MICROBIAL BIOTECHNOLOGY

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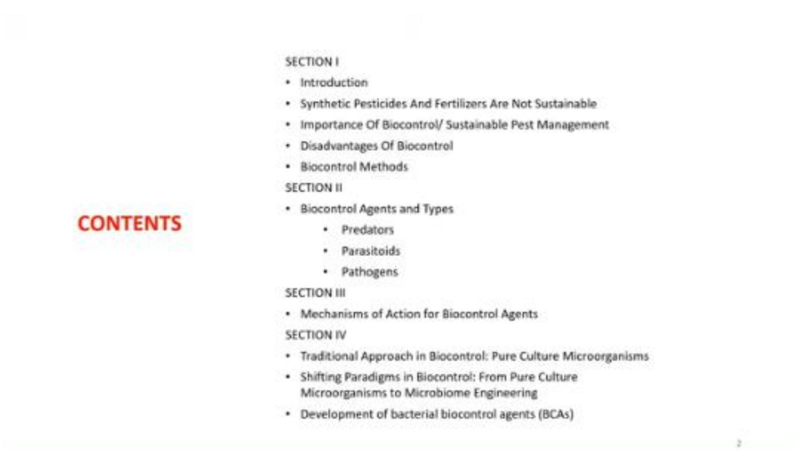
Lecture-24
Lec 24: Microbial Biocontrol

Welcome, friends. Let us continue our lecture in this course on microbial biotechnology. We are in Module 7, and in this particular lecture, we will be speaking about microbial biocontrol. So, it is divided into various sections. We will be speaking about synthetic pesticides and fertilizers and how they are

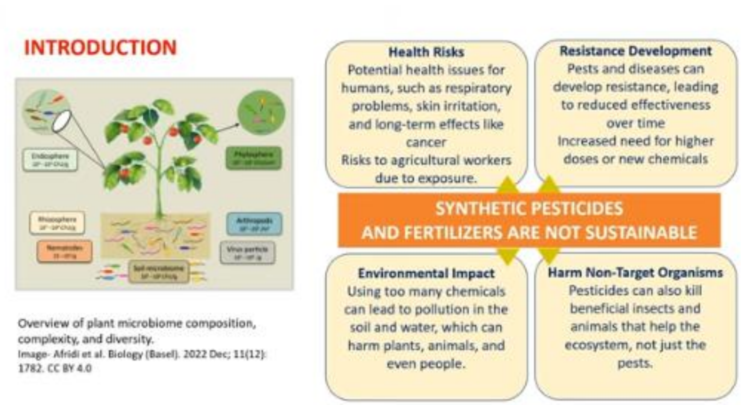
not that suitable, and then the importance of biocontrol, sustainable pest management, and the disadvantages of biocontrol. We will also discuss the different biocontrol methods adopted. Additionally, we have two or three more sections, which we will cover in detail as we proceed. So, before we start, let us revisit the overall plant microbiome composition, which is quite complex and diverse. As you can see in this picture, these microbes are ubiquitous, present almost everywhere—in the leaf, in the soil, and even in the fruits and flowers. And you can see the various counts of them in the endosphere. You have as many as 10 to the power of 4 CFU per gram, and then in the phyllosphere, a similar number—10 to the power of 5 to even 10 to the power of 6. And then you have them in the rhizosphere, along with other organisms like nematodes, arthropods, and virus particles.



So, a plant is exposed to so many different kinds of organisms—microorganisms, slightly submicroscopic particles like viruses, and even higher organisms like arthropods. Many of these are beneficial, while some are harmful to the plant ecosystem. To control the harmful organisms, we use synthetic pesticides and synthetic fertilizers to help boost plant nutrition. However, the long-term use of these pesticides and artificial fertilizers has been found to be harmful and unsustainable. For example, they contribute to health risks for humans, such as respiratory problems, skin irritation, and long-term effects like

Cancer and the agricultural workers who apply them and work on those fields contaminated with these pesticides and artificial fertilizers have chronic exposure and are thereby the most vulnerable group. Even as a consumer, the pesticide residue in agricultural plant food products poses health risks to consumers as well. The continuous application of pesticides will result in resistance development, thereby reducing effectiveness over time, and then farmers try to overcome this resistance by increasing the dose of pesticides, which is actually much more harmful in the long term. These artificial fertilizers and pesticides impact the environment in various ways by polluting the soil and water, ultimately harming plants, animals, people, and soil microorganisms.

One of the problems with these pesticides is that they harm non-target organisms. We aim to target a particular disease-causing organism or a specific pest insect. But when we apply these, they may be broad-spectrum and kill many beneficial insects as well as microbes. They may also be toxic to animals and therefore harm non-target organisms. To overcome many of these shortcomings, one idea is to use biocontrol as a pest management strategy, employing natural enemies or organisms to manage and reduce

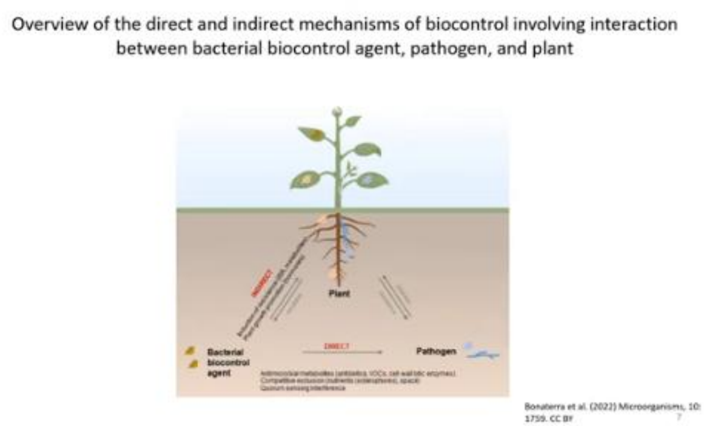


harmful pests, diseases, and weeds in agriculture and natural ecosystems. Here, you can see these Polistes wasps feeding on some of the harmful organisms colonizing cotton. Biocontrol minimizes reliance on chemical pesticides and fosters a more eco-friendly and sustainable approach to pest management. In brief, what is the importance of biocontrol for sustainable pest management? First, there is less chemical use by employing natural predators and other organisms.

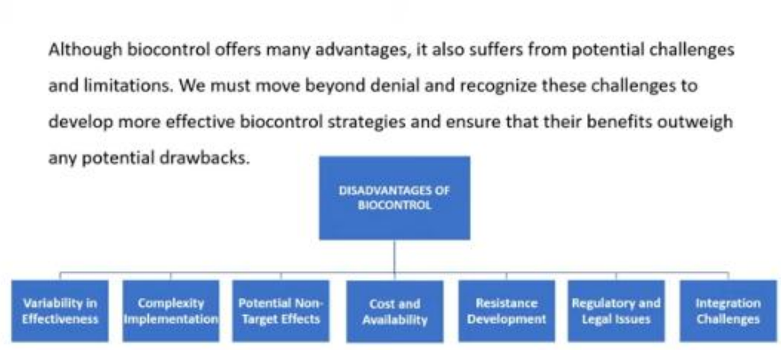
Farmers can use fewer synthetic pesticides, and this is important because too much pesticide can harm the environment, other animals that are not pests, and also us humans. Then it provides long-term solutions. Biological control can keep help keep pest populations low over a long time, and instead of just killing pests quickly, it helps create a balance in the ecosystem. This means pests are kept under control naturally, and it promotes biodiversity by introducing or enhancing the populations of beneficial organisms. Biological control helps increase the variety of life in a particular area, and this is beneficial for the environment because a diverse ecosystem is usually healthier and more resilient.

Biocontrol agents are essential for sustainable and effective pest and disease management. There is no doubt in it, and it offers significant benefits in both agricultural and environmental contexts, with various merits or advantages. For example, it helps in pest and disease management, and it has targeted actions that do not kill non-target organisms. Enhanced agricultural sustainability, safety, and health benefits—it does not harm humans or animals—and it helps in economic benefits, reduced environmental impact, and resilience to environmental changes. So, we have direct and indirect mechanisms of biocontrol, which involve interactions between bacterial biocontrol agents, pathogens, and plants, for example.

So, here when we apply a bacterial biocontrol agent, it will help directly by inducing resistance and also by promoting plant growth. indirectly. Then we have direct action, where it will kill the pathogen. So, antimicrobial metabolites, competitive exclusion, and quorum sensing interference will help us directly in controlling the pathogen, either by killing it or restricting it. Although biocontrol offers many advantages, it also suffers from potential challenges and limitations.



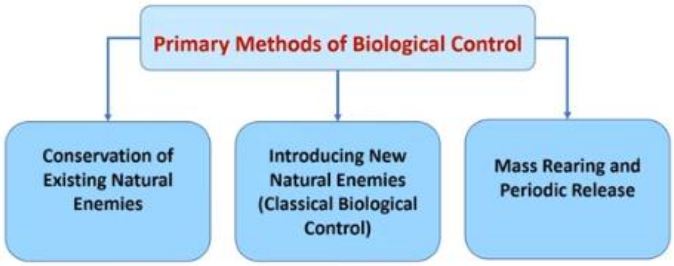
We must move beyond denial and recognize these challenges to develop more effective biocontrol strategies and ensure that they have benefit outweigh any potential drawbacks. Disadvantages are variability in effectiveness. So, there may be batch to batch variation or there may be also variation due to environmental conditions. Then it is very complex when it comes to application or implementation. And there can be potential non-target effects although we have told that it overcomes the disadvantage of synthetic pesticides.



To some extent there may be potential non-target effects which need to be examined and optimized. Cost and availability may sometimes be high. Because we need to grow these biocontrol agents under artificial conditions and there is chances of resistance development as well in this case if we are not following standard operating procedures or package of practices. There can be also certain regulatory and legal issues which do not allow the deployment of certain biocontrol agents in certain countries or certain areas and localities. And overall, there is a challenge of integration, particularly when it comes to integrated paste management.

Nevertheless, it has been found to be very, very effective because the disadvantages are actually compared to the advantages are much lesser. So, there are various methods of biological control. If you look into the primary methods, number one is conservation of existing nature. So, every ecological niche or agro-ecosystem may have, you know, naturally present enemies, which we need to preserve and enhance and enrich. Another method is introducing new natural enemies into an agro-ecosystem or an environmental niche, which is a most, you know, practiced method.

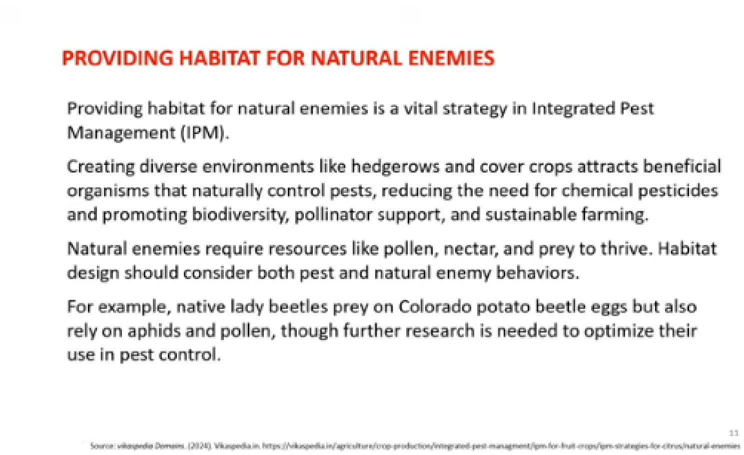
So, we call it classical biological control. And then there is mass rearing and periodic release. We will rear them in artificial conditions and keep on releasing these enemies of various pests. So, how do we conserve existing natural enemies? For example, we have here two examples: the natural parasitoids Trichogramma and Brachymeria, and then we have here natural predators, the Coccinellid and Lacewing.



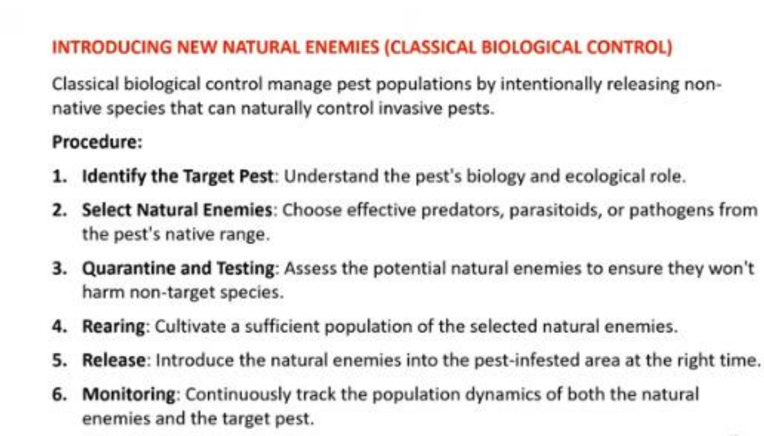
Conserving natural enemies is a key strategy. In protecting our ecosystems and ensuring a sustainable future for our planet. Natural enemies such as predators, parasitoids, and pathogens are crucial for controlling pest populations and reducing the need for chemicals. By protecting and encouraging these beneficial organisms, we improve pest control, support biodiversity, strengthen ecosystems, and promote sustainable farming. Natural enemies also provide important services like pollination and nutrient cycling, making our agricultural system more eco-friendly and resilient to climate change.

These overall reduce production costs and pesticide pollution and help prevent pesticide resistance. How do we provide a habitat for natural enemies, which is a vital strategy in integrated pest management? We need to create diverse environments, for example, like hedgerows and cover crops, to attract beneficial organisms that naturally control pests, reducing the need for chemical pesticides and promoting biodiversity, pollinator support, and sustainable farming. Natural enemies require resources like pollen, nectar, and prey to thrive. Habitat design should consider both pest and natural enemy behaviors.

For example, native lady beetles prey on Colorado potato beetle eggs but also rely on aphids and pollen, though further research is needed to optimize their use in pest control. Now, let us look into the method of classical biological control, where we rely on introducing new natural enemies. This is done intentionally by releasing non-native species that can naturally control invasive species or pests. We begin by identifying the target pest, understanding the pest biology and its ecological role. Then, we select natural enemies for their effectiveness as predators, parasitoids, or pathogens from the pest's native range.



Then, we need to follow quarantine procedures and undergo testing. Assess the potential natural enemies to ensure they won't harm non-target species. Then, rear and cultivate a sufficient population of the selected natural enemies, releasing them into the pest-infested area at the right time. Monitor continuously to track the population dynamics of both the natural enemy and the target pest. While doing this, we need to observe certain precautions, such as carrying out ecological impact assessments to evaluate potential effects on non-target species and ecosystems.



We also need to ensure host specificity, meaning the natural enemy is highly specific to the target host and does not affect non-target organisms. We must comply with local, national, and international regulations regarding non-native species introductions. Additionally, we need continuous public engagement, informing and involving stakeholders to gain support, address concerns, and make them partners in the entire process. We should always have contingency plans for possible negative outcomes, such as invasiveness. Long-term monitoring is very essential.

We implement ongoing assessments to evaluate effectiveness and ecological impact. Some successful examples of controlling the alfalfa weevil with introduced parasitoids can be seen in this picture. You have the adults of Oomyzus attacking an alfalfa weevil larva. And then you have the pupa of these, which I just dissected from a mummy formed from the integument of the alfalfa weevil larva. Then, in this case, you have the Bathplectes anurus.

Precautions:

1. **Ecological Impact Assessment**: Evaluate potential effects on non-target species and ecosystems.
2. **Host Specificity**: Ensure the natural enemy is highly specific to the target pest.
3. **Regulatory Compliance**: Follow local and international regulations regarding non-native species introductions.
4. **Public Engagement**: Inform and involve stakeholders to gain support and address concerns.
5. **Contingency Planning**: Prepare for possible negative outcomes, such as invasiveness.
6. **Long-Term Monitoring**: Implement ongoing assessments to evaluate effectiveness and ecological impact.

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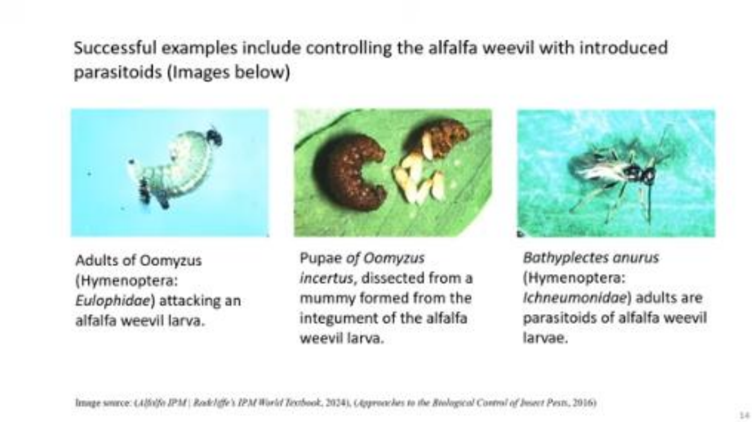
These adults are parasitoids of alfalfa weevil larvae. Mass rearing and periodic release. Here, seasonal inoculative release is one of the methods. This is useful when natural enemies cannot overwinter, requiring annual reintroduction. So, we have introduced them, but then due to changes in weather and lowering temperatures,

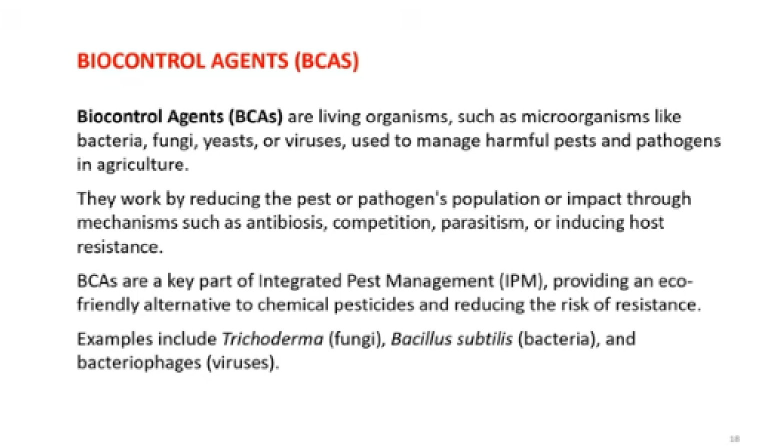


they will all die. So, we need to introduce them again next season. There are case studies in European greenhouses for controlling pest resistance to insecticides, and here we have the example of Pediobius against Mexican bean beetles and Edovum puttleri against Colorado potato beetles. Then, we also use biological insecticides and inundative release. This focuses on immediate pest reduction rather than establishing long-term natural enemy populations.

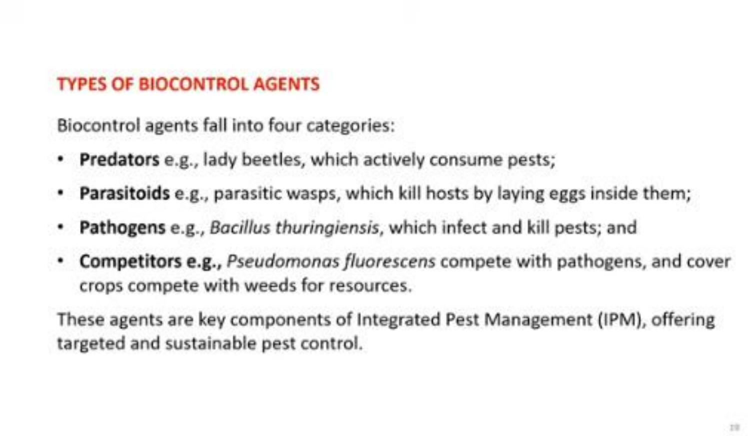
So, we have biological pesticides like Bacillus thuringiensis and beneficial nematodes. They target specific pests without creating lasting populations of natural enemies. In inundative release, we go for mass releasing natural enemies such as lady beetles or parasitic wasps for rapid control, but these do not establish sustained populations. While inundative release can be costly, ongoing research aims to optimize rearing methods and reduce expenses, enhancing its accessibility in integrated pest management. Let us now discuss biocontrol agents.

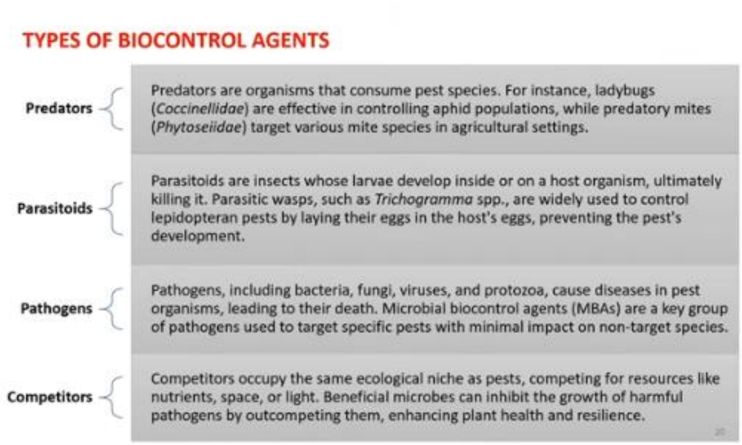
So, biocontrol agents, as we have understood by now, are living organisms such as microorganisms like bacteria, fungi, yeast, or even viruses—which are not living organisms, of course—and they are used to manage harmful pests and pathogens in agriculture. They work by reducing the pest or pathogen's population or impact through mechanisms such as antibiosis, competition, parasitism, or inducing host resistance. We will be discussing each and every one of them in the next few slides. Biocontrol agents are a key part of IPM, providing an eco-friendly alternative to chemical pesticides and reducing the risk of resistance.

So, some examples are like Trichoderma fungi, Bacillus bacteria, and then we also have bacteriophages, which are viruses. What are the various types of biocontrol agents? They may be predators like lady beetles, which actively consume pests. Then we have parasitoids like parasitic wasps, which kill hosts by laying eggs inside them. Then we have pathogens like Bacillus thuringiensis, which infect and kill pests, and competitors like Pseudomonas fluorescens, which compete with pathogens. Cover crops compete with weeds for resources.



These agents are key components of integrated pest management, offering targeted and sustainable pest control. Our discussion will focus more on microbial agents because this course is on microbial biotechnology. So, we have predators, parasitoids, pathogens, and competitors, as we have discussed in earlier cases. So, when it comes to predators, these are animals that hunt and eat other animals. In this context, we are talking about small creatures like ladybeetles and lacewings.





PREDATORS

Predators are animals that hunt and eat other animals. In this context, we are talking about small creatures like lady beetles and lacewings. These predators are called "free-living" because they do not rely on a host or a specific environment to survive; they can move around freely. Insect predators can be found in many places on a plant. They can be on the leaves, stems, and even in the soil below the plant. This means they can hunt for food in various environments, making them very adaptable.

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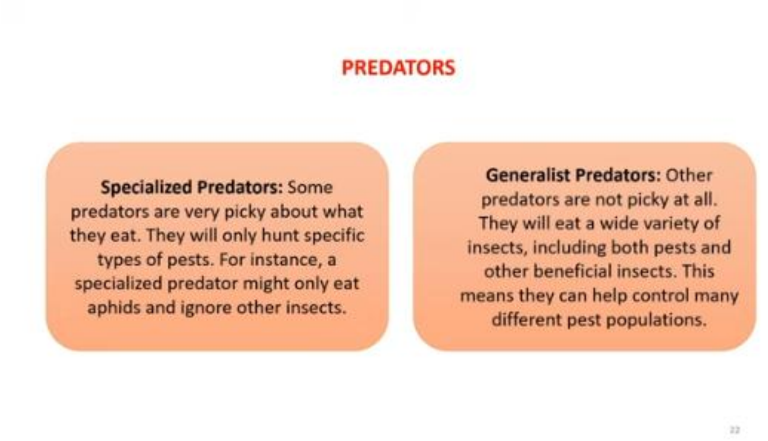
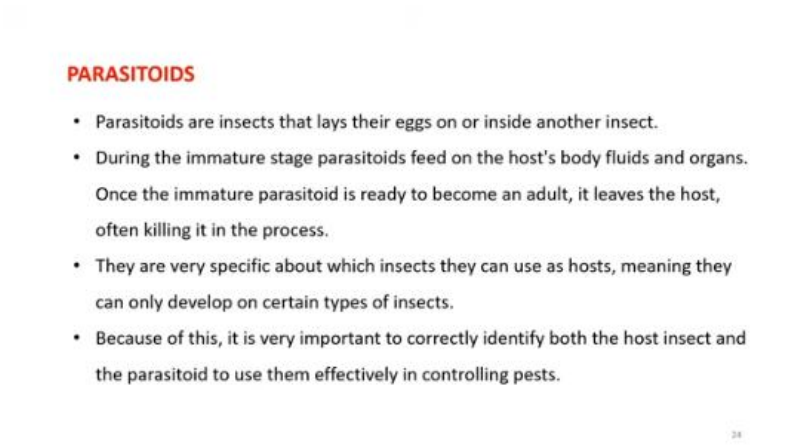
These predators are called free-living because they do not rely on a host or a specific environment to survive. They can move around freely. Insect predators can be found in many places on a plant. They can be on the leaves, stems, and even in the soil below the plant. This means they can hunt for food in various environments, making them very adaptable.

So, we have certain specialized predators which are very choosy or picky about what they eat. They will only hunt specific types of pests. For instance, a specialized predator might eat only aphids and ignore other insects. Then there are generalist predators, which are not choosy. They will eat a wide variety of insects, including both pests and other beneficial insects.

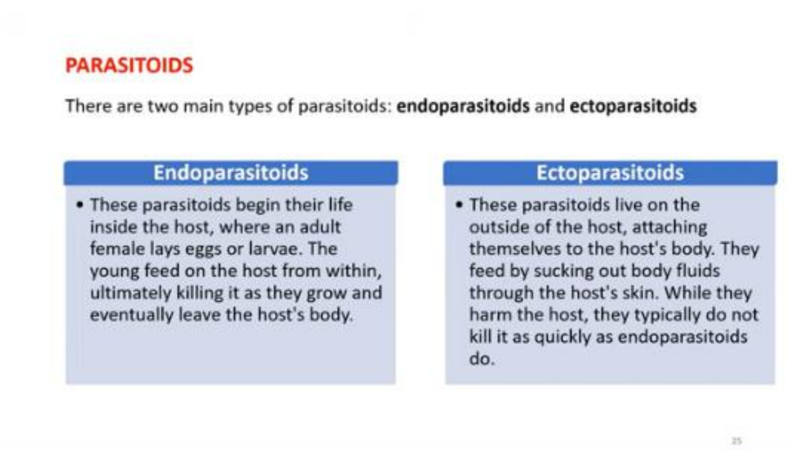
This means they can help control many different pest populations but may also be harmful when it comes to the consumption of beneficial insects. So, we have some of these examples, like the rove beetle. Then we have the hoverfly and the sawfly. And then we have the minute pirate bug, or insidious flower bug. The next type is the parasitoids—the insects that lay eggs on or inside another insect.



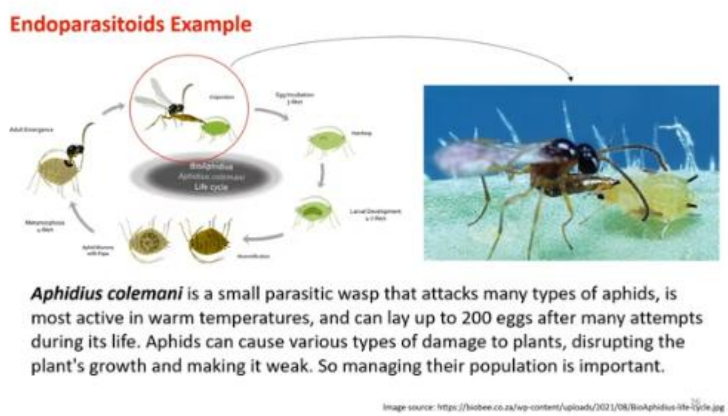
During the immature stage, parasitoids feed on the host's body fluids and organs. Once the immature parasitoid is ready to become an adult, it leaves the host, often killing it in the process. They are very specific about which insect they can use as a host, meaning they can only develop on certain types of insects. Because of this, it is very important to correctly identify both the host and the insect parasitoid to use them effectively in controlling pests. So, there may be two main types.



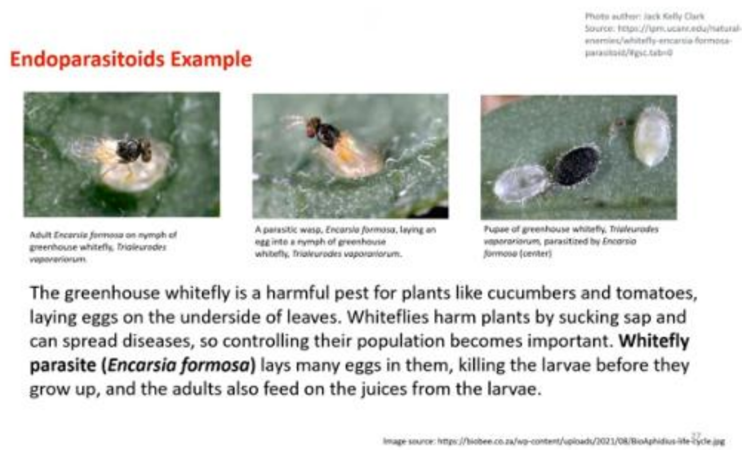
One is the endoparasitoids, which begin their life inside the host, where an adult female lays eggs or larvae. And then the young feed on the host from within, ultimately killing it as they grow and eventually leave the host's body. And then the ectoparasites live on the outside of the host, attaching themselves to the host's body. They feed by sucking out the body fluids through the host's skin. While they harm the host, they typically do not kill it as quickly as endoparasitoids do.



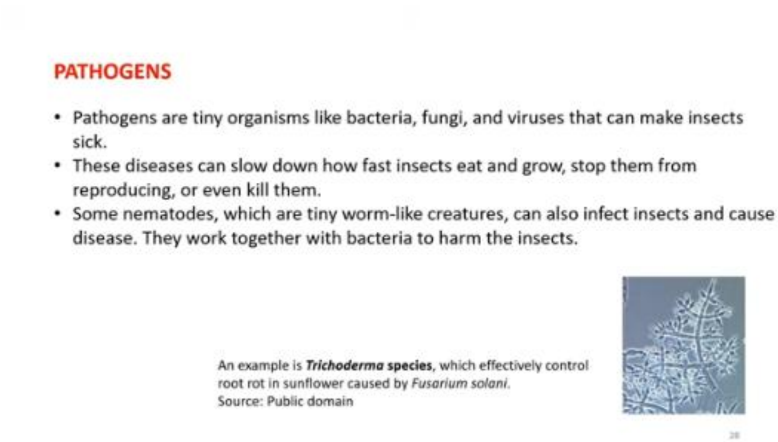
So, some examples are the Aphidius or Colomini, the small parasitic wasps that attack many types of aphids. It is mostly active in warm temperatures and can lay up to 200 eggs after many attempts during its life. Then you have the endoparasitoids. For example, the greenhouse whitefly is a harmful pest for plants like cucumbers and tomatoes, laying eggs on the underside of leaves while whiteflies harm plants by sucking sap. and can spread diseases.



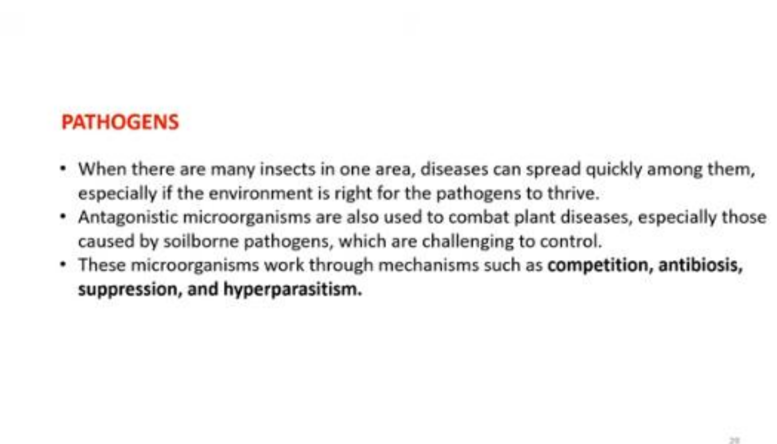
So, controlling their population becomes very important. Whitefly parasite Encarsia formosa lays many eggs in them, killing the larvae before they grow up, and the adults also feed on the juices from the larvae. The next class are the pathogens. These are tiny organisms like bacteria, fungi, and viruses that can make insects sick. These diseases can slow down how fast insects eat and grow, stop them from reproducing, or even kill them.



Some nematodes, tiny worm-like creatures, can also infect insects and cause disease. They work together with bacteria to harm the insects. So we have here an example of Trichoderma species, which effectively control root rot in sunflowers caused by Fusarium solani. When there are many insects in one area, diseases can spread quickly among them, especially if the environment is right for the pathogen to thrive.



Antagonistic microorganisms are also used to combat plant diseases, especially those caused by soil-borne pathogens, which are challenging to control. These microorganisms work through mechanisms such as competition, antibiosis, suppression, and hyperparasitism. Fungal biocontrol. Fungi are used to control harmful pests and pathogens in agriculture and horticulture. Fungi either directly attack pests or pathogens or outcompete them, offering an eco-friendly alternative to chemical pesticides.



Some examples of fungal biocontrol agents are Trichoderma species. These are widely used for controlling soil-borne plant pathogens such as Fusarium, Rhizoctonia, and Phytophthora. They work through competition, antibiosis, and parasitism. Beauveria bassiana is an entomopathogenic fungus that infects and kills a variety of insect pests like aphids, beetles, and caterpillars.

Then we have Metarhizium anisopliae. These target various insect pests, including termites and locusts, by infecting and killing them. Then there is Gliocladium virens. This is effective against damping-off diseases caused by fungi in seedlings. Then we have others like Aureobasidium pullulans.

FUNGAL BIOCONTROL

Fungi are used to control harmful pests and pathogens in agriculture and horticulture. Fungi either directly attack pests or pathogens or outcompete them, offering an eco-friendly alternative to chemical pesticides.

Examples of Fungal Biocontrol Agents:

- ***Trichoderma species***: Widely used for controlling soil-borne plant pathogens such as *Fusarium*, *Rhizoctonia*, and *Phytophthora*. They work through competition, antibiosis, and parasitism.
- ***Beauveria bassiana***: An entomopathogenic fungus that infects and kills a variety of insect pests like aphids, beetles, and caterpillars.
- ***Metarhizium anisopliae***: Targets various insect pests, including termites and locusts, by infecting and killing them.
- ***Gliocladium virens***: Effective against damping-off diseases caused by fungi in seedlings.

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These fungi combat pathogens like Botrytis cinerea and Penicillium species through nutrient competition and antifungal metabolite production. Effectively protecting fruits like apples and grapes and extending their shelf life. We also have Candida oleophila; these yeasts prevent rot in citrus and apples by competing for nutrients and inducing host resistance against pathogens like Penicillium species. It offers a non-toxic solution for post-harvest protection. Others, such as Saccharomyces cerevisiae and P.C.

- ***Aureobasidium pullulans***: This dematiaceous fungus combats pathogens like *Botrytis cinerea* and *Penicillium* spp. through nutrient competition and antifungal metabolite production, effectively protecting fruits like apples and grapes and extending their shelf life.
- ***Candida oleophila***: This yeast prevents rot in citrus and apples by competing for nutrients and inducing host resistance against pathogens like *Penicillium* spp. It offers a non-toxic solution for post-harvest protection.
- Other yeasts, such as ***Saccharomyces cerevisiae*** and ***Pichia anomala***, also produce antimicrobial compounds and enhance plant defenses, making them promising alternatives to chemical pesticides for controlling fungal diseases and post-harvest spoilage.

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anomala, also produce antimicrobial compounds and enhance plant defenses, making them promising alternatives to chemical pesticides for controlling fungal diseases and post-harvest spoilage. So, what are the advantages of using fungi as biocontrol agents? Number one, they are eco-friendly. Fungal agents are natural and degrade harmlessly in the environment, reducing chemical pollution. They have specific targeting.

They typically target specific pests or pathogens, minimizing harm to beneficial organisms like pollinators. They have reduced resistance. Pests and pathogens are less likely to develop resistance to fungal biocontrol agents compared to chemical pesticides. They help soil and plant health. Some fungi, like Trichoderma

Also promotes plant growth and improves soil health by enhancing nutrient uptake. They are important in IPM. They are valuable components of integrated pest management strategies contributing to long term sustainable pest control. We have also certain viruses and bacteriophages which are used in biocontrol. They serve as effective biocontrol agents, leveraging their unique properties to protect plants from harmful pathogens.

Advantages of Fungal Biocontrol Agents:

1. **Eco-Friendly**: Fungal agents are natural and degrade harmlessly in the environment, reducing chemical pollution.
2. **Specific Targeting**: They typically target specific pests or pathogens, minimizing harm to beneficial organisms like pollinators.
3. **Reduced Resistance**: Pests and pathogens are less likely to develop resistance to fungal biocontrol agents compared to chemical pesticides.
4. **Soil and Plant Health**: Some fungi, like *Trichoderma*, also promote plant growth and improve soil health by enhancing nutrient uptake.
5. **Integrated Pest Management (IPM)**: They are a valuable component of IPM strategies, contributing to long-term, sustainable pest control.

They offer innovative methods for sustainable agriculture by reducing reliance on chemical pesticides. Continued research and development are essential to fully realize their potential and address application challenges. Let us discuss some of the viruses like papaya ring spot virus. This is a well documented instance of cross protection which involves using a mild strain of papaya ring spot virus. So, to safeguard papaya plants against severe strains.

VIRUS AND BACTERIOPHAGES IN BIOCONTROL

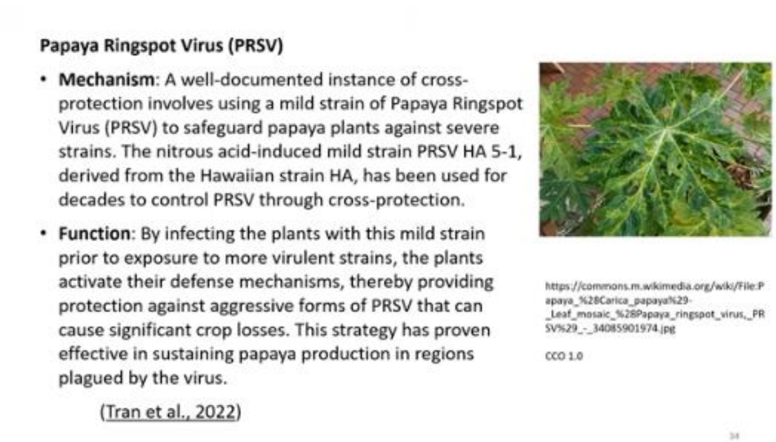
Viruses and bacteriophages can serve as effective biocontrol agents, leveraging their unique properties to protect plants from harmful pathogens.

They offer innovative methods for sustainable agriculture by reducing reliance on chemical pesticides.

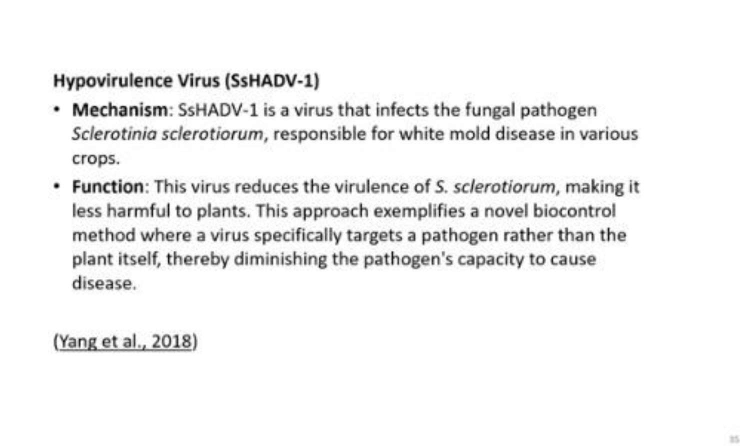
Continued research and development are essential to fully realize their potential and address application challenges.

So, it causes some disease as you can see over here. And if we use a milder strain, that will cause very mild disease or no disease. And that will actually protect the plant from further infection or colonization by a much more harmful strain. So, here we have a nitrous acid induced mild strain called as PRSVHA5-1. This is derived from the Hawaiian strain and this has been used for decades to control Papaya Ring Sport Virus through cross protection.

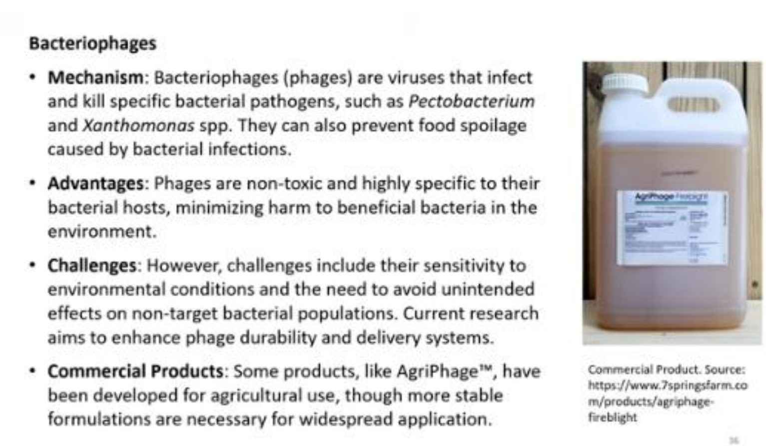
Then we have certain functions of these particular strains. By infecting the plants with this mild strain prior to exposure to a more virulent strain, the plants activate their defense mechanisms, thereby providing protection against aggressive forms of PRSV that can cause significant crop losses. This strategy has proven effective in sustaining papaya production in regions plagued by the virus. Then there is the hypovirulence virus. Here, this particular virus infects the fungal pathogen Sclerotinia sclerotiorum, which is responsible for white mold disease in various crops.



So, when we introduce this particular virus, it reduces the virulence of this Sclerotinia species, making it less harmful to plants. This approach exemplifies a novel biocontrol method where a virus specifically targets a pathogen rather than the plant itself, thereby diminishing the pathogen's capacity to cause disease. Then we have bacteriophages, which are viruses that infect and kill specific bacterial pathogens, such as Pectobacterium and Pseudomonas species. They can also prevent food spoilage caused by bacterial infections. So, some of the advantages are

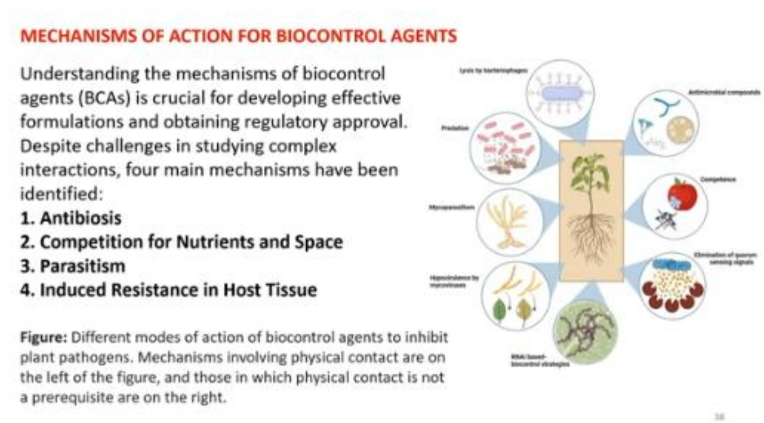


Phages are non-toxic and highly specific to the bacterial host, minimizing harm to beneficial bacteria in the environment. However, they suffer from certain challenges like their sensitivity to environmental conditions and the need to avoid unintended effects on non-target bacterial populations. Current research aims to enhance phage durability and delivery systems. Commercial products available, like AgriPhage, as you can see over here, given as an example, have been developed for agricultural use, though more stable formulations are necessary for widespread applications. Now let us discuss the various mechanisms of action of biocontrol agents.



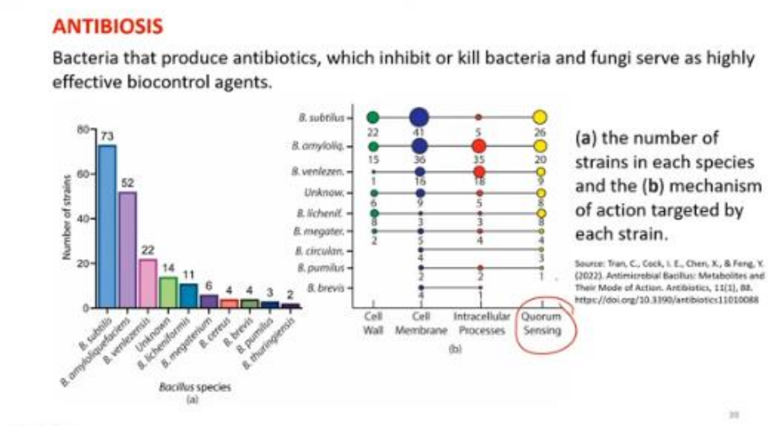
Some of these have actually been mentioned while discussing certain examples. Now, let us look into them in a little bit more detail. So, understanding the mechanism of biocontrol agents is crucial for developing effective formulations and obtaining regulatory approval. Despite challenges in studying complex interactions, four main mechanisms have been identified, which we have already heard about to some extent, such as antibiosis, competition for nutrients and space, parasitism, and induced resistance in host tissue. So, in this figure, you can see the different methods of action of biocontrol agents to inhibit plant pathogens.

Mechanisms involving physical contact are on the left side of the figure, and those in which physical contact is not a prerequisite are on the right side. Let us start with antibiosis. So, bacteria that produce antibiotics that inhibit or kill bacteria and fungi serve as highly effective biocontrol agents. So, you can see here the number of strains in each species, for example, given in this diagram A. You can see Bacillus subtilis is the most dominant one, followed by Bacillus amyloliquefaciens, and then we have others like Bacillus pumilus and Bacillus thuringiensis.



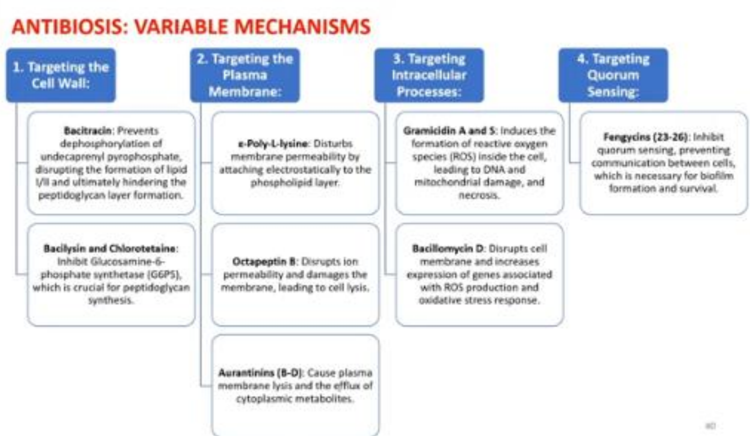
So, different organisms have different mechanisms of action targeted by each strain. For example, some of them will act on the cell wall. So, for example, you have Bacillus subtilis here, roughly around 22. Then, some of these will affect the cell membrane, some are involved in intracellular processes, while others interfere with quorum sensing.

So, some of these we will try to discuss in some of the other slides later. So, let us start with targeting the cell wall. So, here you have bacitracin, which prevents dephosphorylation of undecaprenyl pyrophosphate, disrupting the formation of lipid 1 and 2 and ultimately hindering peptidoglycan layer formation. Then there are bacilysin and chlorotetaine. These inhibit glucosamine 6-phosphate synthesis, which is crucial for peptidoglycan synthesis.

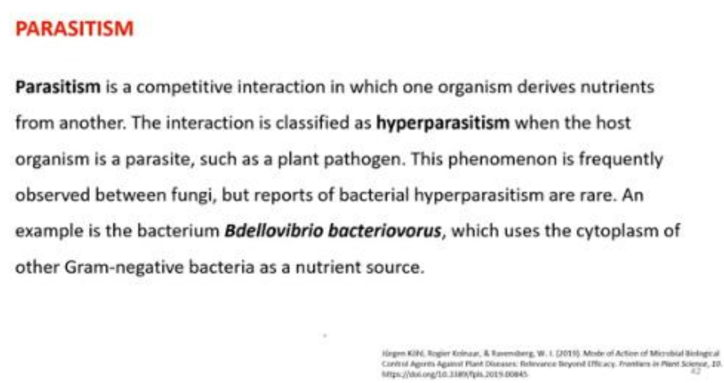


For those that target the plasma membrane, for example, epsilon-poly-L-lysine disrupts membrane permeability by attaching electrostatically to the phospholipid layer. Octapeptin B disrupts ion permeability and damages the membrane, leading to cell lysis. Then there are aurantinins, which cause plasma membrane lysis and the efflux of cytoplasmic metabolites. Then, when it comes to targeting intracellular processes, gramicidin A and S induce the formation of reactive oxygen species inside the cell, leading to DNA and mitochondrial damage and necrosis. And bacillomycin D disrupts the cell membrane and increases the separation of genes associated with ROS production and oxidative stress responses.

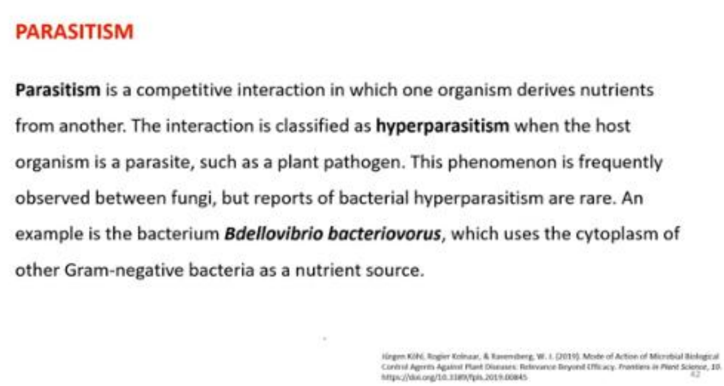
In targeting quorum sensing, fengycins inhibit quorum sensing, preventing communication between cells, which is necessary for biofilm formation and survival. Next comes the competition for nutrients and space. Microorganisms compete for nutrients and space with pathogens, limiting their growth. In the case of yeast, GuillierMondii competes against pathogens like Penicillium digitatum, particularly in nutrient-scarce environments.



Then you have the Enterobacter cloaceae bacteria which competes with Rhizophus stolonifer on peaches. And then yeast often form extracellular polysaccharide capsules to enhance their adhesion to surfaces like food skins, boosting their competitive ability. Then, in the rhizosphere, there are microorganisms like Pseudomonas jessenii RU 47, which compete with pathogens like Rhizoctonia solani, in crops like lettuce for nutrients and space in the rhizosphere, limiting the resource essential for the pathogen's growth. And in the case of Bacillus subtilis, this particular strain SRB28 colonizes sorghum roots, forming microcolonies and enhancing root development in seedlings.

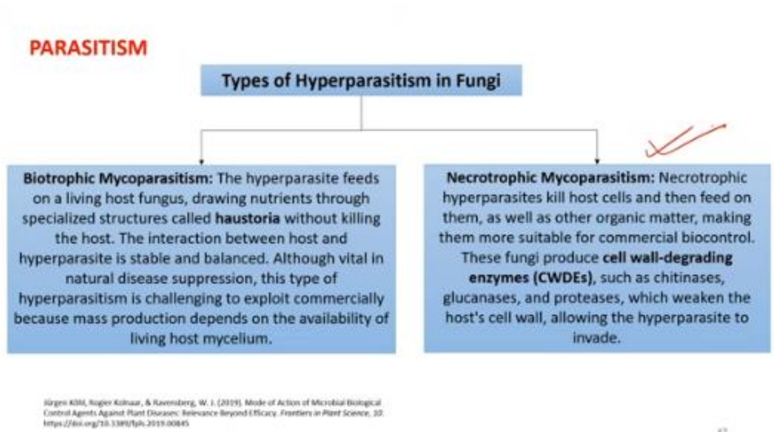


The next most important mechanism is parasitism which is a competitive interaction in which one organism derives nutrients from another. The interaction is classified as hyperparasitism when the host organism is a parasite such as a plant pathogen. These phenomena is frequently observed between fungi, but reports of bacterial hyperparasitism are also there. An example is the bacterium Bdellovibrio bacteriovirus, which uses the cytoplasm of other gram-negative bacteria as a nutrient source.

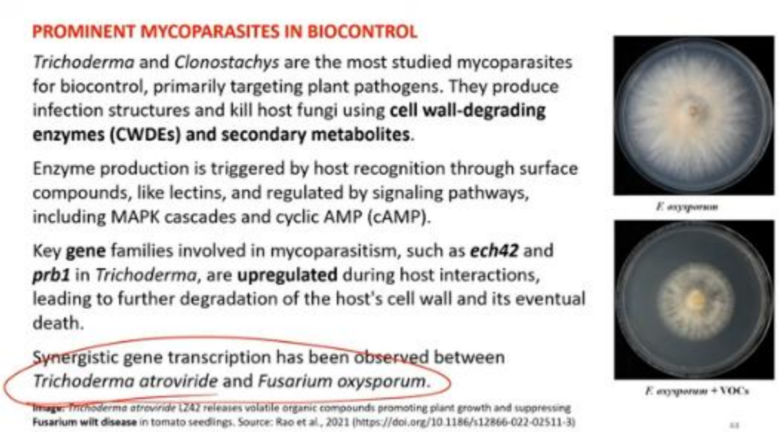


So, what are the types of hyperparasitism in fungi? We may have biotrophic mycoparasitism or we may have necrotrophic mycoparasitism. In the biotrophic, the hyperparasite feeds on a living host fungus, drawing nutrients through specialized structures called haustoria without killing the host. The interaction between host and hyperparasite is stable and balanced. Although vital in natural disease suppression, this type of hyperparasitism is challenging to exploit commercially because the mass production depends on the availability of living host mycelium.

In necrotrophic hyperparasitism, the hyperparasite kills host cells and then feeds on them as well as other organic matter, making them more suitable for commercial biocontrol. These fungi produce cell wall-degrading enzymes such as chitinases, glucanases, and proteases, which weaken the host cell wall, allowing the hyperparasite to invade. What are the prominent mycoparasites in biocontrol? Trichoderma and Clonostachys are the most studied mycoparasites for biocontrol. Primarily targeting plant pathogens, they produce infection structures and kill host fungi using cell wall-degrading enzymes and secondary metabolites.

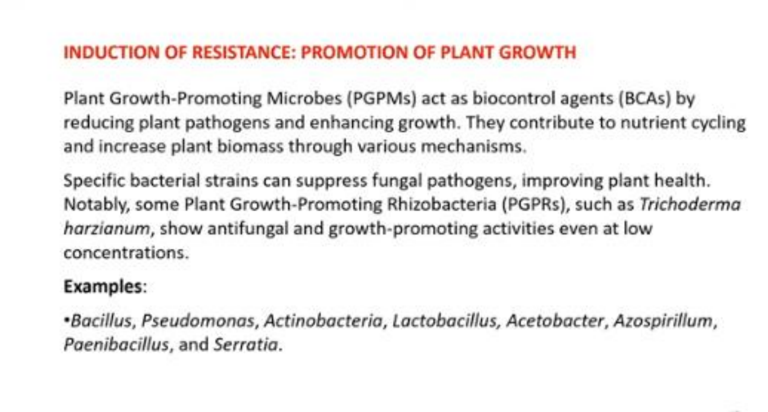


Enzyme production is triggered by host recognition through surface compounds like lectins and regulated by signaling pathways, including MAPK cascades and cyclic AMP. Key genes in families involved in mycoparasitism, such as ech42 and prb1 in Trichoderma, are upregulated during host interactions, leading to further degradation of the host cell wall and its eventual death. Synergistic gene transcription has been observed between Trichoderma atroviride and Fusarium oxysporum. In this figure, you can see Trichoderma atroviride releasing volatile organic compounds, promoting plant growth and suppressing Fusarium wilt disease in tomato seedlings.



Induction of resistance and promotion of plant growth. In these cases, plant growth-promoting microbes act as biocontrol agents by reducing plant pathogens and enhancing growth. They contribute to nutrient cycling and increase plant biomass through various mechanisms. Specific bacterial strains can suppress fungal pathogens, improving plant health, notably some plant growth-promoting rhizobacteria such as Trichoderma harzianum. So, antifungal and growth-promoting activities occur even at low concentrations.

Some examples include Bacillus, Pseudomonas, Actinobacteria, and several others. These PGPMs enhance nutrient uptake in various ways. Number one: nitrogen fixation. Rhizobial bacteria convert atmospheric nitrogen into a usable form, improving soil nitrogen and essential nutrients like iron and zinc. Next is phosphate solubilization; some of these convert insoluble phosphate into soluble forms for plant use.



Then there is siderophore production. These compounds sequester iron, making it more available. Then there is hydrogen cyanide production. Certain PGPMs produce hydrogen cyanide, which inhibits pathogen growth. The mechanism of induced resistance: microbial biocontrol agents enhance plant defenses through two main mechanisms—induced resistance and priming.

PGPMs enhance nutrient uptake by:

- **Nitrogen Fixation**: Rhizobial bacteria convert atmospheric nitrogen into a usable form, improving soil nitrogen and essential nutrients like iron (Fe) and zinc (Zn).
- **Phosphate Solubilization**: Some PGPMs convert insoluble phosphate into soluble forms for plant use.
- **Siderophore Production**: These compounds sequester iron, making it more available.
- **Hydrogen Cyanide (HCN) Production**: Certain PGPMs produce HCN, which inhibits pathogen growth.

Microbial biocontrol agents trigger both induced resistance and priming by releasing signaling molecules like MAMPs, siderophores, antibiotics, and volatile organic compounds. By promoting plant health without directly attacking pathogens, MBCAs help reduce the risk of resistance development in pathogens. Induced resistance is activated by specific stimuli recognized by plant receptors, such as pathogen-associated molecular patterns. Systemic acquired resistance, triggered by necrotizing pathogens, spreads different signals throughout the plant and to neighbors. Then there is systemic resistance initiated by beneficial microbes.

The ISR enhances defense against various pathogens. In the case of priming, it sensitizes the plant for a quicker, stronger defense response to future attacks, offering longer-lasting protection with lower energy cost than higher. There is transgenerational priming, which allows defense capabilities to be passed to offspring, which is quite an interesting phenomenon. Then there is the mechanism of microorganisms producing microbe-associated molecular patterns (MAMPs), recognized by plant receptors that include bacterial proteins, flagellin, and elongation factor TU. Then there are the fungal compounds, glucans and chitins.

Induction of Resistance: Mechanism

Microbial Biocontrol Agents (MBCAs)

MBCAs enhance plant defenses through two main mechanisms: Induced Resistance (IR) and Priming. **MBCAs** trigger both IR and priming by releasing signaling compounds like MAMPs, siderophores, antibiotics (e.g., pyocyanin), and volatile organic compounds (VOCs).

By promoting plant health without directly attacking pathogens, MBCAs help reduce the risk of resistance development in pathogens.

1. **Induced Resistance (IR)**: Activated by specific stimuli recognized by plant receptors, such as pathogen-associated molecular patterns (PAMPs).

- **Systemic Acquired Resistance (SAR)**: Triggered by necrotizing pathogens, SAR spreads defense signals throughout the plant and to neighbors.
- **Systemic Resistance (ISR)**: Initiated by beneficial microbes, ISR enhances defense against various pathogens.

So, in the case of the mechanism involving this defense, this induces defenses which involve producing reactive oxygen species, then phytoalexins, phenolic compounds, pathogenesis-related proteins, and physical barriers like cell wall modifications. So we have various biocontrol agents which can help us in controlling pest attacks on plants. So, in this table we saw, we list here some of the selected bacterial biocontrol agents for certain plant diseases. So, you have many microorganisms listed here in this column.

Induction of Resistance: Mechanism

2. Priming: Sensitizes plants for a quicker, stronger defense response to future attacks, offering longer-lasting protection with lower energy costs than IR.

- **Transgenerational Priming**: Allows defense capabilities to be passed to offspring.

Mechanism: Microorganisms produce microbe-associated molecular patterns (MAMPs) recognized by plant receptors (PRRs), including:

- **Bacterial Proteins**: Flagellin and elongation factor Tu (EF-Tu).
- **Fungal Compounds**: Glucans and chitins.

Defense Mechanisms: Induced defenses involve producing reactive oxygen species (ROS), phytoalexins, phenolic compounds, pathogenesis-related proteins, and physical barriers like cell wall modifications.

Then you have the target pathogens or the diseases, for example, the synonyms have been Given the full form here, AC stands for Alternaria citri, then you have Botryosphaeria B, and so on. So, for understanding the full meaning of these pathogens, please refer to the footnote below. Then these particular organisms in column 1 are used against certain plant species in vivo or in trials, as you can see here, for example, orange fruits and then you have the serif fruits, and so on. And then for reducing the disease, the dose that is to be applied is in this particular column, which is CFU per ml.

So, this is 20 to 70 CFU in this particular case. And then the mechanism involved is mentioned here. Mostly, these are antibiotics. Then, in certain cases, there is competitive exclusion, and in some cases, it is nutrient composition or induced resistance. So, to know more about them, kindly refer to the slides provided along with this lecture material.

Table: Selected bacterial biocontrol agents of plant diseases

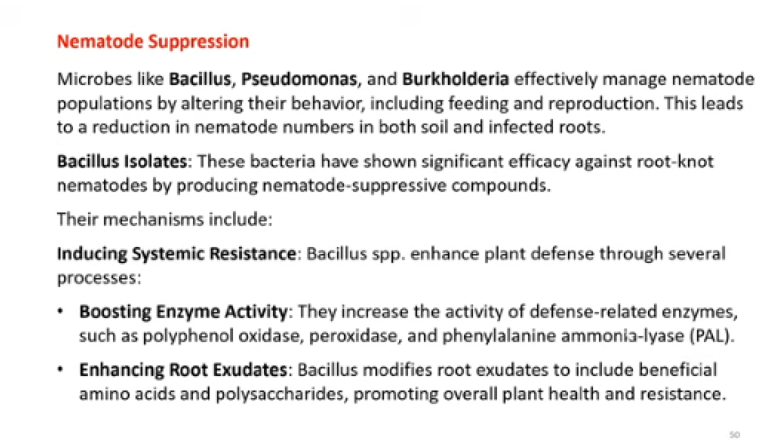
Microorganism and Strain	Target Pathogen or Disease*	In Vivo/In Planta Trials	Disease Reduction (%)/Application Dose/(CFU mL^{-1})	Mechanism Involved/Trait
B. amyloliquefaciens PPCB004	Ac, B, Cg, Fa, Lt, Pc, Pp	orange fruits	20-70/10^8	antibiosis
B. amyloliquefaciens CPA-8	Bc, Mf, Ml	cherry fruits	24-62/10^7	antibiosis
Bacillus subtilis UMAF6614 and UMAF6639	Pf	detached melon leaves	67-74/10^8	antibiosis
Bacillus velezensis A17	Ea, Ps, Xa	-	-	antibiosis
Leuconostoc mesenteroides CM160	BFV	-	-	antibiosis
Pantoea agglomerans EPS125	PF	apricot, peach, and nectarine fruits	49-61/10^7	competitive exclusion
P. agglomerans CPA-2	PF	pear fruits	50-95/10^7	competitive exclusion
Pseudomonas chlororaphis PCL1606	Rn	avocado plants	40/10^8	antibiosis
P.fluorescens EPS62e	Ea	detached flowers, and pear plants	31-98/10^8	competitive exclusion, nutrient competition
P. fluorescens EPS817, EPS894	Pc	strawberry plants	76-80/10^8	antibiosis
Pseudomonas simiae PICF7	Vd	olive plants	20-28/10^8	competitive exclusion /Induced resistance
Pseudomonas pseudoalcaligenesAVO110	Rn	-	-	competitive exclusion
Streptomyces strains CBQ-EA-2, CBQ-B-8	Mp,Rs	bean plants	60-75/10^8	Extracellular enzyme activities
Weissella cibaria TM128	PBF	apple fruits	50/10^8	antibiosis

* BFV, bioprotection of fresh fruits and vegetables; PBF, phytopathogenic bacteria and fungi; PF, postharvest fungi; Ac, Alternaria citri; B, Botryosphaeria sp.; Bc, Botrytis cinerea; Cg, Colletotrichum gloeosporioides; Ea, Erwinia amylovora; Fa, Fusicoccum aromaticum; Lt, Lasidiplodia theobromae; Mp, Macrophomina phaseolina; Mf, Monilia fructicola; Ml, Monilia laxa; Pc, Penicillium crustosum; Pp, Phomopsis persea; Pc, Phytophthora cactorum; Pf, Podosphaera fusca; Ps, Pseudomonas syringae; Rn, Rosellinia necatrix; Rs, Rhizoctonia solani; Vd, Verticillium dahliae; Xa, Xanthomonas arboricola

(Source: Bonaterra et al. 2022)

Now, one of the important pests is nematodes, and when it comes to biocontrol, microbes like Bacillus, Pseudomonas, and Burkholderia effectively manage nematode populations by altering their behavior, including feeding and reproduction. This leads to a reduction in nematode numbers in both soil and infected roots. Bacillus bacteria have shown significant efficacy against root-knot nematodes by producing nematode-suppressive compounds. The mechanism includes inducing systemic resistance through processes like boosting enzyme activity and enhancing root exudates.

In the case of boosting enzyme activity, they increase the activity of defense-related enzymes such as polyphenol oxidase, peroxidase, and phenylalanine ammonia lyase. In the case of enhancing root exudates, Bacillus modifies root exudates to include beneficial amino acids and polysaccharides, promoting overall plant health and resistance. Now, let us move to Section 4, where we will discuss traditional processes in biocontrol, emerging paradigms from pure culture to microbiome engineering, and the development of bacterial biocontrol agents. The traditional approach in biocontrol is to use pure-culture microorganisms. Here, we use single, well-characterized microbial strains that are isolated and cultivated in controlled environments.



Nematode Suppression

Microbes like **Bacillus**, **Pseudomonas**, and **Burkholderia** effectively manage nematode populations by altering their behavior, including feeding and reproduction. This leads to a reduction in nematode numbers in both soil and infected roots.

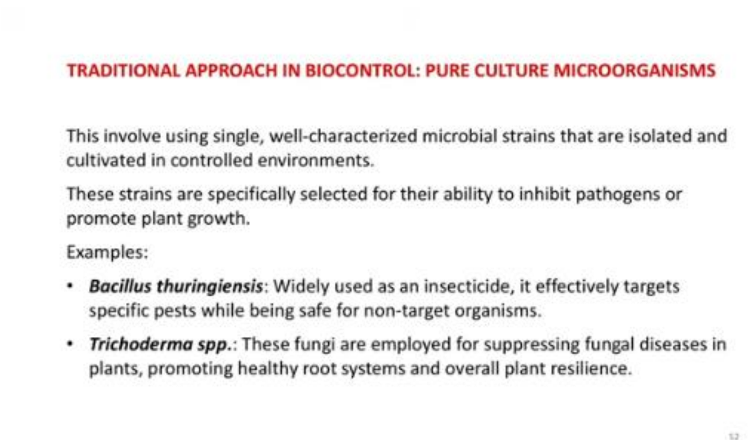
Bacillus Isolates: These bacteria have shown significant efficacy against root-knot nematodes by producing nematode-suppressive compounds.

Their mechanisms include:

Inducing Systemic Resistance: Bacillus spp. enhance plant defense through several processes:

- **Boosting Enzyme Activity**: They increase the activity of defense-related enzymes, such as polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase (PAL).
- **Enhancing Root Exudates**: Bacillus modifies root exudates to include beneficial amino acids and polysaccharides, promoting overall plant health and resistance.

These strains are specifically selected for their ability to inhibit pathogens and promote plant growth. Examples are Bacillus thuringiensis and Trichoderma species. In the traditional approach, the various advantages are control outcomes, easier predictability, and management due to well-understood mechanisms of action, specificity, known targets, and the ability to focus on controlling specific pests or diseases. However, there are certain limitations due to the narrow scope; pure cultures often have a limited range of activities and may not address complex pest and disease interactions. Additionally, there are adaptation issues: single strains may not perform well under diverse environmental conditions or in the presence of varying soil and plant microbiomes.



TRADITIONAL APPROACH IN BIOCONTROL: PURE CULTURE MICROORGANISMS

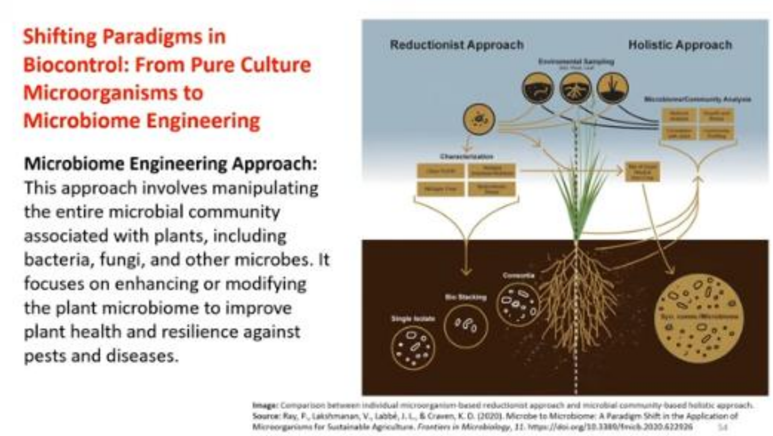
This involve using single, well-characterized microbial strains that are isolated and cultivated in controlled environments.

These strains are specifically selected for their ability to inhibit pathogens or promote plant growth.

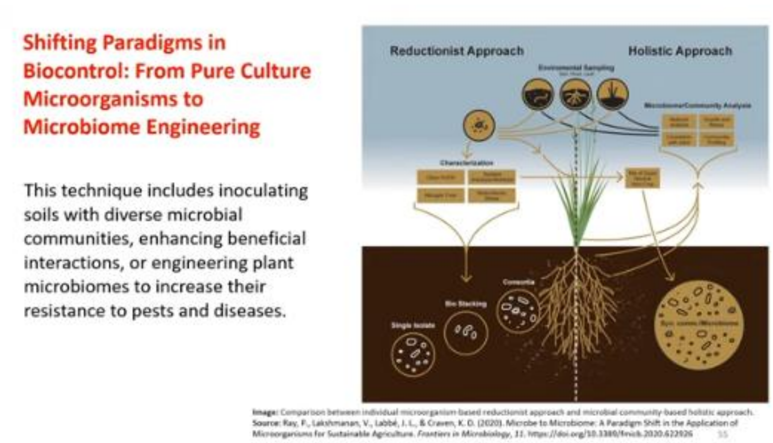
Examples:

- ***Bacillus thuringiensis***: Widely used as an insecticide, it effectively targets specific pests while being safe for non-target organisms.
- ***Trichoderma spp.***: These fungi are employed for suppressing fungal diseases in plants, promoting healthy root systems and overall plant resilience.

Thus, there is a shift in the biocontrol paradigm, where instead of using pure cultures, the focus is on microbiome engineering. This approach involves manipulating the microbial community associated with plants, including bacteria, fungi, and other microbes. It focuses on enhancing or modifying plant microbiomes to improve plant health and resilience against pests and diseases. Here, we compare the individual microorganism-based reductionist approach (using only one) with the microbial community-based holistic approach (using many), with a current focus on microbiome engineering. This technique includes inoculating soils with diverse microbial communities, enhancing beneficial interactions, or engineering plant microbiomes to increase resistance to pests and diseases.

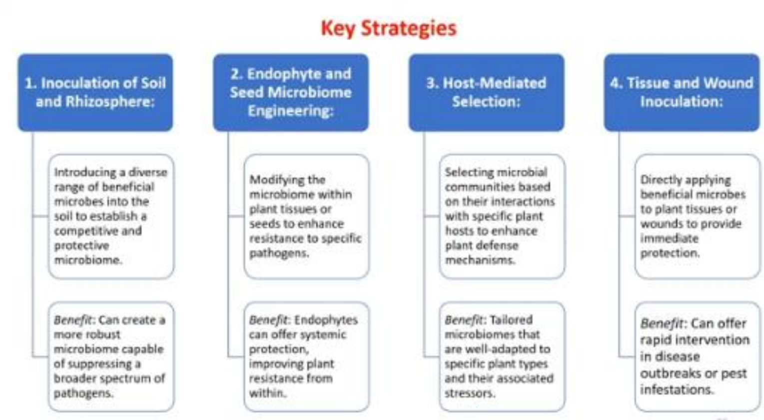


Here, you may first use a single isolate, then biostacking, and finally deploy a consortium. So, what are the key strategies? Inoculation of soil and rhizosphere. Introducing a diverse range of beneficial microbes into the soil to establish a competitive and protective microbiome. The benefit is that it can create a more robust microbiome capable of suppressing a broader spectrum of pathogens.



Then, endophyte and seed microbiome engineering involves modifying the microbiome within plant tissues or seeds to enhance resistance to specific pathogens. The benefit here is that endophytes can offer systemic protection, improving plant resistance from within. Then, there is the host-mediated selection. Here, we select microbial communities based on their interactions with specific plant hosts to enhance plant defense mechanisms. Here, the benefit is the tailored microbiomes that are well adapted to specific plant types and their associated stressors.

And then, finally, we have tissue and wound inoculation, where we directly apply beneficial microbes to plant tissues or wounds to provide immediate protection. This helps us in rapid intervention during disease outbreaks or pest infestations. So, what are the advantages of microbiome engineering? It's a holistic management approach, addressing the entire microbial ecosystem rather than focusing on individual organisms. There's enhanced resilience, creating a more resilient plant-microbe system capable of managing complex pest and disease interactions.



The adaptability to varying environmental conditions is potentially greater, as well as against more plant types. But there are certain challenges in the approach of microbiome engineering. The complexity: managing and understanding interactions within a diverse microbiome is more complex than working with a single microbial strain. Then, stability and persistence: ensuring that engineered microbiomes remain stable and functional over time and under different environmental conditions. Then, the problems of regulatory and safety issues: addressing potential ecological impacts and ensuring that engineered microbiomes do not adversely affect non-target organisms or ecosystems.

ADVANTAGES OF MICROBIOME ENGINEERING

- **Holistic Management:** Addresses the entire microbial ecosystem rather than focusing on individual organisms.
- **Enhanced Resilience:** Creates a more resilient plant-microbe system capable of managing complex pest and disease interactions.
- **Adaptability:** Potentially more adaptable to varying environmental conditions and plant types.

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CHALLENGES IN MICROBIOME ENGINEERING

- **Complexity:** Managing and understanding the interactions within a diverse microbiome is more complex than working with single microbial strains.
- **Stability and Persistence:** Ensuring that engineered microbiomes remain stable and functional over time and under different environmental conditions.
- **Regulatory and Safety Issues:** Addressing potential ecological impacts and ensuring that engineered microbiomes do not adversely affect non-target organisms or ecosystems.

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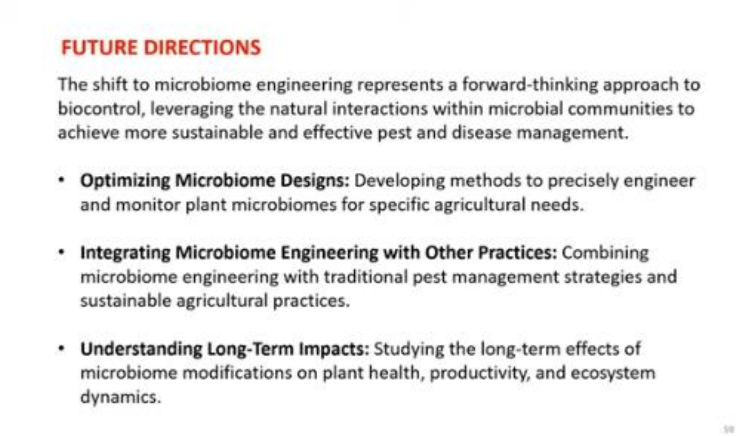
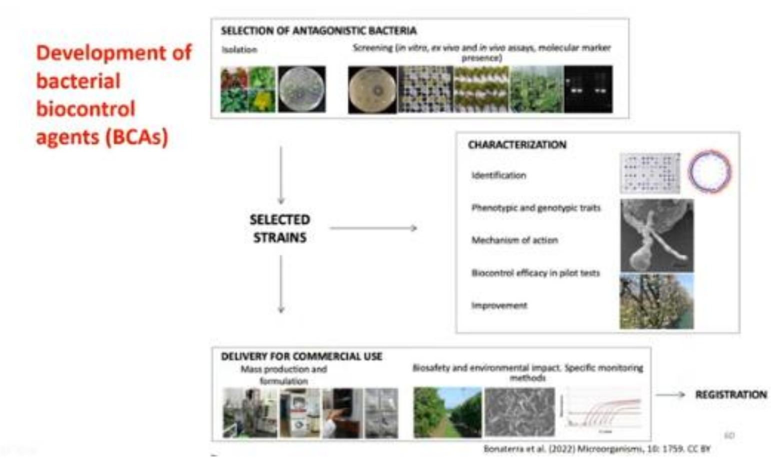
So, some of the emerging directions in the shift towards microbiome engineering represents a forward thinking approach to biocontrol, leveraging the natural interactions within microbial communities to achieve more sustainable and effective pest and disease management. So, we need to focus on optimizing microbiome design, develop methods to precisely engineer and monitor plant microbiomes for specific agricultural needs, then integrate microbiome engineering with other practices combining with traditional pest management strategies and sustainable agricultural practices. Understanding long-term impacts and studying the long-term effects of microbiome modification on plant health, productivity and ecosystem dynamics. So, here is a flow chart diagram for development of bacterial biocontrol agents. So, we have selection of antagonistic bacteria, we go for isolation and screening, then we finally select these.

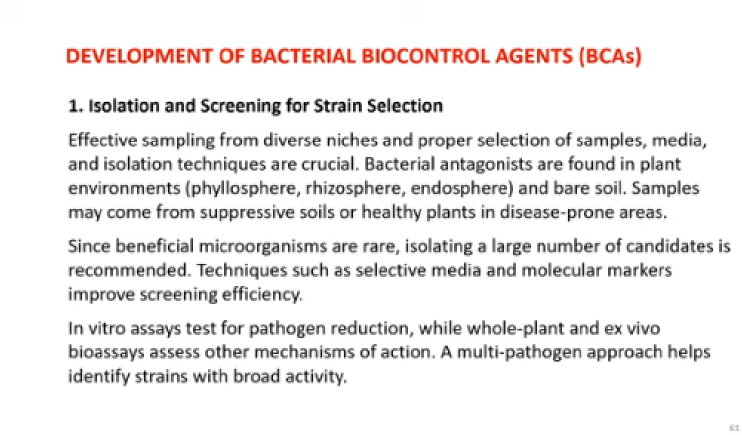


We need to be characterized that includes identification, phenotypic, genotypic traits, mechanism action, biocontrol efficacy in pilot test and their improvement. Once this is completed, we can go for delivery for commercial use. At this stage, we go for mass production and formulation. We need to go for biosafety and environmental impact assessment and here specific monitoring methods are required and of course we need to register these biocontrol agents under regulatory approval. So, in the first case, we go for the isolation and screening of the strain selection.

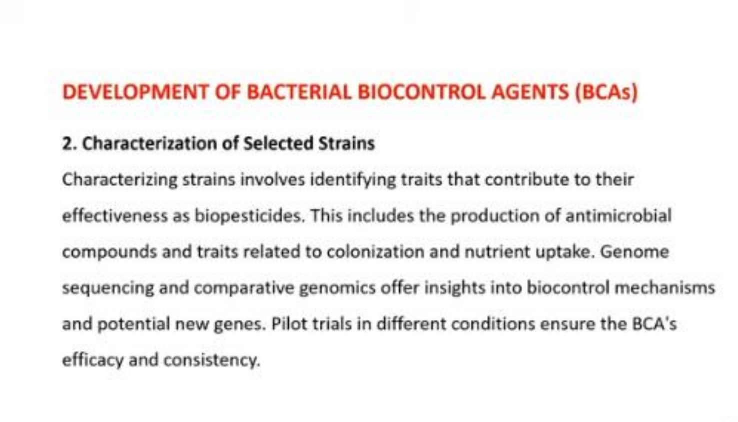


Effective sampling from diverse niches and proper selection of samples, media and isolation techniques are crucial. At this stage, bacterial antagonists are found in plant environments and bare soil. Samples may come from suppressive soils or healthy plants in disease-prone areas. Some beneficial microorganisms are rare. Isolating a large number of candidates is recommended.

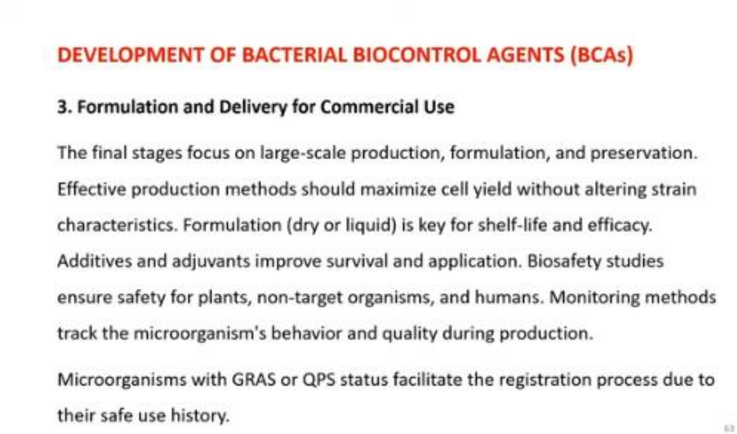
Techniques such as selective media and molecular markers improve the screening efficiency. In vitro assays test for pathogen reduction while whole plant and eggs vivo a bioassay assays other mechanisms of action. A multi-pathogen approach helps identify strains with broad activity. So, we go for the development of BCAs at the next stage where characterization of the selective strains are very, very important. That involves identifying traits that contribute to the effectiveness of biopesticides and includes the production of antimicrobial compounds and traits related to colonization and nutrient uptake.



Genome sequencing and comparative genomics offer insights into biocontrol mechanisms and potential new genes. There are pilot trials in different conditions which ensured a BCAS efficacy and consistency. Then the final development of bacterial control agents is the formulation and delivery for commercial use. This focuses on large scale production, formulation, preservation. Effective production methods should maximize cell yield without altering strain characteristics because if the characteristics are altered, the outcome will be altogether different and maybe not beneficial.



Formulation, whether in dry or liquid, is key for shelf life and efficacy. Additives and adjuvants improve survival and also the ease of application. Biosafety studies ensure safety for plants, non-target organisms, and humans. Monitoring methods track the microorganisms' behavior and quality during production. Microorganism suite generally regarded as safe status facilitated the registration process due to their safe use history.



DEVELOPMENT OF BACTERIAL BIOCONTROL AGENTS (BCAs)

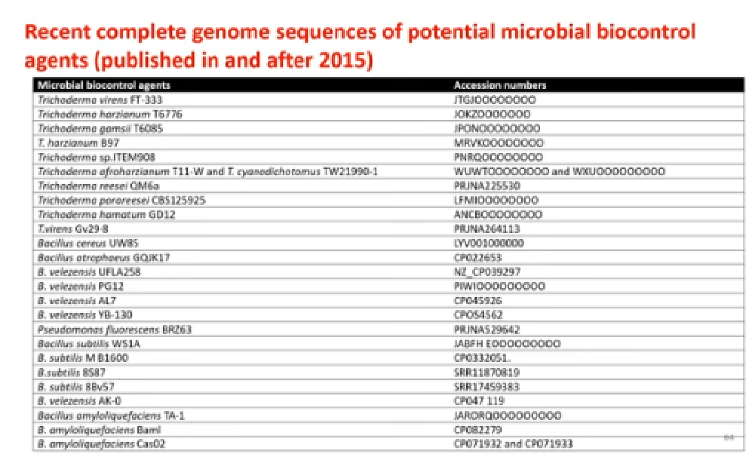
3. Formulation and Delivery for Commercial Use

The final stages focus on large-scale production, formulation, and preservation. Effective production methods should maximize cell yield without altering strain characteristics. Formulation (dry or liquid) is key for shelf-life and efficacy. Additives and adjuvants improve survival and application. Biosafety studies ensure safety for plants, non-target organisms, and humans. Monitoring methods track the microorganism's behavior and quality during production.

Microorganisms with GRAS or QPS status facilitate the registration process due to their safe use history.

So, this table lists some of the recently completed genome sequences of potential microbial biocontrol agents, which were published in and after 2015. There are many, like Trichoderma virens, and you can use these accession numbers to mine them from biological databases. Then, in Trichoderma, there are so many different species: harzianum, gamsii, reesei, hamatum, and so on. Then, in Bacillus, you have cereus and atrophaeus. And velezensis with different strains.

You also have Pseudomonas fluorescens and other species like Bacillus subtilis here, with different strains. So, with this, we come to the end of our lecture. Thank you for your patient hearing. Amen.



Recent complete genome sequences of potential microbial biocontrol agents (published in and after 2015)

Microbial biocontrol agents	Accession numbers
Trichoderma virens FT-333	JTGJ00000000
Trichoderma harzianum T6776	JOKZ00000000
Trichoderma gamsii T6085	JPON00000000
T. harzianum B97	MRVK00000000
Trichoderma sp.ITEM908	PNRQ00000000
Trichoderma afroharzianum T11-W and *T. cyanodichotomus* TW21990-1	WUWT00000000 and WXUO00000000
Trichoderma reesei QM6a	PRJNA225530
Trichoderma parareesei CBS125925	LFMI00000000
Trichoderma hamatum GD12	ANCB00000000
T.virens Gv29-8	PRJNA264113
Bacillus cereus UW85	LYV001000000
Bacillus atrophaeus GQJK17	CP022653
B. velezensis UFLA258	NZ_CP039297
B. velezensis PG12	PIWI00000000
B. velezensis AL7	CP045926
B. velezensis YB-130	CP054562
Pseudomonas fluorescens BRZ63	PRJNA529642
Bacillus subtilis WS1A	JABFH E00000000
B. subtilis M B1600	CP0332051.
B.subtilis 8S87	SRR11870819
B. subtilis 8Bv57	SRR17459383
B. velezensis AK-0	CP047 119
Bacillus amyloliquefaciens TA-1	JARORQ00000000
B. amyloliquefaciens Baml	CP082279
B. amyloliquefaciens Cas02	CP071932 and CP071933