For BioResire students

Life sciences Material

Elite Batch

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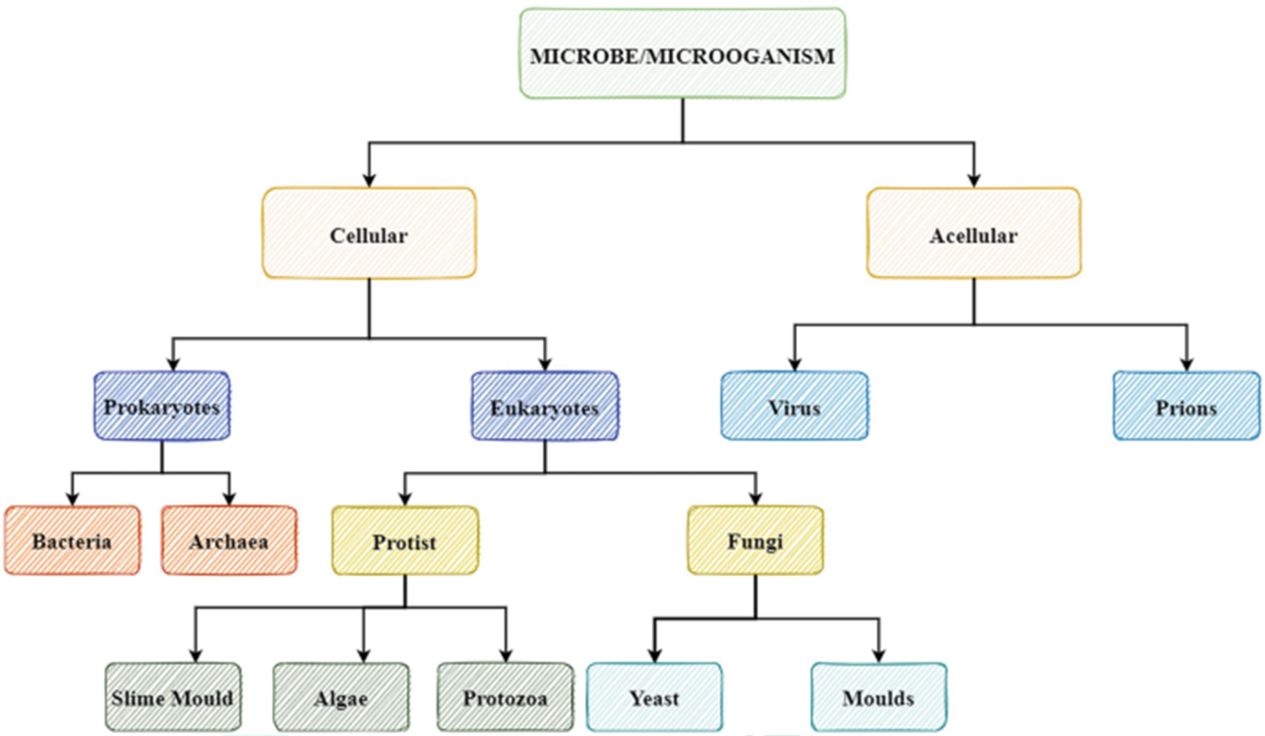
MICROBIOLOGY: A CSIR NET PERSPECTIVE

Microbiology is the study of microscopic organisms, including bacteria, viruses, fungi, and protozoa. This field is crucial for understanding health, disease, ecology, and biotechnology.

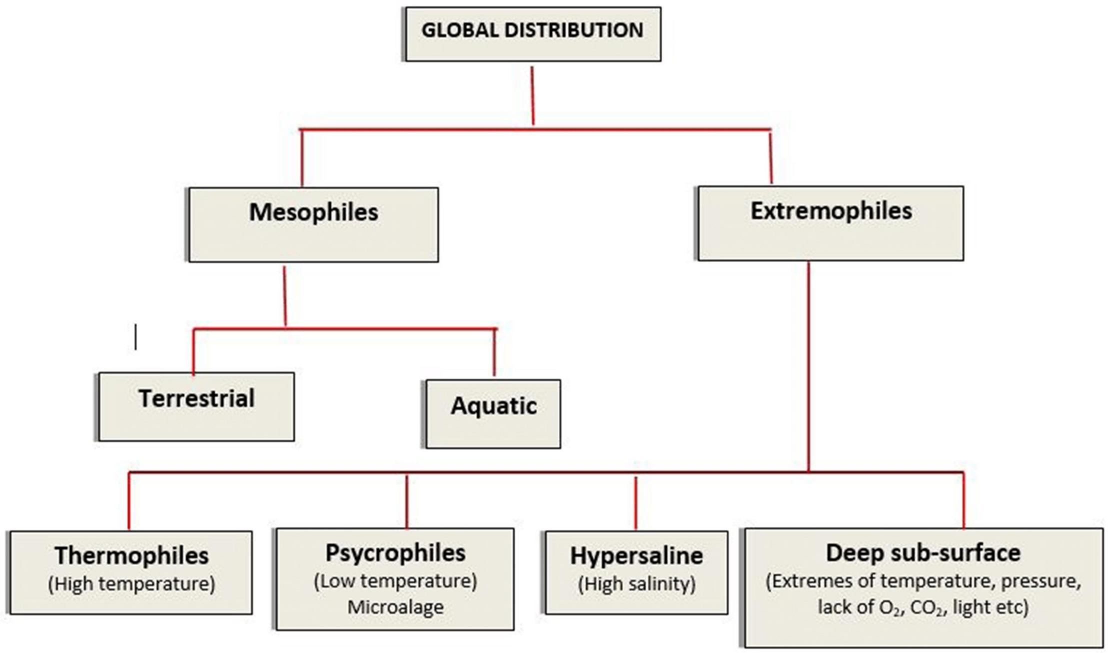
I. Microbes: Types, Distribution, and Biology

Microbes are ubiquitous and inhabit every conceivable environment on Earth.



Distribution of Microbes: Ubiquity and Adaptability

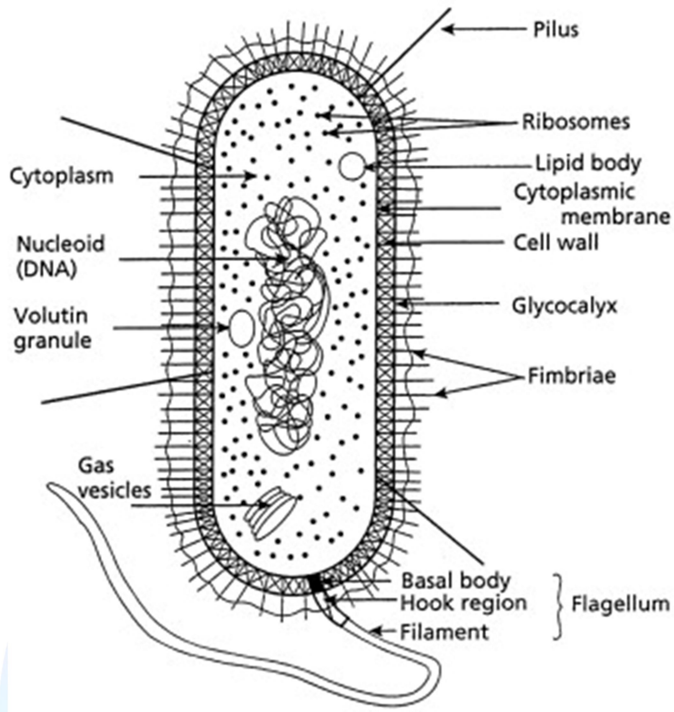
Microorganisms are the most ubiquitous life forms on Earth. Their immense metabolic diversity allows them to colonize every conceivable environment, from the most benign to the most extreme. Their distribution is not random; it is governed by the availability of nutrients, water, energy, and suitable physicochemical conditions.



Unique Features of Prokaryotic Cell Biology

- **Cell Wall:** Provides shape and rigidity. **Gram-positive** bacteria have a thick peptidoglycan layer. **Gram-negative** bacteria have a thin peptidoglycan layer and an outer membrane containing **lipopolysaccharide (LPS, endotoxin)**.
- **Cell Membrane:** Phospholipid bilayer lacking sterols (except in *Mycoplasma*). Site of respiration and photosynthesis.
- **Genome:** Typically a single, circular chromosome located in the **nucleoid**. May also contain smaller, circular **plasmids** that carry accessory genes (e.g., for antibiotic resistance).
- **Ribosomes: 70S** type (50S + 30S subunits), a target for many antibiotics.
- **Specialized Structures:**
    - **Flagella:** For motility.
    - **Pili/Fimbriae:** For attachment and conjugation.
    - **Capsule/Slime Layer:** Polysaccharide layer for protection and adhesion.

- **Endospores:** Highly resistant, dormant structures formed by *Bacillus* and *Clostridium* for survival under harsh conditions.



Respiratory Pathways

- **Aerobic Respiration:** Final electron acceptor is O_2. Highest energy yield.
- **Anaerobic Respiration:** Final electron acceptor is an inorganic molecule other than O_2 (e.g., NO_3^-, SO_4^{2-}, CO_2). Lower energy yield.
- **Fermentation:** Uses an internal organic molecule as final electron acceptor. No electron transport chain involved. Low energy yield (e.g., lactic acid fermentation, alcoholic fermentation).

III. Microbial Genetics & Molecular Biology

- **DNA Transfer Mechanisms:**
  1. **Transformation:** Uptake of free environmental DNA. A key technique in genetic engineering.

2. **Transduction:** Virus-mediated (bacteriophage) transfer of DNA from one bacterium to another.
3. **Conjugation:** Direct cell-to-cell contact via a **sex pilus**; transfer of plasmid DNA (e.g., F plasmid, R plasmid).

- **Gene Regulation:** Allows microbes to rapidly adapt to environmental changes.
  - **Operon Model:** Coordinated regulation of genes (e.g., **Lac operon** for lactose metabolism, **Trp operon** for tryptophan synthesis).
- **Mutation & Evolution:** High mutation rates and rapid generation times allow for quick evolution (e.g., development of antibiotic resistance).

Isolation and Cultivation

A. Cultivation of Bacteria

- **Principle:** To provide optimal physical and chemical conditions (pH, temperature, O_2) and nutrients for growth.
- **Culture Media:**
  - **Based on Consistency:** Liquid (Broth), Semi-solid (for motility), Solid (Agar plates - allows colony formation).
  - **Based on Composition:**
    - **Defined/Synthetic Media:** Exact chemical composition is known.
    - **Complex Media:** Contain extracts of unknown exact composition (e.g., Nutrient Broth, Luria Bertani (LB) Agar).
  - **Based on Function:**
    - **General Purpose:** Supports growth of many bacteria (e.g., Nutrient Agar).

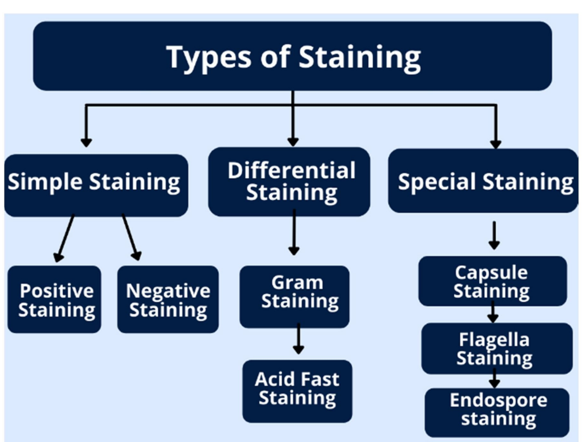
- **Selective Media:** Inhibits unwanted bacteria and encourages desired microbes (e.g., **MacConkey Agar** for Gram-negatives; contains bile salts and crystal violet).
- **Differential Media:** Allows distinction between different bacteria based on colony appearance (e.g., **Blood Agar** for hemolysis; **MacConkey Agar** also differentiates lactose fermenters (pink) from non-fermenters).

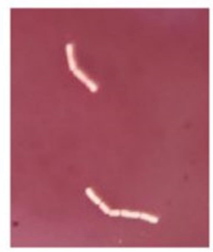
- **Isolation Technique: Streak Plate Method.** The goal is to thin out cells on an agar plate to obtain isolated, pure colonies derived from a single cell.

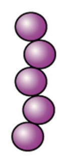
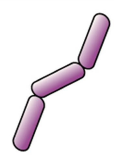
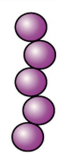
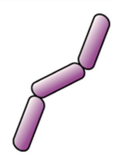
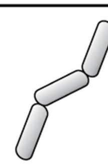
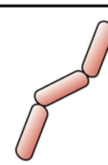
B. Cultivation of Viruses

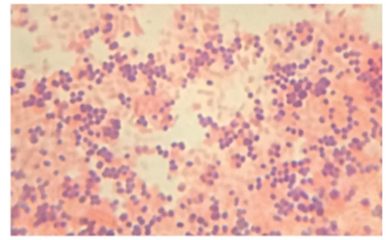
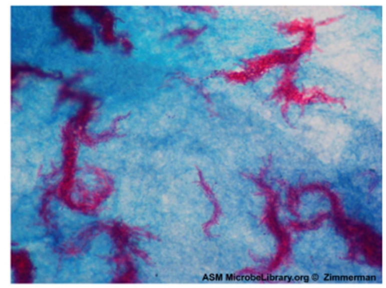
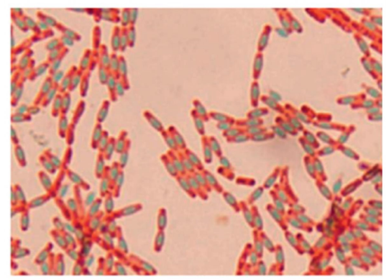
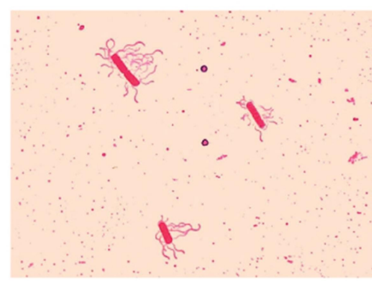
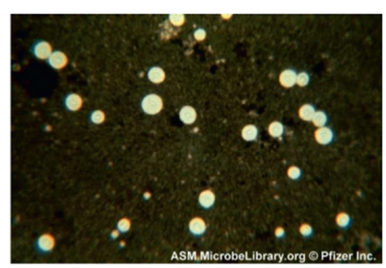
- Viruses **cannot be grown on inert culture media.** They require living host cells.
- **Methods:**
  1. **Laboratory Animals:** Historically used (e.g., mice for rabies virus).
  2. **Embryonated Eggs:** Used for vaccine production (e.g., influenza virus grown in chorioallantoic membrane).
  3. **Cell Culture:** The most common method today. Uses monolayers of cultured animal or plant cells.
     - Viruses often cause a **Cytopathic Effect (CPE)** – visible changes in the host cells (rounding, detachment, syncytia formation).

III. Staining Techniques



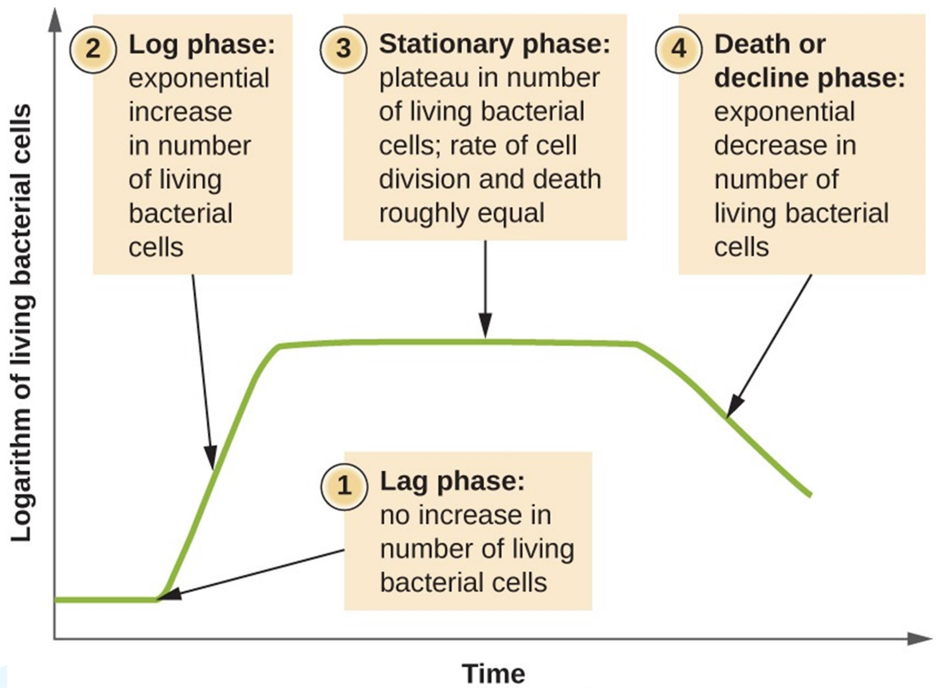
SIMPLE STAINS				
Stain Type	**Specific Dyes**	**Purpose**	**Outcome**	**Sample Images**
Basic stains	Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin	Stain negatively charged molecules and structures, such as nucleic acids and proteins	Positive stain	
Acidic stains	Eosin, acid fuchsin, rose bengal, Congo red	Stain positively charged molecules and structures, such as proteins	Can be either a positive or negative stain, depending on the cell's chemistry.	
Negative stains	India ink, nigrosin	Stains background, not specimen	Dark background with light specimen	

Gram stain process			
Gram staining steps	**Cell effects**	**Gram-positive**	**Gram-negative**
Step 1 **Crystal violet** *primary stain added to specimen smear.*	Stains cells purple or blue.		
Step 2 **Iodine** *mordant makes dye less soluble so it adheres to cell walls.*	Cells remain purple or blue.		
Step 3 **Alcohol** *decolorizer washes away stain from gram-negative cell walls.*	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 **Safranin** *counterstain allows dye adherance to gram-negative cells.*	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

DIFFERENTIAL STAINS				
Stain Type	**Specific Dyes**	**Purpose**	**Outcome**	**Sample Images**
Gram stain	Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.	
Acid-fast stain	After staining with basic fuchsin, acid-fast bacteria resist decolorization by acid-alcohol. Non acid-fast bacteria are counterstained with methylene blue.	Used to distinguish acid-fast bacteria such as *M. tuberculosis*, from non–acid-fast cells	Acid-fast bacteria are red; non–acid-fast cells are blue.	
Endospore stain	Uses heat to stain endospores with malachite green (Schaeffer-Fulton procedure), then cell is washed and counterstained with safranin.	Used to distinguish organisms with endospores from those without; used to study the endospore.	Endospores appear bluish-green; other structures appear pink to red.	
Flagella stain	Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaline or basic fuchsin.	Used to view and study flagella in bacteria that have them.	Flagella are visible if present.	
Capsule stain	Negative staining with India ink or nigrosin is used to stain the background, leaving a clear area of the cell and the capsule. Counterstaining can be used to stain the cell while leaving the capsule clear.	Used to distinguish cells with capsules from those without.	Capsules appear clear or as halos if present.	

Bacterial Growth Curve

When bacteria are inoculated into a fresh liquid medium, they follow a predictable growth pattern with four distinct phases.



Generation Time: The time required for a population to double in number. Calculated from the exponential phase.

Microbial Diseases in Humans

A. Modes of Transmission

Mode	Mechanism	Example Diseases
Food & Water Borne	Ingestion of contaminated food/water.	**Bacterial:** Cholera (*Vibrio cholerae*), Typhoid (*Salmonella Typhi*). **Viral:** Hepatitis A, Polio. **Protozoan:** Amoebiasis (*Entamoeba histolytica*).

Mode	Mechanism	Example Diseases
Air Borne	Inhalation of aerosolized droplets or spores.	**Bacterial:** Tuberculosis (*Mycobacterium tuberculosis*), Diphtheria. **Viral:** Influenza, Common Cold (Rhinovirus), COVID-19 (SARS-CoV-2), Measles. **Fungal:** Aspergillosis.
Insect Borne (Vector)	Transmission via an arthropod vector.	**Mosquito:** Malaria (*Plasmodium*), Dengue, Chikungunya, Zika. **Flea:** Plague (*Yersinia pestis*). **Louse:** Typhus (*Rickettsia*).
Contact Diseases	Direct or indirect contact.	**Direct (STD):** HIV/AIDS, Syphilis (*Treponema pallidum*), Gonorrhea (*Neisseria gonorrhoeae*). **Indirect (Fomites):** Tetanus (*Clostridium tetani*), Ringworm (Fungal).

VI. Microbial Diseases in Plants & Plant-Microbe Interactions

A. Plant Diseases

- **Bacterial:** Cause spots, blights, wilts, galls. Often enter through wounds or stomata.
  - *Example:* **Crown Gall** caused by *Agrobacterium tumefaciens*, which transfers a Tumor-inducing (Ti) plasmid into the plant genome. This is a cornerstone of **plant genetic engineering**.
- **Fungal:** Cause rusts, smuts, mildews, rots. Largest group of plant pathogens.
  - *Example:* **Late Blight of Potato** caused by *Phytophthora infestans* (an Oomycete, not a true fungus), which led to the Irish Potato Famine.

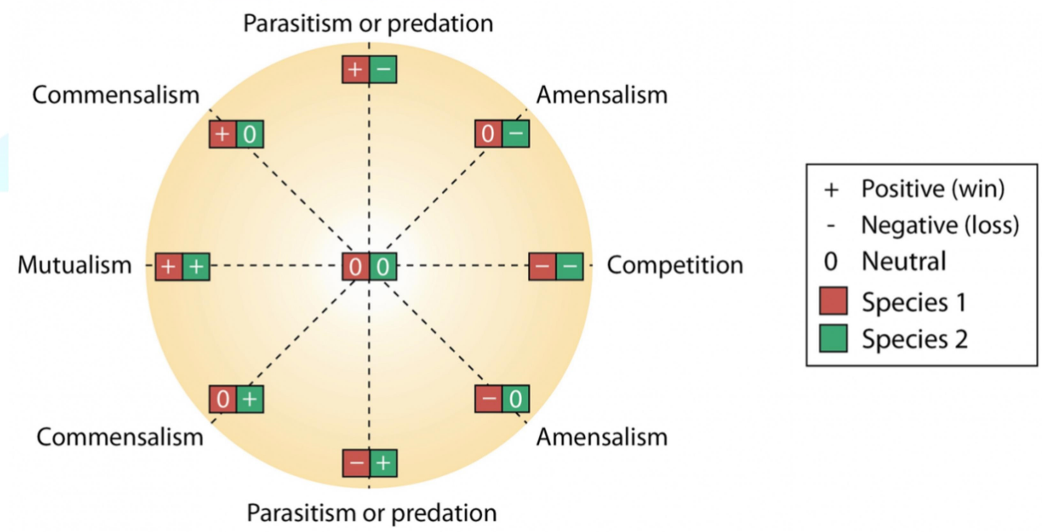
- **Viral:** Cause mosaics, stunting, leaf curling. Transmitted by insects, nematodes, or mechanically.
    - *Example:* **Tobacco Mosaic Virus (TMV)** – the first virus ever discovered.

PLANT-MICROBE INTERACTIONS: A CSIR NET PERSPECTIVE

Plant-microbe interactions are continuous and dynamic relationships that can be either beneficial (mutualistic), harmful (pathogenic), or neutral (commensal). These interactions are fundamental to plant health, ecosystem functioning, and agricultural productivity.

I. Types of Interactions

The interaction between a plant and a microbe exists on a spectrum, defined by the outcome for each partner.



Beneficial Interactions (Mutualism)

A. Rhizobia-Legume Symbiosis (Nitrogen Fixation)

This is a classic example of highly specific mutualism where bacteria fix atmospheric nitrogen (N_2) into ammonia (NH_3) for the plant.

- **Players: Rhizobia** bacteria (e.g., *Rhizobium*, *Bradyrhizobium*) and **Legume** plants (e.g., peas, beans, clover).

- **Mechanism:**
    1. **Signal Exchange:** Plant roots release **flavonoids**. Bacteria respond with **Nod factors** (lipochitin oligosaccharides).
    2. **Root Hair Curling:** Nod factors cause root hairs to curl and entrap the bacteria.
    3. **Infection Thread:** Bacteria enter the root hair and form an infection thread that extends into the root cortex.
    4. **Nodule Formation:** Cortical cells divide to form a **nodule**, a specialized organ where bacteria differentiate into **bacteroids** (N_2-fixing form).
    5. **Nitrogen Fixation:** The enzyme **nitrogenase** (O_2-sensitive) in bacteroids catalyzes $N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$. The plant provides the bacteroids with carbon (malate, succinate) and maintains low O_2 tension via **leghemoglobin** (a pink pigment that buffers O_2).
- **Significance:** Reduces the need for synthetic nitrogen fertilizers in agriculture.

B. Mycorrhizal Symbiosis

An association between **fungi** and the **roots** of over 90% of land plants.

- **Players: Mycorrhizal fungi** (e.g., Glomeromycota) and most plants.
- **Types:**
    - **Endomycorrhizae (Arbuscular Mycorrhizae, AM):** Fungal hyphae penetrate the root cortical cells and form highly branched **arbuscules** for nutrient exchange. Most common type.
    - **Ectomycorrhizae:** Fungal hyphae form a dense sheath (**mantle**) around the root and penetrate between cortical cells (**Hartig net**), but not into them. Common in trees (e.g., pine, oak).
- **Mechanism:** The extensive fungal mycelial network acts as an extension of the root system, dramatically increasing the surface area for absorption.
    - **Fungus to Plant:** Provides **water**, **phosphate (P)**, and other immobile minerals.

    - **Plant to Fungus:** Provides **photosynthates (sugars)** and a niche to live.
- **Significance:** Crucial for plant nutrition, especially in phosphorus-limited soils; enhances drought resistance.

C. Plant Growth-Promoting Rhizobacteria (PGPR)
A diverse group of free-living soil bacteria (e.g., *Pseudomonas*, *Bacillus*, *Azospirillum*) that colonize the rhizosphere (root zone) and benefit plants.

- **Mechanisms of Promotion:**
    - **Direct:**
        - **Nutrient Solubilization:** Secreting organic acids to solubilize **phosphate**.
        - **Nitrogen Fixation:** Some fix N_2 (e.g., *Azospirillum*).
        - **Phytohormone Production:** Producing **IAA** (auxin) to stimulate root growth.
        - **Siderophore Production:** Secreting iron-chelating molecules to make Fe available to the plant, while depriving pathogens.
    - **Indirect (Biocontrol):**
        - **Antibiosis:** Producing antibiotics that inhibit pathogens.
        - **Induced Systemic Resistance (ISR):** Priming the plant's defense system for faster and stronger response to future pathogen attack.

About us

BioResire (NEET | CSIR NET | Biotech Internships) is a life sciences research and training organization dedicated to bridging the gap between academic learning and industry skills. We provide internships, projects, and programs in Bioinformatics, Biotechnology, Molecular Biology, Cancer Research, Neuroscience, and related fields, helping students build job-oriented scientific careers.

"The future belongs to those who explore the unseen — where biology meets innovation and discovery begins."