Virtual University of Pakistan

(Bt102)

(Microbiology)

Final term solved Past Papers

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Good luck for exams

Paper pattern

MCQs = 40

SQs (2 marks) = 4

SQs (3 marks) = 4

LQs (5 marks) = 2

LQs (10 marks) =1

Total marks = 80

1. **Peripheral proteins----------**
   loosely connected and soluble in water
2. **Plasmid--------**
   extrachromosomal circular DNA

3. **Mesophils optimum temp. is**
   37
4. **Unicellular fungi is**
   yeast
5. **Asexual spores are formed by**
   mitosis
6. **Mycobiont is---------**
   fungi
7. **ELISA is used for**
   Antigen and antibody
8. **Western blotting is used for**
   proteins
9. **Consistently present but with low incidence**
   endemic
10. **Which is not belong to innate immunity**
    specific
11. **Which is not lymphocyte**
    monocytes
12. **Which is not belong to ELISA**
    Probe
13. **Extreme halophiles require high salt concentration**
    30%
14. **pH of mold is**

5-6

15. **which do not grow in the presence of o2**
    Obligate anaerobes

16. **the process in hot air sterilization is**
    oxidation
17. **which is not a method of direct count**
    Spectrophotometer
18. **resolution of microscope is**
    0.2µm

19. **persisitent infection --------**
    Viruses replicate slowly

20. **cell wall is absent in**
    mycoplasma
21. **Resolution of Electron Microscope**

0.2nm

22. **Ribosomes made up of**
    mRNA nd protein
23. **Rings of basal body of gram negatives**

4

24. **Gram negative appears on stain..**

red

25. **Basic dye**

methylene blue

26. **Rat bite fever caused by**

Spirillum minus

27. **High salt concentration used by obligat halophiles....**

30%

Short Questions marks 2 ,3

1. Differentiate between horizontal and vertical gene transfer.

- **Vertical Gene Transfer**
  - This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

- **Horizontal Gene Transfer**

 When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant

molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include:

1. Transformation 2. Conjugation 3. Transduction

2. Define plasmogamy, kariogamy and meiosis.

•Sexual spores are produced in three phases:

1. **Plasmogamy:** A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-).

Both cells are haploid (one set of chromosomes)

2. **Karyogamy:**The (+)and (-) nuclei fuse to form a diploid zygote nucleus.

3. **Meiosis:** The diploid nucleus gives rise to haploid nuclei (sexual spores).

3. Define buffers.

○ Buffers are used to maintain desired pH.
○ Bacteria grown in lab produce acids. ○ Growth inhibited by acids. ○ Peptones and amino acids are used as buffers. ○ Phosphates are also used for buffers.

4. Define immunity, innate immunity and adaptive immunity.

- **Immunity: Ability of the body to protect itself from harmful effects of diseases.**
- **Innate (Natural):**

○ Present at birth: One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific. ○ Rapid to respond: Because it is already there, it interacts with the microbes as soon as they enter the body. ○ Nonspecific: It means that it does not differentiate if it is an E. coli or a Staph or Salmonella. Body defense system against these entire pathogens act in a similar fashion. In other words, the same macrophage can phagocytize E. coli, Staph or Salmonella at the same time (if these pathogens are present there). ○ Does not increase in response with repeated exposures to the same agent. Therefore, it has no memory.

○ **First line of defense:** Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside. ○ **Second line of defense:** Phagocytic cells and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaching or breaking the first barrier, macrophages, neutrophils and such other cells phagocytize these microbes and kill them before these can establish infection in the body.

 Adaptive Immunity:
The **adaptive immune** system, also known as the acquired **immune** system or, more rarely, as the specific **immune** system, is a subsystem of the overall **immune**system that is composed of highly specialized, systemic cells and processes that eliminate pathogens or prevent their growth.
 Adaptive (Acquired): It is the third line of defense. Acquired by experience, specific to an organism.

5. What is epidemiology? Describe its tyes.

- The study of where and when diseases occur and how they are transmitted in a population. There are three basic types of investigations relating to epidemiology:

- **Descriptive Epidemiology**
- **Analytical Epidemiology**
- **Experimental Epidemiology**:

6. Enlist microbial identification methods.

- Classical or Conventional Method
- Serological Methods
- Nucleic Acid based Methods

7. Write economical uses of fungi.

- Saccharomyces are used for alcoholic beverages under anaerobic conditions for alcohol production (fermentatively). However, if Saccharomyces are incubated aerobically (for bread making), they metabolize glucose to produce CO_2 (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise. This yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from *Aspergillus niger*; cellulase from Trichoderma, antibiotics from many fungi and anticancer Taxol from Taxomyces are some other examples of useful fungal products. Entomophaga (kills gypsy moth), hence is used as a biological pest control. Mushrooms can be cooked and eaten as a source of proteins for humans.

8. What is sporulation?

• Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.

9. Differentiate between viable and direct count.

10. What is flagellum? Write its functions.

Flagellum: Long filamentous structure that propels bacteria

What is meant by gene transfer? Name the types.

Vertical Gene Transfer
Horizontal Gene Transfer

1. Transformation 2. Conjugation 3. Transduction

12. Describe classification system proposed by Robert H Whittaker.

In 1969, five kingdom classification was proposed by Robert Whittaker as under:

•Plantae: plants

•Animalia: Animals

•Fungi: Yeasts, molds and mushrooms

•Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.

•Prokaryotae or Monera: Bacteria

13. What is taxonomic hierarchy?

All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy.
Specie:
A bacterial **species** represents —a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics, and is diagnosable by a discriminative phenotypic property‖
Genus
Order
Family
Group
Class
Phylum
Kingdom
Domain

14. What is western blotting?

• **Western Blotting:** We can use this technique for the detection of antigen in the serum. Microbial proteins can be separated on SDS-PAGE by electrophoresis and the presence of these proteins can be detected by enzyme-tagged antibodies specific to those proteins. A color band will be seen where the specific protein (antigen) is present on the gel. Please remember that proteins in the gel are first transferred to a paper strip before they could be detected by specific antibodies

15. Enlist types of passive transport.

Simple Diffusion

Facilitated Diffusion
Osmosis

16. Name types of spiral bacteria.

These are curved shaped bacteria. They are further divided into 3 more subgroups.

- **Vibrio:** curved rods
- **Spirillum:** Helical but **rigid**
- **Spirochete:** Helical but **flexible**

 Spirochetes move by axial filaments which are enclosed by the outer membrane.

17. Define passive movement.

• **Passive Movement**

 Movement of substances with the concentration gradient. This means that substances will move from higher concentration of a substance to lower concentration. Gradient provides the force for movements of molecules and this happens with no energy expenditures.

18. What is function of lysozymes?

A lysosome is a membrane-bound organelle found in nearly all animal cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules. Lysosomes are organelles that contain digestive enzymes. They digest excess or worn out organelles, food particles, and engulfed viruses or bacteria.

19. What are fimbriae? Give location and function.

• Hair-like small appendages on G-neg cells Composed of pilin, a protein Can be at one pole or around the entire cell Used for attachment to surfaces or epithelial cells Fimbria: Singular
 Fimbriae help bacteria attach to surfaces before they can secrete biofilm.
• Fimbriae also help attach to epithelial cells e.g *Neisseria gonorrhoeae* E.coli O157: Diahrrea

20. Name types of culture medium on the basis of function.

- Differential medium
- Selective medium
- Enriched medium
- Basal medium
- Reducing medium
- Selective medium
- General purpose medium

21. Write functions of cell wall, flagella and ribosomes.

Cell wall gives support and protection against plasmolysis.

Flagella help bacteria in movement.

Ribosomes are organelles that assist in protein synthesis during translation.

22. What are types of microscopes?

- Light microscope
- Electron microscope
- Scanning electron microscope
- Transmission electron microscope
- Dark field microscope
- Bright field microscope
- Fluorescent microscope

23. What do you know about types of techoic acid?

G-positive cell wall contains teichoic acid which may be divided into wall teichoic (remains within the cell wall) acid and lipoteichoic acid (is inserted into the cell membrane).

 Teichoic acid is antigenic in nature and helps the cell wall by providing rigidity.

24. Write characteristics of integral proteins.

i. Integral proteins act as channels or carriers in facilitated diffusion. Integral proteins are called transporters or permeases

ii. No energy is required during transportation of substances through these integral proteins.

iii. Two kinds of transporters are known:
i. Nonspecific transporters
1. Ions
ii. specific transporters
1. Change in shape 2. Sugars, vitamins

25. What are benefits of solid medium?

Solidification of the medium is done for purifying organisms from each other as solid medium provides surface for individual colonies to grow well separated from each other. A well isolated colony is assumed to be the progeny of a single cell. Secondly, the solid media are used to study the colony characteristics.

26. Define refractive index.

Refractive Index: Ability of a medium to bend the light
- Light rays move in a straight line through a single medium
- Light rays bend when the pass from one medium to another

27. Define medical microbiology.

Medical microbiology is a branch of **medical** science concerned with the prevention, diagnosis and treatment of infectious diseases. In addition, this field of science studies various **clinical** applications of microbes for the improvement of health.

28. What is resolution?

Resolution is the ability of the lenses to distinguish between two closely lying objects as separate.

$$d = \frac{0.5\lambda}{n \sin \theta}$$

- Light microscope resolving power is 0.2 μm.

29. Write three differences between prokaryotes and eukaryotes.

Prokaryotes

• DNA is not enclosed in a nuclear membrane.
• Chromosome: mostly Single, circular
• DNA not associated with histones
• No membrane enclosed organelles
• Cell wall has peptidoglycan, a complex

carbohydrate.
• Divide by binary fission.

Eukaryotes

• DNA is enclosed in a membrane-bound nucleus.
• DNA is found in multiple chromosomes.
• Chromosomes are linear, thread-like structures.
• DNA is associated with histones.
• Cell wall, if present, is chemically simple. Cellulose or Chitin
• They have membrane enclosed organelles. Mitochondria chloroplasts Endoplasmic reticulum Golgi apparatus Lysosomes etc

30. Define virulence.

Virulence: The extent of pathogenicity

31. Write methods for

1. Plate Counts
a. Pour Plate Method:
b. Spread Plate Method:
2. Filtration:
3. The most Probable Number Method:
4. Direct Microscopic Count

32. Write steps of germination of spores.

• Spore germinates when it finds a conducive environment for its growth. Germination has 3 phases or stages:

- **Activation:** It prepares the spore for germination.
  - Heat can activate the spore when appropriate moisture and nutrients are present in the environment.
- **Germination:** Spore starts swelling and loosing its coats etc.
  - It becomes metabolically active.
- **Outgrowth**: New components are made.

33. Write types of lipopolysaccharides.

34. Define culture, media and inoculum.

Microbial growth in the lab is called a **culture**.
A nutrient material that supports the growth of microbes in the lab is called a **culture medium**.
Microbes introduced into a culture medium that initiate growth of organisms are known as **inoculum.**

35. Define antiseptics.

Compounds that are used to remove pathogens from living tissue are known as antiseptics.

36. Define phosphorylation.

The addition of an inorganic phosphate group to a chemical compound is called phosphorylation. e.g conversion of ADP into ATP in living tissues.

37. Explain phases of growth curve.

In a batch culture, organisms go through various phases of growth. If we follow this growth with respect to time, and make a graph of this growth pattern, it will give us what we call as a growth curve. There are four distinct phases of this curve. **1-Lag phase 2- Log phase 3- stationary phase 4- Death phase.**

38. What is high frequency of combination?

In some cells that carry F^+, F^+ (Fertility factor) gets incorporated in the chromosomal DNA which converts F^+ cells to high frequency of recombination cells (Hfr cells). When conjugation occurs between an Hfr cell and an F^- cell, the Hfr cell's chromosome (with its integrated F factor) replicates, and a parental strand of the chromosome is transferred to the recipient cell. Since this transfer starts from the middle of F factor gene, and most of the time, this transfer is not complete; the whole chromosome of Hfr cell is not transferred. However, some genes can be transferred during this process. When these genes become integrated into the genomic DNA of the recipient cells, the recipient cells acquires new versions of genes that were not part of its genome previously. It may be noted that F^- cells remains F^- negative cell because F factor is not transferred completely. This whole process is illustrated in the accompanying diagram.These newly acquired genes can be mapped easily with respect to the time they get transferred to F^- cells.

39. Differentiate between clone and strain?

Clone: Population of cells derived from a single cell that are genetically identical.
Strain: A genetic variant of a clone is called a strain.

40. Differentiate between positive sense and negative sense RNA.

41. What is zoonosis?

Zoonosis: Primarily an animal infection, but can be transmitted to humans. It can assume epidemic proportions in humans and is difficult to control or eradicate.

42. What are interferons? Give their function.

Interferons are antiviral proteins, produced by macrophages and lymphocytes in response to viral infections. Fibroblasts can also secrete it. These proteins interfere with viral replication. Thereare two types of interferons:

Species specific: This means that interferon produced by humans cannot be used in dogs or cats or vice versa.

Not viral specific: This means that interferons produced in response to virus A can also be effective against virus B.

Long Questions 5 & 10 marks.

1. Explain characteristics of aerophiles.

Describe conjugation, transduction and transformation.

Horizontal gene transfer:

When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three

processes are known by which genes can be transferred horizontally from one cell to the other, and they include:
1. Transformation 2. Conjugation 3. Transduction

1. Transformation

- Naked DNA in solution is transferred from one bacterium to another during this process. Frederick Griffith observed this process in 1928 when he was working with *Strep pneumonia* in his lab. This bacterium has two strains: one is capsulated and is called a Smooth strain (which is pathogenic) and the other is Rough strain (non-pathogenic).
- When live encapsulated bacteria are injected into a mouse, the mouse gets killed by the microbe. When rough strain is injected, the mouse is not killed. Moreover, when heat-killed encapsulated bacteria are injected, it also does not kill the mouse. However, when heat-killed strain is mixed with rough live strain, and a mouse is injected with this mixture, the mouse dies. If you isolate the bacterium from these dead mice, the bacteria resemble with encapsulated strain of *Strep pneumonia*. The interpretation of this experiment led to the mechanism that rough live bacteria take up DNA from the heat-killed encapsulated strain and becomes pathogenic by acquiring the ability to form a capsule. Very few bacteria have this property of accepting foreign DNA and most of it is degraded (only small fragments of DNA get incorporated in the genome of the recipient. The cells that can take up DNA are called competent cells. We can create such microbes in the lab that can accept a given DNA during gene transfers that we undertake in our DNA work. The cells that take up the DNA and incorporate this in their genome successfully are called transformed cells.

2. Conjugation

- Genes transfer process can be mediated by plasmids. Plasmids are extra- chromosomal circular DNA fragments that replicate independently of bacterial chromosome. In other words, a given bacterial cell can have multiple copies of plasmid with the same cell. Plasmids often have genes that are not strictly needed by the microbe for its growth. However, the genes on the plasmids provide some advantages to the bacterium. For example, antibiotic resistant genes offer a mechanism for the bacterium to resist the presence of that antibiotic. In other words, bacterium that has a plasmid with an antibiotic gene on it can still multiply in the presence of that antibiotic.
- Conjugation requires direct cell to cell contact and cells have to be opposite mating types. Plasmids in G –ive cells have genes that code for sex pili because pili are needed to make a bridge between the two mating type cells. Since there is no pilus present in G +ve bacteria, they secrete sticky surface molecules instead which create contact between the two cells. The pilus along with two mating cells is shown in the diagram below:
- These plasmids are transmissible from one bacterium to another during conjugation.
- Plasmid is replicated and a single copy of the plasmid is transferred to the recipient bacterium through the pilus.
- A complementary copy is then made in both the cells to make the DNA of the plasmid double stranded molecule.
- In E. coli, fertility factor (F factor) plasmid was the first plasmid observed to be transferred from one organism to the other, hence those bacteria that have this plasmid are called F^+ cells to differentiate from those that do not have one (F^-). However, once F^- cells acquire F^+ plasmid, they also become F^+.

3. Transduction

- Transferring of a gene from a bacterium to another bacterium via a virus is called transduction. ○ When a bacteriophage infects a bacterium such as E. coli, it replicates inside E. coli and also produces a protein coat (which is called a capsid) in which viral DNA is packed before the virus is released. What happens is that when phages cause lysis of infected cells, bacterial chromosomal DNA also gets fragmented and such fragments can sometimes be packed into the viral capsids much like the viral DNA. Now, the capsid carrying the bacterial gene or genes in that fragment can infect another E. coli and can

inject its DNA (bacterial DNA fragment) into it resulting in the transfer of genes from one bacterium to another.

3. Explain methods of microbial control in detail.

These methods can be broadly classified into four groups: Physical, Mechanical, Chemical and Biological

Physical methods of microbial control

Heat: It is the cheapest sources of all and easily available to control microbial growth. There are a few heat related concepts that we must appreciate. Heat denatures enzymes.

 Thermal Death Point: It is the lowest temperature at which all cells in a culture are killed in 10 min. It will be a specific temperature for a specific species of organism.
 Thermal Death Time: Time (minimum) during which all cells in a culture are killed at a given temperature. This will vary from temperature to temperature for the same organism. Obviously, higher temperatures will take less time to kill the organisms than low temperatures.
 Methods that use heat can further be classified into **dry and moist** heat methods.

- Heat kills by oxidation
- Form of dry heat include:

1. Dry heat (hot-air oven),

2. Flaming (Platinum loop sterilization in the flame of Bunsen burner),

3. Incineration (burning to ashes).
 Similarly, moist heat can also be grouped into autoclaving (15psi), boiling at 100$_o$C, and Tyndallization (100$_o$C for 3 consecutive days to sterilize sugar solution that can be degraded by autoclaving) and pasteurization (usually at low temperature than 100$_o$C).

Pasteurization: This technique typically employs low heat for killing pathogenic and food spoiling bacteria in milk. However, it does not kill thermoduric organisms. Thermoduric organisms are not pathogenic though. Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. Dairy industry tests phosphatase to determine if pasteurization of dairy product has occurred effectively because phosphatase present in raw milk gets inactivated with effective pasteurization temperatures. The following three equivalent heat treatments can be given to achieve pasteurization of milk:

- 63°C for 30 min
- High-temperature short-time: 72°C for 15 sec
- Ultra-high-temperature: 140°C for 4 sec

 Other Physical Methods

- Low temperature inhibits microbial growth by slowing down chemical reactions.
  - **Refrigeration:** Materials stored at 2-8°C can last for a day or two.
  - **Deep-freezing:** Long term storage of materials is possible at minus temperatures in the deep freezers.
  - **Lyophilization**: Freeze drying is another method for long term storage of food and other materials.
- **High pressure** deshapes and denatures proteins: This technique is used for preserving juices and wines.
- **Desiccation** prevents metabolism: This method is very old and still in use today. Food stored in high concentrations of salt or sugar solution can keep for long.

○ **Osmotic pressure** causes plasmolysis. High salt concentrations for example can take the water content of the cells out leaving them starved of water.

• **Filtration:** It is also a physical method. It can be used to filter air (using high efficiency particulate air filter) or liquid medium using membrane filters.

 Radiation:

○ Ionizing and non-ionizing radiation ○ Ionizing Radiation: High energy waves that destroys microbes.

• Ionization radiations include gamma rays, X rays, or high-energy electron beams. The ionizing radiation possesses a wavelength shorter than that of nonionizing radiation, less than about 1 nm. All these short wavelengths cause ionization of water generating high reactive hydroxyl radicals which are damaging to the cells as they bind to DNA and results in mutations. Medical plastic supplies, medicines and meat products can be sterilized by radiation.

 Non-ionizing Radiation: wavelengths longer than 1nm fall into non-ionizing radiation. The best example is UV light. UV light causes mutations in the DNA by dimerization of thymidine bases. These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The most useful wavelength is 260 nm. This wavelength is specifically absorbed by DNA. UV radiation is used to control organisms in the air mostly. However, surfaces are also sterilized by UV radiation. UV light cannot penetrate deep into the fluids, so only surface bacteria are killed.

ETHYLENE OXIDE FOR MICROBIAL CONTROL

○ **Ethylene Oxide:** Many heat-sensitive items such as disposable plastic, petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized by ethylene oxide. Ethylene oxide is a strong alkylating agent that kills by reacting with functional groups of DNA and proteins to block replication and enzymatic activity. It rapidly penetrates packing materials, even plastic wraps. EtO is explosive, supplied in a 10% to 20% concentration mixed with either CO_2 or dichlorodifluoromethane. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/L.

CHEMICAL METHODS FOR MICROBIAL CONTROL

Chemicals are used to control microbial growth. Factors that influence the efficacy of a chemical disinfectant include the concentration of the disinfectant, presence of organic matter in the environment (matrix) where the disinfectant is being used, pH of the environment (disinfectants are more effective at acidic pH), and time of exposure to the disinfectant.

4. How infectious diseases are transmitted? Explain in detail.

○ **Direct Transmission:** person to person physical contact is a direct contact. Influenza spread is a typical example.

○ **Indirect Transmission:** A nonliving object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.

○ **Droplet Transmission:** Mucus droplets are created when you sneeze for example.

These droplets carry organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.

○ **Vehicle Transmission:** Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.

 Vectors

○ A **vector** is a vehicle (animate or inanimate) that carries a pathogen from one host to another.

o Arthropods are vectors for many diseases. There are two ways these arthropods can transmit organisms to susceptible hosts:

- **Mechanical Transmission**: In this type of transmission, organisms stick to the body parts of insects and get transmitted passively from one location to another.
- **Biological Transmission**: Microbes multiply in the insect and is transmitted actively by insect bites from one individual to another.

Nosocomial Infections

o Hospital acquired infections are called nosocomial infections.

5. Explain salient features of viruses, virions and viroids.

VIROIDS

These are infectious agents that consist only of RNA and found in plants only. No capsids, no envelopes, just RNA. They are covalently closed circular ssRNAs about 250 nucleotides long. The circular RNA normally exists as a rodlike shape due to intrastrand base pairing, which forms double-stranded regions with single-stranded loops. They do not encode any gene products, so they are replicated by cellular DNA dependent RNA polymerase. A plant infected with such viroids may not show any signs of illness. However, in other plants, they may cause severe disease. They cause the disease by RNA silencing process.

VIRUSOIDS

o They are similar to viroids; however, they encode one or two gene products and need a helper virus to infect the host cells. The helper virus supplies gene products and other materials needed by the virusoid for completion of its replication cycle. Hepatitis D virusoid is a typical example which uses Hepatitis B virus as a helper.

PRIONS

o These are proteinaceous infectious particles that cause neurodegenerative diseases such as scrapie in sheep, bovine spongiform encephlopthy, and Kuru in humans. These are abnormal form of cellular proteins. How these proteins accumulate in the cell is not clear, although, some genetic components are known to be involved.

6. Explain methods of microbial identifications.

7. How fungi can be identified by biochemical testing?

• Yeast if grown on solid medium grows like bacteria. In other words, colonies of yeast look like bacterial colonies but are bigger in size.

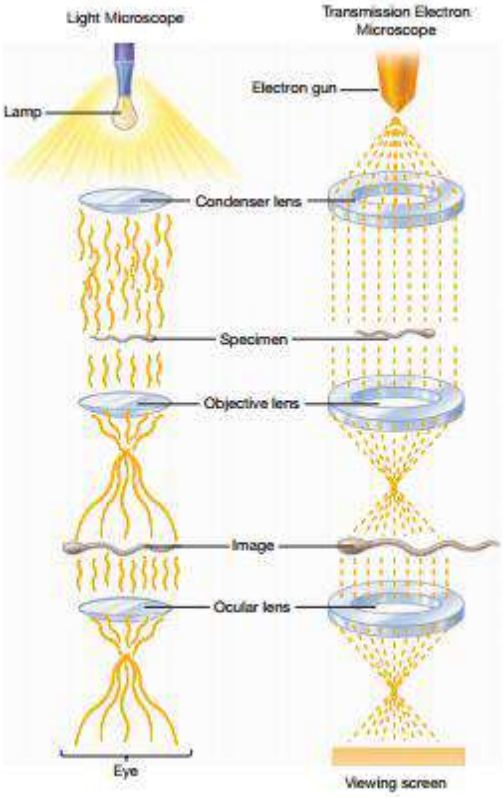
 Yeasts can grow as facultative anaerobes. Saccharomyces if given oxygen to grow, it converts carbohydrates aerobically and produces CO_2 and water; however, if oxygen is denied, carbohydrates get converted fermentatively into ethanol and CO_2.

IDENTIFICATION OF SELECTED FUNGI

 Filamentous fungi are mainly identified by morphology, and not by biochemical testing. Fungi are cultured using a slide (slide culture) on top of Sabouraud's agar. Spores are placed on top of the medium or embedded in the medium. A block of the medium can be used to grow fungi or a glass coverslip can be used to grow spores into hyphae and mycelium and spore arrangements can be studied under the microscope by staining with lactophenol cotton blue. Slides stained with lactophenol cotton blue can

also be seen under the microscope.Stained slides can show specific arrangements of spores, their sizes and other morphological features as seen in the figure for Aspergillus.

8. Label the diagram of Electron microscope with numbers mentioned.



9. Write functions of cell membrane of bacteria.

- Selective permeable barrier
- Passive and Active Transport
- Respiration in microbes (bacteria)
- Photosynthesis in microbes
- Lipid synthesis
- Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane.
- PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

10. Write names of 5 types of bacteria with respect to temperature.

- Organisms are basically classified into three groups based on the temp requirements:

1. **Psychrophiles:** These are further divided into strict psychrophiles and psychrotrophs:
   - **Psychrotrophs:** Cold loving: 15 ^{0}C

○ **Psychrotrophs:** Optimum temp is 20-30 ^{0}C. Food spoilage bacteria that can spoil food during refrigeration.

2. **Mesophiles:** 25 – 40 ^{0}C: These are the ones that cause diseases in animals and humans.

○ Moderate temp loving organisms ○ Optimum: 37 ^{0}C as this is the body temperature of humans and animals.

3. **Thermophiles:** 50 – 60 ^{0}C

○ Heat loving ○ Important in organic compost piles ○ These are further divided into hyperthermophiles that grow optimally at 80 ^{0}C. They live in hot springs.

• Every group of these organisms has a:

○ Minimum growth temp ○ Optimum growth temp. ○ Maximum growth temp.

11. Write characteristics of metachromatic granules.

• Metachromatic Granules

• Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different color as methylene blue gives blue color but the color on these granules is red). • Large inclusions • These inclusions contain inorganic phosphates. Inorganic phosphates are used up in ATP synthesis. • Characteristics of *Corynebacterium diphtheria: This bacterium can be identified by the presence of these granules in it.*

12. Explain five kingdom classification systems.

In 1969, five kingdom classification was proposed by Robert Whittaker as under:

•Plantae: plants

•Animalia: Animals

•Fungi: Yeasts, molds and mushrooms

•Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.

Prokaryotae or Monera: Bacteria and Archea

13. Explain layers of endospore.

• Endospore and its various parts/structures

• **Exosporium:** A thin delicate outermost covering of the spore

• **Spore coat:** 2nd layer underneath the exosporium. It is thick and composed of several protein layers.

- Resistant to chemicals
- It contains enzymes for germination. Germination of spores into vegetative form occurs when environment becomes favorable for their growth.

• **Cortex:** It is the 3rd layer from outside in. It has peptidoglycan in it.

• **Spore cell wall or core wall:** Surrounds the protoplast or spore core

• **Spore core:** Contains nucleoid and ribosomes

14. Enlist types of bacteria on the basis of oxygen.

- Based on the use and concentration of Oxygen, microbes can be classified into five groups:
  - **Obligate Aerobes**: Oxygen must be present for their growth. These organisms do not grow if O_2 is not present in the medium. If you test these organisms in a tube containing nutrient agar, the growth will remain confined to the surface only (see the diagram for the concept). ▪ Mycobacterium tuberculosis is an example of such an organism.
  - **Facultative Anaerobes:** These organisms prefer aerobic respiration; however, if oxygen is not available, they can use anaerobic respiration or fermentative modes for generating ATPs. These organisms will be present throughout the tube, however, more growth will be seen on the top (close to the surface) because these organisms prefer using oxygen if it is available.
  - **Obligate Anaerobes:** Although, these organisms do not use oxygen, they cannot grow in the presence of oxygen. The reason is that oxygen creates toxic compounds which can kill these cells. Normally, oxygen toxic compounds are eliminated by the cells, however, strict anaerobe do not have a system to get rid of these toxic compounds when they are made in the cells. So using or not using oxygen is one thing and growing in the presence of oxygen (and not utilizing it) is another. The growth will remain confined to the bottom which has little oxygen.
  - **Aerotolerant Aerobes:** These organisms do not use oxygen and also are not bothered by the presence of oxygen. In other words, they have a better system to dispose of toxic oxygen compounds. They will grow evenly throughout the tube of medium

15. Name five types of microscopy.

Dark field microscopy

- This microscope makes use of a special condenser that provides illumination from the sides only as depicted in the diagram below:

- This special arrangement of the condenser throws light rays at an oblique angle so that if the specimen is not present on the slide, there will be no image formed. However, if the the organisms are present on the glass slide, those organisms will reflect the light into the objective lens and we will see the cells as bright objects in a dark background much like we see stars in the sky at night.

- The beauty of this microscope is that one does not have to stain the organisms to see them under the microscope.

Phase contrast microscopy

This instrument is built on the concept of phases of light waves. The light ray that passes through an organism gets retarded about ¼ of its wavelengths than the light that passes outside the organism (through the glass slide only). To further retard the retarded light ray, a phase plate is inserted into the body of the microscope (above the objective lens) which is thicker in the center than the ends. This arrangement causes the retarded light to further slow down about ¼ of its wavelength, which when recombines with the un-retarded light creates a contrast which makes the cells/organisms visible.

- This method for visualizing organisms also does not involve staining.

Fluorescent microscopy

- This microscope is equipped with UV light that provides the source of light for illumination. However, this light source does not pass through the slide, it instead falls over the slide (having specimen) and creates fluorescence.

- Fluorochromes are substances that absorb short wavelength of light and emit longer wavelength (visible light spectrum)

o Fluorochromes are attached to antibodies that are specific to organisms. So, if an organism is present in the specimen, we can use specific antibodies to determine the presence of those organisms by use of fluorochrome tagged antibodies. See the accompanying diagrams for details. Look for UV lamp (Mercury Lamp).

Transmission electron microscope

o Electron microscope (EM) makes use of electron beam as an alternative to light rays. Since electron beam has much shorter wavelength, resolution of EM is 0.1nm compared with light microscope which is 200nm.

o Second difference of EM with light microscope is that focusing of electron beam is done not by glass lenses but by electromagnets that acts like lenses.

o The third difference in EM is that the image of the specimen is formed on a fluorescent screen like a TV screen.

Scanning electron microscope
This is a modified version of electron microscope. Electron beam is allowed to fall on to the specimen and the image is formed from the reflected electrons that are collected and assembled into an image by the detector. It gives a three dimensional picture.

16. Explain ELISA.

• There are some interactions (Antigens and Antibodies) that are not directly evident. Interactions may have taken place, but there is no visible direct clue if the interaction has taken place or not. For such interactions, we use indirect methods. Say, there is big body of smoke visible to you from a kilometer ahead of you. What is your guess? Yes, you guessed it right. There is a big fire there. Did you see the fire? Probably not. You guessed on the basis of smoke that there is a fire there. This is called indirect way of interpretations. A beautiful example of such a test is ELISA where we do not know if the interaction has taken place or not. However, we can see it indirectly. We will see that in the following paragraph which discusses ELISA.

 Enzyme-Linked Immunosorbent Assay: This test is done by coating the ELISA plastic plates either with the antigen or the antibodies. Suppose we have Brucella antigen coated ELISA plates that can detect antibodies from the serum of a Brucella infected animal. You take the serum and put into the wells of ELISA plate. You allow time for antigen and antibody to interact and then wash these wells with some buffer to remove unbound antibodies. These bound antibodies are not visible at this step. So, how do we see this interaction? Well, we use another antibody that has been made against the antibody bound to the antigen. This special antibody has been tagged with an enzyme. After allowing time for interaction of this 2nd antibody with the 1st antibody, 2nd antibody is also given a washing to remove unbound antibody molecules. Again, we cannot say if interaction has taken place or not. Here comes an indirect way of detecting this interaction. Remember that the 2nd antibody has a molecule of an enzyme attached to it. Now, if we provide substrate to the enzyme in the wells, enzyme will convert the substrate into a color compound visible to the eyes. The color change can be measured by a spectrophotometer as well which gives a quantitative data

17. Explain types of conidiophores.

18. Explain types of algae.

- **Phaeophyta (Kelp):** Commonly known as brown algae, brownish in color, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll *a* and *c*, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow upto 20 cm in one day.

- **Rhodophyta:** Red Alage, these are reddish in color and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).

- **Chlorophyta:** Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.

- **Bacillariophyta:** Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid with which human can be intoxicated by eating contaminated mussels.

  - Diatoms: Come in beautiful colors and shapes.

- **Dinoflagellata:** These are unicellular algae collectively called as plankton (free floating organisms). These cells have cellulose in their cytoplasmic membrane which gives the membrane rigidity. They have chlorophyll *a* and *c*, and carotene. These algae store starch in them. Dinoflagellates in the genus Alexandrium produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of Alexndrium gives ocean a deep red color commonly known as red tide.

- **Oomycota (Water molds):** They are basically decomposers of dead matter. Superficially, these resemble with fungi; however, these are motile with flagella and their cell wall is composed of cellulose not chitin. Their spores are called zoospores.

- Terrestrial members of water molds are also plant parasite. *Phytophthora infestans* was responsible for Irish potato blight during mid-1800s.

- *P. ramorum* causes sudden oak death