PUNE
SEMESTER END EXAMINATION
B. Sc. (Hons.) Hort.

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Course No. : H/MIBO-121 Title : Introductory Microbiology
Credits : (1+1) 2

Total Marks : 40

MODEL ANSWER PAPER

Q. 1. Enlist major groups of microorganisms and describe characteristics of bacteria and fungi.

Answer:

Major groups of microorganisms:

Bacteria
Fungi
Actinomycetes
Algae
Protozoa
Viruses
Mycoplasmas
Nematodes

Characteristics of bacteria and fungi:

Bacteria

1. Bacteria are prokaryotic, unicellular, achlorophyllous minute microorganisms with rigid cell wall.
2. Their size varies from 0.50 to 1.5 µm in thickness to several µm in length and 1.0-3.0 µm in diameter.
3. Bacterial cells vary in shapes but usually three types of shapes have been recognized: spherical/ ellipsoidal, cylindrical / rod-shaped and spiral /helical.
4. The spherical bacteria are called as Cocci (sing. coccus) rod-shaped/cylindrical as Bacilli (sing. Bacillus) and helical/ spiral as Spirillum (pl. spirilla).
5. Bacteria which exhibit variety of shapes are called as pleomorphic (e.g. *Arthrobacter*).
6. Bacteria can be grown on artificial media in laboratory.
7. Bacteria multiply/reproduce asexually by transverse binaryfission, budding, fragmentation and formation of spores and sexually by conjugation, transduction and transformation.
8. Bacteria are either motile by flagella or non-motile.
9. Flagella (Locomotory organs) are mostly present in rod-shaped and spiral bacteria and are almost absent or rarely present in cocci.

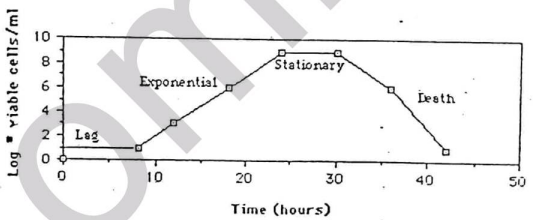
Fungi

1. Fungi are eukaryotic, spore-bearing achlorophyllous microorganisms.
2. They have rigid cell-wall and may be either unicellular (e.g. yeasts) or multicellular and filamentous (moulds).
3. Some are microscopic in nature and others are much larger such as mushrooms, puffballs and bracket fungi
4. Fungi do not ingest food but absorb dissolved nutrients from the environment.
5. Cell wall of fungi is composed of chitin, rather than cellulose.
6. The body of fungus is called as "thallus" which is without root, stem and leaves.
7. A single thread like filament of the fungal body is known as hypha and the hyphal mass which forms the thallus of fungus is called "mycelium.
8. Fungal hyphae may be septate or non-septate/coenocytic
9. They are heterotrophic in nutrition and obtain energy by oxidation of organic compounds.
10. When they feed/grow on dead organic matter, they are known as saprophytes; fungi growing in or on another organism are known as parasites causing diseases in plants. animals and human.
11. Fungi reproduce asexually and sexually.
12. Asexual/somatic/vegetative reproduction does not involve the union ofgametes/nuclei, sex cells or sex organs.
13 .Sexual reproduction involves the union of two nuclei of different parents

Q. 2(a) Explain in detail various phases of bacterial growth with suitable diagram

Answer:

02



1. Lag Phase. Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity. The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

2.Exponential (log) Phase. The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as generation time, also the doubling timeof the bacterial population.

Generation time (G) is defined as the time (t) per generation (n = number of generations). Hence, G=t/n is the equation from which calculations of generation time derive.

3.Stationary Phase. Exponential growth cannot be continued forever in a batch culture (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space". During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce secondary metabolites, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4.Death Phase. If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed.). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Q.2 (b) Discuss the differences between prokaryotic and eukaryotic cells.

Answer:

02

FEATURE	PROCARYOTIC CELLS	EUCARYOTIC CELLS
Groups where found as unit of structure	Bacteria	Algae, fungi, protozoa, plants and animals.
Size range of organism	1-2 by 1-4 μm or less	Greater than 5 μm in width or diameter.
Genetic system location	Nucleoid, chromatin body or nuclear material	Nucleus, mitochondria, chloroplasts.
Structure of nucleus	1.Not bounded by nuclear membrane, one circular chromosome. 2. Chromosome does not contain histones; no mitotic division. 3. Nucleolus absent;functionally related genes may be clustered.	1.Bounded by nuclear membrane; more than one chromosome. 2.Chromosomes havehistones; mitotic nuclear division. 3.Nucleolus present;functionally related genes not clustered.
FEATURE	PROCARYOTIC CELLS	EUCARYOTIC CELLS
Sexuality	Zygote nature ismerozygotic (partial diploid)	Zygote is diploid.
Cytoplasmic nature and structures: 1.Cytoplasmic streaming 2.Pinocytosis	 Absent Absent	 Present Present

Q. 3. (a) Explain in brief general properties of viruses.

Answer:

02

Viruses are ultramicroscopic, nucleoproteinous, self-replicating, filterable, acellular organisms. They areobligate intracellular parasites, smaller than bacteria.Their size varies from 20 to 300 nm (1 nm = 1/1000um) and hence can be seen only through electronmicroscope, scanning microscope or transmissionmicroscope. Despite their simple structure, plantviruses exist in several shapes, such as rod shaped(helical), spherical, polyhedral, bacilliform or pleomorphic. The virus particles arecalled as "virions". Each virion/virus particle is chemically composed of nucleic acidand protein (nucleoproteinous). Nucleic acid in a virus particle is either RNA or DNA. Most of theplant viruses are rod-shaped with RNA type of nucleic acid/genome and only a feware with DNA genome (e.g. cauliflower mosaic virus, dahlia mosaic virus, Maizestreak virus). RNA may be single stranded (ss RNA) or double-stranded (ds RNA) andDNA is mostly double-stranded. The protecting protein coat of virion/virus is knownas Capsid", smaller subunits of protein as "Capsomers" and nucleic acid enveloped with the capsid is called "nucleocapsid".They are the obligate intracellularinhabitants of the living hosts like plants.animals, fungi. bacteria etc. and therefore, they can not be grown/cultured on theartificial /synthetic media in laboratory. Because viruses lack the cellular componentsnecessary for metabolism or independent reproduction, they can multiplyreproduce only within living hosts/cells by the process of replication of genome(RNA/DNA). They do not have any independent metabolic machinery and enzymesystems to generate energy or to synthesize proteins, hence, they depend on hostcells for protein synthesis. They do not have any cytoplasm,cytoplasmicorganellesand plasma membrane.

Q. 3 (b) Discus in brief steps involved in bacteriophage replication
Answer:

02

The steps involved in bacteriophage replication are:

(i) Adsorption:
The first step in infection of a host bacterial cell by a phage isadsorption. The tip of phage tail attaches to the bacterial cell at specific sites on cell surface.

(ii) Penetration:
The enzyme lysozyme present within phage tails weakens thebacterial cell wall. Then the sheath contracts driving the tail core into the cell membrane. Phage injects its DNA into the periplasmic space between cellmembrane and the cell wall and the capsidremains outside the bacterium.

(iii) Replication (Reproduction):
Once the phage DNA enters the host cell,bacterial DNA is disrupted and degraded to small fragments and nucleoid region ofthe bacterium becomes dispersed. Phage DNA is transcribed to mRNA using the hostcell machinery. The mRNA gets translated on host ribosomes and directs thesynthesis of capsid proteins and viral enzymes. Enzyme DNA polymerase replicatesthe phage DNA.

(iv) Maturation:
The head of phage is assembled in the host cell cytoplasm fromnewly synthesized capsid proteins. Then, a viral dsDNA molecule is packed into eachhead and at the same time phage tails are assembled.

(v) Release:
Bacterial cells that contain matured phage particles usually break/burst to release the phage progeny into the environment. This state is termed aslysis and the phage causing lysis of bacterial cell is termed as lytic/ virulent phage.The released phage can now infectmore susceptible bacteria starting the infectionprocess again, such infections by virulent phages represent a lytic cycle of infection.The lytic cycle of virulent phages normally is completed within an hour after infection.

Q. 4. Enlist different methods of quantitative measurement of bacterial growth and explain in brief membrane-filter count and turbidimetric methods.

Answer:

List of different methods of quantitative measurement of bacterial growth:

1. Direct microscopic count
2. Electronic enumeration of cell numbers
3. The plate-count method
4. Membrane-filter count
5. Turbidimetric methods
6. Determination of nitrogen content
7. Determination of the dry weight of cells
8. Measurement of specific chemical change produced on a constituent of the medium
9. The relation of turbidity measurement to direct expressions of growth

Membrane-filter count method:
These filters have a known uniform porosity of predetermined size sufficiently small to trap microorganisms. This technique is valuable in determining the number of bacteria in a large sample that has a very small number of viable cells. The membrane, with its trapped bacteria, is then placed in a special plate containing a pad saturated with the appropriate medium. Special media and dyes can be used to make it easier to detect certain types of organisms than with the conventional plate count. During incubation, the organisms grow into colonies which appear on the membrane surface.

Turbidimetric method:
Bacteria in suspension absorb and scatter the light passing through them, so that a culture of more than 10^7 to 10^8 cells/ml appear turbid to the naked eye. A spectrophotometer or colorimeter can be used for turbidimetric measurements of cell mass. This is simple and rapid method. Culture must be dense enough to register the turbidity. Dead as well as living cells contribute to the turbidity.

Q. 5. Enlist different types of biopesticides along with suitable examples.
Answer:
Types of biopesticides along with suitable examples:

1. **Fungal Insecticides (Mycoinsecticides)**

   Among the insect pathogens, fungi constitute the largest group with more than 500 species.The fungi pathogenic to insects are known as entomogenous or entomopathogenic fungi. Most of the entomopathogenic fungi are from sub-division Deuteromycotina and order Entomophthorales. In India, *Metarrhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii* and *Nomuraea rileyi* are the intensively studied and commercially exploited entomopathogenic fungi. The insect hosts of

these entomopathogenic fungi are: aphids, scales (Homoptera), moths and butterflies (Lepidoptera), beetles (Coleoptera), bees (Hymenoptera), flies (Diptera) and mosquitoes (Isoptera).

2. **Plant disease controlling fungi: *Trichoderma***

Trichoderma is one of the bio-agents used for management of different plant diseases. *Trichoderma* are the common inhabitants of soil and other natural habitats containing organic matter.Rifai (1969) has described nine species of *Trichoderma* of which *T. harzianum* and *T. viride* are most effective and commercially exploited antagonist. *Trichoderma* spp. are common inhabitants of almost every soil and are antagonistic to other fungi. Through the action of antibiosis and mycoparasitism, *Trichoderma* sp. suppress/destroy/lyse the phytopathogenic soil borne as well as foliage fungi. *Trichoderma* spp. has been reported as most potent antagonists and mycoparasites of the pathogenic fungi, such as: *Pythium, Phytophthora, Rhizoctonia, Verticillium, Fusarium, Sclerotium, Sclerotinia, Macrophomina, Armilaria, Botrytis* etc.

3. **Fungal Nematicides**

More than 200 species of fungi (sub-division Deuteromycotina and Mastigomycotina) have been reported as predators and parasites of different genera of nematodes. Predaceous fungi belong to family Moniliaceae (Deuteromycotina) in the genera *Arthrobotrys, Dactylella* and *Monacrosporium*. These nematophagous fungi develop nematode trapping structures like adhesive network, adhesive knob and constricting and non-constricting rings. Fungi belonging to Phycomycetes and Deuteromycetes *Hirsutella, Catenara, Nematophthora* are endoparasites of root knot nematodes (*M. incognita, Heterodera avenae, H. cruciferae, H. Sachachti* etc.). Saprophytic fungi *Fusarium, Gliocladium, Phoma, Verticillium, Paecilomyces* etc. are reported to parasitize females and eggs of nematodes of the genera *Globodera, Heterodera* and *Meloidogyne*.

4. **Bacterial Insecticides/ Biopesticides**

Among a large number of bacterial pathogens used for control of crop pests, two bacterial genera viz., *Bacillus* and *Pseudomonas* could achieve major success as biopesticides. More success as bacterial insecticide has been achieved with the species of the genus *Bacillus*. In fact, four species of *Bacillus* (*B. thuringensis, B. papilliae, B. moritai* and *B. lentimorbus*) account for nearly one-half of all the commercial biopesticides in existence. Out of various biopesticides used in world for suppression of pests on farmers' fields, the share of bacterium, *Bacillus thurningensis* (Bt.) is about 80%. *P. fluorescens*is an effective bacterial antagonist for the management of soil borne (rhizosphere) and foliar diseases (phyllosphere).

5. **Viral Biopesticides**

Insect viruses especially baculoviruses comprising Nuclear Polyhedrosis Virus (NPV) and Granulosis Virus (GV) are the strong regulators of pests (Lepidoptera). Among the baculoviruses, NPVS have been exploited most commercially for the management of pests (e.g. *Helicoverpa*) of several major commercial crops (cotton, arhar, gram, soybean, corn, fruits and vegetables).

Q. 6. Enlist the industrial uses of bacteria and describe in detail vinegar production

Answer:

1. Lactic acid production:
2. Vinegar production
3. Amino acid production

Vinegar Production:

The production of vinegar involves two types of biochemical changes:
(1) Analcoholic fermentation of a carbohydrate and
(2) Oxidation of the alcohol toacetic acid.
There are several kinds of vinegars, and the differences among themare primarily associated with the kind of material used in the alcoholic fermentation. e. g. fruit juices, sugar-containing syrups, and hydrolyzed starchy materials.Yeast fermentation is used for production of the alcohol. The alcoholconcentration is adjusted to between 10 and 13 percent and then exposed to the action of acetic acid bacteria. Many types of equipment have been designed for industrial production of vinegar. The essential features of one of the industrial processes for vinegar production, the Frings method are summarized as follows. A mix is prepared which consists of an adjusted solution of alcoholacidified with acetic acid and special nutrients for the growth of acetic acid bacteria. Acetic acid bacteria, species of the genus *Acetobacter*, are inoculated onto the beechwood shavings. The mixis applied in a trough at the top of the chamber and allowed to trickle downover the shavings. As the alcohol solution passes over the shavings, the acetobacters oxidize some of the alcohol to acetic acid. The mix is collected at thebottom of the unit and may be recirculated over the shaving, resulting in more oxidation of alcohol until vinegar of the desired strength is produced. Since this is an aerobic process, oxygen is required as shown in the following reaction accounting for the formation of acetic acid:

$$2CH_3CH_2OH+2O_2\text{----------}2CH_3COOH+2H_2O$$

Q. 7. How did each scientist disprove spontaneous generation?

Answer:

The belief in the spontaneous formation of living beings from non-living matter is known as Doctrine of spontaneous generation (SG). This controversy existed for a long time. It became difficult to disprove this doctrine, because of lack of experimental proof.

Later Francesco Redi: In 1665 showed that maggots that develop in putrefying meat are the larval stages of flies and will never develop in putrefying meat if it is protected from flies laying eggs. He was the first to disprove SG of animals.

Lazzaro Spallanzani (1729-99) was the first to provide evidence that microorganisms do not develop spontaneously. He boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation.

John Needham (1713-81) insisted that air was essential for SG of microbes. By sealing the flasks, the air had been excluded. This argument was answered after 60 – 70 years independently by two other scientists.

Franz Schulze (1815-73) passed air through strong acid solutions into boiled infusions.

H. Schroder and T. Von Dusch (About 1850) passed air through cotton into flasks containing heated broth. Thus the microbes were filtered out of the cotton fibers and no microbial growth. Basic technique of plugging bacterial culture tubes with cotton stoppers was initiated.

Louis Pasteur (1822-1895) In 1862, he prepared flasks, with long, narrow, goose-neck openings heated the nutrient broth in the flask and thus the air carrying the germs were

allowed to settle in the goose-neck. When the flasks were cooled, the air entering through the gooseneck retained the germs, and under these conditions the broth remained clear.

John Tyndall (1820-1923) proved that dust carried the germs. He showed that sterile infusions placed in a dust free chamber could remain sterile indefinitely even if kept exposed to air. Pasteur and Tyndall's experiments finally disproved the Doctrine of Spontaneous generation (S.G.).

Q. 8. Write short note (ANY TWO)
Answer:

(a) Different types of culture media:

Numerous media are available on the basis of their application or function and are classified as follows:

1. Selective media: These media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not enhance other types of organisms that may be present.
2. Differential media: Certain reagents, when incorporated into culture media, may allow differentiation of various kinds of bacteria.
3. Assay media: such media are used for assay of vitamins, amino acids and antibiotics.
4. Media for enumeration of bacteria: Such media are used for determining the bacterial content of such materials as milk and water
5. Maintenance media: Satisfactory maintenance of the viability and physiological characteristics of a culture over time may require a medium different from that which is optimum for growth.
6. Solid and semisolid media: these media are widely used for cultivation of bacteria.

(b) Contributions of Robert Koch:

1. Robert Koch a German physician, while studying the anthrax disease of cattlediscovered the typical Bacilli with squarish end in the blood of cattle that diedof anthrax. He isolated the bacterium in pure culture and identified theorganism as *Bacillus anthracis*. Hence, he established the bacterial etiology of anthrax of cattle (1876) and thus, for the first time he proved the role ofbacteria in causing animal diseases.
2. He developed the technique of bacterial smearing and staining smears withaniline dyes for better microscopic observations and also developed pure-culture techniques.
3. He discovered the causal agent of tuberculosis i.e. *Mycobacterium tuberculosis* and in 1883 discovered the *Spirillum* bacterium responsible for causing cholera.
4. He used gelatin as solidifying agent in culture media which was then replaced by agar-agar (1881) extracted from sea weed/red algae (*Gelidium* sp.).
5. While dealing with anthrax disease of cattle, Robert Koch had conducted a seriesof experiments and formulated four basicrules/ criteria to establish causalrelationship between a specific causal agent and a specific infectious disease. Theserules/criteria are known as "Koch's Postulates".

(c) Advantages of biological control:

1. Environment friendly and leave behind no toxic residues.
2. Target specific pathogen and avoids unnecessary affect on beneficial microflora and microfauna.
3. Most of them are easily culturable in the lab, with minimum space.
4. Inexpensive to produce large quantities of inoculum.
5. It is mimicry of nature by releasing them into an open environment.

6. Biological control could reduce the use of many pesticides and herbicides hence, which could eliminate the overuse of chemicals by farmers and further reduces cost of cultivation.

Q. 9. Comment on importance and scope of microbiology in agriculture and allied fields..

Answer:

1. Agriculture:

In agriculture microorganisms play vital role in:

(a) Biological Nitrogen Fixation:

- Microorganisms involved in nitrogen fixation are categorized in to: Symbiotic and non-symbiotic
- *Rhizobium*- Symbiotic with leguminous
- *Anabaena azollae*- Symbiotic with non-legume
- *Azotobacter sp., Clostridium, Klebsiella, Beijerinckia, Azospirillum*- non-symbiotic

(b) Decomposition of organic matter:

- Fungi, bacteria, actinomycetes, protozoa etc. play role
- Organically bound plant nutrients in plant and animal residues are converted to simpler nutrients
- Soil fertility is improved
- Fungi: *Aspergillus, Penicillium, Chaetomium, Trichoderma, Humicola, Rhizopus*
- Bacteria: *Bacillus, Pseudomonas, Clostridium, Micrococcus, Arthrobacter*
- Actinomycetes: *Nocardia, Micromonospora, Streptomyces, Thermonospora*

(c) Biogeochemical cycling of elements:

- Play role in conversion of essential elements (N, P, K, S, C, Iron) in the biosphere
- Mineralization: Plant nutrients are made available through mineralization

1. Animal Husbandry and Dairy Technology:

- Complete food for human beings as well as microorganisms
- Good source of nutrients with ideal pH- hence readily gets contaminated
- Beneficial effects of microorganisms are:
- Rumen inhabiting microbes play role in digestion of cellulose
- Synthesize proteins and vitamins and also alter fats in the rumen
- Bacteria and yeasts are employed for production of fermented milk products:

Curd: *Lactobacillus bulgaricus, Streptococcus thermophilus*
Cheese : *S. lactis, S. cremoris*
Acidophilus milk: *L. acidophilus*
Flavoured milk: *Candida albicans*
Bulgarian butter milk: *L. bulgaricus*

3. Industrial microbiology:

Microorganisms are used to produce number of products like alcohol, beverages, drugs, pharmaceuticals, enzymes, vitamins, organic acids, antibiotics etc. e.g.- alcohol

(*Saccharomyces cerevisiae*), Lactic acid (*Lactobacillus delbrukii*), enzymes (*Aspergillus niger*), antibiotics (*Penicullium chrysogenum*) etc.

4. Microorganisms in Mineral, Petroleum and Fuel Industry:

b) Role in recovery of low grade minerals
c) Bioleaching: Microbial process of recovery of minerals or metals from oree.g. *Thiobacillus ferroxidans* for extraction of copper from low grade sulphide ores
d) Formation of petroleum deposits: Xanthom gums produced by *Xanthomonas campestris* are useful in the tertiary recovery of petroleum oils
e) Methane (biogas) : produced from anaerobic digestion of animal and plant wastes by methanogenic bacteria e.g. *Methanobacterium, Methanosarcina, Methanococcus, Methanothrix, Methanospirillum*

5. Sanitation and Health:

- Pathogenic microorganisms: diseases in human beings:

  a. Bacteria: e.g. tuberculosis (*Mycobacerium tuberculosis*), Diphtheria (*Corynebacterium diphtheria*), Pneumonia (*Streptococcus pneumonia*), Leprosy (*Mycobacterium leprae*)
  b. Protozoa: Malaria (*Plasmodium malariae*), Amoebiasis (*Entamobea histolytica*)
  c. Viruses: Small pox (Variola virus), Common cold (Rhinovirus), AIDS (HIV virus)

- Impair the quality of water e. g. *E. coli &Enterobacter aerogenes*
- Digest and oxidize organic matter from sewage, purify sewage water and can be reused for irrigation
- Recycling of both solid and liquid wastes from sewage
- Biodegradation of organic material from sewage: e.g. *Pseudomonas, Alcaligens, Flavobacterium*

6. Microbes as tools for Biological Research:

Useful in studying basic laws of science, they are used for academic studies and research work because:

- Require less space for growth, reproduction and maintenance
- Have very high rates of multiplication
- Metabolic processes, enzyme system and synthetic abilities of microbes are similar to that of higher plants and animals
- Useful for understanding host-pathogen interactions

7. Microbes as Biocontrol Agents:

- Management of plant diseases, insect pests, weeds
- Seed and soil borne diseases: *Trichoderma viride, T. harzianum, Gleocladium, Pseudomonas*

- Insect pests: Fungi: *Beauveria, Entomophthora, Hirsutella, Metarrhizium, Paecilomyces.* Bacteria: *Bacillus thuringiensis, B. papilliae, B. lentimorbus.* NPV-viruses: *Heliothis* bollworms

8. Microorganisms in space technology:

- To explore the space and furnish required information from space
- Used in the space vehicles to maintain O_2 and CO_2 balance
- Source of energy and food in space
- Biological warfare

9. Biodegradation of Agrochemicals and pollutants:

- Act as scavengers and degrade harmful chemicals in soil: physical, chemical and biological forces are exerted and toxic chemicals are degraded to non-toxic
- Bacteria: *Pseudomonas, Clostridium, Bacillus, Thiobacillus, Achromobacter*
- Fungi: *Trichoderma, Aspergillus, Penicillium, Rhizopus, Fusarium*

10. Production of Biofertilizers:

- Carrier based preparation of living/latent cells of efficient strains of nitrogen fixing, phosphate solubilizing and cellulose decomposing
- Seed/soil application for improving plant growth and soil fertility
- Nitrogen fixing: Symbiotic: *Rhizobium.* Non-symbiotic: *Azotobacter, Azospirillum, Azolla, BGA*
- Phosphate solubilizing : *Bacillus, Aspergillus, Penicillium*

Q. 10. Enlist various physical and chemical methods of sterilization and describe in detail sterilization by temperature.

Answer:

List of physical and chemical methods of sterilization:

I. Physical agents:

1) Temperature:

   (a) High temperature:
      i. Moist heat: Steam under pressure, Fractional sterilization, Boiling water, Pasteurization
      ii. Dry heat: Hot-air sterilization, Incineration

   (b) Low temperature

2) Desiccation
3) Osmotic pressure
4) Radiation:
      i. Ultraviolet light
      ii. X-rays
      iii. Gamma rays
      iv. Cathode rays (Electron beam radiation)
5) Surface tension and interfacial tension
6) Filtration:
      i. Bacteriological filters
      ii. Fiberglass filters (HEPA)

II. Chemical agents:

1) Phenol and phenolic compounds
2) Alcohols
3) Halogens: Iodine, Chlorine and Chlorine compounds
4) Heavy metals and their compounds: Triphenylmethane dyes, Acridine dyes
5) Synthetic Detergents
6) Quaternary ammonium compounds
7) Aldehydes: Formaldehyde, Glutaraldehyde

Description of temperature and radiation methods:

2. Temperature:

(a) High temperature: Microorganisms have an optimum, minimum and maximum growth temperature. Temperatures above the maximum generally kill, while those below the minimum usually stasis and may even be considered preservative.

i) Moist heat:

Steam under pressure: Heat in the form of saturated steam under pressure is the most practical agent for sterilization. It has the advantages of rapid heating, penetration and moisture in abundance, which facilitates the coagulation of proteins. The laboratory apparatus designed to use steam under pressure is called an autoclave. Many media, solutions, discarded cultures and contaminated materials are sterilized with this apparatus. Autoclave is operated at 15 lb/in^2 (121^{0}C). The time of operation depends on the nature of material being sterilized, type of container and the volume.

*Fractional sterilization:*Some media, solutions of chemicals and biological materials can not be heated above 100^{0}C without being damaged. It is possible to sterilize them by fractional sterilization (tyndallization). This method involves heating the material at 100^{0}C on three successive days with incubation periods in between. Resistant spores germinate during the incubation periods; on subsequent exposure to heat, the vegetative cells will be destroyed. The apparatus is known as Steam Arnold.

Boiling water: All vegetative cells are destroyed within minutes by exposure to boiling water, but some bacterial spores can withstand. This method is not used in laboratory.

Pasteurization: Milk, cream and certain alcoholic beverages are subjected to a controlled heat treatment, which is called as Pasteurization. This kills microorganisms of certain types but does not destroy all organisms.

ii) Dry-heat:

*Hot air sterilization:*This is recommended where it is either undesirable or unlikely that steam under pressure will make direct contact with the materials to be sterilized. This is true of certain items of laboratory glassware as well as oils, powders and similar substances. For laboratory glassware, a 2-h exposure to a temperature of 160^{0}C is sufficient.

Incineration: Destruction of microbes by burning is practiced when the transfer needle is introduced into the flame of the Bunsen burner. This method is used for the destruction of carcasses, infected laboratory animals and other infected materials to be disposed of.

SECTION "B"

Q. 11. Do as directed

1. Robert Hook developed first compound microscope
2. Culture medium which enhances the growth of particular bacterium is called as selective medium
3. The microscopic field is brightly lighted and microorganisms appear dark is known as bright field microscopy.
4. Gram-positive

Q. 12.Define the following:

Answer:

1.Disinfection: It is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects.

2.Pure culture: A culture containing only one species of organism

3.Tyndallization: A process of fractional sterilization with flowing steam

4.Biological control: The use of natural/ or modified organisms, genes/ gene products to reduce the effects of diseases/disease causing organisms".

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