

MICROBIAL BIOTECHNOLOGY

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Lecture-23 **Lec 23: Microbes in Agriculture**

Hello friends, welcome to my course on microbial biotechnology. Today we are starting module number 7, which will deal with microbes in agriculture, biofertilizers, microbial pesticides, and finally the concept of integrated pest management. So, let us start with the overall role of microbes in agriculture. So, we have divided this lecture into various sections. We start with microbes, the frenemies of agriculture.

They are both friends and enemies of agriculture at the same time. Then we'll discuss the microbiome in soil, then microbial groups in soil and their interactions, then microbes in soil fertility and crop production, and plant growth promotion and phytohormones. We'll talk about sections two and three as we go to them in the subsequent slides. Before we begin, let us make some comparisons between the health of an individual and the health of the ecosystem. So, all of you know that our gut is full of microbes, and so is our skin.

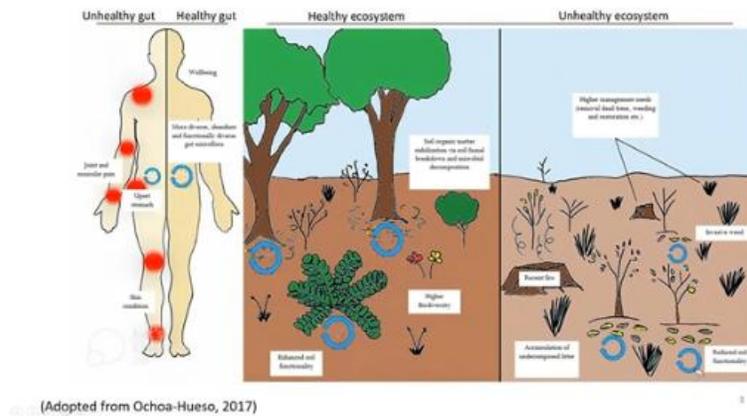
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Now, we have two types of microbes that inhabit us in the gut as well as on our skin. Some are beneficial, and others are harmful. The harmful ones cause us diseases like upset stomach, skin infections, and other disease conditions, but there are many beneficial gut microflora that help us in our well-being. Similarly, if you look into the ecosystem, We have diverse groups of microbes inhabiting every niche of these ecosystems.

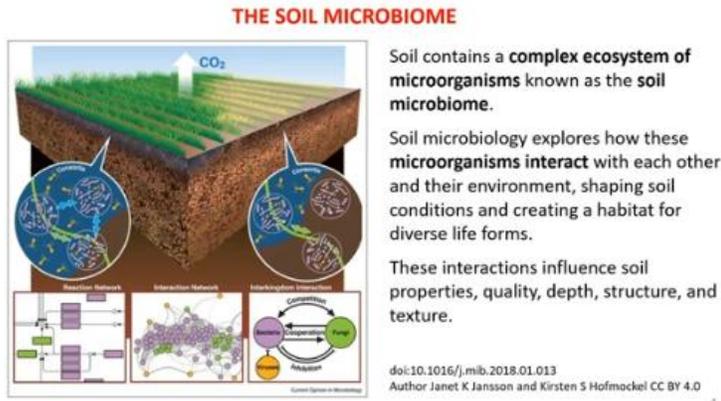
Some of them are beneficial and some of them are harmful. So, the beneficial ones help the ecosystem by recycling nutrients. We have learned about this in earlier slides as well as maintaining the homeostasis of the ecosystem. Now, the health and well-being of an individual is the balance between the good and the bad microbes and many of these good microbes actually help us in controlling the bad microbes and it is also the other way around sometimes the bad microbes dominate over the good microbes depending on the ecological balance whether it is a micro environment or the macro environment. And in an unhealthy ecosystem where there is some kind of unhealthy human interventions, the bad microbes may dominate and the good microbes may altogether disappear further deteriorating the environment.

So similar would be the case if we do not maintain good hygienic practices or food habits or even when we consume a lot of antibiotics, a similar condition may happen to us in the individual level. Now you already have some idea about the soil being inhibited by both beneficial and harmful microbes. So, this whole soil environment contains a complex ecosystem of microorganisms and together we call them as the soil microbiome in the way we call the gut flora as the gut microbiome. The science of soil microbiology explores how these microorganisms interact with each other in the environment, shaping soil conditions and creating habitat for diverse life forms. So, these are the most lowest forms of organisms which inhabit and actually make the soil amenable for other higher organisms like plants to survive on them.



And these interactions influence the soil properties, the quality of the soil, the depth of the soil structure and even the texture. So, what the microbes do in the soil, what are their functions? There are various functions. Number one, they act as decomposers, so helping in the decompositions of organic matter and then some of them produce phytohormones or

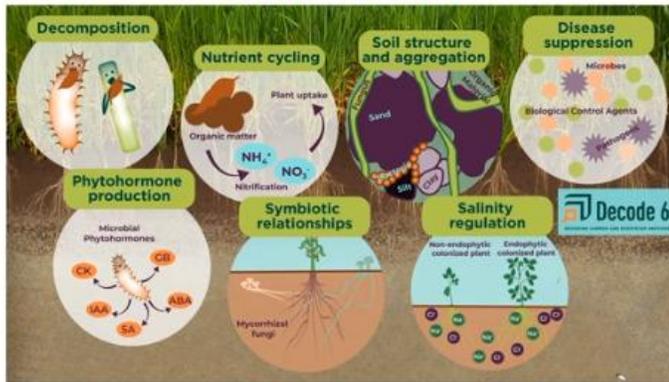
microbial phytohormones and these phytohormones help the plants in their growth and development. And they also do nutrient recycling, cycling where they decompose the organic matter into simple substances and nutrients which is taken up by the plants.



And then they sometimes develop; some of these microbes develop symbiotic relationships. And that is where both organisms are benefited and are of mutual interest. And then they also help in soil structure and aggregation. For example, the sand may be aggregated with the help of certain microbes like fungi and bacteria. And these may be bound to the clay particles, or two sand particles may be bound to one another, thereby helping the aggregation and solidification of the soil structure.

And then many microbes help in regulating the salinity of soil, thereby making the soil, you know, conducive for the growth of plants. The microbes also help in disease suppression, and we can use many of these as biocontrol agents against certain pathogens. So overall, we can see that without microbes in the soil, the soil would actually become very, very uninhabitable or, you know, non-friendly to higher organisms like plants. So, what are the different groups of microbes in soil? So, we may have bacteria, actinomycetes, fungi, soil algae, protozoa, and bacteriophages.

ROLE OF SOIL MICROBES



You can see the various examples of these, like *Agrobacterium*, *Actinomyces*, then *Aspergillus* fungi, and *Anabaena* algae. And then you have nematodes, and then Colpoda, and then, of course, we have bacteriophages, which are not microbes. They are also present in soil and contribute to the ecological balance. So, when it comes to bacteria, they are the most dominant group of microorganisms in soil and are involved in organic matter decomposition, biogeochemical cycling of elements, and antibiotic production.

Actinomycetes are capable of degrading complex organic substances, contributing to soil fertility, and they are notable for antibiotic production. Fungi are the second most dominant organisms after bacteria. They prevail in acidic soils but can thrive in all types of soils, influenced by environmental factors and organic matter content. Soil algae are ubiquitous, visible as green scum where moisture and sunlight are present. Cyanophyceae fix nitrogen, and these are influenced by environmental factors, as in the case of other microbes. The protozoa are unicellular organisms with cysts in their life cycle.

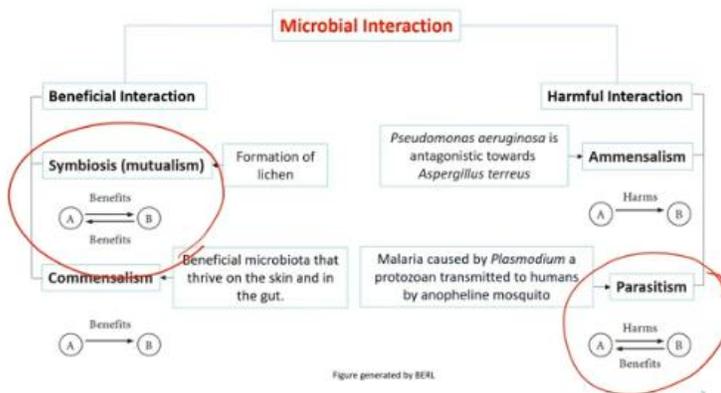
They prefer specific bacterial species for nutrition, abundant in the upper soil layer, depending on the bacterial population. How do microbes interact with one another? One type of interaction is beneficial, while the other is harmful. In beneficial interaction, they form a symbiotic relationship, which is called symbiosis or mutualism. Here, microbe A and B or organism A and B benefit each other, as you can see by the two-directional arrows here.

MICROBIAL GROUPS IN SOIL

Soil Microorganisms	Examples	Description
Bacteria	<i>Agrobacterium</i> <i>Bacillus</i> <i>Clostridium</i> <i>Pseudomonas</i>	Most dominant group of soil microorganisms, involved in organic matter decomposition, biogeochemical cycling of elements, and antibiotic production.
Actinomycetes	<i>Actinomyces</i> <i>Nocardia</i> <i>Streptomyces</i>	Capable of degrading complex organic substances, contributing to soil fertility. Notable for antibiotic production.
Fungi	<i>Aspergillus</i> <i>Fusarium</i> <i>Alternaria</i> <i>Cladosporium</i>	Second dominant group after bacteria. Prevalent in acidic soils but can thrive in all types of soil. Influenced by environmental factors and organic matter content.
Soil algae	<i>Anabaena</i> <i>Oscillatoria</i> <i>Nostoc</i>	Ubiquitous, visible as green scum where moisture and sunlight are present. Cyanophyceae fix nitrogen; influenced by environmental factors.
Protozoa	<i>Cilipoda</i> <i>Nematodes</i> <i>Pleurotricha</i> <i>Heteromita</i>	Unicellular organisms with cysts in their life cycle, preferring specific bacterial species for nutrition. Abundant in the upper soil layer, depending on bacterial population.
Bacteriophages	T4 Bacteriophages	

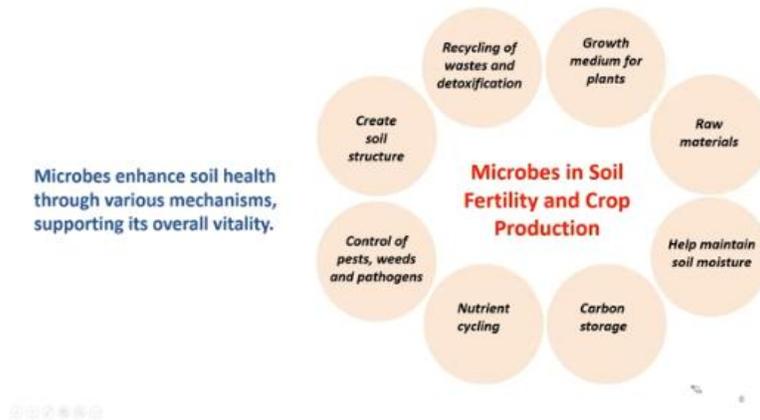
And in certain cases, this benefit is only one-directional. So, A benefits B, but B does not benefit A. Thus, this relationship or interaction is known as commensalism. We have, for example, microbiota that thrive on the skin and in the gut. They help us in many ways. Then there are harmful interactions like amensalism, where A harms B. *Pseudomonas aeruginosa* is antagonistic towards *Aspergillus terreus*, and there is parasitism, where A harms

B, but B actually benefits A. For example, malaria is caused by *Plasmodium*, a protozoan transmitted to humans by *Anopheles* mosquitoes. So, we have to observe parasitism and symbiosis a little more clearly, where symbiosis is the case where both organisms benefit. But in parasitism, one organism benefits while the other is harmed. So, what are the different advantages of having soil microbes? They basically play a major role in soil fertility and, thereby, in crop production.



They enhance soil health through various mechanisms and support its overall vitality. For example, they create soil structure, which we have just studied, recycle waste, and detoxify

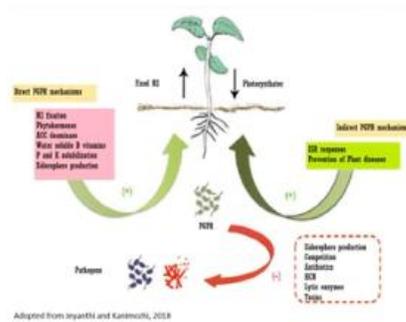
harmful compounds. They serve as a growth medium for plants, provide raw materials, and help maintain soil moisture. They also act as carbon storage, aid in nutrient recycling, and often control pests, weeds, and pathogens. This is very important for us in this particular lecture.



Let us now look into plant growth-promoting rhizobacteria, which we call PGPR, that enhance plant growth, particularly in pathogen-free conditions. They act as eco-friendly biocontrol agents by competing for resources, excluding pathogens, and producing antifungal metabolites like hydrogen cyanide, phenazines, and pyrrolnitrin. They also induce systemic resistance in plants through jasmonate and ethylene signaling, bolstering plant defenses. Key bacterial components such as lipopolysaccharides, flagella, siderophores, and volatiles play crucial roles in ISR, promoting plant health and resilience. So, you can see here that PGPR can help directly by carrying out nitrogen fixation, releasing phytohormones, ACC deaminase, water-soluble B vitamins, P and K solubilization, mineral solubilization, and siderophore production. They also help indirectly by generating ISR responses, preventing plant diseases, and controlling pathogens.

PLANT GROWTH-PROMOTING RHIZOBACTERIA(PGPR)

PGPR enhance plant growth, particularly in pathogen-free conditions.



They act as eco-friendly biocontrol agents by competing for resources, excluding pathogens, and producing antifungal metabolites like hydrogen cyanide (HCN), phenazines, and pyrrolnitrin.

PGPR also induce systemic resistance (ISR) in plants through jasmonate and ethylene signaling, bolstering plant defenses. Key bacterial components such as lipopolysaccharides (LPS), flagella, siderophores, and volatiles play crucial roles in ISR, promoting plant health and resilience.

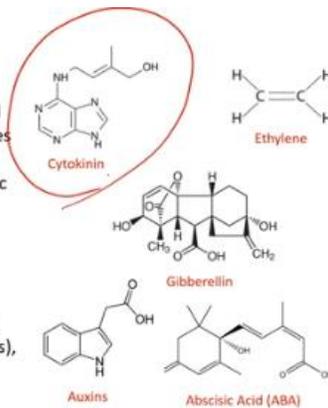
Let us now have a small discussion on phytohormones produced by microbes which we call as microbial phytohormones. So, they plant physiological functions like growth, development and responses to environment stimuli are regulated by various phytohormones such as abscisic acid, salicylic acid, cytokinins, gibberellins, brassinosteroids, auxines, jasmonic acid and ethylene. These hormones interact cooperatively or antagonistically. So, plant growth promoting bacteria, PGPB, can synthesize or decrease many phytohormones and produce volatile organic compounds which enhance plant growth and influence phytohormone expression. phytohormone structures like cytokinin, ethylene, then gibberellin, auxin and abscisic acids which are also produced by plant and many other organisms.

MICROBIAL PHYTOHORMONES

Plant physiological functions like growth, development, and responses to environmental stimuli are regulated by various phytohormones such as abscisic acid, salicylic acid, cytokinins, gibberellins, brassino-steroids, auxins, jasmonic acid, and ethylene.

These hormones interact cooperatively or antagonistically.

Plant growth-promoting bacteria (PGPB) can synthesize or degrade certain phytohormones and produce volatile organic compounds (VOCs), which enhance plant growth and influence phytohormone expression.



Azospirillum, brasilense and bacillus species produce these cytokinins. which promotes cell division and overall plant growth especially in the shoot and the root portion. Rhizobacteria like azospirillum and bacillus produce indole acetic acid that helps root growth grow longer and stronger improving the plant's ability to absorb water and nutrients. Bacillus licheniformis and Bacillus pumilus produce gibberellins which help plant grow

taller, develop more balanced branches and form healthy roots. So, here we can see plant phytohormones which some of which act as growth promoters like auxin, gibberellin, cytokinin, salicylic acid, ACC deaminase,

And then we have growth inhibitors, ethylene abscisic acid and jasmonate. And there are bacterial substances that affect the hormonal regulation of plant by producing growth promoters like auxins, and then gibberellins, and then cytokinins, salicylic acids, ACC, and also volatile organic compounds. And it also produces growth inhibitors like ethylene and abscisic acid. The role of plant microbiome, phytohormones in different plant growth activities. So, plant microbiomes producing, for example, gibberellic acid, auxin, and brassinosteroid.

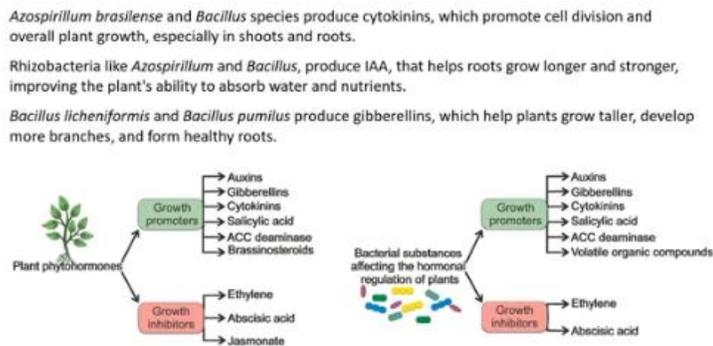
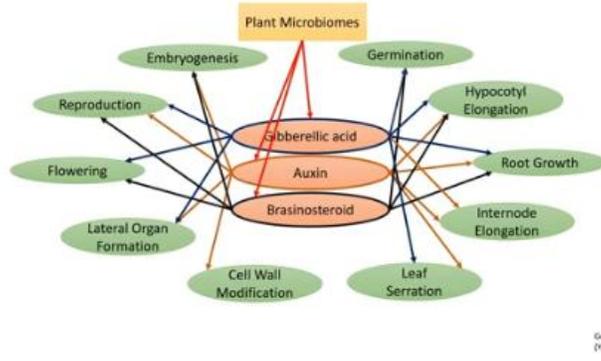


Fig. Classification of plant phytohormones and bacterial substances based on their impact on plant growth. (Plants 2024, 13(17), 2371, CC BY 4.0)

So, they help in different ways. Gibberellic acid, for example, helps in reproduction, flowering, and lateral organ formation. Auxin helps in reproduction. And then lateral organ formation and cell wall modification. Similarly, gibberellic acid may also play a role in germination, leaf serration, and root growth.

Auxins also help in root growth. So, these are, as you can see, many roles played by the phytohormones released by microbes. Overall, microbial phytohormones enhance plant stress tolerance through various mechanisms as well. So, root-associated microbes produce hormones which enhance the plant's antioxidant defenses, reducing oxidative stress. They improve photosynthesis, stabilize cell membranes, promote cell division, regulate stromal function, enhance root growth, and increase water and nutrient uptake.

Role of plant microbiome phytohormones in different plant growth activities

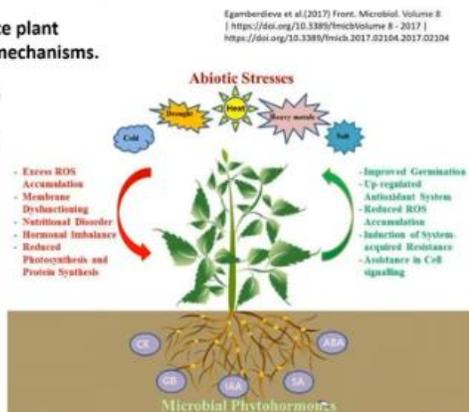


So, when a plant is under abiotic stress like heat, drought, heavy metal salt, or even cold, it will have excess ROS accumulation, membrane dysfunction, nutritional disorder, and hormonal imbalance. It reduces photosynthesis and protein synthesis, and the phytohormones released by microbes help in improved germination, upregulated antioxidant systems, and reduced ROS, which was increased due to this stress. Then, the induction of system-acquired resistance and assistance in cell signaling occur. So, we see that microbial phytohormones play a very crucial role in enhancing stress tolerance in plants. So, this is a list of certain bacteria which produce phytohormones, and it also shows the phytohormones they produce, which overall mitigate abiotic stress in certain selected plants listed here.

Microbial phytohormones enhance plant stress tolerance through various mechanisms.

Root-associated microbes produce hormones which:

- Enhance the plant's antioxidant defenses, reducing oxidative stress.
- Improve photosynthesis and stabilize cell membranes.
- Promote cell division and regulate stomatal function.
- Enhance root growth and increase water and nutrient uptake.



These *Pseudomonas* species and *Bacillus* species produce indole acetic acid in certain plants like *Sulla carnosa*. And then we have *Bacillus licheniformis*, which also produces IAA in *Triticum aestivum*. And then we have others and a host of other microorganisms which produce indole acetic acid from here to here in diverse plant systems like *Cucumis*, *Hordeum*, *Acacia*, *Zea mays*, and so on. Then we also have some other organisms like

Achromobacter, which produces an assay that is helpful in providing Helianthus annuus stress tolerance. Then we have Micrococcus luteus and several others like Bacillus subtilis, which produce cytokinin.

And then we have Azospirillum, which produces gibberellic acid, and others which produce abscisic acid. There are some organisms which produce a host of phytohormones together, whether it is indole acetic acid, gibberellic acid, or abscisic acid. Let us now discuss an important topic: climate resilience and how microbes help in adapting to climate resilience challenges. So, let us start with the role of microbes in stress response against climate resistance. We will also discuss bioremediation and certain biochemical cycling of nutrients in this particular section.

Microorganism	Phytohormone	Host plant, abiotic stress
Pseudomonas sp., Bacillus sp.	IAA	Salt tolerant (Dahl), Salt stress
Bacillus thuringiensis	IAA	Triticum aestivum L., Salt stress
Bacillus subtilis, Azospirillum sp.	IAA	Triticum aestivum L., Salt stress
Pseudomonas putida, Bacillus megaterium	IAA	Triticum aestivum L., Drought stress
Methylobacterium sp., Pseudomonas sp.	IAA	Triticum aestivum L., Salt stress
Flavobacterium sp., Streptococcus sp.	IAA	Cucumis sativus, Salt stress
Serratia glauciflava, Streptococcus pneumoniae, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas		
zooeciolans		
Zinobacterium terrae, Bacillus cereus, Enterobacter formosensis, Pantoea agglomerans	IAA	Triticum aestivum L., Salt stress
Curtobacterium flaccumfaciens, Erwinia carotovora	IAA	Hordium vulgare, Salt stress
Streptomyces coelicolor, Streptomyces griseoviridis	IAA	Triticum aestivum L., Salt stress
Bacillus subtilis	IAA	Azadirachta indica, Salt stress
Pseudomonas sp.	IAA	Zea mays, Salt and heat stress
Serratia sp.	IAA	Citrus aurantium L., Nutrient stress
Achromobacter anophageles	IAA	Zea mays, Co stress
Pseudomonas putida	IAA	Citrus aurantium L., Salt stress
Lactobacillus sp., Bacillus sp.	IAA	Zea mays, Co stress
Burkholderia sp.	IAA	Solanum tuberosum L., Co stress
Bacillus subtilis	IAA	Zea mays, Co stress
Bacillus megaterium	IAA	Wheat roots L., Ni stress
Achromobacter anophageles, Bacillus pumilus	IAA	Helianthus annuus, Drought stress
Serratia marcescens	IAA	Zea mays, Salt stress
Micrococcus luteus	CK	Zea mays, Drought stress
Achromobacter sp., Bacillus sp., Azospirillum sp.	CK	Citrus aurantium L., Salt stress
Bacillus subtilis	CK	Phaseolus vulgaris, Drought stress
Aspergillus fumigatus	GA	Citrus aurantium L., Salt stress
Aspergillus nidulans	GA	Triticum aestivum L., Drought stress
Phoma glomerata, Penicillium sp.	GA	Cucumis sativus, Drought stress
Bacillus amyloliquefaciens	ABA	Dryas octopetala, Salt stress
Bacillus thuringiensis, Pseudomonas fluorescens	ABA	Wheat roots L., Ni stress
Trichoderma reesei	IAA, GA, ABA	Cucumis sativus, Salt stress
Bacillus aryabhattae	IAA, GA, ABA	Citrus aurantium L., Heat stress

Table: Few phytohormone-producing bacteria and their ability to mitigate abiotic stress in selected plants.

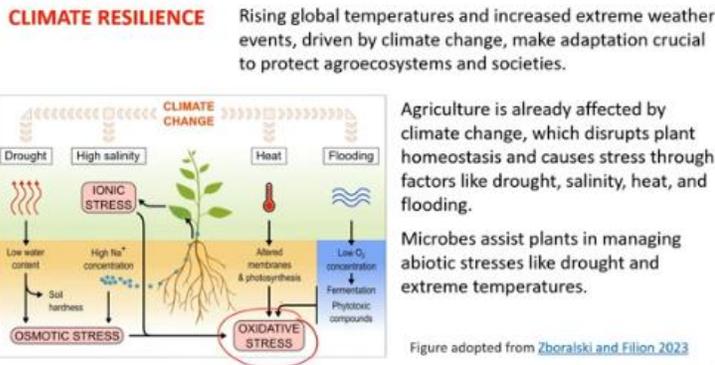
Indole-3-acetic acid (IAA)
Gibberellic acid (GA)
Abscisic acid (ABA)
Cytokinin (CK)

Egamberova et al. (2017) Front. Microbiol., Volume 8
| <https://doi.org/10.3389/fmicb.2017.02104>
<https://doi.org/10.3389/fmicb.2017.02104> | 11

So, we know that currently, rising global temperatures and increased extreme weather conditions and events are driven by climate change, making adaptation crucial to protect agro-ecosystems, forest ecosystems, and ocean ecosystems, as well as to safeguard societies and nations. Agriculture is largely affected by climate change already. It disrupts plant homeostasis and causes stress through factors like drought, salinity, heat, and flooding. Microbes assist plants in managing abiotic stress, such as drought and extreme temperatures. For example, we have here the effect of climate change, which will induce drought, cause high salinity, and result in excessive heat as well as flooding.

Now under these different stress conditions, the plants will have different kind of stresses like osmotic stress and oxidative stress as you can see in this diagram. Whenever there is a low water content, there will be osmotic stress because the soil will become very hard and then there is also ionic stress due to high salinity for example high sodium concentration and all these ionic stress and osmotic stress would lead to oxidative stress and due to flooding there is a low oxygen concentration and then there is a fermentation phytotoxic

compounds are produced these are also lead to the oxidative stress and due to heat there is an altered membrane and photosynthesis which also adds to this oxidative stress and in the earlier slide we have discussed how oxidative stress is harmful and how plant microbial phytohormones help in overcoming this oxidative stress. So, there are numerous mechanisms that help in promoting growth in stress conditions induced by climate change.



Two of them are reduction of ethylene levels in plants and countering the effects of high salinity in plants. So, we know that high salinity causes ionic stress leading to oxidative stress and we will try to discuss how these help. So, pseudomonas species support plant growth under stress by producing hormones, emitting beneficial compounds, reinforcing root barriers, reducing oxidative stress and secreting exopolysaccharides. These actions mitigate stress effects and enhance growth, ultimately improving yields despite adverse conditions. So, this is the pseudomonas species.

There are numerous mechanisms that help in promoting growth in stress conditions induced by climate change. Two of them are

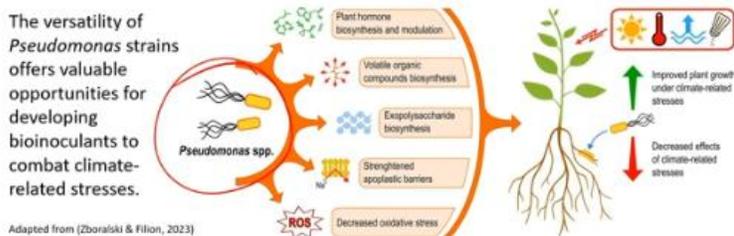
- i. **Reduction of ethylene levels in plants and**
- ii. **Countering the effects of high salinity in plants**

So it is carrying out plant hormone biosynthesis and modulation, releasing volatile organic compounds and also producing exopolysaccharides and it is strengthening apoplastic

barriers and also helping in the decreased oxidative stress which helps in you know adapting against various climate change things like heat, water, flooding and so on. So, this leads to improved decreased effects of climate related stress and improved plant growth under climate related stresses. So, the versatility of pseudomonas strains offers valuable opportunities for developing bioinoculants to combat climate related diseases. So, we know the beneficial effects of pseudomonas, so many.

And then, if we apply these to crops, we can take advantage of all these beneficial properties. So, let us discuss the reduction of ethylene levels in plants. For example, Pseudomonas strains promote plant growth under stress by producing the enzyme ACC deaminase. So, AcdS, which we call in salt, reduces levels of 1-aminocyclopropane-1-carboxylate, which is the ACC. It is a precursor to the stress-related hormone ethylene by breaking it down into ammonium and alpha-ketobutyrate.

Pseudomonas spp. support plant growth under stress by producing hormones, emitting beneficial compounds, reinforcing root barriers, reducing oxidative stress, and secreting exopolysaccharides. These actions mitigate stress effects and enhance growth, ultimately improving yields despite adverse conditions.



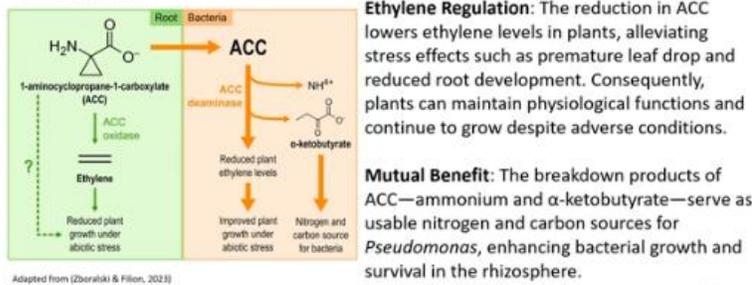
So, this is the ACC compound, and this particular enzyme, ACC oxidase, produced by Pseudomonas, will reduce plant growth under stress because it won't help in the production of ethylene, as it breaks it down into ammonium and alpha-ketobutyrate, as you can see here. So, in the root, this product is available, the compound is available, which is taken by the bacteria, and here it is converted into compounds, thereby stopping the production of ethylene. So, there is ethylene regulation: the reduction in ACC lowers ethylene levels in plants, eliminating stress effects such as premature leaf drop and reduced root development.

Consequently, plants can maintain physiological functions and continue to grow despite adverse conditions. Now, there is a mutual benefit here: the breakdown products of ACC, ammonium and alpha-ketobutyrate, serve as usable nitrogen and carbon sources for Pseudomonas, enhancing bacterial growth and survival in the rhizosphere. So, these

ultimately serve as nitrogen and carbon sources for the bacteria, so they have a mutualistic benefit here. Second is countering the effects of high salinity in plants. So, we can see here a figure: A, root apoplastic barrier without *Pseudomonas* inoculation, and in B, we can see root apoplastic barriers reinforced by *Pseudomonas* inoculation under high soil salinity conditions.

i. Reduction of ethylene levels in plants

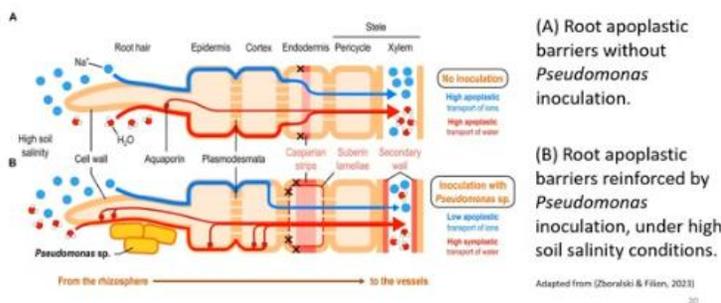
Pseudomonas strains promote plant growth under stress by producing the enzyme ACC deaminase (AcdS). AcdS reduces levels of 1-aminocyclopropane-1-carboxylate (ACC), a precursor to the stress-related hormone ethylene, by breaking it down into ammonium and α -ketobutyrate.



So, for example, in *Pseudomonas mandelii* strain IBKI 14, improved salt tolerance in wheat and pea by enhancing suberin and lignin deposition in xylem cell walls, suberin lamellae, and Casparian strips. So, you can see here the suberin lamellae here, and then there is the Casparian. So, when there is no inoculation, there is high apoplastic transport of ions and high apoplastic transport of water. When there is inoculation by this particular *Pseudomonas* strain, there is low apoplastic transport of ions and high symplastic transport of water. Let us now discuss another important topic: bioremediation.

ii. Countering the effects of high salinity in plants

Pseudomonas mandelii IB-Ki14 improved salt tolerance in wheat and pea by enhancing suberin and lignin deposition in xylem cell walls, suberin lamellae, and Casparian strips.



Microbes can remediate soil contaminated with pollutants and chemicals, contributing to soil health restoration. So, we call bioremediation an eco-friendly approach. So, you can

see here bacteria non-flagellated and bacteria flagellated. This is the flagellated one, and this is the non-flagellated one. We can see fungal hyphae over here, and then we can also see the biosurfactants. Here, you can see these are released biosurfactants.

Now, how does it help? There is natural attenuation, less cell count, and then slow degradation. Native microbes degrade pesticides at a very slow rate. So, then we do bioaugmentation where We have enriched degraders and engineered degraders.

So, introducing enriched or genetically engineered microbes can accelerate pesticide degradation in the environment. Although there is a lot of reservation regarding introducing genetically engineered microbes, which we will discuss in later modules. Then there is biostimulation, which increases growth and enhances bioavailability. This involves the addition of nutrients and biochemicals to boost the activity of existing microorganisms.

So, here we have naturally occurring microbes. So, in biostimulation, we will add nutrients and chemicals to boost the activity of existing microorganisms. In bioaugmentation, we will introduce genetically enriched microbes instead of adding nutrients. One of the most important roles of microbes in soil is the cycling of nutrients, which you call biogeochemical cycling. This involves the recycling of essential elements such as carbon, nitrogen, phosphorus, and sulfur.

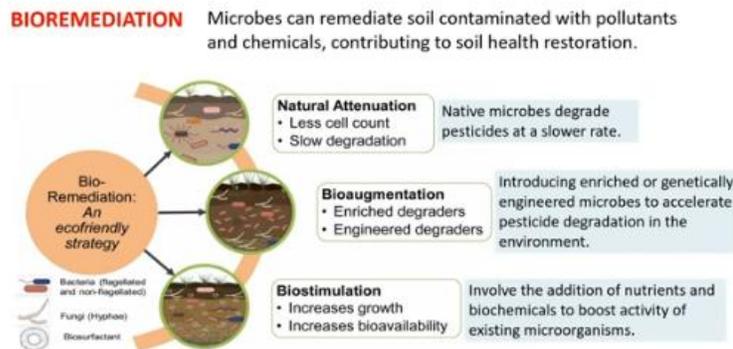


Figure adapted from (Bokade et al., 2023)

through biological activities by plants, animals, microorganisms, and also geological processes. So, for example, here is a tree, and leaves are shed—I mean, you know, dropped—and sometimes the tree dies or falls down due to storms or wind conditions. And also, animals die. So, we have dead plant and animal matter. These are decomposed or broken down into organic matter in the soil by various organisms.

And then these release minerals and other nutrients into the soil, helping in plant growth. That is one cycle, and also the rock present below the soil gets broken down. And in this entire decomposition and breaking down of both the organic matter as well as mineral rocks, microbes play a big role. And this helps in maintaining the ecosystems where both plants and animals survive. So, microbes are crucial in these cycles.

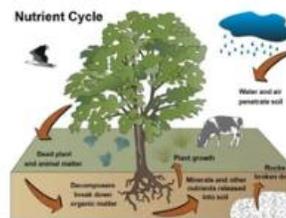
They transform and recycle nutrients, which are then released back into the environment. Sub-cycling maintains ecosystem health, supports plant and animal growth, and ensures a balanced nutrient supply, contributing to ecological balance, agricultural productivity, and environmental protection. So, what are the various soil microorganisms which take part in nutrient cycling and maintaining soil fertility? So, for sustainable agricultural productivity, we need good and healthy soil which is fertile, and this is significantly influenced by soil microorganisms like bacteria, fungi, archaea, and protozoa. So, these microbes are crucial for the biogeochemical cycling of essential nutrients like nitrogen, phosphorus, sulfur, as well as others, as we will be discussing in this module and also in another module.

BIOGEOCHEMICAL CYCLING OF NUTRIENTS

Biogeochemical cycling involves the recycling of essential elements such as carbon, nitrogen, phosphorus, and sulfur through biological activities by plants, animals, microorganisms, and geological processes.

Microbes are crucial in these cycles, transforming and recycling nutrients, which are then released back into the environment.

Such recycling maintains ecosystem health, supports plant and animal growth, and ensures a balanced nutrient supply, contributing to ecological balance, agricultural productivity, and environmental protection.



Nutrient cycle
Image Credit: Adapted from USDA NRCS & NITA (2016) with NIA/UMCES Symbols

So, this enhances nutrient availability, supports plant growth, and enables sustainable land management. Microbial transformation and mobilization of nutrients for plant accessibility. So, one important aspect is nitrogen fixation, and then nitrification and also denitrification in the case of nitrogen recycling. So, in nitrogen fixation, soil bacteria like Rhizobium species and free-living species like Azotobacter convert atmospheric nitrogen into ammonia, making it accessible to plants. In nitrification, ammonia is oxidized here to nitrite and then to nitrate by bacteria, with nitrate being more readily absorbed by the plants.

Soil Microorganisms in Nutrient Cycling and Soil Fertility

Sustainable agricultural productivity depends on soil health and fertility, significantly influenced by soil microorganisms including bacteria, fungi, archaea, and protozoa.

These microbes are crucial for the biogeochemical cycling of essential nutrients like

- nitrogen (N)
- phosphorus (P)
- sulfur (S)

This enhances nutrient availability, supports plant growth, and aids sustainable land management.

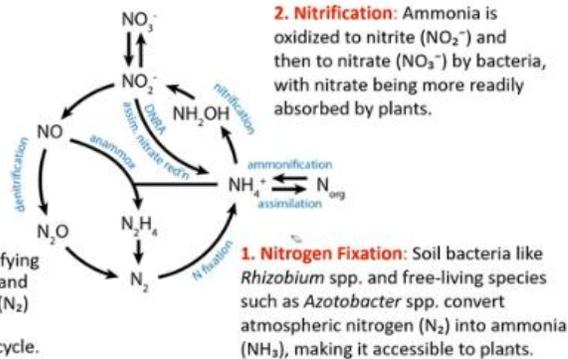
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In the denitrification process, denitrifying bacteria convert nitrates and nitrites into nitrogen gas or nitrous oxide here, completing the nitrogen cycle. So, if you start from nitrogen, we go through nitrogen fixation, nitrification, then finally denitrification and we are back to where we started. And in this end process, the nitrogen is recycled between organisms and different environmental niches. Let us look into the recycling of phosphorus. So, we have mineralization in the case of phosphorus.

Microbial Transformation and Mobilization of Nutrients for Plant Accessibility

Nitrogen Recycling:

Figure Author: Microbalinnert CC
https://commons.wikimedia.org/wiki/File:Microbial_nitrogen_cycle.png



Microorganisms decompose the organic matter to release inorganic phosphate from organic phosphorus. So, here you can see this is inorganic phosphorus and microorganisms act on it. Then by the process of mineralization it sends it into solution form and then the reverse is immobilization where microbes temporarily store phosphorus in their biomass here which is later released back into the soil upon microbial death and decomposition. So, solubilization is held by certain bacteria and fungi that dissolve insoluble phosphorus compounds making them available to plants. So, ultimately phosphorus which is absorbed in you know hematite and other minerals and then here they will actually come to this solution form.

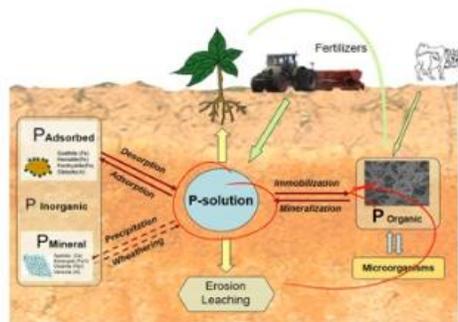
and also those from the organic matter will come to the solution from and these become available to the plants. And of course when we apply fertilizers artificially that also gets solubilized and finally it enters the plant system. And the remaining which cannot be absorbed or even before absorption by plants will be lost by erosional leaching but they will end up somewhere in the environmental, some environmental reaches and then it will enter this cycle. And then it will again become available to some other plants. The next important thing is sulfur recycling.

Phosphorus Recycling:

1. Mineralization: Microorganisms decompose organic matter to release inorganic phosphate (PO_4^{3-}) from organic phosphorus.

2. Solubilization: Certain bacteria and fungi dissolve insoluble phosphorus compounds, making them available to plants.

3. Immobilization: Microbes temporarily store phosphorus in their biomass, which is later released back into the soil upon microbial death and decomposition.

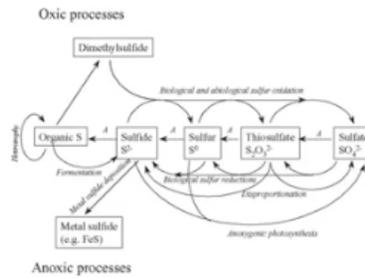


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So, we have two processes over here: the oxic processes and the anoxic processes. So, in sulfur oxidation, sulfur-oxidizing bacteria convert sulfur and hydrogen sulfide to sulfate, which plants can absorb easily. In the sulfur reduction cycle, sulfate-reducing bacteria reduce sulfate to sulfide, which can be reoxidized or used by microbes. So, in certain cases, you have biological and abiological sulfur oxidation, and in others, you have anoxygenic photosynthesis. Once these are converted to sulfate, it can easily go to plants or be absorbed by them. Apart from these, we have many other geological biogeochemical cycles. Due to time constraints for this particular lecture, we will discuss them in another module in this lecture series.

Sulfur Recycling:

- Sulfur Oxidation:** Sulfur-oxidizing bacteria convert sulfur and hydrogen sulfide (H_2S) to sulfate (SO_4^{2-}), which plants can absorb.
- Sulfur Reduction:** Sulfate-reducing bacteria reduce sulfate to sulfide (S^{2-}), which can be reoxidized or used by microbes.



Walter K. Dodds, in Freshwater Ecology, 2002, Academic Press

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Let us now discuss the use of microbes as biofertilizers. We will also discuss the various classes and types, as well as a little about arbuscular mycorrhizal fungi as biofertilizers and certain endophytic fungi as biofertilizers. Biofertilizers, or microbial inoculants, are microorganisms that enhance plant growth and yield by colonizing the rhizosphere and plant roots. They improve soil fertility, protect against pests, and support stronger root development. Common biofertilizers include nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and fungi. These microbes provide essential nutrients and growth-promoting substances, contributing to soil health and sustainable crop production.

MICROBES AS BIOFERTILIZERS

Biofertilizers, or microbial inoculants, are microorganisms that enhance plant growth and yield by colonizing the rhizosphere and plant roots. They improve soil fertility, protect against pests, and support stronger root development. Common biofertilizers include nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and fungi. These microbes provide essential nutrients and growth-promoting substances, contributing to soil health and sustainable crop productivity.

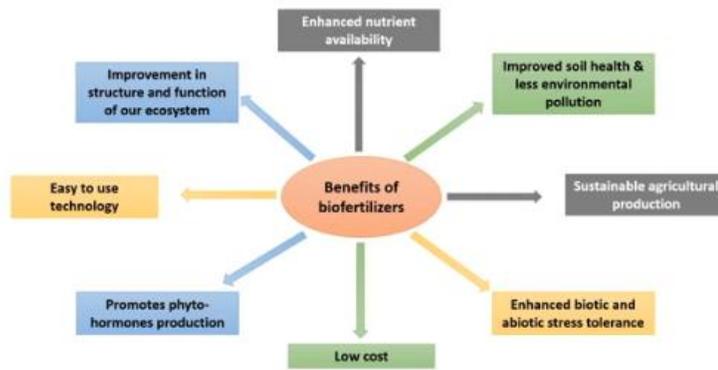


Author: Edward Scott
<https://www.linkedin.com/pulse/unlocking-soil-potential-power-biofertilizers-edward-scott-ia81f/>

(Noehren et al., 2021)

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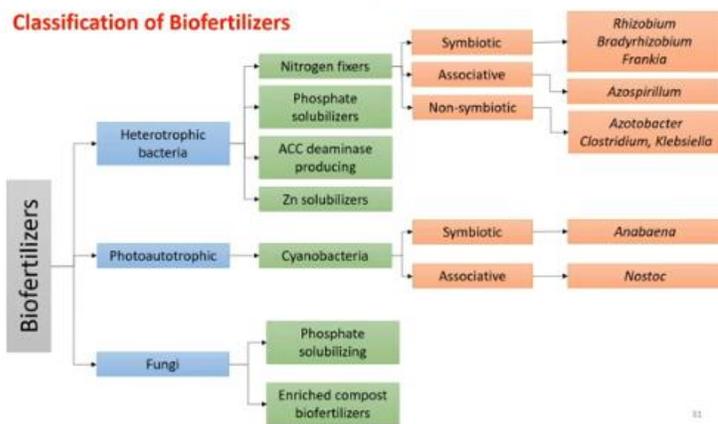
So, what are the benefits of using biofertilizers? They enhance nutrient availability, improve soil health, cause less environmental pollution, and are very important for sustainable agricultural production. They enhance biotic and abiotic stress tolerance, improve the structure and function of our ecosystem, are easy to use, promote phytohormone production, and are comparatively low-cost and can be produced at the farm level. So, let us look into the various types of biofertilizers. So, biofertilizers are produced by heterotrophic bacteria, photoautotrophic organisms, and fungi.



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So, in heterotrophic bacteria, we may have nitrogen fixers or phosphate solubilizers. Then we have ACC deaminase-producing bacteria and then zinc solubilizers. In autotrophic, photoautotrophic, we have cyanobacteria, which may be symbiotic and associative. And then we have, under fungi, phosphate solubilizing and mobilizing bacteria. And also, we have enriched compost biofertilizers.

Nitrogen fixers may be symbiotic, associative, or non-symbiotic. For example, we have Rhizobium, Azospirillum, and Azotobacter, respectively. So, if you look into the various mechanisms of various biofertilizers in nitrogen fixation, we can see that an increase in soil nitrogen content happens by fixing atmospheric nitrogen and making it available to plants. As already discussed, they may be free-living, symbiotic, or associative symbiotic, and these are the examples for these various types. Then we have phosphorus-solubilizing ones, which solubilize the insoluble forms of phosphorus in the soil into soluble forms by secreting organic acids and lowering soil pH to dissolve bound phosphates. This can be carried out by both bacteria and fungi, like Bacillus and Penicillium, respectively. We have phosphate-mobilizing biofertilizers.



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These transfer phosphorus from the soil to the root cortex, and these are broad-spectrum biofertilizers. For example, you have mycorrhiza, arbuscular mycorrhiza, and *Glomus* species. Then you have potassium-solubilizing. Biofertilizers solubilize potassium silicates by producing organic acids that decompose silicates and help in the removal of metal ions, making it available to plants. They may be bacteria or fungi, like *Bacillus* and *Aspergillus niger*, respectively. Then we have potassium-mobilizing biofertilizers.

They mobilize the inaccessible forms of potassium in the soil. There may be bacteria like *Bacillus* and fungi like *Aspergillus niger*. Additionally, there are certain micronutrient-providing biofertilizers that oxidize sulfur to sulfates, which are usable by plants, or solubilize zinc through proton-chelated ligands, acidification, and oxidoreductive systems. These may include mycorrhizae, *Pseudomonas*, and *Bacillus* species. Then there are certain biofertilizers that provide plant growth promoters or hormones, which promote root growth, improve nutrient availability, and enhance crop yield. We refer to them as plant growth-promoting rhizobacteria, for example, *Pseudomonas* and *Agrobacterium* species. Let us now discuss biofertilizers and their nitrogen-fixing abilities.

CLASSIFICATION OF BIOFERTILIZERS

Biofertilizers	Mechanism	Groups	Examples
Nitrogen fixing	Increase soil nitrogen content by fixing atmospheric N and make it available to the plants	Free-living	<i>Azotobacter</i> , <i>Anabaena</i>
		Symbiotic	<i>Rhizobium</i> , <i>Frankia</i> ,
		Associative symbiotic	<i>Azospirillum</i> spp., <i>Enterobacter</i> ,
Phosphorus solubilizing	Solubilize the insoluble forms of P in the soil into soluble forms by secreting organic acids and lowering soil pH to dissolve bound phosphates	Bacteria	<i>Bacillus circulans</i> , <i>B subtilis</i> ,
		Fungi	<i>Penicillium</i> spp., <i>Aspergillus awamori</i>
Phosphorus mobilizing	Transfer phosphorus from the soil to the root cortex. These are broad spectrum bio-fertilizers.	Mycorrhiza	Arbuscular mycorrhiza, <i>Glomus</i> spp., <i>Gigaspora</i> spp.,
Potassium solubilizing	Solubilize potassium (silicates) by producing organic acids that decompose silicates and help in the removal of metal ions and make it available to plants.	Bacteria	<i>Bacillus. mucilaginosus</i> , <i>B. circulanscan</i> , <i>B. edaphicus</i> ,
		Fungi	<i>Aspergillus niger</i> .
Potassium mobilizing	They mobilize the inaccessible forms of potassium in the soil.	Bacteria	<i>Bacillus</i> spp.
		Fungi	<i>Aspergillus niger</i> .
Micronutrient	Oxidizing sulfur to sulfates which are usable by plants.	Sulfur oxidizing	<i>Thiobacillus</i> spp.
	Solubilize the zinc by proton, chelated ligands, acidification, and by oxidoreductive systems.	Zinc solubilizing	Mycorhiza <i>Pseudomonas</i> spp., and <i>Bacillus</i> spp.
Plant growth Promoting	Produce hormones that promote root growth, improve nutrient availability, and improve crop yield	Plant growth promoting rhizobacteria	<i>Pseudomonas</i> spp. <i>Agrobacterium</i> , <i>Pseudomonas fluorescens</i> ,

As already discussed, nitrogen fixers may be symbiotic organisms, free-living, or associated symbiotic. We also have cyanobacteria-based biofertilizers and *Frankia*-based biofertilizers. In the case of symbiotic nitrogen fixers, microbes like *Rhizobium leguminosarum* form symbiotic relationships with leguminous plants, fix atmospheric nitrogen in root nodules, and provide essential nutrients. Then we have free-living nitrogen fixers like *Azotobacter crococom*, a free-living bacterium that fixes nitrogen in non-leguminous plants like cotton and vegetables, reducing the need for chemical fertilizers. We also have associated symbiotic nitrogen fixers like *Azospirillum*-based *Azospirillum*

brasilense and other species associated with grasses to fix nitrogen and promote plant growth, fixing approximately 20 to 40 kg per hectare per year in non-leguminous plants.

Additionally, we have cyanobacteria-based biofertilizers, which are photosynthetic bacteria that fix atmospheric nitrogen in aquatic and semi-aquatic environments, such as in paddy fields, for example, *Anabaena* and *Nostoc*. And then we have *Frankia*-based biofertilizers. *Frankia* is a genus of nitrogen-fixing bacteria that forms a symbiotic relationship with actinorhizal plants, similar to *Rhizobium*'s role with legumes. It helps initiate root nodule formation and fixes atmospheric nitrogen, for example, *Frankia alni*.

Biofertilizers and their nitrogen-fixing abilities	
1. Symbiotic Nitrogen Fixers: Rhizobium-based: <i>Rhizobium leguminosarum</i> forms symbiotic relationships with leguminous plants, fixing atmospheric nitrogen in root nodules and providing essential nutrients. Azolla-based: <i>Azolla</i> associates with blue-green algae to fix nitrogen, benefiting rice crops.	
2. Free-Living Nitrogen Fixers: Azotobacter-based: <i>Azotobacter chroococcum</i> is a free-living bacterium that fixes nitrogen in non-leguminous plants like cotton and vegetables, reducing the need for chemical fertilizers.	3. Associative Symbiotic Nitrogen Fixers: Azospirillum-based: <i>Azospirillum brasilense</i> and other species associate with grasses to fix nitrogen and promote plant growth, fixing approximately 20-40 kg/ha/year in non-leguminous plants.
4. Cyanobacteria-based Biofertilizers: Photosynthetic bacteria that fix atmospheric nitrogen in aquatic and semi-aquatic environments, useful in paddy fields. Example: <i>Anabaena spp.</i> , <i>Nostoc spp.</i>	5. Frankia-based Biofertilizers: <i>Frankia</i> is a genus of nitrogen-fixing bacteria that forms symbiotic relationships with actinorhizal plants, similar to <i>Rhizobium</i> 's role with legumes. It helps initiate root nodule formation and fixes atmospheric nitrogen. Example: <i>Frankia alni</i>

Then we have phosphate-mobilizing microbes. Microorganisms that mobilize native and added insoluble phosphorus sources, including organic phosphorus through enzymatic hydrolysis, are termed phosphate-mobilizing microorganisms. The phosphate-solubilizing microorganisms are also helpful in facilitating phosphate translocation and are thus included as PMM or referred to as PMM. Microbial phosphorus solubilization involves the organic acid theory with two mechanisms: the pH-lowering mechanism, which directly dissolves organic mineral phosphorus, and the organic acid anion-releasing theory, which focuses on the ligand exchange.

PHOSPHATE MOBILIZING MICROBES

Microorganisms that mobilize native and added insoluble phosphorus sources, including organic P through enzymatic hydrolysis, are termed phosphate-mobilizing microorganisms (PMM). The "phosphate-solubilizing microorganisms" also facilitate phosphate translocation and thus are also called PMM.

Microbial P solubilization involves the 'organic acid theory', with two mechanisms:

- pH lowering (directly dissolving mineral P)
- organic acid anions releasing (ligand exchange).

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So, PMM may directly mobilize both inorganic and organic phosphorus. Indirect mobilization of phosphorus can also occur. In the case of organic acid production, microorganisms produce organic acids like gluconic acid that lower the pH, dissolving inorganic phosphates. Then there is chelation, where acyclic agents such as 2-ketogluconic, humic, and fulvic acids bind to metal ions like calcium, iron, and aluminum, aiding phosphate solubilization. Then there is inorganic acid production.

Acids like sulfuric and nitric acids are produced by nitrifying and sulfur-oxidizing bacteria, though they are less effective than organic acids for phosphate solubilization. Then there is proton extrusion, where phosphates can be solubilized by proton extrusion, lowering the pH without organic acid production, influenced by nitrogen sources. And then there is exopolysaccharide production, where microbial exopolysaccharides, which bind to metals in the soil, indirectly aid phosphate solubilization alongside organic acids. Then there is siderophore production, where siderophores, which solubilize iron, can enhance the solubility of iron-bound phosphates. And there is also enzymatic action of enzymes like phosphatases, phytase, and phosphonates, which hydrolyze organic phosphates, releasing phosphate ions for plant uptake.

PMM may directly mobilize both P_i and P_o , indirect mobilization of P also occurs through:

- **Organic Acid Production:** Microorganisms produce organic acids like gluconic acid that lower the pH, dissolving inorganic phosphates.
- **Chelation:** Chelating agents such as 2-keto-gluconic, humic, and fulvic acids bind to metal ions like calcium, iron, and aluminum, aiding phosphate solubilization.
- **Inorganic Acid Production:** Acids like sulfuric and nitric acids are produced by nitrifying and sulfur-oxidizing bacteria, though less effective than organic acids for phosphate solubilization.

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- **Proton Extrusion:** Phosphates can be solubilized by proton extrusion, lowering the pH without organic acid production, influenced by nitrogen sources.
- **Exopolysaccharide Production:** Microbial exopolysaccharides, which bind to metals in the soil, indirectly aid phosphate solubilization alongside organic acids.
- **Siderophore Production:** Siderophores, which chelate iron, can enhance the solubility of iron-bound phosphates.
- **Enzymatic Action:** Enzymes like phosphatases, phytase, and phosphonatase hydrolyze organic phosphates, releasing phosphate ions for plant uptake.

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Let us now discuss potassium solubilizing and mobilizing biofertilizers. Potassium is the most important plant nutrient after nitrogen and phosphorus. It is abundant in soil, but only 1-2% is available to plants, requiring continuous replenishment for plant growth. Deficiency of potassium leads to slow growth, poorly developed roots, and low yields. Various bacterial and fungal strains employ mechanisms like acid production, chelation, and complex analysis to solubilize insoluble potassium into soluble forms.

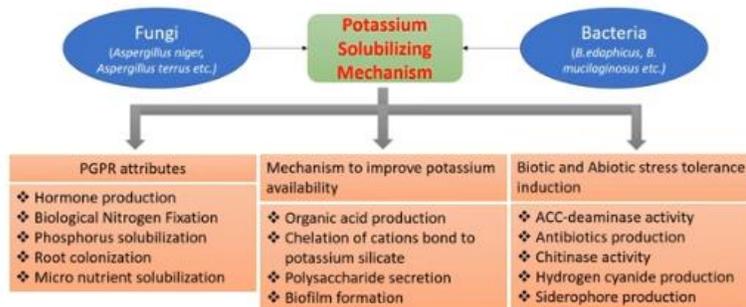
POTASSIUM SOLUBILIZING AND MOBILIZING BIOFERTILIZERS

Potassium (K) is the most crucial plant nutrient after nitrogen and phosphorus. Though abundant in soil, only 1-2% is available to plants, requiring continuous replenishment for plant growth. Deficiency of K leads to slow growth, poorly developed roots, and low yields. Various bacterial and fungal strains employ mechanisms like **acid production, chelation, and complexolysis** to solubilize insoluble K into soluble forms. Examples of potassium-solubilizing biofertilizers include *Bacillus* spp. and *Aspergillus niger*, which improve solubilization and mobilization.

FIGURE 3

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Examples of potassium solubilizing biofertilizers are *Bacillus* species and *Aspergillus niger*, which improve solubilization and mobilization. So, we have fungi and bacteria that can solubilize potassium, and there are different mechanisms. One is the PGPR attributes, where there is hormone production, biological nitrogen fixation, phosphorus solubilization, root colonization, and micronutrient solubilization. Then there is the biotic and antibiotic stress-tolerance induction, where ACC deaminase activity, antibiotic production, chitinase activity, hydrogen cyanide production, and siderophore production take place. There are certain mechanisms to improve potassium availability, such as organic acid production, chelation of cations bound to potassium silicate, polysaccharide secretion, biofilm formation, etc.



Generated by BERT.
(Chaitan et al., 2022)

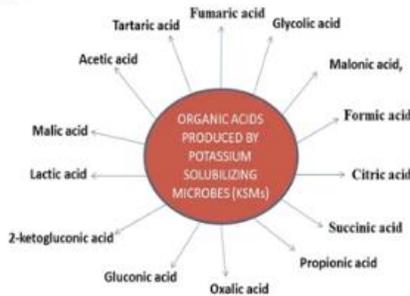
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So, one of the mechanisms of potassium solubilization is to release potassium nutrients by the production of different organic acids, which acidify the rhizosphere and enhance potassium solubilization from minerals. Organic acids produced by potassium solubilizers act on different kinds of sources and make potassium available. These organisms can be called potassium solubilizing microbes or KSMs. The various acids may include oxalic,

gluconic, 2-ketogluconic acid; some produce lactic, others produce malic, acetic, tartaric, fumaric, glycolic, malonic, formic, citric, succinic, and propionic acids. Low pH due to the release of these acids facilitates the production of organic acids and protons, which directly dissolve potassium minerals, releasing potassium ions via protonation.

Potassium Solubilizing Mechanism

One of the mechanisms is to release potassium nutrients by the production of different organic acids which acidify the rhizosphere and enhancing potassium solubilization from minerals.



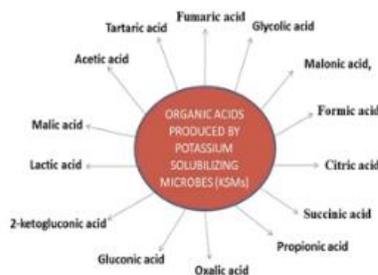
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(Danayan et al., 2022)

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These organic acids can also release potassium ions from potassium-bearing minerals by chelating aluminium, silicon, calcium, and ferrous. Disrupting the lattice structure and releasing potassium into the soil solution. The next important type of microbes are those which can solubilize zinc, or zinc-solubilizing microbes. For example, zinc-solubilizing bacteria efficiently convert complex inorganic zinc into available forms for plants, with examples like *Pseudomonas fragi*, *Pantoea dispersa*, and *Pantoea agglomerans*. Silicate-solubilizing bacteria play a crucial role in solubilizing insoluble silicates as well as potassium and phosphates, enhancing soil fertility and plant production.

Potassium Solubilizing Mechanism

Low pH facilitates the production of organic acids and protons, which directly dissolve potassium minerals, releasing potassium ions via protonation. These organic acids can also release potassium ions from potassium-bearing minerals by chelating Al^{3+} , Si^{4+} , Ca^{2+} , and Fe^{2+} ions, disrupting the lattice structure and releasing potassium into the soil solution.



Generated by BERN
(Danayan et al., 2022)

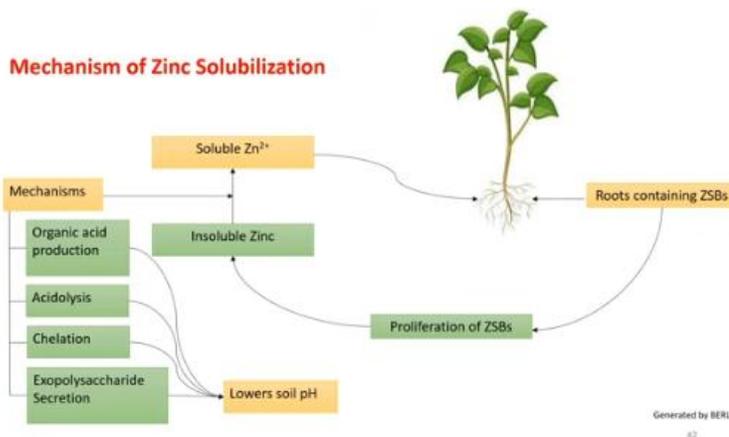
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The silicate-solubilizing bacteria and hints by fertilizers like *Bacillus* species improve the photosynthetic efficiency of plants like *Brassica juncea*. So, if you look into the mechanism

of zinc solubilization, we have organic acid production, then acidolysis, then chelation, and then exopolysaccharide secretion—all these will lower the soil pH, and then the insoluble zinc will be solubilized by the action of these different mechanisms, and then these are made available for the plants to absorb. Overall, these roots will contain the zinc-solubilizing bacteria, and due to availability, the proliferation of these organisms will take place.

ZINC SOLUBILIZING MICROBES

Zinc solubilizing bacteria efficiently convert complex inorganic zinc into available forms for plants, with examples like *Pseudomonas fragi*, *Pantoea dispersa*, and *Pantoea agglomerans*. Silicate solubilizing bacteria (SSB) play a crucial role in solubilizing insoluble silicates, as well as potassium and phosphates, enhancing soil fertility and plant productivity. SSB-enriched biofertilizers, like *Bacillus* sp., improve the photosynthetic efficiency of plants like *B. juncea*.



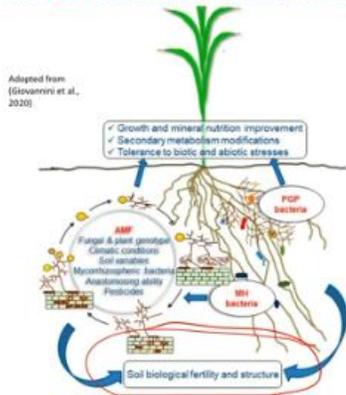
Let us now discuss arbuscular mycorrhizal fungi. Here in this diagram, you can see the effects of arbuscular mycorrhizal fungi. So, here we have these mycorrhizal fungi species over here. Basically, the AMF or arbuscular mycorrhizal fungi—here, fungal and plant genotype plays an important role in climatic conditions as well as soil variables and mycorrhizospheric bacteria. The anastomosing ability of the pesticides also plays a critical role.

So, we have certain species known as the mycorrhizal helper which influence in many of these important events. Then we have plant growth promoting bacteria which

synergistically interact with these AMF. So, it is a little bit of complex interaction as you can see. Overall these AMF will lead to the growth and mineral nutrition improvements and then secondary metabolism, modifications and also tolerance to biotic and abiotic stresses as you can see in this diagram.

So, and then overall they help in improving the soil biological fertility and the soil structure. So, these are the arbuscular mycorrhizal colonization has positive effects extending the hyphal network beyond the phosphate depletion zone. So, you can see here the grey area which is the phosphate depletion zone and then it helps in assessing a larger soil area for phosphate uptake. Mycorrhizal phosphate depletion zones, which are the purples one, form around the arbuscular mycorrhizal hyphae, as you can see in this picture and shows improved assimilation of nutrients like nitrogen in the form of ammonium and then also zinc. Benefits of these colonization include enhanced tolerance to abiotic stress through systematic acquired resistance induction.

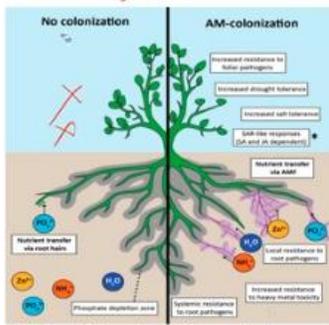
ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AS BIOFERTILIZER



The diagram illustrates the effects of **arbuscular mycorrhizal fungi (AMF)** and beneficial bacteria on plant performance and soil fertility. On the left, the AMF life cycle and factors influencing its developmental stages are depicted. On the right, mycorrhizal helper (MH) and plant growth-promoting (PGP) bacteria are shown to synergistically interact with AMF.

So, due to these two figures, you can see there are on the left side no colonization and here there is arbuscular mycorrhizal colonization. In this case, Here there is increased resistance to foliar pathogens, then increased drug tolerance which is absent over here, then it also has increased salt tolerance not available in case of no colonization and there is the systemic acquired responses or resistance in this particular case. So, overall arbuscular mycorrhizae are highly beneficial to overall plant growth. So, how do mycorrhizae establish? So, plant defenses signaling is modulated during mycorrhizal establishment in the following stages.

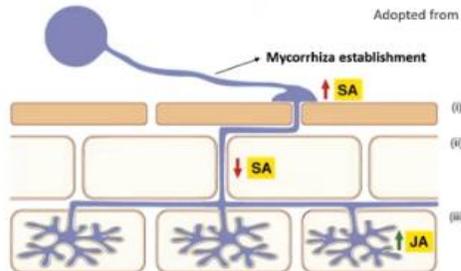
Adopted from (Jacott et al., 2017)



Arbuscular mycorrhizal (AM) colonization has positive effects, extending the hyphal network beyond the phosphate depletion zone (grey) and accessing a larger soil area for phosphate uptake. Mycorrhizal-phosphate depletion zones (purple) form around AM hyphae. AM-roots also show improved assimilation of nutrients like nitrogen (ammonium) and zinc. Benefits of colonization include enhanced tolerance to abiotic and biotic stresses through systemic acquired resistance (SAR) induction.

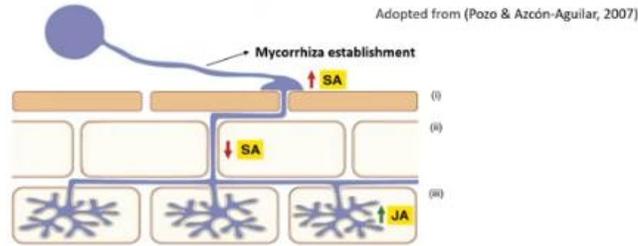
Number one, during germination, arbuscular mycorrhizae find growth towards the root and form appressoria. Okay, on the root surface at this stage, the plant responds with an increase in salicylic acid levels. In a compatible interaction, these salicylic acid levels decrease as the fungus colonizes the cortex. Then, in this particular zone, you can see that there is jasmonic acid biosynthesis, which takes place in arbuscule-containing cells. When mycorrhizae form, they prepare the plant tissue to respond more quickly and effectively when an attacker strikes. This means that the plant can activate its defenses faster, which helps it resist damage better.

Adopted from (Pozo & Azcón-Aguilar, 2007)



Plant defense signaling is modulated during **mycorrhiza establishment** in the following stages:

- (i) During germination, AM fungi grow towards the root and form appressoria on the root surface. At this stage, the plant responds with an increase in salicylic acid (SA) levels.



(ii) In a compatible interaction, SA levels decrease as the fungus colonizes the cortex.

(iii) Jasmonic acid (JA) biosynthesis takes place in arbuscule-containing cells.

When mycorrhiza forms, it prepares the plant's tissues to respond more quickly and effectively when an attacker strikes. This means that the plant can activate its defenses faster, which helps it resist damage better.

Let us now discuss endophytic fungi as biofertilizers. Endophytic fungi, which live within plant tissues without causing harm, significantly enhance plant growth and resilience. As in the case of *Piriformospora indica*, we know it for its ability to promote nutrient uptake. This enhances the availability of essential nutrients such as nitrogen, phosphorus, and potassium by improving root function. It also helps increase biomass production and contributes to higher yields in various crops. And it accelerates early flowering and seed production.

It stimulates hormonal activity that supports flowering and seed development. Additionally, *P. indica* helps plants tolerate abiotic stress, including drought and heavy metal toxicity, and boosts resistance to biotic stresses from pathogens and herbivores. So, we can see here the endophytes that we can use as biofertilizers. So, it can have a direct effect or an indirect effect. In the case of a direct effect, it helps in nutrient acquisition, plant growth, and development. In the indirect effect, they also help as biocontrol agents.

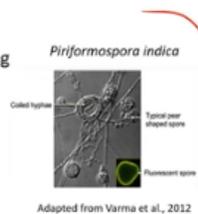
ENDOPHYTIC FUNGI AS BIOFERTILIZER

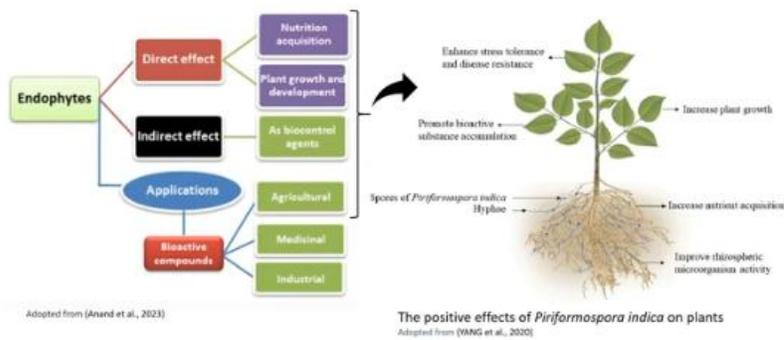
Endophytic fungi, which live within plant tissues without causing harm, significantly enhance plant growth and resilience.

A well-studied example is *Piriformospora indica*, known for its ability to:

- **Promote Nutrient Uptake:** Enhances the availability of essential nutrients, such as nitrogen, phosphorus, and potassium, by improving root function.
- **Increase Biomass Production:** Contributes to higher yields in various crops.
- **Accelerate Early Flowering and Seed Production:** Stimulates hormonal activity that supports flowering and seed development.

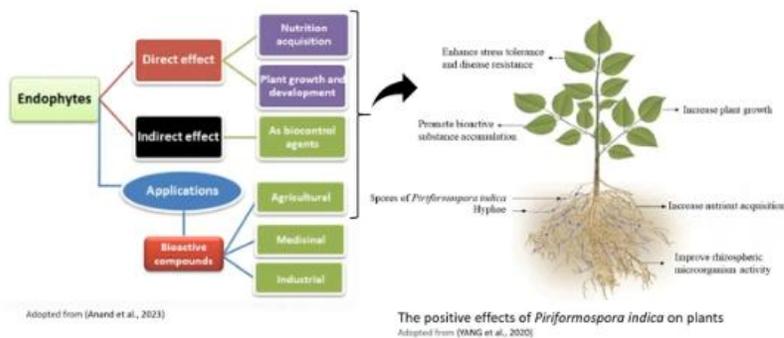
Additionally, *P. indica* helps plants tolerate abiotic stresses, including drought and heavy metal toxicity, and boosts resistance to biotic stresses from pathogens and herbivores.





And then, from the application point of view, they release bioactive compounds which can have importance in agriculture, medicinal value, as well as in certain other industrial applications. So, leaving these two aside, overall, the endophytes directly and indirectly help in enhancing stress tolerance and disease resistance in plants. Then, they promote bioactive substance accumulation and increase plant growth. So here, we can see the spores of *Piriformospora indica* colonizing the roots and, overall, the increase in nutrient acquisition which helps in many of these outcomes. And they improve rhizospheric microorganism activity.

So, these are the positive effects of *Piriformospora indica* on overall plant growth as an endophyte. Then, we have other endophytes which are used in agriculture. For example, we have Clavicipitaceous endophytes. Particularly, *Epichloë* species are mainly used for biocontrol in pasture grasses. These *Epichloë coenophiala* enhance pest tolerance in tall fescue by producing alkaloids that deter herbivores.



Then, we have ryegrass varieties, which have many of these Clavicipitaceous endophytes that exhibit reduced damage from insect herbivores in countries like New Zealand and Australia. However, the application has been largely restricted to grasses, limiting broader use in other crops. Endophytic fungi also help plants in overcoming cold stress effects or aid in cold stress resistance. For example, these same species, *Piriformospora indica*, help plants withstand low temperatures by enhancing ROS scavenging. This improves the plant's ability to detoxify.

Clavicipitaceous Endophytes in Agriculture

Clavicipitaceous endophytes, particularly *Epichloë* species, are mainly used for biocontrol in pasture grasses. For instance:

- *Epichloë coenophiala* enhances pest tolerance in tall fescue by producing alkaloids that deter herbivores.
- Ryegrass varieties with Clavicipitaceous endophytes exhibit reduced damage from insect herbivores in New Zealand and Australia.

However, their application has been largely restricted to grasses, limiting broader use in other crops.

Reactive oxygen species reduce cellular damage, and boosting photosynthetic efficiency ensures continuous energy production and metabolism in cold conditions. Increasing protective enzyme activity elevates levels of enzymes that neutralize ROS, protecting against oxidative stress and promoting osmoprotectant. Accumulation increases soluble sugars and amino acids that stabilize cellular structures under stress. It stimulates cold-responsive genes, enhancing the expression of genes involved in cold acclimation and improving resistance and resilience, as seen in crops like bananas. So with this, we come to the end of this lecture.

Thank you for your patient hearing. Amen.