kind of spoilage. Readily degradable carbohydrates favour vegetable spoilage by bacteria, especially bacteria that cause soft rot, such as *Erwina carotorora*, which produces hydrolytic enzymes. Bacteria do not seem important in the initial spoilage of whole fruits, instead such spoilage often is initiated by molds. These organisms have enzymes that contribute to the weakening and penetration of the protective outer skin.

Food spoilage problems occur with minimally processed, concentrated frozen citrus products. These are prepared with little or no heat treatment, and major spoilage can be caused by

Lactobacillus and *Leuconostoc* sp. Which produce diaceryl-butter flavours. *Saccharomyces* and *Candida* also spoil juices. Concentrated juice has a decreased water activity, and when kept frozen at about -9ºC, the juice can be stored for long periods. However, when concentrated juices are diluted with water that contains spoilage organisms, or if the juice is stored in improperly washed containers, problems can occur. Also, microorganisms in the frozen concentrated juices can begin the spoilage process after addition of water. Ready-to-serve juices present other problems as the water activity values are sufficiently high to allow microbial growth. This is especially true with extended storage at refrigerated temperatures.

Molds are special problems for tomatoes. Even the slightest bruising of the tomato skin, exposing the interior will result in rapid fungal growth. Frequently observed genera include *Cladosporium*, *Fusariun* and *Stemphylium*. This growth affects the quality of tomato products, including tomatoes juices and ketchups. Molds can rapidly grow on grains and corn when the products are held under moist conditions. Infection of grains by the ascomycete *Claviceps pupura* causes ergotism, a toxic condition. Hallucinogenic alkaloids produced by this fungus can lead to altered behavior, abortion and death if infected grains are eaten. More recently discovered fungal contaminants of corm are the fumonisins, first isolated in1988. These are produced by *Fusarium moniliforme* and cause leukoencephaalomalacia in horses, pulmonaryedema in pigs and eosophageal cancer in humans. The fumonisins function by disrupting the synthesis and metabolism of sphingolipids, an important biochemically active compound, which influence a wide variety of cell functions.

Controlling Food Spoilage

Food can be preserved by physical, chemical and biological methods. Contamination often occurs after a package or a can is open and just before the food is served. This can provide an ideal environment and opportunity for growth and transmission of pathogens. Pasteurization results in a pathogen-free product with a longer shelf life. Chemicals can be added to food to reduce microbial growth. Such chemicals include salt, sugar and other chemicals that affect specific groups. Microbial products such as bacteriocins can be added to foods to control food spoilage.

Basic Approaches to Food Preservation

1. **Removal of Microorganisms:** Microorganisms can be removed from water, wine, beer, juices, soft drinks and other liquids by filtration. This can keep bacterial populations low or eliminate them entirely.
2. **Low Temperatures:** This is based on slowing down of microbial activities such as metabolic reactions.
3. **High Temperatures:** High temperatures refers to all temperatures above ambient (room) temperature. Two types of heat treatment are available for use in food preservation, which are pasteurization and sterilization.
4. **Water Availability:** Dehydration, such as lyophilization, to produce freeze-dried foods is a common means of eliminating microbial growths. The modern method of lyophilization is simply an update of older procedures in which grains, meats, fish and fruits were dried.

5. **Chemical-Based Preservation:** Various chemical agents can be used to preserve foods, and these chemicals are closely regulated by food and drug administration. These chemicals are listed as generally regarded as safe (GRAS). They include simple organic acids, sulfites, ethylene oxide as a gas sterilant, sodium nitrate and ethyl formate. The effectiveness of these chemical preservatives depends on the food's pH.

Role of Microorganisms in Soil

The microbial populations of the soils can be very high. In a surface soil, bacterial populations can approach 10^8 to 10^9 cells per gram dry weight of soils as measured microscopically. Fungi can be present at up to several 100 meters of haphae per gram of soil. It is important to remember when discussing soils and their microorganisms that only a minor portion (approximately 10%) of the microscopically observable organisms making up this biomass have been cultured. Microorganisms in soil play a key role in the overall health and productivity of plants and crops. Beneficial soil bacteria and fungi like *Trichodema* are key elements in the food web. Microbial plant interaction is very important.

Soil formation is in large part due to the activity and metabolism of soil microorganisms. It is also as a result of a complex network of biological as well as chemical and physical processes. The role of soil microbes is of high interest since they are responsible for most biological transformations and drive the development of stable and liable pools of carbon, nitrogen and other nutrients which facilitates the subsequent establishment of plant communities.

Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups has characteristics that define them and their functions in soil.

Bacteria: Bacteria and alchaea are the smallest organisms in soil apart from viruses. They are the most abundant microorganisms in soil and serve many important purposes, including nitrogen fixation. Pseudomomas can metabolise a wide range of chemicals and fertilizers. Clostridium can grow in the absence of oxygen, respiring anaerobically. Nitrogen fixation is the conversion of atmospheric nitrogen into nitrogen-containing compounds such as ammonia, that can be used as plants.

Actinomycetes: Actinomycetes are type of bacteria, but share some characteristics with fungi that are most likely a result of convergent evolution due to a common habitat and lifestyle. One of the most notable characteristics of actinomycetes is their ability to produce antibiotics. Streptomycin, Neomycin, Erythromycin and Tetracycline are few examples of these antibiotics. Streptomycin is used to treat tuberculosis and infections caused by certain bacteria, and neomycin is used to reduce the risk of bacterial infection during surgery. Erythromycin is used to treat certain infections caused such as bronchitis, pertussis (whooping cough), pneumonia and ear, intestine, lung, skin and urinary tract infections.

Fungi: Fungi are abundant in soil. They are important in the soil as food sources for other larger organisms, pathogens beneficial symbiotic relationships with plants or other organisms and soil health. The quality and quantity of organic matter in the soil has a direct correlation to the growth of fungi because most fungi consume organic matter for nutrition.

Algae: Algae can make their own nutrients through photosynthesis. For algae to grow, they must be exposed to light, because photosynthesis require light. They don't have to bedirectly exposed to sun, but can live below the soil surface given uniform temperature and moisture conditions.

Protozoa: Protozoa are eukaryotic organisms that were some of the first microorganisms to reproduce sexually. Rgey can be split into three categories:

i. **Flagellate:** smallest members of the protozoa groups. Flagellates of certain chlorophyll typically occur in aquatic conditions.
ii. **Amoebae:** amoebae are larger than flagellate and move in a different way.
iii. **Cillates:** these are the largest in the protozoa groups and move by means of short, numerous cilia that produce beating movements.

Identification and Economic Importance of Selected Microbial Groups

Pathogens, particularly bacteria and yeast coexist with harmless microorganisms in the host. These pathogens must be properly identified as the actual cause of infectious diseases. Microorganisms are identified based on morphological, biochemical, immunologic and molecular procedures. Time is a significant factor in the identification process. Once isolated and identified, the organisms can then be subjected to antimicrobial sensitivity tests.

Specimens

A specimen represents a portion of human materials or other materials that is tested, examined or studied to determine the presence or absence of particular organisms. During the collection of samples, safety for the patients, hospital and laboratory staff is very important. Important concerns concerning specimens include:

i. The specimen should adequately represent the diseased areas in order to isolate and identify potential agents of particular disease.
ii. Sufficient amounts of the specimens should be collected to allow a variety of diagnostic testing.
iii. In order to avoid contamination from many varieties of organisms, attention must be given to specimen collection.
iv. The specimen should be obtained, if possible, before the administration of the antimicrobial agents.

Collection

Specimens may be collected by several methods using aseptic techniques. Aseptic techniques refer to specific procedures used to prevent unwanted organisms from contaminating the clinical specimens. The most common method used to collect specimens from the anterior nares or throats is a sterile **swab**. **Needle aspiration** is used to collect specimens aseptically from cerebrospinal fluids, pus and blood. To prevent blood from clotting and entrapping organisms, like heparin and sodium citrate are included within the specimen bottles. **Intubation** is the inserting of tubes into a body canal or hollow organ. For instance, intubation can be used to collect specimens from the stomach. **Catheter** is a tubular instruments used for withdrawing or introducing fluids from or into a body cavity.

Handling

Immediately after collection, the specimen must be properly handled and labeled. The name, hospital, registration number, location in the hospital, diagnosis and type of specimens should be correctly and legibly written on the culture request form. This information must correspond to that written on the specimen container. The choice of test to be performed must also be specified on the request form.

Transport

Speed of transporting the specimen to the laboratory after collection is of prime importance. Specimen may be transported by various means. Certain specimens should be transported in a medium that preserves the organisms and helps maintain the ratio of the organisms to another. Special treatment is required for specimens when the organism is thought to be anaerobic,

transport of these specimens should take no more than 10 minutes, otherwise the specimens must be injected immediately into an anaerobic transport vial.

Identification of microorganisms from specimens

Bacteria

Characteristics

1) Bacteria are pathogenic organisms and as such they lack membrane bound organells.
2) They are unicellular organisms.
3) They lack a true nucleus, chloroplasts and the other organells present in eukaryotic cells such as the golgi apparatus and endoplasmic reticulum.
4) Bacteria increase in cell size, and then reproduce through binary fission, a form of asexual reproduction.
5) They are usually microscopic.

Economic importance

1) Soil bacteria play an important role in bringing about decomposition of organic matter.
2) They help in maintaining and increasing fertility.
3) The saprophytic bacteria break down the proteins and other nitrogen containing remains of plant and animal origin in the soil to amino acids by secreting enzymes.

Identification

1) Isolation and growth of bacteria are required before many diagnostic tests can be used to confirm the identification of pathogens. Media used for the initial isolation of many bacteria include Nutrient Agar, Eosin Methylene Blue, Nutrient Broth, MacConkey Agar, MacConkey Broth, etc.
2) The temperature of incubation depends on the type of bacteria.
3) Petri dish cultures are used primarily to study colonial characteristics, including size, colour, texture, etc.
4) After the microscopic and growth characteristics of a pure culture of bacteria are examined, specific biochemical tests can be performed. Examples of common biochemical tests used to identify isolates include carbohydrate fermentation, coagulase, urease, oxidase, methyl red, etc.

Protozoa

Concentrated wet mounts of blood, stool or urine specimens can be examined microscopically for the presence of eggs, cysts, larvvae or vegetative cells of parasites. Blood smears for sporozoans (malaria) and flagellate (trypanosomes) parasites are stained with Giemsa. Some serological tests are also available.

Fungi

The study of fungi is known as Mycology.

Characteristics

1) They are eukaryotic organisms, that is they have microbial bound organell.
2) They lack chlorophyll, that is they cannot carry out photosynthesis.
3) Most fungi are aerobic, though some are facultative.

4) Most fungi are saprophytes, that is in the cause of nature, they decompose dead matters into usable forms.
5) Most fungi grow best at temperature between 20-30ºC.

Economic importance

1) Fungi are used as foods, eg mushrooms.
2) They help in decomposition.
3) They help to detoxify the environment.
4) They are used in beer production.
5) They are useful in the production of enzymes.

Identification

1) Media used in the initial identification of fungi include malt extract agar, potato dextrose agar and sabaraud dextrose agar.
2) The temperature of incubation depends on the type of fungus.
3) Petri dish cultures are used primarily for studying colonial characteristics such as size, colour, texture, edge, etc.
4) Most fungi require light for growth and sporulation.
5) Wet mounts can be done by using an inoculating needle to pick a little portion of the growing medium on a slide. Add lactophenol to stain and cover with a slide. Then view under the microscope.
6) A dissecting microscope is preferable for making initial observations. Light microscopes can also be used at lower power magnification.
7) Record the details of your observations, and if possible, make sketches.

Tools and techniques used in Microbiology

Microscopic techniques are methods used for the study of microbes, including bacteria and microscopic fungi and protists. They include methods to survey, culture, stain, identify, engineer and manipulate microbes. Bacteria will grow on practically any source of organic foods which provides carbon compounds to be respire for energy and nitrogen compounds to be incorporated into proteins for growth. These substances are normally provided dissolved in water. However, in nature, bacteria can break down solids and insoluble substances by releasing enzymes into the substrates in which they are growing. The two normal media used in bacteriology are a clear soup-like liquid nutrient broth usually in tubes and nutrient agar which is set into a jelly by the addition of seaweed extracts called agar, and when melted into glass or plastic petri dishes, also known as plates.

Microbial techniques (sterilization, aseptic techniques, inoculation, incubation)

Sterilization : Similar techniques are used to grow fungi such as molds and yeast. All media used in the isolation of organisms must be sterilized by heating in an autoclave at 121ºC for 15minutes, which kills all living things, including spores. All apparatus used from this point onwards must be sterilized by heat (glassware, -160ºC for 2 hrs) or exposure to radiation.

Aseptic techniques must be used to reduce the likelihood of bacterial contamination. This usually involves disinfection of working areas, minimizing possible access by bacteria from the air to exposed media and use of flames to kill bacteria which might enter vessels as they are opened.

Inoculation. Bacteria may be introduced into the media (inoculate) by various means. Usually the bacteria from a drop in a heat sterilized loop on the surface of agars. A similar technique is used with broth cultures. Sometimes, bacteria in a liquid are introduced using a sterile pipette before the agar medium is poured on top (pour plate).

Incubation. The petri dishes containing agar or tubes containing broth are incubated, that is put in an autoclave at fixed temperatures (usually 37ºC, human body temperatures or 25ºC for bacteria from the environment).

It is usual to invert the petri dish when growing organisms so as to prevent condensation droplets from falling into the agar surface from the agar.