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An Instructional Material On ASS-307- Biopesticides and Biofertilizers Production (Elective course)

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An Instructional Material
On
ASS-307- Biopesticides and Biofertilizers
Production
(Elective course)



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CONTENTS

Objective of the course: The aim of the course contents is to provide a comprehensive understanding of the Biopesticides and Biofertilizers Production.

Lecture outlines

Sl. No.	Topic covered	Pg. No.
1.	Aim of the course and learning out of the course contents Biopesticides and Biofertilizers	4-6
2.	History and concept of biopesticides, Importance, scope and potential of biopesticide	7-8
3.	Definitions, concepts and classification of biopesticides viz. pathogen, botanical pesticides, and biorationales	9-11
4.	Virulence, pathogenicity and symptoms of entomopathogenic pathogens and nematodes.	12-13
5.	Mass production technology of bio-pesticides	14-15
6.	Methods of application of biopesticides.	16-17
7.	Methods of quality control and techniques of biopesticides	18-19
8.	Impediments and limitation in production and use of biopesticide	20-21
9.	Bio-Pesticides-a special attention	22-31
10.	Biofertilizers - Introduction, status and scope	32- 36
11.	Instruments Used in Microbiology Lab with Principle and Uses	37-49
12.	Structure and characteristic features of different types of biofertilizers like bacterial biofertilizers - <i>Azospirillum</i> , <i>Azotobacter</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Rhizobium</i> and <i>Frankia</i>	50- 55
13.	Structure and characteristic features of - Cynobacterial biofertilizers - <i>Anabaena</i> , <i>Nostoc</i> , <i>Hapalosiphon</i>	56- 59
14.	Structure and characteristic features of - Fungal biofertilizers - AM mycorrhiza and ectomycorrhiza, endomycorrhiza and ectendomycorrhiza	60-63
15.	Biochemistry of biological nitrogen fixation- Nitrogen fixation -Free living and symbiotic nitrogen fixation.	64-86
16.	Mechanism of phosphate solubilization and phosphate mobilization	87-91
17.	Mechanism of K solubilization by Potassium Solubilizing Bacteria (KSB)	92-96
18.	FCO (Fertilizer Control Order) Specifications of Biofertilizers	97-99
19.	Quality Control of Biofertilizers	100-101
20.	Mass Production of Biofertilizers carrier based and liquid based biofertiizers	102-104
21.	Biofertilizers-Storage, shelf life and marketing	105-107
22.	Application technology of biofertilizers for seeds, seedlings, tubers, sets etc.	108-110
23.	Factors influencing the efficacy of biofertilizers	111-113



Reference Books

1. Biofertilizers in Agriculture and Forestry- N S Subba Rao, 3rd Ed. (1993) Oxford & IBH
2. Soil Microbiology- N S Subba Rao, 1999, Oxford & IBH
3. Microbial-based biopesticides: Methods and Protocols -T. R. Glare and M. E. Moran-Diez.
4. Biopesticides Handbook- Leo M.L. Nollet, Hamir Singh Rathore, 2015, CRC Press

Aim of the Course Contents

The aim of the course contents is to provide a comprehensive understanding of the following key aspects related to Biopesticides and Biofertilizers:

Biopesticides

1. History and Concept of Biopesticides:

- Explore the historical development and conceptual foundations of biopesticides.
- Understand the significance, scope, and potential applications of biopesticides in modern agriculture.
- Definitions, Concepts, and Classification of Biopesticides:

2. Define and classify Biopesticides

- including pathogen-based, botanical, and biorational categories.
- Examine the various concepts associated with biopesticides and their classification.

3. Botanical Pesticides and Their Uses:

- Investigate the types and applications of botanical pesticides.
- Explore the uses of botanicals in biopesticides and their effectiveness.

4. Entomopathogenic Pathogens and Nematodes:

- Understand virulence, pathogenicity, and symptoms associated with entomopathogenic pathogens and nematodes.
- Explore the potential of these biopesticides in pest management.

5. Mass Production Technology of Biopesticides:

- Learn the techniques and technologies involved in the large-scale production of biopesticides.
- Understand the factors influencing the mass production of biopesticides.

6. Methods of Application of Biopesticides:

- Explore different methods for the effective application of biopesticides in agricultural systems.

7. Quality Control and Techniques of Biopesticides:

- Examine the methods and techniques used for quality control in biopesticide production.
- Understand the importance of maintaining high-quality standards.

8. Impediments and Limitations in Production and Use of Biopesticides:

- Identify and analyze the challenges and limitations associated with the production and utilization of biopesticides.

Biofertilizers

1. Introduction, Status, and Scope of Biofertilizers:

- Provide an overview of biofertilizers, their current status, and potential scope in agriculture.

2. Structure and Characteristics of Bacterial Biofertilizers:

- Explore bacterial biofertilizers such as Azospirillum, Azotobacter, Bacillus, Pseudomonas, Rhizobium, and Frankia.
- Understand the structural and characteristic features of these biofertilizers.

3. **Cyanobacterial and Fungal Biofertilizers:**
 - Explore the role and characteristics of cyanobacterial biofertilizers (Anabaena, Nostoc, Hapalosiphon) and fungal biofertilizers (AM and ectomycorrhiza).
 4. **Nitrogen Fixation and Biochemistry:**
 - Understand the mechanisms of free-living and symbiotic nitrogen fixation.
 - Explore the biochemistry involved in biological nitrogen fixation.
 5. **Phosphate and Potassium Solubilization:**
 - Investigate the mechanisms of phosphate and potassium solubilization by biofertilizers.
 6. **Production Technology and Quality Control of Biofertilizers:**
 - Learn the production technology, strain selection, and quality control measures for biofertilizers.
 7. **Application Technology and Factors Influencing Efficacy:**
 - Explore various application technologies for biofertilizers on seeds, seedlings, tubers, and sets.
 - Understand the factors influencing the efficacy of biofertilizers.
 8. **Storage, Shelf Life, and Marketing of Biofertilizers:**
 - Examine the storage conditions, shelf life, and marketing strategies for biofertilizers.
- By covering these topics, the course aims to equip participants with a comprehensive understanding of biopesticides and biofertilizers, fostering knowledge and skills applicable to sustainable and environmentally friendly agricultural practices.

Learning outcomes

Upon completing the course on the history, concepts, and applications of Biopesticides and Biofertilizers, participants should achieve the following learning outcomes:

Learning outcomes of Biopesticides

1. **Understanding of Biopesticides:**
 - Explain the historical development and foundational concepts of biopesticides.
 - Evaluate the significance, scope, and potential applications of biopesticides in modern agriculture.
2. **Classification and Application of Biopesticides:**
 - Classify biopesticides into pathogen-based, botanical, and biorational categories.
 - Apply different methods of biopesticide application for effective pest management.
3. **Botanical Pesticides Knowledge:**
 - Identify various botanical pesticides and describe their uses in biopesticides.
 - Evaluate the effectiveness of botanicals in pest control.
4. **Knowledge of Entomopathogenic Pathogens and Nematodes:**
 - Analyze the virulence, pathogenicity, and symptoms associated with entomopathogenic pathogens and nematodes.
 - Assess the potential of these biopesticides in pest management strategies.
5. **Mass Production Technology:**

- Apply techniques and technologies for the large-scale production of biopesticides.
 - Identify and overcome challenges associated with mass production.
6. **Application Methods and Quality Control:**
- Implement various methods for the application of biopesticides in agricultural systems.
 - Employ quality control measures to ensure the efficacy and safety of biopesticides.
7. **Awareness of Impediments and Limitations:**
- Identify and analyze impediments and limitations in the production and use of biopesticides.
 - Propose potential solutions to address challenges in the biopesticide industry.

Learning outcomes of Biofertilizers

1. Biofertilizers Understanding:

- Summarize the introduction, status, and scope of biofertilizers in agriculture.
- Differentiate between various bacterial, cyanobacterial, and fungal biofertilizers based on their structure and characteristics.

2. Nitrogen Fixation and Biochemistry Knowledge:

- Explain the mechanisms of free-living and symbiotic nitrogen fixation.
- Understand the biochemistry involved in biological nitrogen fixation.

3. Phosphate and Potassium Solubilization:

- Describe the mechanisms of phosphate and potassium solubilization by biofertilizers.
- Apply knowledge to enhance nutrient availability in soil through biofertilizers.

4. Production Technology and Quality Control of Biofertilizers:

- Apply production technology, strain selection, and quality control measures for bacterial, cyanobacterial, and fungal biofertilizers.
- Ensure the production of high-quality biofertilizers for agricultural use.

5. Application Technology and Efficacy Factors:

- Implement various application technologies for biofertilizers on seeds, seedlings, tubers, and sets. Analyze and understand the factors influencing the efficacy of biofertilizers.

6. Storage, Shelf Life, and Marketing:

- Determine appropriate storage conditions and shelf life for biofertilizers.
- Develop marketing strategies for biofertilizers, considering factors such as product efficacy and market demand.

7. Integration of Knowledge for Sustainable Agriculture:

- Synthesize knowledge from both biopesticides and biofertilizers to promote sustainable and environmentally friendly agricultural practices.
- Formulate comprehensive strategies for integrated pest and nutrient management.

By achieving these learning outcomes, participants will be well-equipped to contribute to the adoption and implementation of bio-based solutions in agriculture, promoting sustainable and eco-friendly practices in the field.

History and Concept of Biopesticides: Importance, Scope, and Potential

Dr. AJEET KUMAR¹

1. Introduction

Biopesticides are biologically-based agents used to control pests, encompassing microorganisms (bacteria, fungi, viruses), biochemical substances (plant extracts, pheromones), and beneficial insects. They offer an eco-friendly and sustainable alternative to synthetic chemical pesticides, aligning with the principles of integrated pest management (IPM) and organic agriculture.

2. History of Biopesticides

2.1 Early Use

- **Ancient Practices:** Use of ash, plant extracts (e.g., neem, pyrethrum), and oil emulsions for pest control.
- **China (1100 AD):** Use of predatory ants to control pests in citrus orchards.

2.2 Emergence of Microbial Biopesticides

- **1835:** Discovery of *Beauveria bassiana* as a silkworm pathogen by Agostino Bassi - foundational to microbial insecticides.
- **1874:** *Bacillus thuringiensis* (Bt) first isolated from infected silkworms.
- **1940s-50s:** First commercial production of Bt formulations.
- **1980s onwards:** Regulatory approvals and increased commercial development of microbial and biochemical pesticides.

3. Concept of Biopesticides

3.1 Definition

Biopesticides are natural-based pest management agents derived from living organisms or their by-products. According to the **EPA (USA)**, biopesticides are classified into three major types:

3.2 Types of Biopesticides

- **Microbial Biopesticides:** Contain microorganisms (e.g., *Bacillus thuringiensis*, *Trichoderma*, *Metarhizium*).
- **Biochemical Biopesticides:** Naturally occurring substances (e.g., neem oil, pheromones, plant volatiles) that control pests by non-toxic mechanisms.
- **Plant-Incorporated Protectants (PIPs):** Genetically engineered plants expressing pesticidal properties (e.g., Bt cotton).

4. Importance of Biopesticides

- **Environmental Safety:** Biodegradable, non-persistent, and target-specific with minimal off-target impacts.
- **Human and Animal Health:** Reduced toxicity compared to synthetic pesticides.

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- **Resistance Management:** Lower chances of resistance development due to complex modes of action.
- **Organic Farming:** Essential components of certified organic crop protection.
- **Sustainability:** Promotes biodiversity and ecosystem balance.
- **Regulatory and Trade Compliance:** Safer residues align with stringent Maximum Residue Limits (MRLs).

5. Scope and Application

5.1 Agricultural Crops

- Used against pests in cereals, pulses, vegetables, fruits, and plantation crops.
- Effective in seed treatment, foliar sprays, and soil applications.

5.2 Horticulture and Greenhouse Cultivation

- Widely used due to enclosed conditions that support microbial efficacy.

5.3 Post-Harvest and Stored Grain Protection

- Entomopathogenic fungi and botanical extracts used to manage storage pests.

5.4 Urban and Public Health

- Larvicidal formulations (e.g., *Bacillus sphaericus*) used in mosquito control programs.

6. Potential and Future Prospects

6.1 Market Growth

- The global biopesticide market is growing at >10% CAGR, driven by demand for residue-free food and sustainability.

6.2 Innovations and Advancements

- **Genetic Engineering:** Development of transgenic crops with biopesticide traits.
- **Nano-formulations:** Enhanced stability and targeted delivery of bioagents.
- **AI and IoT:** Precision application and pest monitoring systems.
- **Microbial Consortia:** Synergistic blends for broader spectrum activity.

6.3 Challenges

- **Shelf-life and Storage:** Limited compared to synthetic pesticides.
- **Field Efficacy:** Variable results under different environmental conditions.
- **Awareness and Adoption:** Limited knowledge among farmers.
- **Regulatory Delays:** Lengthy registration processes in many countries.

7. Conclusion

Biopesticides represent a critical tool for achieving sustainable agriculture and ecological pest management. Their importance lies in minimizing environmental impact, promoting biodiversity and ensuring food safety. With advancing research, favourable policies and increasing consumer awareness, the scope and potential of biopesticides are poised to expand significantly.

Definitions, Concepts, and Classification of Biopesticides: Pathogens, Botanical Pesticides, and Biorationals

Dr. AJEET KUMAR²

1. Introduction to Biopesticides

Biopesticides are a class of pest management agents derived from natural materials such as animals, plants, bacteria, and certain minerals. They offer environmentally friendly alternatives to conventional chemical pesticides and are often target-specific, biodegradable, and less harmful to beneficial organisms.

2. Definitions

- **Biopesticide:**
A formulation made from natural sources (microorganisms, botanicals, or other biologically active substances) used to control pests through non-toxic or biologically based mechanisms.
- **Pathogenic Biopesticides:**
These are formulations containing living organisms (bacteria, fungi, viruses, or protozoa) that cause disease or inhibit the development of pest organisms.
- **Botanical Pesticides:**
Plant-derived substances that exhibit pesticidal properties, such as extracts, essential oils, and secondary metabolites (e.g., neem, pyrethrin, rotenone).
- **Biorational Pesticides:**
Pesticides that are biologically based and cause minimal ecological disruption. This includes insect growth regulators, sex pheromones, and natural enzyme inhibitors.

3. Key Concepts

- **Eco-friendliness:**
Biopesticides are typically non-toxic to non-target organisms and decompose quickly without leaving harmful residues.
- **Specificity:**
Most biopesticides are highly specific, targeting only a particular pest or group of pests.
- **Resistance Management:**
Biopesticides play a crucial role in integrated pest management (IPM) programs by delaying resistance development in pests.
- **Mode of Action:**
Varies from microbial infection, growth disruption, repellent effects, to interference with reproductive behaviours.

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4. Classification of Biopesticides

A. Microbial Biopesticides (Pathogens)

Type	Example	Target Pests
Bacteria	<i>Bacillus thuringiensis</i> (Bt)	Lepidopteran larvae
Fungi	<i>Beauveria bassiana</i> , <i>Metarhizium anisopliae</i>	Aphids, beetles
Viruses	Nucleopolyhedrovirus (NPV), Granulovirus (GV)	Caterpillars
Protozoa	<i>Nosema locustae</i>	Grasshoppers

B. Botanical Biopesticides

Source Plant	Active Component	Pest Type
Neem (<i>Azadirachta indica</i>)	Azadirachtin	Insects (all stages)
Chrysanthemum spp.	Pyrethrins	Mosquitoes, flies
Derris spp.	Rotenone	Insects, fish
Garlic	Sulfur compounds	Insects, fungi

C. Biorational Pesticides

Type	Description	Mode of Action
Insect Growth Regulators (IGRs)	Disrupt development	Juvenile hormone analogs
Sex Pheromones	Disrupt mating	Confusion/trapping
Repellents	Deter pest approach	Olfactory stimulation
Enzyme Inhibitors	Interfere with digestion/metabolism	Botanical alkaloids

5. Advantages of Biopesticides

- Environmentally safe
- Decomposes quickly
- Specific to pests
- Reduces chemical pesticide use
- Promotes biodiversity

6. Limitations

- Short shelf life
- Sensitivity to UV light and temperature
- Slow action compared to chemicals
- Requires frequent application
- Narrow target range

7. Conclusion

The classification of biopesticides into microbial (pathogens), botanical, and biorational categories provides a structured approach to their use in sustainable agriculture. They form an integral part of IPM and future pest management strategies due to their eco-compatibility and specificity.

Virulence, Pathogenicity, and Symptoms of Entomopathogenic Pathogens and Nematodes

Dr. AJEET KUMAR³

1. Definitions

- **Virulence:** The degree of damage caused by a pathogen to its host. In the context of entomopathogens, it refers to the severity of disease or mortality induced in insects.
- **Pathogenicity:** The ability of a microorganism or nematode to cause disease in an insect host. It is a qualitative trait (presence or absence of pathogenic effect).

2. Major Groups of Entomopathogenic Organisms

Type	Examples	Mode of Action
Fungi	<i>Beauveria bassiana</i> , <i>Metarhizium anisopliae</i>	Penetrate cuticle, colonize hemocoel
Bacteria	<i>Bacillus thuringiensis</i> (Bt), <i>Serratia</i> spp.	Produce toxins, disrupt midgut epithelium
Viruses	Nuclear Polyhedrosis Virus (NPV), Granulosis Virus (GV)	Replicate inside host cells, lysis
Nematodes	<i>Steinernema</i> spp., <i>Heterorhabditis</i> spp.	Release symbiotic bacteria in hemocoel

3. Virulence Mechanisms

A. Fungi

- Produce cuticle-degrading enzymes (proteases, chitinases).
- Hyphal bodies proliferate in the hemocoel, producing toxins.
- Rapid colonization leads to insect death within 3–7 days.

B. Bacteria

- Bt produces crystal (Cry) toxins → ingestion by larva → pore formation in midgut → septicemia.
- Other bacteria release endotoxins and hemolysins disrupting tissue integrity.

C. Viruses

- Infect midgut epithelial cells after ingestion.
- Viral replication → cell lysis → systemic spread → death.

D. Nematodes

- Infective juveniles (IJs) enter insect body via natural openings.
- Release symbiotic bacteria (*Photorhabdus*, *Xenorhabdus*).
- Insect killed within 24–72 hours by toxemia/sepsis.

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4. Pathogenicity Traits

Parameter	Importance for Pathogenicity
Host range	Determines spectrum of affected insects
Toxin production	Key for killing or weakening hosts
Invasion & replication rate	Influences speed of mortality
Immune evasion ability	Ensures survival within host

5. Symptoms in Infected Insects

Pathogen Type	Symptoms
Fungi	White, green or red mold on cadaver, shriveling, mummification
Bacteria	Cessation of feeding, flaccid body, liquefied internal tissue
Viruses	Milky white hemolymph, body disintegration post-mortem
Nematodes	Rigid body, brown/red discoloration, bacterial ooze

6. Diagram: Infection Cycle of Entomopathogenic Nematodes

[Infective Juvenile] → [Entry into Insect Host] → [Release of Symbiotic Bacteria]
→ [Toxin Production & Host Death] → [Nematode Reproduction] → [Emergence of IJs]

7. Factors Affecting Virulence and Pathogenicity

- Environmental conditions (temperature, humidity)
- Host species and developmental stage
- Genetic variability among pathogen strains
- Dose and mode of application

8. Applications in IPM (Integrated Pest Management)

- Eco-friendly alternatives to chemical pesticides.
- Useful against soil-dwelling and foliage-feeding insect pests.
- Compatible with many biocontrol and cultural practices

Mass Production Technology of Biopesticides

Dr. AJEET KUMAR⁴

1. Introduction

Biopesticides are formulations of living organisms or their by-products that are used to manage pests through natural mechanisms. Mass production of biopesticides involves the cultivation of large quantities of entomopathogenic fungi, bacteria, viruses, or beneficial nematodes under controlled conditions to ensure their viability, virulence, and field effectiveness.

2. Steps in Mass Production

A. Selection of Potent Strain

- High virulence, broad spectrum activity.
- Stable under environmental stress.
- Non-toxic to humans and non-target organisms.

B. Optimization of Culture Media

- Use of cost-effective substrates like agricultural by-products.
- Formulated for high spore/toxin yield and viability.

C. Fermentation Process

Type	Description	Suitable for
Submerged (SmF)	Growth in liquid nutrient broth	Bacteria (<i>Bt</i>), Fungi (toxins)
Solid State (SSF)	Growth on solid substrate (e.g., rice, wheat bran)	Fungi (<i>Metarhizium</i> , <i>Beauveria</i>)
Surface Culture	Culture grown on the surface of solid/liquid medium	Virus production (NPV, GV)

3. Mass Production of Different Biopesticide Types

A. Bacterial Biopesticides (e.g., *Bacillus thuringiensis*)

- Inoculation into nutrient-rich broth (e.g., nutrient yeast extract medium).
- Incubated at 28–30°C for 48–72 hours under agitation.
- Harvesting by centrifugation or filtration.
- Formulated as wettable powder, suspension concentrate, or granules.

B. Fungal Biopesticides (e.g., *Metarhizium anisopliae*, *Beauveria bassiana*)

- Grown on solid substrates (e.g., broken rice, sorghum).
- Incubated for 10–15 days in sterile trays at 25–28°C.
- Spores harvested by sieving or scraping.
- Dried and mixed with talc/lignite for formulation.

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C. Viral Biopesticides (e.g., NPV)

- In vivo production using host larvae (e.g., *Helicoverpa armigera*).
- Infected larvae reared under controlled conditions.
- Virus harvested from larval cadavers.
- Purified and formulated as aqueous suspension or powder.

D. Entomopathogenic Nematodes (e.g., *Steinernema*, *Heterorhabditis*)

- In vivo production in *Galleria mellonella* larvae or in vitro liquid fermentation.
- Harvesting of infective juveniles (IJs).
- Stored in aqueous suspensions or alginate formulations.

4. Formulation and Shelf Life

Component	Role
Carriers	Talc, peat, lignite, rice bran for ease of handling
Additives	Stabilizers, UV protectants, stickers
Packaging	Moisture-proof, breathable pouches
Storage	Refrigerated or ambient depending on shelf life

- **Target shelf life:** 6–12 months
- **Viability tests:** CFU count, spore count, IJ survival

5. Quality Control Parameters

Parameter	Standard Requirement
Viable count (CFU/IJ)	Minimum 1×10^7 per gram/ml
Contamination	Must be <0.1%
Moisture content	<10% in dry formulations
pH and Toxin Profile	Within effective bioactive range

6. Flow Diagram: Fungal Biopesticide Production

[Mother Culture] → [Inoculum Development] → [Mass Production on Substrate] → [Incubation & Sporulation] → [Harvesting] → [Formulation] → [Packaging & Storage]

7. Challenges in Mass Production

- Contamination risk during large-scale fermentation.
- Lack of consistent quality carriers.
- Temperature and humidity control during drying and storage.
- Cost-effectiveness for smallholder farmers.

8. Future Innovations

- Bioreactors with automated control systems.
- Use of agro-industrial waste as media.
- Nano-formulations for increased shelf life and stability.
- AI-based process optimization for yield enhancement.

Methods of Application of Biopesticides

Dr. AJEET KUMAR⁵

1. Introduction

Biopesticides are biological formulations containing living organisms or their derivatives used for pest management. Their success depends not only on the formulation and viability but also on **proper application techniques** to reach the target pest in the most effective manner.

2. Criteria for Choosing Application Method

- Target pest and life stage
- Site of infection (foliar, soil, stored grain)
- Nature of biopesticide (fungal, bacterial, viral, or nematode-based)
- Formulation type (liquid, powder, granule, etc.)
- Environmental factors (UV exposure, humidity, rainfall)

3. Major Application Methods

A. Foliar Application (Spraying)

- **Target:** Leaf-feeding insects and pathogens (e.g., *Bt*, fungal spores)
- **Formulation:** Liquid suspensions or wettable powders
- **Equipment:** Hand sprayer, mist blower, drone-based sprayer
- **Advantages:** Direct contact with insect; easy to apply
- **Limitations:** Sensitivity to UV and wash-off by rain

B. Soil Application

- **Target:** Soil-dwelling larvae, termites, root grubs
- **Organisms:** Fungi (*Metarhizium*, *Beauveria*), nematodes (*Steinernema*, *Heterorhabditis*)
- **Formulation:** Granules, talc-based powders, IJs in aqueous suspension
- **Method:** Mixing with compost/FYM or irrigation water
- **Advantages:** Long-lasting effect; safe from UV exposure

C. Seed Treatment

- **Target:** Seed and seedling-borne pests
- **Agents:** *Pseudomonas fluorescens*, *Trichoderma*, *Bt*
- **Method:** Coating seeds with slurry before sowing
- **Formulation:** Wettable powder or liquid formulations
- **Benefits:** Early-stage protection; low dose requirement

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D. Trunk Injection / Wound Dressing

- **Target:** Borers and wood-infesting pests
- **Organisms:** Fungal or bacterial formulations
- **Method:** Injecting suspension into tree trunk or wound dressing
- **Use:** Orchards, plantations

E. Bait Application

- **Target:** Fruit flies, cockroaches, ants, termites
- **Organisms:** *Metarhizium*, *Beauveria*, or bacterial toxins
- **Method:** Mixing biopesticide with attractants and bait substrate
- **Application:** Field edges, bait stations

F. Drip Irrigation / Drenching

- **Target:** Nematodes, soil-borne insects, and pathogens
- **Method:** Injecting liquid formulation into irrigation lines
- **Suitable For:** High-value crops under drip systems

4. Formulation Types and Compatibility

Method	Common Formulations	Compatible Biopesticides
Foliar spray	WP, SC, SL	<i>Bt</i> , <i>Beauveria</i> , <i>Metarhizium</i>
Soil drenching	Granules, suspension	<i>Trichoderma</i> , nematodes
Seed treatment	WP, DS, LS	<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Trichoderma</i>
Bait	Granules, paste	<i>Metarhizium</i> , <i>Bt</i> toxin

5. Diagram: Matching Method to Pest Type

[Seed Treatment]	→ Seed-borne pests
[Foliar Spray]	→ Caterpillars, aphids
[Soil Application]	→ Root grubs, white grubs
[Drip Irrigation]	→ Nematodes
[Bait]	→ Fruit flies, termites

6. Considerations for Effective Use

- Apply during **early pest stage** for best results
- Avoid **UV exposure** and apply during **cooler times of day**
- Maintain **adequate moisture** for spore/nematode survival
- Avoid mixing with **incompatible agrochemicals**

7. Future Trends

- Drone-assisted precision spraying
- Nano-carriers for foliar delivery
- Encapsulation for slow release
- AI-guided pest detection and smart application

Methods of Quality Control and Techniques of Biopesticides

Dr. AJEET KUMAR⁶

1. Introduction

Quality control (QC) in biopesticide production ensures **efficacy**, **safety**, and **shelf-life** of microbial agents before commercial use. Standardized QC techniques are essential to comply with **regulatory guidelines** and maintain **bioefficacy** across production batches.

2. Importance of Quality Control in Biopesticides

- Ensures **purity**, **viability**, and **correct strain identity**
- Prevents contamination with unwanted microorganisms
- Assures **consistency** in field performance
- Necessary for **registration** under Insecticide Act (1968), CIB & RC (India)

3. Quality Parameters for Common Biopesticides

Biopesticide Type	Minimum Viable Count	Purity (Contaminants)	Moisture (%)	pH Range
<i>Bacillus thuringiensis</i>	1 × 10 ⁹ CFU/g (solid) or ml (liquid)	< 0.1%	< 10%	6.5–7.5
<i>Trichoderma spp.</i>	2 × 10 ⁶ CFU/g	< 0.1%	< 20%	6.0–7.5
<i>Beauveria bassiana</i>	1 × 10 ⁷ spores/g	< 0.1%	< 10%	6.0–7.5
NPV (<i>HaNPV</i>)	1 × 10 ⁹ PIB/ml	No bacterial/fungal growth	Liquid	5.5–6.5

4. Quality Control Techniques

A. Viability Testing

- **Bacteria:** Serial dilution and plate count method on Nutrient Agar
- **Fungi:** Spore germination on PDA plates (viable if ≥ 90% spores germinate)
- **Nematodes:** Visual movement under microscope in distilled water
- **Viruses:** Occlusion bodies counted using a hemocytometer

B. Purity Testing

- Check for contamination using general growth media:
 - **Nutrient Agar, Potato Dextrose Agar (PDA)**
- Observe colony morphology and identify contaminating species
- Should not exceed 0.1% contamination

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C. Moisture Content Analysis

- **Gravimetric Method:** Dry 1g sample at 105°C for 6 hrs
- Moisture content = $(\text{Initial weight} - \text{Dry weight}) / \text{Initial weight} \times 100$
- Lower moisture ensures longer shelf-life

D. pH Testing

- Use **digital pH meter** or indicator strips
- Adjusted with buffer solutions or stabilizers

E. Pathogen Identification & Authentication

- **Microscopy:** Spore or bacterial morphology
- **Molecular Techniques:**
 - PCR for strain-specific genes; DNA barcoding or sequencing
 - RAPD or AFLP fingerprinting

F. Bioassay for Efficacy (Potency Test)

- Example: *Bt* tested against 3rd instar *Helicoverpa armigera* larvae
- Parameters:
 - LC₅₀ (lethal concentration)
 - LT₅₀ (lethal time)
- Compare with reference strain

5. Packaging and Labeling Standards

- Must be moisture-proof, UV-protective, and properly labeled with:
 - Active ingredient
 - Viable cell/spore count
 - Expiry date
 - Batch number
 - Application method

6. Regulatory Guidelines in India

- **CIB & RC** – Central Insecticides Board and Registration Committee
- Must meet BIS (Bureau of Indian Standards) specifications
- QA/QC labs should be accredited under **ISO/IEC 17025**

7. Diagram: Quality Control Workflow

[Raw Strain] → [Media Optimization] → [Fermentation] → [Harvesting] → [Viability Test] → [Contamination Check] → [Packaging] → [Labeling] → [Release]

8. Challenges in Quality Control

- Variation in raw materials; Lack of trained personnel in bioassay
- Inadequate storage conditions; Field inconsistency despite lab QC

9. Future Technologies in QC

- **Automated colony counters** for viable counts
- **Real-time PCR** for rapid identity testing
- **Digital imaging** for nematode movement
- **Portable bioassay kits** for field evaluation

Impediments and Limitations in the Production and Use of Biopesticides

Dr. AJEET KUMAR⁷

1. Introduction

Biopesticides offer eco-friendly alternatives to chemical pesticides. However, despite their proven benefits, **production bottlenecks** and **field-level limitations** hinder their widespread adoption. These challenges must be addressed to ensure scalability and success in Integrated Pest Management (IPM) programs.

2. Major Impediments in Production

A. Technical Challenges

- **Strain instability:** Genetic or phenotypic drift over subcultures
- **Low shelf-life:** Limited viability under ambient storage
- **Contamination risks:** Especially in solid-state and nematode production
- **Requirement of specific substrates:** Cost and availability issues

B. Infrastructure Limitations

- Lack of **automated fermentation units** or **bioreactors** in small-scale setups
- Inadequate **cold storage** for live formulations
- Poor quality control in unregulated labs

C. Regulatory Hurdles

- Delays in **registration and licensing**
- Lack of **clear, crop-specific guidelines** for use
- Absence of **standardized protocols** for QA/QC across different organisms

3. Field-Level Limitations

A. Environmental Sensitivity

- Susceptibility to **UV radiation, high temperatures, and desiccation**
- Need for **moist microclimates** for successful infection (e.g., fungal spores)

B. Limited Spectrum and Speed

- Narrow **host range** in many biopesticides (e.g., NPV for *Helicoverpa*)
- **Slower action** than chemical pesticides; not suitable for emergency control

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C. Application Difficulties

- Lack of awareness about **correct timing, dosage, and methods**
- Incompatibility with common **chemical pesticides or adjuvants**
- Limited availability of **drone or drip delivery systems**

4. Market and Socio-Economic Limitations

- **Higher unit cost** than synthetic pesticides in many cases
- Weak **supply chains and distribution networks**
- **Farmer skepticism** due to past inconsistent performance
- Lack of **government subsidies** or insurance coverage

5. Limitations in R&D and Extension

- **Inadequate funding** for basic and applied biopesticide research
- Absence of **location-specific field validation trials**
- **Limited integration** with mainstream extension services and IPM programs

6. Summary Table: Impediments at Different Stages

Category	Key Limitation
Production	Strain instability, contamination, shelf life
Infrastructure	Lack of cold storage, automation
Regulatory	Delayed approvals, no crop-specific protocols
Environmental	UV sensitivity, need for humidity
Application	Poor farmer training, slow action
Market	High cost, poor supply chain, low adoption
R&D/Extension	Low field support, poor awareness programs

7. Suggestions for Overcoming Limitations

- Develop **stress-tolerant strains and formulations**
- Provide **financial incentives** and subsidies to producers and farmers
- Strengthen **extension services** and demonstration programs
- Promote **PPP models** for biopesticide manufacturing
- Integrate with **digital tools** (apps, IoT, AI) for targeted delivery and pest tracking

Bio-Pesticides-a special attention

Dr. AJEET KUMAR⁸

A fascinating and economically important area of applied microbial ecology is the biological control of pests and disease-causing agents using microorganisms. Biological control using microorganisms to initiate disease in pest populations has the potential of reducing agricultural reliance on chemical pesticides. The negative interactions (amensalism, predation and parasitism) among microbial populations and between microbes and higher organisms have always formed a natural basis for the biological control of pests and pathogens, and biological control methods take advantage of those relationships. Biological methods to control populations of disease causing organisms and pests are based on modification of host and or vector population, modification of reservoirs of pathogens, and the direct use of microbial pathogens and predators. Biotechnology may improve approaches for controlling pest and disease causing populations.

Biological Control

Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms. This is accomplished naturally or through manipulation of the environment/host/antagonist or by mass introduction of one or more antagonists.

Principles of Biocontrol

Biocontrol is based on antagonism. Which is further based on competition (for nutrients and space), parasitism (by production of volatile non-volatile antibiotics) and hyperparasitism. Competition In the soil, the dormant propagules of fungi are subjected to intense microbial competition when nutrients become available, e.g. Ammonium sulphate solution improves the biocontrol of *Heterobasidium annosum* by *Trichoderma viridae*.

Hyperparasitism It is the attack of a secondary parasite on primary parasite (parasitism of one microbe by another) observed among airborne pathogens. Rusts are often parasitised by *Enderluca carices* and powdery mildews by *Ampelomyces quisqualis*. *Cladosporium* species parasitise both rust and powdery mildews.

Antibiosis It depends on competitive saprophytic ability of a fungus. Sterile soil does not produce antibiotic substances. Several phylloplane fungi such as *Aureobasidium*, *Alternaria*, *Botrytis* and *Helminthosporium* have been shown to produce antibiotics.

Microbial Insecticides:

Pests and diseases can be effectively controlled without chemical pesticides by good crop management practices.

Integrated Pest Management (IPM):

It is a law that emphasises the need to restrict insect population rather than to eradicate them so that the pest population is kept at a non-injurious level. IPM is a pest management system

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that in the context of the associated environment and the population dynamics of the pest species utilises all suitable techniques and methods in as compatible a manner as possible to maintain the pest population at levels below those causing economic injury.

Population of pathogenic or predatory microorganisms that are antagonistic towards a particular pest population provide a natural means of controlling pest population and preparations of such antagonistic microbial populations are called microbial pesticides or microbial insecticides. An effective microbial pesticide possesses the following characteristics:

1. The microbial pathogen must be virulent and cause disease in the pest population when properly applied at the recommended concentration.
2. Pathogen should not be susceptible to environmental conditions. After application, it should survive long until the infection is established within the pest population.
3. Resistant stages of the pathogen like spores or cysts are best for use.
4. The pathogen should be rather specific for the pest population and must not cause disease in the non-pest population. It should rapidly establish disease in the pest population so as to minimise destruction caused by the pest.
5. The microbial pesticide should be harmless to humans and other valued plant and animal population.

The various types of microbial insecticides include

- (a) Bacterial pesticides
- (b) Viral pesticides and
- (c) Fungal pesticides.

(a) Bacterial Pesticides

Several bacterial pathogens that have been used as insecticides include

- a) Endospore forming: *Bacillus* and *Clostridium* species
- b) Non-endospore forming: species of *Pseudomonas*, *Enterobacter*, *Proteus*, *Serratia*, *Xenorhabdus*.

Of the potential bacterial pesticides, *Bacillus thuringiensis* has been most extensively studied. *Bacillus thuringiensis* has been successfully tested against more than 140 insect species (Lepidoptera, Hymenoptera, Diptera and Coleoptera). At present, there are 12 groups of *B. thuringiensis*. All strains produce protein crystal inclusion bodies which act as endotoxin. They are toxic factors and are called parasporal bodies. These crystals dissolve under alkaline condition. They are not soluble in water under neutral or acidic condition. The midgut contents of the caterpillar larvae (pests) are alkaline. On ingestion, the crystal dissolves in the midgut fluid and gets digested particularly by the proteolytic enzyme present in the midgut fluid. This digested protein crystal attacks the cementing substances which are present in the gut wall, thus loosening the epithelial gut wall which helps continuous diffusion of liquid from the gut into the blood making the blood of the insect highly alkaline and leading to gut paralysis. The parasporal bodies are highly toxic for caterpillars with an LD50 value of < 0.9 mg/ g of larvae. The process of crystal synthesis and spore formation proceed simultaneously. The toxin production by a culture can be

enhanced by controlling many factors. *Bacillus thuringiensis* toxin genes have been introduced via recombinant DNA technology into the genome of plants or plant associated microorganisms.

Method of Production Submerged fermentation

Preparation of inoculum

The inoculum is prepared using media containing beet molasses, corn steep solids and calcium carbonate.

Production medium

Production medium enhances the production of toxins within the cell which happens during sporulation. The fermentable carbohydrate and nitrogen available for growth are exhausted at the same time after the commencement of sporulation. Submerged fermentations have been mainly used to produce flowable formulations of spore crystal complex to be used as sprays.

Semisolid fermentation

Medium consists of dextrose, yeast autolysate and a potassium source. This medium is used for propagating the culture in flasks. The production medium to produce spores and toxins contain wheat bran and solid substrate along with other nutrients. This is an aerobic fermentation with an incubation period of 36-48 hours. This fermentation gives a wettable powder or dust form which is got by just drying and grinding the bran cake. This product is more stable than flowable one but it cannot be used in the wet form since bran begins to swell when wetted.

Bacillus thuringiensis in the Field

Insecticides based on *B. thuringiensis* are used for short-term control. They do not persist from year to year. During crop spraying, it is essential to make sufficient coverage on a leaf surface. But the *B. thuringiensis* spray must be protected from inactivation by sunlight since it is sensitive to ultra-violet light. In order to avoid this, *emulsifying agents*, stickers or binders and UV protectants can be used in the formulation. Some commercial names of *B. thuringiensis* are **DIPEL**, **DOOM**.

Bacillus popilliae and *B. lentimorbus* are potent insecticides causing Milky disease in Japanese beetle. Following the ingestion of viable spores by larvae, germination takes place in the gut and the vegetative cells penetrate the gut wall and enter the body cavity where they multiply. During this time, they remove all the nutrients from the blood and lead to the death of the larva.

(b) Viruses As Insect Pests

Insect viruses are encased in protein coats which are insoluble in water. These protein crystals serve to protect the virus particles and due to the presence of these protein crystals, the infective viruses remain active even under normal storage condition for many years. Specificity of the virus-host relationship makes viruses an ideal candidate for use in the control of specific pest population. There are two major groups of insect viruses:

1. **Polyhedrosis virus**: where many virus particles are embedded in each protein crystal. When they occur in the host cell nucleus, they are called nuclear polyhedrosis virus (**NPV**), and when they occur in the host cytoplasm they are called cytoplasmic polyhedrosis virus (**CPV**).

2. **Granulosis virus:** where only one virus is contained in each protein crystal. They either develop in the nucleus or cytoplasm of the host cells (NGV or CGV). NPV and CGV come under the group of **Baculoviruses** and they are the most extensively studied insect viruses.

Infection is caused by ingestion of contaminated food containing NPV / GV followed by cell invasion beginning in the midgut. The GV are sprayed over the leaves and they enter the larvae feeding on the foliage. The viruses multiply fast in the larvae and make them lethargic. Affected larvae become sluggish and stop feeding and die. For example, NPV controls the pest *Helicoverpa armigera*. The affected larvae hang upside down from the leaves and twigs in a characteristic way and a brownish fluid oozes from them. This is a highly infective fluid and is readily disseminated amongst the healthy insect population. The spread takes place by wind, rain, etc. **TAIVIRIDAE** is a **commercial NPV** pesticide used to control **cotton pest bollworm** and **budworm**.

Production:

The specificity and obligate parasitism of insect viruses makes their mass production possible only on live insects, e.g. production of bollworm-budworm NPV (*Baculovirus heliothis*). Bollworm larvae are raised on a semi-synthetic diet containing a water-based mixture of casein, sucrose, wheat germ, growth factors, etc. Agar is used to solidify the diet. Chemicals like formalin, sorbic acid, aureomycin are used to inhibit bacteria, yeasts and fungi. The diet is dispersed hot from a large tank to plastic trays and sealed. These are inoculated by caterpillars from an insectary and incubated under controlled conditions to produce a mass of larvae. A known volume of virus is sprayed on the diet which replicates on the caterpillars to the extent of 5000-10000 times in 5-7 days. The infected caterpillars are suctioned and then treated with water, filtered, centrifuged, precipitated and spray dried. After quality control testing, the preparation is packaged and sold.

(c) Fungal Pesticides

Insect mycoses are caused by Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes, e.g. *Beauveria*, *Metarrhizium*, *Entomophthora*, *Hirsutella*.

Biological Control Agents

Trichoderma is a cosmopolitan biological control agent which is a saprophyte.

- ❖ It has rapid growth rate.
- ❖ It sporulates abundantly.
- ❖ It produces antibiotics (**gliotoxin** and **viridian**).
- ❖ It is a well-known antagonist.

Commercial product **BINABTSEEPIC** can be easily applied in soil in the form of granules for control of soil-borne diseases.

Fungi used as insecticides include the following:

1. ***Beauveria bassiana*:** This common soil fungus has a broad host range that includes many beetles and fire ants. It infects both larvae and adults of many species. One or more companies currently are developing preparations of this fungus for EPA registration. Understanding the interactions between *Beauveria bassiana* and other soil microorganisms may be the key to successful use of this fungus.

2. *Nomuraea rileyi*: In soybeans, naturally occurring epidemic infections of *Nomuraea rileyi* cause dramatic reductions in populations of foliage-feeding caterpillars.
3. *Vericillium lecanii* : This fungus (once sold under the trade name Vertelec®) has been used in greenhouses to control aphids and whiteflies.
4. *Lagenidium giganteum*: This aquatic fungus is highly infectious to larvae of several mosquito genera. It cycles effectively in the aquatic environment (spores produced in infected larvae persist and infect larvae of subsequent generations), even when mosquito density is low.
5. *Hirsutella thompsonii*: Although preparations of this pathogen were once registered by the US EPA and marketed under the trade name Mycar®, it is no longer available commercially. *Hirsutella thompsonii* is a pathogen of the citrus rust mite.

Some examples of fungal agents

Trichoderma harzianum is successfully used as a bio-control agent for diseases caused by *Rhizoctonia solanii* on beans and tomato. *T. harzianum* is the most active antagonistic agent in the case of *Fusarium* species.

Control of Airborne Diseases

Botrytis cinerea was controlled when treated with *Trichoderma*. Their colonisation prevented the saprophytic establishment of *B. cinerea* on the vine (hyperparasitism). Their establishment on the floral parts limit the development of disease on the branches. Spore suspension of *Trichoderma* decrease the number of rust pustules caused by *Puccinia*.

Seed-borne diseases

T. harzianum and *T. koningii* when applied to pea seeds resulted in reduction in the incidence of pre-emergence of damping off of the pea.

Fruit diseases

Botrytis rot of strawberry was controlled with *Trichoderma*. Anthracnose by *Colletotrichum* was also effectively controlled by *Trichoderma sp.*

Mechanism of Control

Antibiotics may play a crucial role in microbial antagonism. Lytic enzymes like chitinase and β -1-3, glucanase also help in controlling the diseases.

Mode of Application

Mycelial preparation of *T. harzianum* is more effective in comparison to spore preparation of biological control caused by *Sclerotinia* spp. Wheat bran/ peat is colonised by *Trichoderma sp.* A liquid medium is used to inoculate the bran/ corn meal along with other nutrient ingredients like glucose, sodium chloride, calcium carbonate and soybean meal.

Semi-solid fermentation is preferable for most of the fungi. Since the dry conidia bran mixture is relatively stable. Embedding propagules of the bio-control agents in a matrix formed by sodium alginate (binder) and clay appear to be an effective method of delivery of *T. viridae*. It can also be applied as a seed coating with adhesives. **commercial products** available are Antagon, Biocure-F, Bioderma, Trichocan, etc. Those commercial formulations can be introduced into organic manures, composts, dry cow dung, farmyard manure, neem cake etc.

Fill in the Blank Questions based on the above contents:

1. Biological control involves reducing inoculum density or disease-producing activities of a pathogen by one or more _____.
2. Biocontrol is based on antagonism, including competition, parasitism, and _____.
3. _____ is the law that emphasizes restricting insect populations rather than eradicating them.
4. Microbial insecticides can be categorized into (a) bacterial pesticides, (b) viral pesticides, and (c) _____ pesticides.
5. An effective microbial pesticide should be specific to the pest population, harmless to humans and other valued organisms, and possess resistant stages such as _____ or cysts.
6. *Bacillus thuringiensis* produces protein crystal inclusion bodies known as _____ bodies, which act as endotoxins.
7. In insect viruses, Polyhedrosis viruses embed many virus particles in each protein crystal, while Granulosis viruses contain _____ virus in each crystal.
8. In the context of Integrated Pest Management (IPM), what does IPM stand for? _____ Pest Management.
9. *Trichoderma harzianum* is a biological control agent used for diseases caused by _____ *solanii* on beans and tomatoes.
10. The mode of application for mycelial preparation of *Trichoderma harzianum* is more effective than spore preparation in controlling diseases caused by _____ spp.

Answer:

1. Organisms
2. Hyperparasitism
3. Integrated pest management (ipm)
4. Fungal
5. Spores
6. Parasporal
7. One
8. Integrated
9. *Rhizoctonia*
10. *Sclerotinia*

Multiple Choice Questions:

1. Biological control is based on antagonism, which includes:
 - a) Symbiosis
 - b) Competition, parasitism, and hyperparasitism
 - c) Mutualism
 - d) Commensalism
2. What is the primary characteristic of an effective microbial pesticide?
 - a) High cost
 - b) Broad-spectrum toxicity
 - c) Harmful to non-pest populations
 - d) Specificity to the pest population and harmlessness to humans and valued organisms
3. Which bacterial pathogen has been most extensively studied as an insecticide?
 - a) *Bacillus cereus*
 - b) *Clostridium botulinum*
 - c) *Bacillus thuringiensis*
 - d) *Pseudomonas aeruginosa*
4. Insect viruses are categorized into:
 - a) Polyhedrosis and Granulosis viruses
 - b) Retroviruses and Coronaviruses
 - c) Adenoviruses and Herpesviruses
 - d) Lytic and Lysogenic viruses
5. What is the law that emphasizes restricting insect populations rather than eradicating them?
 - a) Eradication Law
 - b) Pest Control Law
 - c) Integrated Pest Management (IPM)
 - d) Insect Limitation Law
6. Which fungal pesticide is used as a bio-control agent for diseases caused by *Rhizoctonia solanii* on beans and tomatoes?
 - a) Beauveria
 - b) Metarrhizium
 - c) Entomophthora
 - d) Trichoderma harzianum
7. How do Polyhedrosis viruses differ from Granulosis viruses?
 - a) Polyhedrosis viruses contain one virus in each crystal, while Granulosis viruses embed many virus particles.
 - b) Granulosis viruses contain one virus in each crystal, while Polyhedrosis viruses embed many virus particles.
 - c) Both Polyhedrosis and Granulosis viruses contain many virus particles in each crystal.
 - d) Both Polyhedrosis and Granulosis viruses contain one virus in each crystal.
8. What is the primary mode of application for mycelial preparation of *Trichoderma harzianum*?

- a) Submerged fermentation
 - b) Spray application
 - c) Seed coating
 - d) Semisolid fermentation
9. Which commercial product is an example of a mycelial preparation of *Trichoderma harzianum*?
- a) DIPEL
 - b) DOOM
 - c) ANTAGON
 - d) TAIIVIRIDAE
10. What is the primary advantage of using fungal pesticides in biological control?
- a) Broad-spectrum toxicity
 - b) Rapid growth rate
 - c) Harmfulness to humans
 - d) Specificity and obligate parasitism

Answers:

- 1) Competition, parasitism, and hyperparasitism
- 2) Specificity to the pest population and harmlessness to humans and valued organisms
- 3) c) *Bacillus thuringiensis*
- 4) Polyhedrosis and Granulosis viruses
- 5) Integrated Pest Management (IPM)
- 6) *Trichoderma harzianum*
- 7) b) Granulosis viruses contain one virus in each crystal, while Polyhedrosis viruses embed many virus particles.
- 8) Semisolid fermentation
- 9) c) ANTAGON
- 10) d) Specificity and obligate parasitism

True or False type Question:

Sl. No.	True or False statement	Answer
1)	Biological control involves the use of chemicals to eliminate pests	False.
2)	The negative interactions among microbial populations are not relevant to biological control	False
3)	<i>Bacillus thuringiensis</i> is a bacterial pathogen extensively studied as an insecticide, primarily due to its resistance to environmental conditions	False
4)	Insect viruses, specifically Polyhedrosis and Granulosis viruses, are encased in protein coats that are soluble in water	False.
5)	<i>Trichoderma harzianum</i> is a fungal biological control agent known for its slow growth rate and inability to produce antibiotics	False
6)	Integrated Pest Management (IPM) aims to eradicate insect populations completely	False
7)	The specificity and obligate parasitism of insect viruses make their mass production feasible only on live insects	True
8)	Antibiotics play a crucial role in microbial antagonism, aiding in the control of diseases	True
9)	Microbial control in agriculture is significant for its potential harm to humans and other valued plant and animal populations	False
10)	Biological control relies on negative interactions, such as amensalism, predation, and parasitism, for managing pests and diseases.	True
11)	<i>Bacillus thuringiensis</i> produces parasporal bodies that act as exotoxins, causing gut paralysis in pests.	False (endotoxins)
12)	Integrated Pest Management (IPM) focuses on eradicating insect populations to ensure agricultural productivity.	False (restricting insect populations)
13)	<i>Trichoderma harzianum</i> is a bacterial biological control agent effective against diseases caused by Rhizoctonia	False (fungus)
14)	Insect viruses, specifically Polyhedrosis and Granulosis viruses, are encapsulated in protein crystals that protect them and remain active for many years.	True
15)	The specificity of viruses makes them ideal candidates for the control of specific pest populations.	True
16)	Microbial insecticides should not be specific for the pest population, allowing a broader range of control.	False (specific for the pest population)
17)	<i>Azolla pinnata</i> is a bacterium commonly found in ponds, ditches, and rice fields, used as a biofertilizer for rice.	False (water fern)
18)	The role of <i>Trichoderma harzianum</i> as a bio-control agent includes rapid growth, abundant sporulation, and production of antibiotics like gliotoxin and viridian.	True
19)	The microbial pesticide <i>Bacillus thuringiensis</i> remains active from year to year and persists in the environment.	False

Short Type Questions:

1. Define Biological Control.
2. What are the three negative interactions that form the basis for biological control?
3. Explain the principles of Biocontrol.
4. Name the three categories of microbial insecticides.
5. What are the characteristics of an effective microbial pesticide?
6. Describe the role of parasporal bodies in *Bacillus thuringiensis*.
7. Differentiate between Polyhedrosis and Granulosis viruses.
8. What does IPM stand for, and what is its main emphasis?
9. Which bacterial pathogen has been extensively studied as an insecticide, and what is its primary toxic factor?
10. How does *Trichoderma harzianum* act as a bio-control agent?

Long Type Question:

1. Explain the role of microbial agents in biological control, detailing the categories of microbial insecticides, their characteristics, and the principles underlying their use. Provide examples and discuss the significance of microbial control in agriculture.

Answer:

Biological control is a method of managing pests and diseases using microorganisms to initiate disease in pest populations, thereby reducing the reliance on chemical pesticides. The negative interactions among microbial populations and between microbes and higher organisms serve as the basis for biological control. There are three main categories of microbial insecticides: bacterial pesticides, viral pesticides, and fungal pesticides.

An effective microbial pesticide should be specific to the pest population, survive environmental conditions, possess resistant stages like spores, and be harmless to humans and valued organisms. *Bacillus thuringiensis*, a bacterium, has been extensively studied. Its parasporal bodies act as endotoxins, causing gut paralysis in pests.

Insect viruses, categorized into Polyhedrosis and Granulosis viruses, are encased in protein crystals that protect them. They cause infections when ingested by pests, leading to lethargy and death. Integrated Pest Management (IPM) emphasizes restricting insect populations rather than eradication. *Trichoderma harzianum*, a fungal biological control agent, is effective against diseases caused by *Rhizoctonia solanii*. It competes, produces antibiotics, and acts as an antagonist, providing a sustainable approach to disease management.

Microbial control in agriculture is significant as it reduces the need for chemical pesticides, minimizes environmental impact, and helps maintain pest populations below economically damaging levels. This approach aligns with sustainable and environmentally friendly agricultural practices.

Biofertilizers - Introduction, status and scope

Dr. AJEET KUMAR⁹

Introduction

Indian economy is an agrarian economy, a reason that makes biofertilizers an essential need for Indian farmers. Though most of our agricultural activities are dependent on monsoon, the use of a good biofertilizer can yield a better crop to the farmers and increase the fertility of the soil. According to an estimate 240 million tons of food grains will be required to feed about one billion expected populations by 2000 AD in India and to achieve this milestone, a sizable quantity of mineral fertilizer will be required. The total fertilizer requirements of our country would be 23 million tonnes as against the present consumption level of 13 million tonnes, which is beyond any single type of nutrient source to accept the challenge of appropriate nutrient supply. Integrated use of all the sources such as mineral fertilizers, organic manures, biofertilizers etc. is the only alternate for improving soil fertility. It has now become possible to meet a large part of our total nitrogen demand through proper husbandry of biological nitrogen fixation by micro-organism in crop production system. Biofertilizers are capable of providing an economically liable level for achieving the ultimate goal of enhanced productivity. The crop microbial soil ecosystem can, therefore, be energized in sustainable agriculture.

India is fourth largest user of chemical fertilizers (12.5million tones of NPK nutrients) in the world even its soils are still being depleted of their inherent nutrients reserve as a result of wide gap between additions (12.5 million tonnes) and removal (18.5 million tones). One tone of produce removes an average of 32 kg nitrogen, 12 kg phosphorus and 58 kg potash. The strategy for sustaining satisfactory yield levels envisages nutrient balance and efficient nutrient cycling. This can be successfully achieved through integrated use of mineral fertilizer, bulky organic manures, compost, green manure, biological inoculant etc. At present, the nutrient use in India is much less compared to that of other countries. Against world average consumption of 95 kg ha⁻¹, and we are using 74 kg ha⁻¹ with national productivity level of merely 1.1 metric ton per hectare. At present India produces about 117 million metric tones of food grain for its growing population of more than 800 million people. Nitrogen fertilizer plays prominent role in increasing this food requirement. At present the vast gap cannot be filled up merely through the production of synthetic nitrogen fertilizers due to scarcity of high cost of raw materials such as fossil fuels. Biological nitrogen fixation is the key to sustain agriculture productivity application of biofertilizer in the field is the liable alternative. In fertilizer manufacturing factories, nitrogen is fixed industrially, by means of the Haber Bosch process requiring H₂ gas, very high temperature and enormous energy. Industrially fixed nitrogen has been used precipitously. It was produced 4.0 lakh metric tones in 1905 and this was increased to 3.5 million tones of nitrogen fertilizer. However, above every

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hectare of land, there are 78,000 tonnes of inert nitrogen gas (N₂). As nitrogen is the most limiting nutrient for increasing crop productivity. Only a few prokaryotic organisms are able to fix nitrogen directly through a biological process. Annual biological nitrogen fixation (BNF) is estimated to be around 175 million tones of which close to 79 % is accounted for by terrestrial fixation

The term biofertilizers or which can be more appropriately called ‘microbial inoculants’ can be generally defined as a preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms used for application of seed, soil or composting areas with the objective of increasing the number of such microorganisms and accelerate certain microbial process to argument the extent of the availability of nutrients in a form which can be easily assimilated by plant. In large sense, the term may be used to include all organic resources for plant growth, which are rendered in an available form for plant absorption through microorganisms or plant associations or interactions. Soil harbours a range of microorganisms, which bring about a number of biochemical reactions in soil. The soil organisms are classified into two broad groups i.e. soil flora and soil fauna, they are again subdivided, depending upon their size such as micro and macro flora. Soil microflora includes bacteria, fungi, actinomycetes, algae etc. of these groups, bacteria are the most abundant followed by actinomycetes and fungi; algae are found under specific situation. The biomass and population of these micro-organisms in soil have been given in Table 1.

Sr. No	Microorganism	Average number per g of soil (in lakhs)	Average biomass (in kg per ha)
1.	Bacteria	1000	500
2.	Fungi	10	1000
3.	Actinomycetes	100	750
4.	Algae	0.01	150

The presence of these microorganisms make the soil a living and active system. These microorganisms play a significant role in the life cycle of plants and animals through a number of process such as decomposition, solubilization, nutrient fixation and supply of plants. At present, in India there is a gap of about 10 million tones of plant nutrients between removal by crops and replenishment through fertilizers. Supply of nutrients from organic manure has not so far been able to fill up this gap and also in years to come nutrient supply form the source is unlikely to improve due to competitive demand for alternate uses like fuel and fodder. Of late, biofertilizer are being promoted as an important component in supplementing plant nutrient need of the country. India is one of the important countries in biofertilizer production and consumption in the world. The present production capacity of different production units in the country is about 4500 tonnes per annum. The maximum production capacity is in Agro Industries Corporation followed by State Agriculture Departments, National Biofertilizer Development Centre, State Agriculture Universities and private sector. Among the different states, the maximum production capacity is in Tamil Nadu followed by M.P. U.P., Gujarat and Maharashtra.

Thrust in Research and Development:

The rising cost of chemically fixed nitrogen fertilizer and the massive inputs of non-renewable resource will be devastating. As an alternative there is urgency of major research effort in biological nitrogen fixation. Nitrogen in the form of ammonia is used by plants and microbes as a building block for the synthesis of amino acids and of other nitrogenous compounds. The conversion of atmospheric Nitrogen into ammonia by the nitrogen fixing microbes, mostly certain bacteria and blue green algae is called biological nitrogen fixation. Although leguminous plants have been used extensively in agriculture for centuries, it is possible to exploit these nitrogen fixing plants meaningfully. The use of new leguminous crops as well as the breeding of more vigorous and effective traditional leguminous crops can be helpful in food production. As the bacteria are the agents of fixation in bacterial plant Rhizobial technology and soil microbiology are the neglected areas. It would be a right type technology that all farmers could use legume – Rhizobial combinations constitute, a built-in nitrogen source for food crop production. Approximately 75 per cent of the nitrogen utilized by legume comes from the soil, with only 25 per cent from BNF. It is necessary to increase the rate of nitrogen fixation achieved, which would have a multiplier effect on the enhancement of crop productivity. Chemical fertilizers have temporary effect while biofertilizers have permanent effect without any production problem. Use of composite biofertilizers has permanent effect without any production problem. Use of composite biofertilizers can increase soil fertility. The cost is too much low for the biofertilizers and its proper use dose not only include a correct application of the inoculant to the seed soil but also good. Considering the prospects of biofertilizers in the country, the biofertilizers development centres are being established both in government and private sectors and It is only possible by establishing joint venture in biofertilizer agro based industry.

Biofertilizers are products that contain microorganisms essential for soil fertility and plant growth when added to the soil. A biofertilizer is a chemical that contains living microorganisms that colonize the rhizosphere or the interior of the plant when given to seeds, plant surfaces, or soil, and encourage growth by controlling the quantity or availability of primary nutrients to the plant host. Biofertilizers supply nutrients to plants through natural processes such as nitrogen fixation, phosphorus solubilization, and the creation of growth-promoting chemicals. They help restore the soil's natural nutrient cycle and increase soil organic matter. Healthy plants can be developed with the application of biofertilizers while also improving the soil's sustainability and health. Biofertilizers will likely decrease the need for synthetic fertilizers and pesticides, but they will not be able to completely replace them.

Importance of Biofertilizers

There are various uses of biofertilizers that prove their importance. They include – improving the soil quality, protecting the plants from pathogens, avoiding environmental pollution, destruction of harmful substances presents in the soil, etc. Thus, biofertilizers are very important.

Types of Biofertilizers

1. Symbiotic Nitrogen-Fixing Bacteria

The symbiotic nitrogen-fixing bacteria like Rhizobium get food and shelter from the plants and provide them with fixed nitrogen in return. One of the most important symbiotic nitrogen-fixing bacteria is Rhizobium. Bacteria seek shelter and food from plants here. In exchange, they assist the plants by delivering free nitrogen.

2. A Loose Association of Nitrogen-Fixing Bacteria

Some bacteria aren't related directly with the plants but live around them. For example, A nitrogen-fixing bacterium called Azospirillum lives near the roots of higher plants but does not form a close bond with them. This is known as rhizosphere association because these bacteria collect plant exudate and use it as food. Associative mutualism is the name given to this phenomenon.

3. Symbiotic Nitrogen-Fixing Cyanobacteria

There are many symbiotic nitrogen-fixing cyanobacteria like liverworts, cycad roots, the bacteria released by fern plant decay, etc. Cyanobacteria or blue-green algae form a symbiotic relationship with numerous plants. Anabaena can be found in the fern's leaf cavities. It's in charge of nitrogen fixation. The fern plants decompose and release nutrients for the rice plants to use. Azolla pinnata is a fern that grows in rice fields.

4. Free-Living Nitrogen-Fixing Bacteria

Free-living bacteria are found in soil, and they also perform nitrogen fixation. These include clostridium, Azotobacter, and bacillus polymyxin. They are nitrogen-fixing bacteria that live in free-living soil. *Clostridium beijerinckii*, *Azotobacter*, and other saprotrophic anaerobes are among them. Rhizobium and Azospirillum are the most extensively utilized forms of biofertilizers.

Components of Biofertilizers

The components of a biofertilizer are:

1. Bio Compost:

It is eco-friendly and is produced from waste products coming from sugar industry. It also includes bacteria, fungi, and some plants.

2. Tricho-Card:

This eco-friendly non-pathogenic product is useful for many crops and plants, as it plays the role of a productive destroyer against the items that are harmful to the crop.

3. Azotobacter:

It plays an important role in atmospheric nitrogen fixation and protects the plant roots from pathogens in the soil.

4. Phosphorus:

To settle the exact level of need for nitrogen for a plant, and to determine the nitrogen level of the soil, phosphorus fertilizers are very helpful.

5. Vermicompost:

Known for quickly improving soil fertility, these are probably the most eco-friendly fertilizers that contain vitamins, sulphur, hormones, organic carbon, and antibiotics required for the growth of the plant.

Applications of Biofertilizers

These are some important applications of biofertilizers:

1. **Seedling Root Dip:** Used for rice crops, the seedlings are planted in a waterbed for 8 to 10 hours, in this method.
2. **Seed Treatment:** In this process, the seeds are dipped in nitrogen-phosphorus mixed fertilizers. After drying them, they are planted as early as possible.
3. **Soil Treatment:** The mixture of biofertilizers and compost fertilizers is kept overnight and spread over the soil the next day. This treatment takes place before sowing the seeds.

Disadvantages of Fertilisers

- Chemical fertilizers are supplemented by biofertilizers, not substituted for them.
- Biofertilizers only improve crop productivity by 20 to 30 percent. Unlike chemical fertilizers, they do not result in a significant improvement in productivity.
- For specific crops, specific fertilizers are necessary. This is more applicable to microorganisms that live in a symbiotic relationship. If non-specific Rhizobium is applied as a fertilizer, root nodulation, and crop production will not rise.
- Strict aseptic precautions are required during the manufacture of microbial fertilizer. During microbial mass manufacturing, contamination is a common problem.
- Microbes are killed when exposed to sunlight for an extended period of time because they are light-sensitive.
- When stored at room temperature, microbial fertilizers must be used within six months, and when stored at chilling temperature, it must be used within two years.

Instruments Used in Microbiology Lab with Principle and Uses

Dr. AJEET KUMAR¹⁰

List of Instruments used in Microbiology Lab

1. Analytical Balance
2. Autoclave
3. Bunsen burner
4. Centrifuge
5. Colony Counter
6. Deep Freezer
7. Homogenizer
8. Hot plate
9. Hot air oven
10. Incubator
11. Laminar Air Flow/ Laminar Hood
12. Magnetic Stirrer
13. Microscope
14. pH Meter
15. Spectrophotometer
16. Vortex Mixture/ Vortexer
17. Water Bath
18. Water Distiller
19. Culture tube
20. Test tube
21. Inoculation needle/rod/loop
22. Laboratory Heat Sealer
23. Vacuum pump
24. Microwave ovens
25. Lab incubators
26. Sand bath
27. Oil bath
28. Fermenter

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1. Analytical Balance

An analytical balance is a type of balance that is commonly used for the measurement of mass in the sub-milligram range.

Working Principle

- ✚ These types of balances are made with a measuring pan enclosed in a transparent covering that prevents small particles or air currents from getting collected on the pan.
- ✚ An electric analytical balance uses the force necessary to counteract the mass rather than measuring the mass itself.
- ✚ An electromagnet is used to create a force required to achieve a balance with the mass of the substance and the resulting force is displayed.

Uses

- As they are highly precise and based on advanced technology, analytical balances are explicitly used in laboratories for the effective completion of tasks like weighing test materials and sampling amounts, formulation, density determination, purity analysis, quality control testing, and material and conformance testing

2. Autoclave

An autoclave is a pressurized chamber used for the process of sterilization and disinfection by combining three factors: time, pressure, and steam

Working Principle

- ✚ Autoclaves use steam as their sterilization agent. The basic principle of an autoclave is that all the items within the autoclave come in direct contact with the steam for a particular period irrespective of the nature of the material- whether it is liquid, plastic ware, or glassware.
- ✚ The amount of time and the temperature depends on the type of material being sterilized and the increase in temperature of the cycle allows for shorter periods.

Uses

- Autoclaves are mostly used for the sterilization of medical or laboratory equipment with the capacity of sterilizing a large number of materials at once.
- They are commonly used for the preparation of culture media during laboratory applications.



3. Bunsen burner

Bunsen burner is a standard tool used in laboratories, named after Robert Bunsen. It is a gas-fueled single open flame.

Working Principle

- ✚ This burner is made with a metal tube on a flat base with a gas inlet at the bottom of the tube, which may have an adjustable valve. On the sides of the tube are openings that can be adjusted with a collar to control the amount of air that can enter.
- ✚ Once the burner is connected to a gas source, the gas is forced by the gas pressure so that the gas reaches the top where the flame is ignited with a match or a lighter.

Uses

- It is commonly used for processes like sterilization, combustion, and heating. In medical or microbiology laboratories, it is commonly used for micro-loop sterilization.

4. Centrifuge

A centrifuge is a device that allows the rotation of an object about a single axis, where an outward force is applied perpendicularly to the axis. A laboratory centrifuge is motor-based and allows the rotation of a liquid sample resulting in the separation of the components of the mixture.

Working Principle

- ✚ A centrifuge works on the principle of sedimentation, where the high speed of the rotation causes the denser particles to move away from the center while smaller, less dense particles are forced towards the center.
- ✚ Thus, the denser particles settle at the bottom while the lighter particles are collected at the top.
- ✚ In a laboratory tabletop centrifuge, the sample tubes are aligned at an angle so that the particles have to travel a shorter distance before they hit the bottom.

Uses

- The primary application of a centrifuge is the separation of particles suspended in a suspension. It can be used for the separation of cell organelles, nucleic acid, blood components, and separation of isotopes.

5. Colony Counter

A colony counter is used to estimate the density of a liquid culture by counting the number of **CFU (colony forming units)** on an agar or culture plates. It is used for counting microorganism which is not visible by naked eye. The magnifying glass (convex lens) is fitted in this equipment.

Working Principle

- ✚ This instrument can accommodate different sizes of plates which are scanned on top with UV, white light and/or fluorescent illumination.
- ✚ One can accomplish the counting either manually with the touch pressure or with a digital counter.

Uses

- A colony counter is primarily used for counting the number of colonies present on a culture plate to estimate the concentration of microorganisms in liquid culture.

6. Deep Freezer

Working Principle

- ✚ Deep freezers are based on the principle that under extremely low temperatures, there is **minimum microbial growth** which allows for the protection and preservation.
- ✚ Based on this principle, we can even preserve cultures over a long period of time without any change in the concentration of the microorganisms.

Uses

- A deep freeze can be used for the preservation of different things used in the laboratories for a very long period of time. Deep freezers are used in laboratories to store and preserve isolated microbes for a more extended period of time.

7. Homogenizer

Homogenizer is a device used in laboratories for the mixing of various liquids and materials like tissue, plant, food, soil, and many others.

Working Principle

- ✚ This instrument is based on the principle that when large globules in coarse emulsion are passed under high pressure through a narrow orifice, they break down into smaller particles giving a more uniform and stable mixture.
- ✚ A homogenizer has a metal rod with narrow parallel openings in the form of a comb at the end which acts as the orifice for the homogenization process.

Uses

- A homogenizer is primarily used to disrupt cells to acquire cell organelles for different microbiological processes.
- It is used in the preparation step before the extraction and purification of different macromolecules like proteins, nucleic acids, and lipids.

8. Hot plate

A hot plate is a stand-alone appliance used in microbiology laboratories as a table-top heating system.

Working Principle

- ✚ Unlike the traditional ways of producing heat through fire, a hot plate produces heat by the flow of electricity.
- ✚ On a hot plate, electricity runs through the coils which have a high level of electrical resistance. The resistance in the coils converts the electrical energy into heat energy which causes the coils to release heat.

Uses

- In a laboratory, hot plates are used to heat glassware and its components.
- They are used over water baths as water baths might be hazardous in case of any spills or overheating.

9. Hot air oven

A hot air oven is an electrical device that is used for sterilization of equipment, dried glassware or samples using dry heat.

Working Principle

- + A hot air oven is a type of dry heat sterilization which is performed on dry materials and on substances that do not melt or catch fire under high temperatures.
- + There are **two types of hot air ovens** based on the working principle
- + **Forced air hot air oven:** In this type of hot air oven, the heated air inside the oven is distributed throughout the oven with a fan. This prevents the rising of hot air towards the top while keeping the cold air at the bottom. This allows for the adequate heating of materials inside the oven.
- + **Static air hot air oven:** In this type of oven, the heat is produced by coils present at the bottom of the oven with no fan. The hot air rises and doesn't allow the effective sterilization of the materials.
- + The equipment inside the oven acquires heat and passes the heat towards the center, one layer at a time which allows for effective dry heat sterilization.

Uses

- A hot air oven can be used to sterilize materials like glassware, metal equipment, powders, etc.
- It allows for the destruction of microorganisms as well as bacterial spores.



10. Incubator

An incubator is a device used in laboratories for the growth and maintenance of microorganisms and cultures. Incubator provides an optimal temperature, pressure, moisture, among other things required for the growth of microorganisms.

Working Principle

- ✚ The incubator is based on the principle of maintaining a proper atmosphere for the growth of microorganisms.
- ✚ Incubators have a heating system that allows for the temperature within the incubator to be adjusted according to the type of organism allowed to grow inside.
- ✚ Similarly, they are provided with adjustments for maintaining the concentration of CO₂ to balance the pH and humidity required for the growth of the organisms.
- ✚ Variation of the incubator like a **shaking incubator**, which allows for the continuous movement of the culture required for cell aeration and solubility studies.

Uses

- Incubators have a wide range of applications including cell culture, pharmaceutical studies, hematological studies, and biochemical studies.
- Incubators can also be used in the stem cell research area.

11. Laminar Air Flow/ Laminar Hood

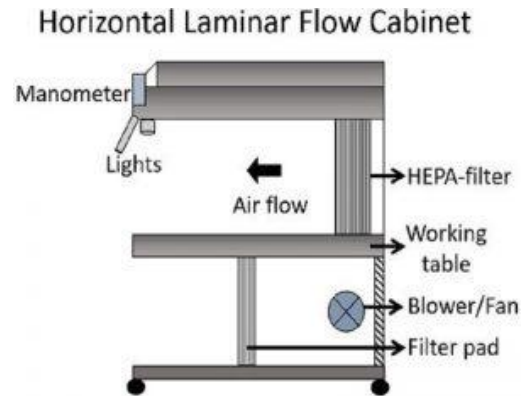
Laminar Hood is a closed device primarily for processes or instruments sensitive to microbial contamination.

Working Principle

- ✚ A Laminar Hood is made up of stainless steel, avoiding joints and corners to prevent the accumulation of bacterial spores.
- ✚ This device creates a sterile environment with the flow of sterile air through a **High-Efficiency Particulate Air (HEPA) filter** and shortwave **ultraviolet germicidal lamp** that sterilizes the workstation.
- ✚ Laminar Air Flow has to **turn on 15 minutes before to ensure complete sterilization** and the workstation should be **cleaned with ethanol** before and after use.

Uses

- Laminar Hood is used to conduct processes that are sensitive to contamination.
- It is used for experiments related to isolation of microorganism, plant tissue culture and for the experiments of genetic transformation.



12. Magnetic Stirrer/culture tube stirrer

Magnetic Stirrer is a device used in microbiology laboratories for the purpose of mixing the liquids/ or the material which is present in culture tube.

Working Principle

- ✚ This device consists of a rotating magnetic or an electromagnet creating a rotating magnetic field that allows the stir bar (a piece of heavy metal) to move around in the vessel.
- ✚ It is **coupled with a heating system** to heat the liquid while it is mixed.

Uses

- It is usually used for mixing various liquid components in a mixture in a chemical or microbiology laboratory.
- This device is used in place of other stirrers as it is noise-free and because the size of the stir bar is so tiny, there is less chance of contamination.



13. Microscope

Microscopes are devices that allow the observer to have an exceedingly close view of minute particles.

Working Principle

- ✚ There are different types of microscopes, each of which works on its respective principles. However, there is some commonality in them.
- ✚ The basic principle in a microscope is magnification. Based on the relative position of the object from the lens or electromagnets, different positions, nature, and magnification of the image can be achieved.
- ✚ Different types of microscopes are developed to cater to the specific needs of the observation. However, the common theme is magnification.

Uses

- Based on the type of microscopes, different microscopes are used for different purposes.
- They are primarily used for the observation of minute particles which cannot be observed with naked eyes.

14. pH Meter

A pH meter is a device used in laboratories that measure the H-ion concentration in water-based solutions to determine the acidity or alkalinity of the solution. A **pH meter** is **often termed** a “**potentiometric pH meter**” as it **measures the difference** in electric potential between the **reference and a pH electrode**.

Working Principle

- ✚ In a potentiometric pH meter, single or multiple glass electrodes, connected to a bulb selective to hydrogen ions, are attached to a metal rod.
- ✚ When the bulb with the electrodes is dipped into a solution, hydrogen ions in the solution exchange with positive charges on the electrode generating an electrochemical potential which is displayed in terms of pH units on display.

Uses

- A pH meter is primarily used to measure the acidity of microbial cultures, soil, and water treatment plant.
- It can be used to measure the acidity level in wine and cheese during their production.

15. Spectrophotometer

The spectrophotometer is an optical instrument for measuring the intensity of light in relation to wavelength. Based on the amount of light absorbed by a coloured solution, a quantitative analysis of the solution can be done.

Working Principle

- ✚ Spectrophotometry is based on the **Beer-Lambert Law**, which states the absorbance of light by a solution (of a particular wavelength) is directly proportional to the concentration of the substance.
- ✚ Different wavelengths of light are passed through a solution as different substances have better absorbance at different wavelengths. Based on the absorbance of a particular wavelength, the quantitative analysis of a solution can be done.

Uses

- In a microbiology laboratory, a spectrophotometer is applied for the measurement of the substance concentration of protein, nucleic acids, bacterial growth, and enzymatic reactions.

16. Vortex Mixture/ Vortexer

A vortex mixture is one of the basic technologies used for the **mixing of samples in glass tubes or flasks** in laboratories.

Working Principle

- ✚ It is based on the simple principle of causing reactions and homogenization by agitating the mixture.
- ✚ Motorized draft shafts present on the mixer oscillate and transfer the movement to the sample tubes causing the sample fluids to undergo turbulent flow.

Uses

- Vortex mixer is mostly used for the mixing of various sample fluids in the sample tubes and also allows for the homogenization of cells and cell organelles.

17. Water Bath

Water Bath is a conventional device that is used for chemical reactions that required a controlled environment at a constant temperature.

Working Principle

- ✚ A sensor in the device transfers water temperature to a reference value which is then amplified and a control system generates a signal for the heating system which heats the water to the desired temperature.

Uses

- Water baths are primarily used for heating samples under a controlled temperature.
- These are suitable for heating chemicals that might be flammable under direct ignition.



18. Water Distiller

A water distiller is a device that purifies water by the process of distillation. This is commonly used in, microbiology laboratories, organic chemistry laboratories and medical laboratories.

Working Principle

- ✚ A water distiller is based on the principle of distillation.

- ✚ According to this process, water is first brought to a boil and then condensed into liquid form to obtain pure distilled water.

Uses

- It is used to obtain distilled water required for lab tests as well as for the preparation of culture media.

19. Culture Tube

Culture tubes are often used in microbiology lab for handling and culturing all kinds of live organisms, such as molds, bacteria, seedlings, plant cuttings, etc. it is also used to store samples and other fluids. Mouth of tube is plain cut because rounded mouth causes problems in removing cotton plug of tube.



20. Test Tube

Test tubes are widely used by chemists to handle chemicals, especially for qualitative experiments and assays. Their spherical bottom reduces mass loss when pouring, make them easier to wash out, and allow convenient monitoring of the contents.



21. Inoculation needle/rod/loop

An inoculation needle is equipment used in the lab of microbiology. It is used in the transfer of microbial organisms from plate culture to needle by first sterilizing the needle to prevent contaminations. Inoculation needle is made up of **Nichrome alloy** {**Nickel, Chromium, Manganese & Fe**} which is heat resistant.

An inoculation loop, also known as a **smear loop** is a simple tool, primarily used by microbiologists **to take and transfer inoculum of a microorganism culture.**

22. Laboratory Heat Sealer

This instrument is used to seal the **pouches, plastic bags, sachets** that are used in packaging of biofertilizer. The apparatus is used to ensure the intactness or band of the plastic bags and other packaging bags that are used to pack different consumable as well as non-consumable goods.

23. Vacuum pump

Vacuum pumps are mechanical devices that enable the removal of air and gas molecules from a sealed area to create an area devoid of air or gas, their purpose is to clean and seal. Vacuum pumps come in wet or dry variants depending on the media being pumped through them.

24. Microwave ovens

Microwave ovens are used for **heating and defrosting in laboratories**. Improper use of a microwave can pose a number of hazards including: Ignition of flammable vapors. Electrical shock from ungrounded or faulty units. Microwave ovens used in laboratories for the rapid heating of material-either to dry them completely or to subject a work piece to sudden thermal stress or electric field stress. **Determinations of the moisture content in soil or leaf tissue samples**, for example, can be made **within tens of seconds** rather than hours. It is often assumed that placing a load within a microwave oven will result in it being heated evenly as well as quickly.

25. BOD incubators

BOD (Biological Oxygen Demand) incubators are essential equipment in the laboratory that provides a temperature-controlled environment to support growth of microbiological cultures. **Here the materials kept in desired temperature for desired period of time.**

26. Sand bath

A sand bath is common laboratory equipment made from a container filled with heated sand. It is used **to provide even heating for another container during a chemical reaction.**



27. Oil bath

An oil bath is a type of heated bath used in a laboratory, most commonly used **to heat up chemical reactions**. It's essentially a container of oil that is heated by a hot plate or (in rare cases) a Bunsen burner. It is used in laboratories for reactions that require heating temperatures up to **200 degrees Celsius**. Oil baths provide uniform heat compared to other heaters. An oil bath is **a heating device which uses boiling oil as the temperature regulator**. Since different oils have different boiling points, it is possible to obtain a temperature near to the desired temperature by selecting oil with a boiling point as close as possible to the desired temperature.



28. Fermenter

A fermenter is the equipment, which provides the proper environment for the growth of a desired organism. It is generally a large vessel in which, the organism may be kept at the required temperature, pH, dissolved oxygen concentration and substrate concentration. Different models of fermenters are available depending upon the necessity. A simple version model contains steam generator, sterilization process devices and agitator. A sophisticated fermenter contains pH regulator, oxygen level regulator, anti-foam device, temperature controller, etc.



True or False type questions with Answer:

1. **True or False:** An analytical balance measures mass by counteracting the mass with an electromagnet.
 - **Answer:** True
2. **True or False:** Autoclaves use dry heat to sterilize equipment in laboratories.
 - **Answer:** False (They use steam for sterilization)
3. **True or False:** A centrifuge works on the principle of sedimentation.
 - **Answer:** True
4. **True or False:** A deep freezer is used to sterilize laboratory equipment.
 - **Answer:** False (It is used to preserve samples by maintaining extremely low temperatures)
5. **True or False:** A homogenizer is used to heat glassware in microbiology laboratories.
 - **Answer:** False (It is used to mix and homogenize materials)
6. **True or False:** A Bunsen burner is commonly used for micro-loop sterilization in microbiology laboratories.
 - **Answer:** True
7. **True or False:** A hot air oven uses moist heat to sterilize equipment.
 - **Answer:** False (It uses dry heat for sterilization)
8. **True or False:** The principle of a spectrophotometer is based on the Beer-Lambert Law.
 - **Answer:** True
9. **True or False:** Laminar Air Flow provides a sterile environment using a HEPA filter.
 - **Answer:** True
10. **True or False:** A pH meter measures the temperature of a solution.
 - **Answer:** False (It measures the hydrogen-ion concentration or pH of a solution)

Structure and Characteristic Features of Different types of Biofertilizers

Dr. AJEET KUMAR¹¹

Bio-Fertilizers

Bio-fertilizers are defined as preparations containing active (living cells) or latent cells of efficient strains of certain microorganisms that help crop plants to utilize the atmospheric nitrogen to increase the nitrogen content of soil, and can dissolve the insoluble phosphate of the soil to release the phosphorus in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

Processing of carrier material

The use of ideal carrier material is necessary in the production of good quality biofertilizers. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. The following points are to be considered in the selection of carrier material; Cheaper in cost, Should be locally available, High organic matter content, No toxic chemicals, Water holding capacity of more than 50%, Easy to process, friability and vulnerability.

Preparation of carrier material

The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron sieve. The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio), since the peat soil / lignite are acidic in nature (pH of 4 - 5). The neutralized carrier material is sterilized in an autoclave to eliminate the contaminants

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

S. No.	Groups	Examples
N₂ fixing Biofertilizers		
1.	Free-living	<i>Azotobacter, Beijerinckia, Clostridium, Klebsiella, Acetobacter, Anabaena, Nostoc,</i>
2.	Symbiotic	<i>Rhizobium, Frankia, Anabaena azollae</i>
3.	Associative Symbiotic	<i>Azospirillum</i>

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P Solubilizing Biofertilizers		
1.	Bacteria	<i>Bacillus megaterium</i> var. <i>phosphaticum</i> , <i>Bacillus subtilis</i> <i>Bacillus circulans</i> , <i>Pseudomonas striata</i>
2.	Fungi	<i>Penicillium</i> sp, <i>Aspergillus awamori</i>
P Mobilizing Biofertilizers		
1.	Arbuscular mycorrhiza	<i>Glomus</i> sp., <i>Gigaspora</i> sp., <i>Acaulospora</i> sp., <i>Scutellospora</i> sp. & <i>Sclerocystis</i> sp.
2.	Ectomycorrhiza	<i>Laccaria</i> sp., <i>Pisolithus</i> sp., <i>Boletus</i> sp., <i>Amanita</i> sp.
3.	Ericoid mycorrhizae	<i>Pezizella ericae</i>
4.	Orchid mycorrhiza	<i>Rhizoctonia solani</i>
Biofertilizers for Micro nutrients		
1.	Silicate and Zinc solubilizers	<i>Bacillus</i> sp.
Plant Growth Promoting Rhizobacteria		
1.	<i>Pseudomonas</i>	<i>Pseudomonas fluorescens</i>

Types of Bio-fertilizers:

There are following types of biofertilizers: Bacterial, fungal, algal, aquatic fern and earthworms

Bacterial Bio-Fertilizers

Bacterial Bio-Fertilizers

Here, live cells of bacteria are used as fertilizers. They are the principal **nitrogen fixers** in the soil and may be either free-living or symbiotic (within the nodules of legume roots, leaf nodules of certain plants and stem nodules of *Sesbania grandiflora*). Some bacterial genera can **solubilize phosphorus** from the bound form (*Bacillus*, *Pseudomonas* sp.).

Bacteria like *Agrobacterium* sp. and *Pseudomonas fluorescens* supply growth hormones like indole acetic acid to the plants. Certain groups of bacteria like the *Pseudomonas fluorescens* living in association with the rhizosphere of most of crop plants (rhizobacteria promoting plant growth) supply all the essential nutrients required for the growth of the crop and in addition, protects the plant roots from the attack by soil-borne pathogens (saprophytic suppression). The nitrogen fixing ability of the bacteria can be studied under the following heads.

Free-living nitrogen fixers: Among the large number of free-living nitrogen fixers, the *Azotobacter* requires oxygen to flourish and *Klebsiella* and *Rhodospirillum* can survive both in the presence and absence of oxygen. Apart from fixing nitrogen, *Azotobacter chroococcum* has the ability to synthesize and secrete B-vitamins, growth hormones and antifungal antibiotics into its environment. *Azotobacter* has one drawback, its nitrogen fixing ability is regulated by the presence of nitrogenous compounds in its environment. Thus it cannot be used along with a nitrogenous

fertilizer. *Klebsiella* is another promising free-living nitrogen fixer, and it has been possible to isolate its 'nif' genes (17 of them) and to introduce them into E.coli.


Benefits of Liquid Bio-fertilizer

The advantages of Liquid Bio-fertilizer over conventional carrier based Bio-fertilizers are listed below:

- Longer shelf life -12-24 months.
- No contamination.
- No loss of properties due to storage upto 45° c.
- Greater potentials to fight with native population.
- High populations can be maintained more than 10⁹ cells/ml upto 12 months to 24 months.
- Easy identification by typical fermented smell.
- Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport.
- Quality control protocols are easy and quick.
- Better survival on seeds and soil.
- No need of running Bio-fertilizer production units through out the year.
- Very much easy to use by the farmer.
- Dosages is 10 time less than carrier based powder Bio-fertilizers.
- High commercial revenues.
- High export potential.
- Very high enzymatic activity since contamination is nil.

Structure and characteristic features of bacterial biofertilizers (Azospirillum, Azotobacter, Bacillus, Pseudomonas, Rhizobium and Frankia)

1. Rhizobium




Rhizobium is a soil habitat bacterium, which is able to colonize the legume roots and fixes the atmospheric nitrogen symbiotically. The morphology and physiology of *Rhizobium* will vary from free-living condition to the bacteroid of nodules. They are the most efficient biofertilizer as per the quantity of nitrogen fixed concerned. They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group. *Rhizobium* inoculant was first made in USA and commercialized by private enterprise in 1930. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is in reality utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of *Rhizobium*. The percentage of nodules occupied, nodules dry weight, plant dry weight and the grain yield per plant the multistrain inoculant was highly promising. **Structure:** Rhizobia are rod shaped bacteria, 0.8 μm in diameter and 2 μm in length, often with flagellae. They assume a different shape when inside their host, being irregularly shaped or often 'Y' -shaped

Physical features of liquid *Rhizobium*

- Dull white in colour
- No foam formation,
- No bad smell
- pH 6.8-7.5

2. Azotobacter



Of the several species of *Azotobacter*, *A. chroococcum* is the dominant inhabitant in arable soils capable of fixing N_2 (2-15 mg N_2 fixed /g of carbon source) in culture media. The bacterium produces abundant slime which helps in soil aggregation. The numbers of *A. chroococcum* in Indian soils rarely exceeds $10^5/\text{g}$ soil due to lack of organic matter and the presence of antagonistic microorganisms in soil. It is well known free living nitrogen fixing aerobic bacterium. It is used as a Bio-Fertilizer for all non leguminous plants especially rice, cotton, vegetables etc. *Azotobacter* cells are not present on the rhizosphere but are abundant in the rhizosphere region.

Physical features of liquid *Azotobacter*

The pigmentation that is produced by *Azotobacter* in aged culture is melanin which is due to oxidation of tyrosine by tyrosinase enzyme. Some of the pigmentation are described below-

- *A. chroococcum* : Produces brown-black pigmentation in liquid inoculum.
- *A. beijerinckii* : Produces yellow- light brown pigmentation in liquid inoculum
- *A. vinelandii* : Produces green fluorescent pigmentation in liquid inoculum.

- *A. paspali* : Produces green fluorescent pigmentation in liquid inoculum.
- *A. macrocytogenes*: Produces, pink pigmentation in liquid inoculum.
- *A. insignis* : Produces grayish-blue pigmentation in liquid inoculum.
- *A. agilies* : Produces green-fluorescent pigmentation in liquid inoculum.

Role of liquid *Azotobacter* as a Bio-control agent

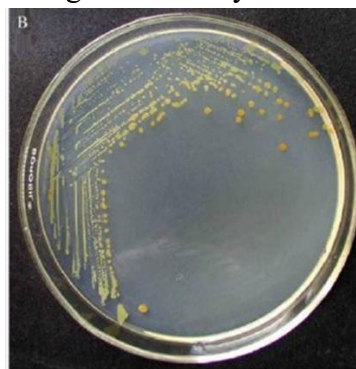
Azotobacter produces antifungal substance which inhibits the growth of soil fungi like *Aspergillus*, *Fusarium*, *Curvularia*, *Alternaria*, *Helminthosporium*, *Fusarium* etc.

3. *Acetobacter*

This is a saccharophilic bacterium and is associated with sugarcane, sweet potato and sweet sorghum plants and fixes 30 kg N/ha/year. Mainly this bacterium is commercialized for **sugarcane** crop. It is known to increase yield by 10-20 t/ acre and sugar content by 10-15 %.

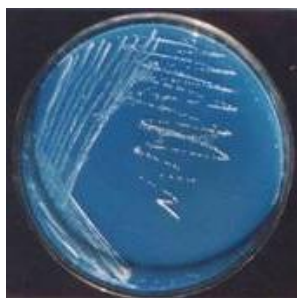


Acetobacter colony



Acetobacter colony

4. *Azospirillum*



Azospirillum lipoferum and *A. brasilense* are primary inhabitants of soil, the rhizosphere, and intercellular spaces of root cortex of graminaceous plants. They perform the associative symbiotic relation with the graminaceous plants. *Azospirillum* are N₂ fixing organisms isolated from the root and above ground parts of a variety of crop plants. They are Gram negative, *Vibrio* or *Spirillum* having abundant accumulation of polybetahydroxybutyrate (70 %) in cytoplasm.

Five species of *Azospirillum* have been described to date

- *A. brasilense*
- *A. amazonense*
- *A. irakense*
- *A. lipoferum*
- *A. halopraeferens*

The organism proliferates under both anaerobic and aerobic conditions but it is preferentially micro-aerophilic in the presence or absence of combined nitrogen in the medium. Apart from nitrogen fixation, growth promoting substance production (IAA), disease resistance and drought tolerance are some of the additional benefits due to *Azospirillum* inoculation.

Phosphatic Bio-fertilizers: Phosphate solubilizing microorganisms (PSM)

Several soil bacteria and fungi, notably species of *Pseudomonas*, *Bacillus*, *Penicillium*, *Aspergillus* etc. secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil. Increased yields of wheat and potato were demonstrated due to inoculation of peat based cultures of *Bacillus polymyxa* and *Pseudomonas striata*. They act in different ways. Some bacteria secrete organic acids such as lactic acid, acetic acid and citric acid which solubilize the bound phosphate to forms which are available to plants. Others produce sulphuric acid by oxidation of sulphur which acts like the organic acids in making phosphate available. Carbonic acid formed by the action of carbon dioxide released by bacteria during respiration and water acts in a similar way. Some organisms give off hydrogen sulphide which reacts with the iron salt, ferrous phosphate, to form ferrous sulphide and thus releases the phosphate. Microbes are also



responsible for decay of dead animal and plant litter in the soil during which humic and fulvic acids are formed which bind the metal ions such as Fe, Al, Mn and Ca and release the phosphate ions for the plants. They are used as seed inoculants.

responsible for decay of dead animal and plant litter in the soil during which humic and fulvic acids are formed which bind the metal ions such as Fe, Al, Mn and Ca and release the phosphate ions for the plants. They are used as seed inoculants.



Structure and characteristic features of Cyanobacterial biofertilizers: (Algae as Bio-fertilisers)

Dr. AJEET KUMAR¹²

Another group of free-living nitrogen fixers are the *cyanobacteria* commonly called the **blue-green algae (BGA)**. More than a hundred species of BGA can fix nitrogen.

Nitrogen fixation takes place in specialized cells called the **heterocysts** (large, thick walled and metabolically inactive cells) which depend on vegetative cells for energy to fix nitrogen while the fixed nitrogen is utilized by the vegetative cells for growth and development. BGA are very common in the rice fields (the micro-aerophilic condition and alkalinity are conducive to the algal population).

If no chemical fertilizers are added, inoculation of the algae can result in 10-14 % increase in crop yields. Unlike *Azotobacter*, the BGA are not inhibited by the presence of chemical fertilizers. On the other hand, presence of inorganic minerals (superphosphate) accelerates their growth. They are easy to produce and usually they are mass produced in cement tanks filled with fresh-water. Since they do not require any processing, they are quite cheap. Cost of 10 kg may be only Rs.30-40. Algal effect can be seen in subsequent years without the use of fresh inoculants.

Algal bio-fertilisers or *cyanobacterial biofertilizers* are *Anabaena*, *Nostoc* and *Oscillatoria*. Algae have proved beneficial in the case of certain crops like vegetables, cotton and sugarcane when added along with chemical fertilizers.

Aquatic Fern (Azolla) as a Bio-fertilizer:

Azolla is a tiny water fern common in ponds, ditches and rice fields. It has been used as a bio-fertilizer for rice. The nitrogen accumulated in the Azolla is made available to the rice crop when the fern decomposes. The nitrogen fixing work is accomplished by the symbiotic relationship between the fern and a BGA, *Anabaena azollae*. The alga inhabits some of the cells on the underside of the Azolla frond and fixes atmospheric nitrogen. It is dependent on the fern for photosynthates which supply the energy for nitrogen fixation. In addition to nitrogen, the decomposed Azolla also provides K, P, Zn and Fe to the crop. It also controls aquatic weeds which would otherwise compete with the crop for nutrients.

AZOLLA (Botanical name is *Azolla pinnata*)

It belongs to the family Azollaceae. Azolla is a water fern. It is considered as aquatic weed commonly found in floating in idle ponds, tanks shallow ditches and canals. Azolla is associated with rice field. A blue green algae (BGA) *Anabaena azollae* living in the epidermal cavity of lower side of the leaf of Azolla. The symbiotic association of *Azolla pinnata* and *Anabaena azollae* is termed as Azolla Anabaena complex. This algae fixes atmospheric nitrogen for Azolla and in

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exchange, the plant provides shelter and food to the algae . As this complex fixes atmospheric nitrogen, it has great potentiality for use, in agricultural field as biofertilizer and can be used as an alternative to nitrogen fertilizers. It is recommended as green manure because of its large biomass and high N content (4-6%N) for sub merged rice fields, with in maximum temperature of 38⁰C. The yield increase is 15-20% Fresh Azolla contains 90-95 water. Decomposed Azolla contains the following plant foods.

S.No.	Nutrient	Per cent (%)
1	Nitrogen	4 to 6
2	Phosphorus	0.5 to 0.9
3	Potassium	2 to 6
4	Calcium	0.4 to 1
5	Magnesium	0.5
6	Manganese	0.11 to 0.16
7	Iron	0.06 to 0.16
8	Total ash	9 to 10

Ideal conditions for growth and multiplication of Azolla

1. Depth of water should be maintained at 8-10 cm in the field
2. Temperature in between 14 to 35⁰C . Optimum for quick growth and multiplication is 20 to 28⁰C.
3. Suitable soil pH is 6.0 to 8.0
4. Single Super Phosphate should be applied at the rate of 20-25 kg ha⁻¹, should be applied
5. Furadon granules @2-3 kg /week should be applied to control leaf eating insects and snails.

Methods of growing Azolla

1. Green manuring
2. Dual cropping
3. Composting
4. Just growing

1. Green manuring

When there is standing water in the field, Azolla is grown by spreading 200-500 kg /ha fresh Azolla. After 25 days of the whole field will be covered by Azolla, if condition is ideal. Green Azolla is incorporated by ploughing after dewatering in the field. This will add 30- 40 kg of N fertilizer /ha.

2. Dual cropping

1. Azolla nursery is prepared simultaneously with the paddy nursery
2. The area of Azolla nursery should be half that of paddy nursery (seed bed).
3. The Azolla nursery should be kept free from other aquatic weeds and there should be 8-10 cm standing water in the Azolla nursery.
4. Where the paddy seeds are sown in the paddy nursery fresh Azolla is spread in the Azolla nursery @40-80 grams per square meter.

5. After 25 days the Azolla will increase an extent of 60 times and super phosphate is applied @ 25 grams/Sq. Meter/week.
6. Within 25 days the paddy seedlings will be ready for transplanting. Before transplantation of paddy seedlings, there will be standing water in the main field .Azolla is taken out from the Azolla nursery and spread in the transplanted field @ 200- 500 kg /ha.
7. Single super phosphate @25-50 kg /ha /week is applied for 3 weeks and water level is maintained 5-10 cm.
8. After another 25 days, the whole field may be covered by Azolla. Azolla is incorporated after dewatering the field .This will add 30-40 kg N /ha besides another nutrients.

3. **Composting:** Azolla is grown in permanent Azolla nursery. The Azolla is collected every week from the nursery and kept in layers on the side of nursery. Within 10 days, the Azolla will decompose and can be used in any crop

4. **Just growing**

Azolla can be grown along with paddy. nitrogen will be added where it is not incorporated. Azolla when grown in one hectare of land throughout the year and ideal conditions are maintained it can fix 800 kg of nitrogen per hectare azolla is suitable for only flooded rice and fixes up to 40-80 kg N/ha. Symbiotically with *Anabaena azollae*. The yield increase is 15-20 %.

Advantages of azolla:

1. It contains higher Nitrogen content
2. Release of nitrogen is easy
3. There is no leaching loss
4. Releases nutrient slowly
5. Weed interference is less where grown as dual crop

V. Blue green algae or Cyanobacteria:

BGA is phototrophic in nature. These are photosynthetic, prokaryotic microorganisms and capable of fixing molecular nitrogen. B G A are found to grown in a paddy filed and marshland. It is used as biofertilizer for irrigated rice crop. The rice field ecosystem provides an environment favourable for the growth of BGA with respect to their requirements viz., light, water and temperature. Nitrogen fixation by BGA have been estimated to be 25-30 kg N/ha. Yield increases by 10-15 %. BGA has some species viz., *Nostoc*, *Anabaena*, *Olocia* , *Calothrix*, *Tolipothix* etc., They fix nitrogen in the presence of sunlight. They also contain growth promoting substances like auxin, Indole acetic acid and Gibberellic acid.

Recommendation: application of 10 kg /ha flakes of BGA. It is applied 10 days after the transplanting of rice crop.

Algal production technology:

Mainly three methods are followed.

- 1) Trough method
- 2) Pit method
- 3) Field scale

1. Trough method:

- ✚ Take 3 m x 2 m x 20 cm size (L x B x h) galvanized iron sheet trough or to the convenient size. Add about 8 to 10 kg of soil and well mixed with 200 grams of SSP.
- ✚ The soil pH should be about neutral. If acidic correct by lime. Add water to the trough 5-15 cm (height) to protect from insects, and add Carbofuran 3.0 G at the rate of 25 grams / tray .
- ✚ Sprinkle the algal culture on the surface of the standing water and keeping it in air (open) and exposed to sunlight.
- ✚ In hot summer months, the growth of algae will be rapid in about 7 to 10 days and form a thick mat, where algal growth becomes sufficiently thick stop watering and allow the water to evaporate completely, the dry algae cracks in to flakes.
- ✚ Collect the dry algal flakes from the trough and store in bags for the use in the field. One trough gives 1.5 to kg mats.

2. Pit- method :

- 🌍 Adopted by number of farmers in Bihar and India
- 🌍 Demark the area in the field for algal production
- 🌍 Flood the area with to a depth of about 12.5 cm
- 🌍 Apply SSP @12.0 kg /40 m² area
- 🌍 Apply the algal culture
- 🌍 In clayey soils good growth of algae takes place in about two weeks in clear sunny weather while in loamy soils it takes about 3-4 weeks
- 🌍 Once algal growing mats are formed, they are allowed to dry up in the field and deed algal flakes are thin collected and stored in bags.
- 🌍 BGA benefit the rice crop up to 25-30 kg N /ha
- 🌍 Dose :Apply BGA @10-15 kg /ha after transplanting .

Actinomycetes as Bio-fertiliser

Frankia is an actinomycete and forms nitrogen fixing nodules in trees and shrubs. The organism invades the cells of a developed lateral root and causes it to fuse into a nodule. Entry into the host changes the structure of the microbe.

Structure and characteristic features of - Fungal biofertilizers

Dr. AJEET KUMAR¹³

Fungi as Bio-fertiliser

Some nonpathogenic fungi help in plant growth by forming associations with the host plant roots called mycorrhizae (myca- fungi, rhiza -root). examples are *Trichoderma*, *Gigaspora*, *Glomus*, etc. One group of mycorrhizae forms a sheath around the fine lateral roots and replaces the root hairs by dichotomous branching of the fungal hyphae. They are called **ectomycorrhizae** because they do not traverse intracellularly. The ectomycorrhizae help the plant by solubilizing nutrients near the plant roots and making it easy for the plants to feed. They also prevent the roots from being attacked by nematodes (by entangling them). Another group called the **endomycorrhizae** penetrate the roots and establish symbiotic relation with the plants.

The fungi helps the roots in obtaining inorganic nutrients while obtaining essential organic nutrients from the host. There is yet another group called **ect-endomycorrhiza** or **vesicular-arbuscular mycorrhiza** (VAM fungi) wherein they are partly outside the host roots and partly intracellular.

The mycorrhizae are of particular significance as they assist plants to absorb phosphates from the soil. Phosphate ions are not mobile in the soil, thus when a plant uses up all the available phosphate near its roots, it starves for want of phosphates. The mycorrhizal mycelia spread far and wide, away from the depleted zone and translocate the phosphates directly to the host. Plants with restricted root system, having short stubby roots and few root hairs are particularly benefited by association with mycorrhiza. Absorption of elements which are required in minute quantities by plants is also facilitated by mycorrhizae. Mycorrhizae are valuable particularly in times of drought. They enable plants to survive and grow in the driest and poorest of soils, even in leached soils that have been mined by deforestation and overcropping.

Cultivation of mycorrhizal fungi

It is easy to mass cultivate ectomycorrhizal fungi since they can grow in an artificial fungal medium. They are mass produced in liquid media, dried and pressed into cakes or cultivated in the form of spores and packaged. The seed inoculation is performed just as that of *Rhizobium*. Cultivation of endo mycorrhizal fungi is quite difficult since they need a live host for survival. Hence the host plants harbouring such mycorrhizal fungi are specially grown in laboratory conditions, their roots excised, powdered and the mixture used as a fertilizer.

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MYCORRHIZA

The term 'mycorrhiza' (literally, fungus root) was first used by A. B. Frank to characterise the association between higher plants and fungi. The symbiotic association between the roots of some plants and some fungi is called mycorrhizal association. The fungus is highly habitat limited and is usually found in the immediate vicinity of or within the roots.

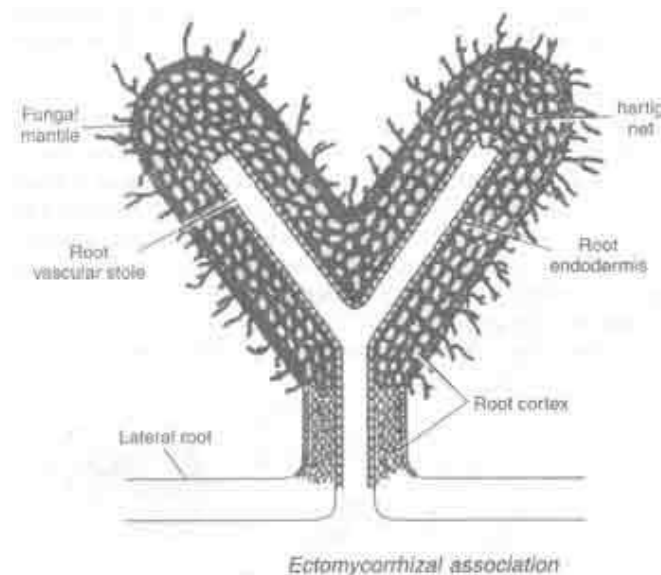
Occurrence of Mycorrhiza

They occur on almost all terrestrial plants though not as specific as the nitrogen fixing symbiosis. Thus a plant may have several mycorrhizae that can form symbiosis with its roots. Extent of symbiosis depends on fertility. High soil fertility leads to low mycorrhizal infection and poor symbiosis and vice versa. Roots supply carbohydrates to the fungi which absorb nutrients from the soil and supply them to the crop.

Types of mycorrhizae

Mycorrhizae are of two kinds:

Ectomycorrhiza Ectomycorrhizal symbiosis is a mutually beneficial union between fungi and the roots of vascular and non-vascular plants. The host of an ectomycorrhizal fungus is usually a gymnosperm (pine). The typical ectomycorrhizal fungi are basidiomycetes (*Agaricus*), ascomycetes or phycmycetes members. Usually more common in the temperate regions, they are capable of growing apart from the host on media containing simple sugars and vitamins. Ectomycorrhizae have poor competitive saprophytic ability hence they have a tough time competing with other microbes in the soil.



During the infection process, ectomycorrhizal fungi in soil are stimulated by the root metabolites to grow toward the root. The hyphae aggregate around the root and penetrate between

the root epidermis and the cortex. A structure called Hartig net is formed which is a fungal sheath surrounding the root in which fungal hyphae penetrate between the root cells.

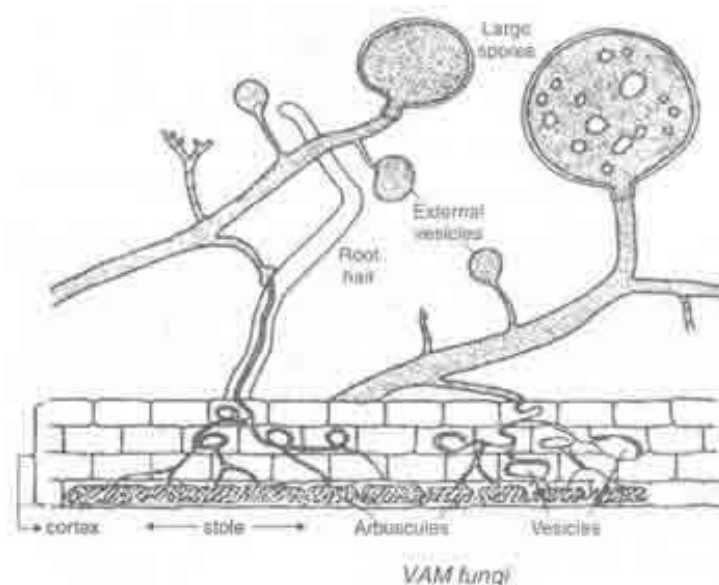
Eventually, the root gets surrounded by a fungal mantle. The fungal hyphae replace the fine lateral root hairs of the host root system thus modifying it structurally. Thus the host root system that is infected with ectomycorrhiza looks stunted and dichotomously branched. The fungal hyphae on the exterior of the roots usually serve as an extension of roots and store large amounts carbohydrates

Endomycorrhiza In this case, the fungal hyphae penetrate the host root cells. They are quite common among the Ericaceae and Orchidaceae members of higher plants as well as fruit trees like citrus, coffee, rubber, etc.

Vam Fungi

Vesicular-arbuscular mycorrhiza (VAM) fungi shown in are geographically ubiquitous. They are commonly found in association with agricultural crops, shrubs, tropical tree species and some temperate trees. Their nutritional requirements are not specific. VAM associations are formed by non septate Zygomycetes and Phycomycetes .fungi. Some examples are *Glomus*, *Gigaspora*, *Acaulospora*, *Entrophospora* and *Scutellospora* of which *Glomus* is the most common fungus.

The fungi are obligate biotrophs and do not grow on synthetic media and hence are classified according to the morphological characteristics of the spores formed in the soil. VAM fungi produce large resting spores (0.2 mm). The spores can survive adverse conditions. The germ tube dies if it is unable to encounter and successfully penetrate the host root. The fungal hyphae traverses and ramifies in the root cortex. Branches from the intercellular hyphae enter cortical cells where further branching results in highly branched hyphal structures called arbuscles. They are short-lived and serve as the nutrient transfer



Mechanism between the fungus and the host. Phosphate transfer possibly occurs across living membranes of the host and the fungus via arbuscles. When the association is well established, hyphal swellings called vesicles form on the mycelium inside and outside the root. The vesicles are sac-like terminal swellings at the tip of hyphae and contain many lipid droplets and function as storage organs. External mycelium form very thick-walled chlamydospores. These fungi are disseminated both actively and passively. Active dissemination is from one root to another by mycelial growth through soil and passive dissemination is through biotic agents like rodents, worms, insects and birds or abiotic agents like wind and water. VAM fungi interact with other soil microbes like the free-living and symbiotic nitrogen fixers and phosphate solubilises to improve their efficiency for the biochemical cycling of elements to the host plants.

Constraints in usage of biofertilizers

1. Farmers acceptance has been far away from satisfaction
2. Difficult to demonstrate striking increase in yield due to biofertilizers unlike chemical fertilizers
3. Strains fail to establish if the soil moisture in the surface in the surface zone
4. Fungicide treatment is a problem in crops like groundnut
5. Inoculants manufacturing is far below the requirement
6. Availability of quality of inoculants is another problem
7. Seed inoculation is no more suitable when seeds are sown on dry soil under
8. When seeds are coated with toxic chemicals and pesticides
9. Soil containing higher number of ineffective native Rhizobia

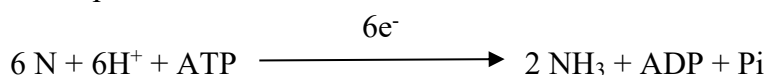
Biochemistry of Biological Nitrogen Fixation

Dr. AJEET KUMAR¹⁴

Nitrogen is an essential element for plant growth and development and a key issue of agriculture. Nitrogen fertilizers contribute to resolving the challenge the world is facing, feeding the human population. The Green Revolution was accompanied by an enormous increase in the application of nitrogen fertilizer. There is however a high heterogeneity of its distribution throughout the world. Biological Nitrogen Fixation (BNF) is known to be a key to sustain agriculture and to reduce soil fertility decline.

Nodulation: From the Infection Process to the Functioning of the Nitrogenase

The production of nitrogen fertilizer by industrial fixation generates large quantities of carbon dioxide, contributing to earth warming. The natural process of BNF offers an economic means of reducing environmental problems and improving the internal resources. It is a process that allows microorganisms to convert atmospheric nitrogen (N₂) to ammonia (NH₃) assimilable by associated plants.



Different types of associations are listed as bellows:

Types of association	Microorganisms	Host plants
Symbiotic	Bacteria(ex. <i>Rhizobium</i>) ^a	Legumes
	Actinomycetes (ex. <i>Frankia</i>)	Actinorhiza
	Cyanobacteria (ex. <i>Anabaena azollaea</i>)	Fern
Non symbiotic	Bacteria (ex. <i>Azotobacter</i> , <i>Azospirillum</i>)	Cereals
Free living systems	Bacteria (ex. <i>Thiobacillus</i> , <i>Clostridium</i>)	

^a: Rhizobium-leguminous plants is the most studied symbiotic association. There are six main genus of rhizobia:

1. Allorhizobium (fast growing)
2. Azorhizobium (fast growing)
3. Bradyrhizobium (slow growing)
4. Mesorhizobium (intermediate growing)
5. Rhizobium (fast growing)
6. Sinorhizobium (fast growing)

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Nodule formation:

Leguminous plants and rhizobia communicate through the gene expression by reciprocally transmitting signals for the activation of the symbiotic genes in two partners. A type of phenolic called flavonoids, are released by host roots plants into the rhizosphere. Flavonoids act as a chemo-attractant for the bacteria to the plant roots, and eventually colonies of rhizobia attach to the root hairs. Flavonoid signal activates expression of nodulation (nod) genes.

In the rhizobial strains there are numerous nodulation genes including the nod genes nodABC and nodD (Figure 1). On the surface of the rhizobial bacteria the flavonoids are recognized by a expressed nodD protein. Then nodD binds to a promoter DNA sequence, and thereby activates transcription of nod genes of the operons. A group of nod genes encode enzymes synthesise the rhizobial nodulation signal, Nod factor (Figure 2), which triggers development of the root nodules by the plant. The plant roots recognise Nod factor, through binding to to a surface protein receptor at the sub apical root tip. Perception of Nod factor induces a development inside the root, producing pronounced curling of the root hairs entrapping the rhizobia which establish infection of root. Bacteria gain access to plants cell membrane. The plasma membrane invaginates to form novel infection structure known as the infection thread, a tubular structure that extends from the root hair tip to the lower cells of the root cortex. Rhizobia enter the infection threads in which they actively multiply. At the same time the underlying root cortex cells are quickly proliferated to constitute the nodule primordia. The infection threads branch out into cells of the nodule primordia. The rhizobia are finally released into the nodule cell and enveloped in a membrane derived from the host cell plasma membrane. At this stage, rhizobial bacteria become bacteroids able to fix nitrogen.

Importance of nitrogen fixation

The process of nitrogen fixation is carried out in microbes.

Key Points

- Nitrogen fixation takes elemental nitrogen (N_2) and converts it into a ammonia, a format usable by biological organism.
- The fixed form of nitrogen (NH_3) is needed as an essential component of DNA and proteins. Therefore, it is needed for all life on earth.
- Nitrogen fixation is carried out by the enzyme nitrogenase, which are found in microbes.

Key Terms

- ❖ **Fixation:** The act of uniting chemically with a solid substance or in a solid form; reduction to a non-volatile condition; — said of gaseous elements.
- ❖ **cofactor:** A substance, especially a coenzyme or a metal, that must be present for an enzyme to function.
- ❖ **Heterometal:** Describing a complex containing two (or more) different metals
- ❖ **symbiosis:** A close, prolonged association between two or more organisms of different species, regardless of benefit to the members
- ❖ **sulfide:** Any compound of sulfur and a metal or other electropositive element or group.
- ❖ **reduction:** A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- ❖ **enthalpy:** In thermodynamics, a **measure of the heat content of a chemical** or physical system.
- ❖ **oxidation:** A reaction in which the atoms of an element lose electrons and the valence of the element increases.
- ❖ **reduction:** A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- ❖ **proteoglycan:** Any of many glycoproteins that have heteropolysaccharide side chains.

Nitrogen fixation:

The conversion of atmospheric nitrogen into ammonia and organic derivatives, by natural means, especially such conversion, by microorganisms in the soil, into a form that can be assimilated by plants.

Nitrogen fixation also refers to other biological conversions of nitrogen, such as its conversion to nitrogen dioxide. Nitrogen fixation is a process by which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). Atmospheric nitrogen or elemental nitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds. Dinitrogen is quite inert because of the strength of its $N\equiv N$ triple bond. To break one nitrogen atom away from another requires breaking all three of these chemical bonds. Fixation processes free up the nitrogen atoms from their diatomic form (N_2) to be used in other ways. Nitrogen fixation, natural and synthetic, is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals, and other life forms, e.g., nucleotides for DNA and RNA and

amino acids for proteins. Therefore, nitrogen fixation is essential for agriculture and the manufacture of fertilizer. Microorganisms that fix nitrogen are bacteria called diazotrophs.

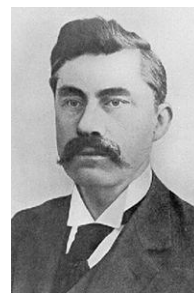
Nitrogenase and Nitrogen Fixation

Some higher plants, and some animals (termites), have formed associations (symbioses) with diazotrophs. Diazotrophs are microbes. Biological nitrogen fixation was discovered by the German agronomist **Hermann Hellriegel** and Dutch microbiologist Martinus Beijerinck.

Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. Nitrogenases are enzymes used by organisms to fix atmospheric nitrogen gas (N_2). There is only one known family of enzymes that accomplishes this process. All nitrogenases have an iron – and sulfur-containing cofactor that includes a heterometal complex in the active site (e.g., FeMoCo). In most species, this heterometal complex has a central molybdenum atom. However, in some species it is replaced by a vanadium or iron atom. Enzymes responsible for nitrogenase action are very susceptible to destruction by oxygen. Many bacteria cease production of the enzyme in the presence of oxygen. Many nitrogen-fixing organisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with proteins.

History of Nitrogen Fixation

For thousands of years farmers were aware that plants belonging to the legume family, promoted crop growth when planted with other non-legumes. Growing a legume crop in a field could also result in the next year's crop of non-legume plants giving a far greater yield. This led to the practice of crop rotation, a practice which can be traced back to techniques recorded in Roman literature.



Martinus Beijerinck: Work done by Martinus Beijerinck was key to the discovery of rhizobia, symbiotic bacteria found on the roots of legumes, responsible for nitrogen fixation.

While the ancient Romans were aware of the improved results gained through crop rotation, they did not know that these benefits were brought about through the replenishment of nitrogen in the soil. Later people knew legumes did replenish nitrogen in the soil, but did not know how atmospheric (N_2) was converted into ammonium (NH_3) by legumes until research done in the 19th century.

Hermann Hellriegel (1831-1895), a noted German agricultural chemist, discovered that leguminous plants took atmospheric nitrogen and replenished the ammonium in the soil through the process now known as nitrogen fixation. He found that the nodules on the roots of legumes are the location where nitrogen fixation takes place.

HELLRIEGEL'S AND BEIJERINCK'S DISCOVERIES

Hellriegel did not determine what factors in the root nodules carried out nitrogen fixation. Martinus Willem Beijerinck (March 16, 1851 – January 1, 1931), a Dutch microbiologist and botanist, explored the mechanism responsible, discovering that the root nodules contained microbes. He further demonstrated that these microbes were bacteria, which he named rhizobia. These rhizobia perform the chemical processes of nitrogen fixation. In addition to having discovered this biochemical reaction vital to soil fertility and agriculture, Beijerinck is responsible for the discovery of this classic example of symbiosis between plants and bacteria. The bacteria in the root nodules are needed to provide nitrogen for legume growth, while the rhizobia are dependent on the root nodules as a environment to grow.

Nitrogen Fixation Mechanism

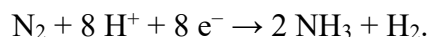
The conversion of N_2 to NH_3 depends on a complex reaction, essential to which are enzymes known as nitrogenases.

Key Points

- ✓ Nitrogen fixation does result in the release of energy, but the activation of this reaction takes energy in the form of ATP hydrolysis.
- ✓ Nitrogenases are metalloenzymes, which are proteins that have metallic molecules as subunits.
- ✓ While a great deal is known about how nitrogenases reduce nitrogen, some steps are unknown.

Biological nitrogen fixation (BNF)

It occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The reaction for BNF is:



This type of reaction results in N_2 gaining electrons and is thus termed a reduction reaction. While the equilibrium formation of ammonia from molecular hydrogen and nitrogen has an overall negative enthalpy of reaction (i.e. it gives off energy), the energy barrier to activation is very high

without the assistance of catalysis, which is done by nitrogenases. The enzymatic reduction of N_2 to ammonia therefore requires an input of chemical energy, released from ATP hydrolysis, to overcome the activation energy barrier.

A General Catalytic Mechanism Scheme for Nitrogenase:

- a) Components I and II are dissociated; II is ready for reduction.
- b) ATP binds to component II, which receives electrons from an electron donor (ferredoxin or flavodoxin); binding of ATP induces an allosteric conformational change which allows association of the two proteins. Electrons flow from the [4Fe-4S] cluster on II to the P cluster on I.
- c) Electrons are further shuttled to the iron-molybdenum cofactor (FeMoco), and ATP is hydrolysed to ADP. This step is repeated several times before a molecule of N_2 can bind to FeMoco.
- d) The protein complex dissociates, and nitrogenase reduces dinitrogen to ammonia and dihydrogen.

(Legend:I: component I (dinitrogenase; MoFe protein); II: component II (dinitrogen reductase; Fe protein); ATP: adenosine triphosphate; ADP: adenosine diphosphate; Fdx: ferredoxin; Fld: flavodoxin).

Nitrogenase is made up of two soluble proteins: component I and II. Component I known as MoFe protein or nitrogenase contains 2 Mo atoms, 28 to 34 Fe atoms, and 26 to 28 acid-labile sulfides, also known as a iron-molybdenum cofactor (FeMoco). Component I is composed of two copies each of two subunits (α and β); each subunit's stability depends on the other in vivo.

Component II known as Fe protein or nitrogenase reductase is composed of two copies of a single subunit. This protein has four non-heme Fe atoms and four acid-labile sulfides (4Fe-4S). Substrate binding and reduction takes place on component I, which binds to ATP and ferredoxin or flavodoxin proteins (Fdx or Fld) (see step B).

The hydrolysis of ATP supplies the energy for the reaction while the Fdx/Fld proteins supply the electrons. Note this is a reduction reaction which means that electrons must be added to the N_2 to reduce it to NH_4 . Thus, the role of component II is to supply electrons, one at a time to component I. ATP is not hydrolyzed to ADP until component II transfers an electron to component I. The association of nitrogenase component I and II and later dissociation occurs several times to allow the fixation of one N_2 molecule.

Nitrogenase ultimately bonds each atom of nitrogen to three hydrogen atoms to form ammonia (NH₃). The nitrogenase reaction additionally produces molecular hydrogen as a side product, which is of special interest for people trying to produce H₂ as an alternative energy source to fossil fuels.

Anaerobiosis and N₂ Fixation

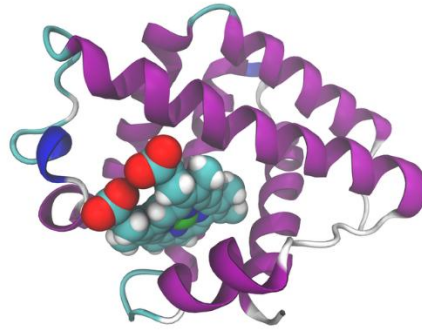
Nitrogen fixing bacteria have different strategies to reduce oxygen levels, which interfere with nitrogenase function.

Key Points

- ✧ The iron (Fe) found in nitrogenases is very sensitive to oxygen, if there is too much oxygen this will in the end disrupt nitrogenase function.
- ✧ Some bacteria produce barriers which protect themselves from oxygen, while others use proteins such as leghemoglobin to bind up oxygen which may interfere with nitrogenases.
- ✧ Portions of leghemoglobin are thought to be produced by rhizobia residing in plant nodules, while other parts are produced by the plant, an elegant example of symbiosis.

Central to nitrogen fixation (N₂ to NH₃) are the enzymes that do the actual fixation, these are known as nitrogenases. Due to the oxidation carried out by oxygen, most nitrogenases, which are essential large reduction complexes are irreversibly inhibited by O₂, which degradatively oxidizes the Fe-S cofactors. In essence, O₂ binds to the iron (Fe) found in nitrogenases and blocks their ability to bind to N₂. To protect nitrogenases, there are mechanisms for nitrogen fixers to protect nitrogenase from oxygen in vivo. One known exception is the nitrogenase of *Streptomyces thermoautotrophicus*, which is unaffected by the presence of oxygen. This is complicated by the fact the bacteria still need the presence of oxygen for proper respiration.

Some microbes have a proteoglycan rich extra cellular matrix which traps a layer of water, often referred to as a **slime layer**. This **slime layer acts as a barrier for oxygen**. The ability of some nitrogen fixers such as azotobacteraceae to employ an oxygen-amendable nitrogenase under aerobic conditions has been attributed to a high metabolic rate, allowing oxygen reduction at the cell membrane.



Leghemoglobin: Leghemoglobin, the protein which binds to oxygen, allowing nitrogenases to function in an oxygen free environment. The ribbons represent protein folds, while the conglomerate of spheres are the position of the iron contain heme group which binds the oxygen.

Many rhizobia, nitrogen fixing bacteria, live in a symbiotic relationship with plants known as legumes. They have an interesting strategy to deal with O_2 . In plants infected with Rhizobium, (legumes such as alfalfa or soybeans), the presence of oxygen in the root nodules would reduce the activity of the oxygen-sensitive nitrogenase. In these situations, the roots of such plants produce a protein known as leghemoglobin (also leghaemoglobin or legoglobin). Leghemoglobin buffers the concentration of free oxygen in the cytoplasm of infected plant cells to ensure the proper function of root nodules. Leghemoglobin is a nitrogen or oxygen carrier; naturally occurring oxygen and nitrogen interact similarly with this protein. Leghemoglobin buffers the concentration of free oxygen in the cytoplasm of infected plant cells to ensure the proper function of root nodules. It has close chemical and structural similarities to hemoglobin, and, like hemoglobin, is red in colour. Leghemoglobin has a high affinity for oxygen, about ten times higher than of human hemoglobin. This allows an oxygen concentration that is low enough to allow nitrogenase to function but not so high as to bind all the O_2 in the bacteria, providing the bacteria with oxygen for respiration.

Leghemoglobin is produced by legumes in response to the roots being infected by rhizobia, as part of the symbiotic interaction between the plant and this nitrogen-fixing bacterium. Interestingly, it is widely believed that leghemoglobin is the product of both the plant and the bacterium in which a protein precursor is produced by the plant and the heme (an iron atom bound in a porphyrin ring, which binds O_2) is produced by the bacterium. The protein and heme come together to function, allowing the bacteria to fix-nitrogen, giving the plant usable nitrogen and thus the plant provides the rhizobia a home.

Genetics and Regulation of N₂ Fixation

Through control of gene expression, nitrogen fixing bacteria can turn on and off the proteins needed for nitrogen fixation.

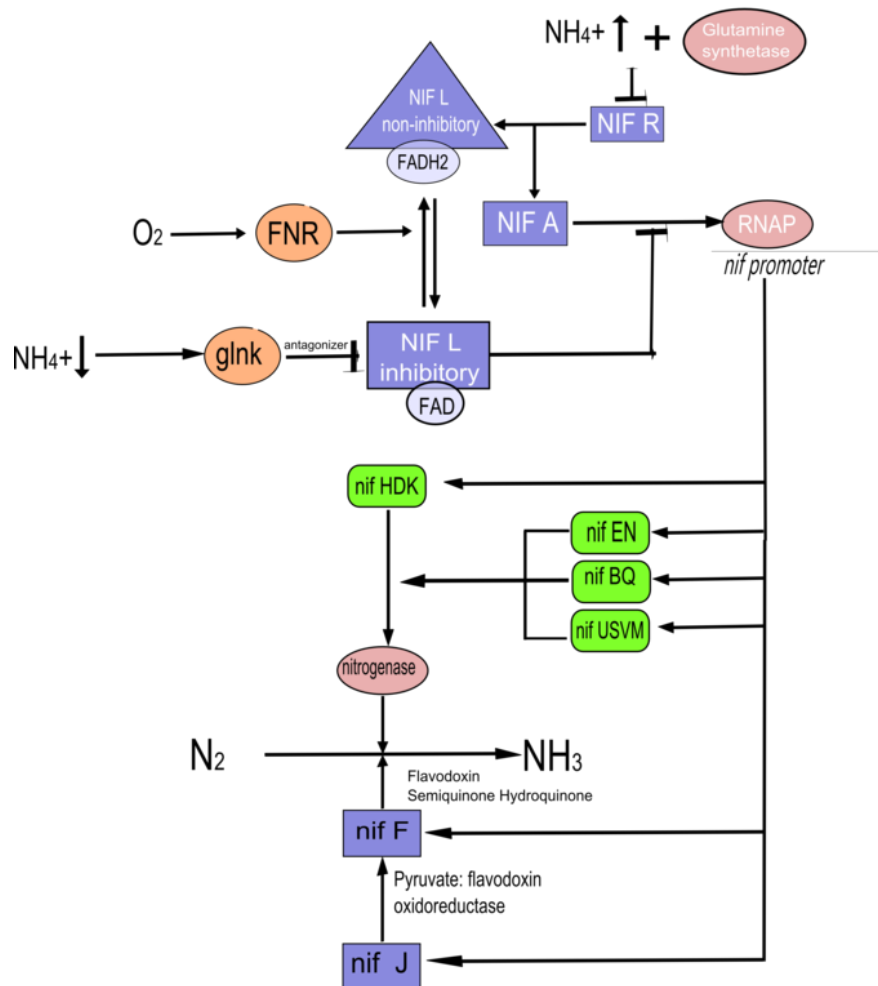
Key Points

- ✧ Nitrogen fixation takes a great deal of energy. If the conditions are not favorable for nitrogen fixation or there is enough ammonia around, nitrogen-fixing bacteria turn off the production of proteins needed for nitrogen fixation.
- ✧ Nitrogen fixing protein production is regulated by the nif regulon.
- ✧ The nif regulon contains factors which both turn on and off the production of proteins needed for nitrogen-fixation.

Key Terms

- ✓ **regulon:** A group of genes that is regulated by the same regulatory molecule. The genes of a regulon share a common regulatory element binding site or promoter. The genes comprising a regulon may be located non-contiguously in the genome.
- ✓ **operon:** A unit of genetic material that functions in a coordinated manner by means of an operator, a promoter, and structural genes that are transcribed together.

NIF REGULON



Nif Regulon: This is a schematic representing many of the proteins in the nif regulon and where they act in the pathway needed for nitrogen fixation.

The fixation of atmospheric nitrogen (N_2) is a very energy intensive endeavor. If there is no need for N_2 fixation, the production of proteins needed for fixation are tightly controlled. The nif genes are responsible for the coding of proteins related and associated with the fixation of atmospheric nitrogen into a form of nitrogen available to plants. These genes are found in nitrogen fixing bacteria and cyanobacteria. The nif genes are found in both free living nitrogen fixing bacteria and in symbiotic bacteria in various plants.

The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen. The primary enzyme encoded by the nif genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen to other nitrogen forms such as ammonia, which plants can use

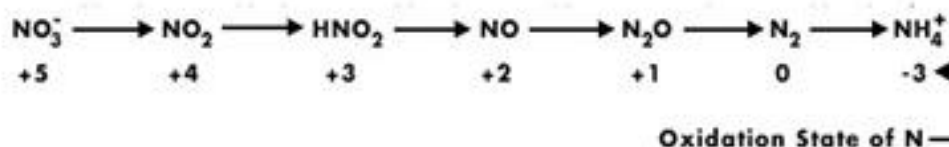
for various purposes. Besides the nitrogenase enzyme, the nif genes also encode a number of regulatory proteins involved in nitrogen fixation. The expression of the nif genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment). Nitrogen fixation is regulated by nif regulon, which is a set of seven operons which includes 17 nif genes. Nif genes have both positive and negative regulators. Some of nif genes are: Nif A, D, L,K, F,H S,U,Y,W,Z.

Activation of nif genes transcription is done by the nitrogen sensitive NifA protein. When there isn't enough fixed nitrogen factor available for the plant's use, NtrC, which is a RNA polymerase, triggers NifA's expression. NifA then activates the rest of the transcription for the nif genes. If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated, NifL. In turn, NifL inhibits NifA activity, which results in the inhibition of nitrogenase formation. NifL is then regulated by other proteins that are sensors for the levels of O₂ and ammonium in the surrounding environment.

The nif genes can be found on bacteria's chromosomes, but many times they are found on bacteria's plasmids with other genes related to nitrogen fixation, such as the genes needed for the bacteria to communicate with the plant host.

Nitrogen in the Biosphere

- Nitrogen is required in large amounts as an essential component of proteins, nucleic acids, and other cellular constituents.
- Nitrogen is the most abundant element in our atmosphere - nearly 79% in the form of N₂ gas.
- In order for nitrogen to be used for growth it must be "fixed" (combined) in the form of ammonium (NH₄⁺) or nitrate (NO₃⁻) ions.
- Plants are able to use nitrogen in the form of nitrate (NO₃⁻) or ammonia (NH₃⁺), but these compounds are present in limited supply in the soil.



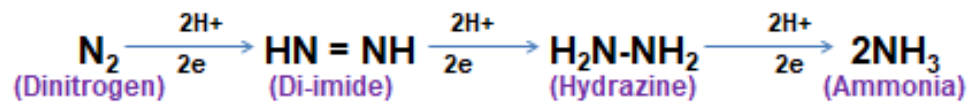
Nitrogen Fixation

- The growth of all organisms depend on the availability of Nitrogen (e.g. amino acids)
- Nitrogen in the form of Dinitrogen (N_2) makes up 80% of the air we breathe but is essentially inert due to the triple bond ($N\equiv N$)
- In order for nitrogen to be used for growth it must be "fixed" (combined) in the form of ammonium (NH_4) or nitrate (NO_3) ions.

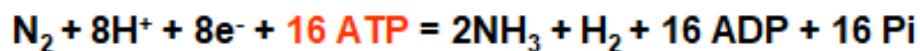
Nitrogen Fixation

Nitrogen Fixation

Enzymatic reduction of atmospheric Nitrogen



Energy intensive process :

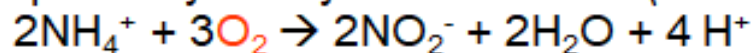


- Performed only by selected bacteria and actinomycetes
- Performed in nitrogen fixing crops (eg: soybeans)

Nitrification

Two step reactions that occur together :

1st step: catalyzed by *Nitrosomonas* (*Slow RKⁿ*)



2nd step: catalyzed by *Nitrobacter* (*Fast RKⁿ*)



Nitrogen Fixation Process

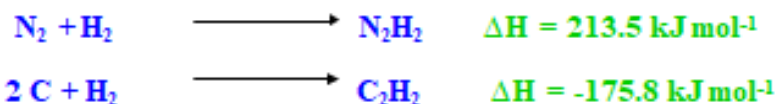
Energetics

- $\text{N}\equiv\text{N}$
- Haber-Bosch (100-200 atm, 400-500°C, **8,000 kcal kg⁻¹ N**)
- Nitrogenase (**4,000 kcal kg⁻¹ N**)

Properties of dinitrogen which makes it inert

- Dinitrogen - two N atoms connected by triple bond
- Breaking the $N \equiv N$ bond is difficult - high dissociation energy of 942 kJ mol^{-1}
- Breaking first π bond requires 540 kJ mol^{-1}

Initial hydrogenation is highly endothermic for N_2



Other important properties

Property	Gas	Nitrogen	Carbon	Oxygen	Argon
Ionization potential (eV)		14.3	11.256	13.614	15.755
Electron affinity (eV)		0.073	1.595	1.461	0
Solubility in water (mole/cm ³)		0.083	Insoluble	0.153	0.140

- High ionization potential and low electron affinity - difficult to reduce and oxidize
- Solubility very less - reactions in solution phase - difficult

Biological fixation of dinitrogen

- Enzyme nitrogenase
- Present in soil bacteria, root nodules and algae
- Two decades of research - mechanism not established
- Enzyme contains Mo and Fe
- Proposed mechanism - complexation of N₂ to metal ions
- Reduces bond strength - breaking 1st π bond easier

Limitations with biological route:

- Nitrogenase - sensitive to O₂ – requires O₂ free environment
- Sensitive to environmental conditions - temperature, pH
- Cannot be used for large scale N₂ fixation

Different types of associations:

Types of association	Microorganisms	Host plants
Symbiotic	Bacteria(ex. <i>Rhizobium</i>) ^a	Legumes
	Actinomycetes (ex. <i>Frankia</i>)	Actinorhiza
	Cyanobacteria (ex. <i>Anabaena azollaea</i>)	Fern
Non symbiotic	Bacteria (ex. <i>Azotobacter</i> , <i>Azospirillum</i>)	Cereals
Free living systems	Bacteria (ex. <i>Thiobacillus</i> , <i>Clostridium</i>)	

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Key Terms

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- **Cofactor:** A substance, especially a coenzyme or a metal, that must be present for an enzyme to function.
- **Heterometal:** Describing a complex containing **two or more different metals**
- **Symbiosis:** A close, prolonged association between two or more organisms of different species, regardless of benefit to the members
- **Sulfide:** Any compound of sulfur and a metal or other electropositive element or group.
- **Reduction:** A reaction in which **electrons are gained** and valence is reduced; often by the **removal of oxygen** or the **addition of hydrogen.**
- **Enthalpy:** In thermodynamics, a **measure of the heat content of a chemical** or physical system.
- **Oxidation:** A reaction in which the atoms of an element **lose electrons** and the valence of the element increases.
- **Proteoglycan:** Any of many glycoproteins that have hetero-polysaccharide side chains.

Genetics of Nitrogenase

Gene	Properties and function
<i>nifH</i>	Dinitrogenase reductase
<i>nifDK</i>	Dinitrogenase
<i>nifA</i>	Regulatory, activator of most <i>nif</i> and <i>fix</i> genes
<i>nifB</i>	FeMo cofactor biosynthesis
<i>nifEN</i>	FeMo cofactor biosynthesis
<i>nifS</i>	Unknown
<i>fixABCX</i>	Electron transfer
<i>fixK</i>	Regulatory
<i>fixLJ</i>	Regulatory, two-component sensor/effector
<i>fixNOQP</i>	Electron transfer
<i>fixGHIS</i>	Transmembrane complex

Nitrogen fixing organisms

- Free living aerobic bacteria
 - *Azotobacter*
 - *Beijerinckia*
 - *Klebsiella*
 - *Cyanobacteria* (lichens)
- Free living anaerobic bacteria
 - *Clostridium*
 - *Desulfovibrio*
 - *Purple sulphur bacteria*
 - *Purple non-sulphur bacteria*
 - *Green sulphur bacteria*
- Free living associative bacteria
 - ❖ – *Azospirillum*
- Symbionts
 - *Rhizobium* (legumes)
 - *Frankia* (alden trees)

Free-living N₂ Fixation

Energy

- 20-120 g C used to fix 1 g N

Combined Nitrogen

- *nif* genes tightly regulated
- Inhibited at low NH₄⁺ and NO₃⁻ (1 μg g⁻¹soil, 300 μM)

Oxygen

- ✓ Avoidance (anaerobes)
- ✓ Microaerophilly Respiratory protection
- ✓ Specialized cells (**heterocysts**, vesicles)
- ✓ Spatial/temporal separation
- ✓ Conformational protection

Associative N₂ Fixation

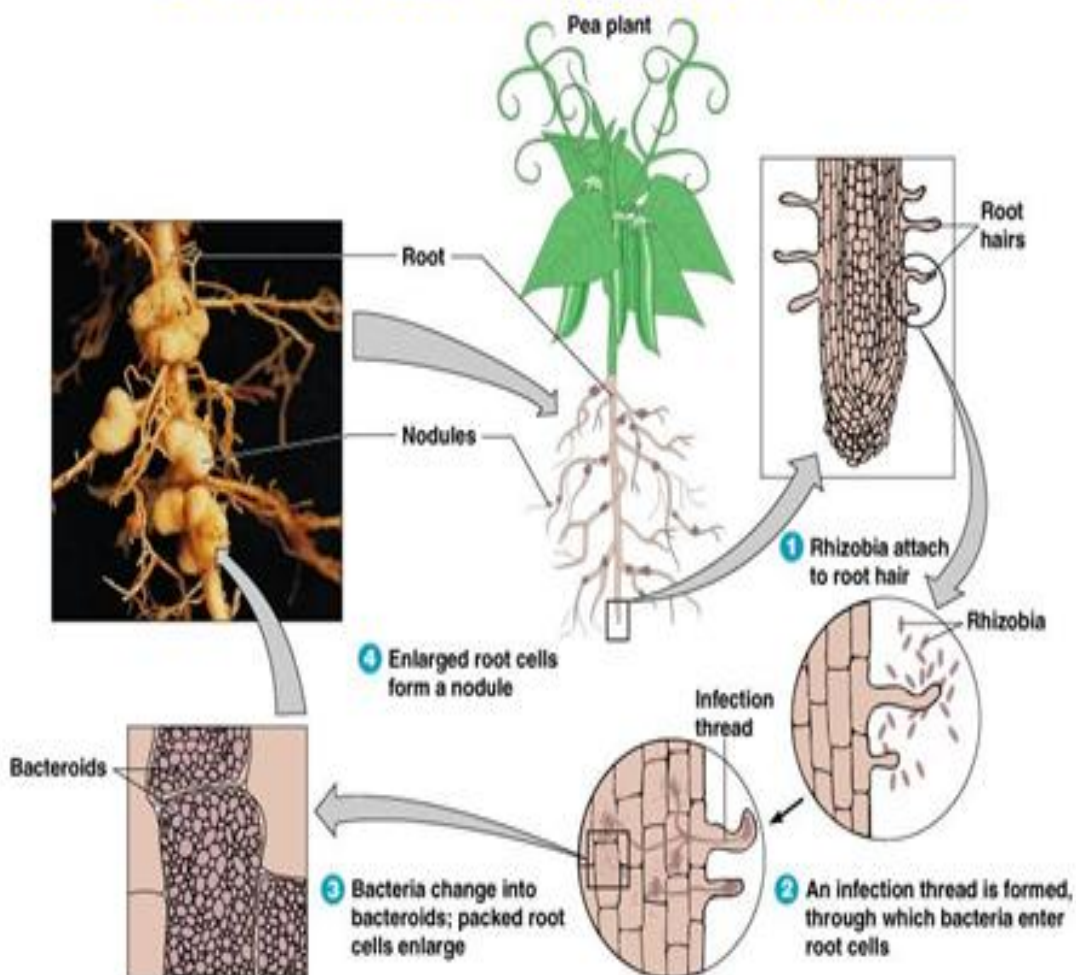
- Phyllosphere or rhizosphere (tropical grasses)
- *Azospirillum*, *Acetobacter*
- 1 to 10% of rhizosphere population

- Some establish within root
- Same energy and oxygen limitations as free-living
- *Acetobacter diazotrophicus* lives in internal tissue of sugarcane, grows in 30% sucrose, can reach populations of 10^6 to 10^7 cells g^{-1} tissue, and fix 100 to 150 kg N $ha^{-1} y^{-1}$

Phototrophic N₂-fixing Associations

- Lichens—cyanobacteria and fungi
- Mosses and liverworts—some have associated cyanobacteria
- *Azolla-Anabaena (Nostoc)*—cyanobacteria in stem of water fern
- *Gunnera-Nostoc*—cyanobacteria in stem nodule of dicot
- *Cycas-Nostoc*—cyanobacteria in roots of gymnosperm

Formation of a Root Nodule



Infection Process:

- Attachment
- Root hair curling
- Localized cell wall degradation
- Infection thread
- Cortical cell differentiation
- Rhizobia released into cytoplasm
- Bacterioid differentiation (symbiosome formation)
- Induction of nodulins

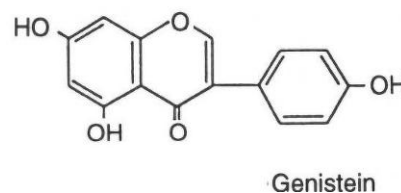
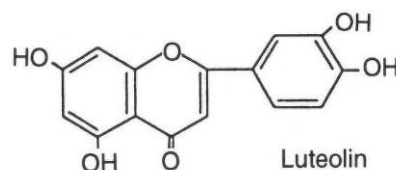
Role of Root Exudates:

General

- Amino sugars, sugars

Specific

- Flavones (luteolin), isoflavones (genistein), flavanones, chalcones
- Inducers/repressors of *nod* genes
- Vary by plant species
- Responsiveness varies by rhizobia species



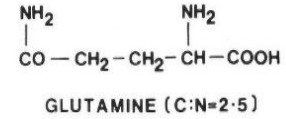
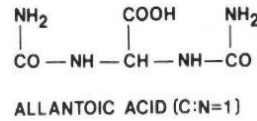
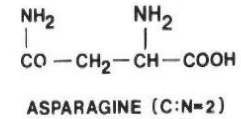
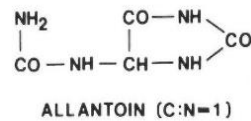
Nodule Metabolism:

✚ Oxygen metabolism

- ✓ Variable diffusion barrier
- ✓ Leghemoglobin

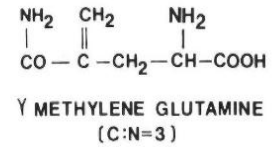
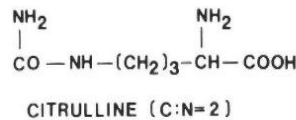
✚ Nitrogen metabolism

- NH_3 diffuses to cytosol
- Assimilation by GOGAT
- Conversion to organic-N for transport



✚ Carbon metabolism

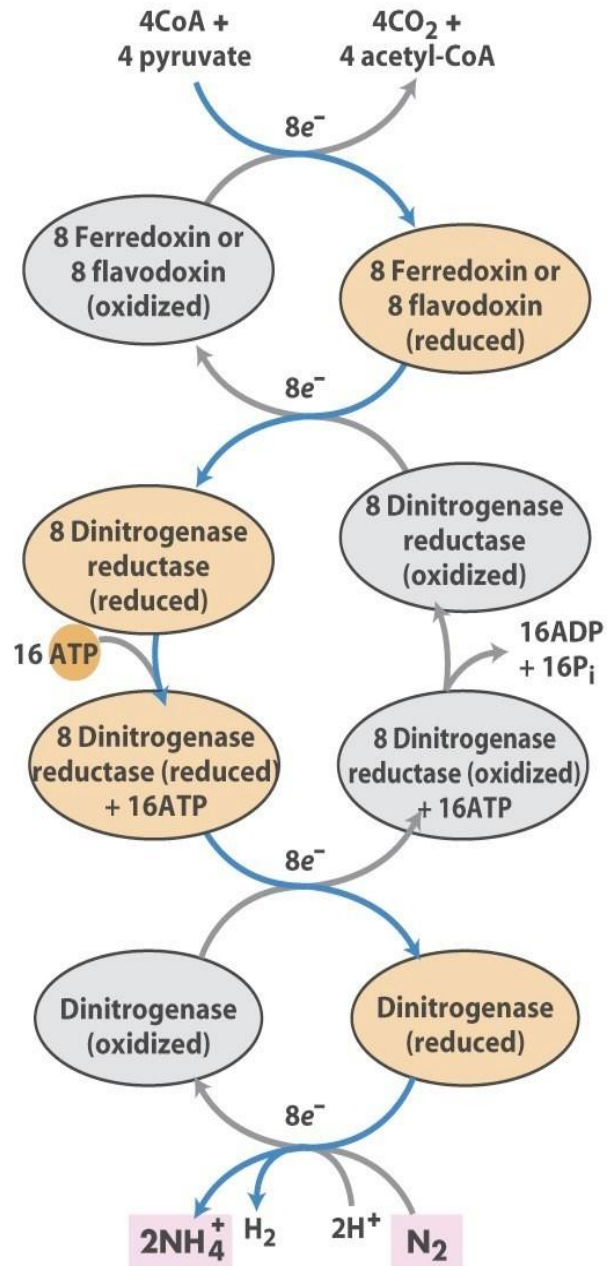
- ✓ Sucrose converted to dicarboxylic acids
- ✓ Functioning TCA in bacteroids
- ✓ C stored in nodules as starch

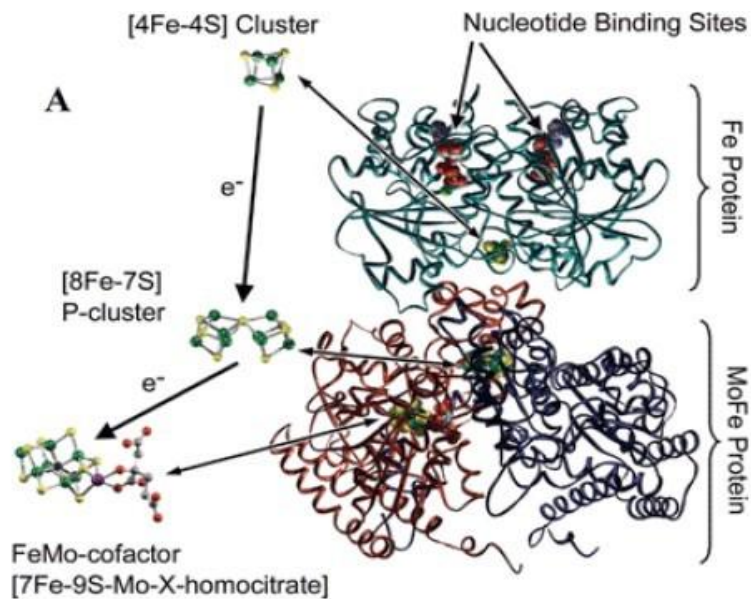
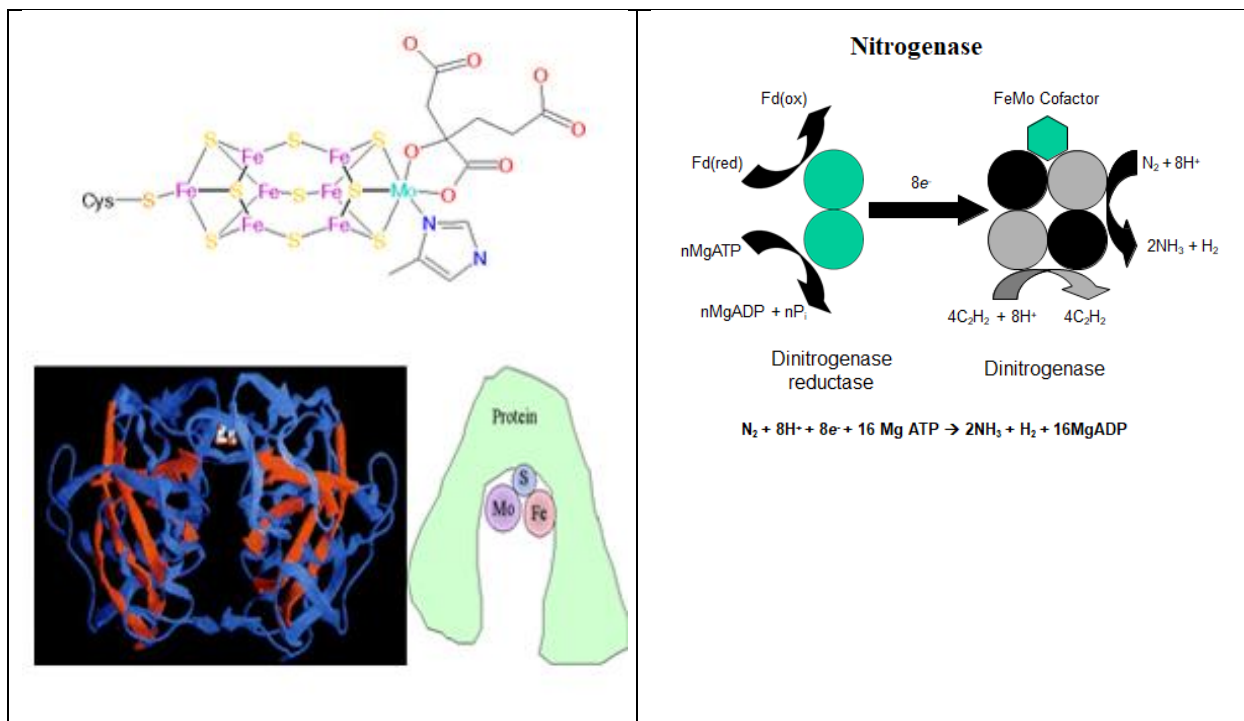


Nitrogen Fixation

- All nitrogen fixing bacteria use highly conserved enzyme complex called **Nitrogenase**

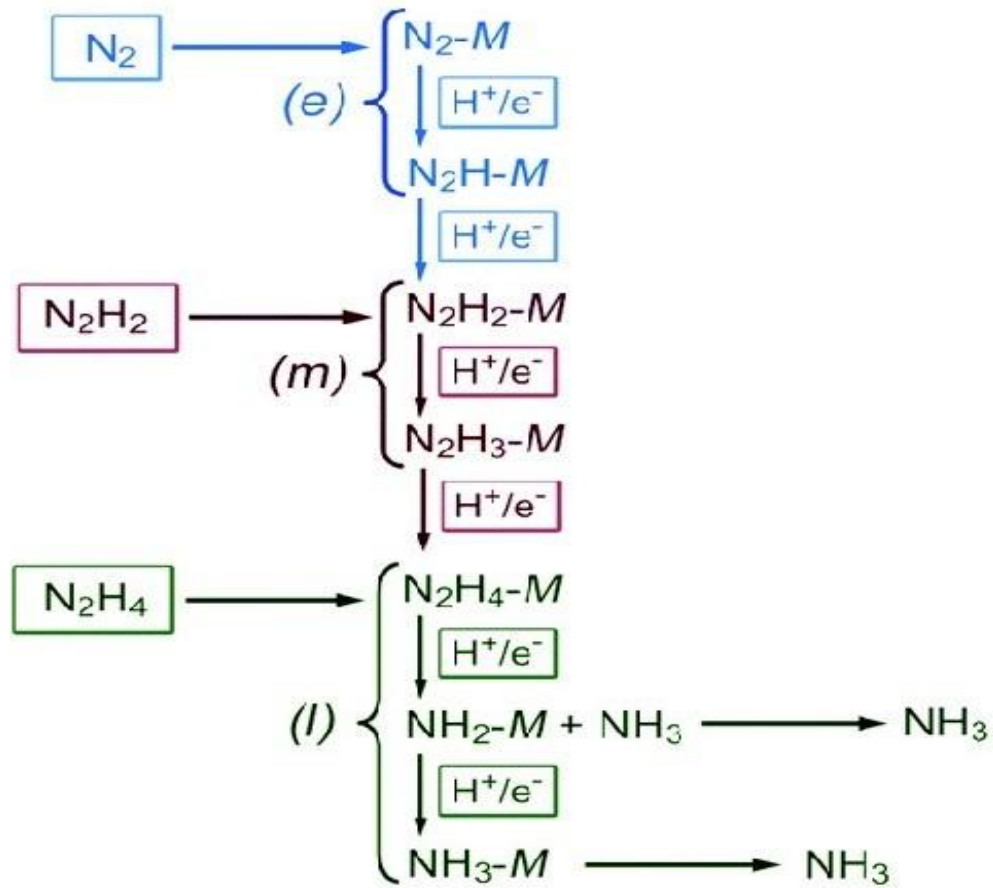
- Nitrogenase is composed of two subunits: an **iron-sulfur protein** and a **molybdenum-iron-sulfur protein**
- Aerobic organisms face special challenges to nitrogen fixation because nitrogenase is inactivated when oxygen reacts with the iron component of the proteins.





Nitrogenase proteins and cofactors. (A) Shown is one half of the nitrogenase complex, with one $\alpha\beta$ pair of the MoFe protein $\alpha_2\beta_2$ tetramer and one bound Fe protein dimer. The location of bound nucleotides and the three metal clusters are also shown.

Barney BM, et al., Dalton Trans. 2006, 2277-84.



Mechanism of phosphate solubilization and phosphate mobilization

Dr. AJEET KUMAR¹⁵

INTRODUCTION

Much of the soluble phosphorus applied as fertilizer to soil is fixed into one of the many sparingly soluble forms (tricalcium phosphate, aluminium phosphate, Iron phosphate and manganese phosphate) and only 10-18 % is utilized by the standing crop. A variety of mechanism have been accounted for the increased mobilization of soil phosphorus in plant rhizosphere such as excretion of organic acids (Moghimi *et al.* 1978), exudation of reducing substances (Gardner, 1982), the production of mucilage and sloughed off root cells which provide an energy substrate for microbial activity in the rhizosphere which subsequently dissolve mineral and organic phosphorus (Banik and Dey, 1982), Hydrolysis of organic phosphorus by phosphatase enzymes (Helal and Sauerbeck, 1984). Many phosphates solubilizing microorganism isolated from the rhizosphere of different crops predominantly of the genera *Bacillus polymixa*, *B. megaterium*, *B. circulans* *Pseudomonas striata*, species of *Aspergillus* and *Penicillium* have shown the ability to solubilise sparingly soluble phosphate *in vitro* as well as *in vivo* condition by secreting the organic acid and inorganic acids. Kumar and Maurya (2006) observed a more rapid initial release of phosphorus from tricalcium phosphate in the presence of the fungal species of *Aspergillus sulfurious*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, and *Rhizopus stolonifer*. Fungi in soils are able to traverse distances more easily than bacteria thus it is more important to phosphorus solubilisation (Kucey, 1983). The solubilizing microorganisms convert insoluble inorganic phosphatic compounds into soluble form. Such microbes not only assimilate phosphorus but they cause a large portion of soluble phosphate to be released in quantities in the excess of their own requirement. With this view an attempt has been made to summarize the information's about the mechanisms of microbial phosphorus solubilisation.

PHOSPHATE FIXATION AND RETENTION

Phosphate ions attracted to soil constituents with such a bond that they become insoluble and difficultly available to plants. This process is called phosphate fixation or phosphate retention.

Phosphate Fixation in Acidic Soil

Acid soils usually contain significant amount of soluble and exchangeable Al^{+3} , Fe^{3+} , and Mn^{2+} ions. These ions serve as bridge in between clay and phosphate ions. **Precipitation and**

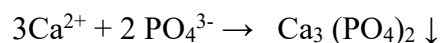
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Adsorption: The phosphate ion enters into a chemical reaction with these free metal ions. $\text{Al}^{3+} + 3 \text{H}_2\text{PO}_4 \rightarrow \text{Al} (\text{H}_2\text{PO}_4)_3 \downarrow$

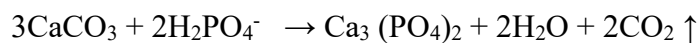
The product formed is difficultly soluble in water and precipitates in solution. With the passage of time the Al phosphate ppt become less soluble consequently less available to plants. Phosphate is fixed through adsorption on the surface of hydrous oxide of iron and aluminium.

Phosphate Fixation in Alkali Soils

An alkaline soil contains high amounts of soluble and exchangeable Ca^{2+} and CaCO_3 . Phosphate is reported to react with the ionic and carbonate form of calcium.



Insoluble



Insoluble

Bacteria (PSB)

Bacillus polymixa

Bacillus subtilis

Bacillus megaterium

Bacillus circulans

Pseudomonas striata

Pseudomonas rathonia

Fungi (PSF)

Aspergillus niger

Aspergillus flavus

Aspergillus sulfuriosus

Aspergillus awamori

Aspergillus wentii

Aspergillus fomitopsis

Penicillium fuscum

Penicillium digitatum

Penicillium bilaii

Penicillium lilacinum

PHOSPHATE SOLUBILISING MICROORGANISM (PSM)

Fungal species are more efficient solubilises than bacteria.

MECHANISM OF PHOSPHATE SOLUBILIZATION

1. Production of organic acids
Lowering of pH
Chelating effect
2. Production of inorganic acid
3. Production of CO₂
4. Production of H₂S
5. The production of mucilage and sloughed off root cells.

Production of organic acids

Phosphate solubilising microorganism produces various kinds of organic acids which are the main cause of solubilisation of insoluble phosphate by lowering the pH. Amount of phosphate dissolution depends up on many factors like types of organic acids (**Monocarboxylic acid**: Formic acid, Acetic acid, Lactic acid, Gluconic acid; **Dicarboxylic acid**: Oxalic acid, Tartaric acid, Malic acid, Succinic Acid; **Tricarboxylic acid**: Citric acid) produced such as aliphatic, aromatic; Structures of organic acids and amount of organic acids.

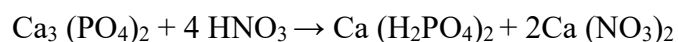
CHELATION

The nonvolatile organic acids mainly responsible for phosphate liberation in solution possibly by their chelating action. These acids were produced as a result of the decomposition of glucose in

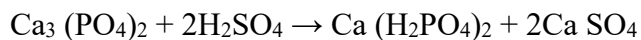
Pikovskaya's medium (1948) by *Bacillus megaterium*, *Bacillus circulans*, *Escherichia freundii*, *Penicillium digitatum* and *Penicillium fuscum*.

Production of inorganic acid

Nitrifying bacteria like *Nitrosomonas* and *Nitrobacter* produces the nitric acids which reacts with the insoluble phosphate and convert into soluble form. The possible chemical reactions are as follows:

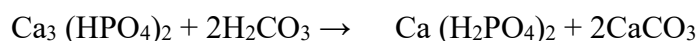
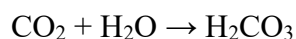


Similarly sulphur oxidizing bacteria like *Thiobacillus* spp. Oxidizes the sulphur containing compounds and produces SO₂ which react with water and form sulphuric acid which reacts with the insoluble phosphate and convert into soluble form.



Production of CO₂

The CO₂ produced by microorganism and plant roots is responsible for solubilization of inorganic phosphate in soil.



Production of H₂S

The H₂S produced by sulphate reducing bacteria (*Desulfovibrio*) particularly in the anaerobic condition.



Exudation of organic compounds from plant root

A variety of organic substances available at the root region, which directly or indirectly influences the quality and quantity of microorganism.

1. Sugars : Glucose, fructose, sucrose, maltose.
2. Amino acids : glutamine, glycine, methionine.
3. **Organic acid** : *Tartaric, oxalic, citric, butyric, Succinic, Fumaric, glycolic.*
4. Fatty acids and sterols: Palamitic, stearic, oleic, linolenic, cholesterol, sitosterol.
5. Nucleotides, flavonones and enzymes: flavonones; Adenine, guanine, uridine, cytidine; phosphatase, invertase, proteinase.

Mucilage and sloughed off root cells provide an energy substrate for microbial activity in the rhizosphere which subsequently dissolve insoluble phosphorus.

Conclusions

- When soluble phosphates are added to soil, it is quickly fixed into insoluble forms, which greatly reduces the efficiency of phosphatic fertilizers.
- In acidic soils, the phosphorus is fixed primarily by Fe, Al, and Mn and in alkaline soils by Ca and Mg.
- Production of organic acid in microenvironment of root zone of plant is the main cause of phosphorus solubilization.
- PSF are more effective in solubilization than PSB.

- ✦ Besides concentration of organic acid, types of organic acid produced by PSM is more important.

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Mechanism of K solubilization by Potassium Solubilizing Bacteria (KSB)

Dr. AJEET KUMAR¹⁶

Potassium is one of the essential macronutrients for plant growth and development, playing a crucial role in various physiological processes such as enzyme activation, photosynthesis, and nutrient transportation. However, a significant portion of soil potassium exists in insoluble forms, making it inaccessible to plants. Enter potassium solubilizing bacteria (KSB) - a group of remarkable microorganisms capable of converting insoluble potassium into forms that plants can readily absorb. The potential of KSB to enhance soil fertility and boost crop yields has garnered significant interest in agricultural research and sustainable farming practices. By harnessing the natural capabilities of these bacteria, farmers can reduce their dependence on chemical fertilizers, leading to more eco-friendly and cost-effective farming solutions. This discovery not only promises to revolutionize traditional farming methods but also contributes to the broader goal of sustainable agriculture. In this article, we delve into the fascinating world of potassium solubilizing bacteria, exploring their mechanisms of action, methods for their screening and their potential applications in agriculture. Join us as we unlock nature's secret to more productive and sustainable farming.

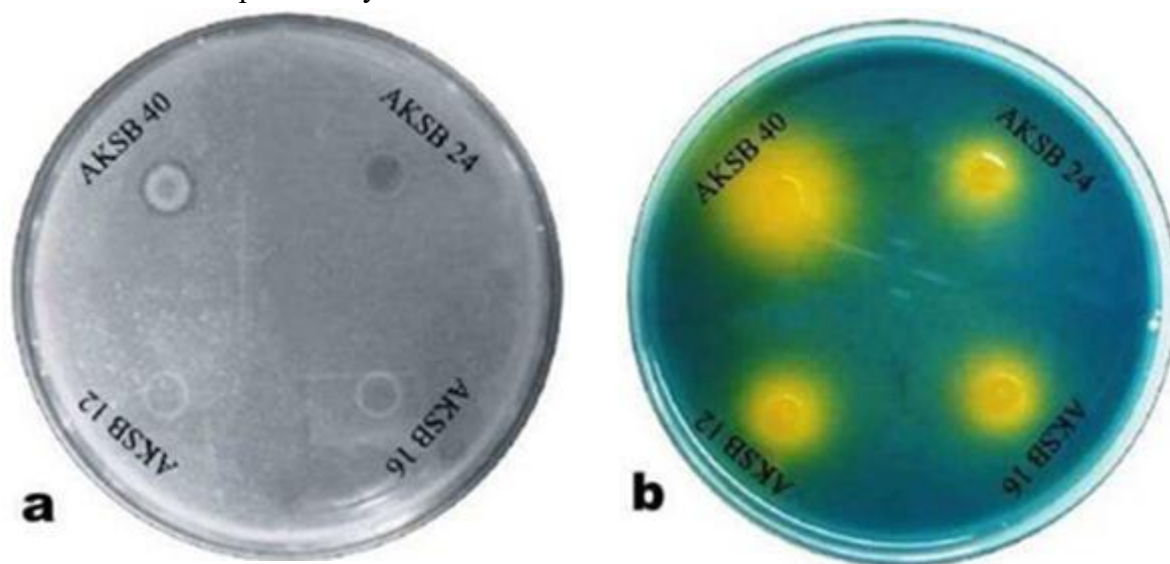
Introduction:

Potassium (K) solubilization is done by a wide range of saprophytic bacteria, fungal strains and Actinomycetes. There are strong evidences that soil bacteria are capable of transforming soil K to the forms available to plant effectively. There is considerable population of KSB in plant rhizosphere. These include both aerobic and anaerobic isolates however the most frequently KSB in soil are aerobic in nature. A considerably higher population of KSB is commonly found in the rhizosphere in comparison with non-rhizosphere soil. Solubilization of K by KSB from insoluble and fixed forms is an import aspect regarding K availability in soils. The ability to solubilize the silicate rocks by *B. mucilaginosus*, *B. circulanscan*, *B. edaphicus*, *Burkholderia*, *A. ferrooxidans*, *Arthrobacter* sp., *Enterobacter hormaechei*, *Paenibacillus mucilaginosus*, *Cladosporium*, *Aminobacter*, *Sphingomonas*, *Burkholderia* and *Paenibacillus glucanolyticus* has been reported. Among the soil bacterial communities, *B. mucilaginosus*, *B. edaphicus* and *B. circulanscan* have been described as effective K solubilizers. KSB are usually present in all soils and have been isolated from rhizosphere soil, non-rhizosphere soil, paddy soil and saline soil. KSB are isolated by serial dilution plate method using modified Aleksandrov medium including 5.0 g glucose; 0.5 g MgSO₄.7H₂O; 0.1 g CaCO₃; 0.006 g FeCl₃; 2.0 g Ca₃(PO₄)₂; 3.0 g potassium aluminium silicate; and 20.0 g agar in 1 L of deionized sterile water. The pH of this medium is adjusted to 7.2 by

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adding 1 N NaOH. The plates are incubated at $28 \pm 2^\circ\text{C}$ in biological oxygen demand incubator for 3-4 days. The colonies exhibiting clear zones are selected and diameter of the solubilization zone is calculated and the values are recorded for each sample.

Recently, a modified plate assay for rapid screening of KSB has been developed. This assay is based on improved visualization of halo zone formation around the colonies on agar plates, through inclusion of an acid-base indicator dye (bromothymol blue, BTB), to modify the Aleksandrov medium. This assay is also time saving, more sensitive and beneficial in comparison to the Aleksandrov plate assay.



K solubilization on Aleksandrov agar plate

K solubilization on modified agar medium plate

Figure 1. Comparison of Potassium Solubilization on Aleksandrov Agar Plate (a) and Modified Agar Medium Plate (b) After 72 Hours of Incubation.

Quantitative estimation of K solubilization is performed by flame photometry wherein culture broth is centrifuged and supernatant is used for precipitation of cobalt nitrite. Standard curve for quantification of K is prepared using various concentrations of KCl solution. In this assay, mica is usually used as a source of insoluble form of K, although other K sources were also used in screening KSB (e.g., insoluble magnesium trisilicate, muscovite, illite powder, montmorillonite, kaolinite, potassium-feldspar, biotite, waste mica, bentonite, wood ash and potassium aluminium silicate). In addition, the amount of K solubilization in different culture media (e.g., different pH, temperature, K source, and carbon source) is different. For example, the amount of K solubilization by *B. edaphicus* in the liquid media was more and a better growth was detected on illite than feldspar. In general, the microbial solubilization of K is strongly influenced by pH, oxygen, the bacterial strains used and kind of K bearing minerals; in fact, moderate alkalinity favours the solubilization of silicate.

Mechanisms of K solubilization by KSB

It is generally believed that microorganisms contribute to the release of K^+ from K-bearing minerals by several mechanisms. Released H^+ can directly dissolve the mineral K as a result of slow releases of exchangeable K, readily available exchangeable K. As occurs in the case of P

solubilization, the major mechanism of K mineral solubilization is by production the organic and inorganic acids and production of protons (acidolysis mechanism) which are able to convert the insoluble K (mica, muscovite, and biotite feldspar) to soluble forms of K, easily taking up by the plant.

The types of various organic acids such as oxalic acid, tartaric acids, gluconic acid, 2-ketogluconic acid, citric acid, malic acid, succinic acid, lactic acid, propionic acid, glycolic acid, malonic acid, fumaric acid, etc. have been reported in KSB, which are effective in releasing K from K-bearing minerals. It has also observed that the type of the organic acid produced by KSB is different. Among the different organic acids involved in the solubilization of insoluble K, tartric acid, citric acid, succinic acid, α -ketogluconic acid and oxalic acid are the most prominent acids released by KSB.

Microbial decomposition of organic materials also produces ammonia and hydrogen sulfide that can be oxidized in the soil to form the strong acids such as nitric acid and sulphuric acid. Hydrogen ions displace K^+ , Mg^{2+} , Ca^{2+} and Mn^{2+} from the cation exchange complex in a soil. In addition to decreasing soil pH, organic acids produced by KSB can release of K ions from the mineral K by chelating (complex formation) Si^{4+} , Al^{3+} , Fe^{2+} and Ca^{2+} ions associated with K minerals. It was reported that KSB weathered phlogopite via aluminum chelation and acidic dissolution of the crystal network. In addition, The *B. altitudinis* strain accelerates weathering of K feldspar, change mineral surface morphology and induce the formation of new mineral complex. This strain dissolved K feldspar and significantly released more Si, Al and Fe by producing more organic acids. The KSB have a considerable role in proving K to plant by storing K in their biomass, which is potentially available to plants. It has been reported that the production of various extracellular polymers (primarily proteins and polysaccharides) can also led to release of K from K bearing minerals. These substances serve as attachment structures to mineral surface. Solution containing fresh microbial exopolysaccharides increases the dissolution rate of feldspars probably by forming complexes with framework ions in solution. KSB also synthesize biofilms, which create controllable microenvironments around microbial cells for weathering. Biofilm formation on aluminosilicate increases the residence time of water as compared to the residence time at the bare mineral surface and enhances the mineral weathering.

It was accepted that the microbial biofilms not only accelerated the weathering process but also regulated denudation losses by acting as a protective layer covering the mineral-water-hyphal/root hair interface in the mycorrhizosphere and rhizosphere of vascular plants. Besides, biofilm formation on mineral surface promoted the corrosion of potassium rich shale and the release of K, Si and Al in the bacteria-mineral contact model. In addition, it is known that the release of organic acids from the plant roots can be effective in enhancing mobilization of mineral K. Therefore, it can be suggested that other PGPRs (e.g., IAA producing bacteria) can also have a role in providing K for plant by increasing root exudates. In general, the most important mechanisms known in K mineral solubilization by KSB are (i) by lowering the pH; (ii) by enhancing chelation of the cations bound to K; and (iii) acidolysis of the surrounding area of microorganism.

Potentialities of KSB

KSB can accelerate weathering of K minerals; especially when they are in direct contact with mineral surfaces by different action mechanisms. Attempts have been made to use of K mobilizing bacteria for solubilizing K from different K bearing minerals and hence to improve plant nutrition. Although KSB could be an alternative and viable technology to solubilize insoluble K into soluble form. The application in agricultural practice is still prevented by several factors, example, (i) lack of awareness about bio-fertilizers amongst the farmers; (ii) slow influence of the K biofertilizer on crop yield; (iii) less interest in scientific community on the development of K biofertilizer technologies; (iv) culture collection banks not yet developed for KSB due to the loss of efficient strains developed by scientists; and (v) and deficiency in technology in respect to product formulations are the major limitations.

Conclusions

K-bearing minerals significantly contribute to potassium (K) fertilization for crop plants, which can only absorb K from the soil solution. While K can be released into the soil solution from insoluble minerals, this release is typically insufficient to meet plant requirements due to the low concentration of soluble K and its relative immobility in the soil. Consequently, K-containing fertilizers are commonly applied in extensive agricultural systems to supply available K. However, these fertilizers are expensive and their long-term use leads to increased input costs, reduced agricultural profitability, and numerous environmental problems due to the accumulation of heavy metals in the soil and plant systems. The use of potassium solubilizing bacteria (KSB) offers a promising alternative to solubilize soil K reserves and make them available to plants, thereby promoting plant growth and reducing the need for K-fertilizers. K solubilization is facilitated by a variety of bacteria, including *B. mucilaginosus*, *B. edaphicus*, *B. circulans*, *Pseudomonas*, *Burkholderia* and *Acidithiobacillus ferrooxidans*. Research has demonstrated that KSB can dissolve K from insoluble K-bearing minerals by secreting organic acids. The primary mechanism through which KSB make K available to plants involves the production of organic acids, which can either directly increase dissolution through proton or ligand-mediated mechanisms or indirectly by forming complexes in solution with reaction products. Thus, applying KSB as biofertilizers not only enhances plant growth and yield but also reduces the need for agrochemicals, supporting eco-friendly crop production. These technologies are becoming increasingly vital in modern agricultural practices, especially given the changing agricultural landscape and environmental hazards associated with chemical fertilizers, which call for a greater role for biofertilizers in the future.

Future Perspectives

To fully realize the potential of KSB, several areas of research and application need to be explored:

1. Conduct field studies to assess the potential of KSB techniques in agricultural production systems and evaluate their impacts on crop growth and soil behaviour.
2. Investigate the role of KSB in increasing the availability of other nutrients affected by pH, such as phosphorus (P), nitrogen (N), iron (Fe), and zinc (Zn).

3. Study the effects of other plant growth-promoting rhizobacteria (PGPRs) such as indole-3-acetic acid (IAA) producers, ACC deaminase producers, phosphate solubilizers, and nitrogen fixers on K availability.
4. Examine the interactions (both positive and negative) between KSB and other PGPRs concerning K availability.
5. Determine the optimal conditions for KSB activity, including the role of organic matter.
6. Evaluate plant species that are effective in K uptake and support K solubilizing microbial populations.
7. Understand the mechanisms of KSB to determine the best bacterial strains for use with specific plants in various geographic regions.
8. Study the stability of KSB inoculants in the soil and assess soil mineralogical properties before inoculation.
9. Conduct further research on the molecular biology of KSB, as there is currently limited information compared to other PGPRs.
10. Addressing these areas will enhance our understanding and application of KSB in sustainable agriculture

FCO (Fertilizer Control Order) Specifications of Biofertilizers

Dr. AJEET KUMAR¹⁷

1. Introduction

The **Fertilizer Control Order (FCO), 1985**, regulates the quality, production, and distribution of fertilizers in India, including biofertilizers. The FCO ensures that biofertilizers meet minimum microbial and physical standards for efficacy and safety.

Learning Objectives:

- ✓ Understand the FCO guidelines for biofertilizers.
- ✓ Learn the microbial and carrier specifications.
- ✓ Discuss labeling, packaging, and storage requirements.

2. Importance of FCO Specifications

- ✓ Ensures **minimum viable microbial count** for effectiveness.
- ✓ Prevents **substandard or contaminated products** in the market.
- ✓ Standardizes **production, testing, and labeling** for farmer trust.

3. FCO-Approved Biofertilizers & Their Specifications

A. Bacterial Biofertilizers

Biofertilizer	Microorganism	Minimum Viable Cells	Contamination Limit
Rhizobium	<i>Rhizobium spp.</i>	1×10^9 CFU/g	No contamination at 10^{-6} dilution
Azotobacter	<i>Azotobacter spp.</i>	1×10^8 CFU/g	No contamination at 10^{-6} dilution
Azospirillum	<i>Azospirillum spp.</i>	1×10^8 CFU/g	No contamination at 10^{-6} dilution

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Biofertilizer	Microorganism	Minimum Viable Cells	Contamination Limit
Phosphate-Solubilizing Bacteria (PSB)	<i>Bacillus</i> , <i>Pseudomonas</i>	1×10^8 CFU/g	No contamination at 10^{-6} dilution

B. Fungal Biofertilizers

Biofertilizer	Microorganism	Minimum Viable Cells	Contamination Limit
Phosphate-Solubilizing Fungi (PSF)	<i>Aspergillus</i> , <i>Penicillium</i>	1×10^8 CFU/g	No contamination at 10^{-6} dilution
Mycorrhiza (AMF)	<i>Glomus</i> , <i>Gigaspora</i>	100 propagules/g	No contamination at 10^{-6} dilution

C. Carrier-Based vs. Liquid Biofertilizers

- ✓ **Carrier-based (Solid):** Peat, lignite, or charcoal (pH 6.5–7.5, moisture <30%).
- ✓ **Liquid Formulations:** Must maintain 1×10^8 CFU/mL for 6 months.

4. Physical & Chemical Specifications (FCO Guidelines)

- ✓ **Moisture Content:** ≤ 30 –40% (carrier-based).
- ✓ **pH:** 6.5–7.5 (neutral range for microbial survival).
- ✓ **Particle Size:** 90% should pass through 150–212 μm sieve.
- ✓ **Contaminants:**
 - No pathogenic bacteria (e.g., *E. coli*, *Salmonella*).
 - Heavy metals (As, Cd, Pb) within permissible limits.

5. Labeling & Packaging Requirements (FCO Compliance)

Mandatory Information:

- Name of biofertilizer & strain (e.g., *Rhizobium leguminosarum*).
- CFU count at production & expiry date.
- Batch number, manufacturing date, and expiry (shelf life: 6–12 months).

- Storage instructions (e.g., "Store in cool, dry place").

Prohibited Claims:

- False promises (e.g., "100% yield increase").
- Unapproved microbial strains.

6. Quality Control & Testing Methods

- **Serial Dilution & Plate Count:** To check CFU/g.
- **PCR & DNA Sequencing:** For strain authentication.
- **Field Trials:** To validate performance under different agro-climatic conditions.

7. Recent Amendments & Challenges

- **New Additions (2023):** *Trichoderma* (biocontrol agent) included under FCO.

Challenges:

- Adulteration with inert materials.
- Poor shelf life due to improper storage.

8. Case Study: Impact of FCO Regulations

- **Before FCO:** Inconsistent biofertilizer quality → low farmer trust.
- **After FCO:** Standardized products → increased adoption in organic farming.

9. Conclusion

FCO specifications ensure **quality, efficacy, and safety** of biofertilizers. Compliance is essential for sustainable agriculture and reducing chemical fertilizer dependency.

10. Discussion Questions

- Why is a **minimum CFU count** necessary for biofertilizers?
- How does **carrier material** affect microbial survival?
- What improvements can be made in FCO regulations for better enforcement?

11. References

- **Fertilizer Control Order (FCO), 1985** (Amended 2023).
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Quality Control of Biofertilizers

Dr. AJEET KUMAR¹⁸

Formulation:

Inoculation of the carrier with the culture broth means the mixing of broth and carrier. This operation must be done in aseptic conditions to avoid any contamination.

Quality control of formulation:

The quality of the carrier-based inoculum depends upon the viable cell count and the presence or absence of contaminants.

- A good culture must contain about 10^7 – 10^8 viable cells or CFUs per g of culture.
- No contaminants are permissible at 10^{-5} – 10^{-6} dilutions. These critical values differ according to the type of biofertilizer.
- As per I.S.I., in case of Rhizobium, the carrier based culture must contain at least 10^7 cell or CFUs per g of culture and no contaminants are permissible below 10^{-6} dilution. In case of Azotobacter, So, the enumeration of cells density and contaminants are important task in the production of carrier based microbial biofertilizer.

Bioactivity of Azotobacter:

Nitrogenase is the enzyme catalyzing the reduction of nitrogen into ammonia. This enzyme can also reduce acetylene into ethylene as well. Acetylene and ethylene can easily be measured by a gas chromatograph. In a closed system, if a portion of gas is substituted by acetylene and acetylene is allowed to be reduced for a certain period, the proportion of acetylene and ethylene can be measured by passing the mixture of gas through the column of gas chromatograph and measuring the peak developed.

The nitrogenase activity (hence, the nitrogen fixation activity) of Azotobacter or other free living bacteria can be extrapolated by **Acetylene Reduction Assay (ARA)** method, but this technique can not be applied in case of Rhizobium as this bacteria can not fix nitrogen in free condition. In this case the plant containing the nodules is to be taken for assay.

Bioefficacy of Rhizobium (Nodulation):

The study of nodulation efficiency of Rhizobium is an important task in Biofertilizer Technology. The number of viable cells in the inoculum can easily be enumerated by dilution plating method but this technique will not show whether the viable cells have retained their nodulation efficiency during the long term laboratory procedure like preservation, production and storage. This technique will enumerate the number of viable cells, which have retained the

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nodulation capability, this is well known that this nitrogen-fixing bacteria can not fix nitrogen unless it is symbiotically harboured in the nodule of leguminous plant.

Application of Microbial Biofertilizers

Application of the microbial biofertilizer is an important step in the Biofertilizer Technology. If the microbial inoculant is not applied properly, the benefits from the biofertilizer may not be obtained. During application one should always remember that the most of the microbial biofertilizers are heterotropic, i.e. they can not prepare their own food and depend upon the organic carbon of soil for their energy requirement and growth. So, they either colonise in rhizosphere zone or live symbiotically within the root of higher plants. The bacteria which are colonised in the rhizosphere zone obtain their organic carbon compounds from the root exudes of the higher plants. The symbiotic ones obtain organic carbon directly from the root. So, microbial inoculants must be applied in such a way that the bacteria will be adhered with the root surface. So, in case of transplanting crops, the inoculant are applied through roots, and in case of the crops in which seeds are sown directly in the field, the inoculants are applied through the seeds so that they can colonize in the rhizosphere region when the young roots are emerged after germination of seed. On the basis of the above principal, the following inoculation methods have been developed:

1. Inoculation of the seeds by slurry inoculating technique
2. Inoculation of seeds by seed pelleting technique
3. Inoculation of the seedlings
4. Inoculation of the soil by solid inoculation technique
5. Inoculation of soil by liquid inoculation technique

Mass Production of Carrier Based and Liquid Based Biofertilizers

Dr. AJEET KUMAR¹⁹

Media Preparation and Starter Culture

Bacteria require different nutrients for their growth. These include: a) organic carbons source, b) nitrogen source and c) a variety of other elements dissolved in water. Blue Green Algae (BGA) that can fix atmospheric carbon dioxide, does not require any carbon source and the nitrogen-fixing bacteria, which can fix atmospheric nitrogen, do not require any nitrogen source. A medium is an aquatic solution of a variety of organic and inorganic compounds that can supplement the above requirements for the growth of different microorganisms. Generally, media are of two types:

- a) General media and
- b) Specific media.

General media is constituted for the growth of most of the microorganisms. Such a medium contains all the ingredients required for the growth of any microorganism. A specific medium is constituted for the growth of specific or specific group of microorganisms. During constitution of such medium, one or few additional components are added and /or one or few components are deleted from the general media depending upon the requirement of the specific microorganism. Again, according to the physical appearance, media are of two types:

- a) Liquid media and
- b) Solid media.

The liquid medium is solidified by the addition of solidifying agent-agar-agar. Liquid medium can harbour bacterial growth suspended in the media, whereas solid medium harbours microbial growth on the surface. Solid media may be prepared as slant or plate.

Sterilization of medium in autoclave:

The media is then autoclaved at 121⁰C temperature and 15lb pressure. The process is as follows

- ✓ Check the water level of the autoclave to note whether the heating coil is completely immersed in water, add water, if necessary.
- ✓ Put the conical flasks, petridishes and the beaker with test tubes inside the basket of the autoclave.
- ✓ Set the lid, tie the screws of the autoclave and switch on the power supply.
- ✓ Keep the outlet of the steam open and wait until the inside air is completely released from the autoclave and only steam is coming out.

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- ✓ When the inside air is completely released close the outlet and observe the meter indicating the inside pressure.
- ✓ When the pressure rises to 15 lbs, adjust the regulating valve to keep the pressure constant.
- ✓ Keep this pressure for 30 min and then close the regulating valve and switch off the power supply
- ✓ When the pressure comes down to zero, open the regulatory valve to release the steam and then open the lid and take out the contents.

Prepare slants and plates:

The slants are necessary for culture storage. The slant preparation process is as follows:

- Put the culture tubes containing medium in slanting position on the table with the help of a wooden blade and allow cooling down. The medium will be solid after cooling down and thus slants are prepared.
- Take the conical flask containing molten media with agar-agar and the petridishes in the laminar flow cabinet and allow the medium to cool down to 50°C.
- Open each petridish by slightly lifting the upper lid, pour 15-20 ml of medium and close the lid.
- Keep to cool down and solidify and thus plates are prepared.

Preparation of Starter Culture:

- ❖ The starter culture is a little amount of bacterial suspension, which is added to the medium to start the growth of that bacterium. Twin flask is a pair of flasks of identical size joined together by a latex tube. For the preparation of starter culture, this type of flask is used. Each flask contains a side arm below the neck position. The latex tube joining the two flasks is held together by this glass tube. The benefit of the use of the twin flask is that contamination can be avoided.

Fermentation:

- ✚ A fermenter is a device in which the optimum conditions for the microbial growth and activity is established artificially. This device may be used for the production of microbial metabolites such as antibiotics or enzymes; it may also be used for the growth of microorganisms i.e. production of microorganism itself.
- ✚ A low-cost production unit has been developed for the production of microbial inoculants to be used as biofertilizers such as *Rhizobium*, *Azotobacter*, and Phosphate Solubilising Bacteria.
- ✚ This device can be prepared by investing small amount of money as compared to the scientific fermenter used in laboratories for research purposes

Sterilization of the fermenter:

Fermenter is a metallic vessel for moist sterilization of any article. The principle of moist sterilization lies in the fact that when water is boiled in a closed system, the water vapour produced due to boiling accumulates within the vessel and increases the inside pressure. Thus, the boiling point of water increases beyond 100°C, which is the boiling point of water in normal atmospheric pressure. In this condition, the steam, released from the boiling water is of higher temperature. If any article placed in this vessel in such condition, the high temperature destroys the microorganisms present in or on the article.

Inoculation, Growth, Quality Testing and Termination of growth:

Inoculation means addition of starter culture to the medium in the fermenter. For the production of microbial biofertilizers a small amount of suspension of the desired bacterium in pure form is inoculated to the medium. Care should be taken to maintain the quality of starter culture, as extent of purity (no contaminants should be allowed), size of the starter culture (in terms of culture volume and density of cell) and stage of growth.

Greater the size of starter culture, lesser the chance of contamination. If the starter culture is inoculated in its log phase, rapid initial growth will occur. Maintenance of proper physical and chemical environment inside the fermenter is essential for proper growth of microorganism.

Quality testing, in this case, is enumeration of cell density and its purity in the broth. When the cell density reaches the desired level, growth is terminated and the culture is ready for mixing with carrier. The time period required for optimum cell density is thus standardized.

Carrier Preparation

Carrier is a medium, which can carry the microorganisms in sufficient quantities and keep them viable under specified conditions and easy to supply to the farmers. A good carrier should have the following qualities:

- Highly absorptive (water holding capacity) and easy to process.
- Non-toxic to microorganisms.
- Easy to sterilize effectively.
- Available in adequate amount and low cost.
- Provide good adhesion to seed.
- Has good buffering capacity.

Different carriers are available in the market like, Charcoal, Peat, Lignite, Rice husk etc.

- But considering all the above qualities Azolla powder is the most suitable carrier in this region. This is due to
 - ♣ It has high water holding capacity (360%).
 - ♣ It has good pH buffering capacity.
 - ♣ It contains nutrient so bacteria can remain viable for a long period.
 - ♣ It is easily available in this region.

Biofertilizers-Storage, Shelf Life and Marketing

Dr. AJEET KUMAR²⁰

Biofertilizers: Storage, Shelf Life, and Marketing

Biofertilizers are microbial inoculants containing living microorganisms that enhance soil fertility and plant growth by fixing atmospheric nitrogen, solubilizing phosphorus, or producing growth-promoting substances. Proper **storage, shelf-life management, and effective marketing** are crucial for maintaining their efficacy and commercial success.

1. Storage of Biofertilizers

Proper storage ensures microbial viability and product effectiveness. Key factors include:

A. Temperature Control

- ✓ **Optimal Storage Temp:** 4°C to 10°C (refrigeration preferred).
- ✓ **Avoid High Temperatures:** >30°C kills microbes.
- ✓ **Avoid Freezing:** Can rupture microbial cells.

B. Moisture & Humidity

- ✓ **Dry Conditions:** Moisture encourages contaminant growth.
- ✓ **Airtight Packaging:** Prevents moisture absorption.

C. Light Exposure

- ✓ **UV Light Harmful:** Store in **dark or opaque containers**.

D. Packaging Material

- ✓ **Polyethylene bags** (for carrier-based biofertilizers).
- ✓ **Glass/Plastic Bottles** (for liquid formulations).

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2. Shelf Life of Biofertilizers

The shelf life depends on the microbial strain, carrier material, and storage conditions.

Biofertilizer Type	Shelf Life (Months)	Storage Condition
Rhizobium	6–12	4–10°C (Refrigerated)
Azotobacter / Azospirillum	6–9	Room Temp ($\leq 30^{\circ}\text{C}$)
Phosphate Solubilizers (PSB)	6–12	Cool & Dry Place
Liquid Biofertilizers	12–24	Room Temp (Avoid Sun)

- ✓ **Liquid formulations** generally last longer than carrier-based ones.
- ✓ **Expiry Date:** Should be clearly mentioned on the package.

3. Marketing of Biofertilizers

- ✓ Effective marketing strategies are essential for farmer adoption and commercial success.

A. Target Audience

- ✓ **Farmers** (organic & conventional).
- ✓ **Horticulturists & Floriculturists.**
- ✓ **Government Agricultural Schemes.**

B. Marketing Strategies

1. Awareness Campaigns

- Farmer training programs, workshops, and demonstrations.
- Collaboration with **Krishi Vigyan Kendras (KVKs)** and NGOs.

2. Branding & Packaging

- Attractive, informative labels with usage instructions.
- Highlight benefits (e.g., "Increases yield by 20%").

3. Distribution Channels

- **Agri-input dealers**, cooperatives, and online platforms.
- Government subsidies (e.g., **Paramparagat Krishi Vikas Yojana - PKVY**).

4. Digital Marketing

- Social media, YouTube tutorials, and e-commerce (Amazon, BigHaat).

5. Certifications & Quality Assurance

- **ISI, FCO (Fertilizer Control Order), or ISO certification.**
- Lab testing reports for credibility.

C. Challenges in Marketing

- 🌐 **Farmer skepticism** (preference for chemical fertilizers).
- 🌐 **Short shelf life** (requires efficient supply chain).
- 🌐 **Competition from synthetic fertilizers.**

Conclusion

- **Storage:** Maintain **cool, dry, and dark conditions** to prolong shelf life.
- **Shelf Life:** Ranges from **6–24 months**, depending on formulation.
- **Marketing:** Requires **awareness, branding, and strong distribution networks.**

Biofertilizers have immense potential in **sustainable agriculture**, but their success depends on proper handling and effective promotion

Application technology of biofertilizers for seeds, seedlings, tubers, sets

Dr. AJEET KUMAR²¹

1. Introduction

Biofertilizer application requires specialized techniques to ensure optimal microbial colonization. This lecture covers precision application methods for different planting materials.

Learning Objectives:

- ✓ Master seed treatment technologies
- ✓ Learn seedling inoculation methods
- ✓ Understand tuber/set treatment protocols
- ✓ Compare traditional vs advanced application systems

2. Seed Application Technologies

A. Slurry Treatment (Most Common)

Procedure:

1. Prepare 10% sugar/jaggery solution (adhesive)
2. Mix biofertilizer @ 500g/kg seed
3. Coat uniformly, shade-dry for 30 mins
4. Sow within 24 hours

Advantages:

- Cost-effective (<₹2/acre)
- Suitable for small seeds (pulses, cereals)

Limitations:

- Short window between treatment & sowing

B. Advanced Seed Coating Methods

Technology	Specifications	Crops
Film Coating	Polymer + microbes (5-20µm layer)	Hybrid maize, vegetables
Encapsulation	Alginate beads (2-3mm)	High-value seeds
Bio-Priming	Hydration (12h)-Dehydration with microbes	Rice, wheat

Efficacy Data:

- Film coating increases rhizosphere colonization by 40%
- Bio-priming enhances germination rates by 15-25%

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3. Seedling Treatment Methods

A. Root Dip Technique

For: Paddy, tomato, cabbage transplants

Protocol:

1. Prepare 10L suspension (1kg biofertilizer + water)
2. Dip roots for 30-60 minutes
3. Transplant immediately

Modified Approaches:

- **Gel Dip:** Carboxymethyl cellulose-based (extends viability)
- **Mycorrhizal Dip:** For perennial crops

**B. Nursery Bed Application

Method:

1. Mix 5kg biofertilizer with 100kg FYM
2. Apply to 1,000m² nursery bed
3. Cover with thin soil layer

Best For:

- Vegetable seedlings
- Forest species propagation

4. Tuber & Set Treatment

A. Tuber Application (Potato, Turmeric)

Step-by-Step:

1. Prepare slurry (1kg biofertilizer + 50L water)
2. Spray on tubers (5kg/treatment for 1 acre)
3. Dry in shade before planting

Innovation:

1. **Wax Coating:** Beeswax + microbes extends shelf-life

B. Set Treatment (Sugarcane, Banana)

Two Approaches:

1. **Furrow Application:**
 - 10kg biofertilizer + 50kg sand per acre
 - Apply in planting furrows
2. **Direct Coating:**
 - Biofilm application on suckers
 - Uses starch-based adhesives

5. Equipment for Large-Scale Application

Equipment	Capacity	Suitable For
Rotary seed treater	500kg/hour	Seed cooperatives
Vacuum coater	Precision coating	Research stations

Equipment	Capacity	Suitable For
Dip-tank systems	10,000 seedlings/hour	Commercial nurseries

Cost Comparison:

- Manual treatment: ₹50/acre
- Mechanical coating: ₹120/acre (but 5× faster)

6. Recent Advances

- ✓ **Nano-film Coatings:** 100nm microbial layers
- ✓ **Drone Spraying:** For field-established crops (5L/ha)
- ✓ **Smart Stickers:** pH-sensitive release on seed surface

7. Field Validation Data

ICAR Trials (2023):

- Proper seed treatment gave 22% yield boost in chickpea
- Root dip increased tomato survival rate by 18%

8. Best Practice Guidelines

1. Always use fresh batches (<3 months old)
2. Avoid chemical fungicide contact for 48 hours
3. Morning/evening application reduces UV damage
4. Combine with organic amendments (FYM, compost)

9. Discussion Questions

1. Compare economics of manual vs mechanical application
2. How would you adapt these methods for arid regions?
3. Design a quality check protocol for treated seeds

10. References

- ✓ FAO (2022) *Microbial Inoculant Application Manual*
- ✓ ICAR Handbook (2023) *Biofertilizer Application Technologies*
- ✓ Journal of Applied Microbiology (2024) *Nano-coating Innovations*

Factors Influencing the Efficacy of Biofertilizers

Dr. AJEET KUMAR²²

1. Introduction

Biofertilizers are microbial inoculants that enhance soil fertility and plant growth by fixing atmospheric nitrogen, solubilizing phosphorus, or producing growth-promoting substances. However, their effectiveness depends on multiple biotic and abiotic factors.

Learning Objectives:

1. Understand the key factors affecting biofertilizer performance.
2. Analyze the role of environmental and management practices.
3. Discuss strategies to optimize biofertilizer efficacy.

2. Key Factors Influencing Biofertilizer Efficacy

A. Microbial Strain Characteristics

1. Strain Selection

- Specificity of strains to host plants (e.g., *Rhizobium* for legumes).
- Competitiveness against native soil microbes.
- Genetic stability and adaptability.

2. Viability and Shelf Life

- Survival during storage and transportation.
- Carrier material quality (e.g., lignite, peat, liquid formulations).

B. Soil Factors

1. Soil pH

- ✓ Most biofertilizers (e.g., *Rhizobium*, *Azotobacter*) prefer neutral pH (6.5–7.5).
- ✓ Acidic soils inhibit microbial activity; liming may be required.

2. Soil Moisture

- Optimal moisture (40–60% water-holding capacity) is crucial.
- Drought reduces microbial mobility, while waterlogging causes anaerobiosis.

3. Soil Temperature

- Mesophilic bacteria (25–30°C) are most common.
- Extreme temperatures (>40°C or <10°C) reduce efficiency.

4. Soil Organic Matter

- Enhances microbial proliferation and nutrient cycling.
- Low organic carbon limits biofertilizer survival.

5. Soil Salinity and Heavy Metals

- High salinity inhibits nitrogen fixation and P-solubilization.
- Heavy metals (Cd, Pb) are toxic to beneficial microbes.

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C. Environmental Conditions

○ Climate

- Rainfall affects leaching and microbial dispersal.
- UV radiation can reduce microbial viability in surface applications.

○ Seasonal Variations

- Kharif (rainy season) vs. Rabi (winter) crops may require different strains.

D. Host Plant Factors

1. Plant Species and Variety

- Specificity (e.g., *Rhizobium* works best with legumes).
- Root exudates influence microbial colonization.

2. Plant Growth Stage

- Early application (seed treatment/seedling dip) improves colonization.
- Late-stage application may be less effective.

E. Agricultural Practices

1. Inoculation Method

- Seed coating, soil application, or foliar spray.
- Liquid formulations show better adherence than carrier-based ones.

2. Chemical Fertilizers and Pesticides

- High N-P-K fertilizers suppress N-fixing bacteria.
- Pesticides/fungicides may be toxic to biofertilizer microbes.

3. Crop Rotation and Intercropping

- Legume-non-legume rotation enhances soil N content.
- Continuous monoculture reduces microbial diversity.

4. Tillage Practices

- Minimum tillage preserves microbial communities.
- Deep plowing may disrupt established biofertilizer networks.

3. Strategies to Enhance Biofertilizer Efficacy

- ✓ **Combined Inoculation:** Co-inoculation of N-fixers + P-solubilizers (e.g., *Rhizobium* + *Pseudomonas*).
- ✓ **Biochar Amendment:** Improves soil structure and microbial retention.
- ✓ **Encapsulation Techniques:** Protects microbes from environmental stress.
- ✓ **Farmer Awareness:** Proper storage, application timing, and dosage.

4. Case Studies/Recent Advances

- **PGPR (Plant Growth-Promoting Rhizobacteria):**
Bacillus and *Pseudomonas* strains enhancing drought resistance.
- **Nano-Biofertilizers:** Improved delivery systems for better microbial survival.

5. Conclusion

The efficacy of biofertilizers is influenced by microbial, soil, plant, and management factors. Optimizing these parameters can maximize their benefits, reducing dependency on chemical fertilizers.

6. Discussion Questions

1. How does soil pH affect the performance of phosphate-solubilizing bacteria?
2. Compare the advantages of liquid vs. carrier-based biofertilizers.
3. What integrated approaches can farmers adopt to improve biofertilizer efficiency?