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FYBSc SEMESTER II
OCTOBER 2018 (ATKT)
PAPER I MICROBIOLOGY
Q.P. CODE : 51279
DATE OF EXAM :

ANSWERS

**Q1. A. Define the following terms:** ( 5)

1. Prion: An infectious agent consisting only of protein; prions cause a variety of spongiform encephalopathics such as scrapie in sheep and goats.
2. Helical symmetry: In virology this refers to a virus with a helical capsid surrounding its nucleic acid.
3. Turbidostat: It is a type of continuous culture system in which the absorbance of the culture adjusts the flow rate of the limiting nutrient and the growth rate of the culture adjusts to this flow rate.
4. Water activity : **Water activity** or $a_w$ is equivalent to the ratio of the solution's vapor pressure (*P*soln) to that of pure water (*P*water). Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it's $a_w$ is low
5. Zygospore – In Zygomycete, after dikaryotic stage the 2 nuclei in fungi fuse to yield spore called zygospore.

**Q I B.State whether the following statement is true or false:** (5)

1. Malaria is caused by Entamoeba. - **FALSE**
2. Algal cell wall contains proteins. - **TRUE**
3. *Rhizopus* is a common food spoilage organism - **TRUE**
4. Archaeal cell membranes have ether linkages: **TRUE**
5. VBNC microbes need special measures of cultivation. **TRUE**

**Q I C. Give one example for each of the following:** ( 5)

1. Fungus causing Plant disease - Ergot, Smut, Chestnut blight
2. Sulfur metabolizing archaea- *Sulfolobus*
3. Algal species used as food. – red algae and brown algae- Porphyra, Chondrus, Palmaria, Gracilaria, Chlorella
4. Enveloped virus: influenza, rabies
5. An organism which is a Psychrophile ***Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium,* and *Shewanella.***

**Q I D. Select the correct alternatives and rewrite the statement.** (5 )

1. Coxiella are causative agents of Q fever
2. Porphyra is a red algae.
3. Cilia is used by *Paramoecium* for locomotion.
4. RMSF is caused by Rickettsia species.
5. The period of adjustment in growth curve is called as lag phase.

**Q2. Answer briefly any two of the following:** ( 20)

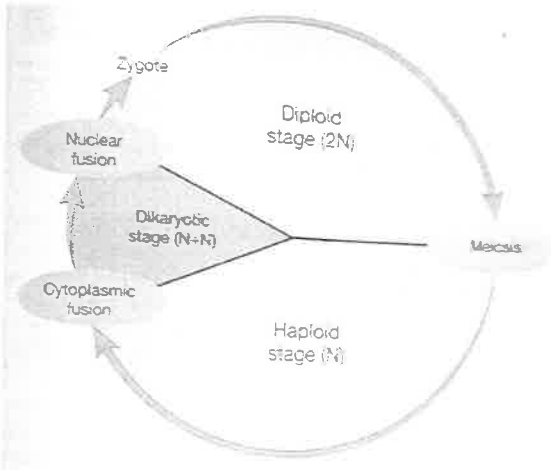
1. Explain the life cycle of lytic bacteriophage. Draw a neat labelled diagram
   **Prescott 428-436**
2. Write a short note on synchronous growth. With a suitable diagram, explain the technique of obtaining synchronous culture.

- Synchronous cultures are cultures composed of cells that are all at the same stage of the cell cycle. Measurements made on such cultures are equivalent to the measurements made on individual cells.

- Synchronous cultures of bacteria can be obtained by a number of techniques. Synchrony can be *induced* by manipulations of environmental conditions, usually cyclic-- repetitive shifts of temperature or furnishing fresh nutrients to cultures that have just entered the stationary phase.
- A synchronous population can be *selected* from a random population by physical separation of cells that are at the same stage of the cell cycle -- differential filtration or by centrifugation.
- For physiological studies, techniques based on selection are preferable to those based on induction.
- An excellent selective method for obtaining synchronous cultures is the Helmstetter-Cummings technique, which is based on the fact that certain bacteria stick tightly to cellulose nitrate (membrane) filters. The technique involves filtering an unsynchronized culture of bacteria through a (membrane) filter, then inverting the filter and allowing fresh medium to flow through it . After loosely associated bacteria have been washed from the filter, the only bacterial cells in the effluent stream of medium are those that arise through division. Hence, all cells in the effluent are newly formed and are therefore at the same stage of the cell cycle

  **Diagram Page 191 Stanier 5th edn.**

3. Discuss asexual and sexual reproduction in fungi. ( Prescott 632- 635)




Explain briefly about –

Sexual reproduction – Mating types, Gametangia, dikaryotic stage, spores- Zygospore, Ascospore, Basidiospore

Asexual reproduction occurs in fungi by – 1) mitosis 2) budding 3) Spore production – Arthrospore, Chlamydospore, Sporangiospore, Conidiospore and Blastospore

**Q3. A. Answer briefly any three of the following:** **(18)**

1. Compare and contrast between *archaea and bacteria*

   Prescott page 98

2. Explain the ecological and commercial significance of genus *Streptomyces.*

   ✓ Streptomycetes are very important, both ecologically and medically. The natural habitat of most streptomycetes is the soil, where they may constitute from 1 to 20% of the culturable population. In fact, the odor of moist earth is largely the result of streptomycete production of volatile substances such as **geosmin.**

   ✓ Streptomycetes play a major role in mineralization. They are flexible nutritionally and can aerobically degrade resistant substances such as pectin, ②

lignin, chitin, keratin, latex, agar, and aromatic compounds. Streptomycetes are best known for their synthesis of a vast array of antibiotics. Stanley Waksman's discovery that *S. griseus* produces streptomycin which was the first drug to effectively combat tuberculosis.

- ✓ Streptomycetes have been found to produce over 10,000 bioactive compounds. Hundreds of these natural products are now used in medicine and industry; about two-thirds of the antimicrobial agents used in human and veterinary medicine are derived from the streptomycetes. Examples include amphotericin B, chloramphenicol, erythromycin, neomycin, nystatin, and tetracycline. Some *Streptomyces* species produce more than one antibiotic.
- ✓ The genome of *Streptomyces coelicolor,* which produces four antibiotics and serves as a model species for research, has been sequenced. At 8.67 Mbp, it isone of the largest procaryotic genomes. Its large number of genes (7,825) no doubt reflects the number of proteins required to undergo a complex life cycle. Many genes are devoted to regulation, with an astonishing 65 predicted RNA polymerase sigma subunits and over 50 two-component regulatory systems.
- ✓ The ability to exploit a variety of soil nutrients is also demonstrated by the presence of a large number of ABC transporters, the Sec protein translocation system, and secreted degradative enzymes. Finally, genes were discovered that are thought to encode an additional 18 secondary metabolites

**3. Discuss the thermophilic and methanogenic archaea.**

- ✓ **Methanogens** are strict anaerobes that obtain energy by converting CO2, H2, formate, methanol, acetate, and other compounds to either methane or methane and CO2. They are autotrophic when growing on H2 and CO2. This is the largest group of archaea.
- ✓ There are five orders (*Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales,* and *Methanopyrales*) and 26 genera, which differ greatly in overall shape, 16S rRNAsequence, cell wall chemistry and structure, membrane lipids, and other features. Several genera have walls with pseudomurein; other walls contain either proteins or heteropolysaccharides.
- ✓ One of the most unusual methanogenic groups is the class *Methanopyri.* It has one order, *Methanopyrales,* one family and a single genus, *Methanopyrus.* This hyperthermophilic, rod-shaped methanogen has been isolated from a marine hydrothermal vent. *Methanopyrus kandleri* has a temperature minimum at 84°C and an optimum of 98°C; it will grow at temperatures up to 110°C. *Methanopyrus* occupies the deepest and most ancient branch of the euryarchaeotes. Perhaps methanogenic archaeal ancestors were among the earliest organisms.
- ✓ Metabolism is unusual. These procaryotes contain several unique cofactors: tetrahydromethanopterin (H4MPT), methanofuran (MFR), coenzyme M (2-mercaptoethanesulfonic acid), coenzyme F420, and coenzyme F430 The first three cofactors bear the C1 unit when CO2 is reduced to CH4. F420 carries electrons and protons, and F430 is a nickel tetrapyrrole serving as a cofactor for the enzyme methyl-CoM methylreductase. T
- ✓ Some methanogens can live autotrophically by forming acetyl-CoA from two molecules of CO2 and then converting the acetyl-CoA to pyruvate and other products
- ✓ Methanogens thrive in anoxic environments rich in organic matter: the rumen and intestinal system of animals, freshwater and marine sediments, swamps and marshes, hot springs, anoxic sludge digesters, and even within anaerobic protozoa.
- ✓ Methanogens often are of ecological significance. The rate of methane production can be so great that bubbles of methane sometimes rise to the surface of a lake or pond.

Rumen methanogens are so active that a cow can belch 200 to 400 liters of methane a day.

- ✓ Methanogenic archaea are potentially of great practical importance since methane is a clean-burning fuel and an excellent energy source. For many years sewage treatment plants have been using the methane they produce as a source of energy for heat and electricity. Anaerobic digester microbes degrade particulate wastes such as sewage sludge to $H_2$, $CO_2$, and acetate. $CO_2$-reducing methanogens form $CH_4$ from $CO_2$ and $H_2$,
- ✓ Methanogenesis also can be an ecological problem. Methane absorbs infrared radiation and thus is a greenhouse gas. There is evidence that atmospheric methane concentrations have been rising over the last 200 years. Methane production may significantly promote future global warming. Recently it has been discovered that methanogens can oxidize $Fe^0$ and use it to produce methane and energy. This means that methanogens growing around buried or submerged iron pipes and other objects may contribute significantly to iron corrosion

**Thermophiles:**

**The Thermoplasms**

- ✓ Procaryotes in the class *Thermoplasmata* are thermoacidophiles that lack cell walls. At present, three genera, *Thermoplasma, Picrophilus,* and *Ferroplasma* are known. They are sufficiently different from one another to be placed in separate families, *Thermoplasmataceae, Picrophilaceae,* and *Ferroplasmataceae.*
  - ✓ *Thermoplasma* grows in refuse piles of coal mines. These piles contain large amounts of iron pyrite (FeS), which is oxidized to sulfuric acid by chemolithotrophic bacteria. As a result the piles become very hot and acidic. This is an ideal habitat for *Thermoplasma* because it grows best at 55 to 59°C and pH 1 to 2.
- ✓ *Picrophilus* is even more unusual than *Thermoplasma.* It originally was isolated from moderately hot solfataric fields in Japan. Although it lacks a regular cell wall, *Picrophilus* has an S-layer outside its plasma membrane. *Picrophilus* is aerobic and grows between 47 and 65°C with an optimum of 60°C. It is most remarkable in its pH requirements: it grows only below pH 3.5 and has a growth optimum at pH 0.7. Growth even occurs at about pH 0!

**Extremely Thermophilic $S^0$-Metabolizers:**

- ✓ This physiological group contains the class *Thermococci,* with one order, *Thermococcales.* The *Thermococcales* are strictly anaerobic and can reduce sulfur to sulfide. They are motile by flagella and have optimum growth temperatures around 88 to 100°C. The order contains one family and three genera, *Thermococcus, Paleococcus,* and *Pyrococcus.*
- ✓ **Sulfate-Reducing *Euryarchaeota:*** Euryarchaeal sulfate reducers are found in the class *Archaeoglobi* and the order *Archaeoglobales.* This order has only one family and three genera. *Archaeoglobus* contains gram-negative-staining, irregular coccoid cells with cell walls consisting of glycoprotein subunits. It can extract electrons from a variety of electron donors (e.g., $H_2$, lactate, glucose) and reduce sulfate, sulfite, or thiosulfate to sulfide. Elemental sulfur is not used as an acceptor. *Archaeoglobus* is extremely thermophilic (the optimum is about 83°C) and has been isolated from marine hydrothermal vents. The organism is not only unusual in being able to reduce sulfate, unlike other archaea, but it also possesses the methanogen coenzymes F420 and methanopterin

4. **Discuss the nucleic acids present in viruses.**

**Viral Genomes**

- ✓ Viruses are exceptionally flexible with respect to the nature of their genomes. They employ all four possible nucleic acid types: single-stranded DNA, double-stranded DNA, single-stranded RNA, and double-stranded RNA. All four types are found in animal viruses.
- ✓ Most plant viruses have single-stranded RNA genomes, and most bacterial viruses contain double-stranded DNA. The size of viral genetic material also varies greatly. The smallest genomes are around 4,000 nucleotides, just large enough to code for three or four proteins. Some other viruses even save space by using overlapping genes. At the other extreme, T-even bacteriophages, herpesvirus, and vaccinia virus have genomes of 1.0 to2.0μm 105 nucleotides and may be able to direct the synthesis of over 100 proteins.
- ✓ Most DNA viruses use double-stranded DNA (dsDNA) as their genetic material. However, some have single-stranded DNA (ssDNA) genomes. In both cases, the genomes can be either linear or circular.
- ✓ Some DNA genomes can switch from one form to the other. For instance, the *E. coli* phage lambda has a genome that is linear in the capsid, but is converted into a circular form once the genome enters the host cell. Another important characteristic of DNA viruses is that their genomes often contain unusual nitrogenous bases. For example, the T-even phages of *E. coli* have 5-hydroxymethylcytosine instead of cytosine, and the hydroxymethyl group is often modified by attachment of a glucose moiety.
- ✓ RNA viruses also can be either double-stranded (dsRNA) or single-stranded (ssRNA). Although relatively few RNA viruses have dsRNA genomes, dsRNA viruses are known to infect animals, plants, fungi, and at least one bacterial species. More common are the viruses with ssRNA genomes. Some ssRNA genomes have a base sequence that is identical to that of viral mRNA, in which case the genomic RNA strand is called the **plus strand** or **positive strand.** In fact, plus strand RNAs can direct protein synthesis immediately after entering the cell. However, other viral
- ✓ Polio, tobacco mosaic, brome mosaic, and Rous sarcoma viruses are all positive strand RNA viruses; rabies, mumps, measles, and influenza viruses are examples of negative strand RNA viruses.
- ✓ Many RNA viruses have **segmented genomes**—that is, the genome consists of more than one RNA strand or segment. In many cases, each segment codes for one protein. Usually all segments are enclosed in the same capsid even though some virus genomes may be composed of as many as 10 to 12 segments. However, it is not necessary that all segments be located in the same virion for successful reproduction. The genome of brome mosaic virus, a virus that infects certain grass species, is composed of four segments distributed among three different virus particles. All three of the largest segments are required for infectivity. Despite this complex and seemingly inefficient arrangement, the different brome mosaic virions manage to successfully infect the same host.
- ✓ Plus strand viral RNA often resembles mRNA in more than the equivalence of its nucleotide sequence. Just as eukaryotic mRNA usually has a 5′ cap of 7-methylguanosine, many plant and animal viral RNA genomes are capped. In addition, most plus strand RNA animal viruses also have a poly-A sequence at the 3′ end of their genome, and thus closely resemble eucaryotic mRNA with respect to the structure of both ends. Strangely enough, a number of single-stranded plant viral RNAs have 3′ ends that resemble eucaryotic transfer RNA. Indeed, the genome of tobacco mosaic virus actually accepts amino acids

5. **Explain cultivation of bacterial viruses and animal viruses using tissue culture.**

✓ Bacterial and archaeal viruses are cultivated in either broth or agar cultures of young, actively growing cells. In some infected cultures, so many host cells are destroyed that turbid cultures clear rapidly because of cell lysis. Agar cultures are prepared by mixing viruses with cool, liquid agar and a suitable culture of host cells. The mixture is quickly poured into a petri dish containing a bottom layer of sterile agar. After hardening, cells in the layer of top agar grow and reproduce, forming a continuous, opaque layer or "lawn." Wherever a virion comes to rest in the top agar, the virus infects an adjacent cell and reproduces. Eventually, lysis of the cells generates a plaque or clearing in the lawn Plaque appearance often is characteristic of the virus being cultivated.

✓ Cultivation of animal viruses in tissue culture:

More recently animal viruses have been grown in tissue (cell) culture on monolayers of animal cells. This technique is made possible by the development of growth media for animal cells and by the use of antimicrobial agents that prevent bacterial and fungal contamination. Viruses are added to a layer of animal cells in a specially prepared petri dish and allowed time to attach to the cells. The cells are then covered with a thin layer of agar to limit virion spread so that only adjacent cells are infected by newly produced virions. As a result, localized areas of cellular destruction and lysis called **plaques** often are formed and may be detected if stained with dyes, such as neutral red or trypan blue, that can distinguish living from dead cells. Viral growth does not always result in the lysis of cells to form a plaque. Animal viruses, in particular, can cause microscopic or macroscopic degenerative changes or abnormalities in host cells and in tissues. These are called **cytopathic effects** . Cytopathic effects may be lethal, but plaque formation from cell lysis does not always occur.

6. Explain the viral envelope and enzymes. State its significance with respect to influenza virus.

**Viral Envelopes and Enzymes**

✓ Many animal viruses, some plant viruses, and at least one bacterial virus are bounded by an outer membranous layer called an **envelope**.

✓ Animal virus envelopes usually arise from host cell nuclear or plasma membranes; their lipids and carbohydrates are normal host constituents.

✓ In contrast, envelope proteins are coded for by virus genes and may even project from the envelope surface as **spikes,** which are also called **peplomers.** In many cases, these spikes are involved in virus attachment to the host cell surface. Because they differ among viruses, they also can be used to identify some viruses.

✓ The envelope is a flexible, membranous structure, so enveloped viruses frequently have a somewhat variable shape and are called pleomorphic. However, the envelopes of viruses like the bullet-shaped rabies virus are firmly attached to the underlying nucleocapsid and endow the virion with a constant, characteristic shape.

✓ In some viruses the envelope is disrupted by solvents like ether to such an extent that lipid-mediated activities are blocked or envelope proteins are denatured and rendered inactive. The virus is then said to be "ether sensitive."

✓ **Influenza virus** is a well-studied example of an enveloped virus. Spikes project about 10 nm from the surface at 7 to 8 nm intervals. Some spikes possess the enzyme neuraminidase, which functions in the release of mature virions from the host cell. Other spikes have hemagglutinin proteins, so named because they can bind the virions to red blood cell membranes and cause the red blood cells to clump together (agglutinate). This is called hemagglutination.

✓ Hemagglutinins participate in virion attachment to host cells. Proteins, like the spike proteins that are exposed on the outer envelope surface, are generally glycoproteins—that is, the proteins have carbohydrate attached to them. A nonglycosylated protein, the M or matrix protein, is found on the inner surface of the envelope and helps stabilize it. It was originally thought that all virions lacked enzymes. However, as just illustrated in the discussion of influenza virus, this is not the case. In some instances, enzymes are associated with the envelope or capsid (e.g., influenza neuraminidase), but most viral enzymes are located within the capsid. Many of theseare involved in nucleic acid replication. For example, the influenza virus uses RNA as its genetic material and carries an enzyme that synthesizes RNA using an RNA template. Such enzymes are called RNA-dependent RNA polymerases. Thus although viruses lack true metabolism and cannot reproduce independently of living cells, they may carry one or more enzymes essential to the completion of their life cycles.

Q III B. **Do as directed any two of the following :** **(2)**

1. Cytopathic effect: The observable change that occurs in cells as a result of viral replication. Examples include ballooning, binding together, clustering, or even death of the cultured cells.
2. bacteria that are able to pass through bacteria proof filters: Mycoplasma
3. One advantage of Lysogeny: Production of toxin, resistance to antimicrobial agents
4. The chemical present in cell wall of archea: Pseudomurein, protein

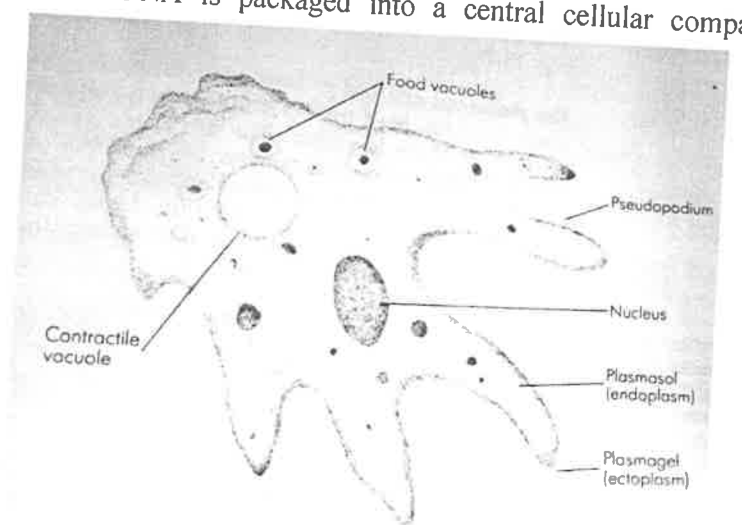
**QIV. A. Answer briefly any three of the following:** **( 18)**

1. Explain the ecological (2M) and commercial importance (4M) of Algae

- Algae are primary producers of organic matter in the ecosystem. They stabilise and improve physical properties of soil.
- The commercial products (in brief) - Agar, alginic acid, carrageenan, Diatomaceous earth. (pelczar 367- 369)

2. Discuss the morphology and characteristic features of Amoeba.(Pelczar -393-394)

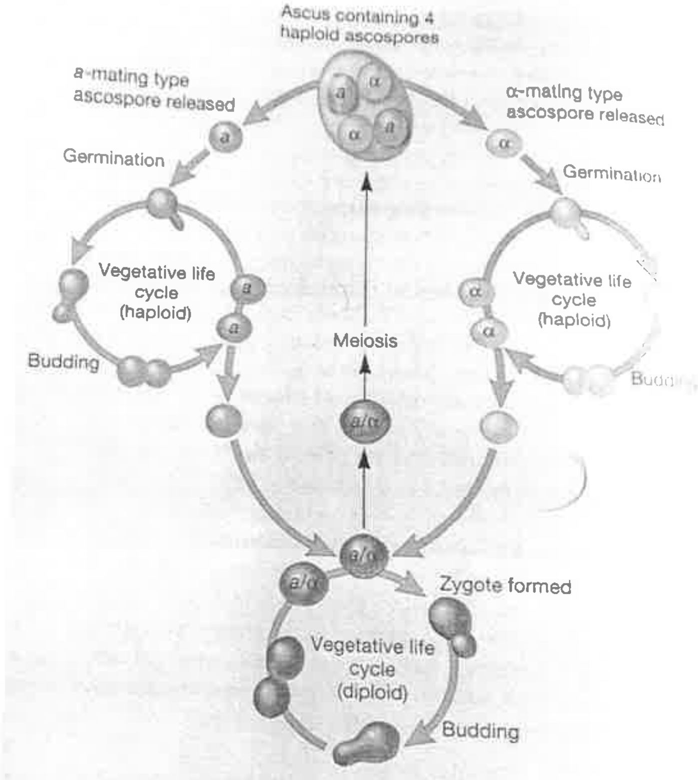
- Amoeba are eukaryotes whose bodies most often consist of a single cell.
- Their cytoplasm is divided as ectoplasm and endoplasm and cellular contents are enclosed within a cell membrane.
- Their DNA is packaged into a central cellular compartment called the




nucleus. Finally, they contain specialized structures called organelles, which execute a range of cellular functions.

- It contains pseudopodia, contractile vacuole and food vacuole.

3. Write a short note on life cycle of *Saccharomyces cerevisiae*. (Prescott 638)



(b) *S.cereviseae* life cycle

4. Comment on differences observed between Algae and Cyanobacteria.

**Algae are** – Eukaryote, unicellular, nucleus and mitochondria present, Sexual or asexual reproduction, Not involved in nitrogen fixation, Do not have heterocyst, Contain chloroplast, Eg. Chlorella, spirogyra

**Cyanobacteria are** – Prokaryote, large form- trichomes, Lacks nucleus and mitochondria, Reproduce asexually by binary fission, fragmentation and spore production, involved in nitrogen fixation, Have heterocyst, Lack chloroplast. eg. Nostoc, Anabena

5. Explain the characteristic features and significance of *Aspergillus*. (Prescott – 638 639)

Aspergillus is defined as a group of conidial fungi—that is, fungi in an asexual state. It has an antheridium and ascogonium. The Asci packed in ascocarp. Members of the genus possess the ability to grow where a high osmotic pressure exists (high concentration of sugar, salt, etc.). Aspergillus species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Aspergillus species are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. *A . fumigates*. It has 8 chromosomes and is pathogenic to humans.

6. Write a short note on asexual reproduction in Protozoa. (pelczar 399-402)

Asexual reproduction in Protozoa occurs by simple cell division, which can be equal or unequal.

- Binary fission – if 2 daughter cells are formed. First division of nuclei-followed by division by cytoplasm. Seen in amoeba with special coverings; both soft and rigid. In flagellates the fission is longitudinal and flagella is regenerated from basal body. Transverse fission in ciliates.
- Multiple fission – if many daughter cells are formed, first multiple nuclei are formed within the mother cell and then cell divides. Eg. Plasmodium
- Budding – unequal cell division, Sessile protozoa produce motile offspring, it can be endogenous or exogenous.

**B. Do as directed any two of the following :** **( 2)**

1. One photosynthetic pigment. – Chlorophyll, Carotenoids, Phycobilins
2. Use of *Agaricus*. – it is an edible mushroom
3. Heterocyst is involved in nitrogen fixation- state true or false. - TRUE
4. Species of slime mold. ***Dictosylelia discoideum, Physarum*** **spp**

**Q5. A. Answer briefly any three of the following:** **(18)**

1. Discuss the plate count method for measuring bacterial growth, its advantages and disadvantages.
   **(Pg 126-127 Pelczar 5th edn)**

*(2 mks)Plate count* -- single viable cells separated from one another in space by dispersion on or in an agar medium give rise through growth to separate, macroscopically visible colonies.

- By preparing appropriate dilutions of a bacterial population and using them to seed an appropriate medium, one can determine the number of viable cells in the initial population by counting the number of colonies that develop after incubation of the plates, and multiplying this figure by the dilution factor. This method of enumeration is termed a *viable count:*
- Numbers are expressed as colony forming units

( 2 mks)Adv: Easy to perform, adapted to measurement of population of any magnitude

- Sensitive- very small nos. can be counted.
- The viable count is by far the most sensitive method of estimating bacterial number, since even a single viable cell in a suspension can be detected.

( 2 mks) Disadv: For mixed culture suspension it measures only those cells that are capable of growth on the plating medium used

- Aggregates of cells give rise to fewer colonies than number of cells
- Significant numbers of colonies must be counted (the standard error is approximately equal to the square root of the number of colonies counted) preferably those on two or three plates with several hundred colonies per plate.

(8)

In a growing culture, increase in cell numbers continues during the process of dilution, even if a medium inadequate for supporting continued growth is employed for the dilutions.

2. Give two distinguishing features of the different phases in a bacterial growth curve.
**Pg 120-121 Pelczar 5th edn.**

The Lag Phase

- Cells transferred from a culture in the stationary phase to a fresh medium of the same composition undergo a change of chemical composition before they are capable of initiating growth. This period of adjustment is called the *lag phase*.
- It is extremely variable in duration; its length is directly related to the duration of the preceding stationary phase.
- The individual cells increase in size beyond their normal dimensions.
- Physiologically the cells are very active.
- New enzymes to adjust to the new environment may be synthesized.
- Organisms are metabolizing but there is a lag in cell division.

The log phase

- The cells divide steadily at a constant rate.
- The log of cells plotted against time results in a straight line.
- The population is nearly uniform in terms of chemical composition of cells, metabolic activity and other physiological characteristics.
- Cells are clearly defined than in any other phase
- Cells in this phase are used for studies of microbial metabolism.

Stationary phase:

- The growth of bacterial populations is normally limited either by the exhaustion of available nutrients or by the accumulation of toxic products of metabolism.
- The rate of growth declines and growth eventually stops. At this point a culture is said to be in the *stationary phase*.
- The population remains constant for a time, either due to cessation of division or reproduction rate is balanced by death rate.
- Consequently, cells in the stationary phase have a chemical composition that is different from that of cells in the exponential phase. The cellular composition of cells in the stationary phase depends on the specific growth-limiting factor.
- Cells in the stationary phase are small relative to cells in the exponential phase (since cell division continues after increase in mass has stopped), and they are more resistant to adverse physical (heat, cold, radiation) and chemical agents.

The Death Phase

- Bacterial cells held in a non growing state eventually die.
- Death results from a number of factors; depletion of the cellular reserves of energy and accumulation of inhibitory products
- Bacteria die faster than new cells are produced.
- Like growth, death is an exponential function and hence in a logarithmic plot
- The death phase is a linear decrease in number of *viable* cells with time.
- The death rate of bacteria is highly variable, being dependent on the environment as well as on the particular organism (e.g., enteric bacteria die very slowly, while vegetative cells of certain *Bacillus* spp. die rapidly).

3. Derive the mathematical expression of growth.

**Pg 184 Stanier 5th edn**

- A bacterial culture undergoing balanced growth mimics a first-order autocatalytic chemical reaction; i.e., the rate of increase in bacteria at any particular time is proportional to the number or mass of bacteria present at that time.
- Rate of increase of cells = $k$ (number or mass of cells) ---------------1

  The constant of proportionality, $k$, is an index of the rate of growth and is called the *growth rate constant.* Since we assume growth to be balanced, $k$ also relates the rate of increase of any given cellular component to the amount of that cellular component,
- In mathematical terms, $\frac{dN}{dt} = kN$ $\frac{dX}{dt} = kX$ $\frac{dZ}{dt} = kZ$ ----------------2)

  where N is the number of cells/ml, X is the mass of cells/ml, Z is the amount of any cellular component/ ml, $t$ is time, and $k$ is the growth rate constant.

  These equations describe the growth of most unicellular bacterial cultures.
- Upon integration Eq. 2, yields $\ln Z - \ln Z_o = k(t - t_o)$------------------------3
- On converting natural logarithms to logarithms to the base 10,

  $\log_{10} Z - \log_{10} Z_o = k / 2.303\ (t - t_o)$ ------------------------------------------------4

  where the values of Z and Zo correspond to the amount of any bacterial component of the culture at times $t$ and $t_o$, respectively.

  By measuring Z and Zo, one can compute the value of $k$, the growth rate constant of the culture. The value of $k$ suffices to define the rate of growth of a culture.
- The mean doubling time or generation time *(g)* defined as the time required for all components of the culture to increase by a factor of 2.
- The relationship between $g$ and $k$ can be derived from Eq. 3, since if the time interval considered *(t - to)* is equal to $g$, then Z will be twice Zo Making these substitutions, one obtains

  $K = \frac{\ln 2}{g} = \frac{0.693}{g}$ -----------------------------------------------------------------------5

4. Discuss the effect of oxygen concentration on the growth of bacteria.

**Pg 125-127 Prescott 6th edn.**

- The importance of oxygen to the growth of an organism correlates with its metabolism—in particular, with the processes it uses to conserve the energy supplied by its energy source.
- Almost all energy conserving metabolic processes involve the movement of electrons through an electron transport system. For chemotrophs, an externally supplied terminal electron acceptor is critical to the functioning of the electron transport system.
- The nature of the terminal electron acceptor is related to an organism's oxygen requirement. An organism able to grow in the presence of atmospheric $O_2$ is an aerobe, whereas one that can grow in its absence is an anaerobe.
- Oxygen serves as the terminal electron acceptor for the electron-transport chain in aerobic respiration.
- Facultative anaerobes do not require $O_2$ for growth but grow better in its presence. In the presence of oxygen they use aerobic respiration.
- Aerotolerant anaerobes such as *Enterococcus faecalis* simply ignore $O_2$ and grow equally well whether it is present or not.

- In contrast, strict or obligate anaerobes (e.g., *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus, Neocallimastix*) do not tolerate O2 at all and die in its presence. They use fermentation or anaerobic respiration for energy generation.
- There are aerobes such as *Campylobacter,* called microaerophiles, that are damaged by the normal atmospheric level of $O_2$ (20%) and require $O_2$ levels below the range of 2 to 10% for growth.

5. How do radiations affect bacterial growth? Discuss briefly.

**Pg 127-128 Prescott 6th edn.**

- Many forms of electromagnetic radiation are very harmful to microorganisms. This is particularly true of **ionizing radiation,** radiation of very short wavelength and high energy, which can cause atoms to lose electrons (ionize).
- Two major forms of ionizing radiation are (1) X rays, which are artificially produced, and (2) gamma rays, which are emitted during radioisotope decay.
- Low levels of ionizing radiation will produce mutations and may indirectly result in death, whereas higher levels are directly lethal. Although microorganisms are more resistant to ionizing radiation than larger organisms, they will still be destroyed by a sufficiently large dose.
- Ionizing radiation can be used to sterilize items. Some procaryotes (e.g., *Deinococcus radiodurans*) and bacterial endospores can survive large doses of ionizing radiation.
- A variety of changes in cells are due to ionizing radiation; it breaks hydrogen bonds, oxidizes double bonds, destroys ring structures, and polymerizes some molecules. Oxygen enhances these destructive effects, probably through the generation of hydroxyl radicals (OH_). Destruction of DNA is the most important cause of death.
- Ultraviolet (UV) radiation can kill all kinds of microorganisms due to its short wavelength (approximately from 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. The primary mechanism of UV damage is the formation of thymine dimers in DNA. Two adjacent thymines in a DNA strand are covalently joined to inhibit DNA replication and function.

6. Write a short note on chemostat

Diag -2 mks, description -3 mks

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- In a chemostat the flow rate is set at a particular value and the rate of growth of the culture adjusts to this flow rate. In the chemostat, maximum stability is attained at low dilution rates.
- The culture medium for a chemostat possesses is an essential nutrient (e.g., an amino acid) in limiting quantities. The final cell density depends on the concentration of the limiting nutrient.
- Both the microbial population level and the generation time are related to the dilution rate
- The generation time decreases (i.e., the rate of growth increases) as the dilution rate increases.
- If the dilution rate rises too high, the microorganisms can actually be washed out of the culture vessel before reproducing because the dilution rate is greater than the maximum growth rate.

B. Do as directed any two of the following: (2)

1. Example of a halophile. *Halobacterium, Dunaliela, Ectothiorhodospira*
2. Define thermophile. Thermophiles are microorganisms which can grow at temperatures of $55^0$C or higher
3. Significance of VBNC in food. VBNC microorganisms could pose a public health threat, as many assays that test for food and drinking water safety are culture-based and these will not be detected through these methods.
4. Reactive oxygen species. Singlet oxygen, Hydroxyl radical, Hydrogen peroxide

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