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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – MICROBIAL ECOLOGY – SMB2101

UNIT:1

MICROBIAL HABITATS

Microbial ecology or environmental microbiology is the ecology of microorganisms: their relationship with one another and with their environment. It concerns the three major domains of life—Eukaryota, Archaea, and Bacteria—as well as viruses.

Microbial Habitats

- Microbes are present in every kind of habitat.
- Microbes are incredibly diverse thriving in environments from the very cold to the extremely hot.
- They are also tolerant of many other conditions such as limited water availability, high salt content and low oxygen levels.
- Not every microbe can survive in all habitats.

Terrestrial Microbial Habitats: Microbes lives in/on soil.

- Only one percent of microbes that live in soil have been identified.
- These organisms take part in the formation of soil and are essential components of their ecosystems.
- Bacteria and fungi that live in soil feed mostly on organic matter such as other plants and animals.
- These microbes are very sensitive to their local environment.
- Factors such as the levels of carbon dioxide, oxygen, pH, moisture and temperature all affect the growth of microbes in the soil.

Microbial Habitats in Other Organisms

- Microbes also live on other organisms.
- As with the ones found on people these microbes can be harmful or beneficial to the host.

Example:

- Bacteria grow in nodules on the roots of pea and bean plants.
- These microbes convert nitrogen from the air into a form that the plants can use.
- In many ways animals and plants have evolved as habitats for the millions of microbes that call them home.

Extreme Microbial Environments

- The microbes living in extreme conditions are called extremophiles.
- This literally means that they love the extreme conditions of their habitat.
- The extremophiles are so well adapted to their own environment.
- Some like the ones in hot springs need extreme temperatures to grow.

ROLE OF MICROBES IN BIOGEOCHEMICAL CYCLE

Nutrients move through the ecosystem in biogeochemical cycles.

- A biogeochemical cycle is a pathway by which a chemical element (such as carbon or nitrogen) circulates through the biotic (living) and the abiotic (non-living) factors of an ecosystem.
- The elements that move through the factors of an ecosystem are not lost but are instead recycled or accumulated in places called reservoirs (or "sinks") where they can be held for a long period of time.
- Elements, chemical compounds, and other forms of matter are passed from one organism to another and from one part of the biosphere to another through these biogeochemical cycles.
- Microorganisms play a primary role in regulating biogeochemical systems in virtually all of our planet's environments. This includes extreme environments such as acid lakes and hydrothermal vents, and even includes living systems such as the human gut.
- The key collective metabolic processes of microbes (including nitrogen fixation, carbon fixation, methane metabolism, and sulfur metabolism) effectively control global biogeochemical cycling.

- Incredibly, production by microbes is so immense that global biogeochemistry would likely not change even if eukaryotic life were totally absent!
- Microbes comprise the backbone of every ecological system, particularly those in which there is no light (i.e. systems in which energy cannot be collected through photosynthesis)

MICROBIAL SUCESSION

- **Ecological succession** is the process of change in the species structure of an ecological community over time. The time scale can be decades (for example, after a wildfire), or even millions of years after a mass extinction
- Succession of micro-organisms including fungi and bacteria occurring within a microhabitat is known as microsuccession or serule.
- Like in plants, microbial succession can occur in newly available habitats (primary succession) such as surfaces of plant leaves, recently exposed rock surfaces (i.e., glacial till) or animal infant guts,
- Growth on disturbed communities (secondary succession) like those growing in recently dead trees or animal droppings.
- Microbial communities may also change due to products secreted by the bacteria present. Changes of pH in a habitat could provide ideal conditions for a new species to inhabit the area.

Membrane biofouling in waste water reactor: An example for microbial succession

- In Phase I (0–2 days), small sludge flocs in the bulk liquid were selectively attached on membrane surfaces, leading to the formation of similar extracellular polymeric substances (EPS) and microbial community composition as the early biofilms.
- Dominant populations in small flocs, e.g., *Nitrosomonas*, *Nitrobacter*, and *Acinetobacter* spp., were also the major initial colonizers on membranes.

- In Phase II (2–4 d), fouling layer structure, EPS composition, and bacterial community went through significant changes. Initial colonizers were replaced by fast-growing and metabolically versatile heterotrophs (e.g., unclassified *Sphingobacteria*).
- The declining EPS polysaccharide to protein (PS:PN) ratios could be correlated well with the increase in microbial community diversity.
- In Phase III (5–14 d), heterotrophs comprised over 90% of the community, whereas biofilm structure and EPS composition remained relatively stable.
- The overall microbial succession pattern from autotrophic colonization to heterotrophic domination implied that MBR biofouling
- In some cases the new species may outcompete the present ones for nutrients leading to the primary species demise. Changes can also occur by microbial succession with variations in water availability and temperature.

SOIL MICROFLORA

- Microflora means, bacteria and microscopic algae and fungi, living in a particular site or habitat.
- 1 g of soil contain
- 100,000,000 bacterial cells
- 11,000 species of bacteria
- Also fungi and larger animals

Types of Microbes in Soil

- Prokaryotic Bacteria, Actinomycetes
- Fungi
- Algae
- Protozoa
- Viruses

BACTERIA

- Tiny 1µm width, one celled
- Single cell division
- 1can produce 5 billion in 12 hours in lab
- (in environment limited by predators, water and food availability)
- Abundant in rhizosphere
- Zone surrounding root –dead root cells and exudate stimulate microbial growth

MAIN TYPES OF SOIL BACTERIA

- Agarobacterium, Alcaligenes
- igenes, Bacillus
- Arthrobacter, Cellulomonas

  Corynebacterium
- Caulobacter, Micrococcus
- Clostridum, Pseudomonas
- Flavobacterium, Achromobacter
- Methanobacterium
- Chromobacterium
- Agarobacterium, Alcaligenes
- igenes, Bacillus
- Arthrobacter, Cellulomonas

  Corynebacterium
- Caulobacter, Micrococcus
- Clostridum, Pseudomonas
- Flavobacterium, Achromobacter

- Methanobacterium
- Chromobacterium

SOIL BACTERIA-ROLE IN SOIL

- Degrade organic matter
- Fix N2-and other steps of N cycle
- Rhizobium-nitrogen fixation
- Nitrification-Nitrosomonas, Nitrobacter
- Organisms involved with vital functions may be in lower aboundance
- Organisms present will depend on many factors-Nutrients, O2, Moisture, pH.

SOIL ACTIOMYCETES

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- Actinomycetes are slow growing compared to bacteria and fungi
- Streptomyces may be up to 65% of soil population
- Nocardia and Micromonospora upto 35% of population
- Other common genera
- Streptosporangium
- Actinoplanes
- Thermoactinomyces

- Thermomonospora
- Role of Actinomycetes in soil1. Organic matter decomposition
- Starch, cellulose, hemicellulose, lignin, humus

2. Antibiotic production

- Streptomycin, tetracycline, chloramphenicol
- 3. Maintain Microbial equillibrium in soil
- Control pathogenic organisms
- Produce proteases lyse cells of pathogens
- Fungi Grow as long threads (hyphe)
- Push through soil particles, roots, rocks
- Often group into masses called mycelium (look like roots)
- Higher fungi have basidium: club shaped structure
- Bearing fruiting body

PREDOMINANT FUNGI IN SOILFUNGI IMPERFECTI: MOST PREDOMINANT GROUP IN SOIL

- Cephalosporium
- Verticillium
- Monilia
- Trichoderma
- Fusarium
- Cladosporium
- Gliocladium
- Role of fungi in soil Organic matter degradation: Degrade starch, hemicellulose, cellulose, lignin,
- Ammonification: degrade proteins, nucleicacids and release NH3

- Control of other organisms: some fungi kill nematodes
- Pathogenicity: Many fungi associated with plant disease-ythium, Sclerotinia, Puccinia
- Formation of mycorrhizae, symbiotic relationship with plant root.
- AlgaeFilamentous, colonial, unicellular
- Photsynthetic
- Most in blue –green group, but also yellow-green, diatoms, green algae
- Form carbonic acid (weathering)
- Add OM(Organic matter) to soil, bind particles
- Aeration
- Some fix nitrogen
- Algal groups in soilChlorophyaceae-Green algae
- Cyabophyaceae-Bluegreen algae
- Bacillariophyaceae: Diatoms
- Xanthophyaceae-Yellow green algae
- Importance of algae in soilProvide organic matter
- Many algae produce polysaccharides, improve soil structure
- Produce oxygen, provide oxygen to rice plants
- Fix nitrogen and provide to rice plants after decomptosition

STRATIFICATION OF FRESHWATER

- Freshwater are areas which having low salt concentration approximately less than 1%, and organisms that can not survive in other regions that have high salt concentration such as sea and oceans.

Types

1. Ponds and lakes

2. Streams and rivers

- Freshwater are areas which having low salt concentration approximately less than 1%, and organisms that cannot survive in other regions that have high salt concentration such as sea and oceans.
- Few square meters to thousands of square kilometers.
- Many ponds are seasonal, lasting for a few months, while lakes may exist for hundreds of years.
- Ponds and lakes may have limited species diversity, except ponds and lakes that connected to other water sources such as river and ocean.

Lakes and ponds are divided into three different zones according to their depth and distance from the shoreline

1- littoral zone

near of the shore either lakes or

ponds, also it is the warmest zone

because of high absorbance to the sun's heat.

- it's contain several species of

algae (like diatoms), rooted and

floating aquatic plants, grazing

snails, clams, insects, crustaceans, and fishes.

- only the egg and larvae stages are found in this zone.
- The vegetation and animals living in the littoral zone are food for other creatures such as turtles, snakes, and ducks.
- **2- limnetic zone**
- the near surface after littoral zone, well lighted, and has a large amount of phytoplankton and zooplankton.

3- profundal zone

This zone is much colder, also there is a Little light penetrates into this zone.

- zone's organisms are heterotrophs, , meaning that they eat dead organisms and use oxygen for cellular respiration.
- For example, dead plankton that fall down from limnetic zone.
- **During the summer,**

the temperature can range from 4° C near the bottom to 22° C at the top.

- **During the winter,**

the temperature at the bottom can be 4° C while the top is 0° C (ice).

- **In between the two layers**, there is a narrow zone called the thermocline where the temperature of the water changes rapidly.
- **During the spring and fall seasons**,

there is a mixing of the top and bottom layers, usually due to winds, which results in a uniform water temperature of around 4° C and 10 °C.

- This mixing also circulates oxygen throughout the lake or ponds.
- new study showed that ,Microorganisms were more active at low temperatures, low dissolved oxygen concentrations and high TN/TP ratios.
- During stratification, the metalimnion (which is a layer of thermal stratification) was observed from 2 to 10 m in spring and then dropped to 16 m to 21 m in summer, and then appeared between 24 and 26 m depths in fall.
- Also, during stratification, temperature, and pH were significantly higher in the epilimnion and rapidly dropping down in the hypolimnion.
- The nutrient concentrations were stable in the water column, except for total phosphorus.
- Specifically, the TN/TP mass ratio was significantly lower in epilimnion

STREAMS AND RIVER

These are bodies of flowing water moving in one direction.

- they get their starts at headwaters, which may be springs, snowmelt or even lakes, and then travel all the way to their mouths .e.g. ocean.
- The temperature is cooler at the source than it is at the mouth.
- the water has high oxygen levels, and freshwater fish such as trout and heterotrophs can be found there
- The middle part of the stream/river, the width increases, as does species diversity, also numerous aquatic green plants and algae can be found.
- The mouth of the river/stream, the water becomes murky from all the sediments that it has picked up upstream, decreasing the amount of light that can penetrate through the water.
- Because of the lower oxygen levels in mouth, fish that require less oxygen, such as catfish and carp, can be found. However, Since there is less light, there is less diversity of flora.

- **Microbial Flora in Fresh water**
  1. Neuston layer is the collective term for the organisms that float on the top of water or live right under the surface.
- Uppermost layer / surface microlayer of the hydrosphere
- Is the interface between the atmosphere and the hydrosphere
- An extreme environment
    - Many adverse factors (i.e. exposure to radiation, temperature fluctuations) can occur
- Insoluble and less dense organic material accumulates in this layer and as a result, is aligned with non-polar organic materials
    - Therefore, is a thin gel-like structure where microbes can live
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Fresh water microbial communities

Depending on composition, organization and functioning as communities, fresh water microbial communities can be divided as follows;

- Planktonic community
- Sediment community
- Microbial mats
- Biofilms

Planktonic community

- Organisms that have little or no control over where they go – Plankton
- Plankton are not essentially needed to be microscopic. But mostly, they are..
- Fish are not plankton, they are nekton.
- Plankton can be;
- Plant-type : Phytoplankton
- Bacteria type: Bacterio-plankton
- Animal type: Zooplankton
- Zoo plankton can be divided in to;

Permanent zoo plankton Temporary zoo plankton

(Example: Barnacle larvae: nauplii)

- Temporary zoo plankton are rare in fresh water ecosystems
- Phytoplankton and Primary production

  Are producers and base of many food webs
- Are very productive
- The principle component is the diatom, a form of single celled alga
- These diatoms have diurnal rhythm in a water column
- And can quickly reproduce and are highly productive

Phytoplanktonic primary production

- Many phytoplankton are single-celled algae.
- Fix dissolved carbon dioxide and produce various organic compounds
- Primary production is dependent upon;
  - Availability of essential nutrients
  - Water temperature
  - pH of the water
- Organic matter produced can be divided as;
  - **P**articulate **O**rganic **M**atter **(POM)**
  - **D**issolved **O**rganic **M**atter **(DOM)**

Diatoms

- Principle component of phytoplankton community
- A form of single-celled algae with an outer wall made out of silica
- Have a **diurnal rhythm** in water columns
  - At nights, sink to lower levels

- At day moves to upper levels to obtain solar energy

Detritus

- All dead organic matter distinguishable from living matter
- POM is about 10%, DOM is about 90%
- Includes bodies and body fragments of dead organisms as well as excreta and fecal material

Microbial Mats

- Together with biofilms, are defined as surface associated layers of microbial cells embedded in **Extracellular Polymeric Substances (EPS)**
- Microbial mats are, multi-layered sheets of microorganisms that are mainly formed by bacteria and archaea

Habitats??

- Mainly grow on submerged or moist surfaces
- Few can survive in deserts
- Few are endosymbionts

- Can colonize environments at -40 to 120 Celsius
- Usually held together by slimy Matrix substances created by inhabitant microbes
- Some inhabitants form tangled web of filaments which makes the mat tougher
- Mats are usually vertically stratified. Aerobic zone on the top is separated from bottom anaerobic zone by a layer of oxidized iron
- Mats can grow to few cm of thickness at most. But still, creates a several layers of different internal chemical environments
- Each layer is composed of microorganisms of the same or closely related species that can tolerate or feed on dominant chemicals at their level

- In each layer, dominant microbes are decided upon their comparative advantage

  or the ability o out perform other microbes to live and survive in that layer

  - It is dependent upon their metabolic capabilities and conditions they can tolerate
  - As metabolic capabilities decided by the phylogeny, several closely related microbes can inhabit the same layer
- However, ecological relationship between microorganisms within the same mat is a combination of both competition and cooperation.
- Hence, different layers are divided based on their individual metabolic contribution to the microbial community within and also by their phylogenetic relationships.
- A microbial mat generally forms its' own food chain where;
  - By-products of one group serve as 'food' for another group of microbes within the mat
  - One or two groups may remain on top of the food chain as their by-products are not

  utilized by others

Biofilms

- An aggregate of microbes in which cells that are frequently embedded within a self-produced matrix of EPS, adhere to each other and/or to a surface.
- Biofilm EPS is a polymeric conglomeration and may contain;
  - Extracellular DNA
  - Polysaccharides
  - proteins

Habitats;

- Found commonly on submerged solid substrates or substrates exposed to an aqueous solution.
- On mats floating on liquid surfaces
- On surfaces of leaves in high humidity

- A biofilm may contain many different types of microbes each of which perform specialized metabolic function within the mat
- Some species are capable of forming single-species biofilms under certain

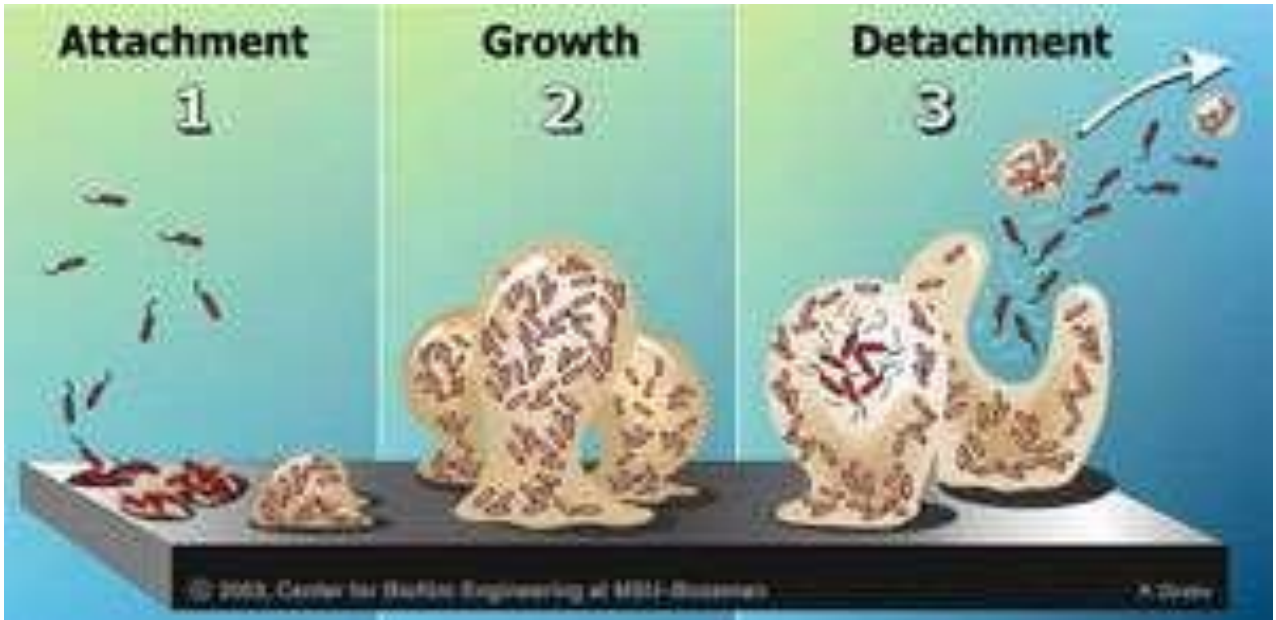
  conditions
- Social structure within a particular biofilm (i.e. interactions within organisms present such as competition and cooperation) is highly dependent on types of organisms present
- However, microbial cells growing in a biofilm are physiologically distinct

  from planktonic cells of the same organism
- A biofilm may grow very quickly, from microscopic to macroscopic when sufficient resources are available

Life cycle of a biofilm

- Biofilm lifecycle can be summarized in to three important steps;

1. Attachment
2. Growth and development
3. Detachment



Step 1: Attachment

- Initial attachment of a free floating microbe to a surface is reversible
    - Occur using weak Van Der Waal bonds
- Secondly it attaches more permanently using;
    - Cell adhesion structures like pili
    - Cell adhesion molecules such as extracellular proteins
    - Hydrophobicity
- When considered, hydrophobicity is the most important of the above three
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- When considered, hydrophobicity is the most important of the above three
- Hydrophobicity determines the ability of microbes to form biofilms
    - Increased hydrophobicity means less repulsion between the EPS matrix and the bacterium
- Bacteria pioneers who initially attaches to the surface starts building the EPS matrix that holds the biofilm together
    - In addition to matrix substances and bacterial cells, EPS matrix may also contain materials from environment such as minerals, soil particles etc.
- This facilitate the arrival of other biofilm bacteria by providing more

adhesion sites

    - If there are species that are unable to attach to a surface on their own, they are often able to anchor the matrix directly to earlier colonists

- When a single bacterium joins a biofilm,
    - Expression of approximately 800 genes have reported to be altered and differentially regulated
    - As a result, cell undergoes a phenotypic shift in behavior
    - This impart different physiological characteristics to the joined bacterium than its planktonic members
- During colonization cells within the biofilm are able to communicate via Quorum Sensing

Step 2: Growth and Development

- Growth of a biofilm occurs through a combination of
    - Division of existing cells
    - Recruitment of new cells
- The next sub-step; is the development of the biofilm.
- At the development state;
    - Biofilm gets well established
    - May only change in size and shape
- Once the biofilm has fully formed,
- It contains channels in which nutrients and also, signaling molecules involved in Quorum sensing can circulate.
- Cells in different regions exhibit different patterns of gene expression
- As a result of above, biofilms often develop their own metabolism
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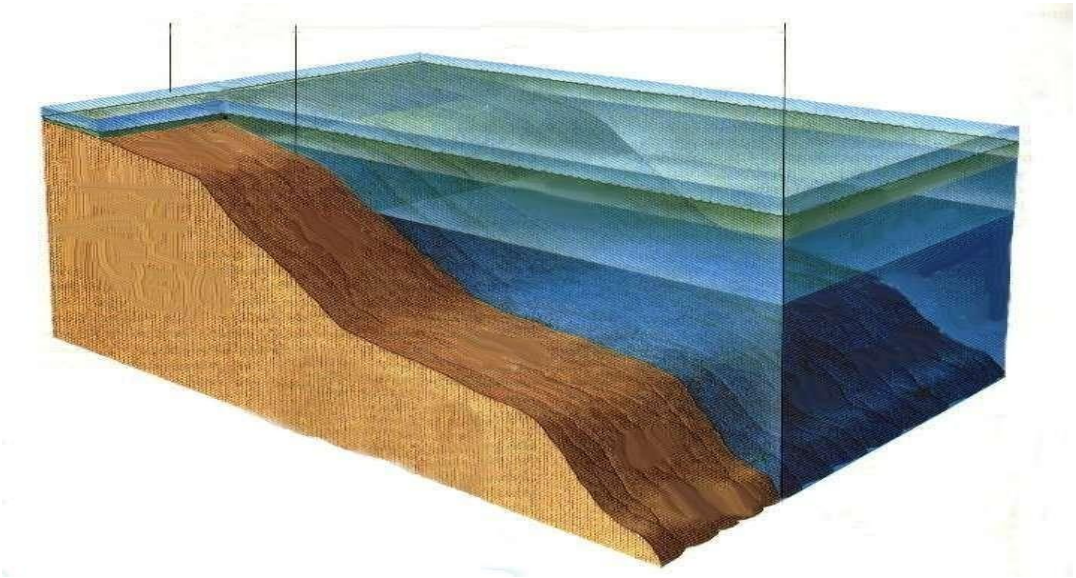
OCEAN ZONES

- The four major zones are :
- Intertidal Zone
- Neritic Zone
- Oceanic Zone
- Benthic Zone



Intertidal Zone

- Area between high tide line and low tide line
- Organisms adapted to harsh, changing environments

- intertidal area (also called the littoral zone) is where the land and sea meet, between the high and low tide zones. This complex marine ecosystem is found along coastlines worldwide. It is rich in nutrients and oxygen and is home to a

Neritic Zone

- Area over the continental shelf
- Area of greatest density and diversity of marine life
- Neritic Zone is located above the contiental shelf which extends along the coastline of the major land masses of the world
- **Producers**

  Primary producers are organisms that convert inorganic carbon in carbon dioxide into organic carbon by autotrophs. There are two types of primary producers. They are phytoplankton, or autotrophic producers, and zooplankton, or heterotrophic producers.

- **Consumers**

  The main pelagic consumers that live along the continental shelf include herring, mackerel, Bluefin Tuna, capelin, and some smaller species which feed on zooplankton and smaller fish. Sea Birds also feed on the fish within the Neritic Zone because it is close to shore.

Oceanic Zones

Epipelagic Zone

- Photic Zone or Sunlight Zone, Enough sunlight for photosynthesis. Primary area of food production. From surface down to about 200 meters

Mesopelagic Zone

- Disphotic Zone or Twilight Zone. From about 200 m down to 1,000 m
- Animals that live in the disphotic zone, like many types of squid, are adapted to life in near darkness, cold water, and high pressure.

Bathypelagic Zone

- Aphotic zone or Midnight Zone. Animals that thrive in the *aphotic zone* are used to living without light!
- No sunlight
- From 1,000 m down to 4,000 m
- Low density and diversity of marine life

Abyssopelagic Zone

- Aphotic Zone or Midnight Zone
- No sunlight
- From 4,000 m down to 6,000 m
- Low density and diversity of marine life

Hadopelagic Zone

- Aphotic Zone or Midnight Zone
- No sunlight
- From 6,000 m down to ocean floor, or around 11,000 m
- Low density and diversity of marine life

Benthic Zone

The benthic zone is home to many different creatures and dead organisms. This zone begins at the end of the intertidal zone through the dark abyss of the deep ocean. In the Benthic Zone there are few different types of environments.

- 2 Basic Types:
    - Sessile - Live attached to the bottom
    - Vagrant - Able to move about
- Sessile:

- Barnacles
- Sponges
- Corals
- Sea Anemones
- Oysters
- Clams

- **Marine microbial diversity**
- In the marine environment, 90% of bacteria are Gram-negative with different characteristics
- and the Gram-negative cell wall is better adapted
- for survival in the marine environment.
- Three domains of Bacterial life exist: Archaea, Bacteria and Eucarya.
- Archaea includes unusual microorganisms which grow under extreme environments and differs from Bacteria due to lack of peptidoglycan.
- Both these domains collectively play a significant role in the marine environment.

Table 1. Different physiological groups of marine bacteria

Group	Physiology	Role in marine environment	Example
Archaebacteria Sulphate-reducing bacteria	Chemoautotrophs, anaerobic, thermophilic and mesophilic.	Contribute over 50% of the carbon turnover of coastal marine sediments; take part in the cycling of sulphur compounds in sea water.	*Desulfomonas*, *Desulfovibrio*, *Desulfobulbus*, *Desulfotomaculum* and *Desulfococcus*
Methanogenic bacteria	Chemoautotrophs, strictest anaerobes, utilize a limited number of simple carbon compounds (hydrogen, carbon dioxide, formate, acetate and methanol) as their carbon and energy sources for methanogenesis.	Utilize trimethylamine in the marine environment as substrate and produce methane as an end-product of their energy-generating metabolism.	*Methanococcus, Methanosarcina*, *Methanomicrobium*, *Methanogenium*, *Methanoplanus*, *Methanococcoides* and *Methanobolus*
Halophilic bacteria	Require at least 12–15% NaCl to survive and grow well even at concentrations up to saturation.	Red colonies formed due to high carotenoid content and dominate in high salt environments, such as salterns and salt lakes; regulate the osmotic pressure thereby resisting the denaturing effects of salt in their environment.	*Haloarcula*, *Halobacterium*, *Haloferax* and *Halococcus*
Eubacteria Luminous bacteria	Produce light by a simple protein-like substance called luciferin in contact with the oxygen molecule; Gram-negative and motile heterotrophic rods.	Bioluminescence in the deep ocean helps the organisms defensively to startle and divert predators (defence), to attract prey (offence) and to camouflage. Luminous bacteria help in cycling of nutrients in the sea and contribute in the nutrition of marine organisms as gut microflora.	*Photobacterium leiognathi*, *Photobacterium phosphoreum*, *Vibrio fischeri* and *Vibrio harveyi*
Nitrifying bacteria	Oxidize either ammonia to nitrite (*Nitrosococcus*) or nitrite to nitrate (*Nitrococcus*) and convert nitrogen to a form readily available for other biological processes.	Extremely important process, since positively charged ammonium ions bind to acidic sediment particles, where they become available for biological processes; more abundant in nearshore waters than in offshore regions.	*Nitrosococcus*, *Nitrococcus*, etc.

ROLE OF BACTERIA IN THE MARINE ENVIRONMENT:

The marine environment is characterized by parameters such as high pressure, salinity, low, temperature, absence of light, etc.

Marine heterotrophic bacteria have adapted themselves to survive in this environment:

- They require Na+ for growth because it is essential to maintain the osmotic environment for protection of cellular integrity.
- Oligotrophy is also one more adaptation because of the small amount of available nutrient.
- Heterotrophic bacterial action promotes organic degradation, decomposition and mineralization processes in sediments and in the overlying water, and releases dissolved organic and inorganic substances.
- By mineralization of organic matter, are again available for primary producers.
- These heterotrophic bacteria comprise the bulk of microbial populations inhabiting the water column of oceans and are responsible for much of

the biological transformation of organic matter and production of carbon dioxide.

- Decomposition of protein takes place by proteolytic bacteria, e.g. Pseudomonas and other eubacteria.
- Cellulose is decomposed by cellulolytic bacteria, e.g. Cytophaga, Sporocytophaga.
- Chitin, which is synthesized by several marine organisms as extracellular material from algae, cell walls of some chlorophytes, exoskeletons, including molts from cope pods and other marine invertebrates is a structural polysaccharide. biopolymer is degraded by chitinolytic or chitinoclastic bacteria, e.g. Bacillus, Pseudomonas and Vibrio, by their exoenzyme chitinase.
- Pectins are also decomposed by numerous bacteria in anaerobic condition, e.g. Clostridium, pectinovorum and the end-products are pectic acid and methanol.
- **Cyanobacteria (blue-green bacteria)**
    - Photosynthetic bacteria which are found in environments high in dissolved oxygen, and produce free oxygen
    - Store excess photosynthetic products as cyanophycean starch and oils
    - Primary photosynthetic pigments are chlorophyll *a* and chlorophyll *b*
    - Accessory pigments include carotenoids and phycobilins

Nutritional Types

- **Cyanobacteria (con't)**
    - Chromatic adaptation—response of pigment composition to the quality of light in the sea
    - May exist as single cells or form dense mats held together by mucilage

        - form associates called stromatolites—a coral-like mound of microbes that trap sediment and precipitate minerals in shallow tropical seas.

- **Other photosynthetic bacteria**
    - Anaerobic green sulfur and purple sulfur and non-sulfur bacteria do not produce oxygen
    - The primary photosynthetic pigments are bacteriochlorophylls
    - Sulfur bacteria are obligate anaerobes (tolerating no oxygen)
    - Non-sulfur bacteria are facultative anaerobes (respiring when in low oxygen or in the dark and photosynthesizing anaerobically when in the presence of light)
- **Chemosynthetic bacter**ia
    - Use energy derived from chemical reactions that involve substances such as ammonium ion, sulfides and elemental sulfur, nitrites, hydrogen, and ferrous ion
    - Chemosynthesis is less efficient than photosynthesis, so rates of cell growth and division are slower
    - Found around hydrothermal vents and some shallower habitats where needed materials are available in abundance
- **Heterotrophic bacteria**
    - Decomposers that obtain energy and materials from organic matter in their surroundings
    - Return many chemicals to the marine environment through respiration and fermentation
    - Populate the surface of organic particles suspended in the water by secreting mucilage (glue-like substance)
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- **Heterotrophic bacteria**
    - Association of heterotrophic bacteria with particles in the water column aids with:
        - Consolidation: adjacent particles adhere
        - Lithification: formation of mineral cement between particles
        - Sedimentation: settling of particles
    - Marine snow: large, cobweb-like drifting structures formed by mucus secreted by many kinds of plankton, where particles may accumulate

NITROGEN FIXATION AND NITRIFICATION

- Nitrogen fixation: process that converts molecular nitrogen dissolved in seawater to ammonium ion
    - Major process that adds new usable nitrogen to the sea
    - Only some cyanobacteria and a few archaeons with nitrogenase (enzyme) are capable of fixing nitrogen
- Nitrification: process of bacterial conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) ions
    - Bacterial nitrification converts ammonium into a form of nitrogen usable by other primary producers (autotrophs)

SYMBIOTIC BACTERIA

- Many bacteria have evolved symbiotic relationships with a variety of marine organisms
- Endosymbiotic theory

    - Mitochondria, plastids & hydrogenosomes evolved as symbionts within other cells.
- Chemosynthetic bacteria live within tube worms and clams
- Some deep-sea or nocturnal animals host helpful bioluminescent bacteria
    - Photophores
    - Embedded in the ink sacs of squid

ARCHAEA

- General characteristics
    - Small (0.1 to 15 micrometers)
    - Prokaryotic
    - Adapted to extreme environmental conditions: high and low temperatures, high salinities, low pH, and high pressure
    - Formerly considered bacteria
    - Differences from bacteria
        - Cell walls lack special sugar-amino acid compounds in bacterial cell walls
        - Cell membranes contain different lipids, which help stabilize them under extreme conditions

NUTRITIONAL TYPES

    - Archaea includes photosynthesizers, chemosynthesizers and heterotrophs
    - Most are methanogens: anaerobic organisms that metabolize organic matter for energy, producing methane as a waste product
    - Halobacteria (photosynthetic), thrive at high salinities, trap light using bacteriorhodopsins, purple proteins
- Hyperthermophiles

- organisms that can survive at temperatures exceeding 100^{o} C, such as near deep-sea vents
- Potential for biomedical and industrial application

EUKARYA

- Eukarya includes all organisms with eukaryotic cells
- Examples:
  - plants
  - animals
  - fungi
  - algae
  - single-celled animal-like protozoa

FUNGI

- History of marine mycology
  - Marine fungi first discovered in 1849
  - Marine fungi's ecological role is difficult to evaluate; biomass needs to be quantified
  - Important in marine ecosystems as decomposers, prey, pathogens and symbionts
- General features of fungi (con't)
  - Store energy as glycogen
  - kingdom Fungi is divided into 4 phyla:
    - Chytridiomycota (motile cells)
    - Zygomycota (e.g. black bread mold)
    - Basidiomycota (club fungi, e.g. mushrooms)
    - Ascomycota (sac fungi)

    - In the sea, ascomycotes are the most diverse and abundant fungi
- Ecology and physiology of marine fungi
    - Can be either obligately marine, requiring ocean or brakish water or facultatively marine (primarily of terrestrial or fresh water origin)
    - Salinity is toxic to fungi, so they must apply energy to removing sodium
    - Most marine fungi live on wood from land
    - Some live on grass in salt marshes
    - Others live on algae, mangroves or sand
    - Fungi decompose the chitinous remains of dead crustaceans in open sea plankton communities
- Marine yeasts reproduce asexually by budding—mitosis that produces daughter cells of unequal size
- Filamentous marine ascomycotes can reproduce sexually by forming a fruiting body called an ascocarp, a structure which produces ascospores
- Lichens: mutualistic associations between a fungus and an alga
    - fungi are usually ascomycotes
    - algae are usually green or blue-green bacteria
- The fungus provides attachment, general structure, minerals, moisture
- The alga produces organic matter through photosynthesis.

Stratification of Atmosphere

- Earth's atmosphere can be divided (called atmospheric stratification) into five main layers.
- Exosphere: 700 to 10,000 km (440 to 6,200 miles)
- Thermosphere: 80 to 700 km (50 to 440 miles)

- Mesosphere: 50 to 80 km (31 to 50 miles)
- Stratosphere: 12 to 50 km (7 to 31 miles)
- Troposphere: 0 to 12 km (0 to 7 miles)

Exosphere

- The exosphere is the outermost layer of Earth's atmosphere.
- Exosphere—contains few particles that move into and from space.
- This layer is mainly composed of extremely low densities of hydrogen, helium and several heavier molecules including nitrogen, oxygen and carbon dioxide closer to the exobase. The atoms and molecules are so far apart that they can travel hundreds of kilometers without colliding with one another.
- Exobase—the lower boundary of the exosphere.

T**hermosphere**

- The thermosphere is the second-highest layer of Earth's atmosphere. This layer is completely cloudless and free of water vapor.
- Temperature increases with height. The temperatures can rise to 1,500 degrees Celsius, but it would not feel warm because of the low air pressure in this layer.
- The International Space Station orbits Earth in this layer.
- Mesopause—the boundary between the mesosphere and the thermosphere; the coldest place on Earth.

Mesosphere

- The mesosphere is the third highest layer of Earth's atmosphere, occupying the region above the stratosphere and below the thermosphere.
- The layer in which most meteors burn up after entering Earth’s atmosphere and before reaching Earth’s surface.

- Stratopause—the boundary between the mesophere and the stratosphere.

Stratosphere

- The stratosphere is the second-lowest layer of Earth's atmosphere.
- Contains the ozone layer; the layer where volcanic gases can affect the climate.
- Tropopause—the boundary between the stratosphere and troposphere.

Troposphere

- The layer closest to Earth's surface in which all weather occurs. It contains 75% of the atmosphere's mass and 99% of the total mass of water vapour and aerosols.

Microflora of atmosphere

- Microbes normally found in atmosphere within 300-10000 feet above from the land.
- Fungal spores which are found in air consist of Alternaria, Cladosporium, Penecillium and Aspergillus found above 4000 feet from the land, found in both polar and non polar air masses.
- Organisms found below 500 feet is mainly in overpopulated area, these include spores of Bacillus and Clostridium, ascospores of yeast and fragments of mycelium, mould, streptomycetaceae, pollen, protozoan cysts, algae, Micrococcus and corynebacterium.
- Air found in school and hospital or living places of the person suffered from infectious disease usually found microbes like tubercle bacilli, streptococci and pneumococci.
- Many different microorganisms can be in aerosol form in the atmosphere, including viruses, bacteria, fungi, yeasts and protozoans. In order to survive in the atmosphere, it is important that these microbes adapt to some of the harsh climatic characteristics of the exterior world, including temperature, gasses and humidity. Many of the microbes that are capable of surviving harsh conditions can readily form endospores, which can withstand extreme conditions.

- Many of these microorganisms can be associated with specific and commonly known diseases.

Airborne Plant pathogens and Diseases

Human Disease	Pathogen
Bacterial diseases	
Pulmonary tuberculosis	Mycobacterium tuberculosis
Pneumonia	Klebsiella pneumoniae
Pulmonary anthrax	Bacillus anthracis
Legionellosis	Legionella spp.
Whopping cough	Bordetella pertussis
Diptheria	Cornebacterium diptheriae
Fungal diseases	
Aspergillosis	Aspergillus fumigatus
Coccidioidomycosis	Coccidioides immitis
Viral disease	
Influenza	Influenza virus
Hantavirus pulmonary synd.	Hantavirus
Hepatitis	Hepatitis virus
Chicken pox	Herpesvirus
Common cold	Picornavirus
Dengue fever	Flavivirus

- **Microorganisms Resist atmospheric stress:**

Bacterial

- One such bacterial microorganism that can resist environmental stresses is *Bacillus anthracis*. It is a gram positive rod shaped bacteria that utilizes spore formation to resist environmental stresses. The spore is a dehydrated cell with extremely thick cell walls which can remain inactive for many years.

- This spore makes *Bacillus anthracis* a highly resilient bacteria, allowing it can survive extreme temperatures, chemical contamination, and low nutrient environments (Gatchalian 2010). This bacteria is associated with Anthrax, which is a severe respiratory disease that infects humans.

- The characteristics of atmosphere as a habitat include extreme temperature variations, light, temperature, low amount of available water and organic water.
- All these characters make the atmosphere unsuitable for growth of micro organisms. Usually most of the organisms are found in the lower region of atmosphere.
- The air in the atmosphere is often exposed to sunlight thus it contains less moisture and higher temperature.
- Thus if the micro organisms are not protected from desiccation, almost most of the organisms will die.
- The origin of the micro organisms takes place through various ways. Soil is one of the source to transfer micro organisms to the air. Whenever the wind blows it disturbs the micro organisms and liberate them into the air and these micro organisms remains suspended in the air for long time.
- Another way of transferring micro organisms to the air is by manmade actions like plugging and digging.

DISPERSAL OF AIR MICROBES

- Though there are significantly less atmospheric microorganisms than there are in oceans and in soil, there is still a large enough number that they can affect the atmosphere (Amato, 2012).
- Once suspended in the air column, these microbes have the opportunity to travel long distances with the help of wind and precipitation, increasing the occurrence of widespread disease by these microorganisms.
- These aerosols are ecologically significant because they can be associated with disease in humans, animals and plants.

Airborne microbial pathogen transmission

1. Airborne droplet nuclei (small particles of 5 mm or smaller in size).

- Airborne droplet nuclei develop when the fluid of pathogenic droplets (1-5 µm in size. They are so small and light they may remain suspended in the air for several hours. Thus, they may also infect persons entering a room which has been left by a patient long ago. Also, airborne droplet

nuclei can be widely dispersed by air currents. Tuberculosis, chickenpox, measles and possibly also influenza may be transmitted this way.

2. Dust particles containing infectious agents

- Microoganisms carried in this manner remain suspended in the air for long periods of time and can be dispersed widely by air currents. Because of this, there is risk that all the air in a room may be contaminated. Some examples of microorganisms that are transmitted by the airborne route are Transmission occurs when droplets containing microorganisms generated during coughing, sneezing and talking are propelled through the air.
- These microorganisms land on another person, entering that new person's system through contact with his/her conjunctivae, nasal mucosa or mouth.
- These microorganisms are relatively large and travel only short distances (up to 6 feet/2 metres).
- M. tuberculosis, rubeola, varicella and hantaviruses
- However these infected droplets may linger on surfaces for long periods of time, so these surfaces (within the range of the coughing/sneezing person) will need additional cleaning.
- For this reason there may be both Droplet and Contact Precautions required at the same time. Examples of microorganisms that are spread by droplet transmission are: influenza, colds, respiratory syncytial virus (RSV) and some organisms causing pneumonia.

EXTREMOPHILES

- Organisms found living in extreme harsh environments.
- Word originated from Greek- ***Extremus*** + ***Philia*** which

means **extreme loving**.

- Most members of this group comes under the **domain Archae.**

- These include **thermophiles**, **hyperthermophiles, thermoacidophiles, alkaliphiles, psychrophiles, halophiles, barophiles, radiation resistant bacteria and endoliths.**
- Organisms lives in **physically/geochemically** extreme conditions that are mostly detrimental for other forms of life.
- In other words, an extremophile is a microorganism, mostly an Achaeon that lives in conditions of **extreme acidity, alkalinity, temperature, salinity, pressure, nutrient scarcities etc.**

HOW DO THEY LIVE SO?

- **Extremozymes**- specialized enzymes that are highly stable.
- Can tolerate extremes of **temperature, pH, salinity** that would inactivate other enzymes.
- Important in industries because of this property.

EXTREME CONDITIONS

- **Temperature**
- **pH**
- **Salinity**
- **Nutritional scarcities**
- **Absence of oxygen**
- **Radiation**
- **Pressure**

Table 6.3
Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkalophile	Growth optimum between pH 8.5 and 11.5	*Bacillus alcalophilus, Natronobacterium*
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Has growth optimum around 20–45°C	*Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Bacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile*
Hyperthermophile	Has an optimum between 80 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O_2 for growth	*Micrococcus luteus, Pseudomonas, Mycobacterium;* most algae, fungi, and protozoa
Facultative anaerobe	Does not require O_2 for growth, but grows better in its presence	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Grows equally well in presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	Does not tolerate O_2 and dies in its presence	*Clostridium, Bacteroides, Methanobacterium, Treponema agilis*
Microaerophile	Requires O_2 levels below 2–10% for growth and is damaged by atmospheric O_2 (20%)	*Campylobacter, Spirillum volutans, Treponema pallidum*
Pressure		
Barophilic	Growth more rapid at high hydrostatic pressures	*Photobacterium profundum, Shewanella benthica, Methanococcus jannaschii*

TYPES

Psychrophiles

- **Temperatue range is -15 to 15^0C**
- Also known as **cryophiles**.
- Have an optimum temperature of **15^0C** or lower
- Isolated from **Arctic and Antarctic habitats** (90% of the ocean is 5^0C or colder)
- Also found in **ice bergs, glaciers, snowfields** etc
- Metabolism is quite normal at colder temperatures.
- Cell membranes-high levels of **fatty acids** which remain fluid at colder temperatures.
- **Proteinaceous antifreeze mechanism** to protect the cell and DNA

- Some of them cause spoilage in refrigerated food materials.
- **Eg:** ***Arthrobacter spp, Psychrobacter spp, Halomonas spp, Pseudomonas, sphingomonas***

FIRMICUTES

- **Gram positive, spore forming** bacterial family that can survive desiccation and can survive extreme conditions.
- This group also is an example for **extremophilic true bacteria (eubacteria).**
- Plays an important role in the spoilage of beer, wine and cider.
- Eg: ***Helicobacterium spp, Mycoplasma, Clostridium spp.***
- Many members of the Family Firmicutes are also thermophiles**.**
- Eg: ***Bacillus stearothermophilus***
- Recently, a DNA polymerase derived from these bacteria, **Bst polymerase** has become important in biotechnology.
- Bst polymerase- **helicase** like activity (making it able to unwind DNA strands.
- Optimum functional temperature is **60-65^{0}C** and get inactivated at temperatures above **80^{0}C**

THERMOPHILES

- Greek- thermotita (heat) and philia (love)
- Temperature loving organisms.
- Most members are **Archae**
- Grows in a temperature range of **55-113^{0}C**
- Mostly found in geothermally heated regions on earth viz., **hot springs, hydrothermal vents** etc.
- As they need extreme temperature, its very hard to study

them under laboratory conditions.

- Also that some members can produce heaty by themselves (compost and garbage landfills).
- Eg : ***Cyanidium caldarium, Chaetomium thermophile***
- **Deinococcus-thermus** is a small group of eubacteria which can thrive environmental hazards.
- Stains Gram positive (thick cell wall) but possesses an

outer membrane, similar to the Gram negative cell wall.

- Several thermophilic bacteria comes under this group.
- It is the source of heat resistant enzyme- **taq polymerase,** which is well used in **PCR.**
- The enzyme is isolated from ***Thermus aquaticus.***

CLASSIFICATION OF THERMOPHILES

1. Obligate thermophiles

- Also known as extreme thermophiles.
- Temperature range is **80-122^{0}C.**
- Membranes and proteins are unusually stable at these extreme temperatures.
- For this reason, most biological processes utilize thermophilic enzymes because of their ability to withstand intense heat.
- Many of this group can resist radiation too.
- Eg: ***Methanopyrus kandleri,*** can survive and reproduce at 122^{0}C, ***Sulfolobus spp , Pyrococcus spp, Pyrodictium spp*** (optimum of 113^{0}C)

- Most of the members require **elemental sulfur** for growth.
- Anaerobic members use sulfur as **electron acceptor**

  instead of oxygen in cellular respiration.
- Some are **lithotrophs** that oxidizes sulfur to sulfururic acid as an energy source.
- Such organisms require a very low pH and hence

  known as **thermoacidophiles**.
- Inhabits regions associated with volcanic eruption viz; hot, sulfur rich, acidic regions such as **hot springs, natural geysers, fumaroles** etc .

Thermoacidophiles

- Requires both high temperature and highly acidic environment for optimum growth.
- Preferred temperature range is **70-80^{0}C** and have an optimum

  pH range of **2-3**.
- All the organisms discovered belongs to the **Domain Archae,**

  so far.
- They can thrive in **acidous and sulfur** rich environments.
- Instead of cell wall, possesses a unique membrane composed of **tetraether lipoglycan**, which gives the unusual stability for the bacteria.
- Eg: ***Thermoplasma acidophilum*** and ***T.volcanium***

Facultative Thermophiles

- Rare group of organisms that can live both in higher temperature and normal temperature are referred to as facultative thermophiles.
- These organisms can live at **20^{0}C**, and have an optimum of **50^{0}C**. Maximum temperature that they can survive is **60^{0}C.**
- Eg: ***Bacillus flavothermus***

ACIDOPHILES

- Microorganisms that lives in highly acidic

  environments are called as acidophiles.

- The pH range is 1-5.
- Some members that mainly found in the drainage of coal mines are able to oxidize **sulfur into sulfuric acid.**
- Mechanism of action is that they have a proton pump machinery to eliminate protons from the cytoplasm of the cell to maintain low pH.
- Eg: ***Pyrodictium, Picrophilus, Ferroplasma, Sulfolobus***

ALKALIPHILES

- These are extremophilic microorganisms which thrives in **roughly alkaline environments (8-11)**, and have an optimum of pH around **10**.
- Organisms which needs high pH to survive are called as **obligate alkaliphiles.**
- There are facultative alkaliphiles and **haloalkaliphiles**

(needs salty environment as well).

- Most of the alkaliphiles possess a **bacillus** morphology.
- Eg**: *Bacillus halodurans* C125, *Bacillus firmus* OF4**
- Two methods for surviving
  - The cell will be having a unique cellular machinery that works best in alkaline range of pH.
  - The cell will have to acidify the cytosol to nullify the effect of the high pH outside the cell.
- Experimental studies revealed that the cytosolic enzymes of alkaliphiles functions best in a neutral pH range (7.5-8.5).
- This shows that for surviving in highly alkaline pH, the cell must have some pH regulatory mechanism to protect the plasma membrane.

- The mechanism is that the cell wall contains acidic polymers composed of residues such as **galacturonic acid, gluconic acid, glutamic acid, aspartic acid, and phosphoric acid.**
- This protects the PM by preventing the entry of hydroxide ions and allowing the entry of s**odium (Na^+) and hydronium ions(H^+)**

XEROPHILES

An extremophilic organism that can grow and reproduce in conditions with a **low availability of water.**

- **Water activity (a_w)** is a measure of the amount of water within a substrate an organism can use to support sexual growth.
- Xerophiles are often said to be "**xerotolerant**", meaning tolerant of dry conditions. They can survive in environments with water activity below **0.8.**
- **Endoliths and halophiles** are often xerotolerant.
- Eg: many molds and yeast,

  Trichosporonoides nigrescens

HALOPHILES

- This group comprises microorganisms that can thrive in high salty environments such as The Great Salt Lake and Dead Sea.
- Most of the halophiles belong to the Domain Archae.
- **Eg: *Salinibacter ruber***
- There are eukaryotic halophiles such as ***Dunaliella salina*** (algae) and ***Wallemia icthyophaga (***fungus).
- Extreme halophiles/obligate halophiles-adapted to

  survive high salt concentrations

- Organisms from Dead Sea often requires nearly 33% salt (sea water has only 3%), and the inoculating loop must be dipped in a saturated salt solution to isolate them.
- Microorganisms live in such high salinity are termed as extreme halophiles

Mechanism

Mainly employ two mechanism to prevent desiccation through osmosis.

Both strategies work by increasing the osmotic

concentration of the cell.

1.In first method (followed my most halophiles including bacteria, archae etc) organic compounds are accumulated in the cytoplasm.

- They are known as **osmoprotectants or compatible solutes.**
- It include **sugars, aminoacids, polyols, betaines** etc.

These compounds can be synthesised or accumulated from the environment.

- Eg**:** ***Ectothiorhodospira halochloris***

2. The second is the selective influx of **potassium ions (K^+)** into the cytoplasm.

- This adaptation is restricted to **moderately halophilic** organisms.
- The entire intracellular machinery (enzymes, structural proteins etc) is highly adapted to withstand the high saline environment.
- Eg: Bacteria comes under the Family

Halobacteriaceae

- The **16S rRNA** studies opens a broad range of information on the field of evolution.

ENDOLITHS

- Endolith is an organism (archae, bacterium, fungus, lichen or algae) that lives in nutritionally poor environments such as inside a rock or something.
- Particularly interesting in the area of astrobiology (exobiology).
- These organisms opens a clue for life beyond earth. There are chances of having life on endolithic environments such as mars and other planets.

Characteristics

- Endoliths have been found in rocks down to the depth of 3 km.
- It is not known that whether this is the limit since digging to the deep is highly expensive.
- The major threats to live in such depth is the high temperature.
- Recently discovered strains can reproduce at **121^0C.**
- All the discovered organisms are **autotrophs.**
- Some utilize gas or dissolved nutrients from water moving through fractured rocks
- Others may incorporate inorganic compounds found in their rock substrate (possibly by excreting acids to dissolve the rock).

Endoliths can be classified into

- **Chasmoendoliths**

  Colonizes fissures and cracks in the rock (chasmo- cleft)

- **Cryptoendolith**

  Colonizes structural cavities within porous rocks, including spaces produced and vacated by euendoliths (*crypto* = hidden)

- **Euendolith**

  Penetrates actively into the interior of rocks forming tunnels that conform with the shape of its body(*eu* = good, true).

Obligate Anaerobes

- Microorganisms which grow strictly in the absence of molecular oxygen are called as obligate anaerobes.
- For these, oxygen is a toxin
- For energy generation, they must employ fermentation or anaerobic respiration pathways.
- The toxic forms of oxygen are **Singlet Oxygen(O_2), Superoxide**
- **radicals (O^-), peroxide anion (O^{2-}), and hydroxyl radical (OH).**
- Some obligate anaerobes are ***Clostridium spp, Methanococcus*** and ***Methanopyrus***
- Microorganisms which can live both in the presence and absence of oxygen are known as **Facultative Anaerobes.**
- They can utilize oxygen if available or, continue their growth by fermentation and anaerobic respiration.
- Eg: ***Bacillus anthracis, Escherichia coli***
- To routinely grow and maintain in pure cultures, **reducing media** which stored in ordinary, tightly packed tubes is been used**.(media containing thioglycollate or cystein)**
- For culturing in petriplates, sealed boxes and jars in which oxygen removed completely is been used.
- Sometimes, certain chemicals which can produce hydrogen and carbon di oxide will be added and the so formed hydrogen will be incorporated with the oxygen present in the container to yield water
- This water can be utilized by the microorganisms.
- The most advanced system is that the media used for culture will be containing an enzyme- **oxyrase** which will bind with oxygen and eliminate as water. No addition of extra chemicals or hydrogen is needed.

Radiation

- Although most living things are sensitive to radiation, there are some microorganisms which can resist high levels of radiation.
- ***Deinococcus radiodurans*** is the radioresistant organism discovered so far which is a eubacteria.
- Their ability to withstand radiation is more than that of

  endospores.
- They can survive exposure to radiation doses as high as **15,000 Grays.** This much radiation is **1500 times** the dosage that would kill a human.
- The mechanism for this extraordinary resistance lies in a unique arrangement of its DNA that facilitates a rapid repair of radiation damage.
- It is similarly resistant to many mutagenic chemicals.

Barophiles

- Microorganisms that can survive under immense hydrostatic pressure.
- Generally found in ocean floors where pressure exceeds

  300 m (38 MPa).
- Some have been found at the bottom of the Pacific Ocean (Mariana Trench-10500 m) where pressure often exceeds 117 MPa.
- These organisms cannot grow in pressure below 400-500atm
- True obligate barophiles also comprises bacteria which present in the gut of holothurians and amphipods (crustaceans).
- Eg: ***Photobacterium, Shewanella, Colwellia***
- Some thermophilc archae such as ***Pyrococcus spp., Methanococcus jannaschii*** are barophiles too.

<u>SOME INTERESTING FACTS</u>

- ***Halomonas titanicae***- the bacterium which is responsible for rusting of RMS Titanic.

- ***Pseudomonas putida*** (superbug) is a genetically engineeredbacteria which literally "eats" petroleum products. These are very much useful in oil spills.

- **GFAJ-1** is a strain of rod shaped bacteria in the family **Halomonadaceae** which is anextremophile, highly resistant to the dangerous poison-**Arsenic.**

- There are chances of life forms beyond earth and the field of study is known as **astrobiology.**

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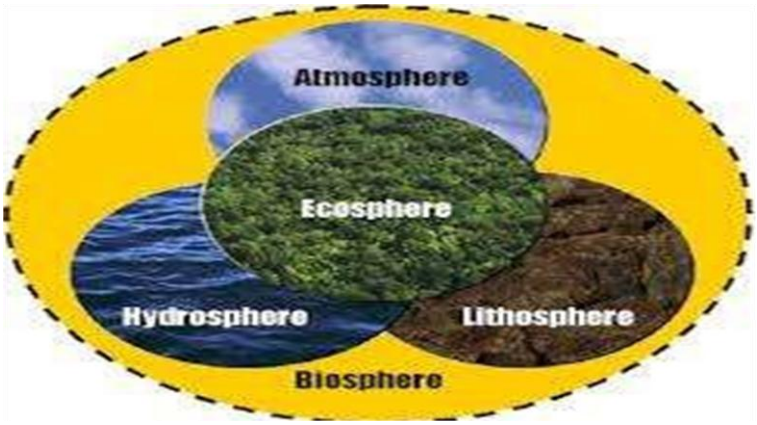
SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – II – MICROBIAL ECOLOGY – SMB2101

UNIT: II

BIOGEOCHEMICAL CYCLES

The term biogeochemical tells us that Biological, Geological & Chemical factors are involved. In earth science, a biogeochemical cycle is a pathway by which a chemical substances moves through both Biotic(Biosphere) & Abiotic(Lithosphere, Atmosphere & Hydrosphere) compartments of earth. A cycle is a series of change which comes back to the starting point & which can be repeated.



" More or less circular pathways, through which the chemical elements, including all the essential elements of the protoplasm, circulate in the biosphere from environment to organisms and back to the environment, are known as the Biogeochemical cycle".

- Biogeochemical cycles always involve Hot equilibrium states: A balance in the cycling of the elements between compartments.
- As biogeochemical cycles describe the movements of substances on the entire globe, the study of these is inherently multidisciplinary.

CYCLINIG ELEMENTS

Macronutrients

Macronutrients are required in relatively large amounts

- Big six": Carbon , Hydrogen , Oxygen , Nitrogen, Phosphorous.
- other Macronutrients:
- Sulfur, Potassium , Calcium , Iron , Magnesium

Micronutrients

Micronutrients are required in very small amounts, (but still necessary)

- Boron Copper Molybdenum

TYPES OF BIOGEOCHEMICAL CYCLES

Biogeochemical cycles can be classed as;

GASEOUS CYCLE

The term gaseous cycle refers to the transformation of gases between various biogeochemical reservoirs; Hydrosphere, Atmosphere & Biosphere Important gaseous cycles are;

- NITROGEN CYCLE
- OXYGEN CYCLE
- CARBON CYCLE
- WATER CYCLE

SEDIMENTARY CYCLE:

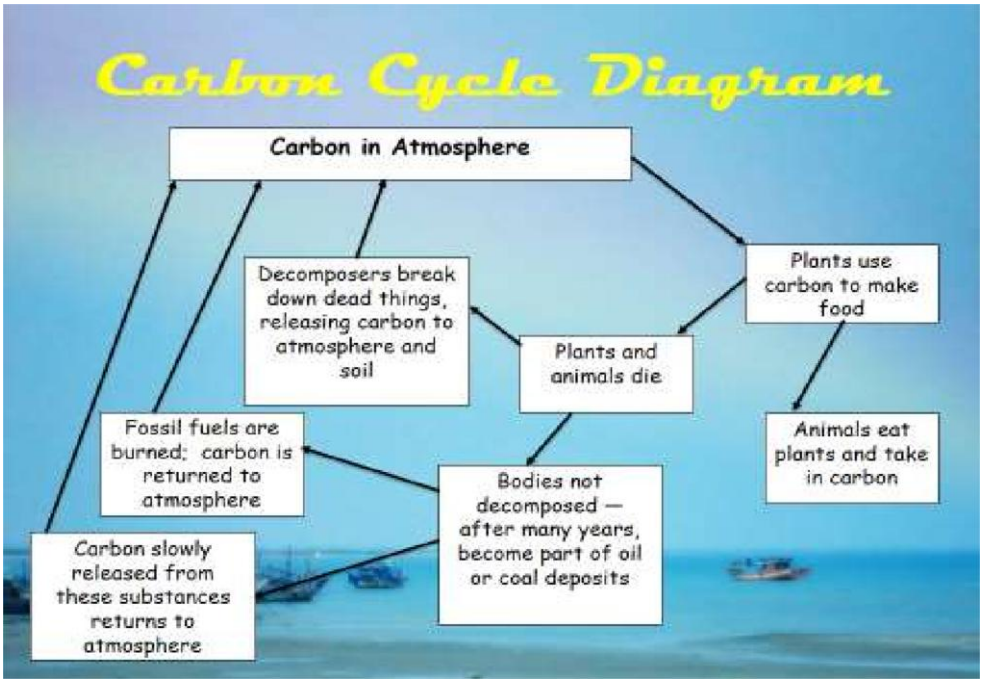
Leaching of minerals & salt's from the earth's crust, which the settle as sediment or rock before the cycle repeats. Sedimentary cycle includes;

- PHOSPHORUS CYCLE
- SULFUR CYCLE
- IRON CYCLE
- CALCIUM CYCLE
- Sedimentary cycles vary from one elements to another, but each cycle consist fundamentally of a solution phase & a sediment phase.

CARBON CYCLE

- **Carbon** is virtually important molecule in the carbon cycle.
- Proteins, nucleic acids, lipids, carbohydrates, and other molecules essential to life contain carbon.

- Carbon is present in the atmosphere as the gas carbon dioxide (CO_2), which makes up approximately <u>0.04% of the atmosphere.</u>
- It is also present in the ocean and fresh water as dissolved carbon dioxide. Carbons are also present in rocks such as limestone ($CaCO_3$).
- The global movement of carbon between the abiotic environment, including the atmosphere and ocean, and organisms is known as the **<u>CARBON CYCLE.</u>**



<u>Step 1: PHOTOSYNTHESIS</u>

- During photosynthesis, plants, algae, and cyanobacteria remove Carbon dioxide from the air and fix, or incorporate it into complex organic compounds such as glucose.
- Photosynthesis incorporates carbon from the abiotic into the biological compounds of producers.

<u>Step 2: DECOMPOSITION, ANIMAL & PLANT RESPIRATION, SOIL MICROORGANISM RESPIRATION.</u>

- ✓ Many of the compounds are used as fuel for cellular respiration by the producer that made them, by a consumer that eats producer, or by a decomposer that breaks down the remains of the producer or consumer.
- ✓ The process of a cellular respiration returns CO_2 to the atmosphere. A similar carbon cycle occurs in aquatic ecosystems between aquatic organisms and dissolved CO_2 in water.
- ✓ The process of photosynthesis incorporates the carbon atoms from carbon dioxide into sugars.
- ✓ Animals eat the plants and use the carbon to build their own tissues.
- ✓ Carnivores eat these animals and then use the carbon for their own needs.
- ✓ These animals return carbon dioxide into the air when they breathe, and when they die,the carbon is returned to the soil during decomposition.
- ✓ **Step 3: PARTLY DECOMPOSED PLANT REMAINS (COAL)**
- ✓ Millions of years ago vast coal beds formed from the bodies of ancient trees that were buried and subjected to anaerobic conditions before they had fully decayed.

Step 4: MARINE PLANKTON REMAINS

- ➢ The oils of unicellular marine organisms probably gave rise to the underground deposits of oil and natural gas that accumulated in the geologic past.
    - ➢ Coal, oil, and natural gas, called **fossil fuels** because they formed from the remains of ancient organisms. Fossil fuels are non- renewable resources. The Earth has a finite or limited supply of these resources.

Step 5: COMBUSTION (HUMAN & NATURAL)

- ✓ The process of burning or combustion, may return the carbon in oil, coal, natural gas, and wood to the atmosphere.

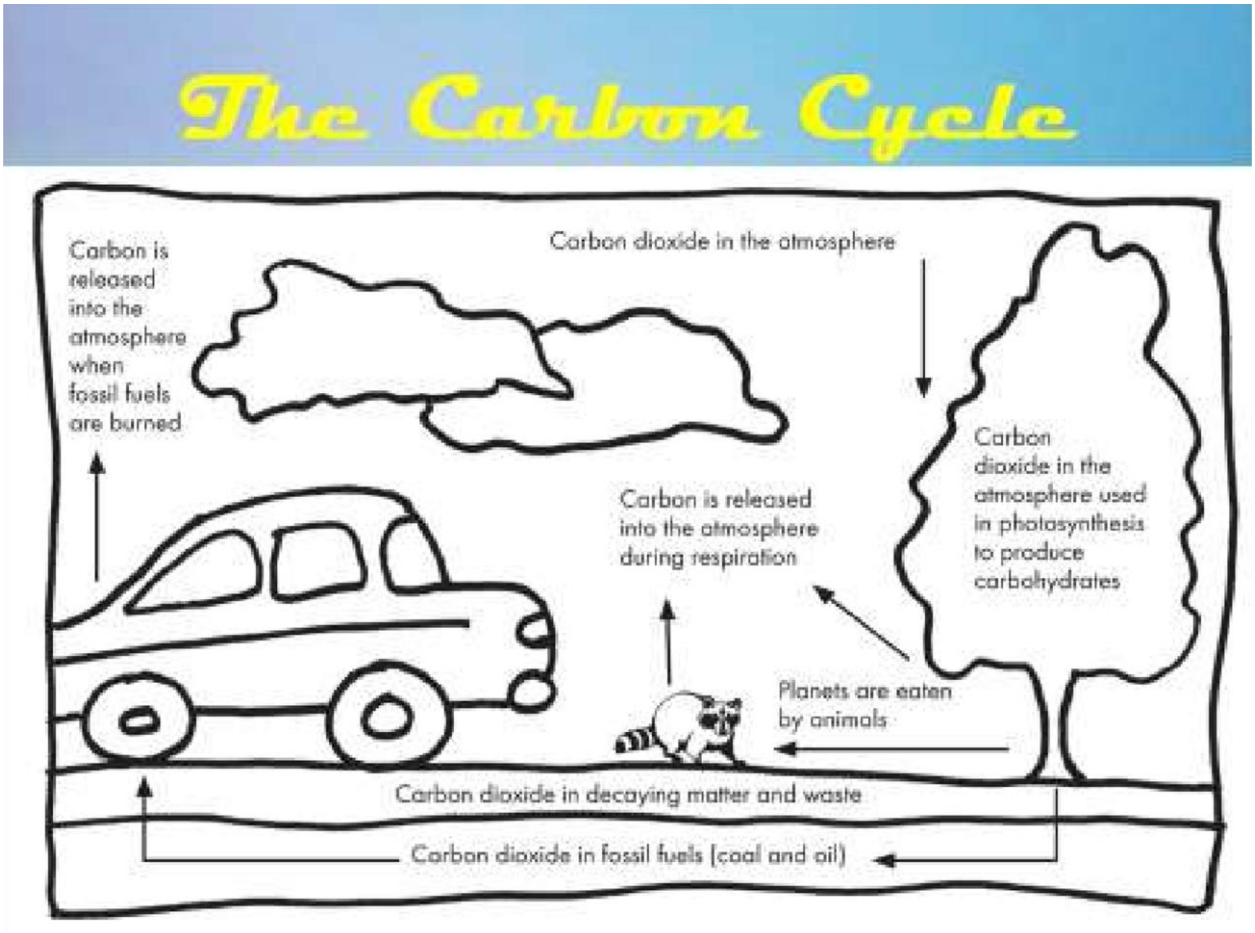
- ✓ In combustion, organic molecules are rapidly oxidized (combined with oxygen) and converted to carbon dioxide and water with release of light and heat.

Step 6: BURIAL AND COMPACTION TO FORM ROCK (LIMESTONE)

- An even greater amount of carbon that is stored for millions of years is incorporated into the shells of marine organisms.
- When these organisms die, their shells sink to the ocean floor and sediments cover them forming cemented together to form limestone.

Step 7: EROSION OF LIMESTONE TO FORM DISSOLVED CO2

- When the process of geologic uplift expose limestone, chemical and physical weathering processes slowly erode it away.
- This returns carbon to the water and atmosphere where it is available to participate in the carbon cycle once again.



- Thus, photosynthesis removes carbon from the abiotic environment and incorporate it into biological molecules.
- While, Cellular respiration, combustion, and erosion of limestone return carbon to the water and atmosphere of the abiotic environment.

Why carbon cycle is important?

- Many elements have cycle, but the cycling of carbon atoms is particularly important because:-
- Through photosynthesis and respiration, it is the way the earth produces food and other renewal resources.
- CO2 plays a key role in trapping heat in the atmosphere - one of the basic mechanisms behind the greenhouse effect.
- Carbon plays a central role in combustion.
- Through decomposition, it serves as the earth's waste disposal system.
- In addition, the carbon cycle is important because carbon-containing gases in the atmosphere affect the earth's climate.
- Increased CO2 in the atmosphere has been responsible for more than half of the climate warming observed in recent decades.

NITROGEN CYCLE

- The majority of earth's atmosphere is **Nitrogen(78%).** However, Atmospheric N2 has limited availability for biological use, and this form is relatively nonreactive and unusable by plants.
- Nitrogen availability can affect the rate of key ecosystem processes including primary production and decomposition
- The Nitrogen(N2) cycle is the process by which N2 is converted between its various chemical forms.
- This transformation can be carried out through both biological & physical processes.

PROCESS OF NITROGEN CYCLE

- Nitrogen is present in the environment in a wide variety of chemical forms including
- Organic nitrogen,
- Ammonium($NH4^+$),
- Nitrite(NO^-),
- Nitrate(NO^-),
- Nitrous oxide(N_2O),
- Nitric oxide (NO)
- Inorganic nitrogen gas.
- Organic nitrogen may be in the form of a living organism, humus or in the intermediate products of organic matter decomposition.
- The process of N2-cycle transform nitrogen from one form to another. Many of those processes are carried by microbes.

NITROGEN FIXATION

Atmospheric nitrogen must be fixed in a usable form to be taken up by the plants mostly fixation is done by free living (eg.Azotobacter and Closteridium or symbiotic (Rhizobium) known as Diazotrophs.

symbiotic nitrogen fixing bacteria such as Rhizobium usually live in the roots noodules of legumes. here they form a mutualistic relationship with the plant, producing ammonia in exchange for carbohydrates. today about 30% of the total fixed N2 is produced industrially using the Haber-Bosh process which uses high temperature and pressure to convert nitrogen gas and a hydrogen source in to ammonia. biological nitrogen fixation can be represented by following equation.

$N2 + 8H+8e^{-}+16ATP= 2NH_2+H_2+16ADP$

NITRIFICAITON

- The conversion of ammonia to nitrate is performed primarily by soil

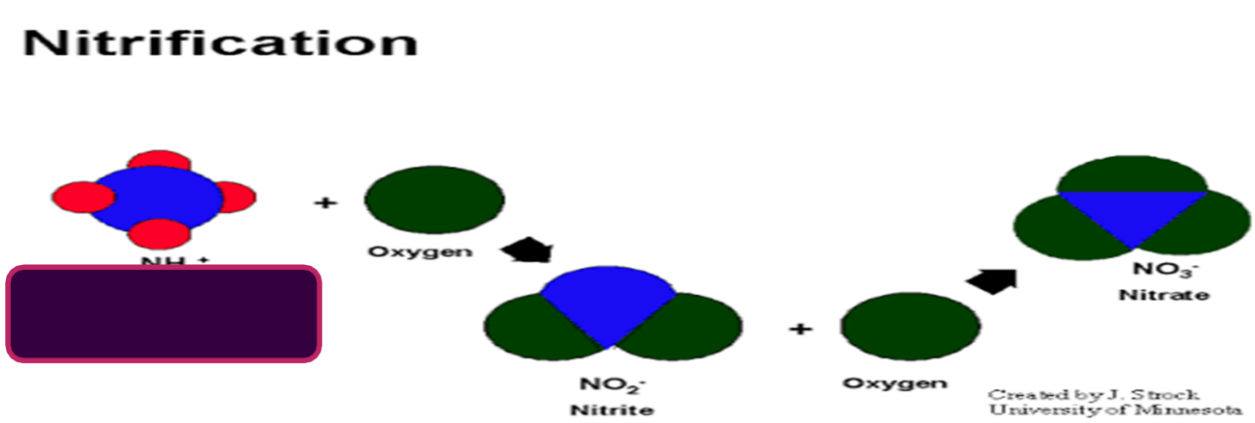
  living bacteria & other nitrifying bacteria.

- In the primary stage of nitrification the oxidation of ammonium is performed by bacteria such as the *Nitrosomonas* species, which convert ammonia to nitrites.

- Other bacterial species such as *Nitrobacter* are responsible for the

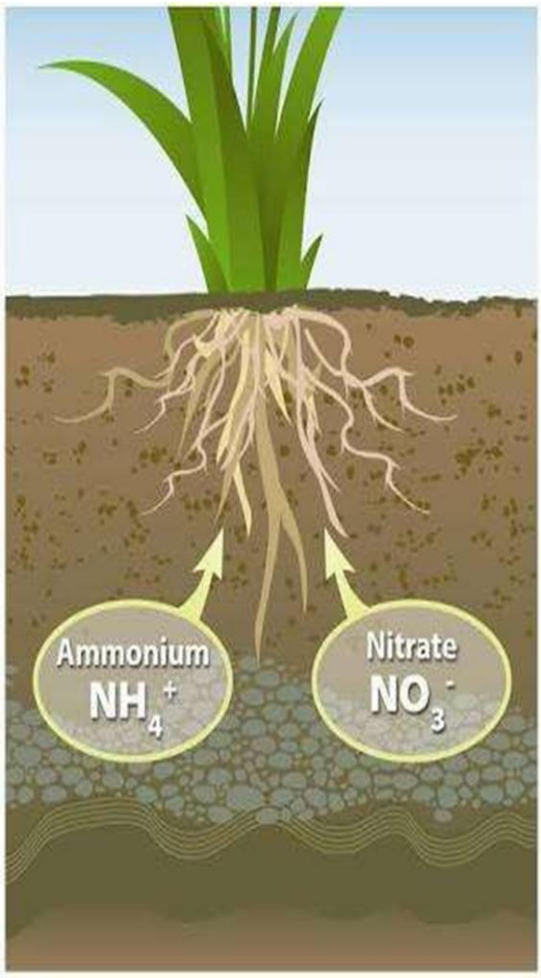
  oxidation of the nitrite into nitrates.

- It is important for the ammonia to be converted to nitrates or nitrites because ammonia gas is toxic to plants.



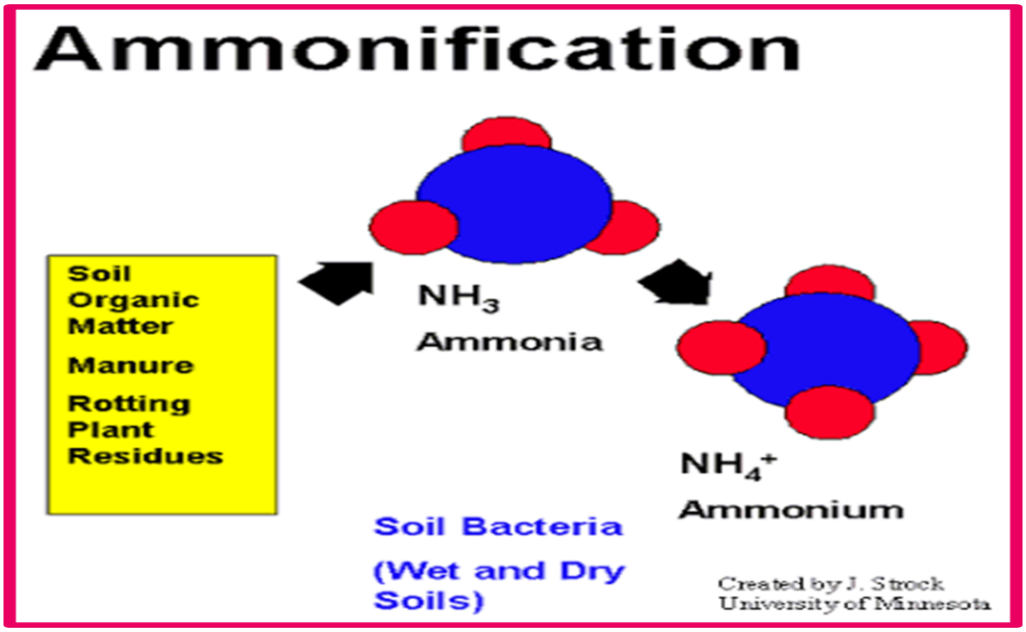
ASSIMILATION

- Plant take nitrogen from soil by absorption through their roots as **Amino acids**, **Nitrate ions**, **Nitrite ions**, or **Ammonium ions**.
- Plants can absorb nitrate or ammonium from the soil via their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporated into amino acids, nucleic acids & chlorophylls.
- In plants that have a symbiotic relationship with Rhizobia, some N2 is assimilated in the form of ammonium ions directly from the nodules.



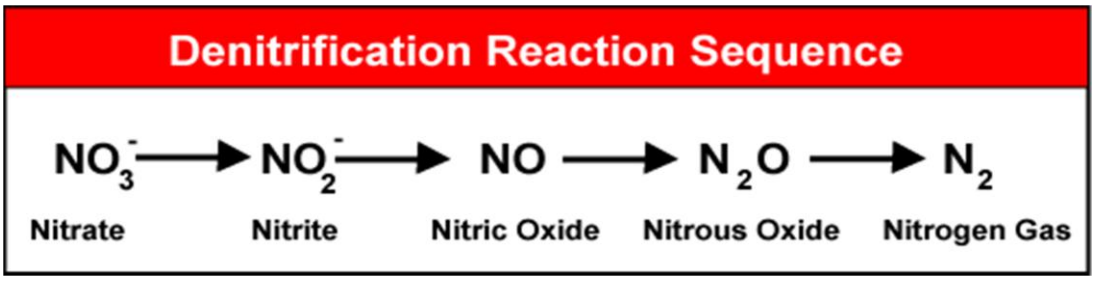
AMMONIFICATION

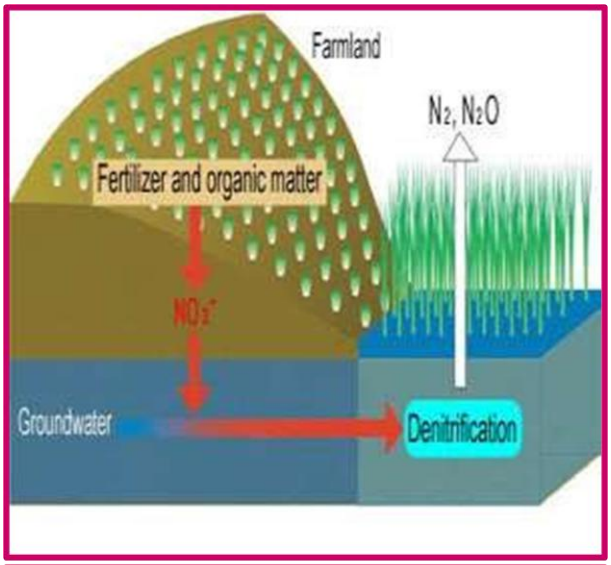
- When a plant or animal dies or an animal expels waste, the initial forms of N2 is organic.
- Bacteria or fungi convert the organic N2 within the remains back to ammonium, a process called **Ammonification** or **Mineralization**
- Enzymes are involved are; **GS : Gln synthetase**, **GOGAT : Glu-2-oxoglutarate GDH : Glu-dehydrogenase**

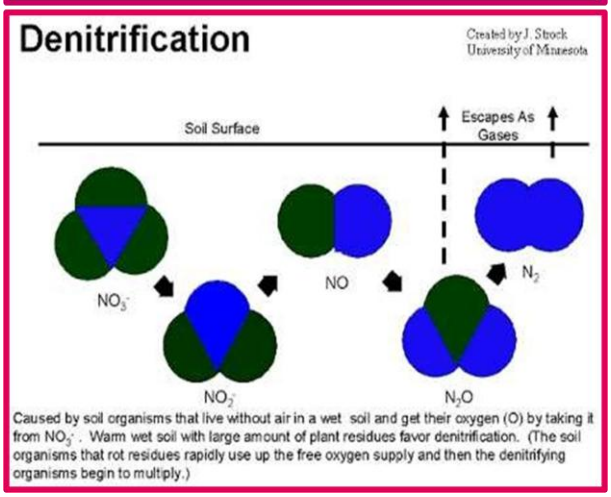


DENITRIFICATION

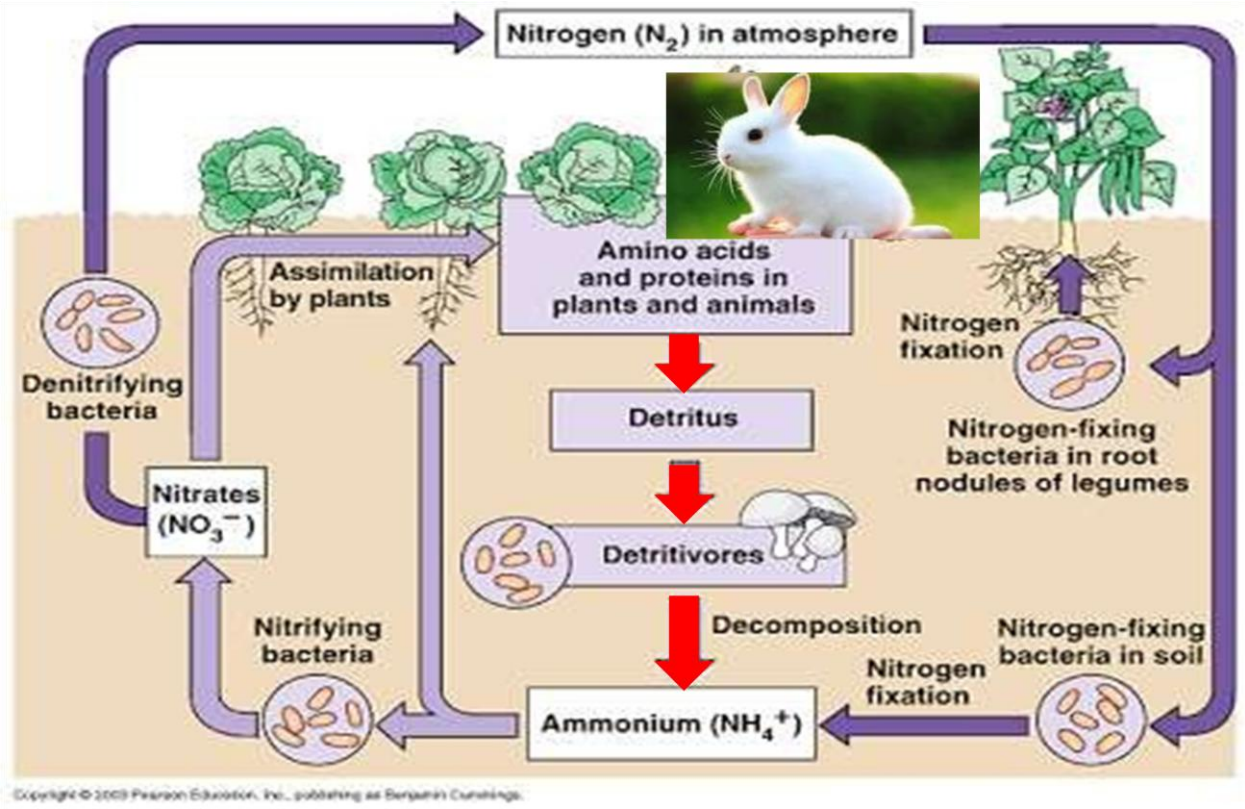
- **Denitrification** is the reduction of nitrates back into the largely inert N2 gas, completing the **N2-cycle**.
- This process is performed by bacterial species such as *Pseudomonas* & *Clostridium* in **anaerobic conditions.**
- They use the nitrate as an electron accepter in the place of oxygen during respiration.
- **Denitrification** happens in anaerobic conditions eg. Waterlogged soils.




Farmland
N_2, N_2O
Fertilizer and organic matter
NO_3^-
Groundwater
Denitrification


Denitrification
Created by J. Stock
University of Minnesota
Soil Surface
Escapes As Gases
NO_3^-
NO_2^-
NO
N_2O
N_2
Caused by soil organisms that live without air in a wet soil and get their oxygen (O) by taking it from NO_3^-. Warm wet soil with large amount of plant residues favor denitrification. (The soil organisms that rot residues rapidly use up the free oxygen supply and then the denitrifying organisms begin to multiply.)

NITROGEN CYCLE



ECOLOGICAL FUNCTIONS

- Nitrogen is necessary for all known forms of life on earth.
- It is a component in all amino acids as it is incorporated into proteins and is present in the bases that make up nucleic acids such as **RNA & DNA**.
- Chemical processing or natural fixation are necessary to convert gaseous nitrogen into compounds, such as nitrate or ammonia which can be used by plants.

USES OF NITROGEN

- Nitrogen is important to the chemical industry, It is used to make **Fertilizers**, **Nitric acid**, **Nylon**, **Dyes** & **Explosives**.
- Nitrogen is present in virtually all pharmacological drugs & In the form of nitrous oxide it is used as anesthetic.
- The CPUs in computers use the N2-gas to keep them from heating up. **X-ray detectors** also rely on this element.

- Cryopreservation also used N2 to conserves blood and other biological specimens.

PHOSPHOROUS CYCLE

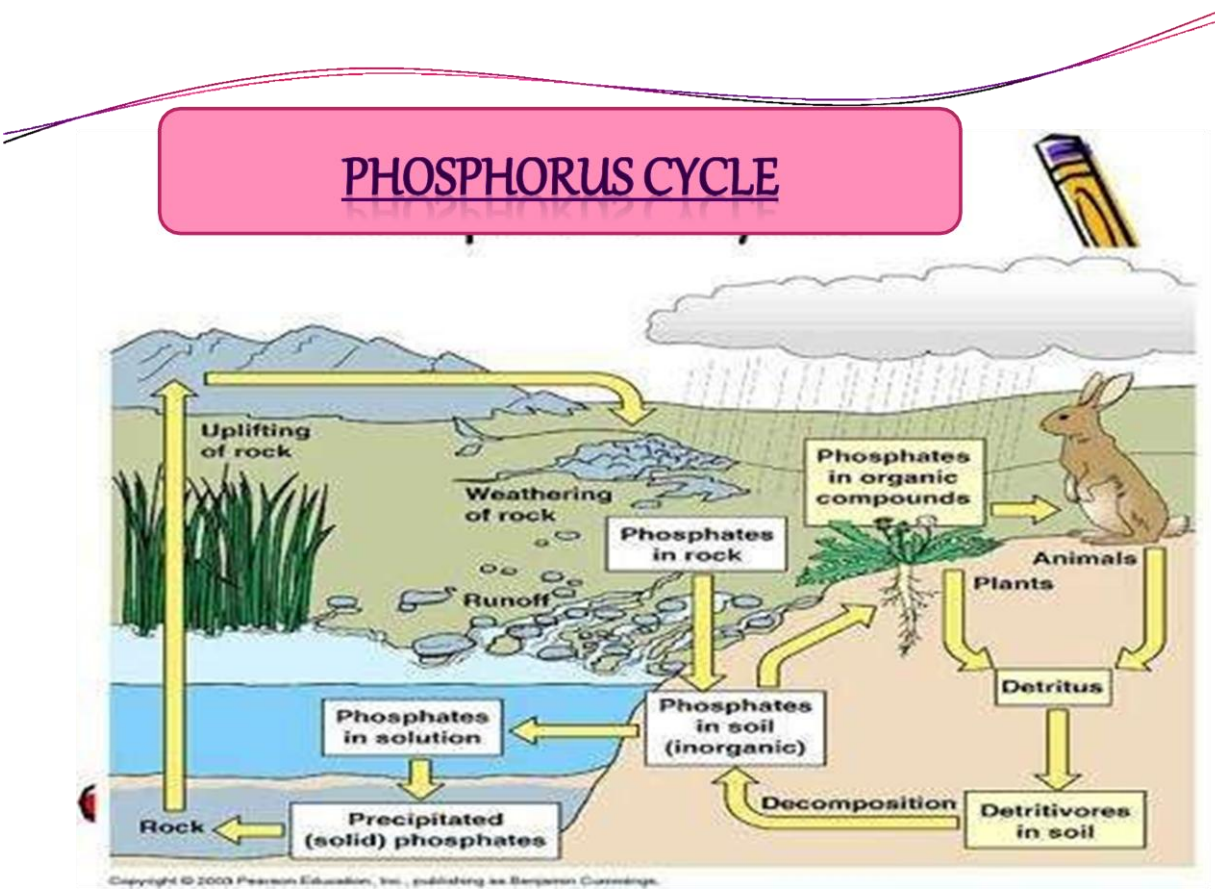
The phosphorus cycle is the **slowest Biogeochemical cycle** that describes the movements of phosphorus(**P**) through the **Lithosphere**, **Hydrosphere** & **Biosphere**.

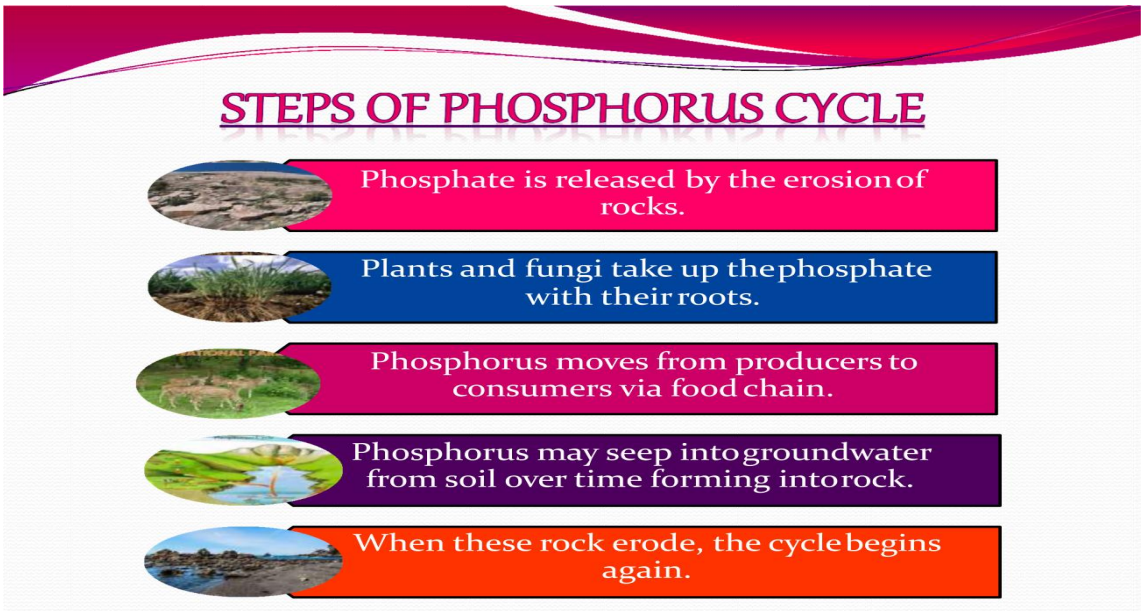
Unlike many other biogeochemical cycles, the atmosphere dose not play a significant role in the movement of **P** because phosphorus and **P** based compounds are usually solids at the typical ranges of temperature & pressure found on earth.Low conc. of **P** in soils reduces plant growth & slows soil microbial growth.

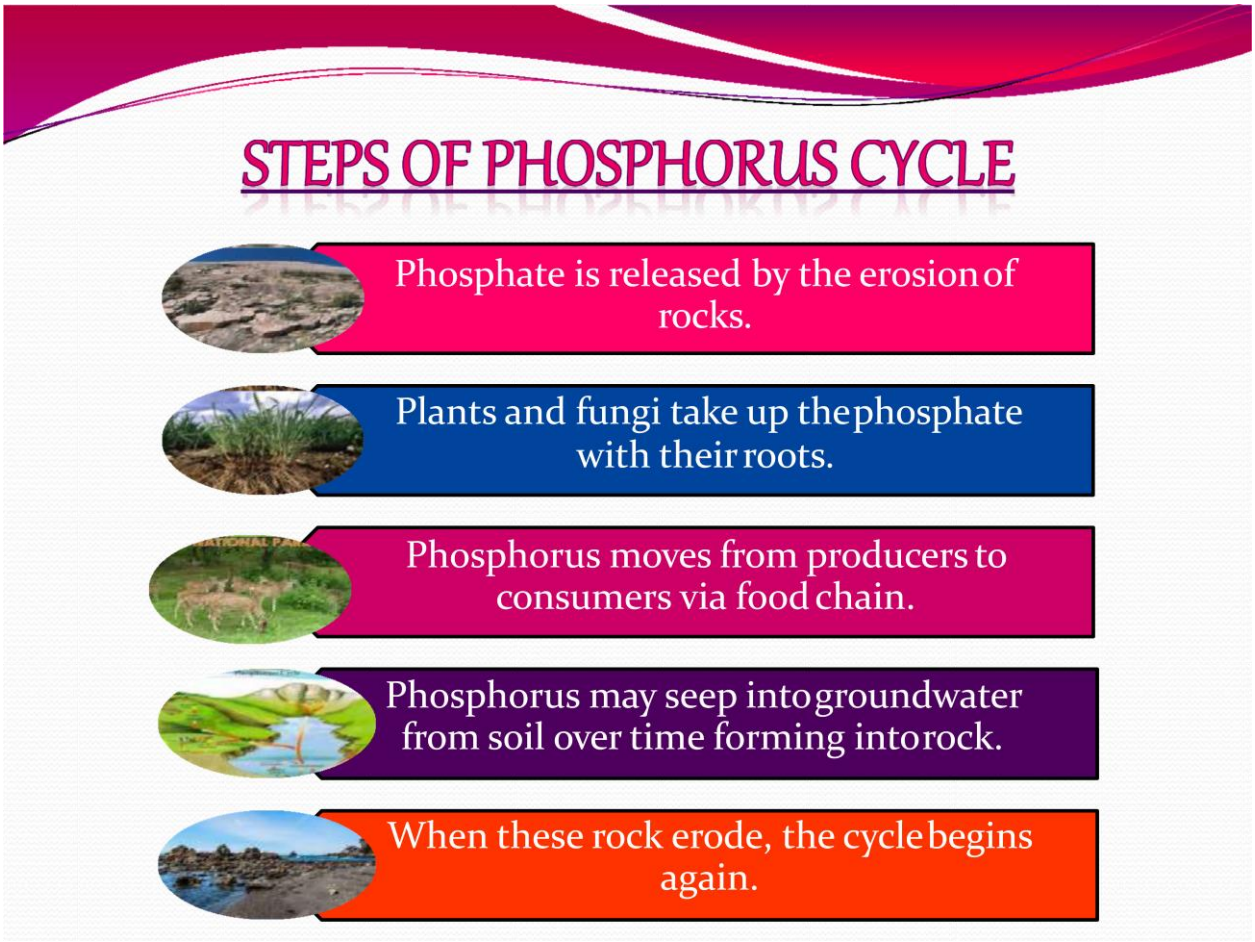
Unlike other cycles P cannot be found in the air as a gas, it only occurs under highly reducing conditions as the gas **Phosphine.** Initially , phosphate weathers from rocks and minerals, the most common mineral being **Apatite** .

Overall small losses occurs in terrestrial environment by leaching erosion, through the action of rain. Weathering of rocks & minerals release phosphorus in a soluble form , where it is taken up by plants & it is transformed into organic compounds.

The plants may then be consumed by herbivores and the phosphorus is either incorporated into their tissues or excreted. After death of animal or plant decays then phosphorus is returned to the soil where a large part of the P is transformed into insoluble compounds. Runoff may carry a small part of the P back to the ocean.

- 
PHOSPHORUS CYCLE
Uplifting of rock
Weathering of rock
Runoff
Phosphates in rock
Phosphates in organic compounds
Animals
Plants
Detritus
Phosphates in soil (inorganic)
Phosphates in solution
Precipitated (solid) phosphates
Rock
Decomposition
Detritivores in soil


STEPS OF PHOSPHORUS CYCLE
Phosphate is released by the erosion of rocks.
Plants and fungi take up the phosphate with their roots.
Phosphorus moves from producers to consumers via food chain.
Phosphorus may seep into groundwater from soil over time forming into rock.
When these rock erode, the cycle begins again.



ECOLOGICAL FUNCTIONS

P is an important nutrient for plants and animals, **P** is also limiting nutrient for aquatic organisms.

- **P** does not enter the atmosphere, remaining mostly on land, in rock & soil minerals.
- 80% of the mined **P** is used to make fertilizers. **P** from fertilizers, sewage can cause pollution in lakes & streams.
- **P** normally occurs in nature as part of a phosphate ion $(PO_4)^{-}$,The most

abundant forms is **Orthophosphate**

- 

IMPORTANCE OF PHOSPHOROUS

BIOLOGICAL FUNCTIONS

The primary biological importance of Phosphates is as a component of nucleotides,

which serves as energy storage within cells (ATP) or when linked together form the nucleic acids DNA & RNA.

- The double helix of two strands of DNA is only possible because of phosphate ester bridge that binds the helix.
- Besides making biomoleculs, P is also found in bone & enamel of mammalian teeth, whose strength is derived from calcium phosphate in the form of Hydroxyl apatite.
- It is also found in the exoskeloton of insects & phospholipids.

OTHER USES

- Phosphorus catches fire readily, Red phosphorus is used in all matches.
- White phosphorus and zinc used as a poison for rats.

- It is used in making incendiary (fire causing) bombs, tracer bullets and for producing smoke screen.
- Many soluble phosphates are used to remove unwanted metal salts from the water.

THE SULFUR CYCLE

The sulfur cycle is the collection of processes by which sulfur moves to and from minerals (including the waterways) and living systems. Such biogeochemical cycles are important in geology because they affect many minerals.

- Found in rocks or buried deep in the ocean in oceanic sediments.
- found in the atmosphere.(enter through both natural and human sources.)
    - occurs in combination with several metals such as, PbS and HgS.
    - a brittle yellow, tasteless and odorless non-metallic element.
- 10th most abundant element in the universe,
- At room temp. it is a solid
- Present in proteins, amino acids, vitamins, and enzymes, necessary for plants and animals

—

—

— USES OF SULFUR

- Important elements of industrial processes
- Sulfur dioxide (SO2) is a bleaching agent and is used to bleach wood pulp
- Sulfur dioxide kills molds and bacteria. (preserve)
- Sulfur is found in every living cell (amino acids)
- Long used as a medicine (Brimstone in the old days)
- Before the advent of antibiotics in the 1940s, sulfur-containing drugs-sulfa drugs-were commonly used to treat infectious diseases.
- Component of penicillin-class antibiotics
- Medications for dandruff, and warts have this ingredient. Combining alcohol and sulfur can be used to treat acne and other skin disorders.

SULPHUR CYCLE MICROORGANISMS CONTRIBUTE GREATLY TO THE SULFUR CYCLE.

Photosynthetic microorganisms transform sulfur by using sulfide as an electron source, allowing *Thiobacillus and* similar *chemolithoautotrophic* genera. In contrast, when sulfate diffuses into reduced habitats, it provides an opportunity for different groups of microorganisms to carry out **sulfate reduction.**

For example, when a usable organic reductant is present, *Desulfovibrio* uses sulfate as an oxidant. This use of sulfate as an external electron acceptor to form sulfide, which accumulates in the environment, is an example of a **dissimilatory reduction process and anaerobic respiration.**

In comparison, the reduction of sulfate for use in amino acid and protein biosynthesis. Other microorganisms have been found to carry out dissimilatory elemental sulfur reduction.

These include *Desulfuromonas, thermophilic archaea* and also

cyanobacteria in hypersaline sediments. Sulfite is another critical intermediate that can be reduced to sulfide by a wide variety of microorganisms, including *Alteromonas* and *Clostridium,* as well as *Desulfovibrio* and *Desulfotomaculum.*

In addition to the very important photolithotrophic sulfur oxidizers such as *Chromatium and Chlorobium, which function under* strict anaerobic conditions in deep water columns, a large and varied group of bacteria carry out **aerobic anoxygenic photosynthesis.** These aerobic anoxygenic phototrophs use bacteriochlorophyll a and carotenoid pigments and are found in marine and freshwater environments; they are often components of microbial mat communities.

Important genera include *Erythromonas, Roseococcus, Porphyrobacter, and Roseobacter.*

"Minor" compounds in the sulfur cycle play major roles in biology.

An excellent example is dimethylsulfoniopropionate (DMSP),which is used by bacterioplankton (floating bacteria) as a sulfur source for protein synthesis, and which is transformed to dimethylsulfide (DMS), a volatile sulfur form that can affect atmospheric processes.

When pH and oxidation-reduction conditions are favorable,

several key transformations in the sulfur cycle also occur as the result of chemical reactions in the absence of microorganisms. An important example of such an abiotic process is the oxidation of sulfide to elemental sulfur.

This takes place rapidly at a neutral pH, with a half-life of approximately 10 minutes for sulfide at room temperature.

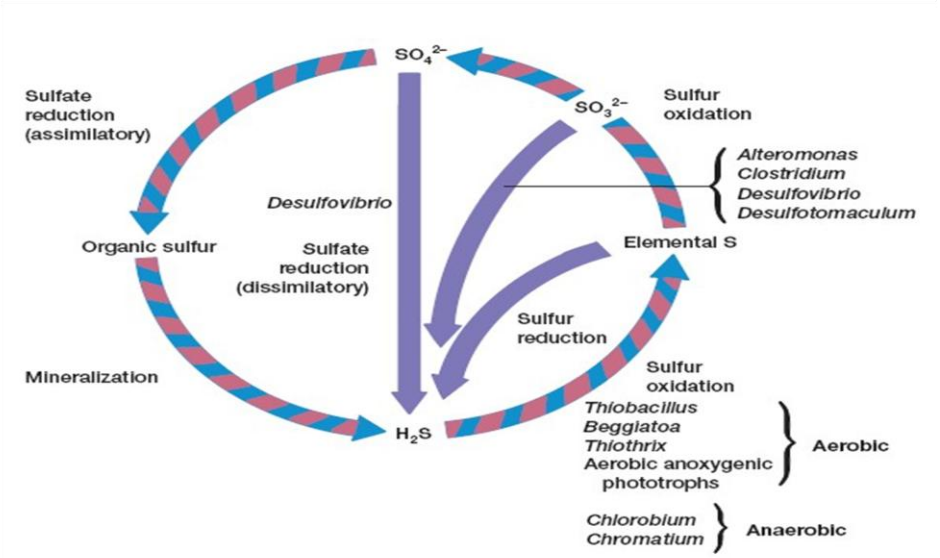


Figure 28.21 The Basic Sulfur Cycle. Photosynthetic and chemosynthetic microorganisms contribute to the environmental sulfur cycle. Sulfate and sulfite reductions carried out by *Desulfovibrio* and related microorganisms, noted with purple arrows, are dissimilatory processes. Sulfate reduction also can occur in assimilatory reactions, resulting in organic sulfur forms. Elemental sulfur reduction to sulfide is carried out by *Desulfuromonas,* thermophilic archaea, or cyanobacteria in hypersaline sediments. Sulfur oxidation can be carried out by a wide range of aerobic chemotrophs and by aerobic and anaerobic phototrophs.

USES:

It is also use to make cements, matches, fireworks, dyes, fungicides.

Powdered sulfur is found in lotions and skin cream ingredients.

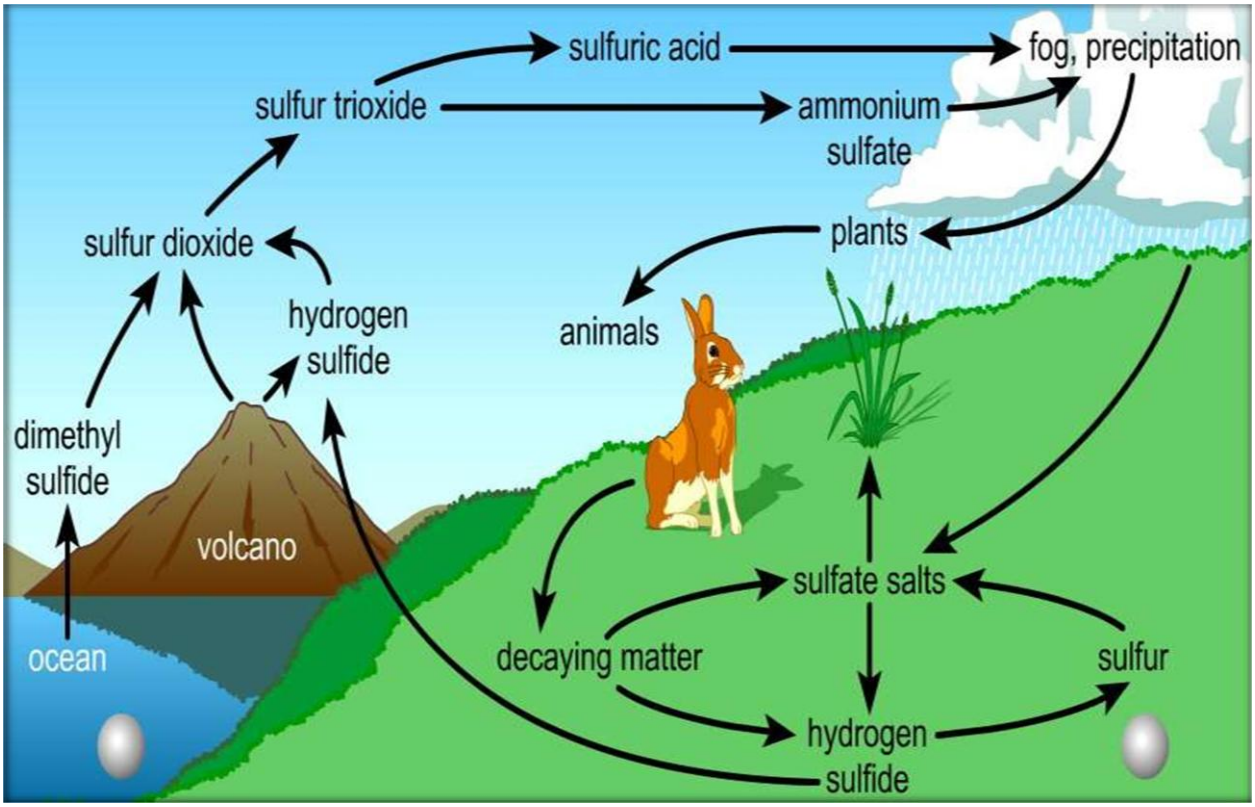
The elements involved in sulfur cycles includes

— S is for sulfur

— SO_4^{-2} is for sulfate

— SO_2 is for Sulfur dioxide ,

— H_2S for Hydrogen sulfide



It includes both atmospheric and terrestrial processes

- Hydrogen Sulfide (H2S) is released into the atmosphere (volcanic eruptions, fossil fuel burning, and the anaerobic decay of sulfur- containg biological material in swamps, bogs)
- Certain marine algae à dimethyl sufide Sulfur dioxide.
- Volcanic eruptions à Sulfur dioxide
- Burning of fossil fuels à Sulfur dioxide
- Sulfur dioxide + Oxygen = Sulfur trioxide, then reacts with tiny water droplets = Sulfuric Acid
- Sulfur Oxides reacts with Ammonia = tiny particles of ammonium salts.

- Wind carries Sulfuric acid and ammonium salts which falls to earth in form of Precipitation.
- Plants get sulfur by taking up ions of sulfate salt from the soil.
- Animals get sulfur by eating plants and all living things release sulfur
- compounds when they decay.
- Decomposition releases sulfate salts (SO4 2-), which can be taken up by plants as well as gaseous hydrogen sulfide.
- Some hydrogen sulfide enters the atmosphere. But when decay occurs in an oxygem-free environment, anaerobic bacteria break down hydrogen sulfide and release sulfur gas (H2)
- Oxygen-requiring bacteria can incorporate sulfure into sulfate salts which can be taken up by plants and enter the food chain once again.
- The remaining sulfur is lost into the oceans depth combining with iron to form Ferrous Sulfide which is responsible for the black color of most marine sediments.
- We burn sulfur containing coal and oil to produce electricity. We refine sulfur containing petroleum.
- Acid rain is corrosive rain caused by rainwater falling to the ground through sulfur dioxide gas, turning it into weak sulfuric acid, which causes damage to ecosystems.
- Transportation - cars are a major contributor to acid rain pollution,
- Alternative fuels - switch over to renewable sources of energy, (solar, wind and water energy)

THE ROLE OF MICRO-ORGANISMS IN THE DECOMPOSITION OF ORGANIC MATTER AND THE MICROBIAL COMMUNIITY SUCCESSION

Two Components of Decomposition Decomposition is described as having two components; **autolysis and putrefaction**.

- **Autolysis** refers to the situation where the body's own enzymes are acting on itself, causing cellular and tissue destruction.
- **Putrefaction** refers to the situation where microorganisms (especially bacteria and fungi) feed on and break down the tissues of the dead body.

Process of Decomposition by Microorganisms

- Within a very short time period microorganisms can breakdown and digest a large amount of soft tissue, resulting in a large production of gas and 'decomposition fluid'
- As decomposition proceeds, the skin begins to darken to various shades of green and brown. This is usually first seen within the right lower abdominal quadrant (image below)
- The body becomes somewhat bloated due to the decomposition gases produced, and the decomposition fluid is frequently expelled from the mouth, nose or other opening in the presence of a red-brown fluid
- Bacteria and fungi contribute to the decay of the body.

Other decomposers

- Insects are not the only organisms involved in decomposition of a body.
- Bacteria also plays a major role.
- Those found in the gut invade the dead tissues after death of a body as well as other fungi and bacteria from surroundings colonising the corpse.
- This in turn leads to decay.
- There is no set succession on the particular sequence of succession however genera often found on corpses include *Bacillus, Candida and Argobacterium*.
- These are collectively known as decomposers.

Microorganisms Found on Corpses

Early Stages of decomposition:

- *Bacillus*

- *Staphylococcus*
- *Candida*
- *Streptococcus*

Followed by:

- *Salmonella*
- *Cytophaga*
- *Agrobacterium*

Microorganisms are Collectively known as Decomposers

- Decomposers obtain a great source of energy from the body such as proteins, fats, organic carbohydrates and nucleic acids which are used as a food source.
- This energy is then released through aerobic/anaerobic respiration.
- This energy allows rapid multiplication which leads to more decomposition.

DECOMPOSITION OF OM

- Definition:

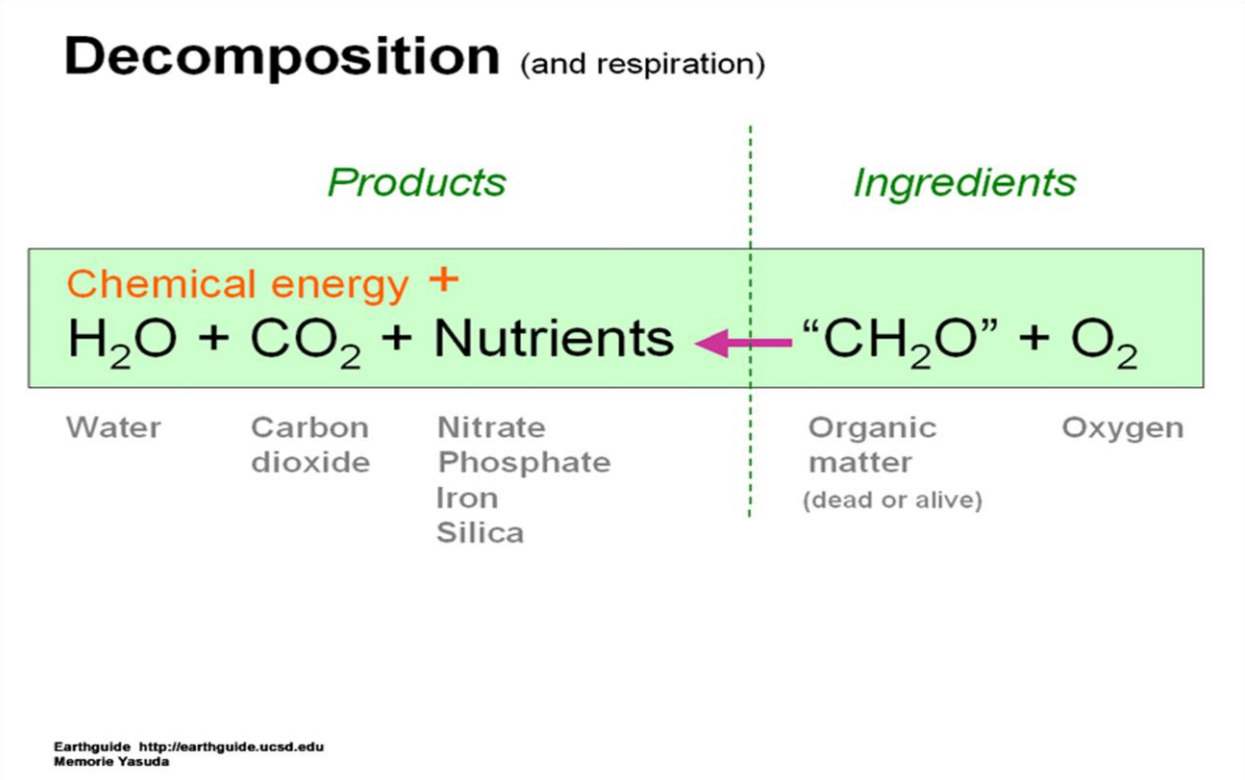
➢ Breakdown of dead plant and animal material and

release of inorganic nutrients

➢ Decomposition is a biological breakdown and biochemical transformation of complex organic molecules of dead material into simpler organic and inorganic molecules (Juma, 1998).

Decomposition (and respiration)

Decomposition (and respiration)



SOURCE OF ORGANIC MATTER

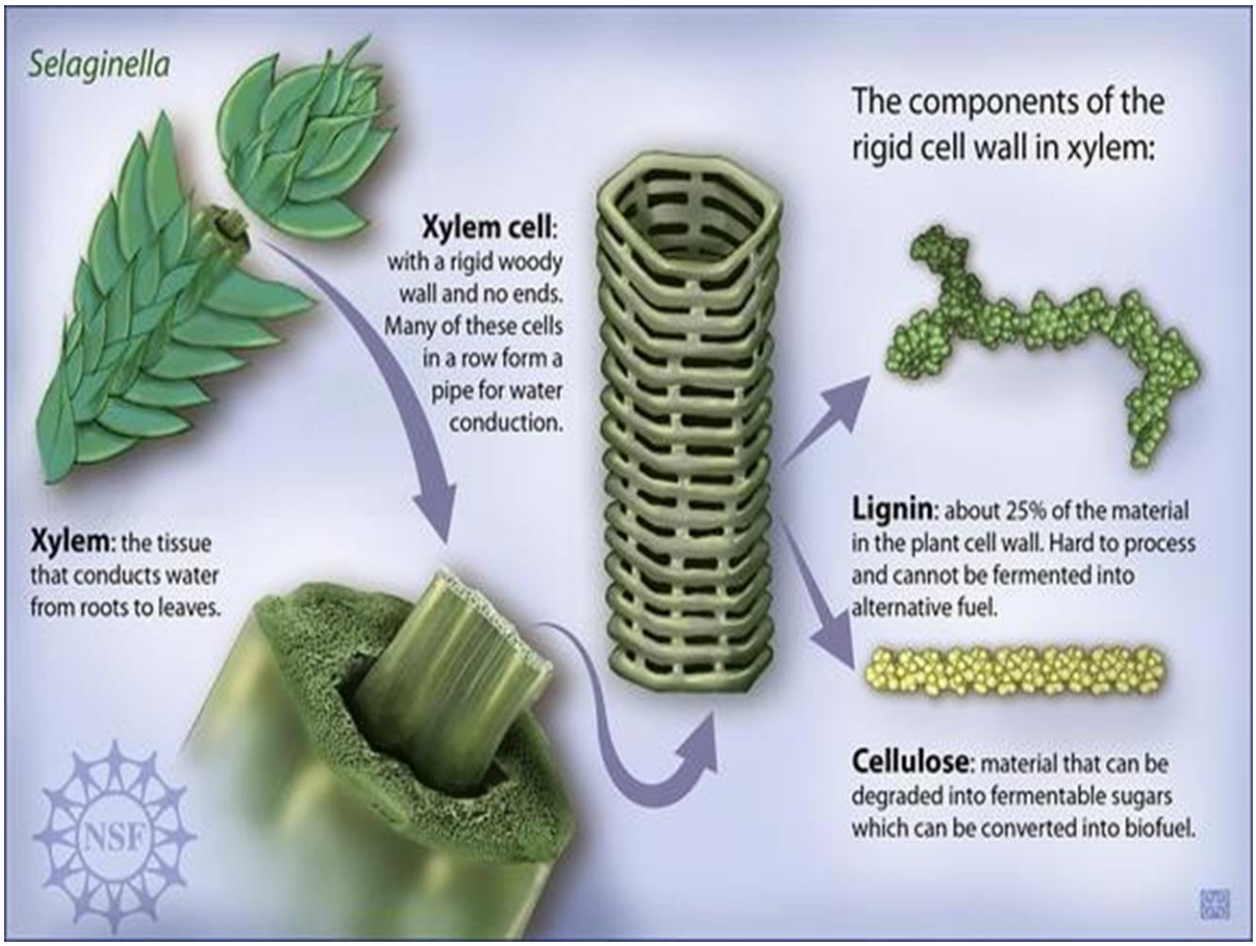
- Plant remains
- Animal tissue and excretory products
- Cells of microorganisms
- However, plant is the main contribution to OM.

ORGANIC CONSTITUENTS OF PLANTS

1) Cellulose most abundant 15 to 60% of dry weight.
2) Hemicellulose, 10 to 30 %
3) Lignin, 5 to 30%
4) Water soluble fraction include simple sugar, amino acids, aliphatic acids, 5 to 30 % of tissue weight

5) Ether and alcohol-soluble constituents; fats, oils,waxes, resins and a number of pigments.

6) Proteins



WHY ORGANISMS DECOMPOSE OM?

- Supplying energy for growth
- Supplying carbon for new cell synthesis
- The cells of most microorganisms commonly contain approximately 50 % carbon. This is derived mainly from the substrates.

WHY DO WE CARE ABOUT DECOMPOSITION?

Decomposition is important in releasing nutrients tied up in dead organic matter and return it back to the soil.

A. <u>SOIL FAUNA</u>

- earthworms, arthropods

- Fragmentation (cominution) increases surface area.
- Distributes OM within soil profile

B. SOIL MICROORGANISM

- Heterotrophic bacteria, fungi
- Derive energy, carbon and nutrients from dead OM in

the process they release CO2 through respiration.

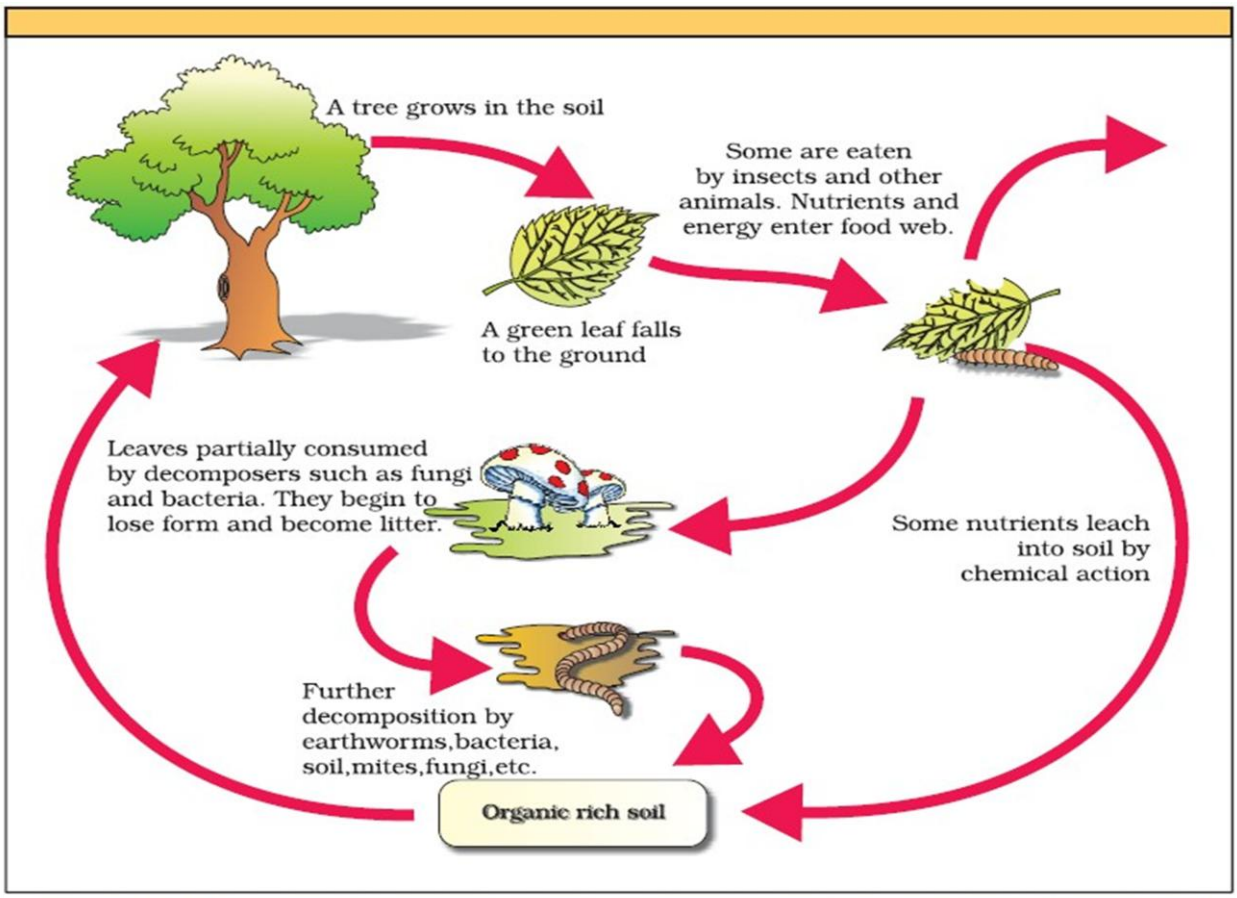


Figure 14.1 Diagrammatic representation of decomposition cycle in a terrestrial ecosystem

DECOMPOSITION PROCESS

- THREE MAIN PROCESSES:

1) ASSIMILATION

- Conversion of substrates materials into protoplasmic

materials

- E.g. OM carbon to microbial carbon

- E.g. protein to microbial protein

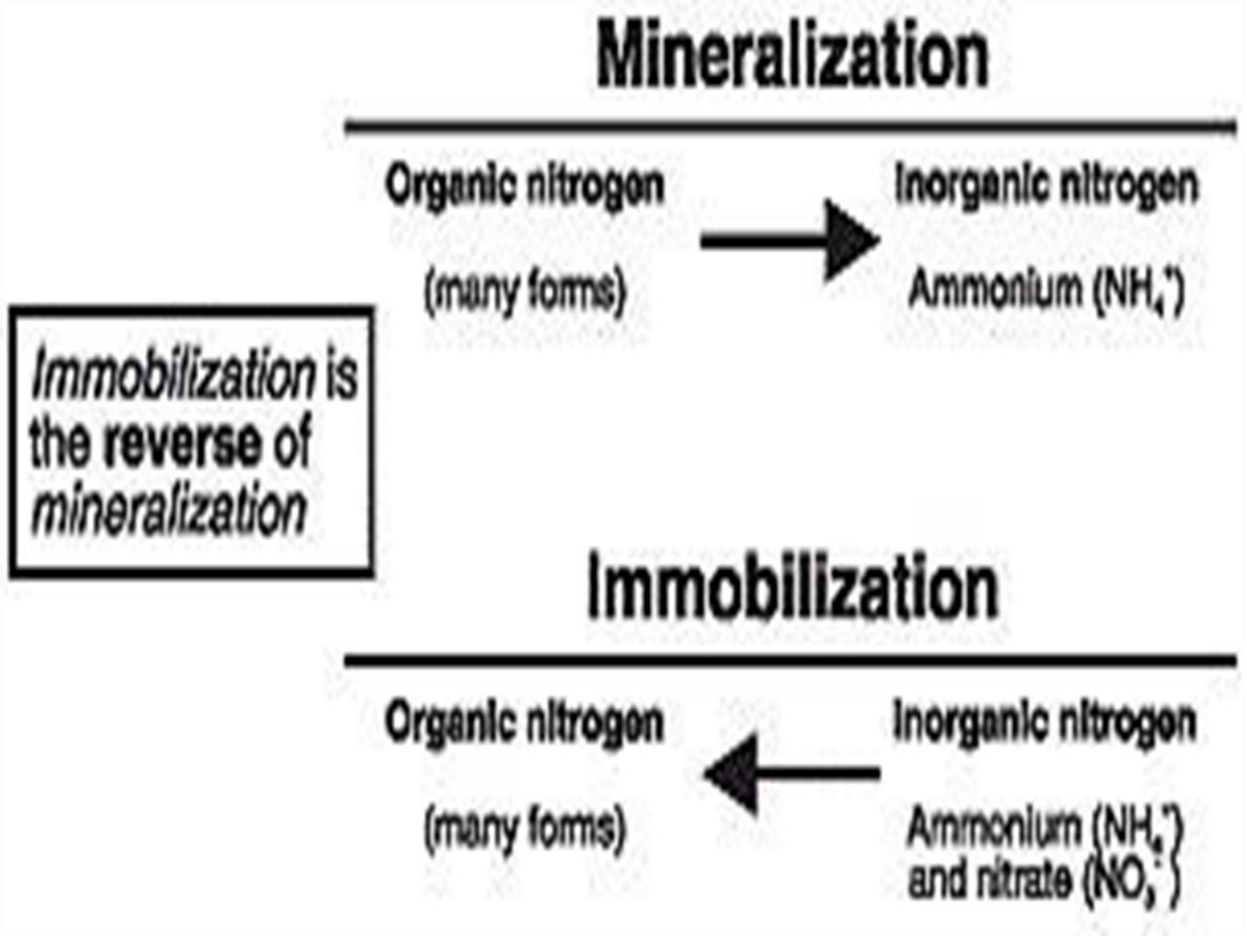
2) <u>MINERALIZATION</u>

- Conversion of organic substance to inorganic form.
- E.g. protein from OM will be converted to inorganic nitrogen

in the soil.

3) <u>IMMOBILIZATION</u>

- Conversion of inorganic form into organic.
- E.g. inorganic nitrogen from the soil converted into microbial protein.



FACTORS AFFECTING RATE OF DECOMPOSITION

TEMPERTURE

- Microbial activity responds exponentially to increased temperature until enzymes denature, etc.

MOISTURE

- Microbial activity has optimum moisture
- Low moisture = dessication, slow diffusion
- High moisture = low O2 availability; no lignin degradation

pH

- Most microbes exhibit optimum activity near pH 7.
- Fungi most active in acid soil and bacteria in moderate soil pH.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – III – MICROBIAL ECOLOGY – SMB2101

UNIT: III

MICROBIAL INTERACTIONS

The inter- and intra-relationships between various microorganisms which can include both positive (like SYMBIOSIS) and negative (like ANTIBIOSIS) interactions. Examples include virus - bacteria and bacteria - bacteria.

The consortium may be in intermitent,cyclic or permanent. These are ubiquitous, diverse and critically important in the function of any biological community.

These includes-

- ❖ Neutralism
- ❖ Mutualism
- ❖ Commensalism
- ❖ Parasitism
- ❖ Cooperation
- ❖ Predation
- ❖ Amensalism
- ❖ Competition
- ❖ Synergism

PARTNERS INVOLVED

- ⦿ MICROBE-MICROBE INTERACTION.
- ⦿ MICROBE-PLANT INTERACTION.
- ⦿ MICROBE-ANIMAL INTERACTION.
- ⦿ MICROBE-HUMAN INTERACTIONS.
- ⦿ MICROBE-ENVIRONMENT INTERACTIONS AND DISEASES.

Mutualism	+	+
Commensalism	+	0
Parasitism	+	-
Predation	+	- (Dies)
Competition	-	-
Amensalism	-	0
Neutralism	0	0

NEUTRALISM

Neutralism describes the relationship between two species (interspecific) which interact but do not affect each other. Microorganisms have no effect on each other. Observed in natural communities if-

- Culture density is low.
- Nutrient level is high.
- Each culture has distinct requirements.
- It describes interactions where the fitness of one species has absolutely no effect whatsoever on that of the other.
- True neutralism is extremely unlikely or even impossible to prove. When dealing with the complex networks of interactions presented by ecosystems, one cannot assert positively that there is absolutely no competition between or benefit to either species.

- Since true neutralism is rare or nonexistent, its usage is often extended to situations where interactions are merely insignificant or negligible.

  Examples of true neutralism are virtually impossible to prove; the term is in practice used to describe situations where interactions are negligible or insignificant

- Neutralism (no interaction)
- implies lack of interaction
- populations have very different metabolic capabilities
- cannot happen between populations having the same or overlapping functional role very difficult to demonstrate experimentally (hard to prove a negative) occurs between populations that are spatially distant from each other

MUTUALISM

In which individuals interact physically or even live within the body of the other mutualist. Frequently the relationship is essential for the survival of at least on member.

eg. Lichens are a fungal-algal symbiosis (that frequently includes a third member, a cyanobacterium). the mass of fungal hyphae provides a protected habitat for the algae and gathers mineral nutrients from rain water and from dissolving the rock underneath. In return the algae and cyanobacteria provides cabohydrates as a sources of energy for the fungus.

- The cyanobacteria (Nostoc, Calothrix), and diatoms (Epithemia, Rhopalodia) containing cyanobacterial endosymbionts.

In these associations the symbiont supplies fixed nitrogen and/or, more rarely, fixed carbon to the host, and the host provides habitat (i.e., optimal oxygen, nutritional, and pH conditions, and optimal oxidation/reduction gradients) for localized growth of the symbiont. A certain kind of bacteria lives in the intestines of humans and many other animals. The human cannot digest all of the food that its eats. The bacteria eat the food that the human cannot digest and partially digest it, allowing the human to finish the job.

DEGREE OF DEPENDENCE

OBLIGATE: At least one species could not grow and reproduce without the other. The species involved are in close proximity and interdependent with one another in a way that one cannot survive without the other.

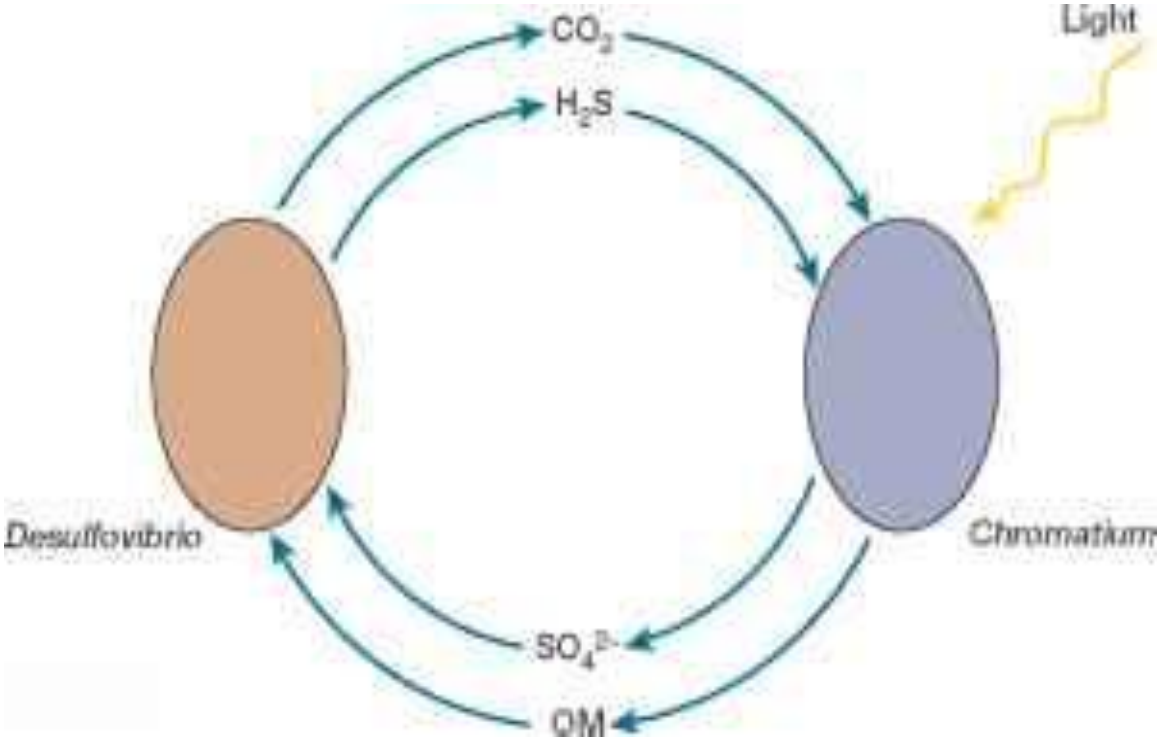
FACULTATIVE: Mutualisms are not essential for the survival of either species. Individuals of each species engage in mutualism when the other species is present. Both organisms do better with their mutualist, but can survive and reproduce without it.

COOPERATION

Cooperation is a mutually beneficial relationship, similar to that which occurs in mutualism, but in cooperation, this relationship is not obligatory:beneficial complementary resources are provided by each of the paired microorganisms. The organisms involved in this type of relationship can be separated, and if the resources provided by the complementary microorganism are supplied in the growth environment, each microorganism will function independently.

- Positive but not obligatery symbiosis
- Can be separated from one another.
- For eg-*Desulfovibrio & Chromatium,Cellulomonas & Azotobactor*.

Eg: 1Desulfovibrio and Chromatium (figure 28.9a), in which the carbon and sulfur cycles are linked.

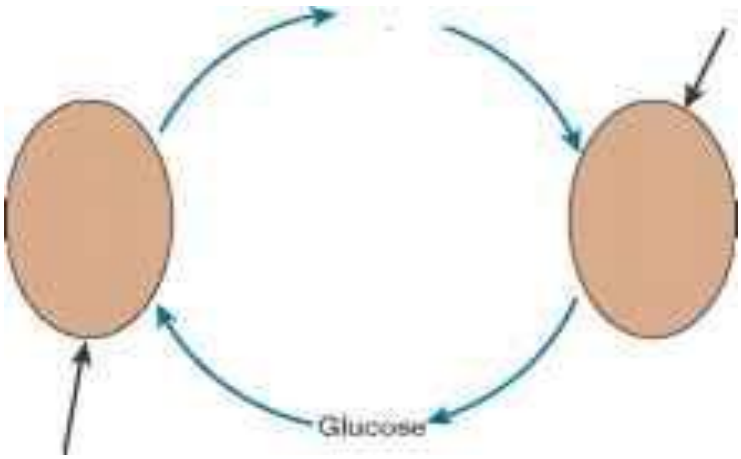


(a) The organic matter (OM) and sulfate required by Desulfovibrio are produced by the Chromatium in its photosynthesis-driven reduction of CO2 to organic matter and oxidation of sulfide to sulfate.

Eg: 2 The interaction of a nitrogen-fixing microorganism with a cellulolytic organism such as Cellulomonas (figure 28.9b). Here the cellulose-degrading microorganism liberates glucose from the cellulose, which can be used by nitrogen-fixing microbes.

Cellulose degrader (Cellulomonas)

Nitrogen fixer (Azotobacter)



Azotobacter uses glucose provided by a cellulose-degrading microorganism such as Cellulomonas, which uses the nitrogen fixed by Azotobacter.

COMMENSALISM

Commensalistic relationships between microorganisms include situations in which the waste product of one microorganism is the substrate for another species.One partner(commensal) benefits. while other(host) remains unaffected.

- – Common among organisms, not obligatory
- – Unidirectional
- – Usually, unaffected population modifies the environment in a way that other population benefits.
- An example is nitrification, the oxidation of ammonium ion to nitrite by microorganisms such as Nitrosomonas, and the subsequent oxidation of the nitrite to nitrate by Nitrobacter and similar bacteria.
- Nitrobacter benefits from its association with Nitrosomonas because it uses nitrite to obtain energy for growth.
- Commensalistic associations also occur when one microbial group modifies the environment to make it more suited for another organism.
- For example, in the intestine the common, non-pathogenic strain of Escherichia coli lives in the human colon, but also grows quite well outside the host, and thus is a typical commensal.
- When oxygen is used up by the facultatively anaerobic E. coli, obligate anaerobes such as Bacteroides are able to grow in the colon.
- The anaerobes benefit from their association with the host and E. coli, but E. coli derives no obvious benefit from the anaerobes.
- In this case the commensal E. coli contributes to the welfare of other symbionts.
- Commensalism can involve other environmental modifications.

- ⦿ The synthesis of acidic waste products during fermentation stimulate the proliferation of more acid-tolerant microorganisms, which are only a minor part of the microbial community at neutral pHs.

PARASITISM

One speices is dependent on a another for nutrition and growth. oIn it, one organism is benefitted and other is harmed. Closly related to predation. oCoexistence between host and parasite.

For eg-Viruses are the highly specialized intracellular *parasites,generally kill the host.*

Microbial parasite may kill the host or can have stable relationship without killing the host.(lysogeny provirus is carried on host chromosome). Pathogenic parasite may attack and kill the plant or animal host.

Obligate parasite *Treponema pallidum(syphilis*),*Rickettsia(Rockey mountain fever)* can't grow without an appropriate host.

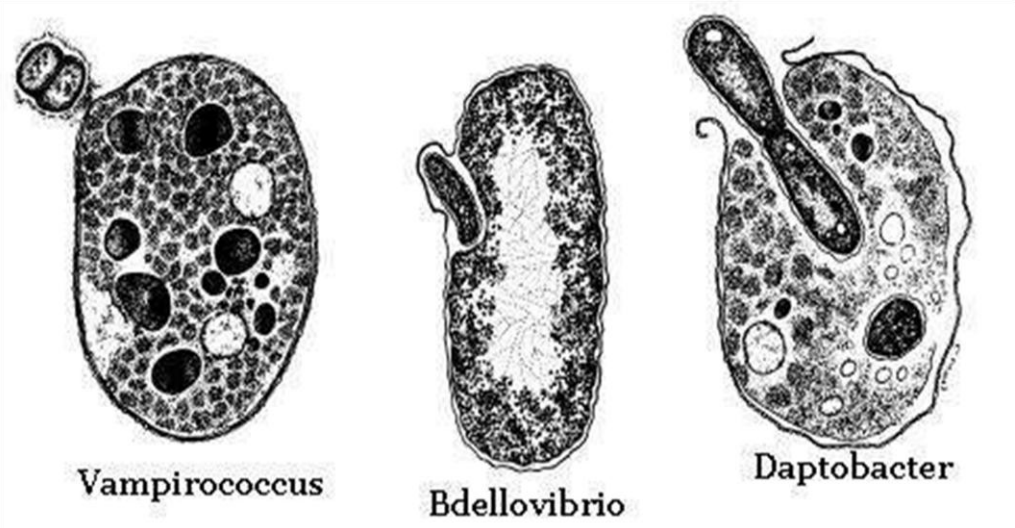
- ❖ Some bacterial viruses can establish a lysogenic relationship with their hosts, and the viruses, in their prophage state, can confer positive new attributes on the host bacteria, as occurs with toxin production by Corynebacterium diphtheriae.
- ❖ Parasitic fungi include Rhizophydium sphaero-carpum with the alga Spyrogyra.
- ❖ Also, Rhizoctonia solani is a parasite of Mucor and Pythium, which is important in biocontrol processes, the use of one microorganism to control another. Human diseases caused by viruses, bacteria, fungi, and protozoa.
- ❖ Myxobacteria are predators that actively kill bacteria of other species to consume their biomass.
- ❖ Myxobacterium *Myxococcus xanthus*, which can access nutrients from a broad spectrum of microorganisms.
- ❖ *M. xanthus* displays an epibiotic predation strategy, i.e., it induces prey lysis from the outside and feeds on the released biomass.

- ❖ This predatory behavior encompasses various processes: Gliding motility and induced cell reversals allow *M. xanthus* to encounter prey and to remain within the area to sweep up its biomass, which causes the characteristic "rippling" of preying populations.
- ❖ Antibiotics and secreted bacteriolytic enzymes appear to be important predation factors, which are possibly targeted to prey cells with the aid of outer membrane vesicles.

PREDATION

It involves predator species which target other microbe for material to survive.

- ⦿ Predator attacks and kills its prey.
- ⦿ They can be obligate or facultative.
- ⦿ Members of predatory bacteria are known as 'Bdellovibrio and like organisms'(BALO).They can be epibiotic,periplasmic,cytoplasmic.
- ⦿ For eg- *Bdellovibrio-E.coli interaction (Vampirococcus,Daptobacter* both attack *Chromatium.*
- ⦿ Predation is a widespread phenomenon where the predator engulfs or attacks the prey,
- ⦿ They can be obligate or facultative
- ⦿ The prey can be larger or smaller than the predator, and this normally results in the death of the prey.
- ⦿ An interesting array of predatory bacteria are active in nature. Several of the best examples including Bdellovibrio, Vampirococcus, and Daptobacter.
- ⦿ Each of these has a unique mode of attack against a susceptible bacterium.
- ⦿ Bdellovib-rio penetrates the cell wall and multiplies between the wall and the



- Bdellovibrio, a periplasmic predator that penetrates the cell wall and grows outside the plasma membrane,
- plasma membrane, a periplasmic mode of attack, followed by lysis of the prey and release of progeny
- Vampirococcus with its unique epibiotic mode of attacking a prey bacterium
- Vampirococcus attaches to the surface of the prey (an epibiotic relationship) and then secretes enzymes to release the cell contents.
- Daptobacter showing its cytoplasmic location as it attacks a susceptible bacterium.
- Daptobacter penetrates a susceptible host and uses the cytoplasmic contents as a nutrient source.

AMMENSALISM

- Focuses on exclusion of an organism from growing on a specific site to prevent the utilization of limiting nutrients.
- Unidirectional process based on the release of a specific compound by one organism that has negative effect on another.
- Product of one impact another i.e one species remains uneffected while other is harmed.
- For eg- microbial production of antibiotics that

can inhibits or kill another.penicillin by fungi inhibit a type of cell wall found

- Amensalism (from the Latin for not at the same table) describes the negative effect that one organism has on another organism as shown in figure 28.1.
- This is a unidirectional process based on the release of a specific compound by one organism which has a negative effect on another organism.
- A classic example of amensalism is the production of antibiotics that can inhibit or kill a susceptible microorganism (figure 28.16a).
- The attine ant-fungal mutualistic relationship is promoted by antibiotic-producing bacteria that are maintained in the fungal garden system (figure 28.16b).
- In this case a streptomycete produces an antibiotic that controls Escovopsis, a persistent parasitic fungus that can destroy the ant's fungal garden. This unique amensalis-tic process appears to have evolved 50 million years ago in South America.
- Other important amensalistic relationships involve microbial production of specific organic compounds that disrupt cell wall or plasma membrane integrity. These include the bacteriocins.
- These substances are of increasing interest as food additives for controlling growth of undesired pathogens .
- Antibacterial peptides can be released by the host and microorganisms in the intestine. These molecules, called ce-cropins in insects and defensins in mammals, recently have been recognized as effector molecules that play significant roles in innate immunity.
- In animals these molecules are released by phagocytes and intestinal cells, and are as powerful as tetracyclines.
- Finally, metabolic products, such as organic acids formed in fermentation, can produce amensalistic effects. These compounds inhibit growth by changing the environmental pH, for example, during natural milk spoilage

Penicillin Antibiotic production by pencillium against Staphylococcus

COMPETITION

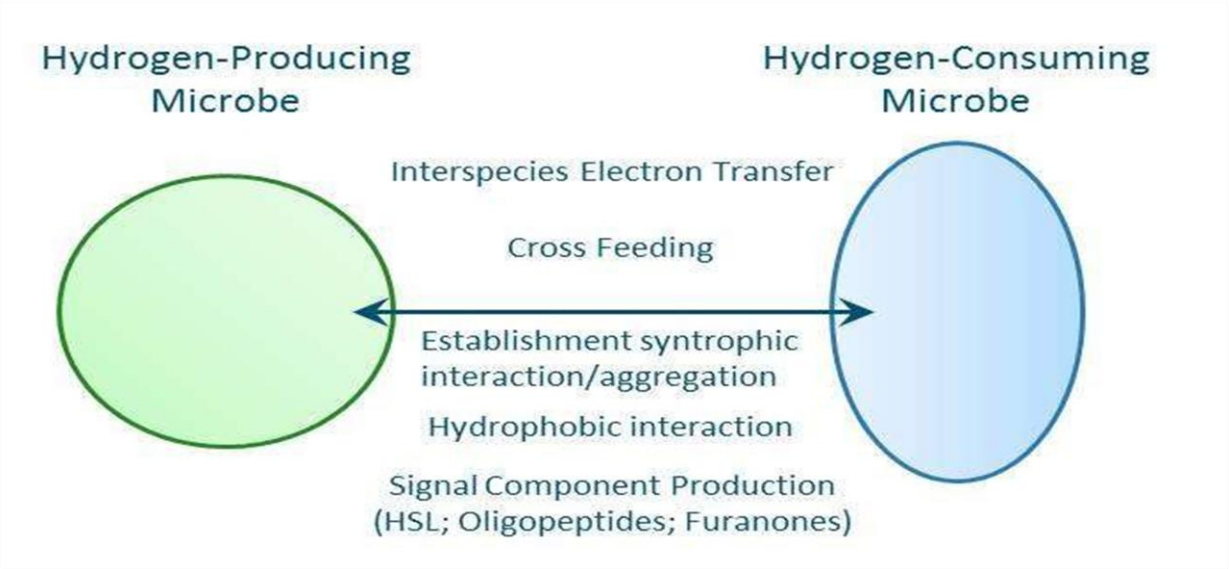
It arises when different organisms within population try to acquire same resources. Both the species are harmed. Competiton within the species or among different species can be attributed to availibility of Nitrogen source,carbon source,electron donors,electron accepter,vitamins,light,water.

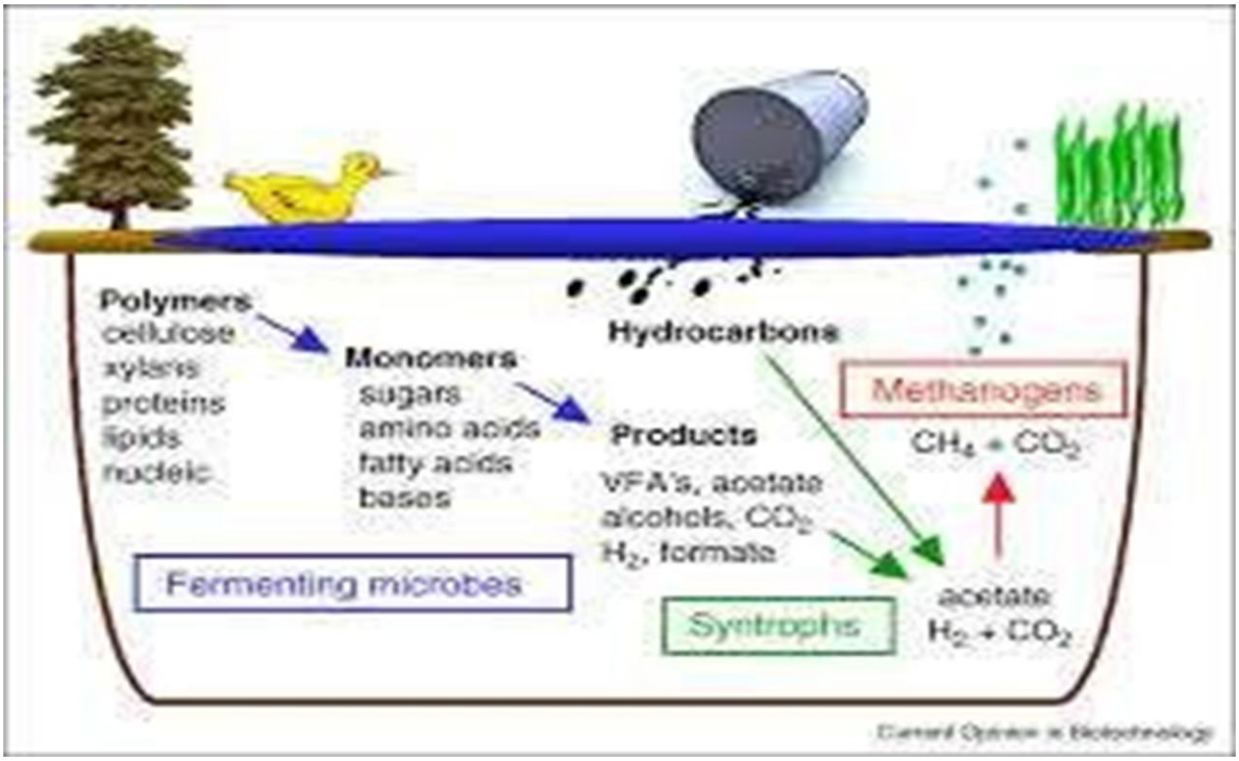
- Competition may result in exclusion of other species or the establishment of a steady state where multiple species coexist.
- Eg- In aquatic environment where extensive phototrophic activity results in blooms of single species of diatoms or cyanobacteria.
- Thermophilic springs chemolithotrophic organisms are dominated.
- Lactic acid fermentation of food.
- Large intestine of animals,a single species doesn't dominate but a mixed population coexist.

SYNTROPISM

Two species are required for growth on a specific electron donor that is not metabolized by either organism alone or one of the organisms remove end products of metabolism from other,which enables both the organisms to grow.

- Both the species are benefited.
- This relationship was discovered by Meyer Wolin and colleagues,when fermentation of propionic acid occurred when there was a coculture.
- *Synophobacter* produces H during fermentation and accumulation of H makes the reaction thermodynamically unstable.
- Pesence of methanogen,*Methanospirillum* makes the oxidation favourable by consuming the H.'





MICROBE PLANT INTERACTIONS

Different interactions between microorganisms and plants have been identified and the most obvious environment for such interactions is soil.

- Microbe-plant association can be mutulistic(a highly specialized interaction where there is considerable specificity found in mutulistic

activities)or it can be commensilistic(secretions from plants benefit bacteria and fungi but no apparent benefit to plant.

SYMBIOSIS WITH CYANOBACTERIA

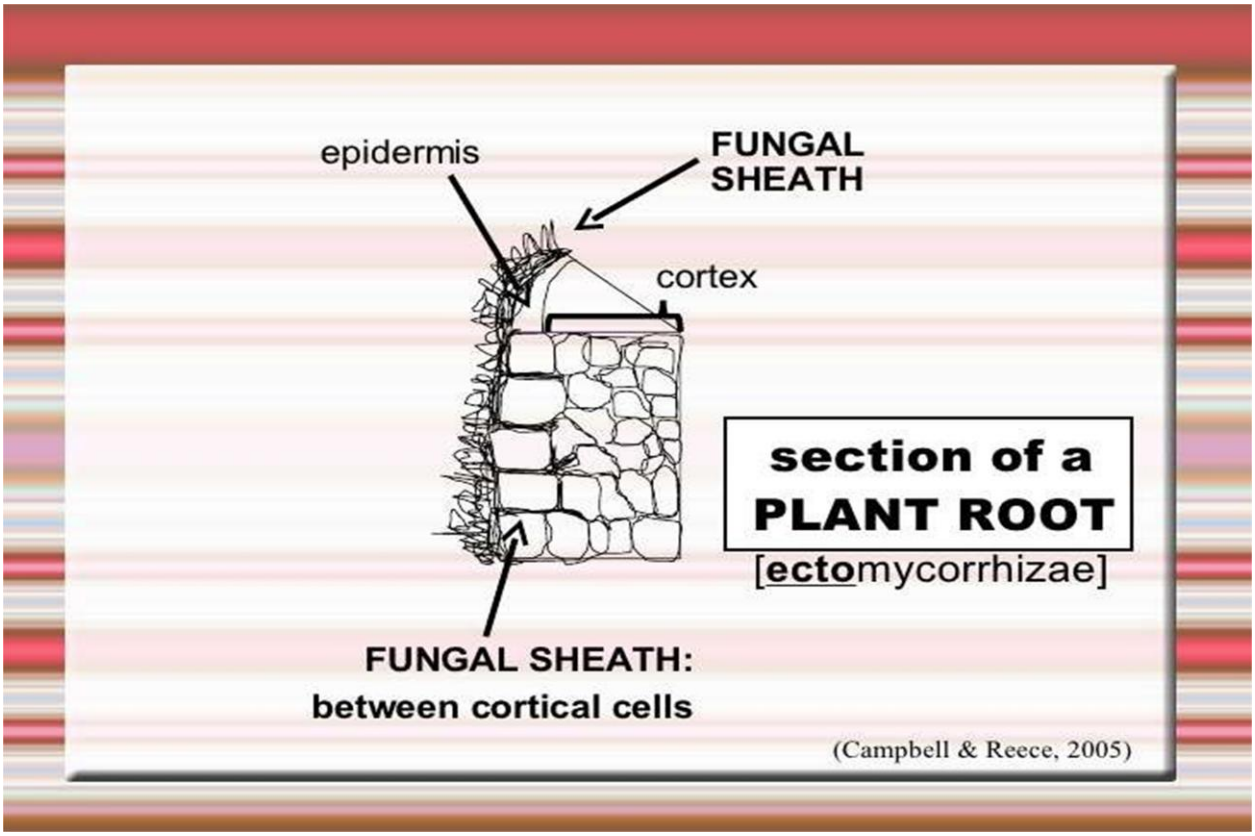
Beneficial aspects of plant-microbe symbiosis are,plants provide c-material to support growth of microbes and microbes promote plant growth by supplying minerals or N_2.

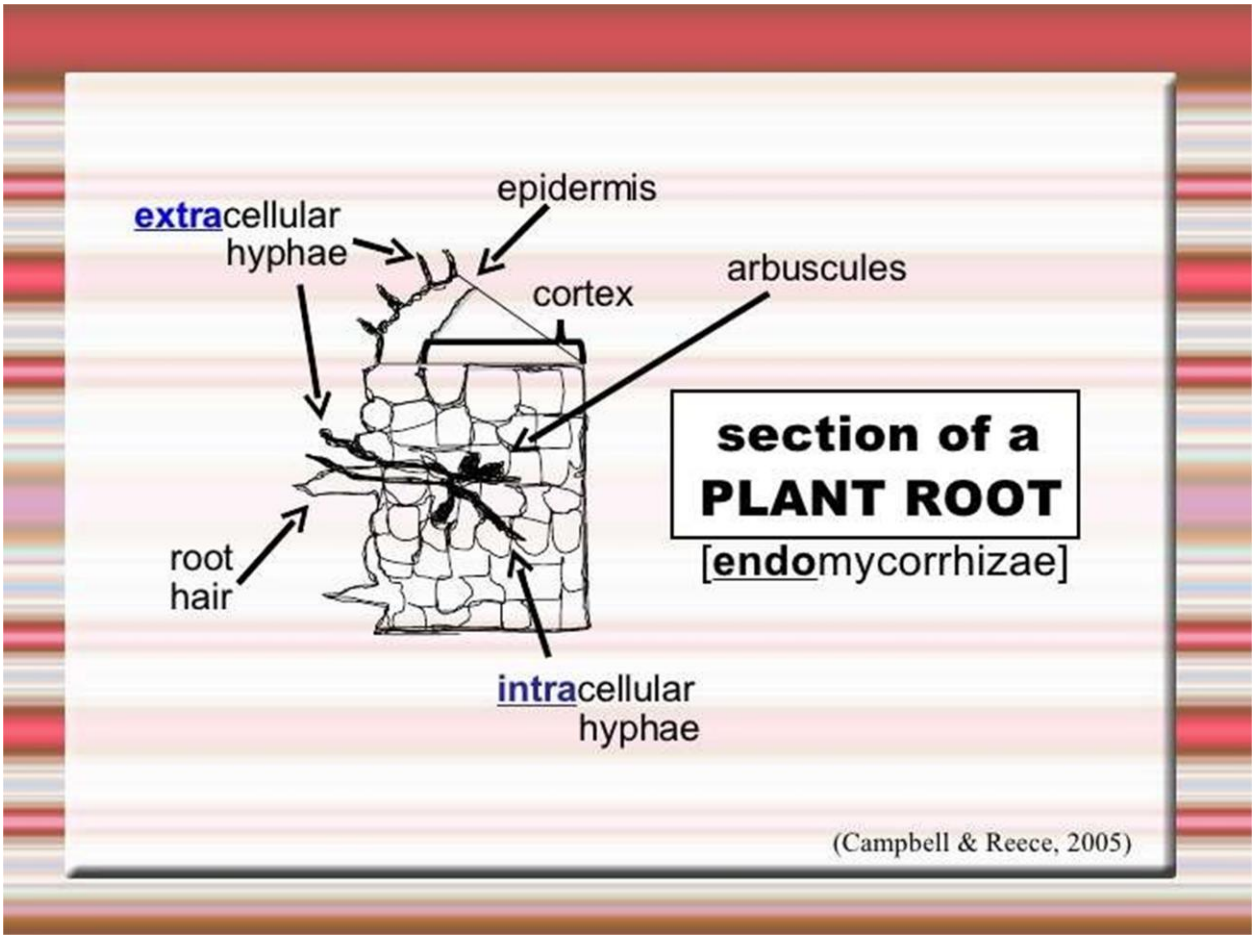
- Eg.*Azolla*(fresh water aquatic fern)lives in symbiotic association with *Anabena azollae* ,where cyanobacteria fixes atmospheric $N_2$and *Azolla* provides carbohydrates.cyanobacteria are present in trichomes and nutrient exchange occurs through tiny fibres extending from plant to cavity.

INTERACTIONS IN RHIZOSPHERE AND SYMBIOTIC SYSTEMS

Fungus-Root system

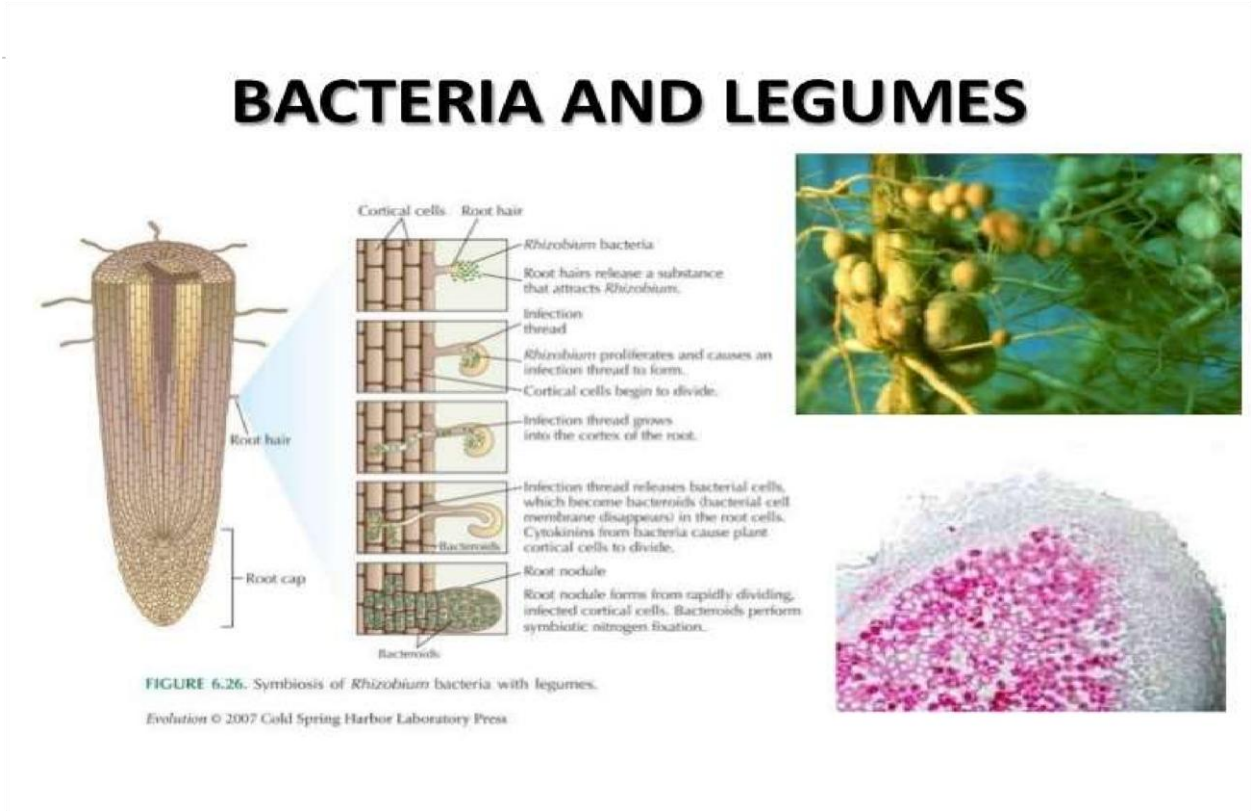
- Mucilage,organic acids(rhizodepozition),dead root cells(nucleic acid,complex carb.,proteins)released by root tip act as c-source for microbes.
- Microorganisms enhance the cycling of c and N compounds,consume rhizospheric O and lower the redox potential of rhizosphere.
- Eg.Mycorrhizae-mutulistic relationship between fungus and plantroot,growth on exterior of the root is the characteristic of ectomycorrhiza while growth inside the root is attributed to endomycorrhiza.
- Plants with mycorrhiza-
- can grow in low nutrient soil.
- display greater growth rates.
- more disease resistent.
- *Boletus elegans* and *larix* sp.
- *Gigaspora margarita* and *cotton*.


epidermis
FUNGAL SHEATH
cortex
section of a PLANT ROOT
[ectomycorrhizae]
FUNGAL SHEATH:
between cortical cells
(Campbell & Reece, 2005)


extracellular hyphae
epidermis
cortex
arbuscules
root hair
intracellular hyphae
section of a PLANT ROOT
[endomycorrhizae]
(Campbell & Reece, 2005)

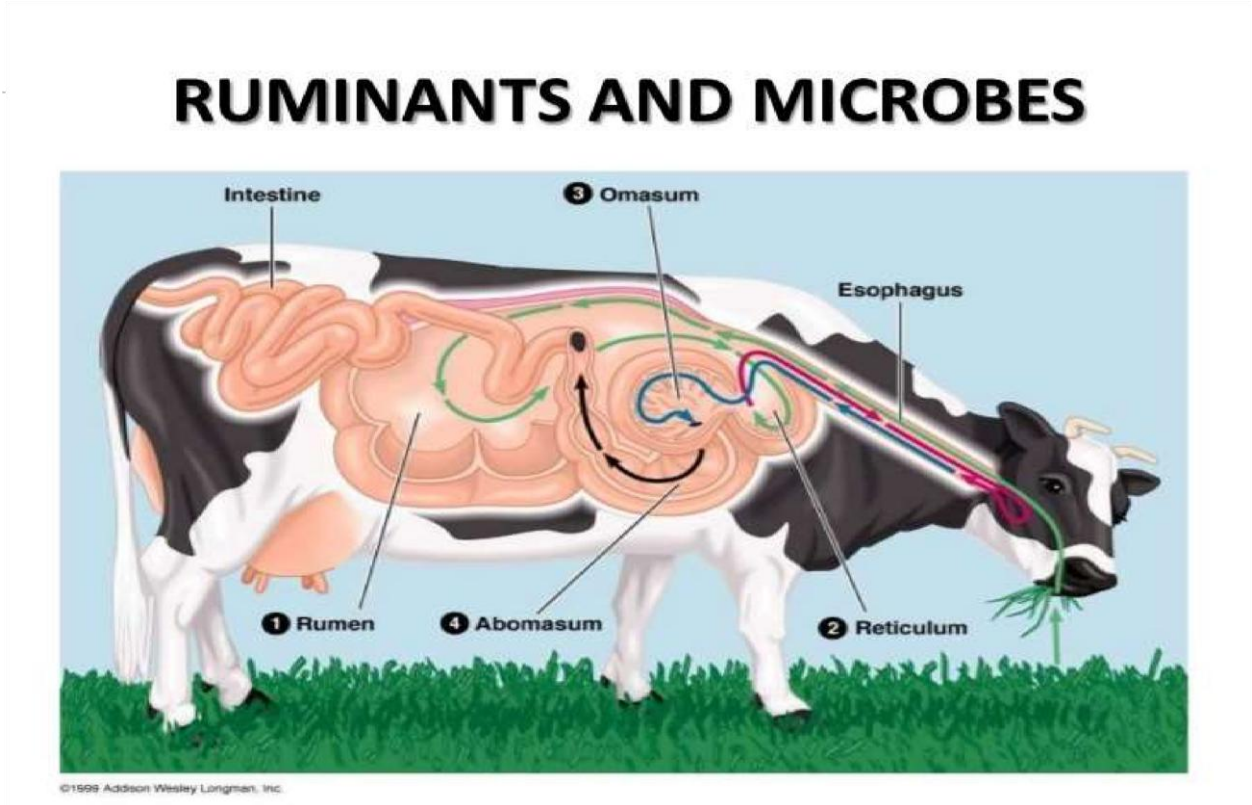
BACTERIA ROOT NODULE SYSTEM

- Enzyme system for N_2 fixation is present only in prokaryotes and nodules are associated with roots of Leguminous plants,bacteria(rhizobia) are specific for a legume species.
- Assciation is beneficial for both,plant provide c and energy source to bacteria and bacteria fix N_2 and provide amino acid to plant.
- *Rhizobium leguminosarum* sp.and pea, beans tropical(root nodules).
- *Azorhizobium caulinodan* and Aquatic tropical legume(stem nodule).



- MICROBE ANIMAL INTERACTIONS Bacteria and fungi interact with humans and other animals and this interaction can be symbiotic, commensalistic or parasitic.
- Symbiotic relationships are widespread and has evolved new metabolic capabilities and cellular structures.(symbiogenesis).

- Evolutionary benefits in a symbiotic relationship are;-provision of dietary needs that their hosts lack including essential amino acids,cofactors,metabolic factors etc.
- N storage and recycling.
- Large alterations in genome of symbionts and adaptations by host to favour the symbiosis occur during long association which can be in form of genome size reduction or increase in AT content in genome.since endosymbionts protect their hosts from pathogens,this may have influenced the evolution of sociality in animals to acquire the endosymbiont through horizontal and vertical transmission.

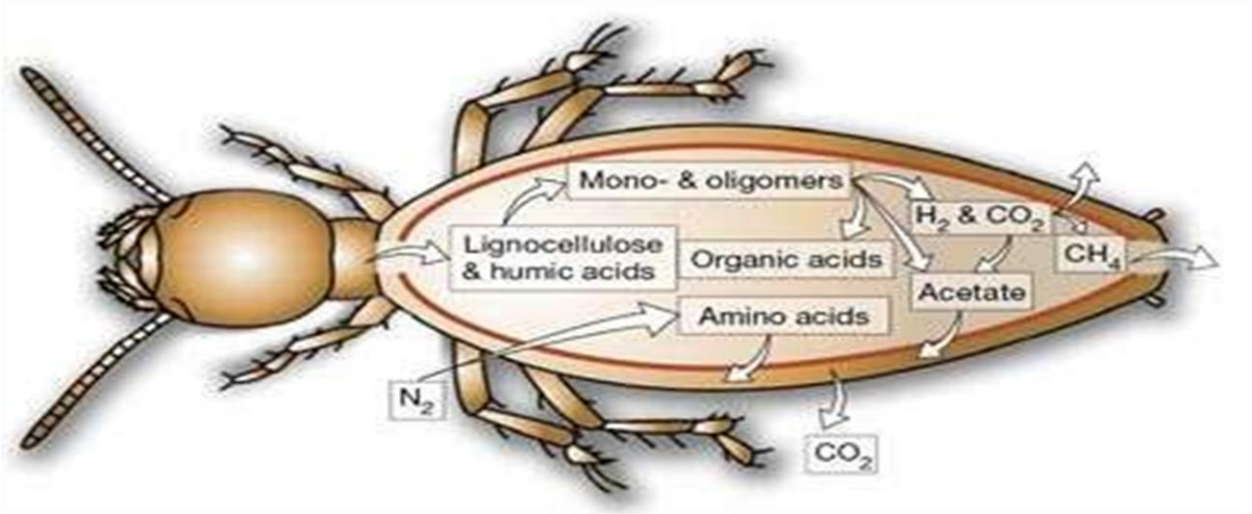


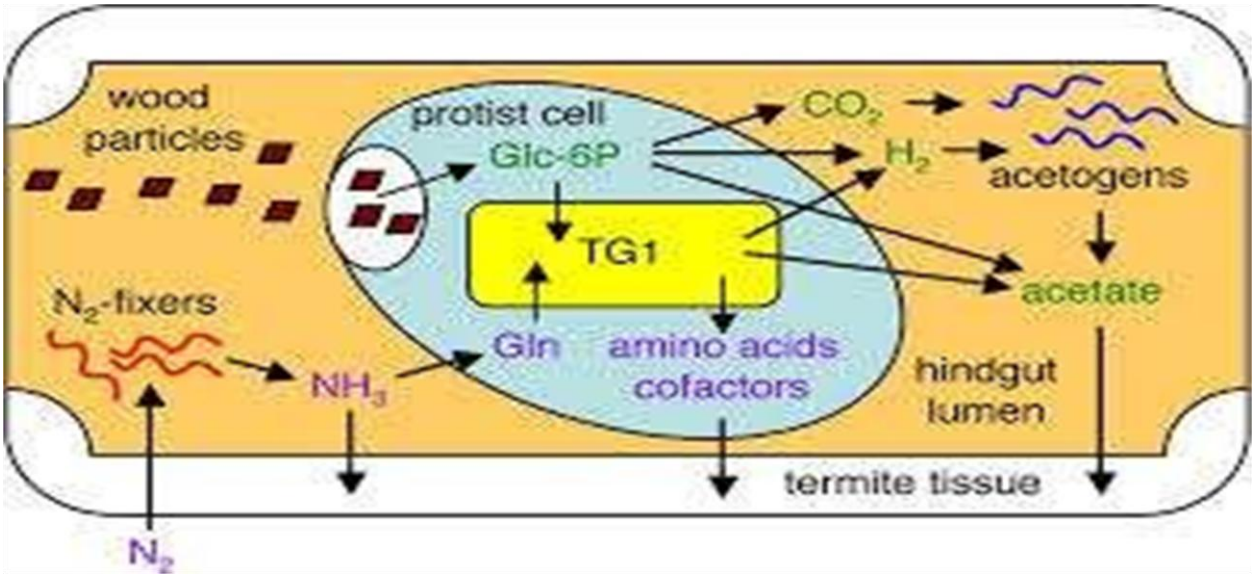
- Primary symbionts are tranmitted through vertical transmission and secondary symbionts through horizontal transmission in addition to maternal transmission.
- *Buchnera aphidicola and Wiglesworthia glossinidia (Bacteria)* are examples of primary symbionts showing marked genome reduction and resultant genome is the commitment to a symbiotic lifestyle.

- ❖ Secondary symbionts may have negative or positive effect on host and are generally facultative and their genomes indicate that they are adapting themselves to an obligate mutulism.

MICROBE ANIMAL MUTUALISM

- ⦿ In vertebrates gut microbial community produce vitamins needed by host,help digetion and colonization resistence.
- ⦿ Termite*(Reticulitermis speratus*) gut community(symbioses within symbiosis) responsible for cellulose degradation include bacteria(spirochaetes,TG1,2,3,bacteroidetes,firmicutes) which provideN-comp. by fixing N_2 and nutrients to host and protists with their bacterial and archeal ecto and endosymbionts degrade cellulose,provide a.acid and cofactors to protists.
- ⦿ Ambrosia beetle carries fungus to a new environment where fungus flourishes beetle uses fungus as food.





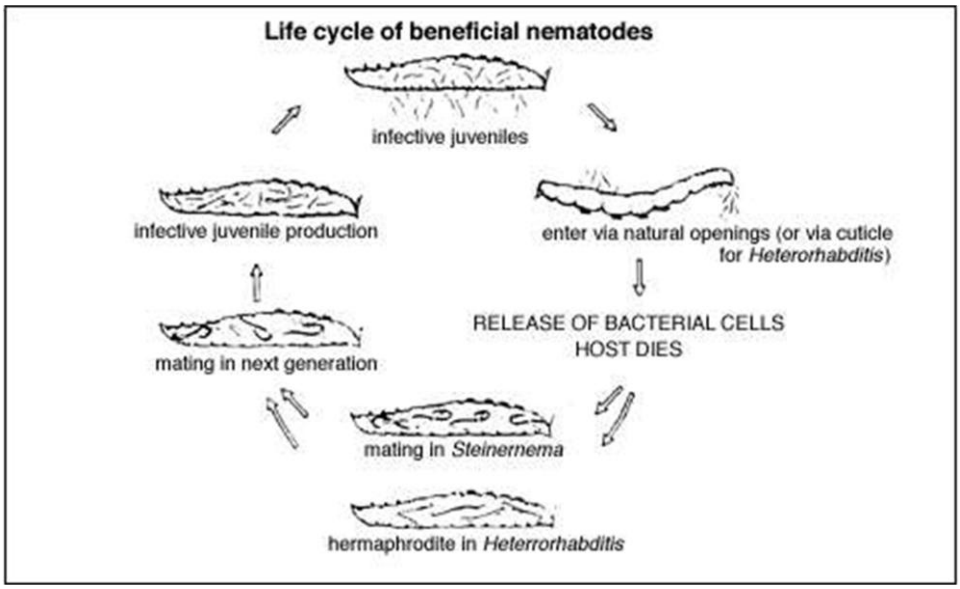
MICROBE ANIMAL PARASITISM

One fascinating parasitic interaction involves nematod(Heterorhabditisbacteriophora harboring a bacterial endosymbiont Photorhabdus luminescens) and parasitize insects and humans.

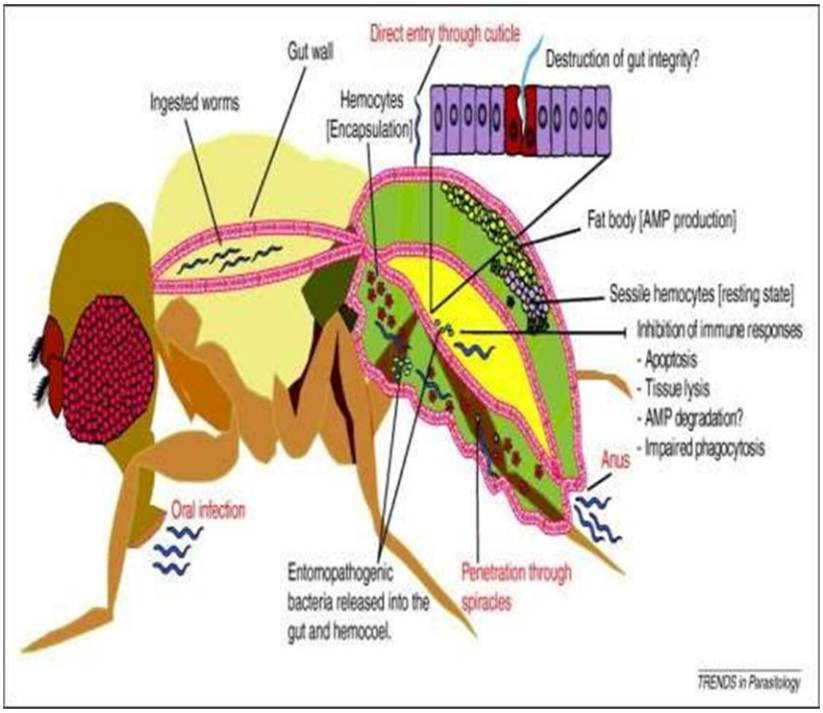
The beneficial nematodes can be used to control a broad range of soil inhabiting insects and above ground insects in their soil inhabiting stage of life. White grubs, Beetle grubs, Japanese beetle

This nematod arrest their development in a phase called infective juvinile larval stage at which it is infected by endosymbiont and when it infects the insect,nematod further development is induced by insects hemolymph.

Endosymbiont secretes proteases supressing insects immune system and damage the insect and nematod parasite feeds on endosymbiont and the insect as well.



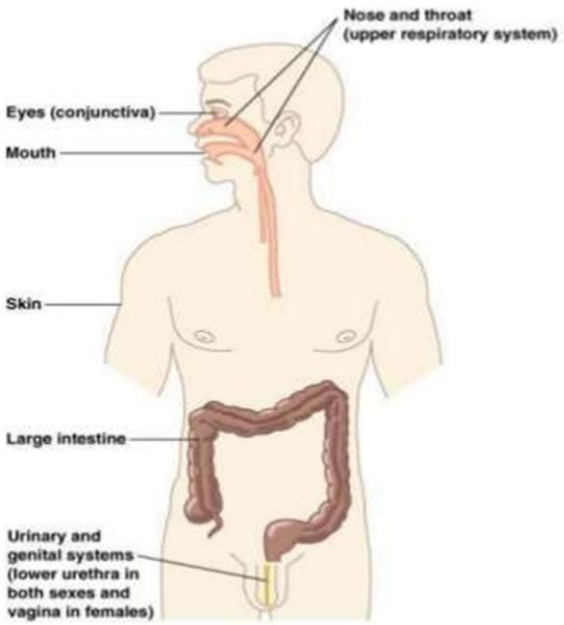
Photorhabdus fluorescent bacteria within a Heterorhabditis nematode


Direct entry through cuticle
Gut wall
Destruction of gut integrity?
Ingested worms
Hemocytes
[Encapsulation]
Fat body [AMP production]
Sessile hemocytes [resting state]
Inhibition of immune responses
- Apoptosis
- Tissue lysis
- AMP degradation?
- Impaired phagocytosis
Anus
Oral infection
Entomopathogenic
bacteria released into the
gut and hemocoel.
Penetration through
spiracles
TRENDS in Parasitology


Larva infected by
Heterorhabditis
bacteriophora
Healthy larva
Healthy and nematode-infected white grubs

Normal Microbiota and the Host:

- Locations of normal microbiota on and in the human body



Normal Microbiota and the Host

- Transient microbiota may be present for days, weeks, or months
- Normal microbiota permanently colonize the host
- Symbiosis is the relationship between normal microbiota and the host

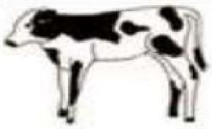
Normal Microbiota and the Host:

- Microbial antagonism is competition between microbes.

- Normal microbiota protect the host by:
  - occupying niches that pathogens might occupy
  - producing acids
  - producing bacteriocins

- Probiotics are live microbes applied to or ingested into the body, intended to exert a beneficial effect.

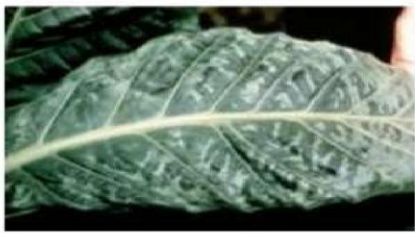
Animal diseases

Animal Reservoir	Top Pathogens to Consider
 Calves	*Cryptosporidium* *E. coli* O157:H7
 Reptiles	*Salmonella*
 Baby Chicks & Ducklings	*Salmonella*
 Adult Poultry	*Campylobacter*
 Puppies & Kittens (particularly those with diarrhea)	*Campylobacter*

Plant pathogens

Xanthomonas
Gram-negative, yellow-pigmented plant pathogenic bacteria

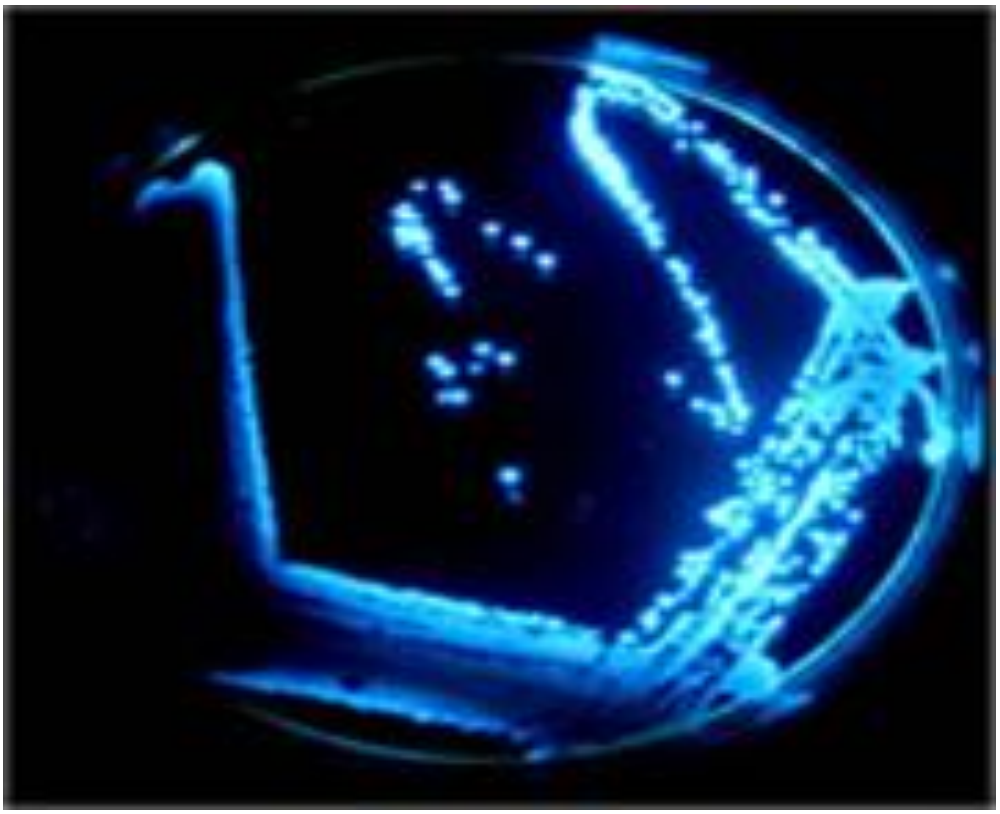
F graminearum causes a disease know as ear and stalk rot in corn and head blight in wheat and barley



Tobacco mosaic virus

Bioluminescent bacteria

- **Bioluminescent bacteria** are light-producing bacteria that are predominantly present in sea water, marine sediments, the surface of decomposing fish and in the gut of marine animals. While not as common, bacterial bioluminescence is also found in terrestrial and freshwater bacteria.
- These bacteria may be free living (such as *Vibrio harveyi*) or in symbiosis with animals such as the Hawaiian Bobtail squid (*Aliivibrio fischeri*) or terrestrial nematodes (*Photorhabdus luminescens*).
- The host organisms provide these bacteria a safe home and sufficient nutrition. In exchange, the hosts use the light produced by the bacteria for concealment, prey and/or mate attraction. Bioluminescent bacteria have evolved symbiotic relationships with other organisms in which both participants benefit close to equally.



Mechanism

- All bacterial luciferases are approximately 80 KDa heterodimers containing two subunits: α and β. The α subunit is responsible for light emission.
- The *luxA* and *luxB* genes encode for the α and β subunits, respectively. In most bioluminescent bacteria, the *luxA* and *luxB* genes are flanked upstream by *luxC* and *luxD* and downstream by *luxE*.
- The bioluminescent reaction is as follows:
- $FMNH_2 + O_2 + R\text{-}CHO \rightarrow FMN + H_2O + R\text{-}COOH + Light\ (\sim 495\ nm)$
- Molecular oxygen reacts with FMNH2 (reduced flavin mononucleotide) and a long-chain aldehyde to produce FMN (flavin mononucleotide), water and a corresponding fatty acid. The blue-green light emission of bioluminescence, such as that produced by *Photobacterium phosphoreum* and *Vibro harveyi*, results from this reaction.
- Because light emission involves expending six ATP molecules for each photon, it is an energetically expensive process. For this reason, light emission is not constitutively expressed in bioluminescent bacteria; it is expressed only when physiologically necessary.
- The symbiotic relationship between the Hawaiian bobtail squid *Euprymna scolopes* and the marine gram-negative bacterium *Aliivibrio fischeri* has been well studied.
- The two organisms exhibit a mutualistic relationship in which bioluminescence produced by *A. fischeri* helps to attract pray to the squid host, which provides nutrient-rich tissues and a protected environment for*A. fischeri*.
- *Euprymna scolopes* lives in a symbiotic relationship with the bioluminescent bacteria *Aliivibrio fischeri*, which inhabits a special light organ in the squid's mantle.
- The bacteria are fed a sugar and amino acid solution by the squid and in return hide the squid's silhouette when viewed from below by matching the amount of light hitting the top of the mantle (counter-illumination)

- Bioluminescence provided by *A. fischeri* also aids in the defense of the squid *E. scolopes* by providing camouflage during its nighttime foraging activity.
- Following bacterial colonization, the specialized organs of the squid undergo developmental changes and a relationship becomes established. The squid expels 90% of the bacterial population each morning, because it no longer needs to produce bioluminescence in the daylight
- This expulsion benefits the bacteria by aiding in their dissemination. A single expulsion by one bobtail squid produces enough bacterial symbionts to fill 10,000m^3 of seawater at a concentration that is comparable to what is found in coastal waters.
- Thus, in at least some habitats, the symbiotic relationship between *A. fischeri* and *E. scolopes* plays a key role in determining the abundance and distribution of *E. scolopes*. There is a higher abundance of *A. fischeri* in the vicinity of a population of *E. scolopes* and this abundance markedly decreases with increasing distance from the host's habitat.
- Bioluminescent *Photobacterium* species also engage in mutually beneficial associations with fish and squid.
- Dense populations of *P. kishitanii*, *P. leiogathi*, and *P. mandapamensis* can live in the light organs of marine fish and squid, and are provided with nutrients and oxygen for reproduction in return for providing bioluminescence to their hosts, which can aid in sex-specific signaling, predator avoidance, locating or attracting prey, and schooling.

NEMATOPHAGUS FUNGI

Some fungi prey on nematodes as a source of nutrients .

By parmer 1964, Nordbring-Hertz and Jansson 1984, Barron 1992

Common Genera of nematode-trapping fungi are:-

1. Arthrobotrys

2. Dactylaria

3. Dactylella

4. Trichothecium

Mechanisms by which the fungi capture nematode prey:-

1. Production of networks of adhesive branches

2. Stalked adhesive knobs

3. Adhesive rings

4. Constrictive rings

MECHANISM

When a nematode prey attempts to move past an adhesive hyphal structure, it is sticks to it and is trapped. When it tries to pass through a constricting ring, a fungal ring contracts by a sudden osmotic swelling and traps the nematode.

Voilent movements and attempts by nematodes to escape generally fails. The fungal hyphae penetrates into nematode which is then enzymatically degraded.

When growing in the absence of nematode appears to induce the formation of morphological structure that traps the nematodes. This is a unique relationship in which presence of prey induces the formation of fungal structures that result in its capture and consumption. When a nematode prey attempts to move past an adhesive hyphal structure, it is sticks to it and is trapped.

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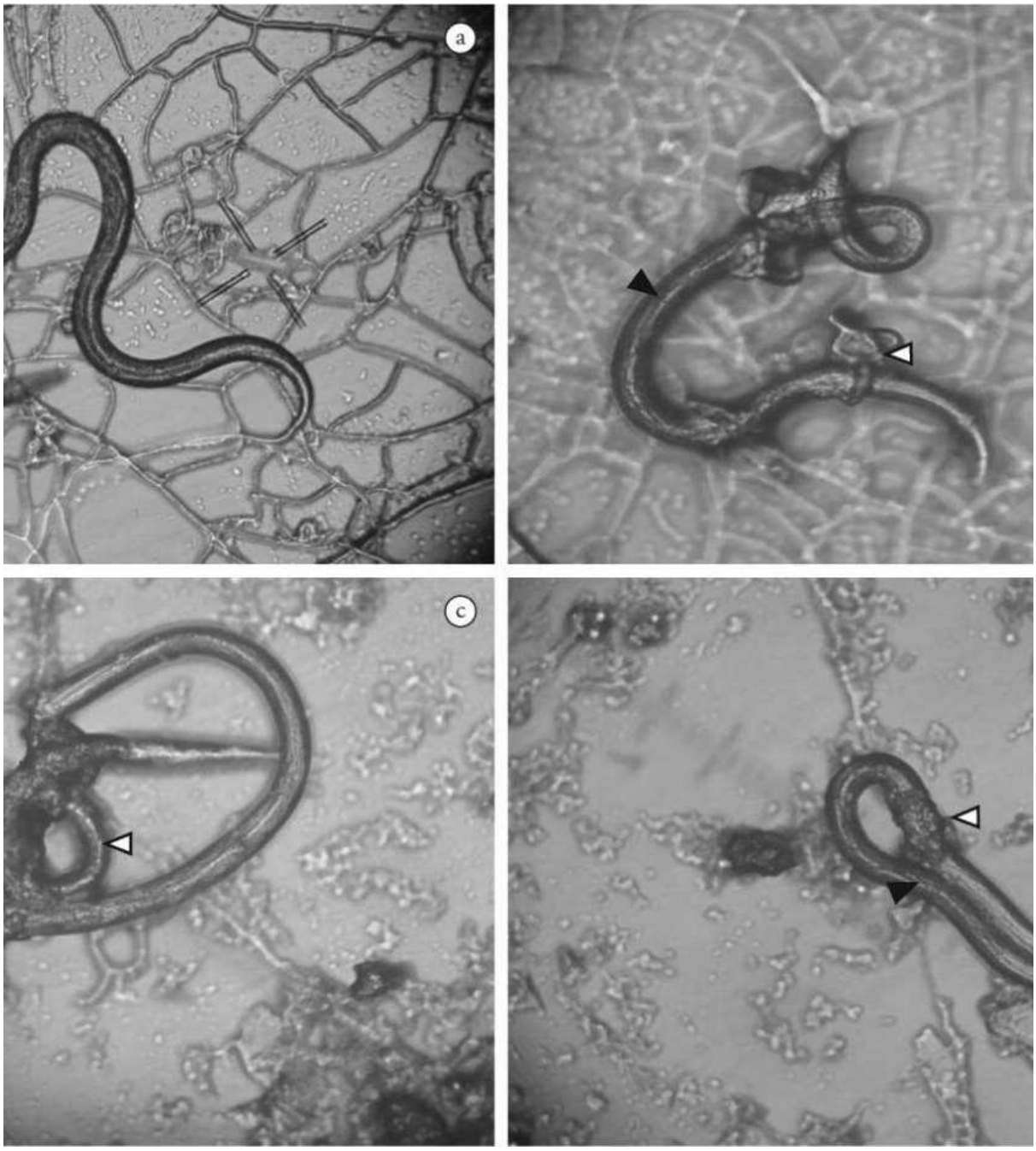
This is a unique relationship in which presence of prey induces the formation of fungal structures that result in its capture and consumption.

MOSTLY DEUTEROMYCOTA ARE FEW BASIDOMYCOTA

EXAMPLE

Hohenbuehelia and Resupinatus- capture nematode by means of adhesive knobs. Pleurotus ostreatus (edible oyster mushroom) & Pleurotus sp. Form no trap structure. By mean of toxin they paralyze nematodes. The described Basidiomycota often grow on decaying wood, a nitrogen poor substrate.It is suggest by Thorn and Barron in 1984 that captured nematodes are source of supplemental nitrogen for fungi.



Infective larvae captured by nematophagus fungi

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DEPARTMENT OF BIOTECHNOLOGY

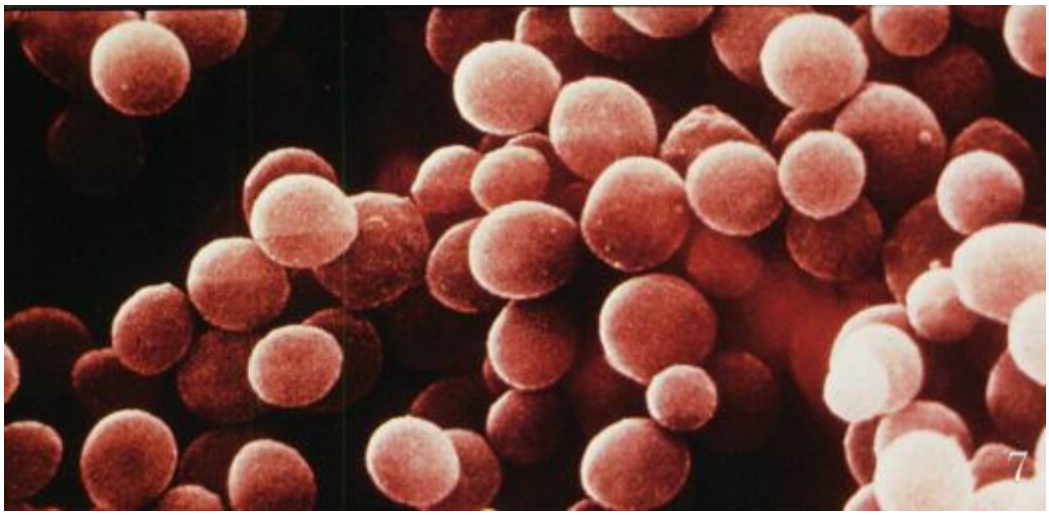
UNIT – IV – MICROBIAL ECOLOGY – SMB2101

UNIT: IV

MECHANISMS OF BACTERIAL PATHOGENESIS

Introduction

A **pathogen** is a microorganism that is able to cause disease in a plant, animal or insect. **Pathogenicity** is the ability to produce disease in a host organism. Microbes express their pathogenicity by means of their **virulence**, a term which refers to the degree of pathogenicity of the microbe. Hence, the **determinants of virulence** of a pathogen are any of its genetic or biochemical or structural features that enable it to produce disease in a host.



***Staphylococcus aureus*, arguably the most prevalent pathogen of humans, may cause up to one third of all bacterial diseases ranging from boils and pimples to food poisoning, to septicemia and toxic shock.**

The Underlying Mechanisms of Bacterial Pathogenesis

Two broad qualities of pathogenic bacteria underlie the means by which they cause disease:

1. Exposure to the host

2. Attachment to the host

3. Colonization in the site of attachment and cause localized infection or follow Invasiveness.

Invasiveness is the ability to invade tissues. It encompasses mechanisms for **colonization** (adherence and initial multiplication), **production of**

extracellular substances which facilitate invasion (**invasins**) and **ability to bypass or overcome host defense mechanisms**.

4.Establishment of disease in specific site by production of toxin or enzymes.

1. Bacterial Adherence to Mucosal Surfaces. In its simplest form, bacterial adherence or attachment to a eucaryotic cell or tissue surface requires the participation of two factors: a **receptor** and an **ligand**. The receptors so far defined are usually specific carbohydrate or peptide residues on the eucaryotic cell surface. The bacterial ligand, called an **adhesin**, is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion. Table 1 is a list of terms that are used in medical microbiology to refer to microbial adherence to surfaces or tissues.

TABLE 1. TERMS USED TO DESCRIBE ADHERENCE FACTORS IN HOST-PARASITE INTERACTIONS

ADHERENCE FACTOR	DESCRIPTION
Adhesin	A surface structure or macromolecule that binds a bacterium to a specific surface
Receptor	A complementary macromolecular binding site on a (eucaryotic) surface that binds specific adhesins or ligands
Lectin	Any protein that binds to a carbohydrate
Ligand	A surface molecule that exhibits specific binding to a receptor molecule on another surface
Mucous	The mucopolysaccharide layer of glucosaminoglycans covering animal cell mucosal surfaces
Fimbriae	Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence
Common pili	Same as fimbriae
Sex pilus	A specialized pilus that binds mating procaryotes together for the purpose of DNA transfer
Type 1 fimbriae	Fimbriae in *Enterobacteriaceae* which bind specifically to mannose terminated glycoproteins on eucaryotic cell surfaces
Type 4 pili	Pili in certain Gram-positive and Gram-negative bacteria. In *Pseudomonas*, thought to play a role in adherence and biofilm formation

S-layer	Proteins that form the outermost cell envelope component of a broad spectrum of bacteria, enabling them to adhere to host cell membranes and environmental surfaces in order to colonize.
Glycocalyx	A layer of exopolysaccharide fibers on the surface of bacterial cells which may be involved in adherence to a surface. Sometimes a general term for a capsule.
Capsule	A detectable layer of polysaccharide (rarely polypeptide) on the surface of a bacterial cell which may mediate specific or nonspecific attachment
Lipopolysaccharide (LPS)	A distinct cell wall component of the outer membrane of Gram-negative bacteria with the potential structural diversity to mediate specific adherence. Probably functions as an adhesion
Teichoic acids and lipoteichoic acids (LTA)	Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence

Specific Adherence of Bacteria to Cell and Tissue Surfaces

Several types of observations provide indirect evidence for **specificity of adherence** of bacteria to host cells or tissues:

1. **Tissue tropism**: particular bacteria are known to have an apparent preference for certain tissues over others, e.g. *S. mutans* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *S. salivarius* which is attached in high numbers to epithelial cells of the tongue but is absent in dental plaque.

2. **Species specificity**: certain pathogenic bacteria infect only certain species of animals, e.g. *N. gonorrhoeae* infections are limited to humans; Enteropathogenic *E. coli* K-88 infections are limited to pigs; *E. coli* CFA I and CFA II infect humans; *E. coli* K-99 strain infects calves.; Group A streptococcal infections occur only in humans.

3. **Genetic specificity within a species**: certain strains or races within a species are genetically immune to a pathogen , e.g. Certain pigs are not susceptible to *E. coli* K-88 infections; Susceptibility to *Plasmodium vivax* infection (malaria) is dependent on the presence of the Duffy antigens on the host's redblood cells.

Although other explanations are possible, the above observations might be explained by the existence of specific interactions between microorganisms and

eucaryotic tissue surfaces which allow microorganisms to become established on the surface.

Mechanisms of Adherence to Cell or Tissue Surfaces

The mechanisms for adherence may involve two steps:

1. **nonspecific adherence: reversible attachment** of the bacterium to the eucaryotic surface (sometimes called "docking")

2. **specific adherence: reversible permanent attachment** of the microorganism to the surface (sometimes called "anchoring").

The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur.

Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eucaryotic cell surface. Possible interactions and forces involved are:

1. hydrophobic interactions

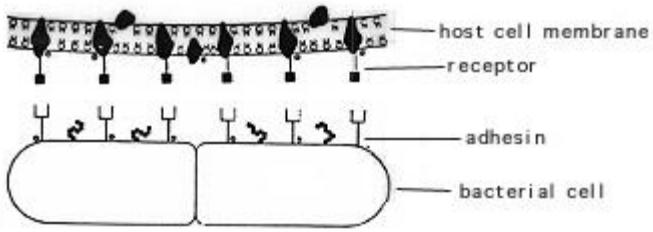
2. electrostatic attractions

3. atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies

4. Brownian movement

5. recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible.



Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the bacterium "docks" itself on the host surface. The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoproteins located on the cell membrane or tissue surface.

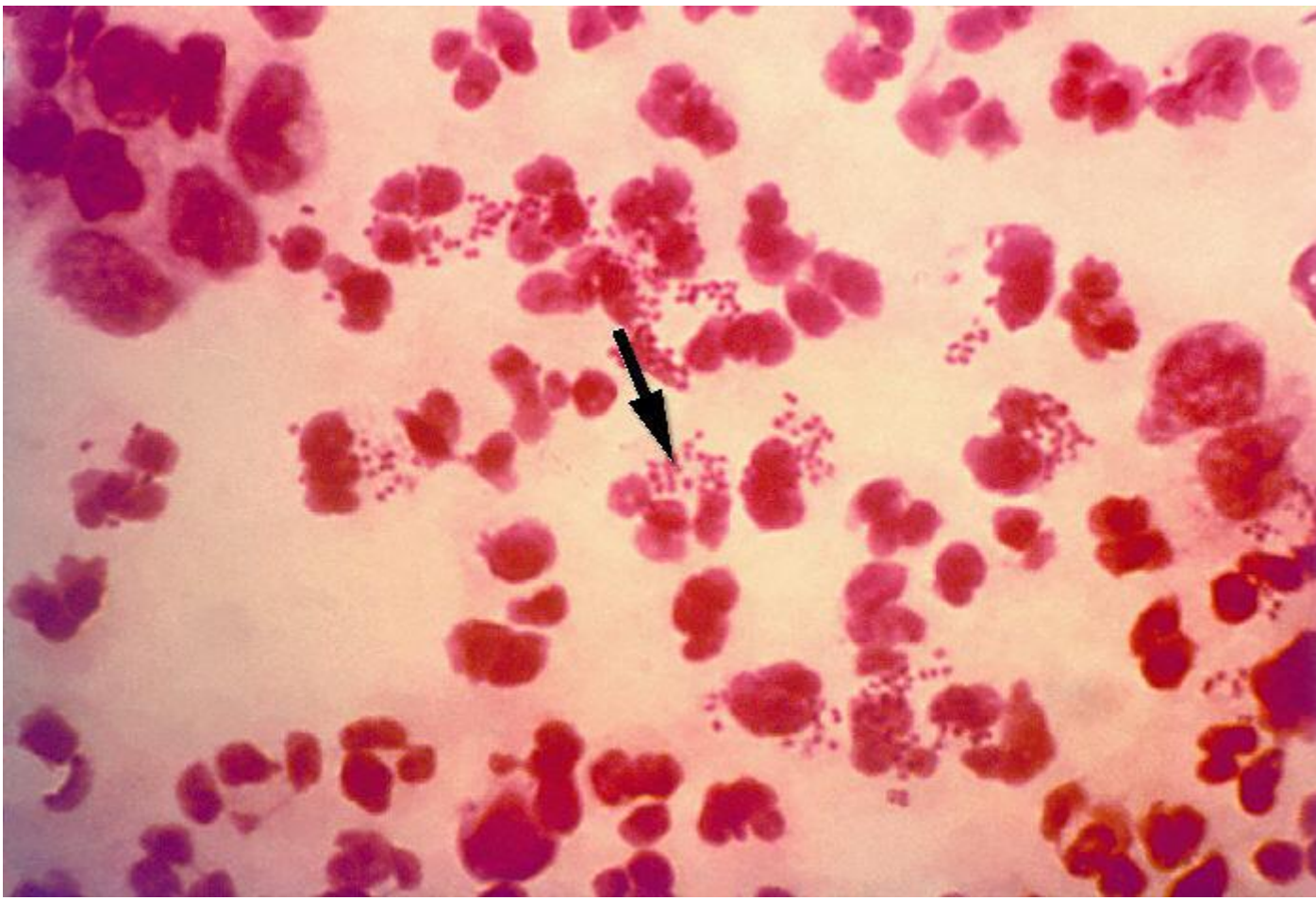
Some Specific Bacterial Adhesins and their Receptors

The adhesins of ***E. coli*** are their common pili or fimbriae. A single strain of *E. coli* is known to be able to express several distinct types of fimbriae encoded by distinct regions of the chromosome or plasmids. This genetic diversity permits an organism to adapt to its changing environment and exploit new opportunities presented by different host surfaces. Many of the adhesive fimbriae of *E. coli* have probably evolved from fimbrial ancestors resembling Type-I and Type IV fimbriae.

Type-I fimbriae enable *E. coli* to bind to D-mannose residues on eucaryotic cell surfaces. Type-I fimbriae are said to be "mannose-sensitive" since exogenous mannose blocks binding to receptors on red blood cells. Although the primary 17kDa fimbrial subunit is the major protein component of Type-1 fimbriae, the mannose-binding site is not located here, but resides in a minor protein (28-31kDa) located at the tips or inserted along the length of the fimbriae. By genetically varying the minor "tip protein" adhesin, the organisms can gain ability to adhere to different receptors. For example, tip proteins on pyelonephritis-associated (pap) pili recognize a galactose-galactose disaccharide, while tip proteins on S-fimbriae recognize sialic acid.

Pseudomonas, Vibrio and ***Neisseria*** possess Type IV pili that contain protein subunit with a methylated amino acid, often phenylalanine, at or near its amino terminus. These "N-methylphenylalanine pili" have been established as virulence determinants in pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. These type of fimbriae occur in *Neisseria gonorrhoeae* and their receptor is thought to be an oligosaccharide. Type IV pili

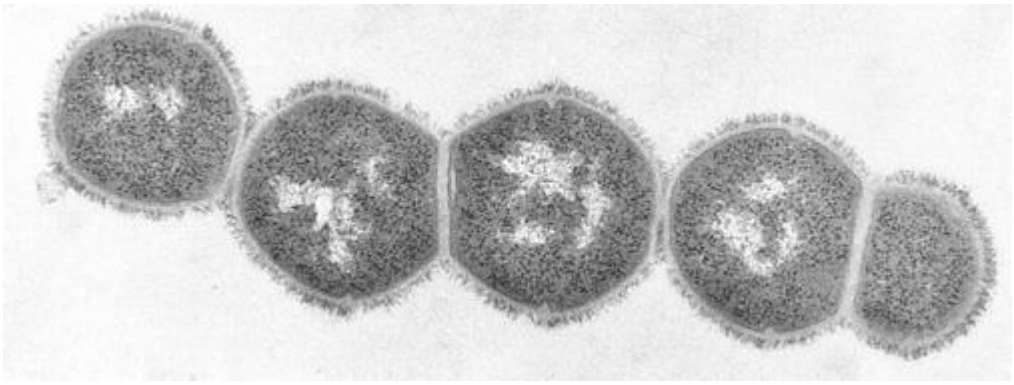
are the tcp (toxin coregulated pili) fimbriae used in attachment of Vibrio cholerae to the gastrointestinal epithelium.



Gram stain of *Neisseria gonorrhoeae,* the agent of the STD gonorrhea. The bacteria are seen as pairs of cocci (diplococci) in association with host pmn's (polymorphonuclear leukocytes). Gonorrhea is the second most prevalent STD in the U.S. behind chlamydia. The bacterium has multiple determinants of virulence including the ability to attach to and enter host cells, resist phagocytic killing and produce endotoxins which eventually lead to an intense inflammatory response. CDC.

The adhesins of ***Streptococcus pyogenes*** are controversial. In 1972, Gibbons and his colleagues demonstrated that attachment of streptococci to the oral mucosa of mice is dependent on M protein. Olfek and Beachey argued that lipoteichoic acid (LTA), rather than M protein, was responsible for streptococcal adherence to buccal epithelial cells. In 1996, Hasty and Courtney proposed a two-step model of attachment that involved both M protein and teichoic acids. They suggested that LTA loosely tethers streptococci to epithelial cells, and then M protein secures a firmer, irreversible association. In 1992, protein F was discovered and found to be a fibronectin binding protein.

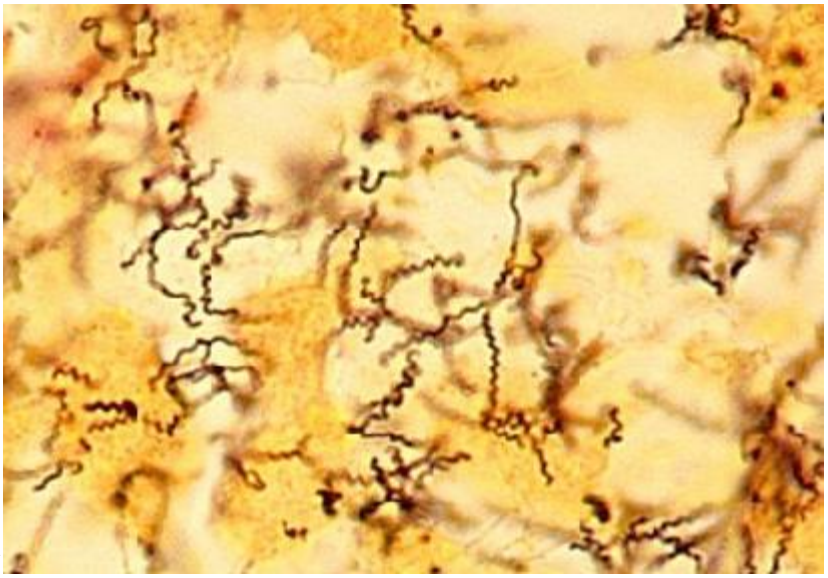
More recently, in 1998, M proteins M1 and M3 were also found to bind to fibronectin. Apparently, *S. pyogenes* produces multiple adhesins with varied specificities.



Electron micrograph of *Streptococcus pyogenes* (Group A strep) by Maria Fazio and Vincent A. Fischetti, Ph.D. with permission. The Laboratory of Bacterial Pathogenesis and Immunology, Rockefeller University. The cell surface fibrils, that consist primarily of M protein, are clearly evident. The M protein has several possible roles in virulence: it is involved in adherence, resistance to phagocytosis, and in antigenic variation of the pathogen.

Staphylococcus aureus also binds to the amino terminus of fibronectin by means of a fibronectin-binding protein which occurs on the bacterial surface. Apparently *S. aureus* and Group A streptococci use different mechanisms but adhere to the same receptor on epithelial surfaces.

Treponema pallidum has three related surface adhesins (P1, P2 and P3) which bind to a four-amino acid sequence (Arg-Gly-Asp-Ser) of the cell-binding domain of fibronectin. It is not clear if *T. pallidum* uses fibronectin to attach to host surfaces or coats itself with fibronectin to avoid host defenses (phagocytes and immune responses).

** 

***Treponema pallidum*, the spirochete that causes syphilis. Silver stain. CDC.**

TABLE 2. EXAMPLES OF SPECIFIC ATTACHMENTS OF BACTERIA TO HOST CELL OR TISSUE SURFACES

Bacterium	**Adhesin**	**Receptor**	**Attachment site**	**Disease**
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexos-amine-galactose disaccharide	Mucosal epithelium	Pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	Type IV pili (N-methylphenyl-alanine pili)	Glucosamine-galactose carbohydrate	Urethral/ cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-I fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea

Uropathogenic *E. coli*	Type I fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenyl-alanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein (fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

COLONIZATION

Colonization: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva. Organisms that infect these regions have usually developed tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses at the surface.

INVASION

The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substances which act against the host by breaking down primary or secondary defenses of the body. Medical microbiologists have long referred to these substances as **invasins**. Most invasins are proteins (enzymes) that act locally to damage host cells and/or have the immediate effect of facilitating the growth and spread of the pathogen. The damage to the host as a result of this invasive activity may become part of the pathology of an infectious disease.

The extracellular proteins produced by bacteria which promote their invasion are not clearly distinguished from some extracellular protein toxins ("exotoxins") which also damage the host. Invasins usually act at a short range (in the immediate vicinity of bacterial growth) and may not actually kill cells as part of their range of activity; exotoxins are often cytotoxic and may act at remote sites (removed from the site of bacterial growth). Also, exotoxins typically are more specific and more potent in their activity than invasins. Even so, some classic exotoxins (e.g. diphtheria toxin, anthrax toxin) may play some role in colonization or invasion in the early stages of an infection, and some invasins (e.g. staphylococcal leukocidin) have a relatively specific cytopathic effect.

A Survey of Bacterial Invasins

Spreading Factors

"Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

Hyaluronidase. is the original spreading factor. It is produced by streptococci. staphylococci, and clostridia. The enzyme attacks the interstitial cement ("ground substance") of connective tissue by depolymerizing hyaluronic acid.

Collagenase is produced by *Clostridium histolyticum* and *Clostridium perfringens*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

Neuraminidase is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

Streptokinase and **staphylokinase** are produced by streptococci and staphylococci, respectively. Kinase enzymes convert inactive plasminogen to plasmin which digests fibrin and prevents clotting of the blood. The relative absence of fibrin in spreading bacterial lesions allows more rapid diffusion of the infectious bacteria.

Enzymes that Cause Hemolysis and/or Leucolysis

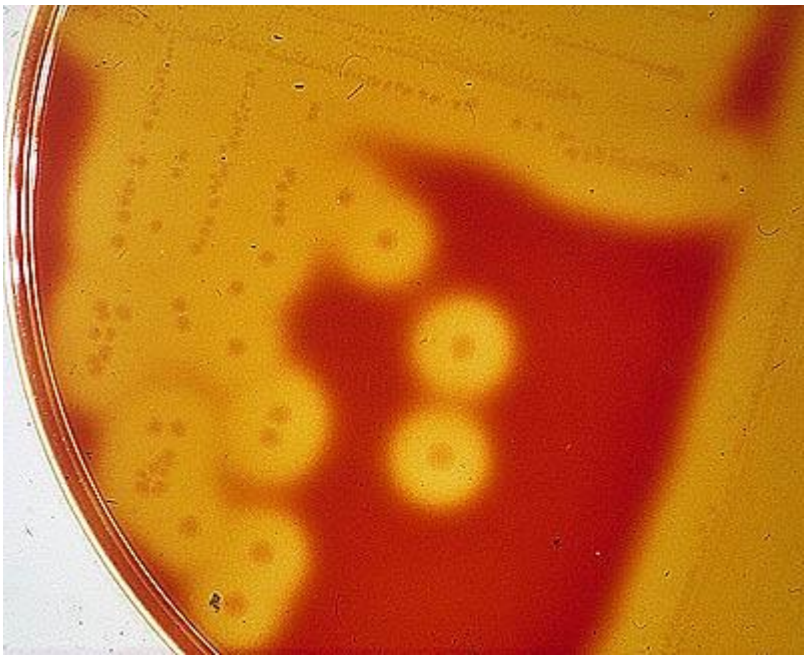
These enzymes usually act on the animal cell membrane by insertion into the membrane (forming a pore that results in cell lysis), or by enzymatic attack on phospholipids, which destabilizes the membrane. They may be referred to as **lecithinases** or **phospholipases**, and if they lyse red blood cells they are

sometimes called **hemolysins**. **Leukocidins**, produced by staphylococci and **streptolysin** produced by **streptococci** specifically lyse phagocytes and their granules. These latter two enzymes are also considered to be bacterial exotoxins.

Phospholipases, produced by *Clostridium perfringens* (i.e., alpha toxin), hydrolyze phospholipids in cell membranes by removal of polar head groups.

Lecithinases, also produced by *Clostridium perfringens,* destroy lecithin (phosphatidylcholine) in cell membranes.

Hemolysins, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e., streptolysin) and various clostridia, may be channel-forming proteins or phospholipases or lecithinases that destroy red blood cells and other cells (i.e., phagocytes) by lysis.



Beta-hemolytic *Streptococcus*. This is the characteristic appearance of a blood agar plate culture of the bacterium. Note the translucency around the bacterial colonies, representing hemolysis of the red cells in the culture medium due to production of a diffusible hemolysin (streptolysin).

Staphylococcal coagulase

Coagulase, formed by *Staphylococcus aureus*, is a cell-associated and diffusible enzyme that converts fibrinogen to fibrin which causes clotting. Coagulase activity is almost always associated with pathogenic *S. aureus* and almost never associated with nonpathogenic *S. epidermidis*, which has led to much speculation as to its role as a determinant of virulence. Possibly, cell bound

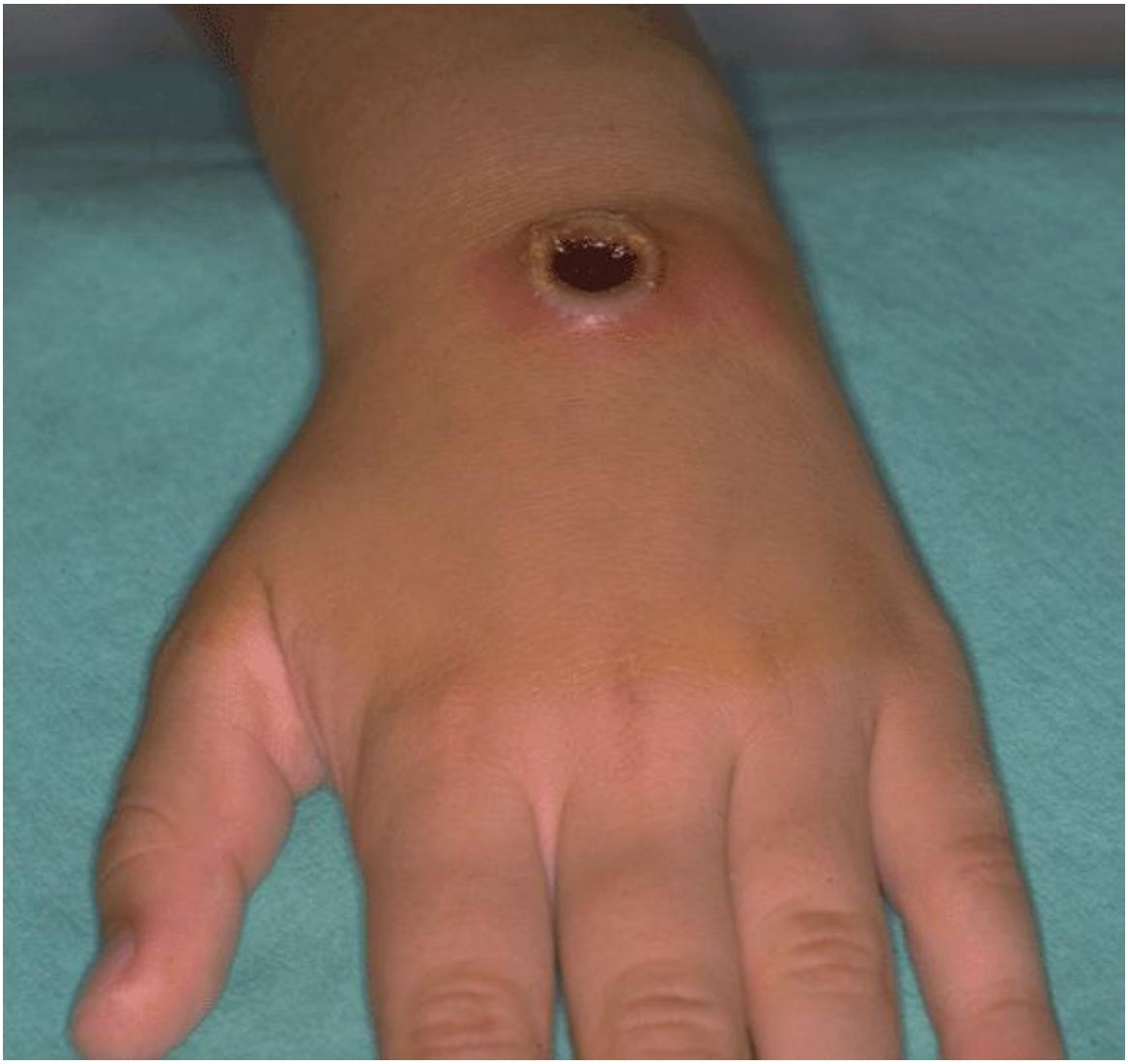
coagulase could provide an antigenic disguise if it clotted fibrin on the cell surface. Or a staphylococcal lesion encased in fibrin (e.g. a boil or pimple) could make the bacterial cells resistant to phagocytes or tissue bactericides or even drugs which might be unable to diffuse to their bacterial target.

Extracellular Digestive Enzymes

Heterotrophic bacteria, in general, produce a wide variety of extracellular enzymes including **proteases**, **lipases**, **glycohydrolases**, **nucleases**, etc., which are not clearly shown to have a direct role in invasion or pathogenesis. These enzymes presumably have other functions related to bacterial nutrition or metabolism, but may aid in invasion either directly or indirectly.

Toxins With Short-Range Effects Related to Invasion

Bacterial protein toxins which have adenylate cyclase activity, are thought to have immediate effects on host cells that promote bacterial invasion. One component of the anthrax toxin (**EF** or **Edema Factor**) is an **adenylate cyclase** that acts on nearby cells to cause increased levels of cyclic AMP and disruption of cell permeability. One of the toxins of *Bordetella pertussis,* the agent of whooping cough, has a similar effect. These toxins may contribute to invasion through their effects on macrophages or lymphocytes in the vicinity which are playing an essential role to contain the infection. For example, since they use ATP as a substrate, they may deplete phagocyte reserves of energy needed for ingestion. Edema is seen as a pathology because the increase in cAMP in affected cells disrupts equilibrium.



Gelatinous edema seen in a cutaneous anthrax lesion. CDC.

The following table summarizes the activities of many bacterial proteins that are noted for their contribution to bacterial invasion of tissues.

TABLE 3. SOME EXTRACELLULAR BACTERIAL PROTEINS THAT ARE CONSIDERED INVASINS

Invasin	**Bacteria Involved**	**Activity**
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue
Collagenase	*Clostridium*	Dissolves

	species	collagen framework of muscles
Neuraminidase	*Vibrio cholerae* and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	*Staphylococcus aureus*	Converts fibrinogen to fibrin which causes clotting
Kinases	Staphylococci and streptococci	Converts plasminogen to plasmin which digests fibrin
Leukocidin	*Staphylococcus aureus*	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	*Streptococcus pyogenes*	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules
Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis
Lecithinases	*Clostridium perfringens*	Destroy lecithin in cell membranes

Phospholipases	*Clostridium perfringens*	Destroy phospholipids in cell membrane
Anthrax EF	*Bacillus anthracis*	One component (EF) is an adenylate cyclase which causes increased levels of intracellular cyclic AMP
Pertussis AC	*Bordetella pertussis*	One toxin component is an adenylate cyclase that acts locally producing an increase in intracellular cyclic AMP

EVASION OF HOST DEFENSES

Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues. For example,

1.The poly-D-glutamate capsule of *Bacillus anthracis* protects the organisms against cell lysis by cationic proteins in sera or in phagocytes.

2. The outer membrane of Gram-negative bacteria is a formidable permeability barrier that is not easily penetrated by hydrophobic compounds such as bile salts which are harmful to the bacteria.

3. Pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides.

4. And intact lipopolysaccharides (LPS) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme.

Most successful pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host internal defense against them, i.e., the phagocytic and immune responses of the host.

Overcoming Host Phagocytic Defenses

Microorganisms invading tissues are first and foremost exposed to phagocytes. Bacteria that readily attract phagocytes, and that are easily ingested and killed, are generally unsuccessful as parasites. In contrast, most bacteria that are successful as parasites interfere to some extent with the activities of phagocytes or in some way avoid their attention.

Microbial strategies to avoid phagocytic killing are numerous and diverse, but are usually aimed at blocking one or of more steps in the phagocytic process. Recall the steps in phagocytosis:

1. Contact between phagocyte and microbial cell

2. Engulfment

3. Phagosome formation

4. Phagosome-lysosome fusion

5. Killing and digestion

Avoiding Contact with Phagocytes

Bacteria can avoid the attention of phagocytes in a number of ways.

1. Invade or remain confined in regions inaccessible to phagocytes. Certain internal tissues (e.g. the lumen of glands) and surface tissues (e.g. the skin) are not patrolled by phagocytes.

2. Avoid provoking an overwhelming inflammatory response. Some pathogens induce minimal or no inflammation required to focus the phagocytic defenses.

3. Inhibit phagocyte chemotaxis. e.g. Streptococcal streptolysin (which also kills phagocytes) suppresses neutrophil chemotaxis, even in very low concentrations. Fractions of *Mycobacterium tuberculosis* are known to inhibit leukocyte migration. *Clostridium ø* toxin inhibits neutrophil chemotaxis.

4. Hide the antigenic surface of the bacterial cell. Some pathogens can cover the surface of the bacterial cell with a component which is seen as "self" by the host phagocytes and immune system. Phagocytes cannot recognize bacteria upon

contact and the possibility of opsonization by antibodies to enhance phagocytosis is minimized. For example, pathogenic *Staphylococcus aureus* produces cell-bound coagulase which clots fibrin on the bacterial surface. *Treponema pallidum* binds fibronectin to its surface. Group A streptococci are able to synthesize a capsule composed of hyaluronic acid.

Inhibition of Phagocytic Engulfment

Some bacteria employ strategies to **avoid engulfment (ingestion)** if phagocytes do make contact with them. Many important pathogenic bacteria bear on their surfaces substances that inhibit phagocytic adsorption or engulfment. Clearly it is the bacterial surface that matters. Resistance to phagocytic ingestion is usually due to a component of the bacterial cell wall, or fimbriae, or a capsule enclosing the bacterial wall. Classical examples of antiphagocytic substances on the bacterial surface include:

Polysaccharide capsules of *S. pneumoniae*, *Haemophilus influenzae*, *Treponema pallidum* and *Klebsiella pneumoniae*

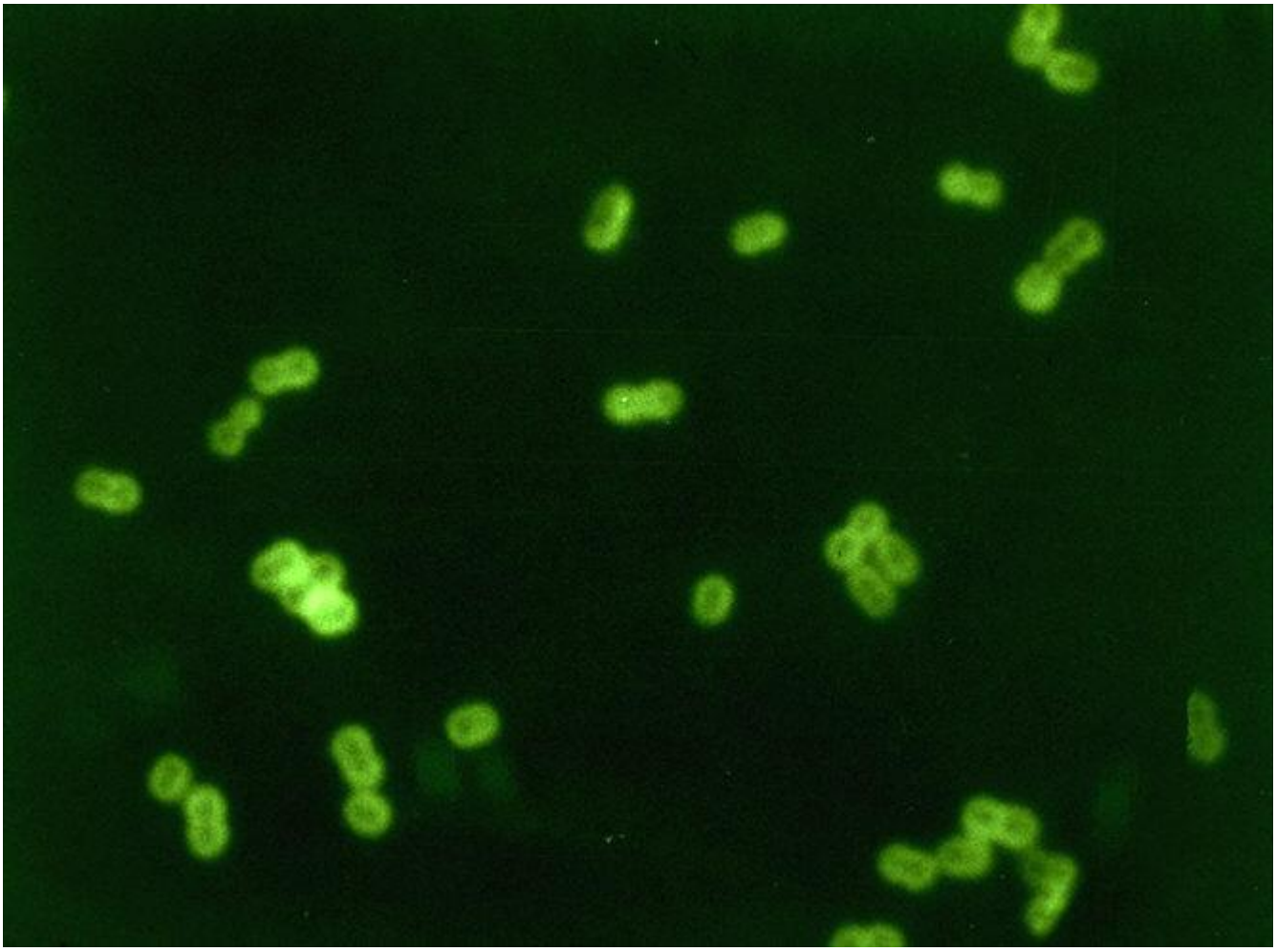
M protein and fimbriae of Group A streptococci

Surface slime (polysaccharide) produced by *Pseudo monas aeruginosa*

O antigen associated with LPS of *E. coli*

K antigen of *E. coli* or the analogous Vi antigen of *Salmonella typhi*

Cell-bound or soluble Protein A produced by *Staphylococcus aureus*



***Streptococcus pneumoniae*, FA stain showing its antphagocytic capsule (CDC). *S. pneumoniae* cells that possess a capsule are virulent; nonencapsulated strains are avirulent. Although *S. pneumoniae* strains possess a variety of determinants of virulence, this illustrates the essential role of their capsule in ability to resist phagocytosis by alveolar macrophages in order to initiate disease.**

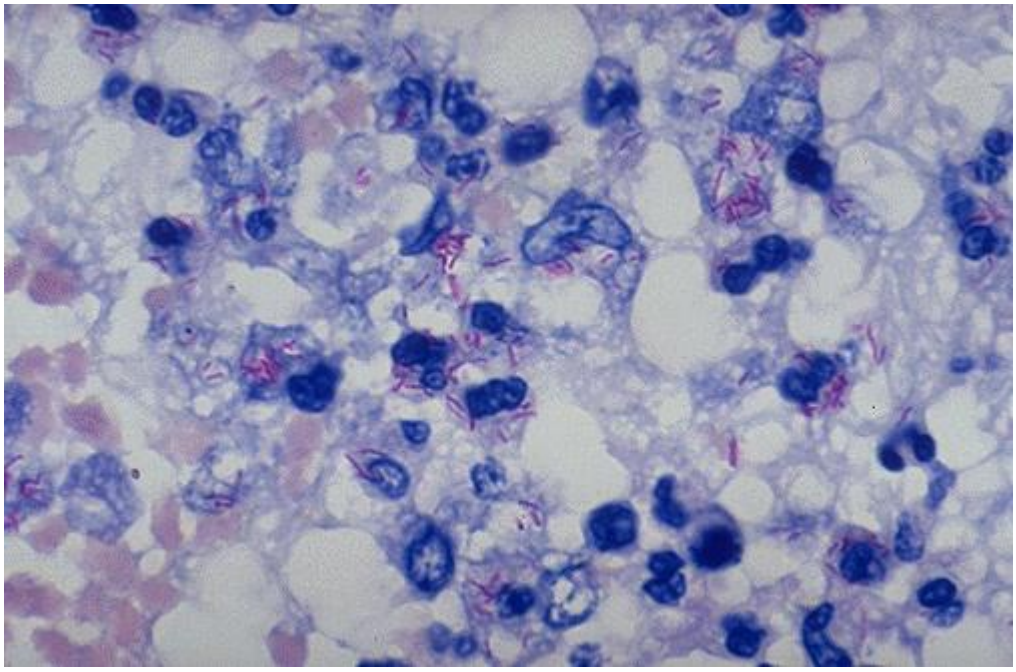
Survival Inside of Phagocytes

Some bacteria survive inside of phagocytic cells, in either neutrophils or macrophages. Bacteria that can resist killing and survive or multiply inside of phagocytes are considered intracellular parasites. The environment of the phagocyte may be a protective one, protecting the bacteria during the early stages of infection or until they develop a full complement of virulence factors. The intracellular environment guards the bacteria against the activities of extracellular bactericides, antibodies, drugs, etc.

Most intracellular parasites have special (genetically-encoded) mechanisms to get themselves into their host cell as well as special mechanisms to survive once

they are inside. Intracellular parasites usually survive by virtue of mechanisms which interfere with the bactericidal activities of the host cell. Some of these bacterial mechanisms include:

1. **Inhibition of phagosome-lysosome fusion**. The bacteria survive inside of phagosomes because they prevent the discharge of lysosomal contents into the phagosome environment. Specifically, phagolysosome formation is inhibited in the phagocyte. This is the strategy employed by *Salmonella*, *M. tuberculosis*, *Legionella* and the *Chlamydiae*.



Intracellular *Mycobacterium tuberculosis* in lung. Ziehl-Neelsen acid fast stain (CDC).

2. **Survival inside the phagolysosome**. With some intracellular parasites, phagosome-lysosome fusion occurs but the bacteria are resistant to inhibition and killing by the lysosomal constituents. Also, some extracellular pathogens can resist killing in phagocytes utilizing similar resistance mechanisms. Little is known of how bacteria can resist phagocytic killing within the phagocytic vacuole, but it may be due to the surface components of the bacteria or due to extracellular substances that they produce which interfere with the mechanisms of phagocytic killing. *Bacillus anthracis*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* all possess mechanisms to survive intracellular killing in macrophages.

3. **Escape from the phagosome**. Early escape from the phagosome vacuole is essential for growth and virulence of some intracellular pathogens. This is a very clever strategy employed by the Rickettsias which produce a

phospholipase enzyme that lyses the phagosome membrane within thirty seconds of after ingestion.

Products of Bacteria that Kill or Damage Phagocytes

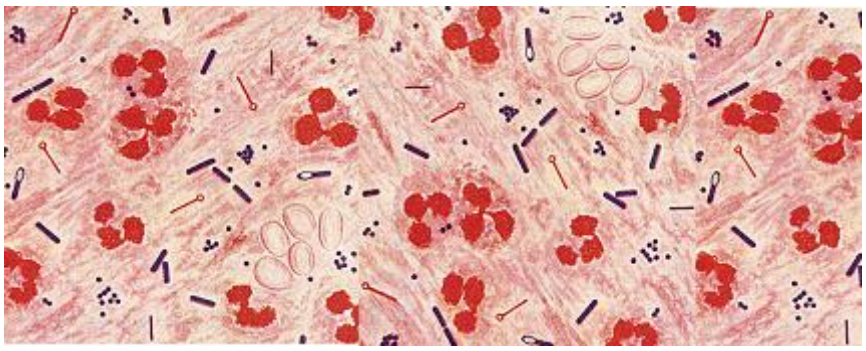
One obvious strategy in defense against phagocytosis is direct attack by the bacteria upon the professional phagocytes. Any of the substances that pathogens produce that cause damage to phagocytes have been referred to as "aggressins". Most of these are actually extracellular enzymes or toxins that kill phagocytes. Phagocytes may be killed by a pathogen before or after ingestion.

Killing phagocytes before ingestion. Many Gram-positive pathogens, particularly the pyogenic cocci, secrete extracellular enzymes which kill phagocytes. Many of these enzymes are called "hemolysins" because their activity in the presence of red blood cells results in the lysis of the rbcs.

Pathogenic streptococci produce streptolysin. Streptolysin O binds to cholesterol in membranes. The effect on neutrophils is to cause lysosomal granules to explode, releasing their contents into the cell cytoplasm.

Pathogenic staphylococci produce leukocidin, which also acts on the neutrophil membrane and causes discharge of lysosomal granules.

Other examples of bacterial extracellular proteins that inhibit phagocytosis include the Exotoxin A of *Pseudomonas aeruginosa* which kills macrophages, and the bacterial exotoxins that are adenylate cyclases (e.g. anthrax toxin EF and pertussis AC) which decrease phagocytic activity.



Gram stain of a pustular exudate from a mixed bacterial infection. Pus is the usual outcome of the battle between phagocytes and bacterial strategies to kill them.

Killing phagocytes after ingestion. Some bacteria exert their toxic action on the phagocyte after ingestion has taken place. They may grow in the phagosome and release substances which can pass through the phagosome membrane and cause discharge of lysosomal granules, or they may grow in the phagolysosome

and release toxic substances which pass through the phagolysosome membrane to other target sites in the cell. Many bacteria which are the intracellular parasites of macrophages (e.g. Mycobacteria, *Brucella*, *Listeria*) usually destroy macrophages in the end, but the mechanisms are not understood.

Evading Complement

Antibodies that are bound to bacterial surfaces will activate complement by the classical pathway and bacterial polysaccharides activate complement by the alternative pathway. Bacteria in serum and other tissues, especially Gram-negative bacteria, need protection from the antimicrobial effects of complement before and during an immunological response.

One role of **capsules** in bacterial virulence is to protect the bacteria from complement activation and the ensuing inflammatory response. Polysaccharide capsules can hide bacterial components such as LPS or peptidoglycan which can induce the alternate complement pathway. Some bacterial capsules are able to inhibit formation of the C3b complex on their surfaces, thus avoiding C3b opsonization and subsequent formation of C5b and the membrane attack complex (MAC) on the bacterial cell surface. Capsules that contain sialic acid (a common component of host cell glycoproteins), such as found in *Neisseria meningitidis*, have this effect.

One of the principal targets of complement on Gram-negative bacteria is LPS. It serves as the attachment site for C3b and triggers the alternative pathway of activation. It also binds C5b.

LPS can be modified by pathogens in two ways that affects its interaction with complement. First, by attachment of sialic acid residues to the LPS O antigen, a bacterium can prevent the formation of C3 convertase just as capsules that contain sialic acid can do so. Both *Neisseria meningitidis* and *Haemophilus influenzae*, which cause bacterial meningitis, are able to covalently attach sialic acid residues to their O antigens resulting in resistance to MAC. Second, LPS with long, intact O antigen side-chains can prevent effective MAC killing. Apparently the MAC complex is held too far from the vulnerable outer membrane to be effective.

Avoiding Host Immunological Responses

On epithelial surfaces the main antibacterial immune defense of the host is the protection afforded by secretory antibody (IgA). Once the epithelial surfaces have been penetrated, however, the major host defenses of inflammation, complement, phagocytosis, Antibody-mediated Immunity (AMI), and Cell-mediated Immunity (CMI) are encountered. If there is a way for a pathogen to

successfully bypass or overcome these host defenses, then some bacterial pathogen has probably discovered it. Bacteria evolve very rapidly in relation to their host, so that most of the feasible anti-host strategies are likely to have been tried out and exploited. Ability to defeat the immune defenses may play a major role in the virulence of a bacterium and in the pathology of disease. Several strategic bacterial defenses are described below.

Immunological Tolerance to a Bacterial Antigen

Tolerance is a property of the host in which there is an immunologically-specific reduction in the immune response to a given Ag. Tolerance to a bacterial Ag does not involve a general failure in the immune response but a particular deficiency in relation to the specific antigen(s) of a given bacterium. If there is a depressed immune response to relevant antigens of a parasite, the process of infection is facilitated. Tolerance can involve either AMI or CMI or both arms of the immunological response.

Tolerance to an Ag can arise in a number of ways, but three are possibly relevant to bacterial infections.

1. **Fetal exposure to Ag**

2. **High persistent doses of circulating Ag**

3. **Molecular mimicry**. If a bacterial Ag is very similar to normal host "antigens", the immune responses to this Ag may be weak giving a degree of tolerance. Resemblance between bacterial Ag and host Ag is referred to as molecular mimicry. In this case the antigenic determinants of the bacterium are so closely related chemically to host "self" components that the immunological cells cannot distinguish between the two and an immune response cannot be raised. Some bacterial capsules are composed of polysaccharides (hyaluronic acid, sialic acid) so similar to host tissue polysaccharides that they are not immunogenic.

Antigenic Disguise

Bacteria may be able to coat themselves with host proteins (fibrin, fibronectin, antibody molecules) or with host polysaccharides (sialic acid, hyaluronic acid) so that they are able to hide their own antigenic surface components from the immunological system.

Immunosuppression

Some pathogens (mainly viruses and protozoa, rarely bacteria) cause immunosuppression in the infected host. This means that the host shows depressed immune responses to antigens in general, including those of the infecting pathogen. Suppressed immune responses are occasionally observed during chronic bacterial infections such as leprosy and tuberculosis.

Persistence of a Pathogen at Bodily Sites Inaccessible to the Immune Response

Some pathogens can avoid exposing themselves to immune forces.

Intracellular pathogens can evade host immune responses as long as they stay inside of infected cells and they do not allow microbial Ag to form on the cell surface. Macrophages support the growth of the bacteria and at the same time give them protection from immune responses.

Some pathogens persist on the luminal surfaces of the GI tract, oral cavity and the urinary tract, or the lumen of the salivary gland, mammary gland or the kidney tubule.

Induction of Ineffective Antibody

Many types of antibody are formed against a given Ag, and some bacterial components may display various antigenic determinants. Antibodies tend to range in their capacity to react with Ag (the ability of specific Ab to bind to an Ag is called **avidity**). If Abs formed against a bacterial Ag are of low avidity, or if they are directed against unimportant antigenic determinants, they may have only weak antibacterial action. Such "ineffective" (non-neutralizing) Abs might even aid a pathogen by combining with a surface Ag and blocking the attachment of any functional Abs that might be present.

Antibodies Absorbed by Soluble Bacterial Antigens

Some bacteria can liberate antigenic surface components in a soluble form into the tissue fluids. These soluble antigens are able to combine with and "neutralize" antibodies before they reach the bacterial cells. For example, small amounts of endotoxin (LPS) may be released into surrounding fluids by Gram-negative bacteria.

Antigenic Variation

One way bacteria can avoid forces of the immune response is by periodically changing antigens, i.e., undergoing antigenic variation. Some bacteria avoid the

host antibody response by changing from one type of fimbriae to another, by switching fimbrial tips. This makes the original AMI response obsolete by using new fimbriae that do not bind the previous antibodies. Pathogenic bacteria can vary (change) other surface proteins that are the targets of antibodies. Antigenic variation is prevalent among pathogenic viruses as well.

Changing antigens during the course of an infection

Antigens may vary or change within the host during the course of an infection, or alternatively antigens may vary among multiple strains (antigenic types) of a parasite in the population. Antigenic variation is an important mechanism used by pathogenic microorganisms for escaping the neutralizing activities of antibodies. Antigenic variation usually results from site-specific inversions or gene conversions or gene rearrangements in the DNA of the microorganisms.

Changing antigens between infections

Many pathogenic bacteria exist in nature as multiple antigenic types or serotypes, meaning that they are variant strains of the same pathogenic species. For example, there are multiple serotypes of *Salmonella typhimurium* based on differences in cell wall (O) antigens or flagellar (H) antigens. There are 80 different antigenic types of *Streptococcus pyogenes* based on M-proteins on the cell surface. There are over one hundred strains of *Streptococcus pneumoniae* depending on their capsular polysaccharide antigens. Based on minor differences in surface structure chemistry there are multiple serotypes of *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae* and an assortment of other bacterial pathogens.

TOXIGENESIS

Two types of bacterial toxins

At a chemical level there are two types of bacterial toxins:

lipopolysaccharides, which are associated with the cell walls of Gram-negative bacteria.

proteins, which may be released into the extracellular environment of pathogenic bacteria.

The lipopolysaccharide (LPS) component of the Gram-negative bacterial outer membrane bears the name endotoxin because of its association with the cell wall of bacteria.

Most of the protein toxins are thought of as exotoxins, since they are "released" from the bacteria and act on host cells at a distance.

BACTERIAL PROTEIN TOXINS

The protein toxins are typically soluble proteins secreted by living bacteria during exponential growth. The production of protein toxins is generally specific to a particular bacterial species (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin (or range of toxins) while nonvirulent strains do not, such that the toxin is the major determinant of virulence. Both Gram-positive and Gram-negative bacteria produce soluble protein toxins. Bacterial protein toxins are the most potent poisons known and may show activity at very high dilutions.

The protein **toxins resemble enzymes** in a number of ways. Like enzymes, bacterial exotoxins:

are **proteins**

are **denatured by heat**, acid, proteolytic enzymes

have a **high biological activity** (most act catalytically)

exhibit **specificity** of action

As enzymes attack specific substrates, so bacterial protein toxins are **highly specific** in the substrate utilized and in their mode of action. The substrate (in the host) may be a component of tissue cells, organs, or body fluid. Usually the site of damage caused by the toxin indicates the location of the substrate for that toxin. Terms such as "enterotoxin", "neurotoxin", "leukocidin" or "hemolysin" are sometimes used to indicate the target site of some well-defined protein toxins.

Certain protein toxins have very specific **cytotoxic activity** (i.e., they attack specific cells, for example, tetanus or botulinum toxins), but some (as produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of tissues (necrosis). Toxins that are phospholipases may be relatively nonspecific in their cytotoxicity because they cleave phospholipids which are components of host cell membranes resulting in the death of the cell by leakage of cellular contents. This is also true of pore-forming "hemolysins" and "leukocidins".

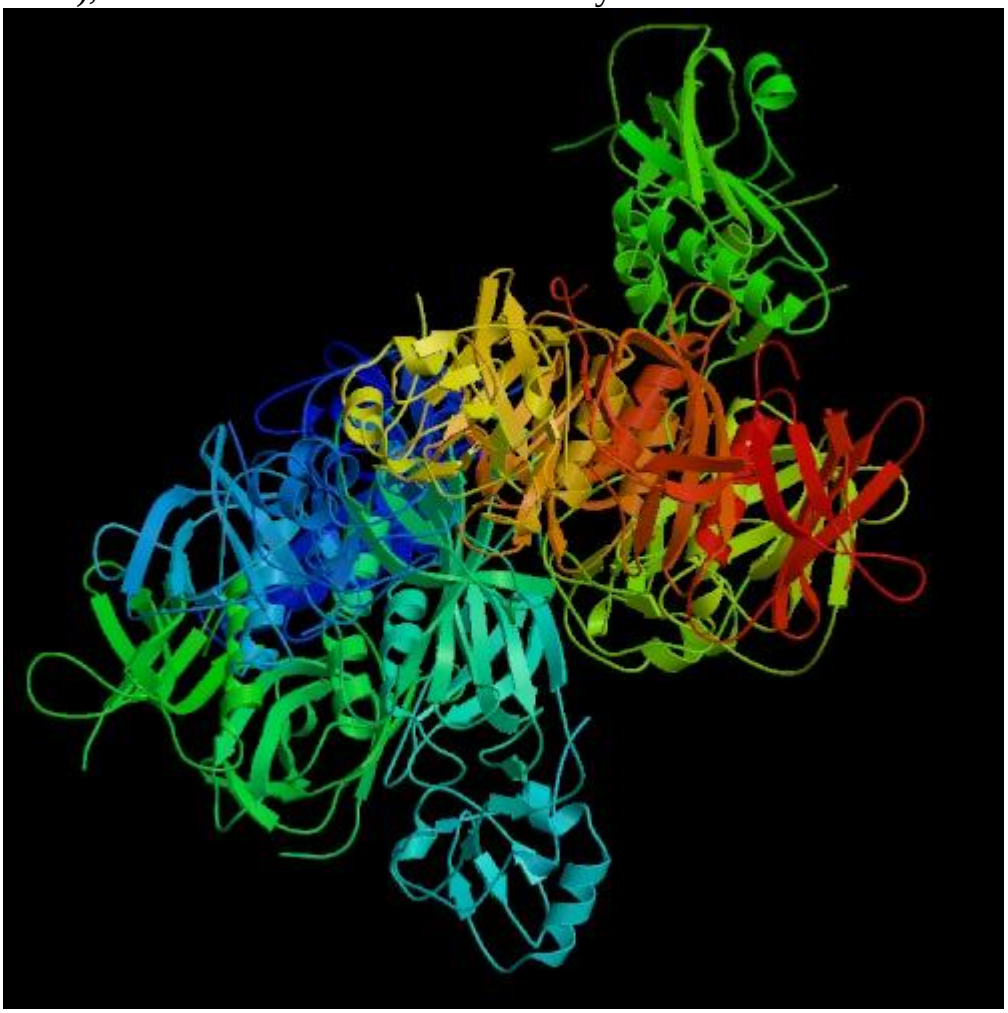
A few protein toxins obviously bring about the death of the host and are known as "lethal toxins", and even though the tissues affected and the target sites may be known, the precise mechanism by which death occurs is not understood (e.g. anthrax toxin).

Protein toxins are inherently unstable: in time they lose their toxic properties but retain their antigenic ones. This was first discovered by Ehrlich and he coined the term toxoid for this product. **Toxoids** are detoxified toxins which retain their antigenicity and their immunizing capacity. The formation of toxoids can be accelerated by treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, etc. The mixture is maintained at 37^{o} at pH range 6 to 9 for several weeks. The resulting toxoids can be use for artificial immunization against diseases caused by pathogens where the primary determinant of bacterial virulence is toxin production. Toxoids are the immunizing agents against diphtheria and tetanus that are part of the DPT vaccine.

A + B Subunit Arrangement of Protein Toxins

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components: one component (subunit A) is responsible for the enzymatic activity of the toxin; the other component (subunit B) is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native toxin. Isolated A subunits are enzymatically active and but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native A+B

toxin), but they are nontoxic.



Tertiary structure of the pertussis toxin produced by *Bordetella pertussis*. Pertussis toxin is a member of the A-B bacterial toxin superfamily. It is a hexameric protein comprising five distinct subunits, designated S1-S5. S2, S3, S4 and S5 comprise the B oligomer, responsible for binding the toxin to the cell surface. Each subunit is translated separately with an amino-terminal signal sequence which is cleaved during transport to the periplasm. S2 and S3 function as adhesins, S2 binds specifically to a glycolipid called lactosylceramide, which is found primarily on the ciliated epithelial cells. S3 binds to a glycoprotein found mainly on phagocytic cells.

Attachment and Entry of Toxins

There are at least two mechanisms of toxin entry into target cells. In one mechanism called **direct entry**, the B subunit of the native toxin (A+B) binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm. In an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis**

(**RME**). The toxin is internalized in the cell in a membrane-enclosed vesicle called an endosome. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. Somehow, the B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface. In both cases, a large protein molecule must insert into and cross a membrane lipid bilayer. This activity is reflected in the ability of most A/B native toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways.

Table 4. SOURCES AND ACTIVITIES OF BACTERIAL TOXINS

NAME OF TOXIN	BACTERIUM INVOLVED	ACTIVITY
Anthrax toxin (EF)	*Bacillus anthracis*	Edema Factor (EF) is an adenylate cyclase that causes increased levels in intracellular cyclic AMP in phagocytes and formation of ion-permeable pores in membranes (hemolysis)
Adenylate cyclase toxin	*Bordetella pertussis*	Acts locally to increase levels of cyclic AMP in phagocytes and formation of ion-permeable pores in membranes (hemolysis)
Cholera enterotoxin	*Vibrio cholerae*	ADP ribosylation of G proteins stimulates adenylate cyclase and increases cAMP in cells of the GI tract, causing secretion of water and electrolytes
E. coli LT toxin	*Escherichia coli*	Similar to cholera toxin
Shiga toxin	*Shigella dysenteriae*	Enzymatically cleaves rRNA resulting in inhibition of protein synthesis in susceptible cells
Botulinum toxin	*Clostridium botulinum*	Zn^{++} dependent protease that inhibits neurotransmission at neuromuscular synapses resulting in flaccid paralysis
Tetanus toxin	*Clostridium*	Zn^{++} dependent protease that

	tetani	inhibits neurotransmission at inhibitory synapses resulting in spastic paralysis
Diphtheria toxin	*Corynebacterium diphtheriae*	ADP ribosylation of elongation factor 2 leads to inhibition of protein synthesis in target cells
Pertussis toxin	*Bordetella pertussis*	ADP ribosylation of G proteins blocks inhibition of adenylate cyclase in susceptible cells
Staphylococcus enterotoxins*****	*Staphylococcus aureus*	Massive activation of the immune system, including lymphocytes and macrophages, leads to emesis (vomiting)
Toxic shock syndrome toxin (TSST-1)*****	*Staphylococcus aureus*	Acts on the vascular system causing inflammation, fever and shock
Pyrogenic exotoxins (SPE) e.g. Erythrogenic toxin (scarlet fever toxin)*****	*Streptococcus pyogenes*	Causes localized erythematous reactions

Superantigens: The "pyrogenic exotoxins" produced by *Staphylococcus aureus* and *Streptococcus pyogenes* have been designated as superantigens. They represent a family of molecules with the ability to elicit massive activation of the immune system. These proteins share the ability to stimulate T cell proliferation by interaction with Class II MHC molecules on APCs and specific V beta chains of the T cell receptor. The important feature of this interaction is the resultant production of IL-1, TNF, and other lymphokines which appear to be the principal mediators of disease processes associated with these toxins.

VIRAL VIRULENCE FACTOR

Although viral pathogens are not similar to bacterial pathogens in terms of structure, some of the properties that contribute to their virulence are similar. Virus virulence factors allow it to replicate, modify host defences, and spread within the host, and they are toxic to the host.

1. **REPLICASES**:

   The **replicases** of viruses like Human immunodeficiency virus 1 and Human immunodeficiency virus 2 (HIV-1 and HIV-2, respectively) are called

reverse transcriptases since they can make DNA copies using a genomic RNA molecule as a template.

In most RNA viruses, the replicase is an RNA-dependent RNA polymerase, or RdRp (RNA --> RNA). In the case of retroviruses (Family Retroviridae) like HIV (genus Lentivirus), however, the replicase is an RNA-dependent DNA polymerase (RNA --> DNA); that is, the said reverse transcriptase.

The enzymes are encoded and used by viruses that use reverse transcription as a step in the process of replication. Reverse-transcribing RNA viruses, such as retroviruses, use the enzyme to reverse-transcribe their RNA genomes into DNA, which is then integrated into the host genome and replicated along with it. Reverse-transcribing DNA viruses, such as the hepadnaviruses, can allow RNA to serve as a template in assembling and making DNA strands. HIV infects humans with the use of this enzyme. Without reverse transcriptase, the viral genome would not be able to incorporate into the host cell, resulting in failure to replicate.

2. **VIRAL ADHESINS OR COAT PROTEIN**

Viruses use adhesins to facilitate adhesion to host cells, and certain enveloped viruses rely on antigenic variation to avoid the host immune defenses. These virulence factors are discussed in more detail in the following sections.

One of the first steps in any viral infection is adhesion of the virus to specific receptors on the surface of cells. This process is mediated by adhesins that are part of the viral capsid or membrane envelope. The interaction of viral adhesins with specific cell receptors defines the tropism (preferential targeting) of viruses for specific cells, tissues, and organs in the body. The spike protein hemagglutinin found on Influenzavirus is an example of a viral adhesin; it allows the virus to bind to the sialic acid on the membrane of host respiratory and intestinal cells. Another viral adhesin is the glycoprotein gp20, found on HIV. For HIV to infect cells of the immune system, it must interact with two receptors on the surface of cells. The first interaction involves binding between gp120 and the CD4 cellular marker that is found on some essential immune system cells. However, before viral entry into the cell can occur, a second interaction between gp120 and one of two chemokine receptors (CCR5 and CXCR4) must occur. Table 6 lists the adhesins for some common viral pathogens and the specific sites to which these adhesins allow viruses to attach.

Table 6. Some Viral Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
Influenzavirus	Influenza	Hemagglutinin	Sialic acid of respiratory and intestinal cells
Herpes simplex virus I or II	Oral herpes, genital herpes	Glycoproteins gB, gC, gD	Heparan sulfate on mucosal surfaces of the mouth and genitals
Human immunodeficiency virus	HIV/AIDS	Glycoprotein gp120	CD4 and CCR5 or CXCR4 of immune system cells

3. ANTIGENIC VARIATION IN VIRUSES

Antigenic variation also occurs in certain types of enveloped viruses, including influenza viruses, which exhibit two forms of antigenic variation: antigenic drift and antigenic shift (Figure 9). Antigenic drift is the result of point mutations causing slight changes in the spike proteins hemagglutinin (H) and neuraminidase (N). On the other hand, antigenic shift is a major change in spike proteins due to gene reassortment. This reassortment for antigenic shift occurs typically when two different influenza viruses infect the same host.

The rate of antigenic variation in influenza viruses is very high, making it difficult for the immune system to recognize the many different strains of Influenzavirus. Although the body may develop immunity to one strain through natural exposure or vaccination, antigenic variation results in the continual emergence of new strains that the immune system will not recognize. This is the main reason that vaccines against Influenzavirus must be given annually. Each year’s influenza vaccine provides protection against the most prevalent strains for that year, but new or different strains may be more prevalent the following year.

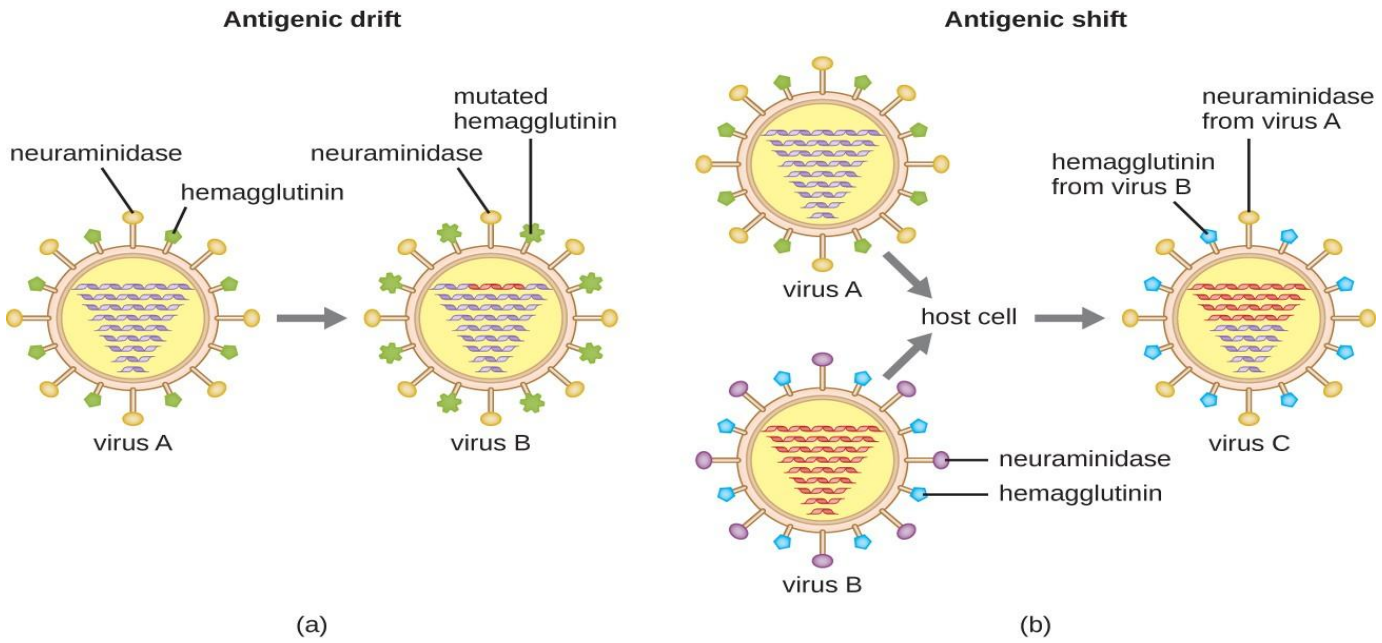


Figure 9. Antigenic drift and antigenic shift in influenza viruses. (a) In antigenic drift, mutations in the genes for the surface proteins neuraminidase and/or hemagglutinin result in small antigenic changes over time. (b) In antigenic shift, simultaneous infection of a cell with two different influenza viruses results in mixing of the genes. The resultant virus possesses a mixture of the proteins of the original viruses. Influenza pandemics can often be traced to antigenic shifts.

4. SILENCING SUPPRESSORS

SUPPRESSORS ACTION OF HOST AGAINST VIRUSES

1. INTERFERON:

Interferons are naturally occurring proteins secreted by cells in response to virus infections. When a cell is infected with a virus, it releases interferon which diffuses to the surrounding cells. After binding to the receptors present on the adjacent or surrounding cells; interferon stimulates the production of antiviral proteins in the cells.

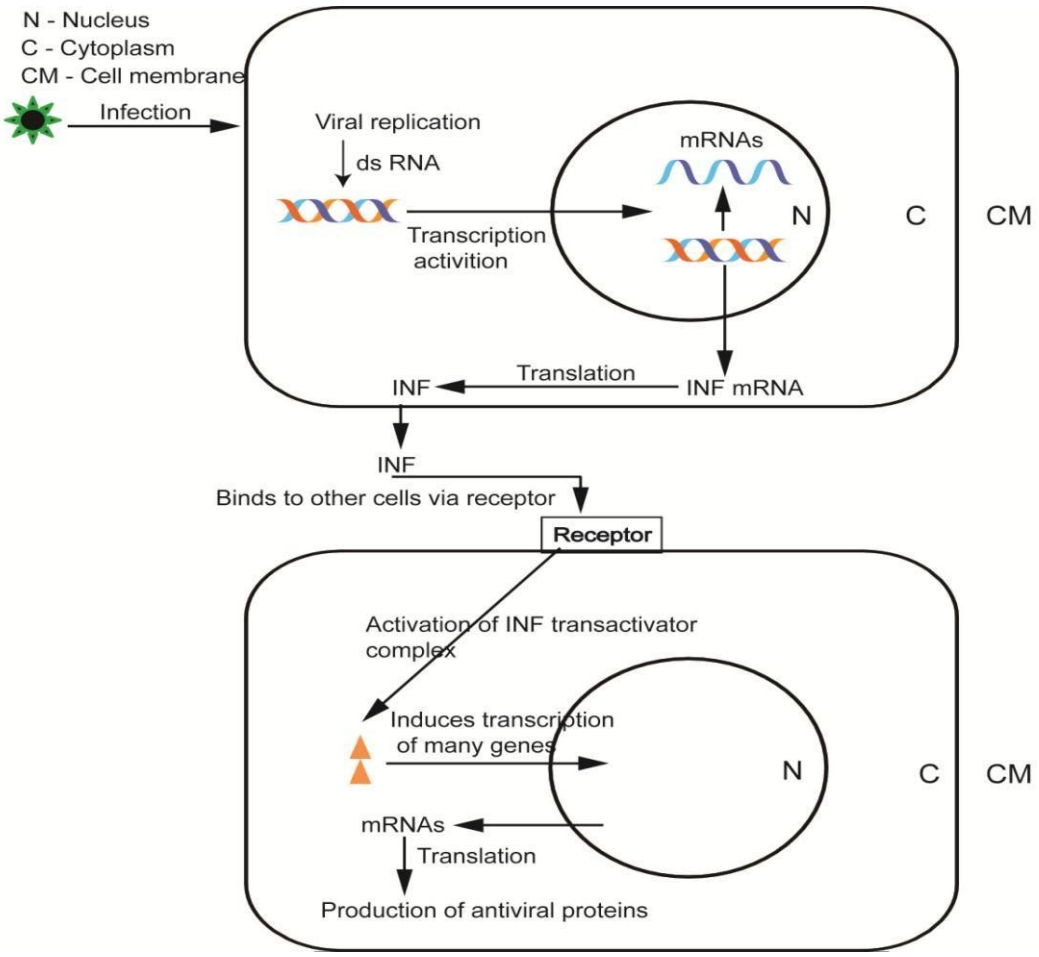


Figure 2.5. Activation of interferon following virus infection:

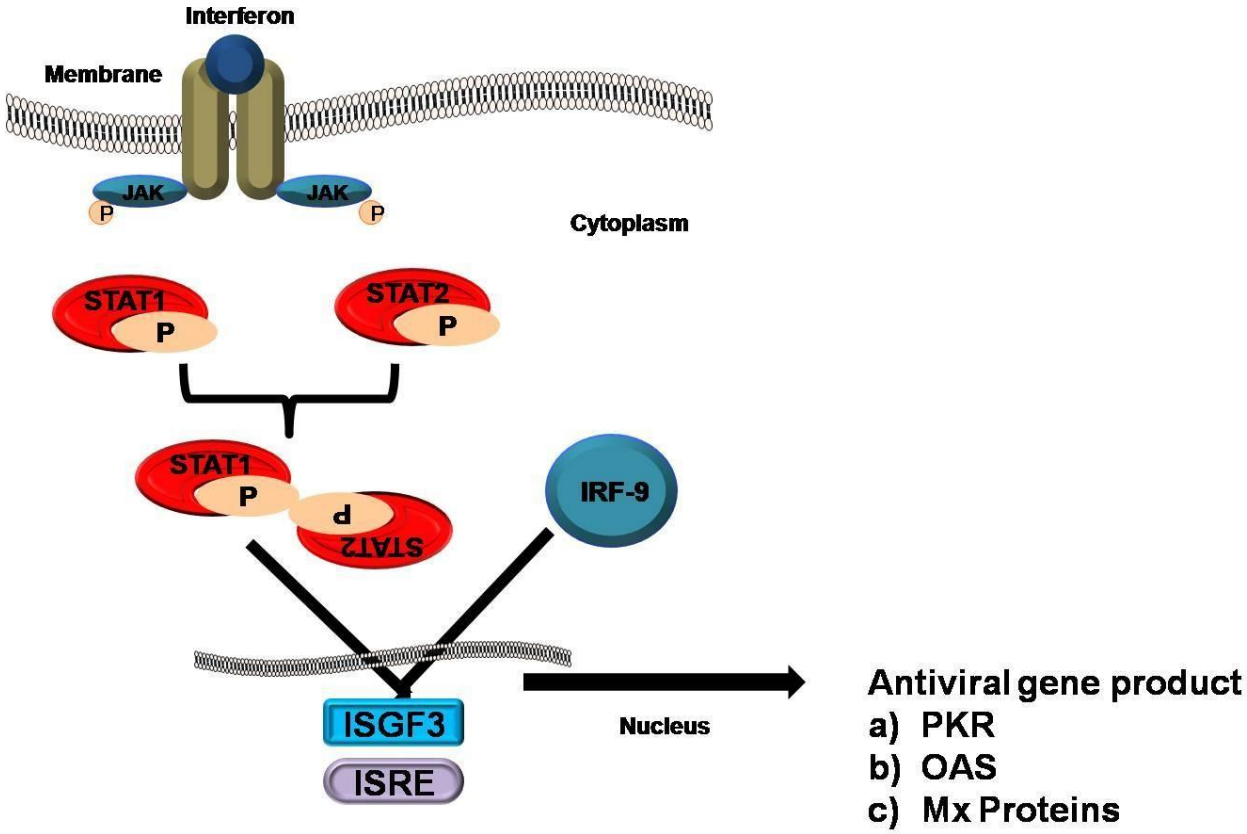
Interferons are of two types

Type I (interferon α and β) and type II (interferon γ). Interferon α is produced by lymphocyte, β by fibroblast, and γ by T lymphocytes upon viral infection. The type of interferon are less or more potent against the class of virus species, for example, interferon α and β inhibits the vesicular stomatitis and encephalomyocarditis viruses better than interferon γ while interferon γ works better in case of vaccinia and reovirus infection.

Many genes are transcriptionally regulated by interferons following virus infection. Among all, three members have been studied extensively for their antiviral activities.

1) dsRNA activated protein kinase (PKR)
2) 2',5'- oligoadenylate synthetase (OAS)
3) Mx proteins

Figure 2.7.Schematic representation of interferon signaling for the activation of antiviral gene



1. dsRNA activated protein kinase (PKR) – During dsRNA virus infection PKR forms the dimer, and is activated following phosphorylation. Eukaryotic translation initiation factor (EIF-2α) is the most important substrate phosphorylated by PKR. EIF-2α gets inactivated following its phosphorylation leading to inhibition of viral protein synthesis. This way PKR exhibits antiviral activity.
2. 2', 5'- oligoadenylate synthetase (OAS) - Interferon inducible OAS is also activated by dsRNA formed during viral infection. It binds to RNase L triggering its dimerization and activation. Activated RNase L degrades the mRNA leading to inhibition of protein synthesis
3. Mx proteins - Interferon induced Mx protein have antiviral activity against several RNA viruses. Mice expressing Mx proteins are more resistant to many virus infections e.g. influenza.

Apoptosis

Apoptosis (programmed cell death) is an interesting way explored by the host cell in order to prevent virus infection. In apoptosis cell must die before virus starts its replication. During apoptosis, cellular DNA undergo fragmentation and apoptotic bodies are formed which are then engulfed by macrophages and other cells of the immune system. Activation of apoptotic cycle involves release of "cytochrome C" from mitochondria and downstream activation of caspases (cysteine-aspartic proteases) cascade.

Role of the immune system against virus attack

Natural killer cells and cytotoxic T cells are the major type of immune cells involved against virus infection in the host. Natural killer cells are activated immediately upon virus infection and produce the cytokines such as tumor necrosis factor (TNF) and interferon γ. They also cause the direct cytotoxicity of the virus infected cells. In later stages of virus infection, the virus surface antigens are presented over the major histocompatibility antigens class I (MHC-I) molecules and activates the cytotoxic T lymphocytes (CTLs). CTLs exert antiviral state by secreting cytokines and apoptosis. Sometime the level of MHC-I gets downregulated in the virus infected cells, in that condition natural killer cells comes at the site of rescue to take over the task and kills the virus infected cells.

EVASION OF INTERFERON SYSTEM BY VIRUSES

Interferons are well studied and established defense system against virus infection. Nevertheless, cohabitation between the host and viruses resulted in the procurement of mechanism to inhibit interferon system by most of the viruses. Viruses inhibit the interferon activation by blocking the different steps involved in the interferon signaling cascade. Some of the unique strategies used by the viruses to decoy the interferon system are enlisted below

Inhibition of protein synthesis

Many viruses hijack the host protein synthesis machinery for their own benefits. This leads to inhibition of cellular protein synthesis and upregulation of viral protein synthesis. As the interferons are also proteins, viral mediated inhibition of host protein synthesis can assist to the inhibition of interferons. Translation inhibition by phosphorylation of eIF2α is a host mediated antiviral mechanism,

many viruses evolved in a way to carryout eIF2α independent translation in order to escape the immune surveillance.

Inhibition of interferon production

Type-I interferon production is activated by dsRNA formed during virus infections. Many viruses encode dsRNA-binding proteins that inhibit the enzymes protein kinases and 2'-5' oligoadenylate synthetase. The sigma protein of reoviruses, and the non structural protein of rotavirus and influenza viruses are some examples of dsRNA- binding proteins.

Inhibition of interferon signaling

Herpes virus and papillomavirus blocks the interferon production by inhibiting the downstream signaling pathway. Adenoviruses, measles virus, and hepatitis viruses were also shown to inhibit the interferon production. All the essential components of interferon signaling pathways, i.e. interferon receptors, JAK/STAT and IRFs have been shown to be involved in virus mediated inhibition.

Despite the identification of the various strategies by which virus interfere with interferon action, little is known on the precise mechanism that exists between viruses and the interferon pathways, and its possible implications on viral pathogenicity, clearance, and viral immunity.

Virus response against apoptosis

Virus inhibits the apoptosis by interrupting the various stages of transcription and translation. Herpes and poxviruses are evolved in a way to modulate the apoptosis by blocking the activation of caspases. SV40 T antigen and E1 protein of adenovirus are known to bind with p53 and target it for proteasomal degradation. Although many viruses prevent apoptosis, herpes virus can selectively cause apoptosis in the lymphocytes in order to delay their removal from the host cell.

Virus response against host immune system

Many viruses come up with a system to reduce the expression of MHC-I molecules over the virus infected host cell surface. This explains the important role of MHC-I towards viral invasion into the susceptible host cells.

HIV, adenovirus, and herpesvirus inhibits the translocation of peptide within the endoplasmic reticulum, which is a necessary step for the loading and trafficking of the peptide over the MHC-I molecules. Cytomegalovirus produces a homologues of MHC-I molecule to decoy the host immune system.Herpes simplex virus express a “glycoprotein E” that binds to the immunoglobulin molecules and prevents the activation of antibody mediated immune response.

EFFECTS OF PATHOGENS ON PLANT PHYSIOLOGICAL FUNCTIONS

Plant pathogens: Organisms which cause diseases in plants called plant pathogens. Pathogens interfere with the different physiological function(s) (photosynthesis, translocation of water, organic compounds, inorganic minerals, transpiration, respiration etc) of the plant and lead to the development of different symptoms (necrosis, chrorosis, stunted growth, no flower, no fruit, no seed).

Eg: 1. Thus a pathogen that infects and kills the flowers of a plant interferes with the ability of the plant to produce seed and multiply.

Eg: 2. A pathogen that injects and kills part or cell of the roots of a plant reduces the ability of the plant to absorb water and nutrients and results in its wilting and death.

2.1 eg: The photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthrachose was found that these diseases assess for the crop damage.

Similarly, a pathogen that infects and kills parts of the leaves or destroys their chlorophyll leads to reduced photosynthesis, growth and yield of the plant and so forth.

In most cases the relationship between the symptoms of the plant and the physiological functions affected is obvious end understandable.

Effect of pathogens on photosynthesis

Photosynthesis is the basic function of green plants. It enables them to transform light energy into chemical energy, which they can utilize in all cell activity. Photosynthesis is the ultimate source of nearly all energy used in all living cells.

In view of the fundamental position of photosynthesis in the life of plants, it is apparent that any interference by pathogens with photosynthesis results in a diseased condition in the plant.

That pathogens do interfere with photosynthesis is obvious from the chlorosis (chlorosis is a condition in which leaves produce insufficient chlorophyll).

The infected plant produces neurotic lesions or large necrotic areas on green plants parts, that leads to reduced growth and amounts of fruits produced by many infected plants.

Chlorosis in leaves

Diseases affect the photosynthesis: the leaf spots, blight and other kinds of diseases in which there is destruction of leaf tissue. The diseases like cereal rusts and fungal leaf spots, bacteial leaf spots, viral mosoics and yellowing and stunting diseases or in defoliations, photosynthesis is reduced because the photosynthetic surface of the plant is declined.

The effects of rust and anthrac nose (Anthracnose Disease Info Anthracnose is a fungal disease that tends to attack plants in the spring when the weather is cool and wet, primarily on leaves and twigs.) on the photosynthetic competence of diseased bean leaves was reported. The overall chlorophyll content of leaves in many fungal and bacterial diseases is reduced, but the photosynthetic activity of the remaining chlorophyll seems to remain unaffected.

Leafy spot

Blight disease

Effect of Aschochyta blight on the decrease in photosynthesizing leaf area and the reduction of photosynthetic efficiency by green leaf area of dried-pea was observed.

Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose was reported to asses crop damage.

In some fungal and bacterial diseases, photosynthesis is reduced because the toxins such as **tentoxin and tabtoxin** produced by these pathogens inhibit some of the enzymes that are involved directly or indirectly in photosynthesis:

In plants infected by many vascular pathogens, tomato remain partially closed, chlorophyll is reduced and photosynthesis stops, even before the plant eventually wilts.

Due to leaf blast of rice, ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis was reported.

Most virus, mollicute (*Mollicutes* are a class of bacteria with no bacterial cell wall) and nematode diseases also induce varying degrees of Chlorosis and stunting. In the majority of such diseases, the photosynthesis of infected plants is reduced greatly. In advanced stages of disease, the rate of photosynthesis is no more than one forth of normal rate.

Effect of pathogens on translocation of water and nutrients in the host plant

All living plant cells require an abundance of water and an adequate amount of organic and inorganic nutrients in order to live and to carry out their physiological functions. Plants absorb water and inorganic (mineral) nutrients from the soil

through their root system. Effect of potassium on growth, water relations, the inorganic and organic solute contents for two maize cultivers grown under saline conditions had been reported. These substances are generally translocated upward through the xylem vessels of the stem and into the vascular bundles of the petioles and leaf veins, from which they enter the leaf cells. All organic nutrients of plants are produced in the leaf cells, following photosynthesis and are translocated down ward and distributed to all the living plant cells passing, for the most part, through the phloem tissues.

A calcium regulated gatekeeper in phloem sieve tube is found. When a pathogen interferes with the upward movement of inorganic nutrients and water or with the downward movement of organic substances diseased conditions results in the parts of plants denied these materials. The comparative physiology of salt and water stress shows that the diseased parts, in turn, will be unable to cary out their own functions and will deny the rest of the plant their services or their products, thus causing disease of the entire plant. Soil water accumulation under different precipitation potential evaporation and straw mulch conditions show reduction in the yield crop.

For e.g. if water movement to the leaves is inhibited, the leaves cannot function properly photosynthesis is reduced

or stopped and few or no nutrients are available to move up to the roots, which in turn become starred and diseased and may die.

Photosynthesis, transpiration and carbohydrate content of apple leaves infected by *Podosphaera leucotricha (fungi).* Interference with upward translocation of water and inorganic

Many plant pathogens interfere in one or more ways with the translocation of water and inorganic nutrients through plants. Some pathogen affect the integrity or function of the roots, causing them to absorb less water; other pathogens, by growing in the xylem vessels or by other means, interfere with translocation of water through the stem; and in some diseases pathogens interfere with the water economy of the plant by causing excessive transpiration through their effects on leaves and stomata.

Effect on translocation of water through the xylem

Fungal and bacterial pathogens that cause damping off, stem rots, and cankers may reach the xylem vessels in the area of the infection and if the affected plants and collapse. Canters is older plants, particularly older trees, may cause some reduction in the translocation of water, but generally do not kill plants unless the cankers are big or numerous enough to encircle the plant.

In vascular wilts, however reduction in water translocation may vary from little to complete. In many cases, affected vessels may be filled with the bodies of the pathogen and with the substances secreted by the pathogen or by the host in response to the pathogen and may become clogged.

Certain pathogens, such as the crown gall bacterium, the club root protozoa on the foot knot nematode induce gall formation in the stem, roots or both. The enlarged and proliforating cells near or around the xylem exert pressure on the xylem vessels, which may be crushed and dislocated, there by becoming less efficient in transporting water.

The most typical and complete dysfunction of xylem in translocating water, however, is observed in the vascular wilts,

caused by the fungi cersatocystis, ophiostoma, fusarium and verticillium and bacteria such as Pseudomonas, Ralstonia and Erwinia. These pathogens invade the xylem of roots and stems and produce diseases primarily by interfering with the upward movement of water through the xylem.

In host combinations with the fastidious bacterium *Xylella fastidiosa* growth, multiplication and spread of bacteria in xylem vessels are clower and instead of causing wilting and rapid death of the plant, a scorching of the margins of the leaves and several other symptoms occur, but rarely does the plant die quickly. In all cases, however in infected hosts the flow of water is reduced through reduction in the size or collapse of vessels due

to infection, development of tyloses in the vessels, release of large molecules compounds in the vessel as a result of cell wall break down by pathogenic enzymes and reduced water tension in the vessels due to pathogen induced alteration in foliar transpiration.

Effect on transpiration

Stomatal transpiration

Lenticular transpiration

Cuticular transpiration

In plant diseases in which the pathogen infects the leaves, transpiration is usually increased.

This is the result of destruction of at least part of the protection afforded the leaf by the cuticle, an increase in the permeability of leaf cells, and the dysfunction of stomata.

- In diseases such as **rusts**, in which numerous pustules form and break up the epidermis.

Rust disease

- In most leaf spots; in which the cuticle epidermis and all the other tissue, inducing xylem may be destroyed in the infected areas
- In the powdery mildews, in which a large proportion of the epidermal cells are invaded by the fungus,

Powdery mildew on leaves

- In apple scab, in which the fungus grows between the cuticle and epidermis
- In all these examples, the destruction of a considerable portion of cuticle and epidermis results in an uncontrolled loss of water from the affected areas.

- If water absorption and translocation cannot keep up with the excessive loss of water, loss of turgor, and wilting of leaves follow.
- The suction forces of excessively transpiring leaves are increased abnormally and may lead to collapse or dysfuction of underlying vessels through the production of tyloses (**balloon like outgrowth of parenchymatous cells to the lumen of tracheids or vessels of the secondary xylem**) and gums.

Interference with translocation of organic nutrients through the phloem

Organic nutrients produced in leaf cells through photosynthesis move through plasmodesmeta into adjoining phloem elements. Plant pathogens may interfere with the movement of organic nutrients from the leaf cells to the phloem, with their translocation through the phloem elements, or possibly, with their movement from phloem into the cells that will utilize them.

Obligate fungal parasites, such as rust and mildew fungi, cause an accumulation of photosynthetic product as well as inorganic nutrients in the areas invaded by the pathogen. In these diseases, the infected areas are characterized by reduced photosynthesis and increased respiration.

In stem diseases of woody plants in which cankers develop, the pathogen attacks and remains confined to the bark for a considerable

time. In diseases caused by phytoplasmas, as well as in diseases caused by phloem-limited fastidious bacteria, bacteria exist and reproduce in the phloem sieve tubes thereby interfering with the downward translocation of nutrients.

Cankers

In several plants propagated by grafting a variety scion onto a rootstock, infection of the combination with a virus e.g. infection of an apple or stone-fruit root stock with (tomato ringspot virus) leads to formation of a necrotic plate at the points of contact of hypersensitive scion variety with the root stock, which leads to the death of scion.

In some viral diseases, particularly the leaf curling type and some yellows diseases, starch accumulation in the leaves, as the result of degeneration (nacrosis) of the phloem of infected plants, which is one of the first symptoms.

It is also possible however at least in some virus diseases, that the interference with translocation of starch stems from inhibition by the virus of the enzymes that break down starch into smaller transposable molecules.

This is suggested by the observation that is some mosaic diseases, in which there is no phloem necrosis, infected discolored areas of leaves contain less starch than 'healthy', greener areas at the end of the day, a period favorable for photosynthesis, but the same leaf areas contain more starch than the 'healthy' areas of the a period of dark, which favors starch hydrolysis add translocation.

This suggests not only that virus infected areas synthesis less starch than healthy ones, but also that starch is not degraded and translocated easily from virus-infected areas, although no damage to the phloem is present.

Effect of pathogens on host plant respiration

Respiration is the process by which cells, through the enzymatically controlled oxidation (burning) of the energy rich carbohydrates and fatty acids, liberate energy in a form that can be utilized for the performance of various cellular processes.

The energy produced through respiration is utilized by the plant for all types of cellular work, such as accumulation and mobilization of compounds, synthesis of proteins, activation of enzymes, cell growth and division, defense reactions, and a host of other processes.

- The respiration is a complex process
- Number of enzymes involved
- Respiration occurs in every single cell
- It affects the functions and existence of the cell

From this we can understand that the respiration of plant tissues is one of the first functions to be affected when plants are infected by pathogens.

Respiration in diseased plants

When plants are infected by pathogen, the rate of respiration generally increases. This means that affected tissues use up their reserve carbohydrates faster than healthy tissues.

- The increased rate of respiration appears shortly after infection certainly by the time of appearance of visible

symptoms and continues to rise during the multiplication and sporulation of the pathogen.

- Stimulation of respiration in *Ulva* /eciuca by high concentration of cadmium and zinc evidence for an alternative respiratory pathway.
- In susceptible varieties, in which no defense mechanisms can be mobilized quickly against a particular pathogen, respiration increases slowly after inoculation, but continues to rise and remains at a high level for much longer periods.
- The effect of Pb^2 and Cd^2 on respiration and mitochondrial electron transport chain in germinating pea seed .

The increased respiration of diseased plants can also be explained as the result of increased metabolism.

- In many plants diseases, growth is at first stimulated protoplasmic streaming increases and materials are synthesized translocated and accumulated in the diseased area.
- It is also possible that the plant because of the infection, utilizes ATP energy less efficiently than a healthy plants.
- A number of chemicals was also found that affects the respiration rates in the diseased plants e.g.- effects of cadmimum on germination, amylasss and rate of respiration on germinating peaseeds was observed by some workers.

- The cations play very prominent role in the regulation *of* electron transport chain.
- The mechanism of the stimulation of state-4 respiration by cadmium in potato tuber mitochondria become the cause of diseases in potato plants.

Effect of pathogen on permeability of cell membrane

Cell membrane consist of a double layer of lipid molecules in which many kinds of protein molecules are embedded; parts of which usually protrude on one or both sides of the lipid bilayer.

- The lipid bilayer is impermeable to most biological molecules changes in all membrane permeability are often the first detectable responses of cells to infection by pathogens,
- They allow most host specific and several non-specific toxins, to certain pathogen enzymes and certain toxic chemicals, such as air pollutants.
- The most commonly observed effect of changes in cell membrane permeability is the loss of electrolytes i.e. of small water soluble ions and molecules from the cell.
- Electrolyte leakage occurs much sooner and at a greater rate when the host pathogen interaction is incompatible and the host remains more resistant than when the host is susceptible and develops extensive symptoms.

- If pathogens cause death by affecting cell membrane permeability directly. It is by stimulating certain membrane bound enzymes, such as ATP ase. Which are involved in the pumping of H* in and K' out through the cell membrane, by interfering with processes required for the maintenance and repair of the fluid film making up the membrane or by degrading the lipid or protein components of the membrane by pathogen produced enzymes.

Effect of pathogens on plant growth

Pathogens that destroy part of the roots of a plant or clog the xylem or phloem elements, thereby severely interfering with the translocation of water and of inorganic or organic nutrients in these plants, often cause a reduction in size and yields by these plants and some times, their death.

In many plant diseases, however, infected tissues or entire plants increase or reduce abnormally in size without a clear-cut explanation of how those changes are brought about.

- The effects of beet yellow virus on the growth and physiology of sugar beet (Beta vulgaris) was reported by Clo'ver, ct *al.* (1999). It is apparent that growth regulators affecting plant cell division and enlargement are involved, but very little is known about the specific compounds and mechanisms involved on the genes that controlled there events.

- Some of the most common diseases in which pathogens use obvious abnormal growth of their host organs and tissues include clubroot of crecifers caused by the plasmodiophoro mycete
- Plasmodiophora brassicae alfalfa wart caused by the fungus *Physoderma alfalfae,*
- potato wart caused by the fungus *Spongospora subterranea,*
- Peach leaf curl and plum pockets caused by the fungus *Taphrina* sps.,
- Black knot canker on chery caused by *Dibotryon morbosum*
- Sphaeropsis gall stone fruits caused by *Sphaeropsis* sps;
- corn smut caused by *Ustilago maydis,*
- dwarf bunt of wheat caused by *Tilletia contraversa,*
- leaf gall of azalea caused by *Exobacidism azoleae*
- Several rusts of pine trees caused by *Cronaritism* sps.
- Some bacterial pathogens also cause abnormal growths such as crown gall of many hosts and hairy root of apple caused by **Agrobacterium tumefaciens** and *A. rhizogenes* respectively,
- olive knot and oleander gall caused by *Pseudomonas sarastanoi* and leafy gall of several host caused by *Rhodococcus* sps.
- Some phytoplasma-infected plants produce shoots that are yellowish, short and bushy and are known as witches brooms.

- The most frequent and unusual effects on plant growth are those caused by viruses and virioids. Many viruses cause stunting

or dwarfing of infected plants. Whereas others cause rolling or curling of leaves, abnormally shaped fruits. Some viruses cause plants to produce galls on their root stems or leaves. Some induce pitting on the roots or stems of infected plants. How the various viruses bring about these effects on their respective hosts is not known.

Bostock, ei *a/.* (1999) studied in spite of growth of the plants pathogen some substances secreted by host plant diminishes the growth of pathogen e.g.- suppression of *Monilinia facticola* cutinose production by peach fruit surface.

Effects of pathogens on plant reproduction

Pathogens that attack various organs and tissues of plants weaker and often kill these organs or tissues, there by weakening the plants; As a result such plants remain smaller in size, may produce fewer flowers and may set fewer fruit and seeds; the latter may be inferior vigor and vitality and therefore, if planted they may produce fewer and weaker new plants. In addition to these indirect effects on pathogens on plant reproduction, many pathogens have a direct adverse effect on plant reproduction because they attack and kill the flowers, fruit or seed directly or interfere and inhibit their

production or the pathogen interfere directly or indirectly with the propagation of their host plants,

Cao, H. *et al.* (2001) reported one of the most common ways by which pathogens interfere with the reproduction of their host is by infecting and killing the flowers of the host; as happens for example with the **brown rot of stone fruits** caused by the fungus *Monilinia* spp. the bacterial canker and gummosis of stone fruit trees caused by *Pseudotnonas syringae* and the fire blight disease of pears and apples caused by the bacterium Erwinia amylovara reported by Chen, Z. et al., (2000), Prusky et al (2001).

In some diseases e.g. in the boost bloom fruit drop of citrus, the fruit after set, drops prematurely as a result of infection by anthracnose fungus *Colletotrlchum acutatutn.*

In several plant diseases, especially in grain crops, the pathogen interferes directly with the reproduction of the plant host by killing the embryo, that would have produced the seed and replacing the contents of the seed with its own fruiting structure or its own spores.

Mareell, ct *al.* (2002) & Gold, S.E. (2003) Examples of such diseases are ergot of grains caused by the fuagus *Claviceps purpurea* core smut and the covered and loose smuts of the

various cereals caused by *Tilletia* and *Utilago* sps. respectively.

Finally, in some diseases caused by viruses, phytoplasmas or plasma-limited bacteria, no flowers are produced or those produced are sterile and therefore few or no fruits and seedes are reproduced.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – V – MICROBIAL ECOLOGY – SMB2101

UNIT: V

PRINCIPLES OF PLANT PATHOLOGY

Introduction to plant pathology

Physiological activities of a healthy plant

1. Normal cell division, differentiation and development.
2. Uptake of water and nutrients from the soil.
3. Synthesis of food from sunlight by photosynthesis.
4. Translocation of water and food to the sites of necessity through xylem and phloem.
5. Metabolism of synthesized material
6. Reproduction

A diseased plant fails to perform one or more of these functions. The effect of a disease on functioning of an organ depends on which cells or tissues were first attacked by the pathogen.

For example, rotting of root tissues will affect the absorption of water and minerals from soil and if vascular tissues have been affected, the translocation of water and photosynthesis will be stopped or reduced.

If leaf tissues are attacked by a pathogen, photosynthesis is affected and plant suffers from deficiency of carbohydrates essential for supplying energy for other activities. Thus, disease can be defined as malfunctioning process that is caused by continuous irritation by a pathogen (Dimond, 1959).

Definitions:

Disease: Any malfunctioning of host cells and tissues that result from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms (G.N.Agrios, 1997).

Pathogens bring about these irritating processes through different but inter-related pathways

1. by utilizing the host cell contents,
2. by causing death of cells or by interfering with their metabolic activities through their enzymes, toxins and growth regulators,
3. by weakening of tissues due to continuous loss of nutrients, and
4. by interfering with translocation of food, minerals and water.

OBJECTIVES OF PLANT PATHOLOGY:

The science of plant pathology has four main objectives:

1. to study the living, non-living and environmental causes of plant diseases,
2. to study the mechanisms of disease development by pathogens,
3. to study the interactions between the plants and the pathogen, and
4. to develop the methods of controlling the diseases and reducing the losses caused by them.

TERMS AND CONCEPTS USED IN PLANT PATHOLOGY

Disease: Any malfunctioning of host cells and tissues that result from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms (G.N.Agrios, 1997).

Disorder: Non-infectious plant diseases due to abiotic causes such as adverse soil and environmental conditions are termed disorders. The common characteristic of non- infectious diseases of plants is that they are caused by the lack or excess of something (temperature, soil moisture, soil nutrients, light, air and soil pollutants, air humidity, soil structure and pH) that supports life. Non-infectious plant diseases occur in the absence of pathogens, and cannot, therefore, be transmitted from diseased to healthy plants.

Pathogen: An entity, usually a micro-organism that can incite disease. In a literal sense a pathogen is any agent that causes *pathos* (ailment, suffering) or damage. However, the term is generally used to denote living organisms (Fungi, bacteria, MLO's, nematodes etc.,) and viruses but not nutritional deficiencies.

Parasite: Organisms which derive the materials they need for growth from living plants (*host or suscept*) are called parasites.

Pathogenicity is the ability of the pathogen to cause disease

Pathogenesis is the chain of events that lead to development of disease in the host (or) sequence of progress in disease development from the initial contact between the pathogen and its host to the completion of the syndrome

Sign: The pathogen or its parts or products seen on a host plant.

Symptom: The external or internal reactions or alterations of a plant as a result of a disease.

Syndrome: The set of varying symptoms characterizing a disease are collectively called a syndrome.

Biotroph: An organism that can live and multiply only on another living organism. They always obtain their food from living tissues on which they complete their life cycle.
Ex: Rust, smut and powdery mildew fungi.

Hemibiotroph (Facultative Saprophyte): The parasites which attack living tissues in the same way as biotrophs but will continue to grow and reproduce after the tissue is dead called as ***facultative saprophytes***.

Perthotrophs or perthophytes (**Necrotroph)**: A parasite is a ***necrotroph*** when it kills the host tissues in advance of penetration and then lives saprophytically
Ex: ***Sclerotium rolfsii***.

Inoculum: It is the part of the pathogen which on contact with susceptible host plant causes infection (or) the infective propagules which on coming in contact with the host plant causes an infection are known as inoculum

Inoculum potential: The energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected (or) The resultant of the action of environment, the vigour of the pathogen to establish an infection, the susceptibility of the host and the amount of inoculum present

Incubation period: The period of time (or time lapse) between penetration of a host by a pathogen and the first appearance of symptoms on the host. It varies with pathogens, hosts and environmental conditions.

Predisposition: It is the action of set of environments, prior to penetration and infection, which makes the plant vulnerable to attack by the pathogen. It is related to the effect of environments on the host, not on the pathogen, just before actual penetration occurs

Hypersensitivity: Excessive sensitivity of plant tissues to certain pathogens. Affected cells are killed quickly, blocking the advance of obligate parasites.

Infection is the establishment of parasitic relationship between two organisms, following entry or penetration (or) the establishment of a parasite within a host plant.

Systemic infection: The growth of pathogen from the point of entry to varying extents without showing adverse effect on tissues through which it passes.

Epidemic or Epiphytotic disease: A disease usually occurs widely but periodically in a destructive form is referred as epidemic or Epiphytotic disease.
Ex: Late blight of potato – Irish famine (1845)

Endemic: Constantly present in a moderate to severe form and is confined to a particular country or district.
Ex: Club root of cabbage in Nilgiris
Black wart of potato – *Synchytrium endobioticum*
Onion smut – *Urocystis cepulae*

Sporadic disease: Occur at very irregular intervals and locations and in relatively fewer instances. Ex: Udbatta disease of rice, Angular leaf spot of cucumber – *Pseudomonas lachrymans*

SURVIVAL OF PLANT PATHOGENS

sources of survival of pathogens:

1) Infected host as reservoir of inoculum (or) survival in vital association with living plants.
2) Survival as saprophytes outside the host.
3) Survival by means of specialized resting structures in or on the host or outside the host.
4) Survival in association with insects, nematodes and fungi.

1) Infected host as reservoir of inoculum:

a) **Seed:** Seed may be externally or internally infected by plant pathogens during the course of development and maturation in fruit or pod.

b) **Collateral hosts / Alternative hosts (wild hosts of same families)**: Collateral hosts are weeds those which are susceptible to the plant pathogens of crop plants and provide adequate facilities for their growth and reproduction of these pathogens during off- season.

Ex: The fungal pathogen for blast disease of rice, *Pyricularia grisea* (Teleomorph: *Magnaporthe grisea*) can infect the grass weeds like *Brachiaria mutica*.

c) **Alternate hosts (Wild hosts of other families):** These alternate hosts are very important for the completion of the life cycle of heteroecious rust pathogens.

The role of alternate hosts is not as important as of collateral hosts. For example in temperate regions the alternate host of *Puccinia graminis tritici* (black or stem rust pathogen of wheat), the barberry bush (*Berberis vulgaris*) grows side by side with the cultivated host. In such areas this wild host belonging to a different family is important for survival of the fungus.

d) **Self sown crops:** Self sown crops, voluntary crops and early sown crops are reservoirs of many plant pathogens. Ex: Self sown rice plants harbour the pathogen (*Rice tungro virus*) as well as vector (*Nephottetix virescens*).

e) **Ratoon crops:** Sometimes ratoon crops also harbour the plant pathogens. Ex: Sugarcane mosaic.

f) **Survival by latent infection:** Latent infection refers to the conditions in which the plant pathogens may survive for a long time in plant tissue without development of visual symptoms. Ex: *Xylella fastidiosa*, the causal agent of pierce's disease of grapevine infect different weeds without developing visible symptoms.

2) Saprophytic survival outside the host:

Many plant pathogens survive in or on the soil in the absence of growing susceptible plants. Waksman (1971) distinguished between soil inhabitants and soil invaders; the former comprise the basic fungal flora of the soil, whereas the later are short lived exotics.

In the absence of the cultivated host plant, fungi are capable of surviving as saprophytes and can be studied under three

categories:

1) **Soil inhabitants**: Those organisms which survive indefinitely in the soil as saprophytes in the absence of the host plant. Ex: Species of *Pythium*, *Rhizoctonia* and *Sclerotium*
2) **Root inhabitants**: These are more specialized parasites that survive in soils in close association with their hosts. The active saprophytic phase remains as long as the host tissue in which they are living as parasites is not completely decomposed. Ex: Species of *Fusarium*, *Verticillium* (vascular wilt causing fungi) and root rot of cotton (*Phymatotrichum omnivorum*)
3) **Rhizosphere colonizers**: Those organisms which colonize the dead substrates in the root region and continue to live like that for a longer period which are more tolerant to soil antagonism. Ex: Leaf mold in tomato: *Cladosporium fulvum*

Differentiate Soil inhabitants and soil invaders:

Soil inhabitants fungi	**Soil invaders / Root inhabiting**
1. These are unspecialized parasites that survive in soils in close association with the soil as saprophytes.	1. These are more specialized parasites that with a wide host range that are able to survive indefinitely in their hosts.
2. Soil inhabitants include obligate facultative saprophytes and facultative parasites which are endo-pathogens they are (root infecting fungi).	2. Soil invaders include saprophytes exo-pathogens
3. Soil and plant debris serve as media for their saprophytic survival.	3. The active saprophytic phase remains as long as the host tissue in which they were living as parasites is not completely decom- posed.
4. They have high competitive saprophytic tic survival ability.	4. They have low competitive survival ability.
5. Species of *Pythium*, *Rhizoctonia*, *Sclerotium*, fungi and bacteria etc., survive as soil inhabitants for considerable length of time in absence of the host.	5. Most plant pathogenic soil invaders. Many vascular wilt causing species of *Fusarium*, *Verticillium*, etc., are soil invaders.

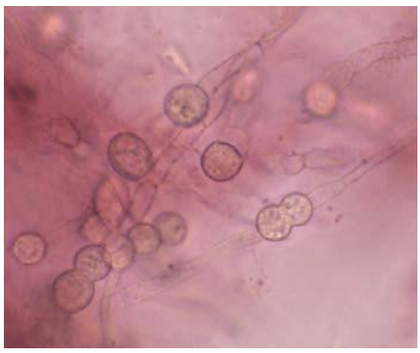
3) Survival as dormant spores or specialized resting structures:

Plant viruses have no resting stage and are transmitted through a continuous infection chain.

Phytopathogenic bacteria: The plant **bacteria** also do not produce resting spores or similar structures. They continuously live in their active parasitic stage in the living host or as active saprophytes on dead plant debris.
Nematodes: They survive in the form of active parasitic phase on a living host and also survive through dormant structures, i.e., eggs, cysts, galls, formed in host tissues. These structures may be present in soil or in seed lots
Phanerogamic parasites: They survive in dormant state for many years through seeds. Ex; Seeds of Orobanchae survive in soil for more than 7 years.

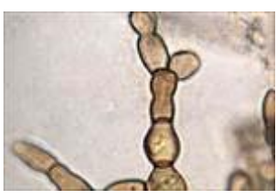
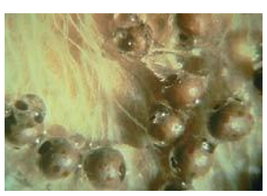
Among plant pathogens, fungi are the only organisms that produce spores, analogous to eggs of nematodes, and other resting structures for their inactive survival. These dormant structures of survival can be classified in the following categories.

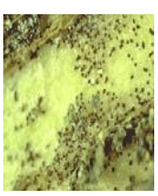
1) ***Soil borne fungi***:
   a) Dormant spores {Conidia (Peach leaf curl pathogen, *Taphrina deformans*), Chlamydospores (Wilt pathogen, *Fusarium* sp.), oospores (Downy mildew fungi), perithecia (Apple scab pathogen, *Venturia inaequalis*) etc.}.




Oospore Chlamydospores Perithecium

   b) Other dormant structures such as thickened hypha, sclerotia (Cottony rot fungus, *Sclerotinia sclerotiorum*), microsclerotia (Verticillium), Rhizomorphs (Armillaria mellea), etc.



Thickened hyphae Sclerotia Rhizomorphs Microsclerotia

   c) Factors affecting the survival of pathogen in the soil are **a)** physical factors (high temperature, irradiation, dessication and anaerobiosis), **b)** chemical factors (antibiotics, antagonistic chemicals produced by other microbes) and **c)** biotic factors (parasitism, predation by microflora and microfauna).

2) Seed borne fungi:

a) **Externally seed borne**: Dormant spores on seed coat Ex: Covered smut of barley, grain smut of jowar, bunt of wheat, etc.

b) **Internally seed borne**: Dormant mycelium under the seed coat or in the embryo Ex: Loose smut of wheat (*Ustilago nuda tritici*)

c) Factors affecting the survival of the pathogen on/in the seed are temperature and moisture.

3) ***Dormant fungal structures on dormant or active host*** Ex: In downy mildew of grapevine, powdery mildew of grapevine, apple etc., The fungus mycelium may be present in dormant state in the affected twigs or its oospores or perithecia may be embedded in the tissues of the affected organs.

Parasitic phanerogams survive in the form of seeds, and in plant parasitic nematodes eggs, cysts and larvae serve as over seasoning structures.

4) Survival in association with insects, nematodes and fungi

Several important plant pathogens may survive within the insect body and over winter therein. The corn flea beetle, *Cheatocnema pulicaria* carries inside its body, the corn wilt pathogen, *Xanthomonas stewartii* and thus helps in over wintering.

Plant viruses like wheat mosaic, tobacco necrosis, tobacco rattle and tobacco ringspot viruses survive with nematodes or fungi found in the soil between crop seasons. Tobacco ringspot is associated with the nematode *Xiphinema americana*. The fungi, *Polymyxa graminis* (Wheat soil borne mosaic & Barley yellow mosaic) and *Spongospora subterranea* (Potato mop top virus) carry the viruses internally and transmit them through the resting spore.

DISPERSAL OF PLANT PATHOGENS

The **second link** in infection chain is the dissemination of plant pathogens. Transport of spores or infectious bodies, acting as inoculum, from one host to another host at various distances resulting in the spread of the disease, is called **dispersal, dissemination** or **transmission** of plant pathogens.

In **fungi**, productions of asexual and sexual spores follow the active vegetative growth of the fungus in or on the host tissues and are dispersed mechanically in time and space by various means. In **bacterial diseases**, the bacterial cells come out on the host surface as ooze or the tissues may be disintegrated so that the bacterial mass is exposed and then dispersed by various physical and biological agencies.

The dispersal of infectious plant pathogens in *space* occurs through two ways:

1. Autonomous or direct or active dispersal.

2. Indirect or passive dispersal.

I) Autonomous or direct or active dispersal:

In this method the dispersal of plant pathogens takes place through soil, seed and planting material during normal agronomic operations. There is no major role of external agencies like insects, wind, water, etc. in this type of dispersal.

1) Seed as the source of autonomous dispersal:

1. The dormant structures of the pathogen (Ex: seeds of Cuscuta, Sclerotia of ergot fungus, smut sori, etc.) are found mixed with seed lots and they are dispersed as **seed contaminants**.
2. The bacterial cells or spores of fungi present on the seed coat (such as in smuts of barley, sorghum, etc.) are transported to long distances.
3. Dormant mycelium of many fungi present in the seed is transmitted to long distances.

There are three types of dispersal by seed, *viz*.,

i. contamination of the seed
ii. externally seed borne
iii. internally seed borne.

i. Contamination of the seed: Seed borne pathogens move in seed lot as **separate contaminants** without being in intimate contact with the viable crop seeds. The seeds of the pathogen or parasite and the host are mixed during harvest of the crop. In many cases, the identity of the seeds of the two entities (host and the pathogens) is difficult to separate.

Ex: Smut of pearlmillet and ergot of rye. Smut sori and ergots mix easily with the seed lots during harvest and threshing.

ii. Externally seed borne: **Close contact between structure of the pathogen and seeds** is established where the pathogen gets lodged in the form of dormant spores or bacteria on the seed coat during growth of the crop or at the time of harvest and threshing.

Ex: Short smut of sorghum, bacterial blight of cotton, loose smut of barley etc.

In many pathogens the externally seed borne structures such as smut spores can persist for many years due to their inherent capacity for long survival. Ex: The spores of *Tilletia caries* (Stinking smut of wheat) remain viable even after 18 years and those of *Ustilago avenae* (Oat smut) for 13 years.

iii. *Internally seed borne*: The pathogen may penetrate into the ovary and cause **infection of the embryo** while it is developing. They become internally seed borne. Ex: **Loose smut of wheat**.

Differentiate Seed infection and infestation

Seed infection: The seed in infected only when the pathogen has grown in or on it for sometime and established its relationship with the seed tissues. Ex: Loose smut of wheat, where the fungus grows

in the embryonic tissues and becomes dormant when the seed enters dormancy.

Seed infestation*:* When the fungus or the pathogen is present on the seed coat and in the seed lot, it is only transport of the pathogen and the seed is infested.

2) Soil as a means of autonomous dispersal: Soil borne facultative saprophytes or facultative parasites may survive through soil. The dispersal may be by movement of pathogen in the soil or by its growth in soil or by movement of the soil containing the pathogen. The former is known as *dispersal in soil* while the later is called *dispersal by soil.*

a) <u>Dispersal in soil:</u> The following are the three stages of dispersal in soil

i) *Contamination of soil*: Contamination of the soil takes place by gradual spread of the pathogen from an infested area to a new area.

ii) *Growth and spread of a pathogen in soil*: Once the pathogen has reached the soil it can grow and spread based on its ability to multiply and spread. Among characters of the pathogen its adaptability to soil environment including its **saprophytic survival ability** are most important. The survival ability of the pathogen is governed by high growth rate, rapid spore germination, better enzymatic activity, capability to produce antibiotics and tolerance to antibiotics produced by other soil-microorganisms.

iii) *Persistence of the pathogen in soil*: The pathogens persist in the soil as dormant structures like **oospores** (*Pythium, Phytophthora, Sclerospora* etc.), **Chlamydospores** (*Fusarium*), smut spores (*Ustilago*) and **sclerotia** (*Rhizoctonia*, *Sclerotium*).

b) <u>Dispersal by the soil</u>: The pathogen is dispersed by the soil during cultural operations through the agricultural implements, irrigation water, workers feet etc. Propagules of fungi and the plant debris containing the fungal and bacterial pathogens thus spread through out the field. The transfer of soil from one place to another along with propagating materials is the most important method of dispersal of pathogen.

For example transfer of papaya seedlings from a nursery infested with *Pythium aphanidermatum* (causal agent of stem or foot rot of papaya) can introduce the pathogen in new pits for transplanting the seedlings. Similarly grafts of fruit trees transported with soil around their roots can transmit pathogens present in the nursery to the orchards.

3) The plant and the plant organs as a means of autonomous dispersal: The plants, plant parts **other than seed** that are used for vegetative propagation, raw field produce and plant debris that accumulates during the course of cropping constitute the third method of autonomous dispersal. Ex: Late blight of potato was introduced in North America and in Europe through seed tubers brought from the native source of the in South America. Citrus canker was

introduced into California from Asia. The climatic conditions favoured its epidemic in California.

II) <u>Passive or Indirect dispersal:</u>

Passive dispersal of plant pathogens happens through animate and inanimate agents.

1) Animate agents:

a) ***Insects***: Insects carry plant pathogens either externally (**epizoic**) or internally (**endozoic**). They can disseminate bacteria, fungi, viruses, mycoplasmas, spiroplasmas, rickettsia, etc.

S.No.	Vector	Viruses
1.	**Aphid transmitted viruses**	
	Myzus persicae	Beet mosaic, Lettuce mosaic, Potato virus Y, Turnip mosaic, Beet yellows
	Acyrthosiphon pisum	Bean common mosaic, Bean yellow mosaic, Soybean mosaic, Pea enation Mosaic
	Toxoptera citricidus	Citrus tristeza
2.	**Leaf hopper transmitted viruses**	
	Nephotettix impicticeps, N. nigropictus, N. virescens	Rice tungro virus
	Nephotettix cincticeps, N. nigropictus	Rice dwarf virus
	Circulifer tenellus	Beet curly top
	Agallia contricta	Potato yellow dwarf
	Graminella nigrifrons	Maize chlorotic dwarf
3.	**Tree hopper transmitted viruses**	
	Micrutalis malleifera	Tomato-pseudo curly top
4.	**Plant hopper transmitted viruses**	
	Perigrinus maidis	Maize mosaic
	Sogatodes oryzicola	Rice hoja blanca
5.	**Whitefly transmitted viruses**	
	Bamesia tabaci	Bhendi yellow vein mosaic, Bhendi leaf curl, Chilli leaf curl, Cotton leaf curl, Papaya leaf curl, Mungbean yellow mosaic
6.	**Thrips transmitted viruses**	
	Thrips tabaci, Frankliniella schultzei, Scirtothrips dorsalis	Tomato spotted wilt virus
7.	**Mealy bugs transmitted viruses**	
	Planococcoides njalensis	Cocoa swollen shoot
	Pseudococcus saccharifolii	Sugarcane spike (Phytoplasma)
8.	**Grass hoppers transmitted viruses**	
	Melanophus differentialis	Potato virus X, Tobacco mosaic virus (Mechanical transmission)
9.	**Lace bugs transmitted**	

	viruses	
	Piesma quadratum	Beet leaf curl virus
	Stephanites typicus	Root (wilt) disease of coconut (Phytoplasma)
10.	**Beetle transmitted viruses**	
	Ceratoma trifurcate	Cowpea mosaic
	Acalymma trivitata	Squash mosaic
	Diabrotica longicornis	Brome mosaic

Mycoplasma diseases: Plant MLO's are phloem inhabitants and those insects which are feeding on phloem of plants transfer the MLO's. Mycoplasmal diseases are mostly transmitted by leaf hoppers. Ex: Sesamum phyllody (*Orosious albicinctus*) and little leaf of brinjal (*Hishimonas phycitis*)

b) *Mites*: Mites belonging to the families Eryophyiidae (eryophyiid mite) and Tetranychidae (spider mite) of class Arachnida transmit plant viruses. The genera *Abacarus, Aceria, Eriophyes* and *Brevipalpus* are important.
Ex: *Aceria cajani* transmits Pigeonpea sterility mosaic virus
Aceria tulipae transmits wheat streak mosaic

c) *Fungi*: Some soil borne fungal plant pathogens carry plant viruses in or on their resting spores and zoospores, and transmit them to susceptible hosts during the infection process. *Tobacco necrosis virus* and *Cucumber mosaic virus* are carried outside the fungi, while lettuce big vein virus is carried inside the zoospores. Many soil borne viruses are transmitted by the members of Chytridiales and Plasmodiophorales.

Fungal transmitted viruses

S.No.	**Fungal vector**	**Disease**
1.	*Olpidium brassicae*	**Tobacco necrosis**, Tobacco stunt, Lettuce big vein
2.	*Olpidium cucurbitacearum*	Cucumber necrosis
3.	*Polymyxa graminis*	Barley yellow dwarf mosaic, Wheat soil borne mosaic, Peanut clump
4.	*Polymyxa betae*	Beet necrotic yellow vein
5.	*Spongospora subterranean*	**Potato mop top**
6.	*Synchytrium endobioticum*	**Potato virus X**

d) *Nematodes*: Several nematodes act as vectors for transmission of fungi, bacteria and viruses.

Nematode transmitted viruses:

S.No.	**Nematode vector**	**Virus**	**Virus group**
1.	*Paratrichodorus* sp. & *Trichodorus* sp.	Pea early browning, **Tobacco rattle**	NETU group
2.	*Xiphenema index*	**Grapevine fan leaf**	NEPO virus
3.	*Xiphenema americanum*	Tobacco ringspot, Tomato ringspot	NEPO virus
4.	*Longidorous elongatus*	Raspberry ringspot	NEPO virus

e) *Human beings:* Human beings role in dissemination of plant pathogens is more direct than indirect. The ways and means in which human beings help in dispersal are as follows.

- **Transportation of seeds (seed trade):** The import and export of contaminated seeds without proper precautions lead to movement of pathogens from one country to another or from one continent to another. The diseases which are amenable to such transmission are mainly those that are carried in or on the propagative parts and seed. Ex: Late blight of potato, Downy mildew of grapevine, Citrus canker, *Fusarium* wilt of banana, etc.

- **Planting diseased seed materials:** Planting diseased bulbs, bulbils, corms, tubers, rhizomes, cuttings, etc., of **vegetatively propagated** plants such as potato, sweet potato, cassava, sugarcane, banana, many ornamentals and fruit trees etc., help in dispersal of pathogens from field to field, orchard to orchard, locality to locality or from one country to another.

- **During adoption of normal farming practices:** Human beings engaged in preparatory cultivation, planting, irrigation, weeding, pruning etc., help in dispersal of plant pathogens. Spores and other external structures of fungi can be carried by workers clothing's, shoes, and hands etc., from plant to plant and from field to field.

- **By use of contaminated implements:** Pathogens are transferred from one area to another through implements used in various cultural operations (weeding, thinning, hoeing etc.) in the field. Ex: Soil borne diseases such as root rot, wilt etc. Cutting knives and pruning knives also help in dispersal from one plant to another. Ex: Bunchy top of banana.

- **By use of diseased grafting and budding material:** Grafting and budding between healthy and diseased plants is the most effective method of distribution of pathogens of horticultural crops.

f) ***Dispersal by phanerogamic parasites:*** Phanerogamic parasites transmit the viruses by acting as a bridge between the diseased and healthy plants. Ex: **Dodder** (*Cuscuta California, C. campesris, C. subinclusa* etc.)

Cuscuta subinclusa – Cucumber mosaic virus

Cuscuta california – Tobacco mosaic virus
Tobacco rattle virus
Tomato spotted wilt virus

Cuscuta campestris - Tomato bushy stunt virus

g) ***Dispersal by birds:*** This mode of dispersal is important in

dissemination of seeds of flowering parasites and certain fungi. In tropics, crows feeding on the fleshy, sticky and gelatinous berries of gaint mistletoe (*Dendrophthoe* sp.) deposit the seeds on the other trees with excreta. Seeds of *Loranthus* are disseminated by birds by sticking on their beaks and also through excreta. Stem segments of dodder are carried by birds for preparing their nests and thus get transported to new areas. Moreover, spores of chestnut blight fungus, *Endothea parasitica* are disseminated by more than 18 species of birds. Cleistothecia of many powdery mildew fungi are carried by feathers of birds.

h) ***Farm and wild animals:*** Farm animals (cattle) while feeding on diseased fodder ingest the viable fungal propagules (spores or oospores or sclerotia) and pass out as such in the dung. This dung when used as manure spread in the field and act as source of inoculum. Further, soil inhabiting fungi especially sclerotia adhere to the hoofs and legs of animals and get transported to other places.

2) Inanimate agents:

a) Wind: The dispersal of pathogens by wind is known as **anemochory**. Wind transmission involves the upward air currents, velocity and the downward movements of the wind. Wind acts as a potent carrier of propagules of fungi, bacteria and viruses.

Fungi: Usually the fungal pathogens are light in weight and are well adapted to wind dispersal. The **adaptations for wind dispersal** in fungal pathogens include production of numerous spores and conidia, discharge of spores with sufficient force, production of very small and light spores so that they can move to long distances. Ex: Powdery mildew, downy mildew, rusts, smuts etc.

Both short and long distance dissemination is possible by means of wind.

i) Spores adopted for **short distance** dissemination- sporangia of downy mildew fungi, conidia of powdery mildew fungi and basidiospores of rust fungi. In the plains of northern India the annual recurrence of cereal rusts is solely due to uredospores brought by wind from the source of survival in the hills in the far north (Himalayas) and south (Nilgiris).

ii) Spores adapted to **long distance** dispersal – uredospores of rust fungi, Chlamydospores of smut fungi and conidia of *Alternaria*, *Helminthosporium* and *Pyricularia*

Uredial stages of the rust fungi travel long distances through air currents and thus are responsible for destructive epidemics over wide areas. Ex: The uredospores of *Puccinia graminis var. tritici* have been detected as high as **14000** feet above infected wheat fields (Stackman and Christensen). Similarly, *Alternaria* spores at 8000 feet, *Puccinia recondita* and *Cronartium ribicola* spores at 12500 feet were reported.

Dispersal distance: In USA, uredospores of this fungus are blown from the far south (Mexico) into Dakota and Minnesota (far north) travelling more than 1000 miles in about two days without losing their viability. If the uredospores reach an altitude of 5000 feet, their distance dispersal in a 30 mile per hour wind could be about **1100 miles**, without loosing viability.

Nematodes: In addition to fungi, it also helps in the dissemination of the cysts of nematodes and also the seeds of phanerogamic parasites. Ex: Cysts of the nematode *Heterodera major*, which causes **molya disease of wheat and barley,** are carried by dust storms from **Rajasthan** to **Haryana**

Bacteria: Some pathogenic bacteria are carried along with the infected material to short distances by wind. Ex: *Erwinia amylovora*, the causal agent of fire blight of apple and pear, produces fine strands of dried bacterial exudates which may be broken off and are transmitted by wind.

Viruses and **phytoplasmas** are not directly transmitted by wind, but the insect and mite vectors that carry the viruses move to different directions and distances based on the direction and speed of the air.

b) **Water**: Transmission of plant pathogens by water is called as **hydrochory**. Water is less important than air in long distance transport of pathogens, but it is more efficient as the pathogens land on the wet surface and can germinate immediately. Water dissemination occurs mainly through **surface running water** and **rain splash**.

The **surface flow of water** after heavy rains or during irrigation from canals and wells carries the pathogens to short distances. Ex: The mycelial fragments, spores or sclerotia of fungi, *Colletotrichum falcatum* **(red rot of sugaecane)**, *Fusarium, Ganoderma, Macrophomina, Pythium*, *Phytophthora, Sclerotium,* etc., are transmitted through rain or irrigation water. Long distance dispersal is also possible by water only when the floods cover larger areas or when the water flows from the sources of survival of pathogens to longer distances.

Dissemination by rain splash is also called as **splash dispersal**. It is one of the efficient methods of dispersal of **bacterial plant pathogens**. Rain drops falling with force on sori, pustules, cankers or even soil surface may splash the propagules in small droplets and enable them to land on neighbouring healthy susceptible surfaces or the water droplets may be carried to long distances by air. Ex: Bacterial leaf spot of rice (*Xanthomonas campestris pv. oryzae*), Bacterial leaf streak of rice (*Xanthomonas campestris pv. oryzicola*), Green ear of bajra (*Sclerospora graminicola*).

Fungal spores and bacteria present in the air or plant surface are washed downward by rain splash or drops from overhead irrigation

and are deposited on susceptible healthy plants. Water not only plays an important role in the dissemination of plant pathogens, but also helps in the growth and spore discharge of many fungi. It also helps in the spore germination and infection process.

Phenomenon of infection/ infection process

It is the **third link** in the infection chain after survival and dispersal of inoculum. Infection process means establishment of pathogen in the host plant. Entry and colonization of pathogen in the host tissues is known as establishment and the infective propagules coming in contact with the host are known as **inoculum**.

Inoculum potential: It is the inoculum needed for successful infection. It is a function of
inoculum density and their **capacity**.

In case of specialized pathogens as rusts and powdery mildews, very few or even one spore is capable of causing infection successfully. In case of non-specialized pathogens such as *Pythium, Phytophthora, Rhizoctonia* and *Sclerotium* require high density of inoculum on the surface of susceptible host for successful infection.

The success of process of infection depends on

1. Host factors

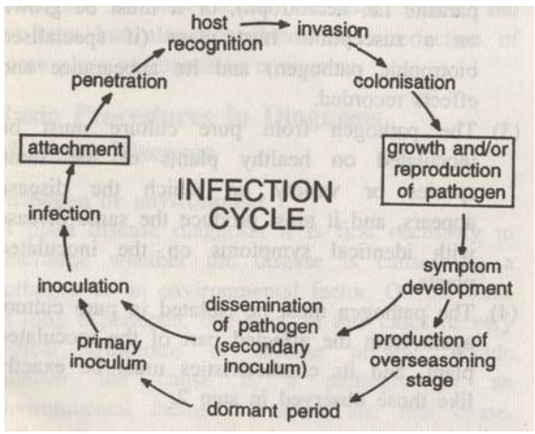
- *Susceptibility of host*: It is genetically controlled by DNA and it is an inheritable character which is transmitted from parents to off springs.
- *Disease proneness of the host*: It is decided by the external factors such as host nutrition, i.e., more nitrogen application makes the host more susceptible and more potash application leads to less susceptibility.

2. Pathogen factors

- *Virulence / aggressiveness of the pathogen*: It is determined by genetic material which is inheritable.
- *High multiplication rate of the pathogen*: Chances of infection increases with high rate of multiplication. High birth rate and low death rate is highly essential for successful infection.
- *Proper inoculum potential*: In case of specialized pathogens very few or even one spore is capable of causing infection successfully, whereas, non-specialized pathogens require high density of inoculum on the surface of susceptible host for successful infection.

3. **Environmental factors**: Environmental conditions such as temperature, relative humidity, moisture, etc., are very important for survival, dissemination and infection process.

Process of infection can be grouped into three stages, *i.e.*, pre-penetration, penetration and post-penetration.



Stages in the development of infection or disease cycle

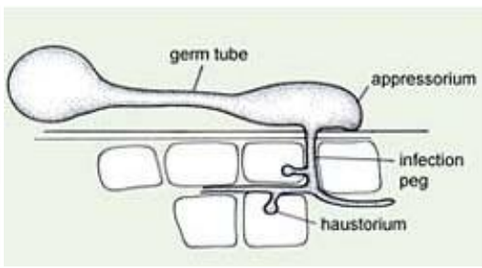
1. PRE-PENETRATION: Depending upon the plant pathogen activity, the plant pathogens are classified in to 2 categories

1. Active invaders and 2.Passive invaders

Active Invaders	Passive Invaders
1. Pathogens which make an aggressive effort to gain entry into intact host cells. 2. They do not require help of any external agency to gain entry into host cells. 3. Eg. Phyto-pathogenic fungi Phanerogamic parasites	1.No aggressive effort 2. Require help of external agencies like insect vectors or wounds caused by agricultural implements. 3. Eg. Plant viruses Phyto-pathogenic bacteria

Plant viruses are particulate in nature and they do not have any capacity to enter the host cell so they do not make any aggressive effort for entry, but depend on different insect vectors for their entry into host cell. Bacteria have no dormant structures; hence no pre- penetration activity except for multiplication in infection drops on the natural openings. However, nematodes show some orientation towards root surface before actual penetration.

In fungal pathogens, pre-penetration includes spore germination and growth of the resulting germ tube on the surface of the host plant. Germination is essentially the change from low metabolic rate to a high metabolic rate and involves a change from near dormancy to intense activity; for this an energy source is needed such as a carbohydrate or fat reserve in the propagule. Fungal invasion is chiefly by germ tubes or structures derived from them. In some fungi like *Rhizoctonia solani* and *Armillariella mellea*, the hypha act in a concerted way to achieve the penetration. In ***Rhizoctonia solani***, the fungus on coming in contact with root surface, first forms infection cushions and **appressoria** and from these multiple infections takes place by means of infection pegs. In ***Armillariella mellea***, the fungus hyphae form the **rhizomorphs** (aggregation of hyphae into rope like strands) and only these can cause infection.



Rhizomorphs Appressorium

2. **PENETRATION:** Pathogens penetrate plant surfaces by direct penetration or indirectly through wounds or natural openings. Bacteria enter plants mostly through wounds and less frequently through natural openings. Viruses, viroids, mollicutes, fastidious bacteria enter through wounds made by vectors. Fungi, nematodes and parasitic higher plants enter through direct penetration and less frequently through natural openings and wounds.

A. Indirect Penetration

1. **Wounds**: Wounds caused by farm operations, hail storms, or insect punctures, etc., will help in the entry of different plant pathogens into the host cells. Organisms which cause storage diseases and ripe rots will enter through the wounds caused by farm operations.

Ex. *Rhizopus, Gloeosporium, Aspergillus, Penicilium, Colletotrichum, Diplodia*, etc. Weak parasites enter through the wounds caused by hail storms and freezing

Ex. *Macrophomina phaseolina*

Pathogen causing brown rot of fruits (*Sclerotinia fructicola*) enters through the wounds caused by insect punctures. Similarly, causal organism of Dutch elm disease (*Ceratostomella ulmi*) enters through the wounds caused by elm bark beetle.

2. Natural openings

a) Stomata: There is variation in the behaviour of germ tube at the time of penetration through the stomata. In *Puccinia graminis tritici*, the uredospore germinates and forms a germ tube which on approaching stoma swells at the tip to form an appressorium in the stomatal aperture. From the appressorium a blade like wedge grows through the stomatal slits and swells inside to form a sub-stomatal vesicle from which the haustoria penetrating the cells are produced.

In *Peronospora destructor* infecting onion leaves, the germ tube continues to grow after the formation of first appressorium. In *Pseudoperonospora cubensis*, the hyphae penetrate the stomatal aperture and swell to form a sub-stomatal vesicle from which in turn other hyphae grow to form haustoria in the adjacent cells of the leaves. *Mycosphaerella musicola* forms a small structure called **stomatopodium** over the pore of the stoma after growing for few days on the surface of the leaf. A hypha then arises from it which grows into the sub-stomatal chamber and swells to form a vesicle, which in turn gives rise to hyphae which invade palaside tissues.

Other examples: *Xanthomonas campestris* pv. *malvacearum* (Black arm of cotton), *Xanthomonas phaseoli* (Bacterial leaf spot of green gram), *Phytophthora infestans* (Late blight of potato), *Albugo candida* (White rust of crucifers) and uredospores of *Puccinia graminis tritici* (Black stem rust of wheat).

b) Lenticels: *Sclerotinia fructicola* (Brown rot of fruits), ***Streptomyces scabies*** (Scab of potato), *Phytophthora arecae* (Mahali disease of arecanut)

c) Hydathodes: ***Xanthomonas campestris pv. campestris*** (Black rot of crucifers)

B) Direct penetration: Most fungi, nematodes and parasitic higher plants are capable of penetrating the host surface directly. However, the plants are provided with different mechanisms of defense which include structural features of the host, presence of chemical coverings on the cell walls, and anti-infection biochemical nature of the protoplasm. Hence, the pathogen should have mechanisms to overcome these barriers for direct penetration.

a) *Breakdown of physical barriers*. Viruses have no physical force or enzyme system of their own to overcome structural or chemical barriers of

the host and therefore come in contact with the host protoplasm only through wounds. Bacteria are mostly weak parasites and cannot employ force to effect penetration. Fungi and nematodes are the only group of plant pathogens that employ force for direct penetration of the host. Fungi penetrate host plants directly through a fine hypha produced directly by the spore or mycelium or through a penetration peg produced by an appressorium. These structures exert pressure on the surface which results in stretching of the epidermis which becomes thin. Then the infection peg punctures it and effects its entry.

b) *Breakdown of chemical barriers*: the host is provided with defense mechanisms against invasion which include i) presence of cuticular layer on the epidermis, ii) lack of suitable nutrients for the pathogen in the host cells, iii) presence of inhibitory or toxic substances in the host cells, iv) exudation of substances toxic to pathogen or stimulatory

to antagonists of the pathogen. Ex: The glands in leaf hairs of begalgram contain maleic acidwhich is antifungal and provide resistance to infection by the rust fungus (Uromyces ciceris arietini). Similarly, protocatecheuic acid and catechol in the red scales of onion provide resistance to onion smudge pathogen, *Colletotrichum circinans*. To overcome these physical and chemical barriers, the fungi produce various enzymes, toxins organic acids and growth regulators.

Through non-cutinized surfaces:

a) Seedlings: Grain smut of jowar (*Sphacelotheca sorghi*), Loose smut of jowar (*Sphacelotheca cruenta*), Downy mildew of jowar and bajra (*Sclerospora graminicola*), Wheat bunt disease (*Tilletia caries*, *Tilletia foetida*)

b) Root hairs: Wilt causing fungi (*Fusarium* sp.), Club root of cabbage (*Plasmodiophora brassicae*), Root rot of cotton (*Phymatotrichum omnivorum*)

c) Buds: Pea rust fungi (*Uromyces pisi*), Witches broom of cherries (*Taphrina cerasi*)

d) Flowers: Loose smut of wheat (*Ustilago nuda tritici*), Long smut of jowar (*Tolyposporium ehrenbergi*), Bunt of rice (*Neovossia horrida*), **Ergot of rye** (*Claviceps purpurea*)

e) Leaves: Basidiospores of white pine blister rust fungus (*Cronartium ribicola*) germinate and grow down into branches and leaves, where aecia are produced.

d) Nectaries: **Fire blight of apple** (*Erwinia amylovora*)

e) Stalk ends: *Penicillium italicum, Theilaviopsis paradoxa* (Post harvest disease fungi)

Through cutinized surfaces:

a) Cuticle: Leaf spot of spinach (***Cercospora beticola***), early blight of solanaceous plants (*Alternaria solani*), Tikka disease of groundnut (***Cercospora personata***)

3. POST PENETRATION

Invasion and colonization: Infection is the process by which pathogens establish contact with the susceptible cells or tissues of the host and derive nutrients from them. A parasitic relationship is formed between host cytoplasm and parasite cytoplasm. During infection, pathogens grow and multiply within the plant tissues. **Invasion** of plant tissues by the pathogen, and growth and reproduction of the pathogen (**colonization**) are two concurrent stages of disease development.

Fungi spread into all parts of host organs, either by growing directly through the cells as an intracellular mycelium or by growing between the cells as an intercellular mycelium. During establishment, pathogen produces different substances which include enzymes, toxins, growth hormones and polysaccharides which will help in colonization of the host.

In **ectoparasites** the main body of the pathogen lies on the surface of the host with only feeding organs (haustoria) penetrating the tissues Ex: Most of the powdery mildew fungi. Some fungal parasites develop both external and internal mycelium Ex: *Rhizoctonia solani*. The endophytic parasites or **endoparasites** grow subcuticularly (*Diplocarpon rosae*, black spot of rose), in parenchyma tissues (most fungal and bacterial pathogens as well as many nematodes) or in vascular tissues (vascular wilt parasites). Some pathogens are **endobiotic**, *i.e.*, mycelium is not produced and the thallus is entirely present within a host cell Ex: *Synchytrium endobioticum*.

Bacteria invade tissues intercellularly, but also grow intracellularly when parts of the cell walls dissolve. Viruses, viroids, mollicutes and fastidious bacteria invade tissues by moving from cell to cell intracellularly.

Infection caused by microbes may be local (involve single cells or few cells or small area) or **systemic** (pathogen spreads and invades most or all susceptible cells and tissues throughout the plant Ex: *Sclerospora graminicola*). The time interval between inoculation and appearance of disease symptoms is called the **incubation period**.

Exit of the pathogen

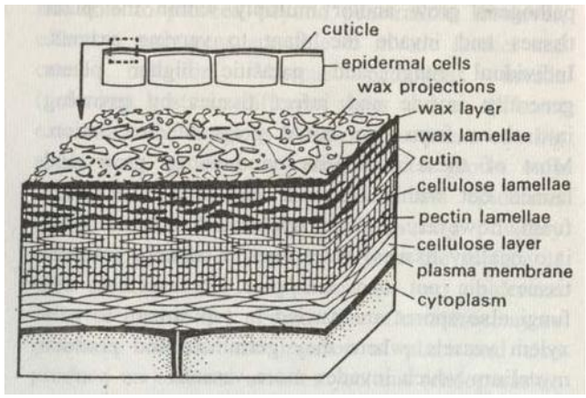
After invasion and colonization of the host, the pathogens come out of the host to maintain the continuity of the infection chain or disease cycle and escape death due to overcrowding. Once the pathogens exit from the host, they survive and are disseminated to other hosts and continue the infection cycle.

Viruses can exist only with the living protoplasm and hence disseminated through their animate vectors like insects, fungi, nematodes, etc. The **bacteria** ooze out in the form of slime on the host surface from where they can be disseminated through water and insects. However, the **fungi** have the most elaborate system of exit. Most plant pathogenic fungi grow out on the host surface and produce repeating spores (secondary inoculum), usually asexually, under favourable conditions. The spores thus formed are disseminated through wind, water, soil, seed, vegetative propagating material, agricultural implements, etc.

ROLE OF ENZYMES IN PATHOGENESIS

Enzymes are large protein molecules which catalyze all inter-related reactions in the living cell. Most pathogens derive energy principally from enzymatic break down of food materials from host tissue.

Composition of the cell wall: Functionally cell wall is divided into 3 regions, *viz*., middle lamella (made of pectins), primary wall (cellulose, pectic substances) and secondary cell wall (entirely cellulose).



Middle lamella acts as intercellular cement which binds the cells together in tissue system. Pectin or pectic substances are major chemical constituents of wall layers and entire middle lamella, where as in other layers, cellulose is found in good amounts.

Besides these two major components, other components such as hemicelluloses, lignin and some amount of protein is also present. Main components of cell wall are pectic substances, cellulose, hemicelluloses, lignin and small quantity of protein.

The epidermis of plants is covered by cuticle, whose major chemical substance is cutin in addition to cuticular wax.

Cuticular wax: Plant waxes are found as granular or rod like projections or as a continuous layer outside / within the cuticle. Wax formation is a continuous process and it is not a terminal phase in the development of leaf. Cuticular waxes are made up of long chain molecules of paraffin, hydrocarbons, alcohols, ketones and acids. Most of the fungi and parasitic higher plants penetrate wax layers by means of mechanical force alone.

Cutin: It is an insoluble polyester of unbranched derivatives of **C_{16} and C_{18} hydroxy fatty acids**. Cutin is admixed with waxes on upper side and with pectin and cellulose on the lower side. **Cutinases** break cutin molecules and release monomers as well as oligomers from insoluble cutin polymer. Cutinases reaches its highest concentration at penetrating point of the germ tube and at infection peg of appressorium forming fungi
Ex: *Colletotrichum gloeosporioides*, *Sphaerotheca pannosa*, *Venturia inaequalis*, *Helminthosporium victoriae*.

Pectic substances: These are major components of middle lamella (intercellular cement that holds in place the cells of plant tissues). They also make up a large portion of primary cell wall in which they form an amorphous gel filling the spaces between cellulose microfibrils. Pectic substances are polysaccharides consisting mostly of **d- galactouronic acid** units with **α-1,4-glycosidic bonds**. These chains are esterified with **methyl** groups or linked with other carboxyl groups in calcium and magnesium salt bridges.
Pectic substances are of three types, namely, **pectic acid** (non methylated units), **pectinic acid** (<75% methylated galacturonan units) and **pectin** (>75% methylated units). Term **protopectin** is used to denote substances which are soluble in water and upon restricted hydrolysis yields pectinic acid.

The enzymes that degrade pectic substances are known as **pectinases** or **pectolytic** enzymes. Pectinases and pectolytic enzymes are pectin methyl esterases (PME's), polygalactouronases (PG's) and pectin lyases (PL's).

1. **Pectin methyl esterases**: Breaks ester bonds and removes methyl groups from pectin leading to the formation of **pectic acid** and **methanol** (CH_3OH).

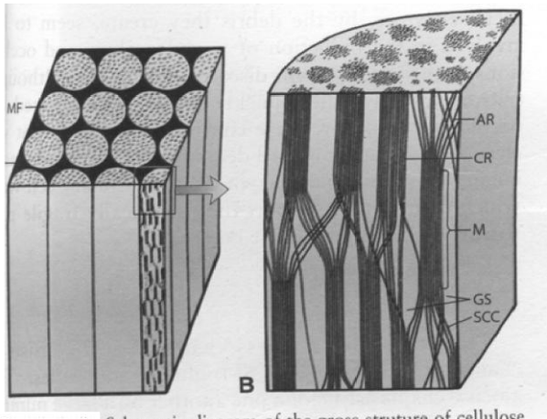
2. **Polygalacturonases**: Split pectin chain by adding a molecule of water and breaks the linkage between two galacturonan units. These enzymes catalyze reactions that break α- 1,4-glycosidic bonds.

3. **Pectin lyases**: Split pectin chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with unsaturated double bonds.

These pectin enzymes can be **exopectinases** (break only terminal linkage) or **endopectinases** (break pectin chain to random sites). Pectin degradation results in liquefaction of the pectic substances and weakening of cell walls, leading to tissue maceration
Ex: Soft rot bacterium, *Erwinia caratovora* subsp. *caratovora* and other fungi like *Botrytis cinerea, Sclerotium rolfsii*, etc.

Cellulose: Cellulose is a polysaccharide, made of chains of **β-D-glucopyranose** units (where C_1 is linked to C_4). Glucose chains are held by **hydrogen** bonds. Cellulose occurs in all higher plants as the skeletal substance of cell walls in the form of microfibrils. Primary and secondary wall consists of a matrix in which a large number of microfibrils are embedded. These microfibrils are like bundles of iron bars in a reinforced concrete building. In some parts of microfibrils the chains are arranged in an orderly fashion attaining crystalline form, when arranged in less orderly fashion, it attains amorphous form. If the proportion of crystalline portion is more, the resistance of the host to pathogen is more. The space between microfibrils and between micelles or cellulose chains is filled with pectins, hemicelluloses and also lignin at maturity.

Cellulose is insoluble in **crystalline** form (native form), and soluble in **amorphous** form (modified cellulose). The enzymatic breakdown of cellulose results in final production of glucose molecules.
Cellulose is degraded by **cellulases**. Cellulase one ($\mathbf{C_1}$) attacks native cellulose by cleaving cross-linkages between chains. A second cellulase ($\mathbf{C_2}$) also attacks native cellulose and breaks into shorter chains. These shorter chains are then attacked by $\mathbf{C_x}$ enzyme, which degrade them into disaccharide, **cellobiose**. Finally cellobiose is degraded by the enzyme, **β-glucosidase** into glucose.



Schematic diagram of the gross struture of cellulose microfibrils (A) and of the arrangement of cellulose molecules within a microfibril (B). MF, microfibril; GS, ground substance (pectin, hemicelluloses, or lignin); AR, amorphous region of cellulose; CR, crystalline region; M, micelle; SCC, single cellulose chain (molecule).

Cellulase degrading enzymes play a role in softening and degradation of cell wall material and facilitate easy penetration and spread of pathogen in the host.
Ex: Basidiomycetes fungi
Hemicellulose: These are the major constituents of **primary cell wall** and also seen in middle lamella and secondary cell wall. The hemicellulose polymers include primarily **xyloglucan** but also glucomannans, galactomannans, arabinogalactans, etc. Hemicelluloses link the ends of pectic polysaccharides and various points of the cellulose microfibrils.
Hemicellulases degrade hemicelluloses and depending on the monomer released from polymer on which they act, they are termed as xylanase, galactanase, glucanase, arabinase, mannose, and so on. Ex: *Sclerotinia sclerotiorum, Sclerotinia fructigena.*

Lignin: Lignin is found in the **middle lamella**, as well as in the secondary cell wall of xylem vessels and the fibres that strengthen plants. It is an amorphous, three-dimensional polymer made up of basic structural unit, **phenylpropanoid**. Lignin forms by oxidative condensation (C-C and C-O bond formation) between phenylpropanoid units or substituted **cinnamyl alcohols** (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol). **White rot fungi** (Basidiomecetes) secrete one or more ligninases which enable them to utilize lignin. Ex: *Xylaria, Chaetomium, Alternaria, Cephalosporium*, etc.

Cell wall proteins: Cell wall proteins are similar to other proteins, except that they are rich in aminoacid, **hydroxy proline**. Five classes of structural proteins are found in cell walls: extensins, proline-rich proteins (PRP's), glycine-rich proteins (GRP's), Solanaceous lectins and arabinogalactan proteins (AGP's). Proteins are degraded by means of enzymes, **proteases** or **proteinases** or **peptidases**.

Lipids: Various types of lipids occur in all plant cells. The most important ones are **phospholipids** and **glycolipids**. These lipids contain fatty acids, which may be saturated or unsaturated. Lipolytic enzymes, called **lipases** (phospholipases, glycolipases) hydrolyze lipids and release fatty acids.

Starch: Starch is the main reserve polysaccharide found in plant cells. It is a glucose polymer and exists in two forms: **amylose**, a linear molecule, and **amylopectin**, a highly branched molecule. Starch is degraded by enzyme,

amylases.

ROLE OF TOXINS IN PLANT PATHOGENESIS

Def: Toxin can be defined as a microbial metabolite excreted (**exotoxin**) or released by lysed cells (**endotoxin**) which in very low concentration is directly toxic to the cells of the suscept (host).

The term toxin is used for a product of the pathogen, its host, or pathogen host interaction which even at very low concentration directly acts on living host protoplasm to influence disease development or symptom expression.

Toxins are different from enzymes in that they do not attack structural integrity of host tissues but affect the metabolism of the host because the toxins will act on protoplast of the cell.

Toxin hypothesis (Luke and Wheeler, 1955):
1. A toxin should produce all symptoms characteristic of the disease
2. Sensitivity to toxin will be correlated with susceptibility to pathogen
3. Toxin production by the pathogen will be directly related to its ability to cause disease. Except, **victorin**, the toxic metabolite of *Cochliobolus victoriae*, the vast majority of toxins associated with plant diseases fail to exhibit all the above characters.

Classification of toxins (Wheeler and Luke, 1963)
According to the **source of origin**, toxins are divided into 3 broad classes namely, ***pathotoxins, vivotoxins*** and ***phytotoxins***.

1. **Pathotoxins**: These are the toxins which play a major role in disease production and produce all or most of the symptoms characteristic of the disease in susceptible plants. Most of these toxins are produced by pathogens during pathogenesis.
Ex: **Victorin**: *Cochliobolus victoriae* (*Helminthosporium victoriae*), the causal agent of Victoria blight of oats. This is a host specific toxin.

2) **Phytotoxins**: These are the substances produced in the host plant due to host-pathogen interactions for which a causal role in disease is merely suspected rather than established. These are the products of parasites which induce few or none of the symptoms caused by the living pathogen. They are non-specific and there is no relationship between toxin production and pathogenicity of disease causing agent.
Ex: Alternaric acid – *Alternaria solani*

3) **Vivotoxins**: These are the substances produced in the infected host by the pathogen and / or its host which functions in the production of the disease, but is not itself the initial inciting agent of the disease.
Fusaric acid – Wilt causing *Fusarium sp.*
Lycomarasmin – *Fusarium oxysporum f.sp. lycopersici*
Piricularin – *Pyricularia oryzae*

Classification based on specificity of toxins
1. **Host specific / Host selective toxins**: These are the metabolic products of the pathogens which are selectively toxic only to the susceptible host of the

pathogen
Ex: Victorin, T-toxin, Phyto-alternarin, Amylovorin

2. Non-specific / Non-selective toxins
These are the metabolic products of the pathogen, but do not have host specificity and affect the protoplasm of many unrelated plant species that are normally not infected by the pathogen
Ex: Ten-toxin, Tab-toxin, Fusaric acid, Piricularin, Lycomarasmin and Alternaric acid

Differentiate host – specific and non-host specific toxins

Host specific	**Non-host specific**
1. Selectively toxic only to susceptible also affect host of the pathogen	1. No host specificity and can the physiology of those plants that are normally not infected by the pathogen
2. Primary determinants of disease	2. Secondary determinants of disease
3. Produce all the essential symptoms symptoms of of the disease	3. Produce few or none of the the disease
4. Ex: Victorin, T- toxin	4. Ex: Tentoxin, Tabtoxin

Effect of toxins on host tissues
A) *Changes in cell permeability*: Toxins kill plant cells by altering the permeability of plasma membrane, thus permitting loss of water and electrolytes and also unrestricted entry of substances including toxins. Cellular transport system, especially, H^+ / K^+ exchange at the cell membrane is affected.

B) Disruption of normal metabolic processes
- Increase in respiration due to disturbed salt balance
- Malfunctioning of enzyme system Ex: Piricularin inhibits polyphenol oxidase
- Uncoupling of oxidative phosphorylation

C) Interfere with the *growth regulatory system* of host plant Ex: Restricted development of roots induced by *Fusarium moniliforme*

ROLE OF GROWTH REGULATORS IN PLANT PATHOGENESIS

Growth regulators
Growth regulators are of two types
1. Growth promoting substances and 2. Growth inhibiting substances
Auxins, gibberellins and cytokinins are growth promoting substances, whereas, dormin, ethylene and abscissic acid are growth inhibiting substances.

The imbalance in growth promoting and growth inhibiting substances causes **hypertrophy** (excessive increase in cell size) and **atrophy** (decrease in cell size). Symptoms may appear as tumors, galls, knots, witches broom, stunting, excessive root branching, defoliation and suppression of bud growth.

1. Growth promoting substances:

a) Auxins: *Indole-3-acetic acid (IAA)* is the naturally occurring auxin. It is continuously produced in young meristematic tissue and moves rapidly to older tissues. If auxin concentration is more, its concentration is reduced by the enzyme, **IAA oxidase**.

Functions: IAA regulates cell elongation and differentiation, also affects permeability of the membrane, increases respiration, and promotes synthesis of mRNA.

How disease is induced?
Increased IAA results in **hypertrophy** and decreased IAA results in **atrophy**. Increased IAA may be due to inhibition of IAA oxidase.
Ex: *Ralstonia solanacearum* (*Pseudomonas solanacearum*), the causal agent of wilt of Solanaceous plants, induces a 100 fold increase in IAA level in diseased plants. Increased plasticity of cell walls as a result of high IAA levels renders the pectin, cellulose and protein components of the cell wall more accessible to pathogen degradation. Increase in IAA levels may also inhibit lignifications of tissues.

b) Gibberellins: First isolated from ***Gibberella fujikuroi*** (Conidial stage: *Fusarium moniliforme*), the causal agent of bakanae or foolish seedling disease of rice. Infected seedlings show abnormal elongation due to excessive elongation of internodes. Best known gibberellin is **Gibberellic acid**.

Functions: Cell elongation, stem and root elongation, promote flowering and growth of fruits. It also induces IAA synthesis. IAA and GA act synergistically. Ex: *Sclerospora sacchari*, the causal agent of downy mildew of sugarcane induces GA production.

c) Cytokinins: Are necessary for cell growth and differentiation. It inhibits breakdown of proteins and aminoacids and thereby inhibit senescence and they have the capacity to direct the flow of aminoacids and other nutrients towards high cytokinin concentration. Cytokinin activity increases in club root, in crown galls and in rust infected bean leaves.

Growth inhibiting substances
a) Ethylene (CH_2=CH_2): Ethylene exerts a variety of effects on plants, viz., chlorosis, leaf abscission, epinasty, stimulation of adventitious roots, fruit ripening and increased permeability of cell membranes.
Ex: Ethylene is involved in premature ripening of fingers in banana infected by *Pseudomonas solanacearum*, the causal agent of moko disease of banana. Ethylene was also detected in leaf epinasty symptom of the vascular wilt syndrome. Ex: *Fusarium oxysporum f.sp. lycopersici* (Wilt in tomato).

b) Abscissic acid: It exerts dormancy in seeds, closure of stomata, inhibition of seed germination and growth and stimulated germination of fungal spores. It is one of the factors involved in stunting of plants.

c) Dormin / Abscissin II: Dormin induces dormancy by converting developing leaf primordia of a bud into bud scales. It acts as an antagonist of gibberellins and masks the effect of IAA. However, the exact role of dormin is not known.

Role of polysaccharides in pathogenesis

Polysaccharides: Fungi, bacteria and nematodes release varying amounts of mucilaginous substances that coat their bodies and provide interface between the outer surface of the micro-organism and its environment. The role of slimy polysaccharides is of utmost importance in wilt diseases. In the vascular wilts, large polysaccharide molecules released by the pathogen in the xylem causes mechanical blockage of vascular bundles and initiate wilting.
Ex: *Ralstonia solanacearum* (Bacterial wilt of Solanaceous plants)

Defense mechanism in plants

In general plants defend themselves against pathogens by two ways

i. Structural or morphological characteristics that act as physical barriers
ii. Biochemical reactions that take place in cells and tissues that are either toxic to the pathogen or create conditions that inhibit the growth of the pathogen in the plant.

I. Structural defense mechanisms: These may be pre-existing, which exist in the plant even before the pathogen comes in contact with the plant or induced, *i.e*, even after the pathogen has penetrated the preformed defense structures, one or more type of structures are formed to protect the plant from further pathogen invasion.

A) Pre-existing structural defense structures
These include the amount and quality of wax and cuticle that cover the epidermal cells and the size, location and shapes of natural openings (stomata and lenticels) and presence of thick walled cells in the tissues of the plant that hinder the advance of the pathogen.

i) Waxes: Waxes on leaf and fruit surfaces form a hydrophobic or water repellent surface preventing the germination of fungi and multiplication of bacteria.

ii) Cuticle and epidermal cells: A thick cuticle and tough outer wall of epidermal cells may increase resistance to infection in diseases in which the pathogen enters its host only through direct penetration. Ex: Disease resistance in Barbery species infected with *Puccinia graminis tritici* has been attributed to the tough outer epidermal cells with a thick cuticle.

iii) Sclerenchyma cells: The sclerenchyma cells in stems and leaf veins effectively blocks the spread of some fungal and bacterial pathogens that cause angular leaf spots.

iv) Structure of natural openings:
a) ***Stomata***: Most of the pathogens enter plants through natural openings. Some pathogens like stem rust of wheat can enter its host only when the stomata are open. The wheat varieties (Cultivar, **Hope**) in which stomata open late in the day are resistant as the germ tubes of the spores germinating in the night dew desiccate owing to evaporation of the dew before stomata begin to open. This can also be called as functional resistance. The structure

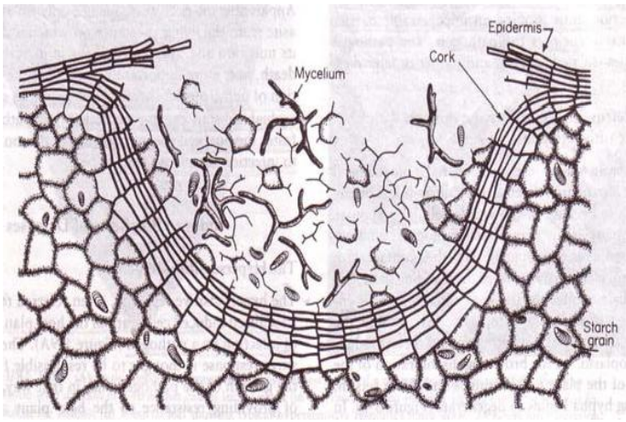
of stomata provides resistance to penetration by certain plant pathogenic bacteria.

b) **Lenticels**: The shape and internal structure of lenticels can increase or decrease the incidence of fruit diseases. Small and suberised lenticels will offer resistance to potato scab pathogen, *Streptomyces scabies.*

B) Post-infectional structural defense mechanisms/Induced structural barriers: These may be regarded as histological defense barriers (cork layer, abscission layers and tyloses) and cellular defense structures (hyphal sheathing).

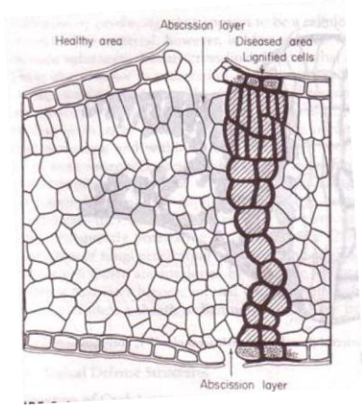
i) Histological defense structures

a) Cork layer: Infection by fungi, bacteria, some viruses and nematodes induce plants to form several layers of cork cells beyond the point of infection and inhibits the further invasion by the pathogen beyond the initial lesion and also blocks the spread of toxin substances secreted by the pathogen. Furthermore, cork layers stop the flow of nutrients and water from the healthy to the infected area and deprive the pathogen of nourishment. Ex: Potato tubers infected by *Rhizoctonia*; *Prunus domestica* leaves attacked by *Coccomyces pruniphorae*.



b) Abscission layers

An abscission layer consists of **a gap formed between infected and healthy cells** of a leaf surrounding the locus of infection due to the disintegration of the middle lamella of parenchymatous tissue.



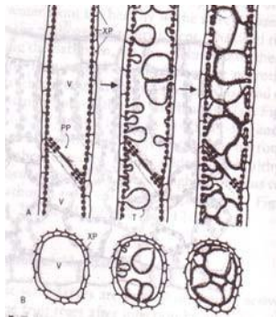
Gradually, infected area shrivels, dies, and sloughs off, carrying with it the pathogen. Abscission layers are formed on young active leaves of stone fruits infected by fungi, bacteria or viruses.

Ex: *Xanthomonas pruni,* and *Closterosporium carpophylum* on peach leaves

c) Tyloses

Tyloses are the overgrowths of **the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits**. Tyloses have cellulosic walls and are formed quickly ahead of the pathogen and may clog the xylem vessels completely blocking the further advance of the pathogen in resistant varieties. In susceptible varieties, few or no tyloses are formed ahead of pathogen invasion.

Ex: Tyloses form in xylem vessels of most plants under invasion by most of the **vascular wilt** pathogens.



ii) Cellular defense structures:

Hyphal sheathing: When a hyphae penetrating the cell wall and growing into the cell lumen are enveloped by a cellulosic sheath (callose) formed by extension of cell wall, which become infused with phenolic substances and prevents further spread of the pathogen.

Ex: Hyphal sheathing is observed in flax infected with *Fusarium oxysporum f.sp*. *lini*.

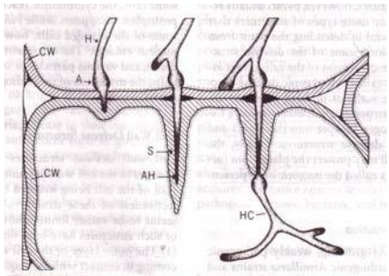


FIGURE 5-3 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, Appressorium; AH, advancing hypha still enclosed in sheath; HC, hypha in cytoplasm; S, sheath.

II) Biochemical defense mechanisms: These can be classified as pre-existing and induced biochemical defenses.

1) Pre-existing chemical defenses:

a) *Inhibitors released by the plant in its environment*:

Plants exude a variety of leaf and root exudates which contain aminoacids, sugars, glycosides, organic acids, enzymes, alkaloids, flavones, toxic materials, inorganic ions and also certain growth factors. The inhibitory substances directly affect micro-organisms or encourage certain groups to dominate the environment which may act as antagonists to pathogen.

- Ex 1: Tomato leaves secrete exudates which are inhibitory to *Botrytis cinerea*
- Ex 2: Red scales of red onion contain the phenolic compounds,

b) Inhibitors present in plant cells before infection:

- Antimicrobial substances pre-existing in plant cells include unsaturated lactones, cyanogenic glycosides, Sulphur containing compounds, phenols, phenolic glycosides and saponins
- Several phenolic compounds, **tannins**, and some fatty acid like compounds such as **dienes**, which are present in high concentrations in cells of young fruits, leaves or seeds are responsible for the resistance of young tissues to *Botrytis*. These compounds are potent inhibitors of many hydrolytic enzymes.
- **Saponins** have antifungal membranolytic activity which excludes fungal pathogens that lack saponinases. Ex: **Tomatine** in tomato and **Avenacin** in oats
- Similarly, **lectins**, which are proteins that bind specifically to certain sugars and occur in large concentrations in many types of seeds, cause lysis and growth inhibition of many fungi.
- Plant surface cells also contain variable amounts of **hydrolytic enzymes** such as **glucanases** and **chitinases** which may cause breakdown of pathogen cell wall.

2) Post inflectional or induced defense mechanisms:

a) *Phytoalexins* (*Phyton* = plant; *alexin* = to ward off)

- **Muller** and **Borger** (1940) first used the term phytoalexins for fungistatic compounds produced by plants in response to injury (mechanical or chemical) or infection.
- Phytoalexins are toxic antimicrobial substances produced in appreciable amounts in plants only after stimulation by phytopathogenic micro-organisms or by chemical or mechanical injury.

- Phytoalexins are not produced by uninfected healthy plants, but produced by healthy cells adjacent to localized damaged or necrotic cells in response to materials diffusing from the infected cells. These are not produced during compatible biotrophic infections.
- Phytoalexins accumulate around both resistant and susceptible necrotic tissues. However, resistance occurs when one or more phytoalexins reach a concentration sufficient to restrict pathogen development.

Characteristics of phytoalexins

1. Fungitoxic and bacteriostatic at low concentrations.
2. Produced in host plants in response to stimulus (elicitors) and metabolic products.
3. Absent in healthy plants
4. Remain close to the site of infection.
5. Produced in quantities proportionate to the size of inoculum.
6. Produced in response to the weak or non-pathogens than pathogens
7. Produced within 12-14 hours reaching peak around 24 hours after inoculation.
8. Host specific rather than pathogen specific.

Synthesis and accumulation of phytoalexins are shown in diversified families, viz., Leguminosae, Solanaceae, Malvaceae, Chenopodiaceae, Convolvulaceae, Compositae and Graminaceae.

S.No.	Phtoalexin	Host	Pathogen
1	Pisatin	Pea	*Monilinia fructicola*
2	Phaseolin	French bean	*Sclerotinia fructigena*
3	Rishitin	Potato	*Phytophthora infestans*
4	Gossypol	Cotton	*Verticillium alboatrum*
5	Cicerin	Bengalgram	*Ascochyta rabiei*
6	Ipomeamarone	Sweet potato	*Ceratocystis fimbriata*
7	Capsidol	Pepper	*Colletotrichum capsici*

b) Hypersensitive response (HR)

- The term hypersensitivity was first used by **Stakman** (1915) in wheat infected by rust fungus, *Puccinia graminis*.
- The hypersensitive response is a **localized induced cell death** in the host plant at the site of infection by a pathogen, thus limiting the growth of pathogen. In the infected plant part, HR is seen as water soaked large sectors which subsequently become necrotic and collapsed.
- HR occurs only in **incompatible** host-pathogen combinations. HR may occur whenever virulent strains or races of pathogens are injected into non-host plants or into resistant varieties, and when avirulent strains or races of pathogens are injected into susceptible cultivars.
- HR is initiated by the recognition of specific pathogen-produced signal molecules, known as **elicitors**. Recognition of the elicitors by the host results in altered cell functions leading to the production of defense related compounds.

The most common **new cell functions** and compounds include:

- A rapid burst of oxidative reactions
- Increased ion movement, especially of K^+ and H^+ through cell membrane
- Disruption of membranes and loss of cell compartmentalization
- Cross-linking of phenolics with cell wall components and strengthening of plant cell wall
- Production of antimicrobial substances such as phytoalexins and pathogenesis-related proteins (such as chitinases)

Cellular responses during HR

- In many host-pathogen combinations, as soon as the pathogen establishes contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates.
- Brown resin like granules form in the cytoplasm, first around the point of penetration of pathogen and then throughout the cytoplasm
- As the browning discolouration of the cytoplasm continues and death sets in, the invading hypha begins to degenerate and further invasion is stopped.

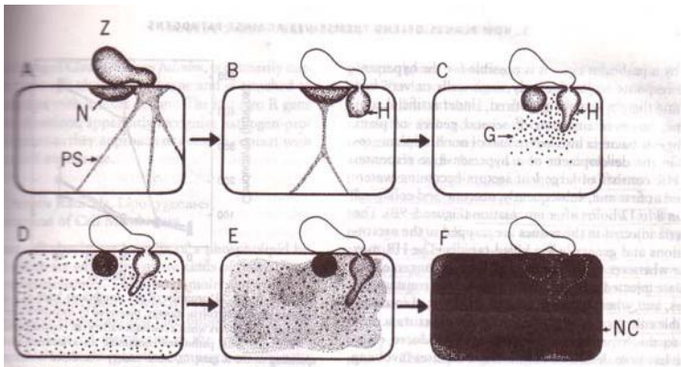


FIGURE 5-8 Stages in the development of the necrotic defense reaction in a cell of a very resistant potato variety infected by *Phytophthora infestans*. N, Nucleus; PS, protoplasmic strands; Z, zoospore; H, hypha; G, granular material; NC, necrotic cell. [After K. Tomiyama (1956), *Ann. Phytopathol. Soc. Jpn.* 21, 54–62.]

PRINCIPLES OF PLANT DISEASE MANAGEMENT

Management: It is based not only on the principle of eradication of the pathogen but mainly on the principle of minimizing the damage or loss below economic injury level.

Importance: Plant diseases are important because of the losses (qualitative and quantitative) they cause. Loss may occur at any time between sowing of the crop and consumption of the produce. The management practices includes

- Measures taken to prevent the incidence of the disease,
- Reduce the amount of inoculum that initiates and spreads the disease
- And finally minimize the loss caused by the disease

Essential considerations in plant disease Management**:**

1. Benefit-cost ratio
2. Procedures for disease control should fit into general schedule of operations of crop production
3. Control measures should be adopted on a **co-operative basis** over large adjoining areas. This reduces frequency of applications, cost of control and increases chances of success of control measures
4. Knowledge aspects of disease development is essential for effective economical control. Information is needed on the following aspects
   a. Cause of a disease
   b. Mode of survival and dissemination of the pathogen
   c. Host parasite relationship
   d. Effect of environment on pathogenesis in the plant or spread in plant population
5. Prevention of disease depends on management of primary inoculum
6. Integration of different approaches of disease management is always recommended

General principles of plant disease management

1. **Avoidance**: Avoiding disease by planting at times when, or in areas where, inoculum is ineffective due to environmental conditions, or is rare or absent
2. **Exclusion of inoculum**: Preventing the inoculum from entering or establishing in the field or area where it does not exist
3. **Eradication**: Reducing, inactivating, eliminating or destroying inoculum at the source, either from a region or from an individual plant in which it is already established
4. **Protection**: Preventing infection by creating a chemical toxic barrier between the plant surface and the pathogen

5. **Disease resistance (Immunization)**: Preventing infection or reducing effect of infection by managing the host through improvement of resistance in it by genetic manipulation or by chemical therapy.

I. Avoidance of the pathogen: These methods aim at avoiding the contact between the pathogen and susceptible stage of the crop. This is achieved by

a. Proper selection of geographical area
b. Proper selection of the field
c. Adjusting time of sowing
d. Disease escaping varieties
e. Proper selection of seed and planting material

II. Exclusion of the pathogen: These measures aim at preventing the inoculum from entering or establishing in the field or area where it does not exist. Different methods of exclusion are seed treatment, seed inspection & certification, and plant quarantine regulation.

a) Seed inspection and certification: Crops grown for seed purpose are inspected periodically for the presence of diseases that are disseminated by seed. Necessary precautions are to be taken to remove the diseased plants in early stages, and then the crop is certified as disease free. This practice will help in the prevention of inter and intra regional spread of seed borne diseases.

b) Plant quarantine regulation: Plant quarantine is defined as " a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delaying the spread of the plant pests and diseases in uninfected areas".

Plant quarantine measures are of 3 types.

1. Domestic quarantine: Rules and regulations issued prohibiting the movement of insects and diseases and their hosts from one state to another state in India is called domestic quarantine. Domestic quarantine in India exists for two pests (Rooted scale and Sanjose scale) and three diseases (Bunchy top of banana, banana mosaic and wart of potato).

Bunchy top of banana: It is present in Kerala, Assam, Bihar, West Begal and Orissa. Transport of any part of *Musa* species excluding the fruit is prohibited from these states to other states in India.

Banana mosaic: It is present in Maharashtra and Gujarat. Transport of any part of *Musa*
species excluding the fruit is prohibited from these states to other states in India.

Wart of potato: It is endemic in Darjeeling area of West Bengal, therefore seed tubers are not to be imported from West Bengal to other states.

2. Foreign quarantine: Rules and regulations issued prohibiting the import of plants, plant materials, insects and fungi into India from foreign countries by air, sea and land. Foreign quarantine rules may be general or specific. General rules aim at prevention of introduction of pests and diseases into a country, where as the specific rules aim at specific diseases and insect pests. The plant materials are to be imported only through the prescribed ports of entry.

Phytosanitary certificate: It is an official certificate from the country of origin, which should accompany the consignment without which the material may be refused from entry.

Plant diseases introduced into India before/after enforcement of plant quarantine laws:

S.No.	Disease	Year	Introduced into	From
1	Late blight of potato	1883	India	Europe
2	Coffee rust	1879	India	Srilanka
3	Flag smut of wheat	1906	India	Australia
4	Downy mildew of grapes	1910	India	Europe
5	Bacterial blight of rice	1964	India	Phillippines
6	Rice blast	1918	India (Madras)	South East Asia
7	Downy mildew of maize	1912	India (Madras)	Java
8	Ergot of bajra	1957	India (Bombay)	Africa
9	Panama wilt of banana	1920	India	Panama canal
10	Bunchy top of banana	1940	India	Srilanka
11	Wart of potato	1953	India	Netherlands
12	Golden cyst nematode of potato	1961	India	Europe

Diseases not entered into India: Swollen shoot of cocoa, leaf blight of rubber and many viral diseases.

III. Eradication: These methods aim at breaking the infection chain by removing the foci of infection and starvation of the pathogen (i.e., elimination of the pathogen from the area by destruction of sources of primary and secondary inoculum). It is achieved by

a) Rouging: Removal of diseased plants or their affected organs from field, which prevent the dissemination of plant pathogens.

Ex: Loose smut of wheat and barley, whip smut of sugarcane, red rot of sugarcane, ergot of bajra, yellow vein mosaic of bhendi,

b) Eradication of alternate and collateral hosts: Eradication of alternate hosts will help in management of many plant diseases.

Ex: Barbery eradication programme in France and USA reduced the severity of black stem rust of wheat

Ex: Eradication of *Thalictrum* species in USA to manage leaf rust of wheat caused by Puccinia recondita.

Eradication of collateral hosts, such as *Panicum repens*, *Digitaria marginata* will help in the management of rice blast disease (*Pyricularia oryzae*)

c) Crop rotation: Continuous cultivation of the same crop in the same field helps in the perpetuation of the pathogen in the soil. Soils which are saturated by the pathogen are often referred as **sick soils**. To reduce the incidence and severity of many soil borne diseases, crop rotation is adopted. Crop rotation is applicable to only root inhabitants and facultative saprophytes, and may not work with soil inhabitants.

Ex: Panama wilt of banana (long crop rotation), wheat soil borne mosaic (6 yrs) and club root of cabbage (6-10 yrs), etc.

d) Crop sanitation: Collection and destruction of plant debris from soil will help in the management of soil borne facultative saprophytes as most of these survive in plant debris. Collection and destruction of plant debris is an important method to reduce the primary inoculum.

e) Manures and fertilizers: The deficiency or excess of a nutrient may predispose a plant to some diseases. Excessive nitrogen application aggravates diseases like stem rot, bacterial leaf blight and blast of rice.

Nitrate form of nitrogen increases many diseases, whereas, phosphorous and potash application increases the resistance of the host. Addition of farm yard manure or organic manures such as green manure, 60-100 t/ha, helps to manage the diseases like cotton wilt, Ganoderma root rot of citrus, coconut, etc.

f) Mixed cropping: Root rot of cotton (*Phymatotrichum omnivorum*) is reduced when cotton is grown along with sorghum. Intercropping sorghum in cluster bean reduces the incidence of root rot and wilt (*Rhizoctonia solani*)

g) Summer ploughing: Ploughing the soil during summer months expose soil to hot weather which will eradicate heat sensitive soil borne pathogens.

h) Soil amendments: Application of organic amendments like saw dust, straw, oil cake, etc., will effectively manage the diseases caused by *Pythium, Phytophthora, Verticillium, Macrophomina, Phymatotrichum* and *Aphanomyces*. Beneficial micro-organisms increases in soil and helps in suppression of pathogenic microbes.
Ex: Application of lime (2500 Kg/ha) reduces the club root of cabbage by increasing soil pH to 8.5
Ex: Application of Sulphur (900 Kg/ha) to soil brings the soil pH to 5.2 and reduces the incidence of common scab of potato (*Streptomyces scabies*).
i***j) Changing time of sowing***: Pathogens are able to infect susceptible plants under certain environmental conditions. Alternation in date of sowing can help avoidance of favourable conditions for the pathogens.
Ex: Rice blast can be managed by changing planting season from June to September/October.

j) Seed rate and plant density: Close spacing raises atmospheric humidity and favours sporulation by many pathogenic fungi. A spacing of 8'X8' instead of 7'X7' reduces sigatoka disease of banana due to better ventilation and reduced humidity. High density planting in chillies leads to high incidence of damping off in nurseries.

k) Irrigation and drainage: The amount, frequency and method of irrigation may affect the dissemination of certain plant pathogens. Many pathogens, including, *Pseudomonas solanacearum, X. campestris pv. oryzae* and *Colletotrichum falcatum* are readily disseminated through irrigation water. High soil moisture favours root knot and other nematodes and the root rots caused by species of *Sclerotium, Rhizoctonia, Pythium, Phytophthora, Phymatotrichum*, etc.

PHYSICAL METHODS: Physical methods include soil solarization and hot water treatments.

i. Soil solarization: Soil solarization or slow soil pasteurization is the hydro/thermal soil heating accomplished by covering moist soil with polyethylene sheets as soil mulch during summer months for 4-6 weeks. Soil solarization was developed for the first time in Israel (Egley and Katan) for the management of plant pathogenic pests, diseases and weeds.

ii. Soil sterilization: Soil can be sterilized in green houses and sometimes in seed beds by aerated steam or hot water. At about 50^0C, nematodes, some oomycetous fungi and other water molds are killed. At about 60 and 72^0C, most of the plant pathogenic fungi and bacteria are killed. At about 82^0C, most weeds, plant pathogenic bacteria and insects are killed. Heat tolerant weed seeds and some plant viruses, such as TMV are killed at or near the boiling point (95-100^0C).

iii. Hot water or Hot air treatment: Hot water treatment or hat air treatment will prevent the seed borne and soil borne infectious diseases. Hot water treatment of certain seeds, bulbs and nursery stock is done to kill many pathogens present in or on the seed and other propagating materials. Hot water treatment is used for controlling sett borne diseases of sugarcane [whip smut, grassy shoot and red rot of sugarcane (52^0C for 30 min)] and loose smut of wheat (52^0C for 10 min).

Biological methods:
Def: Biological control of plant disease is a condition or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except human beings), with the result that there is reduction in incidence of the disease caused by the pathogen (Garett, 1965).

Def: Biological control is the reduction of inoculum density or disease producing activity of a pathogen or a parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of the environment of host or antagonist by mass introduction of one or more antagonists (Baker and Cook, 1974)

<u>Mechanisms of biological control</u>

1. Competition: Most of the biocontrol agents are fast growing and they compete with plant pathogens for space, organic nutrients and minerals. Most aerobic and facultative anaerobic micro-organisms respond to low iron stress by producing extracellular, low molecular weight (500-1000 daltons) iron transport agents, designated as **Siderophores**, which selectively make complex with iron (Fe^{3+}) with very high affinity. Siderophore producing strains are able to utilize Fe^{3+} - Siderophore complex and restrict the growth of deleterious micro-organisms mostly at the plant roots. Iron starvation prevents the germination of spores of fungal pathogens in rhizosphere as well as rhizoplane. Siderophores produced by
Pseudomonas fluorescens (known as **pseudobactins** or **pyoveridins**) helps in the control of soft rot bacterium, *Erwinia caratovora*.

2. Antibiosis: Antagonism mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other

toxic substances is known as antibiosis.

a. **Antibiotics**: Antibiotics are generally considered to be organic compounds of low molecular weight produced by microbes. At low concentrations, antibiotics are deleterious to the growth or metabolic activities of other micro-organisms.
Ex: *Gliocladium virens* produces **gliotoxin** that was responsible for the death of *Rhizoctonia solani* on potato tubers.
Ex: Colonization of pea seeds by *Trichoderma viride* resulted in the accumulation of significant amount of the antibiotic **viridin** in the seeds, thus controlling *Pythium ultimum*.

Ex: Some strains of *Pseudomonas fluorescens* produce a range of compounds, viz., 2,4- diacetyl phloroglucinol (DAPG), phenazines, pyocyanin, which have broad spectrum activity against many plant pathogenic bacteria and fungi

b. **Bacteriocins**: These are antibiotic like compounds with bactericidal specificity closely related to the bacteriocin producer. Ex: The control of crown gall (caused by *Agrobacterium tumefaciens*) by the related *Agrobacterium radiobacter* strain K 84 is by the production of bacteriocin, **Agrocin K84**.

c. **Volatile compounds**: Antibiosis mediated by volatile compounds has been observed in the management of soil borne pathogens, viz., *Pythium ultimum, Rhizoctonia solani* and *Verticillium dahlia*, by *Enterobacter cloacae*. The volatile fraction responsible for inhibition was identified as ammonia.

3. ***Hyperparasitism***: Direct parasitism or lysis and death of the pathogen by another micro-organism when the pathogen is in parasitic phase is known as hyperparasitism.
Ex: *T. harzianum* parasitize and lyse the mycelia of *Rhizoctonia* and *Sclerotium*.

Biocontrol agents for the management of plant pathogens

Biocontrol agent	**Pathogen/disease**
1. *Ampelomyces quisqualis*	Powdery mildew fungi
2. *Darluca filum, Verticillium lecanii*	Rust fungi
3. *Pichia gulliermondii*	*Botrytis, Penicillium*

Biocontrol agent	**Nematode**
1. *Pasteuria penetrans* (Bacteria)	Juvenile parasite of root knot nematode
2. *Paecilomyces lilacinus* (Fungus)	Egg parasite of *Meloidogyne incognita*

Important fungal biocontrol agents:
Most of the species of Trichoderma, viz., *T. harzianum, T. viride, T. virens* (*Gliocladium virens*) are used as biocontrol agents against soil borne diseases, such as, root rots, seedling rots, collar rots, damping off and wilts caused by the species of Pythium, Fusarium, Rhizoctonia, Macrophomina, Sclerotium, Verticillium, etc.

Formulations of biocontrol agents available: *T. viride* (**Ecofit**, **Bioderma** in India), *G. virens* (**GlioGard** in USA), *T. harzianum* (**F-Stop** in USA) and *T. polysporum* (**BINAB- T**)

Important bacterial biocontrol agents:
1. *Pseudomonas fluorescens* (**Dagger-G** against damping off of cotton seedlings in USA)
2. *Bacillus subtilis* (**Kodiak** against damping off and soft rot in USA)
3. Agrobacterium radiobacter K-84 (**Gallex** or **Galltrol** against crown gall of stone fruits caused by *Agrobacterium tumefaciens*)

Plant growth promoting Rhizobacteria (PGPR):
Rhizosphere bacteria that favourably affect plant growth and yield of commercially important crops are designated as plant growth promoting

rhizobacteria. The growth promoting ability of PGPR is due to their ability to produce phytohormones, Siderophores, Hydrogen cyanide (HCN), chitinases, volatile compounds or antibiotics which will reduce infection of host through phyto-pathogenic micro-organisms.

Many bacterial species, viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, etc., are usually used for the management of plant pathogenic microbes. *Bacillus* has ecological advantages as it produces endospores that are tolerant to extreme environmental conditions. *Pseudomonas fluorescens* have been extensively used to manage soil borne plant pathogenic fungi due to their ability to use many carbon sources that exude from the roots and to compete with microflora by the production of antibiotics, HCN and Siderophores that suppress plant root pathogens.

PROTECTION: Use of chemicals for the control of plant diseases is generally referred to as protection or therapy.

Protection: The prevention of the pathogen from entering the host or checking the further development in already infected plants by the application of chemicals is called protection and the chemicals used are called **protectants**.

Therapy means cure of a disease, in which fungicide is applied after the pathogen is in contact with the host. Chemicals used are called **therapeutants**.
Fungicide: Any agent (chemical) that kills the fungus

Fungistat: Some chemicals which do not kill fungi, but simply inhibit the fungus growth temporarily.

Antisporulant: The chemical which inhibits spore production without affecting vegetative growth of the fungus.

Fungicides are classified into three categories: Protectants, eradicants and therapeutants.
1. ***Protectants***: These are the chemicals which are effective only when used before infection (prophylactic in behavior). Contact fungicides which kill the pathogen present on the host surface when it comes in contact with the host are called protectants. These are applied to seeds, plant surfaces or soil. These are non-systemic in action (i.e, they cannot penetrate plant tissues). Ex: Zineb,sulphur, captan, Thiram, etc.

2. ***Eradicants***: Those chemicals which eradicate the dormant or active pathogen from the host. They can remain on/in the host for some time. Ex: Lime sulphur, Dodine.

3. ***Therapeutants***: These are the agents that inhibit the development of a disease syndrome in a plant when applied after infection by a pathogen. Therapy can be by physical means (solar and hot water treatment) and chemical means (by use of systemic fungicides, i.e., chemotherapy).

CLASSIFICATION OF FUNGICIDES BASED ON METHOD OF APPLICATION

The fungicides can also be classified based on the nature of their use in managing the diseases.

1. Seed protectants: Ex. Captan, thiram, carbendazim, carboxin etc.
2. Soil fungicides (preplant): Ex. Bordeaux mixture, copper oxy chloride, Chloropicrin, Formaldehyde, Vapam, etc.
3. Soil fungicides: Ex. Bordeaux mixture, copper oxy chloride, Captan, PCNB, thiram etc.
4. Foliage and blossom: Ex. Capton, ferbam, zineb, mancozeb, chlorothalonil etc.
5. Fruit protectants: Eg. Captan, maneb, carbendazim, mancozeb etc.
6. Eradicants: EX. Lime sulphur
7. Tree wound dressers: Ex. Boreaux paste, chaubattia paste, etc.
8. General purpose sprays and dust formulations.

HOST PLANT RESISTANCE (IMMUNIZATION)

Disease resistance: It is the ability of a plant to overcome completely or in some degree the effect of a pathogen or damaging factor.

Susceptibility: The inability of a plant to resist the effect of a pathogen or other damaging factor.

Types of resistance:

1. **Vertical resistance**: When a variety is more resistant to some races of the pathogen than others, the resistance is called vertical resistance (race-specific resistance, qualitative resistance, discriminatory resistance). Vertical resistance is usually governed by single gene and is unstable.

2. **Horizontal resistance**: When the resistance is uniformly spread against all the races of a pathogen, then it is called horizontal/generalized/non-specific/field/qualitative resistance. Horizontal resistance is usually governed by several genes and is more stable.

3. **Monogenic resistance**: When the defense mechanism is controlled by a **single gene pair**, it is called monogenic resistance.

4. **Oligogenic resistance**: when the defense mechanism is governed by a **few gene pairs**, it is called oligogenic resistance.

5. **Polygenic resistance**: When the defense mechanism is controlled by **many genes** or more groups of supplementary genes, it is called polygenic resistance.

Cross protection: The phenomenon in which plant tissues infected with mild strain of a virus are protected from infection by other severe strains of the same virus. This strategy is used in the management of severe strains of *Citrus Tristeza **virus***