CHAPTER 3

Microbial Agents of Disease And Microbes in Extreme Environment

LONG ANSWER QUESTIONS

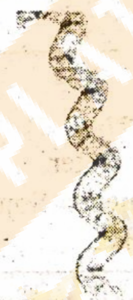
Q.1. Discuss about genetic material(s) in a bacterial cell.

Ans. **Genetic Material in Bacterial Cell**

Bacteria have been on the Earth for millions of years. Is wasn't until the late 1600s that scientists discovered bacteria. In fact, bacteria were discovered by accident. Anton van Leeuwenhoek accidentally noticed them while looking at scrapings from his teeth through a very simple microscope. He did not know what they were, but he was essentially the first person to see bacteria. Bacteria are prokaryotic cells. Bacteria cells come in a variety of shapes and sizes. For our purposes, however, we will focus on the three main shapes : spherical, rodlike and spiral.

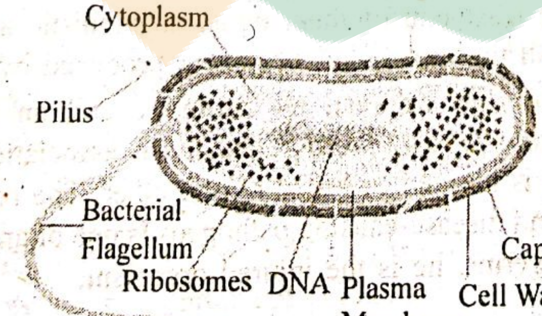
Spherical

Rodlike

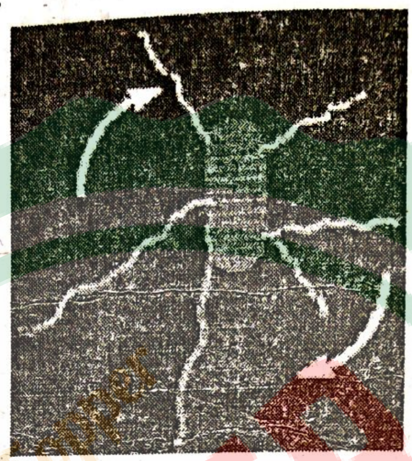


Spiral

All bacteria have the same basic structure. Most bacteria cells have a cell wall. The cell wall is rigid and provides protection for the bacteria cell. Beneath the cell wall is the cell membrane. Since bacteria cells do not have a nucleus, the genetic material simply floats around in the cytoplasm. Remember, the genetic material contains the instructions for all of the bacteria cell's functions.



Some bacteria cells have flagella or tiny whip-like structures. A bacteria may have one flagellum or many flagella. A bacteria will use its glagella to move in its environment. Bacteria that do not have flagella cannot move on their own. They rely on air or water currents, clothing, or other objects to carry them from place to place.



Two Kingdoms of Bacteria

Archaebacteria	Eubacteria
Very old bacteria, i.e. evolve a long time ago	More recently envolved bacteria.
Many live in extreme environments such as hot springs, salty water, and acidic environments. Some live in or on organism.	Live in or on organism.
Autotrophic or heterotrophic.	Autotrophic or heterotrophic.
Can be harmful or helpful	Can be harmful or helpful.

☛ **Q.2. Comment upon Antibiosis in Microbes.**

Ans.

Antibiosis in Microbes

Antibiosis is a biological interaction between two or more organisms that is detrimental to at least one of them or an antagonistic association between an organism and the metabolic substances produced by another.

The general relationship between an antibiotic and an infectious organism is one of antibiosis. The word refers to an association of two organisms in which one is harmed or killed by the other. The relationship between human beings and disease-causing pathogens is one of antibiosis. If a person is affected by germs, he is the injured organism.

"Antibiosis resistance affects the biology of the insect so pest abundance and subsequent damage is reduced comapred to that which would have occurred if the insect was on a susceptible crop variety. Antibiosis resistance often results in increased mortality or reduced longevity and reduction of the insect." ●

Q.3. Discuss in brief different defence mechanisms operative against microbes.

Ans. Different Defence Mechanisms Operative against Microbes

Under standard laboratory conditions, the human beta-defensin 1 (hBD-1), a human antibiotic naturally produced in the body, had always shown only little activity against microbes. Nevertheless the human body produces it in remarkable quantities. The solution to the puzzle was the investigation process itself, as the research group led by Dr. Jan Wehkamp at the Dr. Margarete Fischer-Bosch Institute for Clinical Pharmacology of the Stuttgard-based Robert Bosch Hospital found out.

Before the research group took a new approach to this research, defensins were usually tested in the presence of oxygen, although little oxygen is present, for example, in the human intestine. Starting out from the discovery that a special antibiotic-activating protein of the human body is diminished in patients with inflammatory bowel diseases, Crohn's Disease and Ulcerative Colitis, the working group investigated how defensins act under low-oxygen conditions. During their investigations the scientists found out the under these conditions hBD-1 unfolds a strong antibiotic against lactic acid bacteria and yeast.

Furthermore the researchers discovered that another human protein, thioredoxin, is able to activate betadefensin 1 even in the presence of oxygen. Moritz Marcinowski and Professor Johannes Buchner from the Department of Chemistry at the Technical University of Munich, used circular dichroism spectroscopy to elucidate the differences between the folded inactive and the unfolded active form of the protein.

Surprisingly, while almost all poteins are active only in their folded form, in the ase of the small defensin the opposite is true. To activate the beta-defensin 1 the thioredoxin opens the three disulphide bridges that hold the molecule together. The molecule then opens up into the active astate.

Using this mechanism the body has the opportunity to selectively activate the defensin.

So far the cause of inflammatory bowel disease is unclear. Genetic as well as environmental factors seem to play a role, finally leading to a weakening of the antimicrobial barrier, which is mainly mediated by defensins. Accordingly the identified mechanism might contribute to the development of new therapies to treat affected patients. ●

☛ Q.4. What are chemotherapeutic agents ? Examplify your answer with a note on their mechanism of action.

Ans. **Chemotherapeutic Agents**

Chemotherapeutic agents i.e., pharmacologic agents that are known to be of use in the treatment of cancer, otherwise known as chemotherapeutic agents. This list is organized by "type" of agent, though the subsections are not necessarily definitive and are subject to revision. Each drug is listed once (at present), though it might fall in more than one subsection.

A full alphabetical listing is included after the categorical listing.

Alkylating agents

- ➢ Cyclophosphamide
- ➢ Mechlorethamine
- ➢ Chlorambucil

Anthracyclines

- ➢ Daunorubicin
- ➢ Doxorubicin
- ➢ Epirubicin

Cytoskeletal disruptors (Taxanes)

- ➢ Paclitaxel
- ➢ Docetaxel

Epothilones

Histone Deacetylase Inhibitors

- ➢ Vorinostat
- ➢ Romidepsin

Inhibitors of Topoisomerase I

- ➢ Irinotecan
- ➢ Topotecan

Inhibitors of Topoisomerase II

- ➢ Etoposide
- ➢ Teniposide
- ➢ Tafluposide

Kinase Inhibitors

- Bortezomib
- Erlotinib
- Gefitinib

Monoclonal Antibodies

- Bevacizumab
- Cetuximab
- Ipilimumab

Nucleotide analogs and precursor analogs

- Azacitidine
- Azathioprine
- Capecitabine

Peptitde antibiotics

- Bleomycin
- Actinomycin

Platinum-based agents

- Carboplatin
- Cisplatin
- Oxliplatin

Retinoids

- Tretinion
- Aliretinoin
- . Bexarotene

Vinca alkaloids and derivatives

- Vinblastine
- Vincristine
- Vindesine

Mechanism of action of Chemotherapeutic Agents : Chemotherapy (often abbreviated to **chemo**) is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms. It is often used in conjunction with order cancer treatments, such as radiation therapy or surgery. Certain chemotherapeutic agents also have a role in the treatment of other conditions, including ankylosing spondylitis, multiple sclerosis, Crohn's disease, psoriasis, psoriatic arthritis, systemic lupus erythematosus, rheumatoid arhtritis, and scleroderma.

Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract and hair follicels. This results in the most common side-effects of chemotherapy : myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the liming of the digestive tract), and alopecia (hair loss).

Some newer anticancer drugs (for example, various monoclonal antibodies) are not indiscriminately cytotoxic, but rather target proteins that are abnormally expressed in cancer cells and that are essential for their growth. Such treatments are often referred to as targeted therapy (as distinct from classic chemotherapy) and are often used alongside traditional chemotherapeutic agents in antineoplastic treatment regimens.

Chemotherapy may use one drug at a time **(single-agent chemotherapy)** or several drugs at once **(combination chemotherapy or polychemotherapy)**. Chemotherapy using drugs that convert to cytotoxic activity only upon light exposure is called **photochemotherapy** or photodynamic theray.

An order and broader use of the world chemotherapy encompassed any chemical treatment of disease (for example, treatment of infections with antimicrobial agents). However, this use has become archaic.

Treatment Strategies

There are a number of strategies in the administration of chemotherapeutic drugs used today. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms.

- Combined modality chemotherapy is the use of drugs with other cancer treatments, such as radiation therapy or surgery.
- Induction chemotherapy is the first line treatment of cancer with a chemotherapeutic drug. This type of chemotherapy is used for curative intent.
- Consolidation chemotherapy is the given after remission in order to prolong the overall disease free time and improve overall survival. The drug that is administered is the same as the drug that achieved remission.
- Intensification chemotherapy is identical to consolidation chemotherapy but a different drug than the induction chemotherapy is used.
- Combination chemotherapy involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side effects. The biggest advantage is minimising the chances of resistance developing to any one agent. Also, the drugs can often be used at lower doses, reducing toxicity.

- Neoadjuvant chemotherapy is given prior to a local treatment such as surgery, and is designed to shrink the primary tumor. It is also given to cancrs with a high risk of micrometastatic disease.
- Adjuvant chemotherapy is given after a local treatment (radiotherapy or surgery). It can be used when there is little evidence of cancer present, but there is risk of recurrence. It is also useful in killing any cancerous cells that have spread to other parts of the body. These micrometastases can be treated with adjuvant chemotherapy and can reduce relapse rates caused by these disseminated cells.
- Maintenance chemotherapy is a repeated low-dose treatment to prolong remission.
- Salvage chemotherapy or palliative chemotherapy is given without curative intent, but simply to decrease tumor load and increase life expectancy. For these regimens, a better toxicity profile is generally expected.

All chemotherapy regimens require that the patient be capable of undergoing the treatment. Performance status is often used as a measure to determine whether a patient can receive chemotherapy, or whether dose reduction is required. Because only a fraction of the cells in a tumor die with each treatment (fractional kill), repeated doses must be administered to continue to reduce the size of the tumor. Current chemotherapy regimens apply drug treatment in cycles, with the frequency and duration of treatments limited by toxicity to the patient. ●

Q.5. Classify the microorganisms on the basis of ecological groups of microorganisms.

Or

Describe in some details the major kinds of microbes in extreme environments.

Ans.

Ecological Groups of Microorganisms

Microorganisms are an important component of an ecosystem. Various ecological categories can be made on certain grounds however grouping the microorganisms.

(A) Based on Carbon Sources as Energy

Carbon is a chief source of energy and a building block of cell wall and other cell components. All living organisms are divided into the two major groups, the autotrophs and the heterotrophs.

(i) Autotrophs : In autotrophs the carbon sources is CO_2. Energy is provided through photosynthesis in photoautotrophs or oxidation of inorganic compounds as in chemotrophs. Examples of autotrophs are cyanobacteria, microscopic algae and photosynthetic bacteria.

(ii) Heterotrophs : In heterotrophs the sources of carbon and energy of biosynthetic mechanism of the cell are the dead organic materials. Such materials are broken down through the heterotrophs for their carbon and energy to form their cell components and cell as well. There is a large number of heterotrophs represented through bacteria fungi, actinomycetes etc.

(B) Based on Oxygen Requirement

On the basis of their ability to grow in the presence or absence of O_2 they can be divided into three distinct categories : *aerobes, anaerobes* and *facultative anaerobes.*

(C) Based on Temperature

If temperature becomes a limiting factor *i.e.*, governs the growth and activities of microorganisms the latter becomes adapted to the new environment. On the basis of temperature regime, microorganisms may be categorised as follows :

(i) Psychrophiles : Psychrophiles are those micro-oraganisms which grow at a very low temperature. Further the microorganisms growing at low temperature can be classified into two groups : (a) *facultative psychrophiles* and (b) *psychrotrophs.* Facultative psychrophiles are capable of growing at temperature ranging from 0°C or less to 20°C with an optimum growth at 15°C. Some isolates from Antarctic waters have been observed to indicate maximal growth at 10°C or less. On the otherhand psychotrophs are those microoraganisms which can grow at 5°C of below, irrespective of their upper or optimum growth temperatures. Singh *et al.* (1984) have reviewed the ecological aspects of microbial life at low temperatures.

In the troposphere where temperature remains below - 40℃, bacteria, fungi and other microorganisms have been isolated. Procter and Parken (1942) have recorded the presence of **Bacillus** over polar Pacific and Atlantic oceans where the temperature remains below - 20℃. Moreover, from some glacial caves for example caves in the Arctic, Lapland the pyrenesis, the Alps in Romania where temperature ranges between - 0.8℃ and 5°C certain bacteria such as **Arthrobacter. Pseudomonas** and **Flavobacterium** have been isolated. The optimal temperature required for growth through **Polaromonas vacuolata** is 4°C.

(ii). Mesaphiles : The microorganisms growing optimally at temperatures between 25°C and 35°C and have capacity to grow at about 15°C to 45°C are called mesophiles. Most of microorganisms are mesoplhiles and constitute the major component of an ecosystem. e.g., cyanobacteria for example *Nostoc, Anabaena, Oscillatoria,* etc. bacteria for example *Rhizobium, Mycobacterium, Corynebacteritan Azotobacter* etc, fungi for example *Fusarium, Trichoderma, Puccinia, Pythnam* etc., amoebae etc.

(iii) Thermophiles : Microorganisms growing readily at temperatures of 45 to 65°A are called thermophiles. The, obligate thermophiles are incapable of multiplying below 40°C. Johri and Satyanarayana (1984) reviewed thermophilic fungi of paddy straw for example *Aspergillus fumigatus, Chateomium Thermophila. Humicola lanuginosa, Thermoascus aurantiacus*, coal mine soil for example *A. fumigatus, C. thermophila, Torula thermophile, Paecilomyces sp.*, etc.

(iv) Hyperthermophiles and super-hyperthermophiles : The microorganisms that prefer to grow above 80°C to 100°C are known as *hyper thermophiles*. Thomas D. Brock of the University of Wisconisin about 30 years ago first discovered the earliest specimens of microbial life in hot springs and other waters. In 1960 Brock with his colleagues identified the first hyperthermophile capable of growing at temperature greater than 70°C. The bacterium is now called *Thermus aquaticus*. From this bacterium an enzyme named *Taq* has been isolated which is widely used in polymerase chain reaction (PCR) technology. Some users of PCR have replaced the *Taq* with *Pfu* polymerase. This enzyme is isolated from *Pyrococcus furiosus*.

In addition, this team observed the first hyperthermophile in an extremely hot and acidic spring which is known as *Sulfolobus acidocaldarius*. This bacterium grows prolifically at temperatures as high as 85°C. To date more than 50 hyperthermophiles have been isolated, manyby Karl O. Stetter and his colleagues at the university of Regensburg in Germany. The most heat resistant of these microbes is *Pyrolobus fumarii* that grows in the walls of smokers. It multiplies at temperature upto 113°C. It stops growing at temperatures below 90°C. Example of the other hyperthermophile is *Methanopyrus* that lives in deep-sea chimneys and produces methane.

(D) Microorganisms Living in Extreme Environment *i.e.,* Extremophiles

Extremophiles thrive under conditions which would kill the other creatures. The molecules that enable extremophiles to prosper are becoming commercially useful now a days. Of the particular interest are the enzymes that help the extremophiles to function in brutal circumstances. Example of other groups of high and low pH loving microorganisms are also not uncommon. Most natural environments on the earth are essentially netural having pH values between 5 and 9. *Acidophiles* thrive in the rare habitats having a pH below 5 and *alkaliphiles* favour habitats with a pH above 9.

(E) Based on Habitat

On the basis of habitat in broad sense, microorganisms are classified groups :

(i) Soil Microorganisms : Microorganisms dwelling is soil subsystem are called soil microorganisms or soil microflora and the branch of microbiology dealing with soil microflora is called as *soil microbiology.*

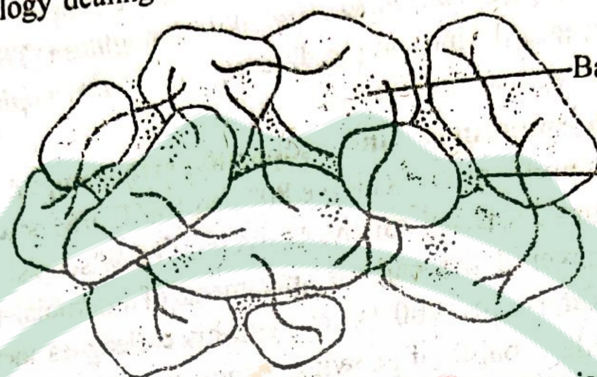


Fig. The Microenvironment. The world of Microorganisms in soil : Bacteria present as isolated microcolonies on surfaces and in pores, that are lovered by thin water films : Filamentous fungi are able to grow on and between these aggregated particles. Protozoa move in water films and graze on bacteria.

(ii) Aquatic Microorganisms : Microorganisms residing in water subsystem irrespective of its quality or physical or chemical nature are in broad sense known as aquatic microorganisms. The branch of microbiology dealing with aquatic micrrorganisms is called *aquatic microbiology.* Most of the human pathogens spread through water.

(iii) Aeromicroflora : Microorganisms living in air are generally known as *aeromicroflora* or microorganisms of air and the branch of microbiology dealing with aeromicroflora is known as *aeromicrobiology* various fungal and viral diseases are transmitted through air.

(F) On the Basis of Mode of Nutrition of Habitat

There are various ways by which the microorganisms derive nutrition for their growth and development.

(i) Saprophytism : Saprophytism is a phenomenon which refers to getting nutrition from dead organic materials and such microorganisms are called saprophytes for example *Aspergillus, Penicillium, Mucor Rhizopus* etc. The saprophytes are equipped with extracellular enzyme-producing capacity according to the available substrate. Some time in the presence of a living host a few saprophytes change their tendency and cause disease. Such saprophytes are known as *facultative parasites.* Facultative parasites are basically saprophytes but have tendency to behave as parasite as well. Such microorganisms are very dangerous. They are also called opportunities microorganisms. For example, some species of *Fusarium, Pythium* etc. are facultative parasites.

(ii) Parasitism : Parasitism refers to deriving nutrition from a living plant or animal host and microorganisms associated with parasitism are known as parasites (living upon). There are certain nutrients that are not found in dead organic materials. For these nutrients the parasites have to infect the plant or animals. On the other hand it can be said that parasitism is a tendency of parasites to infect living hosts. It a parasite is very virulent and cannot live without a living host, it is known as *obligate parasite* e.g., *Puccinia*, powdery mildews etc. Obligate parasitism is the highest level of specialization for deriving nutrition. On the other hand a parasite, in the absence of a suitable living host can pass its life as saprophyte. This is a second mode of leading the life and survival mechanism. Such types of parasites are known as *facultative saprophytes*. It means the parasites that have faculty to live as saprophyte in the absence of a suitable host, for example *Phytophthora infestans*. *Taphrina deformans* some smut fungi etc.

(iii) Symbiosis : In parasitism advantages are only to the microorganisms. The hosts are the losers. Consequently, there develops disease. While in other case both the microbes and hosts are benefited as far as nutrition is concerned. These association of mutual benefit is known as *symbiosis*. Symbiosis can be seen in lichens, mycorrhiza, root nodules of legumes and non-leguminous plants.

Q.6. What do you know about Microbial Interaction?

Ans. **Microbial Interactions**

Microorganisms are ubiquitous in their occurrence. While in natural environment they interact among themselves with plants, with animals and moreover, with their inches. Finally, various types of interrelations are established. Reasons for microbial interactions are the competition for nutrients and space in an ecological niche. Baker and Cook (1974) pointed out that a microbe may not affect the other, or may affect by one or more of the following ways : (a) by inhibition of growth and development of the associate, (b) by stimulation of growth and development of associate, (c) by stimulating the formation of resting bodies by the associate, (d) by directly benefitting the plants, (e) by enforcing the dormancy of the associate, (f) by causing lysis of the associate, (g) by harming the population of plants, (h) by inhibiting the formation of resting bodies by the associate, (i) by getting influenced by its own micro environmental factors. Some of the possible microbial interactions have been discussed in this section.

While, on the basis of relative advantage to each partner. It means hosts and microorganisms, the relationships are basically of three types : (a) *neutralism* (where host remains unaffected by the microbe), (b) *mutalism* and (c) *parasitism*.

(a) Clay-Humus-Microbe Interaction

Clay mineral (and humic substances) affect the activity, ecology and population of microorganisms in soil. Clays modify the physico-chemical environment of the microbes that are either enhance or atten[illegible] the growth of individual microbial population. After release from clays, the organic material is either degraded through microorganisms or again bind to clays. Microorganisms have a negative charge at the pH of most microbial habitats. The magnitude of electronegativity on cell walls of bacteria and fungi is regulated through pH, amino acid residues and changes in wall composition.

Clay minerals get adsorbed and bind with proteins, amino acids small peptides and humic substrates. Microorganisms utilize the nutrients for their growth and activity directly from clayprotein clay-amino acids or peptides and clay-humic substrate complexes. However, high levels of clay (for example montomorillonite) soil interferes and restricts infection of banana rootlets by *Fusarium exysporum* f.sp *cubense*, and hence exerts natural biological control of panama disease. The clays and humic colloids influence the distribution and activity of *Streptomyces, Nocardia* and *Micromonospora*. Clay particles (for example kaolinite) is known to reduce the toxicity of cadmium (Cd) on *Macrophomina phaseolina*.

(b) Plant-Microbe Interactions

The above ground and below ground portions of plants are constantly interact with a large number of microorganisms for example bacteria, nematodes, actionmycetes, fungi, amoebae and algae and viruses and develop different types of interrelationships. While, considering the result of interactions, it may develop neutral destructive, symbiotic or beneficial association with plants.

Interactions on afore said Ground Parts

Microbial interactions on above ground part of plant occur in a varieties of ways where the foliage especially leaf surface works as microbial niche.

1. Destructive Associations

Plants provide a substantial ecological niche for microorganisms. While, the abundance of this potential niche with respect to any individual microbe in more apparent than real, as a few are able to grow on a wide range of plant species. Mciro-ogranisms show specificity with the hosts, pathogens.

Disease development is governed by the resultant of three important factors : (a) virulent pathogen in the presence of resistant host, unfavourable environment or avirulent pathogen disease will not develop. (b) congenial environment and (c) host susceptibility.

Plant-microbe interaction found at molecular level. In this interaction 'gene-for-gene relationship' of H.H. For implies. A gene-for-gene relationship exists when the presences of a gene in one population is contingent on the continued presence of a gene in another population and where the interactions between the two genes lead to a single phenotypic expression through which the presence or absence of the relevant gene in either organism may be recognised.

2. Beneficial Association

The excellent example of plant-microbe interaction causes beneficial association visualised on above ground part is the development of stem nodules. There are three known genera of legumes which are known to bear stem nodules are *Aeschynomene, Sesbania* and *Neptunia.* The stem nodules develop due to interaction between these plants and *Azorhizobium* species. Rhizobia develop symbiotic association with host, fix atmospheric nitrogen and benefit the plants *S. aculeata* is the most popular green manure in north India that contributes about 70 kg of nitrogen and 15-20 tonnes/has wet biomass to the soil. *A. americana* is a wild annual legume which is also used as green manure. *S. rostrata* bears both stem as well as root nodules.

Anabaena azoliae establishes symbiotic association with *Azolla* that is a member of pteridophyta. Species of *Nostoc* establishes symbiotic relationship with *Anthoceros* and *Blasia,* members of Bryophyta.

Interactions on Below Ground Parts

Similar to above ground part plant root-microbe interactions occur in soil as well which lead different types of associations, for example destructive, associative or symbiotic. One of the interesting point is that the microbe has to pass the rhizosphere region before the start of interaction with plant roots.

1. Destructive Associations

Like destructive association of above ground parts, the roots also result in a destructive associations. The symptoms developed through the pathogens on root are damping off, wilt, rot, knot, scab etc. Root diseases occurs by various groups of pathogens.

The pathogens infect roots. Entry of patho genes takes place through wounds results by fungi or nematodes, cracks or root hairs. In most of the cases penetration is preceded through the formation of a specific cushion like structure that exerts mechanical pressure on root surface. Some pathogens directly penetrate the root tissues. In *Rhizoctonia solani* multicellular cushions are found on the roots or hypocotyl of infected plants. Nematodes, directly inflict a slight mechanical injury on plant root. Their saliva is toxic for host tissues that causes in cellular hypertrophy and hyperplasia, suppression of mitosis, cell necrosis and growth stimulation. Second stage larvae of *Meloidogyne* and *Heterodera* normally enter the root at or just behind the root tip. *Meloidogyne* larvae enter through the ruptures trade through emerging roots, cracks on root surfaces, sodular-tissues etc. and causes in development of root knots.

Certain wilt causing species of *Fusarium* for example *udum. Foxysporum* f.sp. *cubense, udum, Fl. oxysporum* fsp. *lycopersici* etc. infect root, enter in vascular supply i.e., xylem bundles and produce mycelia that block the xylem vessels. These works as mechanical plug for xylem vessels. Consequently plants show wilting symptoms. Interestingly, *Macrophomina phaseolina* enters in roots and gets established in root tissues. It produces intraxylem sclerotia. Sclerotia are produced in this high amount that impart sprinkling charcoal like symptoms. Thus, root rot caused by this pathogen is called charcoal-rot. Certain fungi e.g., *Pythium, Rhizoctonia* etc., results damping-off of seedlings of different crop plants. *Synchytrium endobioticum* causes wart of potato tubers.

A member of actinomycetes for example (*Streptomyces scabies*) *causes scab disease of potato. Agrobacterium tumifaciens,* a soil borne bacterium, results crown gall of fruit trees including roots. Affected plants become stunted with restricted growth of plant part having a poor fruit set. *Pseudomonas solanacearum* resulting brown-rot and bacterial wilt of tomato, potato and other solanaceous plant is a popular pathogen. After cutting open the affected tubers and creamy, viscous exudation from open surface is observed and the dark brown discolouration of the vascular region becomes distinct. As a result, tuber formation is affected and size of tubers is greatly reduced.

2. Beneficial Associations (Symbiosis)

It is the phenomenon of living together where both the partners are benefitted. The microsymbionts derive freshly prepared food from the host plant which lack in soil. The macrosymbionts get certain nutrients from soil that are not readily available such as trace elements, nitrogen phosphorus etc., while, as a result of interaction of microorganisms with plant roots there may or may not develop apparent symbiotic structure. Symbiotic associations with various groups of microorganisms are given as follows :

(i) Cyanobacterial Symbiosis : The term cyanobacteria is of recent origin which includes the members of cyanophyceae. They may be both heterocystous and non-heterocystous forms. Heterocyst is the site of nitrogen fixation. The non-heterocystous forms also fix nitrogen. *Anabaena cycadae* is associated with the corralled roots of *Cycas.* It is found in cortex in a well defined region which is known as algal zone.

(ii) Bacterial Symbiasist : Among bacteria there are two categories of symbiosis, one that does not form apparent symbiotic structure it means root nodules and the second group which forms root nodules. While, there is a third group which enhances plant growth without entering in symbiosis.

(a) Associative symbiosis : The first group includes the species of *Azospirillum* which are intimately associated with their host. These have been isolated from the rhizoplane region. Due to infection root nodules are not formed but pictures of root hair deformation are known. Again, *Azospirilum* also invades cortical and vascular tissues of host and enhances the number of lateral root hairs. It causes in an increase in mineral uptake which are probably due to phytochrome production rather than N_2 fixation. Host specificity of *Azospirillum* differs form that of *Rhizobium.* Because of intimate association of *Azospirillum* with roots of several non-leguminous plants, *Azospirillum* and the other such bacteria are known *associative symbiont.* The other non-nodule forming associative symbionts are *Azotobacter paspali Beijerinckia. Azospirillum* etc.

Much work has been done on *Azospirillum.* It is associated with roots in such a way that gentle washing do not dislodge the nitrogen metabolizing activity. It has been found that *A. paspali* contributes 15-93 kg N/ha/annum on sugarcane root. It saves nitrogen fertilizer equivalent to 20-40 kg/ha.

(b) **Legume-Rhizobium symbiosis :** *Rhizobium,* a soil bacterium, enters in symbroiosis with leguminous plants. It develops root nodules which are the site of N_s fixation.

(c) **Plant growth promoting rhizobacteria :** The bacteria which colonize the rhizosphere of root are rhizobacteria. The non-symbiotic beneficial rhizobacteria which affect the plant growth favourably are called PGPR. The PGPR have been discovered by Kloepper. PGPR belong to genera of *Pseudomonas, Bacillus* and *streptomyces* and most of them are fluorescent pseudomonads. The other types are non-fluorescent pseudomonads, for example *Serratia* and *Arthobacter.* The most common species of *bacillus* are *B. polymyxa, B. circulans* and *B. macerens.*

These bacteria increase the growth of host plants. The increase in plant growth is due to (a) release of nutrients form soil, (b) control of pathogens and other harmful microorganisms in the rhizosphere. (c) production of growth hormones like gibberellin and indole acetic acid, (d) changes in balance of rhizosphere microflora producing an indirect effect on the crop, (e) possible nitrogen fixation by rhizobacteria, (f) possible production of vitamins or conversion of materials to a usable form by the host.

(iii) Fungal Symbiosis (Mycorrhiza) : In 1885, it was a German Forest pathologist, A.B. Frank, who for the first time coined the term mycorrhiza to denote plant-fungus association. Mycorrhiza has been defined as an apparent structure developed due to symbiotic association between fungi and plant roots. Mycorrhizal associations are diverse in both structure and physiological function. Garrett grouped the mycorrhizal fungi into the ecological category of root-inhabiting fungi as shown in fig. This indeed be regarded as end terms in the specialization of root-inhabitants i.e., is, of an ecological group that includes many important soil-bome plant pathogens.

Frank divided the mycorrhizae into *ectotrophic* and *endotrophic* ones on the basis of trophic levels. While, on the basis of strictly morphological and anatomical feature mycorrhizae are divided into the three broad groups : *ectomycorrhiza, endomycorrhiza* and *ectendo- mycorrhiza* that correspond to the older and still commonly used terms ectotrophic, endotrophic, and ectendotrophic mycorrhizae. That is literally by *outside, inside* and *outside-inside feeding,* respectively.

Harley and Smith (1983) have recognised the endomycorrhizae into five distinct types : (a) ericoid mycorrhiza, (b) arbutoid mycorrhiza, (c) monotropoid mycorrhiza, (d) vesicular-arbascular (VA) mycorrhiza, (e) orchid mycorrhiza. Marks (1991) has recognised the seven forms of mycorrhiza (Table), the special features of which are briefly described as follows :

Table : Mycorrhizal types, their characters and distribution within the plant kingdom (after Marks, 1991)

S. No.	Mycorrhiza	Host range	Types of relationships
1.	Arbutoid mycorrhiza	Very restricted, Ericales	Sheath, inter-and-coiled intra-cellular hyphae
2.	Ectomycorrhiza	Gymnosperms and angiosperms	Sheath, intercellular hyphae
3.	Ectendomycorrhiza	Gymnosperms and angiosperms	Sheath optinal, inter and intra-cellular hyphae
4.	Ericoid mycorrhiza	Very restricted cricales	No sheath, no inter-cellular hyphae, long coiled
5.	Monotropoid mycorrhiza	Very restricted, Monotropaceae	Sheath, inter-and coiled intra-cellular hyphae
6.	Orchid mycorrhiza	Very restricted	Only coiled, intracellular hyphae
7.	Vesicular-arbuscular mycorrhiza	All groups of plant kingdom	Coiled intracellular hyphae, vesicle and arbuscules present

(iv) Actinomycete-Non-legume Symbiosis : From this class the species of *Frankia* are known to develop nodules which are called *actinorhiza.* Nitrogen fixing nodulated non-legumes are the species of *Alnus, Casuarina. Cercocarpus Hippophae, Discaria, Comptonia, Dryas Elaeagnus, Myrica, Purshia, Shepherdia.* etc. These plants grow in such a condition where the concentration of nitrogen is low. One of the most extensively studied plant is the alder trees, *Alnus nepalensis.* That grows in nitrogen-deficient soil. The extent of nitrogen gain by such angiosperms varies with soil types, climatic conditions and plant age. The nitrogen gain with *Alnus* is 12-200 kg/ha/annum, and with *Hippophae* 27-179 kg/ha/annum.

(a) Ectomycorrhiza : Which 5% vascular plants develop ectomycorrhiza that predominates in family Pinaceae, Fagaceae, Betulaceae, Juglandaceae and Myrtaceae and in other trophical and temperate families. Fungi which participate in ectotrophic assciation include agaric Basidiomycetes Gasteromycetes, Ascomycetes, fungi imperfeccti and occasionally phycomycetes.

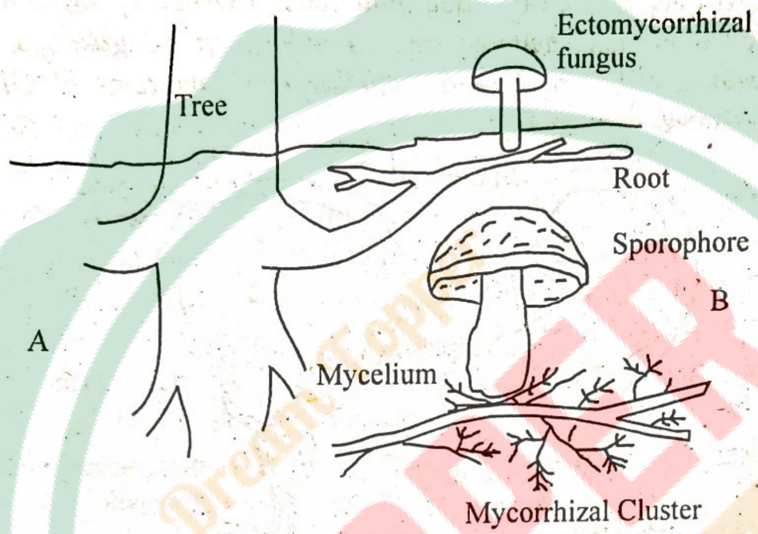


Fig. Ectomycorrhizal symbiosis. A, a tree root showing symbiosis of an ectomycorrhizal fungus; B. an enlarged view of a sporophore (of fungal symbiont) growing on mycorrhizal root of host tree.

Strict host specificity is rare and therefore, one plant may form mycorrhizae with several fungi simultaneously. Thus, over 5,000 fungi of Basidiomycetes-Ascomycetes involved in forming ectomycorrhizae on 2000 woody plants. The fungi interact with feeder roots which in turn, undergo morphogenesis. The mycorrhizae may be unforked, bifurcated, nodular, multiforked or coralloid. Outside the root surface fungal mycelia form a compact and multilayered covering called *mantle* (Photoplate 25.1 A,B). It prevents the direct contact of root tissues with rhizosphere.

Thickness of mantle varies from 20-40 mm depending on mycorrhizal fungi, temperature, nutritional factors etc. The fungus forms a network of mycelia in cortex which is called *hartig net.* The mycelia never enter the endodermis.

The fungi forming ectomycorrhiza are *Amanita muscaria, Boletus edulis, Russula spp., Pisolithus tinctorius, Cenococcus geophilus, Inocybe rimosa. Laccaria laccata. Leccinum, Lepiota, Suillus spp., Rhizopogon* spp., *Scleroderma citrinum.*

(b) Vesicular-arbascular Mycorrhiza (VAM) : Over 90% of vascular plants of world floara form VA mycorrhiza. The mycosymbionts are widespread among both cultivated and wild plants, and found in bryophytes, pteridophytes, gymnosperms and angiosperms. The fungi forming VAM belong to family Endogonaceae of Zygomycotina. Hyphae are aseptate, inter-and intra-cellular in cortex. The intracellular hyphae either become coiled or differentiated into densely branched arbuscules. Arbuscules function like haustoria and perhaps involved in interchange of materials between plant and fungus. In addition, large, multinucleate, terminal or intercalary oil-rich vesicles may be produced on both inter-and intra-cellular hyphae. VAM are formed by about hundreds of fungal species. All of them belong to only six genera *viz., Acaulospora, Gigaspora, Glomus, Entrophospora, Sclerocystis* and *Scutellospora.* Diagrams of *Glomus* and *Gigaspora* are shown in Fig.

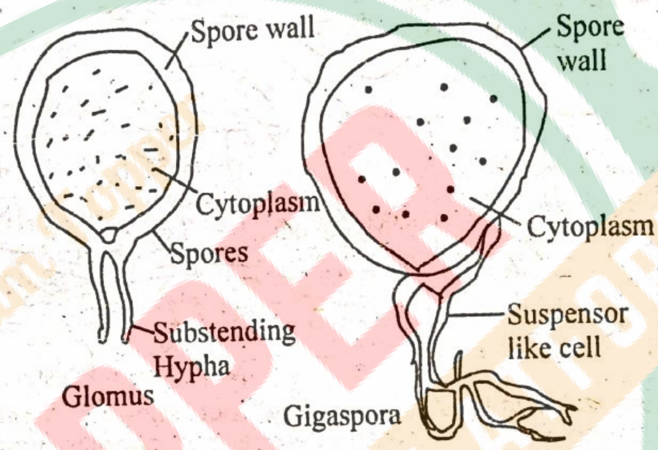


Fig. Spores of vesicular-arbuscular mycorrhizal (VAM) fungi. Glomus has globose spore with constricted substending hypha without septum. Gigaspora has a bulbous substending hypha.

(c) Ectendomycorrhiza : Ectendomycorrhiza shares the features of both ecto-and endomycorrhiza. They have less developed external mantle. The hyphae within the host penetrate its cells as well as grow within them. These are found in both gymnosperms and angiosperms. Very little is known about the fungi involved in this types of association due to little researches on them.

(d) Ericoid mycorrhiza : Ericoid mycorrhiza occurs throughout the fine root systems (hair roots) in the tribe Ericoidae of family Ericacease. Many genera such as *Epachris, Leucopogon, Monotoa, Rhododendron, Vaccinum* etc., develop ericoid mycorrhiza. Plants are woody shrubs or small trees observed in open or acid peaty soil. They have usually fine roots on which the fungus established to outermost layer of cortical cells forming dense intracellular-cells. The fungi may all be ascomycetes. e.g., *Pezizella, Clavaria* spp. etc.

(e) Arbutoid mycorrhiza : Mycorrhiza of the tribe Arbutoidae of family Ericaceae was first described from *Arbutus unedo.* The host plants are mostly woody shrubs and trees. Roots are typically herorhizic the fungus penetrates cortical cells where it forms extensive coils of hyphae. The mycosymbionts are Basidiomycetes. Many fungal symbionts that form symbiosis in these plant, also form mycorrhiza with conifers. It has been suggested that a transition between ecto-and endo-mycorrhizae exists in the arbutoid type of mycorrhiza, accounting for the term ectendomycorrhiza sometimes applied to this phenomenon.

(f) Orchid mycorrhiza : In nature, orchids germinate mere with infected endomycorrhizal fungi that subsequently colonize the host plants. The fungi are mostly the form genus *Rhizoctonia* with perfect state *Ceratobasidium. Sebacina* and *Tulasnella* occurring in Basidiomycetes and Ascomycetes.

(g) Monotropoid mycorrhiza : The family Monotropaceae which includes achlorophyllous plants for example *Monotropa hypopitys*, develops monotropoid mycorrhiza. These plants completely depend on mycorrhizal fungi for carbon and energy. Roots form ball throughout which fungal mycelium ramifies enclosing the mycorrhizal roots of neighbouring green plants. The root ball is the survival organ of *Monotropa* during winter and after return of favourable conditions it gives rise to flowering shoots. With the root growth, a sheath and Hartig net are formed. From the hyphae a peg like haustroia push into epidermal and cortical cells. In the beginning lost cell wall invaginates to include fungal pegs, but finally pegs penetrate cell wall and emerge into cells. The structure and function of monotropoid mycorrhiza change with seasonal development of the host plants. ●

Q.7. What do you know about microbe-mirobe interactions? Write in detail their antagonism.

Ans.

Microbe-mirobe Interactions

Microorganisms interact themselves and lead to beneficial and harmful relationships. Some of the interactions and interrelationships have been discussed as follows :

1. Symbiosis between Alga and Fungus (Lichens)

Lichen is a thallus of dual organism. It means a fungus and an alga that form a self supporting combination. The fungal component is known as *mycobiont* and the algal partner as *phycobiont*. The two groups of organisms live in close proximity and look like a single plant. The fungus forms the thallus of the lichen, whereas the alga occupies mere 5-10% mass of the thallus.

Mycelium of the fungal partner forms a close network that appears as tissue. Inside this compact mass of mycelium algal cells are embedded. Generally, fungi derive nutrition saprophytically from dead organic materials or parasitically from a living host. In lichen fungal mycelium derives nutrition from the alga. The algal cells form food by themselves and/or Fix N_2 from the atmosphere which then are diluted into fungal hyphae. This type of mode of

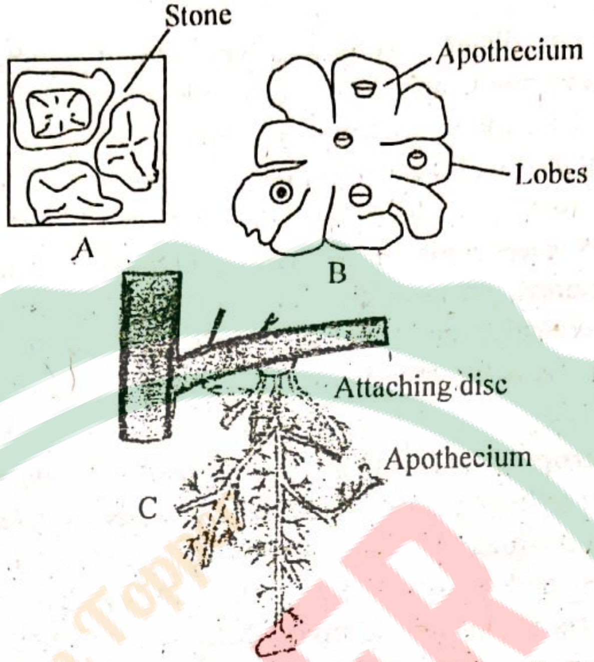


Fig. A. Lichens. A, crustose Lichen; B. Foliose lichen; C. Fruticose lichen.

nutrition is called *biotrophic nutrition* which is found in lichen. The members of algae forming lichen belong to Cyanophyla or Calorophyta. However, it may be unicellular or filamentous forms. The genera of blue-green algae are *Glaeocapsa, Rivularia* and *Stigonema*. Of the green algae, species of *Trebouncia* are the most common unicellular green algae. The fungal partners forming lichen are mostly the members of Ascomycetes and 2-3 genera of Basidiomycetes. No fungus of Phycomycetes enters into lichen formation. Symbiosis is based on the facts that alga provides food to fungus and fungus give shelter to alga.

(i) Classification : On the basis of nature of fungal partner and fructification types lichen are divided into two groups : *ascolicheus* (in which fungal component is an Ascomycetes and *basidiolichen*. In which the fungal component is Bosidiomyutes). While on the basis of the habitat lichens are divided into three groups : *saxicolous, corticolous* (growing on leaves and bark of trees epiphytically) and *terricolous* (growing on soil).

(ii) Lichen Thallus : As in lower plant, in lichens also the plant body is known as thallus. Lichen thalli are grey trees or greyish green in colour. On the basis of structure of thalli, lichens are of three main types *(a) crustose lichens, (b) foliose lichens* (thalli are flat, much lobed and leaf-like appearing as twisted leaves have distinct lower and upper surface, attached to substrate with rhizoid-like structure known as *rhizinae e.g., Chaudhuria, Cetraria, Parmelia, Peltigera, Physcia* and *Xanthoria),* and *(c) fruticose lichens* (thalli are most conspicuous, most complex and slender and freely branched, the branches are cylindrical, flattened and form thread like tuft, thalli not differentiated into upper and lower surfaces *e.g., Cladonia, Ramalina* and *Usnea.*

2. Antagonistic Interactions (Antagonism)

The composition of the microflora/microfauna of any habitat is governed through the biological balance created through interactions and associations of all individuals present in a community. While, the environmental conditions upset the equilibrium. Any inhibitory effect of an organism created by any means to the other organism(s) is called antagonistic interaction and the phenomenon of this activity is known as *antagonism.* Antagonism is the balancing wheel of the nature.

Through this mechanism some sorts of biological equilibrium is maintained. Antagonism has three facets, *competition, amensalism* and *parasitism* and *predation.*

(i) Amensalism : Amenalism is the phenomenon where one microbial species is adversely affected by the other species, whereas the other species is unaffected through the first one. Generally, amensalism is accomplished by secretion of inhibitory substances such as antibiotics etc. Antibiosis is a situation where the metabolites secreted by organism A inhibits the organism B, but the organism A is unaffected. Metabolites penetrate the cell wall and inhibit its activity by chemical toxicity. Generally, antimicrobial metabolites produced by microorganisms are antibiotics, siderophores, enzymes etc. the potent antagonists for example *Trichoderma harzianum* and *t. viride* are known to secrete cell wall lying enzymes, β-1, 3-glucanase, chitinase etc. Lysis of fungal mycelium occurs because of secretion of enzymes.

Siderophores : Siderophores are the other extracellular secondary metabolites which are secreted by bacteria for example *Aerobacter aerogenes, Arthrobacter pascens, Pseudomonas cepacia, P.fluorescens),* actinomycetes (*Streptomyces* spp.), Fungi *(Penicilium* spp), yeast *(Rhodotorula* spp.) and dinoflagellates *(Prorocentrum minimum)* east *(Rhodotorula* spp.). Siderophores are commonly known as microbial iron-chelating compounds as these have a very high chelating affinity for Fe^{3+} ions and very low affinity with Fe^{2+} ions. Siderophores are low molecular weight compounds. These after chelating iron (III) transport it into bacterial cells. Kloepper were the first to demonstrate the importance of siderophore production through PGPR in enhancement of plant growth. Siderophores chelate Fe^{3+} and make Fe^{3+} deficient condition for other microorganisms. Consequently growth of microbe is inhibited. If the siderophore producing PGPR is present on root surface, it supplies iron to plant. Therefore, plant growth is stimulated. Role of siderophores in biological control of plant pathogens is of much importance in recent years.

(ii) Competition : Among the microorganisms, competition exists for nutrients, including oxygen and space but not for water potential temperature or pH. Success in competition for substrate through any particular species is determined by competitive saprophytic ability and inoculum potential of that species. Garrett has suggested four characteristics which are likely to contribute to the competitive saprophytic ability : (a) secretion of fungistatic and bacteriostatic growth products including antibiotics, (b) appropriate enzyme equipment for degradation of carbon constituents of plant tissues, (c) rapid germination of fungal propagules and fast growth of young haphae towards a source of soluble nutrients, (d) tolerance of fungistatic substances produced through competitive microorganisms.

Hence competition exists for limiting resources. The inadequate quantity of readily available carbon compounds is more likely basis for competition. At low level of carbon, the fast growers will often hold slow growers in check when both are added to sterilized soil. But there is no such check on the less active heterotroph when carbon supply is adequate. Under these conditons, competitiveness is directly correlated with growth rate.

(iii) Parasitism and Predation : Parasitism is a phenomenon where one organism consumes another organism, often in a subtle and non-debilitating relationship. Predation is an apparent mode of antagonism where a living organism is mechanically attacked through the other with the consequences of death of the former. It is often violent and destructive relationship. These phenomena are dealt with the example of fungi, amoebae and nematodes as shown in Table.

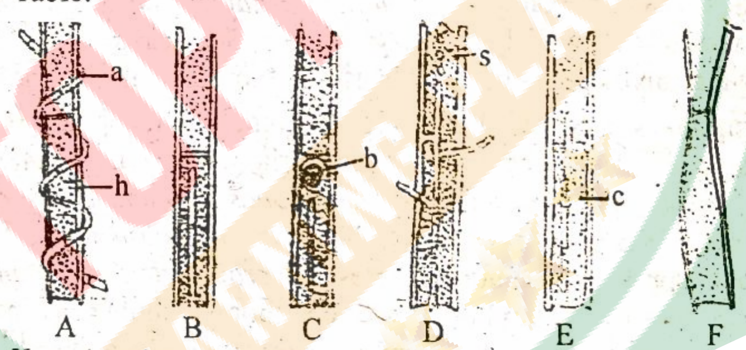


Fig. Mycoparasitism showing post-interaction events. A, coiling; B, penetration; C. barrier formation (a) by host; D, branching and sporulation (s) by antangonist; E, chlamydospore (c) formation; F, lysis of host hyphae (diagrammatic, after Dubey and Dwivedi, 1986).

(a) Mycoparasitism Fungus-fungus interaction : If one fungus is parasitized by the other fungus, this phenomenon is known as mycoparasitism. The parasitizing fungus is known as hyper parasite and the parasitized fungus as hypoparasite. Mycoparasitism commonly occurs in nature. As a result of inter-fungus interaction, several events take place which lead to predation viz., coiling, penetration, branching, sporulation, resting body formation, barrier formation to check the entry of pathogen and lysis of host cell(s).

In coiling event (A) the hyperparasite *i.e.,* antagonist (a) recognises its host hypha *i.e.,* hypoparasite (b) among the microbial community, comes in its contact and coils around the host hypha. Host recognition through the antagonist has been discussed on molecular basis. Manocha has given the basis of host-recognition by mycoparasites. Cell walls surface of host and non-host microbes contains D-glucose and N-acetyl-D-galactosamine residues as lectins present on the cell wall, an antagonist recognises the suitable sites and binds the host hypha. As a result of coiling the host hypha loses its strength. Antagonist dissolves cell wall of host and enters inside the lumen of the later.

Table : Examples of Predation and Parasitism (Dubey, 2005)

S. No.	Mode of antagonism	Plant Pathogens	Antagonists	Post-infection events
1.	Mycophagy	*Cochliobolus sativus*	soil amoebae	Perforation in conidia
		Gaeumannomyces graminis var. tritici	soil amoebae	Penetration and hyphal lysis
2.	Mycoparasitisn	*Botrytis olli*	*Gliocladium roseum*	Penetration of hyphae
		Cochliobolus salivas	*Myrothecium verrucaria*	Antibiosis and penetration
		Rhizoctonia solani and Fomes annosus	*Trichoderma viride*	Coiling, cytoplasm coagulation
		Sclerotium rolfsii	*T. harziamum*	Coiling, penetration lysis
3.	Nematophagy	*Heterodera rostochiensis*	*Phialospora heteroderae*	Cyst penetration and egg killing

Some times host develops a resistant barrier stop the penetration and proliferation inside the lumen. Host's cytoplasm accumulates to form an irregular spherical. or elongated structure, so that the hypha of antagonist could not pass towards the adjacent cells of the hypha (C). Depending upon nutrition, the antagonist forms branches and sporulates (s) inside the host hypha (D). Until the host's nutrients deplete, the antagonist produces resting bodies e.g., chlamydospores (c) inside the host hypha (E) due to loss of nutrients and vigour for survival.

(b) Nematophagy : The phenomenon of eating upon nematodes by fungi is called nematophagy and the fungi as *predaceous fungi*. Fungi are mechanically involved in attaching and killing the nematodes resulting in consumption of nematodes. The predaceous fungi are widely distributed in the surface litter and decaying organic matter. Over 50 species of fungi are known that attack nematodes, various developmental stges of nematodes are susceptible to attack by different types of fungi.

As early as 1869, for the first time M.S. Woronin established the fact that the predanceous fungi capture and destruct the nematodes with certain specialised trapping organs. In 1930s. C. Drechsler added greatly to the list of predaceous fungi and unravelled the mechanism of trapping. Duddington reviewed the work of fungi that attack microscopic animals and contributed significantly to the knowledge of nematophagous fungi.

(c) Mycophagy : Mycophagy is the phenomenon of feeding upon fungi through amoebae. Many amoebae are known to feed on pathogenic fungi. The antagonistic soil amoebae are *Arachnula, Archelle, Gephyramoeba, Geococcus, Saccamoeba, Vampyrella* etc. These amoebae interact with fungal hyphae and make perforations. The fungi on which perforations have been found are *Cochliobolus sativus, Gaeumannomyces graminis* var, *tritici, Fusarium oxysporum, Phytophthora cinnamomi*. On the lysed hyphase of these fungi amoebae develop round cysts.

Chakraborty have described the following three major steps of feeding on fungal propagules through soil amoebae.

Attachment : As a matter of chance trophozoites of amoebae attach to fungal propagules *i.e.,* conidia, hyphae etc. The attachment occurs by chemotaxis or thigmotaxis.

Engulfment : The fungal propagules according to its size are fully engulfed through amoebae. But the small trophozoites attached to the hyphal wall or spore make perforations on it.

Digestion : The completely or partially engulfed propagules/ cytoplasm of the host fungi are digested in a large central vacuole formed inside the cysts.

☛ **Q.8. Write a detailed account on the application of halophiles microorganism.**

Ans.

Halophiles

Halophiles are the Gram-negative, non-spore forming, non-motile bacteria that reproduce through binary fission. They appear red pigmented due to the presence of carotenoids but sometimes they are colourless. They consists of the largest plasmid so far known among all the known bacteria.

Halophiles are able to live in salty conditions through a fascinating adaptation. Since water tends to flow from the areas of high to low solute concentrations. A cell suspended in a very salty solution will lose water and become dehydrated unless its cytoplasm contains a higher concertration of salt than its environment. Halophiles contend with this problems by producing large amounts of an internal solute or by containing a solute extracted solute or by containing a solute extracted from outside. *e.g. Halobacterium salinarum* concentrates KCl in the interior of the cell. The enzymes in its cytoplasm will function only when a high concentration of HCl is found. But their cellular proteins contacting the environment require a high concentration of NaCl.

This group of bacteria lives in highly saline environment such as neutral salt lakes or artificial saline source like salted food, fish etc. Extreme halophilic organisms require at least 1.5 M NaCl but most of them have optimum growth at 2-4 M NaCl. Some examples of prokaryotic extremely halophilic bacteria occurring in nature are given in Table.

Table : Prokaryotic genera of extremely halophilic species.

Halabacteria	Methanogens	Bacteria
Halobacterium salinarium	*Methanobacterium sp.*	*Acetohalobium sp.*
Halobacterium halobium		
Haloferax mediterranei		*Actinopolyspora sp*
Haloarcula sp.		*Ectothiorhodospira sp.*
Halococcus acetoinfaciens		
Halococcus agglomeratus		
Natronobacterium gregoryi		
Natronococcus sp.		

(i) Molecular Adaptation : In such bacteria K^+ ions inside the cell is more than Na^+ ion outside the cell which act as its solute. Thus the cell maintain cellular inegrity. Halobacteria lack peptidoglycans in their cell walls and contain ether-linked lipids and archaean type RNA polymerases which maintain the rigidity at salty conditions. These changes in cytoplasmic membrane permit such bacteria to survive.

(ii) Physiology : Halophilic bacteria lack peptidoglycan in cell walls and have ether-linked lipids and archaean type RNA polymerases but *Natrobacterium* is extremely alkalophilic as well, Former also contains diether lipids not present in other extreme halophiles. They are chemoorganotrophic bacteria that need amino acids, organic acids and vitamins for optimum growth. Some times they oxidize carbohydrates as energy source. Cytochromes *a*, *b* and *c* are present but membrane mediated chemiosmosis generates proton motive force. They also need sodium for

Na^+ *Halobacterium* exceptionally thrives in osmotically stressful environment and does not produce compatible solutes. Peptidoglycon is absent in their cell wall. Aspartate and glutamate are present. The negative charges of carboxyl groups of these amino acids are shielded through Na^+ ions. The ribosomes of *Halobacterium* requires high K^+ ions for stability, which is a unique feature as no other group of prokaryotes need it for internal components.

The membrane lipids of these archaea are composed of diphytanylglycerol, diether analogues of glycerophospholipids. The extreme halophiles contain high intracellular concentration of Na^+ and K^+ and their proteins seem to have adapted to this high salt concentration through having a higher fraction of acidic amino acid residues and more compact packing of a polypeptide chain than protein from non halophilic bacteria. In the halophilic bacteria generally a Na^+ / H^+ antiporter is used to pump Na^+ outwards and solute uptake has been shown to be Na^+ coupled in several halobacterial species.

(iii) Applications : Certain extreme halophiles synthesize a protein known as bacteriorhodopsin into their membrane. Some produce polyhydroxy alkanoates and polysaccharides, enzymes and compatible solutes. They are also used in oil recovery cancer detection, drug screening and biodegradation of residue and toxic compounds. Kushner defined the halobacteria based on utilization of optimum salt concentration for their growth. In this system, non-halophiles are those that grow best in media containing <0.2 M NaCl, slight halophiles grow best at 0.2 to 0.5 M NaCl, moderate halophiles at 0.5 to 2.5 M NaCl and extreme halophiles grow in media containing 2.5 M to 5.2 M NaCl.

It is interesting to note that all extreme halophiles are archaea except for two species of the photosynthetic *Ectothiorhodospira,* one of the *Acetohalobium* and one actinomycete *Actinopolyspora.* Some actinomycete species of the genus *Methanohalobium* has been described. The bright red colour water of the salterns is now known to be because of the bacterioruberin pigments of the halobacteria. The biotechnological potential of halobacteria with commercial interest following.

(a) Polysaccharides : Microbial exopolysacchrides are used as stabilizers, thickness, gelling agents and emulsifiers in the pharmaceutical industries, paint and oil recovery, paper, textile and food industry *Halof mediterranei* produces a highly sulphated and acidic heteropolysaccharides that contain mannose as a major component. Such a polymer combines excellent rheological properties with a remarkable resistance to extreme of salinity, temperature and pH.

(b) Bioplastic or polyhydroxy alkanoates : This kind of heteropolymer is biodegradable. It shows total resistance to water and degraded in human tissues; hence it is biocompatible. It has pharmaceutical and clinical importance, including the use in delayed drug release, bone replacement and surgical structures. Production of PHA is always higher through using *Halof. mediterranei.* In addition, these halobacteria possess high genomic stability which is a pre-requisite for industrial purposes.

(c) Bacteriorhodopsin : The retinal proteins of halobacteria have been observed as integral proteins of the purple membrane, containing one of the proteins known as bacteriorhodopsin. This protein is light-driven, proton translocator and converts sunlight to electricity. The bacteriorhodopsin absorbs light at 570 nm. It found in two forms. The trans configuration after excitation converted to the cis form following the absorption of light as shown in Fig. In this case, ATP synthesis is prevented and the electrical potential arising from the porton gradient will be the source of electricity. It is used in optical data processing and as light sensors. A photographic film based on purple membrane displays the interesting properties as it does not require developing. Holographic films of this type are suitable for computer memory i.e. parallel processing Recently, *biochips* have been introduced in new generation of computers. In future, robots with vision may have biosensors based on this protein. Desalination of water is also demonstrated through the application of bacteriohodopsin.

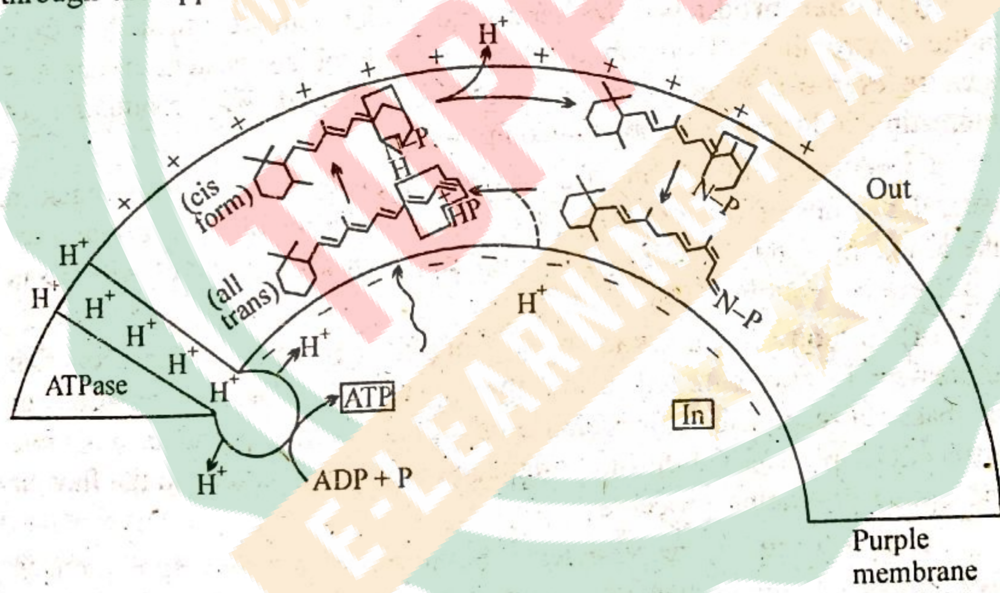


Fig. bacteriorhodopsin proton pump working under the influence of light.

(d) Microbially enhanced oil recovery : Residual oil in natural oil fields can be extracted by injection of pressurized water down in a new well. The bacterial biopolymers are of interest in enhanced oil recovery because of their bio-surface activity and properties of bio-emulsifiers.

(e) Liposomes : Ether-linked lipid of the halobacteria is used in liposome preparation having great value in the cosmetic industry. Such liposomes would be more resistant to biodegradation, good shelf-life and resistance to other baceria.

(f) Drug screening : Plasmid, pGRB-1 of *Halobacterium* strain GRB-1 used in the prescreening of new antibiotics and anti-tumor drugs affect eukaryotic type II DNA topoisomerase and quinotone drugs which act on DNA gyrase. Such drug results DNA cleavage of small plasmid from halophilic archaea *in vivo.*

(g) Cancer detection : A protein has been used from *halobacterium halobium* as an antigen to detect antibodies against the human *e-myc* oncogene product in the sera of cancer patient suffering from pyrolytic leukaemia cell line. The use of halobacterial antigens as probe for some types of cancer seems to be promising.

(h) Bioremediation : Bertrand *et al.* (1990) observed that the halobacterial strain EH4 isolated from a salt-mark was found to degrade alkanes and other aromatic compounds in the presence of salt.

(i) Enzymes : Proteases and amylases from *Halobacterium salimarium, H halobium* and lipases from several halobacteria have been reported. A site-specific endonuclease activity has been reported in *H. halobium.*

(j) Other products : Moderate halophiles remove phosphate from saline environment. Isolation of stable antimicrobial-resistent mutants is because of the presence of cloning of the genes for over-production of interesting industrially important compounds.

Large scale cultivation of *Spirulina platensis* in Israel uses brackish water that is unsuitable for agriculture and the *Spirulina* biomass is marketed like a healthy food. *Spirulina* grows optimally in alkaline lakes with a salt concentration ranging from 2 to 7%.

(k) In food : A sauce known as 'nam pla' is prepared in Thai from fish fermented in concentrated brine that contains a large population of halobacteria responsible for aroma production. As they produce salt-stable extracellular proteases. It has importance in the fermentation and the flavour and aroma producing processes.

(l) Gas vacuoles or vesicles : Some *Halobacterium* spp. produce intracellular gas filed organelles called vacuoles of gas vesicles which give buoyancy. In the future, the genes of such properties are possible to engineer in other microorganisms to produce gas vacuoles to float in water. ●

Q.9. Write a brief account on the psychrophilies.

Ans.

Psychrophiles

Temperature is an important environmental factor which influences the different groups of microorganisms. Various groups of microorganism based on different temperature regime are shown in Fig.

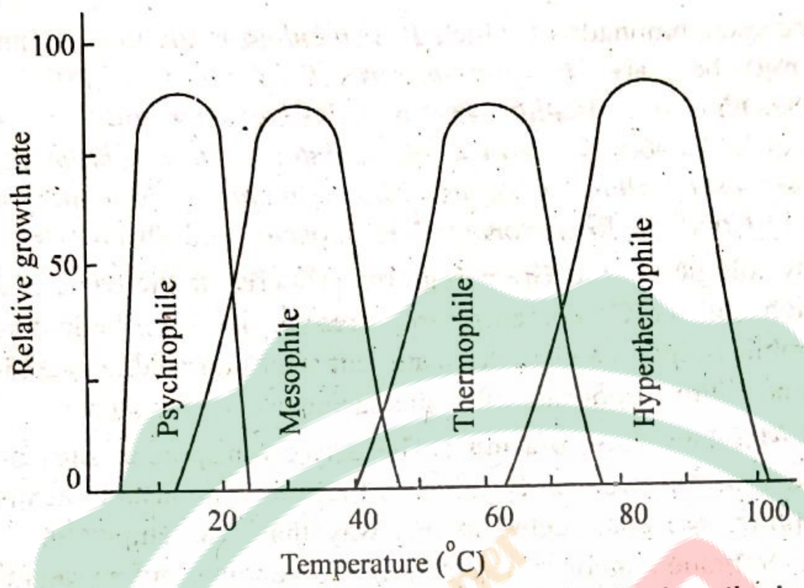


Fig. (A) : Different groups of microorganisms categorized on the basis of different temperature regime.

Cold environments are actually more common similar to hot environment in summer. The oceans which maintain an average temperature of 1-3°C make up our half the earth's surface. The vast land areas of the Arctic and Antarctica are permanently frozen or unfrozen for more a few weeks in summer.

James T. Staley and his colleagues at the University of Washington have represents that microbial communities populate ice ocean water of Antarctic sea that remains frozen for much of the years. These communities include photosynthetic eukarya, notably algae and diatoms as well as variety of bacteria. *Polasomonas vacuolata* found through Staley's groups is a prime representative of a psychrophile. Psychrotolerant can be isolated from more widely distributed habitat than psychrophiles. They can be isolated from soil, water in temperate climates as well as meat, milk and other dairy products, vegetables and fruits under refrigeration.

They grow best between 20 and 40°C but cannot grow at 0°C. After many weeks of incubation their visible growth can be observed. Its optimum temperature for growth is 4°C and 12°C for reproduction. The cold-loving microorganisms have begins to interest manufacturers who need enzymes that work at refrigerator temperature such as food processors, makers of fragrances and producers of cold-wash laundry detergents.

Some psychrophiles can be dangerous organisms for man for example *Pseudomonas syringae, Erwinia* sp., *Yersinia enterocolitica* etc. Most of the foods or food products are stored at freshing temperature so that the pathogenic or saprophytic microbes cease to grow.

A majority of marine microbes is psychrophiles because of their habitat (ocean). Generally, these are Gram-negative rod shaped bacteria. Among them are pseudomonads of which *P. geniculata* is the most common. The other microbes are *P. putrefaciens, P. fragi* and *P. fluorescens, Flavobacterium* spp. *Alcaligenes* spp, *Achromobacter* and a few strains of *Escherichia, Aerobacter, Aeromonas, Serratia, proteus, Chromobactor* and *Vibrio are psyhrophilic in nature. The common psychrophilic yeasts are species of Condida, Rhodotorula Cryptococcus,* and *Torulopsis.*

Physiologically the Gram-negative property of the bacteria and high proportion of G+C contents are present in such microorganisms. Psychrophiles contain an increased amount of unsaturated fatty acids in their lipids. Flagellum disappears after increasing the temperature.

(i) Molecular Adaptation : The active transport in such organisms occurs at low temperature. It indicates that the cytoplasmic membranes of psychrophiles are constructed in this way that low temperature does not inhibit membrane function. The membrane contains polyunsaturated fatty acids in their lipids that maintains the rigidity at low temperature and organisms thus are able to survive.

(ii) Physiology : Psychrophiles gives enzymes that function optimally in the cold. Its cell membrane contains high content of unsaturated fatty acid which maintains a semi-fluid-state at low temperature. The lipids of some psychrophilic bacteria also contain polyunsaturated fatty acids and long chain hydrocarbons with multiple double bonds.

(iii) Applications : Psychrophiles and their products have many applications as described below :

(a) Fermentation industry : Mesophilic yeasts containing unsaturated fatty acids in membranes have been found to be resistant between –80 and –20°C. These are preferred for its storage in baking and other processing industries. Fermentation at 6 – 8°C reduces the inhibitory effect oif ethanol on cell membrane of the yeast cells.

(b) Bacterial ice nucleating agents : There are various uses for ice-nucleating agents produced by bacteria. They are being used in artificial snow-making, in the production of ice creams and other frozen foods. These are also used in immunodiagnostic kits as a conjugate to antibodies and like a substitute for silver iodide in cloud seeding. Among several organisms bacterial INAs have attracted much attention because of its ability to form ice nuclei at relatively high temperature in comparison to other sources.

(c) Source of pharmaceuticals : Many psychrophiles *e.g. Stteptmyces, Aiteromonas, Bacillus, Moraxella, Micrococcus, Pseudomonas* and *Vibrio* have been isolated from deep-sea sediments. They found at temperature between 3 and – 30°C. Aquatic plants and animals are highly prone to infestation by pathogenic microorganism. An *Alteromonas* sp. has been reported to synthesize 2, 3-indolinedione. This compound protects *Palaeoman macrodactylus* form pathogenic fungus *Lagenidum callinectes.* Similarly, another strain of *Alteromonas* sp. is intimately associated with the marine sponge *Halichondria okada* and gives a tetracycline alkaloid for example alterimide. There is wide scope for the discovery of novel biologically active compounds in marine microbiology. An antitumor polysaccharide has been isolated as narinactin from marine actinomycetes. A mixture of protease and amylase isolated from *Bacillus sabtilis* removes the dental plaque. Mainly lipases are used as stereo-specific catalysts and in the biotrans-formations of different high value compounds such as flavouring agents and pharmaceuticals. Trehalose is formed by an enzyme trehalase present in several psychrophilic bacteria.

(d) In bioremediation : Psychrophiles have ability to degrade many compounds in their natural habitat. They are used in bioremediation of different pollutants at low temperature. The bacterial strains were observed to mineralize dodecane, hexadecane, naphthalene, toluene. It has been demonstrated in laboratory and field experiments using specific bacterial strains. A psychrophilic bacterium, *Rhodococcus* sp. strain has been studied for its ability to degrade *n*-alkanes and diesel fuel at low temperature.

(e) In microbial leaching : Currently microbial leaching operations involve oxidative solubilization of copper and uranium ores. Leaching operation in temperate countries is carried out at low ambient temperature. Microbial leaching operation from sulfide ores is carried out at 4-37°C.

(f) Anaerobic digestion of organic wastes : The obligate anaerobes which convert organic acids to CH_4 and CO_2 *i.e.* methanogens are highly sensitive to low temperature. The rate of methanogenesis can be increased several times through low temperature adaptation by methanogens. The process can be made possible through selective enrichment of psychrophilic methanogens through long term laboratory trials. *Methanogenium frigidum* isolated from Ace lake grows optimally at 15°C. This bacterium is found to produce methane from hydrogen and carbon dioxide.

(g) Denitrification of drinking water sources : The presence of high nitrate concentration in water has become a major problem in many countries. The most widely used practices for removal of NO_3 is the biological denitrification. Most of the denitrification processes are carried out at 10°C. The rate of denitrification in these cases case be enhanced through employing psychrophilic bacteria isolated from permanently cold habitat. ●

Q.10. Write a detailed account on the application of thermophile and hyperthermophiles-microorganism.

Ans. **Thermophiles and Hyperthermophiles**

Hyperthermophilic bacteria are archaea that represent the organism at the upper temperature border of life. Neutrophilic and slightly acidophilic hyperthermophiles are found in terrestrial solfataric fields, and deep oils reservoir. These show specific adaptations to their environments and most of the bacteria are strictly anaerobic.

Various factors both abiolic and biotic, that control the growth of all living organisms are called *biotope*. The moderate thermophiles are known as *extreme thermophiles* which grow optimally between 80 and 100°C. The hyperthermophiles are unable to grow below 80°C but adapted to high temperature as they do not even grow at 80°C.

The hyperthermophiles can grow in natural and in artificial environment conditions. Natural suplphur-biotopes are usually asociated with active volcanism. In such situation, soil and surface waters from S-containing acidic fields (pH 0.5-6.0) and neutral to slightly alkaline hot spring environment persists. Well-known biotopes of hyperthermophiles are volcanic areas such as hot springs and solfataric fields *i.e.* high temperature fields located within volcanic zones with much sulphur acidic soil, acidic hot springs and boiling mud. Few of hyperthermophiles live in shallow submarine hydrothermal systems and abyysal hot vent systems. known as "black smokers" having temperature of about 270-380°C. The black smokers are mineral-rich hot water which makes cloud of precipitated material on mixing with sea water. Other biotopes are smouldering coal refuse piles having acidic pH and geothermally heated soil reservoirs.

Most of the hyperthermophiles are anaerobic because of low solubility of oxygen at high temerature and the presence of red gases. Anaerobic chemolithoautotrophic hyperthermophiles completely independent on sun, but they could even found in other planets also. Hydrothermal vents in the bottom of the ocean have temperature of 350°C or greater and also indicate the existence of hyperthermophiles. The recently discovered non-volcanic biotope embedded in deep geo-thermal heated oil stratification of extracted fluids evidenced for here microbial communities.

For the cultivation of these bacteria, samples are brought to the laboratory without temperature control. They are isolated through enrichment culture technique with variation in composition of substrate and control of *in situ* temperature. Agar is not suitable hence more heat-stable polymer like gellan gum or polysilicate gels are used for solidification.

Many taxonomic types of cultured hyperthermophiles are already known so far. They shown 52 species belonging to 23 genera and 11 orders of hyperthermophilic bacteria and archaea known in literature. The organisms whose optimum growth temperature is < 45°C are known as thermophiles and those above 80°C are known as hyperthermophiles.

(i) Molecular Adaptation : These bacteria contain heat-stable enzymes and proteins which regulate various macromolecular functions at high temperature. The critical amino acids substituted in one or more locations in these enzymes permits them to fold in a different manner and thereby withstand the denaturing effect of heat resulting into the survival of these organisms. Further the cytoplasmic membrane contains lipids rich in saturated fatty acids, hence allow the membrane to remain stable and functional at high temperature. The thermophilic archaea do not contain fatty acids in their lipids, neither its membrane has ester linkages with glycerol phosphare. It imparts more rigidity to its membrane systems.

(ii) Physiology : The enzymes and proteins are much more stable than the other forms and these macromolecules function at high temperature. Thermophilic porteins have various amino acid sequences that catalyse the same reaction in a mesophile which allow it to fold in a different way and thereby show heat tolerant effect. All thermophiles contain reverse gyrase, a unique type 1 DNA topoisomerase which stabilizes DNA.

Heat stability of proteins from hyperthermophiles is also due to increased number of salt bridges present and densely packed highly hydrophobic interior of the protein which have membranes rich in saturated fatty acids. This allows the membrane to remain stable and function at high temperature. Most of the hyperthemophiles are archaea which do not contain fatty acids. the lipids in their membranes but instead have hydrocarbons of different lengths composed of repeating units of 5-6 compound phytans bonded by ether linkage to glycerophosphate. With increase in temperature of growth an increase in degree of saturation, chain length and/or iso-branching of the acyl chains are observed. Sometimes, special lipids are present in thermophiles. These may also affect an adaptation to life at high temperature 1 making the membrane more rigid.

(iii) Applications : Most of the microorganisms that thrive above the boiling point of water belong to archaea. The enzymes of thermophiles are great interest. Hyperthermophiles have focused on thermostable enzymes from vent. The proteins were also discovered. Those proteins are expressed under stress conditions and involved in protein foldings.

Enzymes New engyms from hyperthermophiles have reduced the number of steps needed to transform starch into fructose syrup. The amylase, glucoamylase, pullunases and glucosidases are the enzymes used in starch industry. Pullunases are observed in anaerobic bacteria. Amylases are widely used in textile, confectionary, paper, brewing and alcohol industries. Similarly, glucosidases are used for hydrolyzing lactose syrup and mixture to glucose and galactose. They may have clinical applications as there is proof of a lactase deficiency in the population which is either inherited or is the cause of ageing. Glucose isomerase is widely used in the food industry which changes glucose to fructose for use as sweetner.

Because of the thermal stability of the enzymes, hyperthermophiles have been the subject of intensive investigation. Thermostable enzymes are more resistant to the denaturing activities of detergents and organic solvents. The amylases have been extracted from *Pyrococcus furious and Pyrococcus woessei.* Enzymes have been exploited from some archaea e.g. *Desulfurococcus mucosus. Thermococcus celer Staphylothermus marinns,* and *Thermococcus litoralis.* A togaassociated amylase has also been detected from *Thermotoga maritima.* This enzyme is active between 70 and 100°C at pH 6. *Fervidobacterium pullunolyticum* has the potentiality of producing thermotolerant enzyme optima at 90°C.

Certain bacteria and archaea e.g. *P woesei, OP. furiosus. Thermococcus litoralis, T celer, F. pennavorans, D. mucosus* etc. are reported to produce pullunase II having 90 kDa molecular weight with temperature optima 105°C and pH 6. Some of these also gives glucosidases with temperature optima 110-115°C. These are useful for the bioconversion of starch into different useful products of industrial significance.

A thermostable exo-4-β-cellobiohydroplase with a half life of 70 minutes at 108°C has been isolated from *Thermotoga* sp. strain FjSS3-B. Similarly, thermostable xylanases have been reported from *T. thermarum. P. furiosus, Thermotoga maritima, T. neoplolitiana* shows β-xylanosuidase activity. The enzymes from *Thermotoga* sp. are extremely stable with half-life of 8h at 90°C.

The protein hydrolyzing enzymes have been isolated, purified and characterized from a number of thermophilic and hyperthermophilic microorganisms specially *Thermococcus, Sulfolobus, Pyrococcus, Staphylothermus* and *Desulfurococus.* Pyrolysin, an enzyme associated with the cell envelope which is a serine-type proteaase has temperature optima of 110°C and a half life of 4h at 100°C. It has been observed and characterized in *P. furiosus* and *P. woesei.* The serine-protease from *Sulfurococcus mucosus* exhibits its activity at 100°C.

A unique protease which hydrolyses keratin of chicken feather, hair and wool has been characterized from a bacterium *F. pennavorans.* A thermophilic glucose isomerase was characterized and purified from *Thermotoga maritima.* Ferredoxins from *Thermoplasma acidophilum, Sulfolobus acidocaldarius* and *Desulfurococcus mobilis* have also been investigated. Hydrogenase, having a half-life of 21 h at 80-85°C has been isolated from *Pyr. furiosus.* A thermoactive pyruvate-ferrodoxin-oxidoreductase that catalyses the oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2 has been detected in *D. amylolyticus, H. butylicus, Thermococcus celer, Pyrococcus woesei, P. furiosus* and *Thermotoga maritima.*

Enzymes involved in amino acid biosynthesis e.g. aromatic aminotransferase from *Thermococcus litoralis* and *Sul. solfataricus* have been detected. An extremely thermostable enzyme with optimum activity at 100°C has also been found from *Methanobacterium thermoformicicum*. The purified enzyme from *P. woesei* and *P. furious* has molecular mass of identical subunits 45 kDs each. The enzymes have heat-stability up to 70% after heat treatment at 100°C for an hour.

Glutamate synthetase (GS) is responsible for the synthesis of glutamine from glutamate and ammonia. The half-life of partially purified GS is 2 hours at 100°C. Two thremo-active atomatic aminotransferases from *Thermococcus lithoralis* has been purified and characterized, that are active at 100°C temperature. The enzyme asparate aminotransferase transferring amino group from glutamate to oxaloacetate has been found in *Sul. solfataricus*.

Taq polymerase is very important enzyme used in molecular biology for the amplification of DNA using polymerase chain reaction (PCR). This enzyme observed in *Thermus aquaticus* is active at 80°C at pH 8. Simpson *et al.* (1990) has investigated the other DNA polymerase from *Thermotoga* sp. Certain archaea e.g. *Sul. acidocaldarius* and *Sul. solfataricus* consists of DNA polymerase of a single polypeptide chain with a molecular mass of 100 kDa. The DNA polymerase from *P. furiosus* has also been purified. The DNA ligase has been characterized from *Thermus thermophilus* Topoisomerases type I purified from *Sulfolobus acidocaldarius. Thermoplasma acidophilum Fervidobacterium islandicum, Desulfurococcus amylolyticus, Thermotoga maritima* and *Methanopyrus kandleri,* while copoisomerase II has so far been isolated from *Sulfolobus acidocaldarius. Thermotgoga maritima* contains of both gyrase and recerse gyrase enzymes. Repair of extensive DNA damage ressults due to ionzing-radiation at 95°C has been demonsrated in *Pyrococctus furiosus*.

(b) Chaperons : The *chaperons* are proteins which express under stress conditions such as elevated temperatures. They are involved in protein folding. These are detected in *Su. shibate* and *Su. sdolfactaricus*. It is known as thermophilic factor which has 55 kDa molecular mass. Due to increase in high concentration of intracellular protein up to 105°C, this protein complex is known as **thermosome**. The thermosome consists of a cylindrical complex of a two stacked identical rings each unit consists of 8 subunits around a central channel. Both subunits contain 56 and 59 kDa molecular mass. They also bind the unfolded proteins similar to chaperons. A thermostable disulfide-bond forming enzyme has been isolated, characterized and purified from *Sul. solfataricus*.

SHORT ANSWER QUESTIONS

Q.1. Write a brief account on the animals microbe interactions.

Ans. **Animal-Microbe Interactions**

There are many kinds of microorganisms that interact various groups of animals and develop a variety of relationships. Some of the relationships have been discussed in this section.

1. Destructive Associations

Pathogenic microbes interact with animals including man and cause many kinds of disease. For detail description of protozon, bacterial and fungal diseases *Medical microbiology.* While destructive associations are found between two microbes such as fungi and amoebae, nematode and fungi etc.

2. Neutral Association (neutralism)

Normal microbiota of human body : There is a large number of microorganisms that normally act as the resident of different body organs of humans e.g. skin, nose and nasopharynx, eropharynx, respiratory tract, mouth, eyes, external ears, stomach, small intestine, large intestine and genito-urinary tract. Reasons of having informations about the normal human microbiota are :

(i) to increase awareness of the role of indigenous microbiota that stimulate host immune response.

(ii) to help the physician investigator so that he can understand the causes and consequences of overgrowth of microorganisms normally absent at a specific body site and

(iii) to have an understanding of microorganisms at specific site so that greater inside into the possible infections can be provided.

Table : Normal microbiota of human body

S. No.	Body Sites	Microorganisms
1.	Ear	Coagulase-negative staphylococci, diphtheroids, pseudomonads, Enterobacteriaceae
2.	Eye	Coagulase-negative staphylococci, *Haemophilus* spp. *Staphylococus aureus, Streptococcus* spp.
3.	Intestine (small)	Species of *Lactobacillus, bacteriodes, Clostridium,* Enterococci, *Mycobacterim*
4.	Intestine (large)	*Actinomyces, Acinetobacter, Bacteroides, Clostridium,* Coagulase-negative staphylococci, *Escherichia coli,* Enterococci, *Lactobacillus, Klebsiella, Mycobacterium, Pseudomonas, Proteus, S. aureus,* streptococci

5.	Mouth	Species of *Actinomyces, Candida* coagulase-negative staphylococci, Diphtheroids, *Fusobacterium, Hemophilus, Neisseria Porphyromonos, Prevetella, S. aureus, Streptococcus*
6.	Nose	Coagulase-negative staphylococci. *Haemophilus, Neisseria, S. aureus. S. pneumoniae, Viridans streptococci*
7.	Skin	Coagulase-negative staphylococci. diphtheroids, *Bacillus, Candida. S aureus,* streptococci, Mycobacterium
8.	Stomach	Species of *Lactiobaciilus, Streptococcus, Staphylococcus, Peptostreptococcus*
9.	Urethra	Cooagulase-nagative staphylocooci diphtheroids, *Bacterioides, Fusobacterium, Mycobacterium Peptostreptococcus.*
10.	Vagina	Species of *Bacteroides, Candida, Clastridum,* dipshheroids, *Lactobacillus. Gardnerella veginalis, Peptostreptococcus streptoccocci.*

3. Symbiotic Associations

Symbiotic associations of bacteria, fungi and protozons with insects, birds and herbivorous mammals are emplained as follows :

(i) Ectosymbiosis of protozoa, Bacteria and Fungi with Insects and Birds : Most of the animals such as insects cannot utilize the cellulose and lignia components of woody tissues of tree because of lack of cellulose and lignin degrading enzymes. Thus various insects develop ectosymbiotic association with cellulose and lignin-decomposing microorganisms that can degrade these substrates. All termites and cockroaches that eat upon wood, harbour flagellated protozoa in their guts. These protozoa digest cellulose. In turn the protozoa develop symbiotic association with certain N_2-fixing bacteria and spirochetes that perhaps also help in cellulose degradation. In addition, in moulting season of cockroaches hormones (*e.g.* ecdysone) are secreted which induce cyst formation in symbiont protozoan.

(ii) Ruminant Symbiosis : The herbivorous mammals for example cattles, sheep, goats, camels etc) are known as ruminants because they have a special region of gut which is known as *rumen.* These animals use plant cellulose as the source of carbohydrate which is not digested in normal gut. The cellulosic material is digested in rumen which works as incubation chamber teeming with protozoa and bacteria. In some animals like cow, tthe size of rumen is very large. Some of anaerobic cellulose-digesting bacteria for example *Bacteroides succinogens, R.albus* and *Botryovibrio Ruminococcus flavofaciens fibrisolvens* develop mutualistic symbiosis and hydrolyse cellulose and other complex polysaccharides to simpler froms which in turn are fermented to fatty acids for example acetic acid, propionic acid, butyric acid) and gases. Some of the bacteria are capable of digesting proteins, lipids and startch as well. Lignin fraction of plant remains undigested. The rumen bacteria ferment proteins and lipids and produce hydrogen and carbon dioxides gase, which in turn is converted into methane through *Methanobacterium ruminantium.* The bacteria of rumen multiply into a large population. While most of them are passed into stomach along with undigested material where they are killed by proteases and other enzymes. The fatty acids in rumen are absorbed and gases are passed out.

☛ **Q.2. Write important pathogens causing diseases on the roots of plant species.**

Ans. **Some Important pathogens causing diseases on roots of several plant species**

S. No.	Diseases	Hosts	Pathogens
1. Bacterial Diseases			
	Scab	Potato	*Streptomyces scabies*
	Crown gall	Fruit trees, dicots	*Agrobacterium tumifaciens*
2. Fungal Diseases			
	Club-root disease	Cruciferous plants	*Plasmodiophora brassicae*
	Powdery scab	Potato	*Spongospora subterranea*
	Wart disease	Potato	*Synchyrium endobioticum*
	Damping off	Nursery seedings	*Pythium aphanidermotion*
	Root-tot	Wheat sugarcane corn etc	*P. graminicolum* / *P. arrhemomones*
	With and foot-rot	Coconent arecanut	*Ganoderma*
	Will	Cotton, arhar, banana. Tomato, okra, soybean	*Species of Fusarium*
	Root rot/charcoal rot		*Macrophomina phaseolina*
	Foot-rot	Barley, soybean	Selerotiam rolfsii
	Root-rot	Many plants	Rhizoctonia solani
3. Nematode disease			
	Root-knots	Many plants e.g. tomato, sugarcane etc.	*Meloidogyne jayanica* / *M. incognita*
	Root lesions	Many plants	*Prarylenchus* spp. / *Heterodera rostochiensis*

Q.3. Give microbial pathogens causing disease on above ground parts of plants.

Ans. **Some microbial pathogens causing disease on above ground parts of plants**

S. No.	Diseases	Hosts	Pathogen
1. Algal Diseases			
	Red rust	Citrus, mango, sapota, cocoa, guava, tea	*Cephaleuros parasitica* and *C. virescens* coffee
2. Bacterial Diseases			
	Stem canker and wilt	Tomato and other member of Solanaceae	*Corynebacterium michi-ganess*
	Fire blight	Apple	*Erwinia amylovora*
	Stem blight	Pea	*Pseudomonas pisi*
	Canker	Citrus	*Xanthomonas citri*
	Angular leaf spot	Cotton	*X. malvacearum*
3. Fungal Diseases			
	Late blight	Potato	*Phytophthora infestans*
	Early blight	Potato	*Altermaria solani*
	Downy mildew	Grape	*Plasmopara viticola*
	Powdery mildews	many plants	Species of *Erysiphe, Phyllactinia Uncinula, Oidium, Podosphaera, Sphaerotheco*
	Ergot	Cereals and grasses	*Claviceps purpurea*
	Smuts	Many plants	*Urocystis, Sphacelotheca Tolyposporium, Ustilago, Tilletia.*
	White rust	Crucifers	*Albugo spp.*
	Rust	Many plants	
		Wheat	*Puccinia graminis tritici, P. recondida*
		Linseed	*Melampsora lini*
	Tikka leaf spot	Peanut and groundnut	*Cercospora personata*
	Blast	Rice	*Pyricularia oryzae*
	Red rot	Sugarcane	*Colletotrichum falcatum*
	Leaf spot	Guava	*C. gloecosporioides*
	Charcoal rot	Soybean, sunflower etc	*Macrophomina phaseolina*

4. Mycoplasma Diseases		
Little leaf	Several plants *e.g.* Brinjal	*Mycoplasma spp.*
Phyllody	Sesame	*Mycoplasma sp.*
Sandle spike	Sandle wood	*Mycoplasma*

Q. 4. Write a short note on the effect of micorrhizal fungi on their hosts.

Ans. Effect of Mycorrhizal Fungi on their Hosts

(a) Mycorhizosphere effect : Similar to rhizosphere, mycorrhizosphere indicare increased microbial community leading to mycorhizosphere effect. The photosynthates flow into soil by roots and mycorrhizae support a diverse community of soil microorganisms. Some of them influence plant growth. The mycorrhizosphere microorganisms may be facultative anaerobes, extracellular chitinase producers, phosphate solubilizers and producers of siderophores, antibiotics, hormones, plant growth-suppressors and promotors.

(b) Nutrient uptake and Translocation : Mycorrhizae increase the absorptive surface of root resulting in increased uptake of water and nutrients with the soil. The ectomycorrhizal fungi translocate phosphorus, nitrogen, calcium and amino acids and increase translocation of Zn, Na and other minerals to the hosts. Their hyphae extract N and transport from soil to plant due to increased absorptive surface area. Plant available phosphorus in soil is in small amount of total P content. The by-products of fungi dissolve several insoluble nutrients. Three mechanism of mycorrhizal activity has been discussed for weathering soil P and transport to host plants; (a) the interaction of mycorrhizal fungi and phosphate solubilizing bacteria, (b) production of phosphatases by the mycorrhizal fungi and (c) production of organic acids by mycorrhizal fungi.

Translocation of P in fungal hyphae takes place through cytoplasmic streaming. P is stored in the form of polyphosphates because of polyphosphate kinase activity. Then P is transferred to host plant after break down of phosphates through phosphatases and release of inorganic phosphate. P is accumulated in mantle and Hartig net and thereafter, transferred from Hartig net to host tissue.

Similarly, the VAM fungi also influence growth, exudation and nutrient uptake in host plants. Polyphosphate granules have been observed in arbuscules, hyphae and vesicles of VAM fungi. Chitin appears to be the main carbhydrate-related material present in vesicle and hyphal walls. *Glomus fasciculatus* translocate P over a distance of at least 7 cm, and *Rhizopogon luteus* to 12 cm.

(c) Plant Protection Biocontrol of Pathogens : Both VAM and ectomycorrhizal fungi make the plants drought and frost resistant, increase tolerance to strees against soil temperature, soil toxins, high acidity, and heavy metal toxicity.

VAM and ectomycorrhizal fungi inhibit the infection of pathogens to plant roots. The fungal mantle works as a passive mechanical barrier influencing either the pathogen or its spread in host tissues.

**(d) Growth-hormone/amibiotic production : ** Some of the ectomycorrhizal fungi produce indole acetic acid (IAA) that possibly is involved in morphogenesis and longevity of roots. Moreover, *Leucopaxillus Cerealis* var *piceina* is known to give growth inhibiting antibiotics.

(e) Transfer of metabolites from Host to Fungal symbiont and the other plants : The products of photosynthesis move from host to the fungal symbiont. While host to host transfer of carbohydrate via a shared fungal symbiont also takes place.

Q.5. Write an account of on works mycorrhizal fungi in India.

Ans. Works on Mycorrhizal Fungi in India

Though the study of mycorrhizal fungi has been a neglected field, yet in India acts on mycorrhizal fungi was started by Bakshi at Forest Research Institute, Dehra Dun. His project report *Mycorrhiza and its role in forestry* published from F.R.I. has served as a mile stone for the beginner. Mishra and Sharma reported the association with *Pinus kesiya* of *Amanita muscaria, Boletus edulis, Cenococcus geophilus*\. *Inocybe rimosa, Russula roseipes, Sclleroderma aurantium, Suillus bovinas* from Meghalaya of North East Himalaya. Moreover, association of ectomycorrhizal fungi with some other gymnosperm has been explored R.B. Mishra and his research group. The work on this aspect done by K.S. Think and his research group. Chandigarh cannot be overlooked. Lakhanpal has reported 72 fungai species forming mycorrhiza in various forest trees in the Western Himalaya. At Forest Research Institute, Dehra Dun, in addition to significant research work done by Bakshi and coworkers, mycorrhizal researches are being strenghthened, *Amanita muscaria. L. laccata* and *Scleroderma citrinus* were recorded from *Pims patula* and *A. muscaria,* and *L. chinensis* were identified from *Eucalyptus globulus*. Ginwal *et al* (1996) surveyed oak forests of Kumaon Himalaya and reported the genera *Agaricus, Amanita, Russula, Entolioma, Laccaria, Scleroderma, Rhizopogon* and *Tricholoma* that form-mycorrhiza. A total of 27 sporophore producing ectomycorrhizal fungi were identified. Variations in genotypes of the host, biotic disturbances in the forest, tree density, soil temperature, soil pH, moisture and the presence of antagonistic microorganisms were the factors that governed the occurrence of ectomyçorrhizal fungi in different oak forests. The western and eastern Ghats of South India have been extensively surveyed by A. Mahadevan and his research group of Madras University. Dubey have reviewed the influence of nutrients on formation and growth of ectomycorrhiza.

In India. B.K. Bakshi (1974) was the first to publish an account of 14 spore types of 5 genera of Endogonaceae such as *Glomus, Gigaspora, Acaulospora, Endogone* and *Sclerocystis* from forest soils. Sharma recorded the species of *Acaulospora, Gigaspora, Glomus* and *Sclerocystis* from rhiziosphere soils of different forest trees of Meghalaya. Negi recorded

Endogone sp. in soils of *Cupressus torulosa* and VA-mycorrhizal fungi in root tissues from Nainital. Vesicles of changing colours and numbers were observed on *C.torulosa* roots throughout the year. The maximum infection was recorded in winter and minimum in summer seasons. In Himachal pradesh 10 districts were periodically surveyed over a wide altitudinal range of 500 to 2500 meters. A total of ten VAM fungi of four genera were recorded.

Many epiphytic and terrestrial orchids are considerably dependent on mycorrhizal fungi for their carbon sources. Katiyar studied the mycoirrhizal status in certaion tropical epiphytic orchids from Khasi and Garo hills of Meghalaya. Diversity of mycorrhizal fungi in the Himalaya with emphasis on their forms, function and management has been reviewed by Dubey and Ginwal (1997).

An important work on VAM fungi has been done by Sudhir Chandra and Kehri of the University of Allahabad especially on management of waste land and introduction in crop field for high yield of crops. D.J. Bagyaraj and his group have done excellent work on VAM fungi as far as crop improvement is concerned. Reena and Bagyaraj screened various VAM fungi for their suitability to use as inoculants for two slow growing tree species, *Acacia nilotica* and *Calliandra calothyrsus.* They found that the inoculated seedlings had greater plant height, leaf number, stem girth, biomass and phosphorus and zinc content. Johri and Mathew successfully produced inocula of VAM fungi in bulk on *Phaseolus mungo* roots of *Gigaspora margarita, G. fasciculatus, G. caledonium* and *G. calospora.* Of these *G. margarita* showed the highest percentage of root infection. Tata Energy Research Institute. New Delhi is contributing significant work on mycorrhizal fungi. In recent years researches on VAM fungi have gained momentum throughout the country.

☛ Q.6. Write a brief account on nematophagous fungi.

Ans. Nematophagous (Predaceous) Fungi

The predaceous fungi are also termed as nematophagous fungi. The nematophagous fungi are of three main types on the basis of ecological habit: (a) endoparasitic fungi and the (b) nematode-trapping fungi, (c) egg parasites.

(A) Endoparasitic Fungi : Unlike nematode-trapping fungi, the edoparastic fungi do not extensively produce mycelium external to nematode body. But they attack nematodes through many modifications brought about in conidia. The endoparasitic fungi are species of *Meria, Veriticillium, Cephalosporium, Catenaria, Meristacrum* etc.

Catenaria anguillulae produces zoospores which track down nematodes through swarming, eventually encyst near nematode body orifice for example anus, vulva and buccal cavity. Penetrate and colonize the prey. The encysted zoospores produce germ tube that penetrate nematode through orifice or by dissolving cuticle. The infectious hyphae grow well inside nematode body, digest content and lyse the prey. Zoosporangia are produced inside body from which numerous uniflagellated zoospores are liberated.

In addition, *Meristacrum asterospermum* forms adhesive conidia that attach to the cuticle of nematode. It germinates to form the hypha which swells and acts as infectious thallus. Similarly, adhesive spores are also produced through species of *Meria, Cephalosporium* and *Verticillium.* In *M. coniospora* an adhesive bud develops at the distal end of tear-drop shaped spores. The conidia attach to nematode body, germinate and penetrate through cuticle. After penetration, an infectious hypha in body cavity of nematode is formed, the amount of which rises eventually with the result to nematode death.

(B) Nematode-trapping Fungi : Fungi capturing nematodes are called nematode-trapping fungi. Such fungi have evolved structural adaptations to trap or penetrate their prey. They may be predatory or endoparasites. There are varieties of ways by which fungi trap nematodes resulting in their death. These methods are explained as follows :

(a) Adhesive branches : The nematode trapping fungi produce the most primitive and simple organ of capture, the adhesive branches, which are few cells in height. From the main prostrate hyphae short laterals grows as erect branches, which are a few cells in height. From the main prostrate hyphae short laterals grows as erect branches on or below the substrate. Over the whole surface of branch a thin film of adhesive material is coated. Examples of adhesive branch producing fungi are *Dactylella cionopaga* and *D. gephyropaga.*

(b) Adhesive hyphae : The fungal hyphae form adhesive which capture nematodes. These hyphae produce adhesive at any point in response to nematode cantact or the hyphae are coated with adhesive along their entire surface. At the point of hyphae where contact is made for capture a thick and yellowish chemical material is secreted e.g. *Stylopage hadra.* There after, an outgrowth of hyphae similar to appressorium develops. WHen the nematode is trapped. It becomes inactive first and killed in the last after penetration of hyphae. After penetration. elongate, unbranched absorptive hyphae grow along the nematode body and completely exploit the contents.

(c) Adhesive nets : Nets are formed by fungal hyphae which-are adhesive in nature. Nets may be in the form of a single hoop-like to a complex multibranched networks. Upon observation with electron microscope it appears that the hyphae are coated with adhesive material. Since the nematode comes in contact of hyphae it is attached at many points resulting in penetration by infectious hyuphae. Initially penetration is accompanied by the formation of infectious bulb which leads to form hyphae which grow inside nematode. Hyphae growth exlpoit nutrients and causes death of the prey.

(d) Non-constricting rings : From the prostrate creeping septate hyphae there arise erect and lateral branches that form non-constricting rings. Initially the branch is slender but widens subsequently and being curved to form a circular structure. At the point where tip of branch makes contact with supporting stalk, cell walls get fused. Hence it results in formation of three-celled ring with a stalk. A nematode enters the ring and moves forward. This results in marked constriction in cuticle. Generally rings are impossible to dislodge. In struggle the rings break from the weak point. Therefore, nematodes containing rings can move. Initially rings do not have any harmful effect on nematode but eventually nematode is penetrated and its body content is consumed. *Dactylaria candida* and *D. lysipage* produce non-constricting rings, inspite of producing the adhesive knobs.

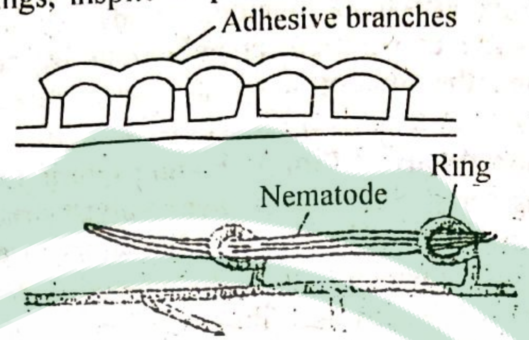


Fig. (A) Predaceous (nematophagous) fungi. A, scalariform adhesive branches of *Dactylaria gephyropaga;* B, constricting rings of *Arthrobotrys dactyloides.*

(e) Adhesive knobs : Morphologically a distinct adhesive cell, globose to sub-globose in structure, is produced at the apex of a slender non-adhesive stalk containing 1-3 cells. A thin film of adhesive material is produced over the surface of knob. When a nematode is caught by a knob, soon it is attacked by several knobs with subsequent penetration. The immobilized nematode is destroyed thereafter *e.g.* adhesive knob-producing fungi are *Dactyleria candida. Dactylella* and *Nematoctonus.*

(f) Mechanism of interaction : The body of nematode consists of a low molecular weight peptide (or possibly a single amino acid) which is called *nemin.* Nemin is water soluble and potential stimulant for trap-formation. It results morphological changes in nematophagous fungi. The process of nematode-fungus interaction is accomplished-through a series of molecular events resulting in nematode death. Pramer and Kuyama identified on the trap of *A. oligospora* the initiator of prey-predator recognition as *lectin.* The lectin of fungus binds especially to the sugar. N-acetyl-D-galactosamine, present on nematode cuticle. The lectin has also been purified. It is a protein of molecular weight 22,000 daltons. The trapped nematodes secrete mucilage that has been identified under electron microscope. The nematode cuticle is lysed at the point where lecting combines with N acetyl-D-glucosamine. Within an hour fungal hyphae penetrate the prey. The enzyme collagenase is secreted through the fungus which dissolves collagen protein of nematode cuticle. The hyphae which have penetrated the nematode digest the body content and translocated to rest of the parts of hyphae for fungal growth and reproduction.

(g) Constricting rings : The constricting rings are produced similar to non-constricting rings but the supporting stalk is shorter and stouter as shown Fig. A. In this case also a three celled ring is formed. It is a most sophisticated ring formed by predaceous fung *e.g. Arthrobotrys anchonia, Dactylaria brachopaga* and *Dactylella* which are abundant in soil.

The nematode is captured by garroting action of the ring cell. By swallowing the ring cell graps the nematode in a single hold when a nematode enters into the ring. friction created through its body induces to swell the rings soon. The cells swell inwardly through three times greater than the original one within 1/10 of a second resulting in body of nematode deeply constricted. Struggle between nematode and fungi goes on for some fine. Thereafter nematode becomes still and hyphae from ring cell penetrate the body and exploit the nutrients of nematode with the consequences of death. The most potential predatory fungi are the species of *Dactylaria*.

(C) Egg Parasites : There are a few saprophytic fungi which attack on nematode eggs. If a fungal hypha comes in contact of an egg. a swollen structure at terminal protion develops at the point of contact. It gets attached to the egg where from a narrow infectious tube develops that penetrates the shell of the egg. After penetration, the infectious hyphae sweel up and form a post-penetration bulb which looks like appressorium. From such structure there develops numerous irregularly branched absorption hyphae that consumes egg nutrients. Examples of egg parasites are *Dactyllela oviparasitica* and *Pacilomyces lilacinus* that penetrate root-knot or cyst nematode.

Q.7. Define Acidophitis.

Ans.

Acidophiles

Most natural environments on the earth are essentially neutra, having pH between 5 and 9. Only a few microbial species can grow at pH less than 2 or greater than 10. Microorganisms which live at low pH are called **acidophiles.** Fungi as a group tend to be more acid tolerant than bacteria. Many fungi grow optimally at pH 5 or below and a few grow well at pH values as low as 2. Various species of *Thiobacillus* and genera of archaea including *Sulfolobus* and *Themoplasma* are acidophilic.

Thi. ferroxidans and *Sulfolobus* sp. oxidize sulfide mineral and gives sulphuric acid. the most important fazctor for obligate acidophily is the cytoplasmic membrane of obligatory acidophilic bacteria which actually dissolves and lyses the cell wall. It suggests that high concentration of H^+ ions are required for membrane stability.

Highly acidic environment is formed naturally from geochemical activities and from the metabolic activities of certain acidophile themselves. Acidophiles are also observed in the debris left over coal mining. Interestingly, acid-loving extremophiles can not tolerate great acidity inside their cells, where it would destory DNA. They survive through keeping the acid out. But the defensive molecules provide this protection as well as others that come in contact with the environment must be able to operate in extreme acidity. Indeed **extremozymes** are able to work at pH below one, more acidic than even vinegar or juice of stomach. These enzymes have been isolated from the cell wall and underlying cell membrane of some acidophiles.

(i) Physiology : Obligate acidophiles have an optimum pH for growth which remains extremely low. To shield the intracellular enzymes and other components from low to medium pH, the organisms maintain a large pH gradient across the membrane. Special forms of lipids are found in their membrane which may minimize the leakage of H^+ down the pH value. e.g., the presence of certain fatty acids has been reported to provide special adaptations to growth and survival at extremely low pH. Acidophiles maintain the cytoplasmic pH around 6.5. In these organisms, the pH remains generally 1-2 that is lower in comparison to neutrophiles and alkalophiles. In acidophiles the pH is compensated by positive inside electric potential which is opposite to which present in neutrophiles. The reversed electric potential is generated by electrogenic K^+ uptake which permits the cells to extrude more H^+ and thus maintain the internal pH.

(ii) Molecular Adaptation : Most critical factor for obligate acidophily lies in the cytoplasmic membrane. If the pH is raised to neutrality, the cytoplasmic membrane of obligately acidophilic bacteria actually dissolve and the cells lyse. It is suggested that the high concentration of hydrogen ions are required for stability of membrane that allows bacteria to survive.

(iii) Applications : Potential applications of acid-tolerant extremozymes range from catalysts for the synthesis of compounds in acidic solutions to additives for animals feed which are intended to work in animal stomach. If added to feed, the enzymes improve the digestibility of expensive grains therefore avoiding the need for more costly food. Rusticyanin proteins from acidophiles help in acid stability. Expression of heterogenous arsenic resistance genes in the iron-oxidizing *Thiobacillus ferrooxidans* has been established like biotechnological approach of bioremediation. ●

☛ Q.8. What are Alkalophilies? What kinds of physiological and molecular adaptations allow it to grow optimally under pressure?

Ans. **Alkalophiles**

Alkalophiles live in soils laden with carbonate and in Soda lakes, e.g. those observed in the Rift Valley of Africa and the west U.S. The first alkalophilic bacterium was reported in year 1968. Most alkalophiolic prokaryotes studied have been aerobic non-marine bacteria and reported as *Bacillus* spp. Krulwich and Guffanti (1989) separated them into two broad categories: alkali-tolerant organisms (pH 7.0-9.0) and alkalophilic organism. Most of the aklaophilic organisms are aerobic or facultative anaerobic. Some alkalophiles are *Bacillus alkalophilus. B. firmus* RAB *Bacillus* sp. No. 8-1 and *Bacillus* sp. No. C-125 which bear flagella and hence are motile. The flagella induced motility is considered by a sodium motive force instead of proton motive force. They are motile at pH 9-10.5 but no motility is seen at pH 8. The Indigo-fermentation process. Their cell wall contains acidic compounds similar in composition to peptidoglycans.

(i) Molecular Adaptation : Alkalophiles contain unusual diether lipids bonded with glycerol phosphate just like other archaea. In these lipids, long chain, branched hydrocarbons, either of the phytanly or biphytanyl type are present. The intracellular pH remains neutral in order to stop alkali-labile macromolecules in the cell. The intracellular pH may very through 1-1.5 pH units from neutrality which helps those organisms to survive in highly alkaline external environment.

(ii) Physiology : The cell surface of alkalophiles can maintain the neutral intracellular pH in alkaline environment of pH 10-13. The recommended concentration of NaOH for large scale fermentation is 5.2% depending upon organism. The pH should remain 8.5-11. Sodium ions are needed for growth, sporulation and also for germination. The presence of sodium ions in the surrounding environment has proved to be essential for effective solute transport through the membranes.

In the Na^+ ion membrane transport system the H^+ is exchanged with Na^+ by Na^+/H^+ antiport system, thus generating a sodium motive force. This drives substrate accompanied through Na^+, ions into the cell. The incorporation of α-aminobutyrate increased two fold as the external pH shifts from 7 to 9 and the presence of Na^+ ions significantly enhance the incorporation. Molecular cloning of DNA fragments conferring alkalophily was isolated and cloned. This fragment is responsible for Na^+/H^+ antiport system in the alkalophily of alkalophilic micro- organisms.

(iii) Applications : Some alkalophiles produce hydrolytic enzymes such as alkaline proteases, which function well at alkaline pH. These are used as supplements for house hold detergents. For example an alkaline protease known as **subtilisin** has been produced from *B. subtilis* which is used in detergent. The stone washed denim fabric is due to the use of these enzymes. These enzymes soften and fade fabric by degrading cellulose and releasing dyes.

Table : Some extremozymes and their applications

S. No.	Extremozyme	Uses
1.	Acidozymes	Sulphate oxygenase, *Thiobacillus* dehydrogenase, rusticyanin (acid stable e^- carrier) and thromopsin.
2.	Alkalozymes	Protease (detergents), amylase (starch industry), cyclomaltodextrin glucanotransferase (chemical and pharmaceutical), pullunases (detergents), xylanses (pulp and paper industry), pectinases (paper production).
3.	Halozymes	Proteases, alkaline phosphatases, lipases and amylases are used in industry for manufacturing of detergents.
4.	Psychozyme	Rectinase, lipase, cellulase, amylase for detergents, Food processing (cheese making), meat tendering, lactate hydrolysis), Biosensors (environmental applications), biotransformations and contact lens cleaning solutions.
5.	Thermozymes	Required for DNA amplification reactions and industrially important product formation.

Q.9. What are Barophilis? Give it physiology, molecular adaptation and Applications.

Barophiles

Ans.

Barophiles are those bacteria that grow at high pressure at 400-500 atmosphere (atm) on 2 to 3°C. These conditions exist in deep-sea habitat about 100 metre in depth. Many are barotolerant and do not grow at pressures above 500 atm. but some live in the gut of invertebrates *Photobacterium, shewanella* and *Cholwelha* inhabit more rapidly. Some thermophilic archaea are barophiles for example *Purococcus* spp. and *Methanococcus jannaschii.* Barophiles adapt the extreme pressure involving macromolecular structures in cells. Increasing pressure makes structures more compacts and this tendency had been the principle of microscopic ordering.

(i) Molecular Adaptation : In the cytoplasmic membranes of high pressure tolerant microbes. The amount of unsaturated fatty acids is more which allows the adaptative significance. Further, the adaptativity may also be because of changes in protein composition of the cell wall outer membrane called OmpH protein, a type of *porin.* The porins are structural proteins meant for diffusion of organie molecules through the outer membrane and into the periplasm. It is found that OompH system is pressure-dependent and needed for growth at high pressure.

(ii) Physiology : There are variations in membrane structure and function. The amount of monounsaturated fatty acids in the membrane increases due to increase in the pressure. The organism is thereby able to circumvent the loss of membrane fluidity imposed by increasing the pressure.. Since the pressure decreases, membrane fluidity presumably increases and the cells respond by decreasing the level of mono-unsaturated fatty acids. it is evidenced that increased pressure decreases the binding capacity of enzymes for their substrates. Hence the enzymes must be folded in such a way as to minimize these pressures in baropiles. It is not known whether H^+, Na^+ or both are used as coupling ions in energy transduction in these organisms.

(iii) Applications : Barophiles are the major source of unsaturated fatty acids or polyunsaturated fatty acid. The microbial barophilism is helpful in enhancing the mining. Underground mining operations usually found at increased prerssures and temperatures and barophilic thermoophiles are better adapted under such situations. Recently, vacant sait-mine area has been worked out as fermenters for the biological gasification of pretreated lignite.