

الجمهورية الجزائرية الديمقراطية الشعبية
DEMOCRATIC AND POPULAR REPUBLIC OF ALGERIA

Ministry of Higher Education
and Scientific Research

University of 20 August 1955
Skikda



وزارة التعليم العالي
والبحث العلمي

جامعة 20 أوت 1955
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Faculty of Sciences

Department of Ecology and Environment

Teaching document

Plant diseases

for Master 2 Applied Microbiology students



Written by Dr. Nora Sakhraoui

MCA

Academic year : 2023/2024

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Official program

Introduction

Microbial populations associated with the plant (rhizosphere): location, diversity, activity

I. Pathogens and parasites

1. Phytopathology (virulence, epidemiology, etc.).
2. Mechanisms of plant resistance to pathogens.
3. The pathogenic bacterium *Agrobacterium* and gene transfer to higher plants.

II. Beneficial microorganisms

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2. Associative symbioses: PGPR, antagonists, inducers of resistance, etc.
3. Soil resistant to disease.
4. Biological control, biofertilization, phytostimulation, revegetation of degraded sites.
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Introduction

1. Plant-microorganism interactions and types of relationships

Plants and microorganisms have shared the same environment for thousands or even millions of years, which has allowed them to develop communication mechanisms through the exchange of substances, which have been able to establish relationships between the two groups. Various microorganisms are therefore associated with different plant species whether they are cultivated or living in wild areas. Below, we give a perception on the diversity and activity of microbial populations associated with plants that will be gradually developed in this document.

Three types of relationships were defined:

- Negative relationships: in this case microorganisms infect the plant and are responsible for the appearance of diseases that can be fatal,
- Positive relationships: in this case plants and microorganisms enter into a symbiotic relationship,
- Neutral relationships: in this case microorganisms living in plant tissues benefit from substances produced by host cells but do not cause any disease or exchange metabolite with the plant.

2. Microbial populations associated with plants (location, diversity and activity)

Different microorganisms are associated with plants through the three types of relationships noted above. These microorganisms can be located on the surface of plants, especially around the roots (rhizosphere), they are called epiphytes, as they can be located inside plant tissues, they are called endophytes.

Fungi, bacteria, viruses, algae and protozoa can interact with plants and act in different ways.

In the mushroom group, for example, we find fungi that are beneficial to plants, such as mycorrhizae associated with roots and that significantly promote plant growth through the use of water and mineral salts, and harmful fungi (phytopathogens), responsible in this case for the appearance of diseases that can endanger the life of the plant, such as the genera *Alternaria*, *Botrytis*, *Fusarium*, *Phytophthora*, *Plasmopara* and *Puccinia*.

Bacteria also undertake various relationships with plants. Phytopathogenic bacteria belong to several genera and causes of various symptoms, several of which are incurable, among the genera including phytopathogenic species, we quote, *Erwinia*, *Ralstonia*, *Pseudomonas*, *Xanthomonas* and *Xylella*. Other bacteria are beneficial to plants and participate clearly as

several studies have shown in the development of these, such as the genera *Azorhizobium*, *Bradyrhizobium* and *Rhizobium*, whose species are associated with the roots of legumes for the fixation of atmospheric nitrogen or bacteria of the genus *Frankia* that can associate with several plant species such as alder and casuarinas to fix atmospheric nitrogen. Some bacteria secrete hormones similar to plant hormones that significantly promote plant growth like *Azospirillum* sp.

Viruses (Phytoviruses) and protozoa on the other hand undertake only negative relationships with plants, they are therefore responsible for the appearance of plant diseases that are most often difficult to treat or even incurable, but when present at low levels of infection, they can give plants resistance properties against different parasites.

I. Pathogens and parasites

1. Phytopathology

Phytopathology is a science that deals with the interaction between pathogens and plants. This science also involves the disease etiology, pathogenic identification and classification, disease cycles, plant disease epidemiology, disease resistance, and the effects of diseases on humans and other organisms. Monitoring plant health and diagnosing different plant diseases is essential to control the diseases in agriculture.

Plant diseases are divided into two types: non-parasitic (=physiological) diseases and parasitic diseases.

- a. Physiological diseases are caused by adverse environmental factors or nutritional deficiencies or excesses. They are generally easy to treat and are not transmissible from one plant to another.
- b. Parasitic diseases are caused by various phytopathogens, they are transmissible from one plant to another and are more difficult to treat. Fungi, bacteria, viruses and protozoa are the microorganisms responsible for the appearance of diseases in plants, they are able to induce various symptoms such as the change of coloration of the infected area, rot, leaf and fruit deformation, gummosis or gumming syndrome (= excessive secretion of gum), dwarfism, canker (= cracking of the bark often accompanied by secretion of gum), tumors (=galls)... etc. Some phytopathogens cause chronic diseases while others cause quick death. The appearance of a disease is under the influence of several factors such as: the physiology of the plant (sensitive or resistant plant), environmental factors (excessive moisture conditions promote the appearance of fungal diseases) and the minimum threshold of the inoculum (=phytopathogen), it must be present in sufficient quantities to be able to induce a disease, a minimum threshold is therefore essential without which, the phytopathogen cannot cause disease); for example, the minimum number of spores necessary to cause appreciable incidence and severity for Stripe rust (*Puccinia striiformis* f. sp. *Tritici*) affecting wheat is 10^5 – 10^6 spores.

1.1. Virulence and virulence factors

The term virulence has a conflicting history among plant pathologists. Here we adopted the definition given by the American Phytopathological Society that defines pathogenicity as the ability of a pathogen to cause disease on a particular host (i.e. a qualitative property), and virulence as the degree of damage caused to the host (i.e. a quantitative property). Therefore,

virulence is the key property of pathogens, and understanding the evolution of virulence has been a major goal in pathology, including plant pathology. The evolution of virulence may determine important phenomena such as the emergence and re-emergence of diseases or pathogens, host range expansion, and overcoming host resistance, which may compromise the success of control strategies for infectious diseases. If virulence, is the amount of damage caused to a host, then a **virulent microorganism** is a pathogenetic microorganism that causes measurable damage to the host. Consequently, **virulence factors** include all those pathogen factors that harm a susceptible host. In principle, any molecule (plant cell-degrading enzyme, toxin, hormone, siderophore, or extracellular polysaccharide) that occurs on the microbial cell surface or is translocated to an extracellular environment where it harms the host cells, is a virulence factor.

1.2. Epidemiology

Epidemiology of plant diseases is the study of disease in plant populations. Moreover, epidemiology is often viewed as an aggregation of statistical tools that could be used to discuss the associations of exposures to health outcomes. However, a thorough appreciation of this science is that of discovering causal relationships.

For a fungal disease for example, epidemiological studies attempt to determine spore dispersal patterns in order to predict the spread of an epidemic disease. Models of dispersal and disease transmission tend to be very complicated because a large number of variables affect spore movement. The concentration of spores at a particular distance from the source is determined by multiple factors including: (i) the number of spores released per minute, (ii) windspeed, (iii) air turbulence, (iv) convection currents, and (v) the sedimentation rate of the spores. When this physical puzzle is scaled up from a single source to an infected stand of trees, or to fields of infected crops, the complexities of disease forecasting become evident. Epidemiologists must also consider genetic variations in plant disease resistance, weather conditions, and other factors in forecasting the severity of the disease once the spores are deposited on the plant surface.

1.3. Diseases cycle

The disease cycle consists of a number of distinct steps that can be summarized into three successive steps (which can be alternated), namely: Conservation, spread and finally infection. **The conservation** of phytopathogens is done in several places, it can be done in the soil or on dead plant organs whether it is the organs of the aerial part or those of the underground part

including bulbs and tubers. Studies have shown that fungal spores of some phytopathogens, are able to withstand several years waiting for the improvement of environmental conditions.

The spread can be done through various vectors, including: air, insects and migratory birds and human being who currently contributes largely in this phenomenon through his cultural and other practices (soil amendments, work tools not disinfected, commercial traffic, introduction of new plants,...etc.).

The infection, meanwhile, is also done in 3 major steps : germination (in case of a fungal spore), penetration and finally the establishment of a trophic relationship. But it should be noted that before these 3 steps, another crucial step must first be done, it is the meeting between the phytopathogens and the adequate plant. Most often this encounter results from pure chance, but in other cases, it is the result of active chemotaxis between the two partners. Several plants secrete by their roots substances of various biochemical nature, these substances can be recognized by certain microorganisms that colonize the rhizosphere and are therefore attracted to the plant following an increasing gradient of the substance.

If the conditions are good, the fungal spore germinates, most often, without any external nutrient additions because it contains all the nutritional reserves that allow its germination.

To be able to feed and multiply, the phytopathogen must enter the plant tissue (case of endophytic phytopathogens: bacteria, viruses, protozoa and many fungi) or make penetrate a part of its body, hyphal extensions (= haustoria) for example in the case of Oidium fungi. The penetration strategies followed by phytopathogens are varied:

- Penetration can be through existing natural openings on plant tissues such as stomata and lenticels;
- It is also done through the plant cell walls, in this case the phytopathogen produces enzymes degrading the cell walls that allow it to weaken and cross them; microorganisms that follow this strategy usually prefer to attack the plant from a weak point at which the cell wall is naturally fragile which will facilitate its work such as absorbent hairs or growing areas root, these areas are devoid of protective tissues and are therefore vulnerable.
- As it can be done via the various injuries that could affect the plant, either injuries caused by man (such as pruning, shearing...) or injuries caused by hail, insects and nematodes. Remember that viruses and certain bacteria can only penetrate into plant tissues through wounds.

Once inside the plant tissue, the phytopathogen begins to feed, so it will establish a trophic relationship with the plant, these relationships can be fatal or not for the plant cell. This is where we define two types of phytopathogens:

Necrotrophic agents: produce pectinases and cellulases that degrade plant cell walls and cause the collapse of the host cell and ultimately death (called necrotrophs), these phytopathogens feed on dead material like degraded pectins. Other agents secrete specific toxins that can have a very harmful effect on the functioning of the plant cell that also ends up dying, such as phaseolotoxin (N-sulphodiaminophosphinyl-ornithyl-alanyl-homoarginine) produced by *Pseudomonas syringae* pv. *phaseolicola* and which has a toxic effect on chloroplasts.

Biotrophic agents, on the other hand, do not cause cell death, they produce very limited amounts of pectinases or cellulases, which have no negative effect on the plant cell wall; these agents (called biotrophs) usually have to feed on a very complex range of substances, which can only be synthesized by the host cell.

1.4. Some examples of plant diseases

1.4.1. Fungal diseases

Are ranked first in relation to the number of species that can cause diseases. Phytopathogenic fungi can be external or internal to plant tissues. External fungi develop mainly outside the tissues of the host plant and remain visible to the naked eye, they form myceliums that colonize external tissues such as the epidermis but send distinctive hyphal extensions, called haustoria, into living epidermal cells to feed (e.g. *Oidium*). Internal fungi develop inside the plant tissues between the intercellular spaces and are not visible to the naked eye, only a histological section and a microscopic observation can highlight their presence, their spores on the other hand are usually external allowing their propagation (e.g. rust fungi). Phytopathogenic fungal species belong to several branches and different classes, we find Basidiomycetes, Zygomycetes, Ascomycetes... etc. Among the most frequently reported fungal diseases in the world but also in Algeria are:

Rusts (black rust of wheat (caused by *Puccinia graminis*, yellow rust of cereals caused by *P. striiformis* such as yellow rust of wheat caused by *P. striiformis* f. sp. *tritici*), smut and bunt diseases of cereal (Loose smut of barley (*Ustilago nuda*) and wheat (*Ustilago tritici*), flag smut of wheat (*Urocystis tritici*), common bunt of wheat (*Tilletia laevis* and *T. caries*), monilia or brown rot of fruit trees (caused by *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia fructicola*), mildew (mildew of vine (caused by *Plasmopara viticola*), mildew of potato (caused by *Phytophthora infestans*)), scab (apple and pear trees caused by *Venturia inaequalis*).

fungal diseases can cause considerable damage in agricultural production but are most often controlled by the use of fungicides which give very positive results.

1.4.2. Bacterial diseases

Are ranked second in relation to the number of species that can cause diseases. These diseases are widespread throughout the world and cause enormous damage because many are not curable. The techniques used to fight these diseases are usually preventive. Antibiotics were also used to fight these diseases, but they were later banned because of the problem of resistance. Many are transmissible by insects. Inside plant tissues, bacteria can colonize several cellular sites, allowing them to take advantage of metabolites developed by the host cell and multiply. The most feared plant pathogens include:

Xylella fastidiosa named the killer of olive trees while it can affect dozens of other plant species; *Erwinia amylovora*, fire blight agent that can also affect several plant species, *Ralstonia solanacearum* (agent of bacterial wilt of plants and Moko disease of Banana), *Xanthomonas campestris pv citri* (the causal agent of Citrus bacterial canker) and many others.

1.4.3. Viral diseases

They also cause considerable damage to agricultural production worldwide, as they are not curable either. Viruses can also be positioned in different sites inside the attacked plant cell, some have a preference for transport tissues including liber where viral particles condense, this in fact allows them to be transported by insects that bite the plant tissues at the point of the vessels to suck the sap. The most feared plant pathogens include:

Citrus tristeza virus (CTV) is the most economically important virus disease of citrus in the world. In Algeria, the disease also exists but it is not widely spread, some niches have been detected for years in the region of Mitidja.

1.4.4. Protozoan diseases

Protozoa, or protists, are single-celled organisms, phytopathogenic protozoa belong to the Trypanosomatidae class. The protozoan diseases are less widespread than the former, is expressed much more in the tropical and subtropical regions, affecting strategic crops of these areas such as the cultivation of coffee, coconut and oil palm. The best known are:

Phytomonas leptovosorum (a flagellate protista, agent of coffee phloem necrosis disease), *Phytomonas stahelii* (responsible for cases of lethal wilt disease in coconut and oil palm trees).

1.5. How to diagnose plant diseases?

While some diseases are easy to identify in the field, especially by an expert, some are difficult and require a more minacious study to identify the phytopathogenic agent.

Plant pathologists use three main methods of detection: microscopy, growth and observation on media and molecular techniques.

- **Microscopy** is an important initial tool in plant disease diagnosis and in many cases, the only standard tool available. The suspected diseased plant tissues or pathogen itself are examined under a dissecting and/or compound microscope, and the pathologist can determine the morphology (shape) and size of the pathogen if it is present in the sample. This method requires highly trained personnel with extensive knowledge of classical pathogen taxonomy. This method generally does not allow the determination of the species of the pathogen
- **Culturing and observing the pathogen on media** in a plate/petri dish is another important traditional tool for disease diagnosis. A small piece of suspected diseased plant tissue is placed on growing media with different nutrients and chemicals in it. After several days of incubation, the pathologist will know more about the pathogen due to how it grows on the media, they will observe the color of the pathogen on the plate, the growth pattern it follows, and many other distinguishing characteristics. Bacteria and Fungi grow very differently on media, and it is easy to distinguish between the two types of pathogens. This method takes a long time and it is ineffective for some pathogens like viruses that cannot grow on their own without a host.
- with recent **technological advances**, rapid disease diagnosis strategies have significantly improved in the last two decades. Many different pathogens – including fungal, bacterial, and viral pathogens, can be identified with PCR (Polymerase chain reaction) tests and many other forms of genomic identification (multiplex PCR, real-time PCR, quantitative PCR, RAPD-PCR, DNA sequencing, etc). These molecular methods are highly effective for identifying a pathogen accurately to the species level.

1.6. Emergence of new diseases in the early 21st century

The end of the 20th century was characterized by the emergence of several diseases, this was mainly due to:

- Increased intercontinental trade,
- The introduction of new cultures in different parts of the world,
- Genetic homogeneity of crop varieties.

Plant diseases are capable of causing considerable damage to agricultural production and significantly influencing our quality of life, which is why we employ different strategies to combat them, including:

- Use of chemicals such as fungicides,
- Sterilization of work tools,
- Removal and incineration of infected plants,
- Quarantine areas or outbreaks of contamination by prohibiting any import or export of plant material,
- The use of resistant varieties of plants, the latter are obtained, via conventional techniques, after a long process of crossing and selection or by the biotechnological process

2. Mechanisms of plant resistance to pathogens

When a plant pathogen attacks a plant, two types of reactions can occur:

- A compatible reaction: in which the phytopathogen manages to develop considerably and colonizes plant tissues, in this case the plant is sensitive and the phytopathogen is virulent (virulent pathogen);
- An incompatible reaction: in which there is an early cessation of the development of the phytopathogen, the latter does not colonize the plant, the plant does not become diseased, and does not develop any symptom, in this case the plant is resistant and the phytopathogen is avirulent (avirulent pathogen).

The mechanisms that can explain an incompatible reaction are multiple, indeed, plants have several defense strategies that allow them to face a microbial attack, above we develop the most important.

2.1. External defence structures

These structures can have a varied composition, constitute a physical barrier to the penetration of phytopathogens, they are the first front of defence in plants, among these structures (= protective tissues) The epidermis can be solidified in several plant species by the presence of a layer of complex chemical substances (cutin polyester and waxes) called cuticle, or the bark most often constituted by specific tissues giving very rigid structure such as suberized cells. Some morphological barriers can also be classified in this category. A recognized example of a morphological barrier is the height of lips of stomatal guard cells. As shown by some studies, certain fungal rust pathogens possess exquisite detection mechanisms that sense the height of stomatal guard cell lips encountered on susceptible plants. Thus, when the hyphae find a lip of the proper height, they are programmed to undergo a developmental process resulting in the formation of invasive structures that enter the stomata and begin colonization of the leaf interior.

2.2. Secreted substances

These substances can be of various biochemical nature and act in different ways.

2.2.1. Substances inducing structural changes

These substances can **induce structural changes** in plant cell walls, making them stronger and more rigid. These substances can also prevent or limit the diffusion of microbial toxins to the cytoplasm and decrease the output of cytoplasmic electrolytes to the extracellular matrix. The

secretion of these substances can be local (limited to the point of attack of the pathogen, = papilla) or generalized (the deposition of these substances is done on the entire cell wall). These include lignin, callose, suberin, protein and pectic substances. The papilla is a local cell-wall fortification formed on the inner side of the plant cell walls at the site of pathogen penetration that is regarded as an inducible structural barrier. In the case of powdery mildews, papillae have been reported to play an important role against fungal invasion.

2.2.2. Substances with a toxic effect

Some substances have a **toxic effect** on the plant pathogen, in this category we find several metabolites.

- a. **Polyphenols**, which can have a double effect: in addition to being toxic compared to the phytopathogen, they induce the rigidity of cell walls, the low molecular weight precursors of lignin and free radicals (nitric oxide) act the same way.
- b. **Phytoalexins** are the most important substances in the plant defence system. They are antimicrobial low-molecular-weight compounds (secondary metabolites) that are synthesized *de novo* following pathogenic attack. Hundreds of Phytoalexins have been characterized (Flavonoid phytoalexins include kievitone and phaseollin from bean, pisatin from pea and glyceollin from soybean). They are especially widespread in the Fabaceae and Solanaceae but are also found in about 15 other families. Phytoalexins are not cosmopolitan; they have not been reported from the Cucurbitaceae and in the Poaceae they occur in oats, sorghum and rice, but apparently not in maize.

Phytoalexins are synthesized through complex biochemical pathways, and are chemically diverse, they are coumarines, isocoumarines, flavonoids, isoflavonoids, stilbenes, terpenoides and poly-acetylenes, but a large number of them are products of the shikimic acid (phenylpropanoid).

Phytoalexins are typically synthesized in moribund cells surrounding the site of infection and accumulate to high levels in dead and dying cells. Careful quantitation of the concentrations of phytoalexins in thin sections surrounding the site of infection has found levels sufficiently high to inhibit growth of fungi and bacteria, as well as to kill plant cells.

The substances inducing the synthesis of phytoalexins are called **elicitors**, the latter are very varied both for their biochemical nature and for the specificity of their action. The elicitors can be:

- **Oligosaccharides**, they can be of parasitic origin (released from the phytopathogen walls under the action of degrading enzymes of the plant) or of endogenous origin (released from the plant cell walls under the action of enzymes of the phytopathogen). Elicitors of an oligosaccharidic nature include: β -glucans, major constituents of microbial walls, chitin oligomers (constituting fungal walls) and oligogalacturonides (constituting plant cell walls). Studies have shown that both types of oligosaccharin elicitors (endogenous and parasitic elicitors) can coexist in a defence action leading to the synthesis of phytoalexins and that even in some cases, a synergistic relationship between the two types can also develop. This was observed in soybeans, where the mixture of these elicitors (endogenous and parasitic) induces a high concentration of glyceollin while each of the components is isolated inactive.
 - **Glycoproteins**: glycoprotein elicitors have been isolated from fungal and bacterial walls.
 - **Proteins**: studies have shown that protein elicitors produced by *Phytophthora* (phytopathogenic fungus) are decisive for an incompatible reaction with tobacco, e.g. cryptogein produced by *Phytophthora cryptogea*.
 - **Lipids**: have been isolated from *Phytophthora infestans* and *Phytophthora capsici*. They can be phospholipids, triglycerides, glycolipids... etc.
- c. **PR proteins**: (Pathogenesis-Related proteins) first identified in 1970 from tobacco leaves infested with the Tobacco mosaic virus (TMV). PR proteins are produced by plants during normal development or as part of an induced defence from fungal pathogens, against which they exert biocontrol. Therefore, their biosynthesis and accumulation is considered a major defence mechanism of plants against fungal pathogens. PR proteins are also induced in response to various environmental stress factors, such as drought, salinity, wounding, heavy metals, endogenous and exogenous elicitor treatment, and plant growth regulators. These proteins are now classified into seventeen classes, numbered in the order in which they were discovered, from PR-1 to PR-17 (**Table 1**).

PR proteins have different modes of action, but their toxic effect on phytopathogens has been demonstrated. Some are hydrolytic enzymes, such as PR-2 (β -1.3-glucanases) and PR-3, 4, 8 and 11 (chitinases) acting respectively against β -glucans and chitin oligomers, components of the cell walls in most higher fungi. These

enzymes allow to stop the growth of the phytopathogen by degrading its walls and on the other hand, they allow to release the elicitors to activate the synthesis of phytoalexins. Others are pectinase inhibitors that prevent the latter from degrading the plant cell walls. The function of many PR proteins remains a mystery.

Table 1. Families of pathogenesis-related proteins.

Family	Type member	Biochemical properties	Molecular mass range (kDa)
PR-1	Tobacco PR-1a	Unknown	15–17
PR-2	Tobacco PR-2	β -1,3-glucanase	30–41
PR-3	Tobacco P,Q	Chitinase class I, II, IV, VI, VII	35–46
PR-4	Tobacco R	Chitin-binding proteins	13–14
PR-5	Tobacco S	Thaumatococcus-like	16–26
PR-6	Tomato inhibitor I	Proteinase inhibitor	8–22
PR-7	Tomato P ₆₉	Endoproteinase	69
PR-8	Cucumber chitinase	Chitinase class III	30–35
PR-9	Tobacco 'lignin forming peroxidase'	Peroxidase (POC)	50–70
PR-10	Parsley 'PR-1'	'Ribonuclease-like'	18–19
PR-11	Tobacco class V chitinase	Chitinase class V	40
PR-12	Radish Rs-AFP3	Defensins	5
PR-13	<i>Arabidopsis</i> THI-2.1	Thionins	5–7
PR-14	Barley LTP4	Lipid transfer proteins	9
PR-15	Barley OxOa (germin)	Oxalate oxidases	22–25
PR-16	Barley OxOLP	Oxalate oxidase-like protein	100 (hexamer)
PR-17	Tobacco PRp27	Unknown	?

2.3. How to explain a compatible reaction ?

A compatible reaction can be explained by several mechanisms, namely:

- The absence of plant defence mechanisms induction (absence of synthesis of phytoalexins), this can be explained in turn by the absence of elicitors on the walls of the pathogen or the absence of receptors on the plant walls. The difference between a virulent microorganism and avirulent microorganism can be quantitative: virulent agent produces few quantities of elicitors inducing a small defensive reaction while an avirulent agent produces many.
- Circumvention of defence mechanisms; this is done by the production of mutant strains resistant to phytoalexins or able to metabolize them into other substances much less toxic.

Virulence of the pea pathogen *Nectria haematococca* is genetically correlated with ability to detoxify the pea phytoalexin pisatin by cytochrome P450-catalyzed demethylation;

- Suppression of defence mechanisms: some fungi secrete substances that have the ability to suppress phytoalexin accumulation and even phytoalexin synthesis. Suppression is the most widespread process among phytopathogenic microorganisms.
- Slowing defence mechanisms: it is clear that a plant that synthesizes and accumulates phytoalexins faster is more resistant than another plant that does it slowly.

3. The pathogenic bacterium *Agrobacterium* spp. and the transfer of bacterial gene to higher plants

Plant tumors are symptoms that can be induced by several phytopathogenic agents such as viruses and bacteria including *Agrobacterium tumefaciens*, which can infect several plant species such as tobacco, tomato or common sunflower. *Agrobacterium tumefaciens* and plant root hairs caused by *A. rhizogenes* involves a permanent genetic change of the plant host cell in which a segment of bacterial DNA is transferred and integrated into the plant genetic material.

3.1. Presentation of *Agrobacterium tumefaciens* (Figure 1)

Agrobacterium tumefaciens is a soil bacterium, flagellated, bearing 1-6 flagella of the family Rhizobiaceae, by this belonging, it is thus close to the *Rhizobium* which associates with the Fabaceae species. Its genetic material is found in two forms, a linear chromatin (non-circular chromosome) and a circular chromatin called plasmid Ti (for tumor-inducing) or pTi, which includes several distinct regions according to their function:

- T region or T-DNA (for Transferred DNA), specific segment of DNA because it can be transferred and incorporated to the genome of the host plant,
- Vir region (virulence region), includes genes responsible for controlling T-DNA transfer,
- Region of the catabolism of bacterial products, includes genes responsible for the absorption and degradation of nutrients (source of carbon and nitrogen).

3.2. Plant-*Agrobacterium* interaction

The plant-*Agrobacterium* interaction can be established through several stages (Figure 2):

- **Attraction of the bacterium to the plant** (positive chemotaxis): studies have shown that this attraction is regulated by the products secreted by the plant during its injury, these products, most often, phenolic, are recognized by the bacteria that colonizes the soil, and that follows an increasing gradient of these substances to arrive near the wounded plant. Membrane proteins, encoded by the genes of the Vir region, are involved in the recognition of these signals and the transmission of an intracellular message leading to the attraction or mobility of the bacteria to the wound area.

- **Penetration and attachment** : the bacteria enters plant tissues from the wound, this is its only penetration strategy, and then attaches to specific sites at the level of plant cells through products encoded by the genes of the bacterial chromosome. Attachment is an essential step for virulence of the bacteria; induced mutations affecting certain components of the bacterial wall and its membrane (including polysaccharides and membrane peptides) making bacteria unable to attach to the plant cell, gave avirulent bacteria or less virulent bacteria.
- **T-DNA transfer**: this step is managed by the virulence region, the products of the specific genes of this region allow the excision of the T region to the border sequences, leading to its release, other products of the Vir region allow the transport, protection and integration of the T region into the plant genome.
- **Results of incorporation of the T-region**: the T region enters the plant nucleoplasm through the nuclear pores. A plant cell can receive about ten copies of T-DNA that will be inserted on several sites of the same chromosome or distributed on several chromosomes. The tomato has 7 sites spread over 5 chromosomes.
Incorporation gives the plant cell two properties:
 - The synthesis of opines (two coupled amino acids) such as nopaline, octopine, agropine, source of carbon and nitrogen for the bacteria.
 - Overexpression of genes encoding the synthesis of plant growth hormones such as Auxin and Cytokinin, the accumulation of which causes the appearance of the tumor.

Infection by *Agrobacterium* is now used routinely to introduce foreign genes, engineered into the T-DNA, into plants. The *Agrobacterium* bacteria used are “disarmed” by removal of the genes responsible for tumor formation and opine production.

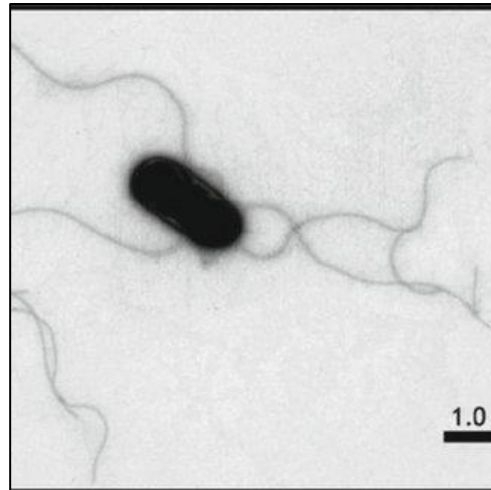


Figure 1. Electron microscopic view of *Agrobacterium tumefaciens*.

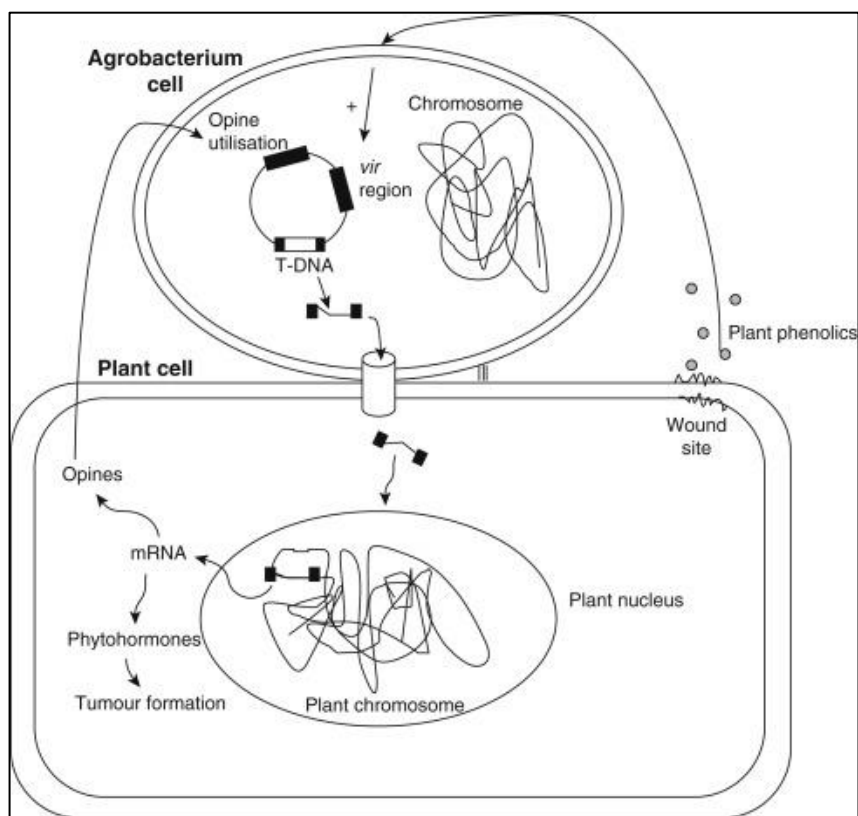


Figure 2. Infection by *Agrobacterium* spp. and the transfer of a bacterial DNA segment to the plant cell nucleus.

II. Beneficial microorganisms

It is estimated that about 20,000 plant species (out of about 300,000) would be fully dependent microbial organisms for growth and survival. This dependence implies the establishment of systems of recognition and functioning between the plant and microorganisms that need to be regulated so that the association takes place; this will therefore lead to a specificity of the associations between the plant and so-called symbiotic microorganisms. symbiotes set up mechanisms to recognition of the appropriate host plant that will provide them with the necessary nutrients.

1. Symbioses: Symbiotic nitrogen fixation and mycorrhizae.

1.1. Symbiotic nitrogen fixation

1.1.1. In Fabaceae (legume family)

Symbiotic fixation of atmospheric nitrogen can be observed in several plants and microorganisms. The classic example of this symbiosis is undoubtedly that of leguminous species (Fabaceae) in association with soil rhizobia (*Rhizobium*, *Bradyrhizobium*, *Azorhizobium*...). It should be noted that naturally, in the free state, the *Rhizobium* species are often flagellated bacteria that do not generally carry out diazotrophy (nitrogen fixation) but, within the plant, these bacteria lose their mobility and present a nitrogen autotrophy (ability to assimilate mineral nitrogen, here N₂) while remaining heterotrophic to carbon (inability to assimilate mineral carbon). In this association, the plant provides the bacteria with an ecological niche and carbon sources that are necessary for their development, and in return, bacteria transfer nitrogen in to the plant in an assimilable form (ammonia (NH₃) or ammonium ions (NH₄⁺)).

- **Fabaceae-*Rhizobium* interaction (Figure 3)**

Attracted by the substances secreted by the roots of leguminous plants (flavonoids generally), *Rhizobium* bacteria in turn secrete, hydrolytic enzymes and penetrate inside the plant through the root hairs after causing their deformation and curvature under the effect of other bacterial substances: Nod factors. These lead to other reactions and responses that are very important for the installation of the symbiosis:

- They induce the synthesis of nodulines (of a protein nature) by the cells of the host plant, which in turn induce the dedifferentiation of the cells of the root bark and the constitution of a specific meristem (plant embryonic tissue) called nodule meristem.

- They also induce the invagination of the plasma membrane of the root hair and the constitution of a tubular infection thread, which will be very quickly colonized by bacteria in intensive division. Rhizobia in the infection thread are in the apoplast and remain physically separated from plant cell cytoplasm, they are surrounded by an exopolysaccharide capsule, which may play an important role in facilitating their movement.

The infection thread will gradually growth into the root structure, until it reaches the nodule meristem. the growth and development of the infection thread are controlled by the host plant with a constant signal exchange between symbionts. Nod factors and low molecular mass exopolysaccharides have been proposed as such signaling molecules. Rhizobia continue to express *nod* genes and synthesize Nod-factor in the infection threads.

- At this level, bacteria are released from infection threads into meristem cells as organelle-like structures termed symbiosomes, which are still surrounded by a plant membrane (symbiosomal membrane) that is structurally equivalent to the plasma membrane. Within the symbiosome compartment, rhizobial cells lose their exopolysaccharide capsule, stop dividing and differentiate into an endosymbiotic, nitrogen-fixing form (bacteroids) thanks to nitrogenase activity.

A vascular connection establishes between the nodule in formation (meristem containing bacteroids) and the vascularization of the root, it allows the import of sugars produced during photosynthesis in the nodule and the export of N₂ fixed from the nodule to the rest of the plant after its reduction in ammonia (NH₃) or ammonium ions (NH₄⁺) by nitrogenase which are used by the plant in the synthesis of amino acids.

- **Nitrogenase activity**

The nitrogenase responsible for the fixation of atmospheric nitrogen is very sensitive to high levels of oxygen, hence the preventive measures found in plant cells allowing the proper functioning of this enzyme, among these measures including :

- The internal cortex of the nodule is often protected by a layer of sclerenchyma that limits gas exchange;
- The respiration of bacteroids, present in large numbers inside the host cell (8000 bacteroids/cell in soybeans), consumes most of the O₂;
- The presence of a specific pigment (leghemoglobin) in the cytosol of the host cell. This pigment, produced by the plant, has a high affinity for O₂, it ensures the bacteroids a sufficient supply of O₂, to allow their respiration, while significantly lowering the

partial pressure of O₂ which would then allow the activity of nitrogenase. It is also thanks to this pigment that the nodules have a pinkish coloration.

Finally, it's must be noted that there is generally a specificity between species of bacteria and species of Fabacea

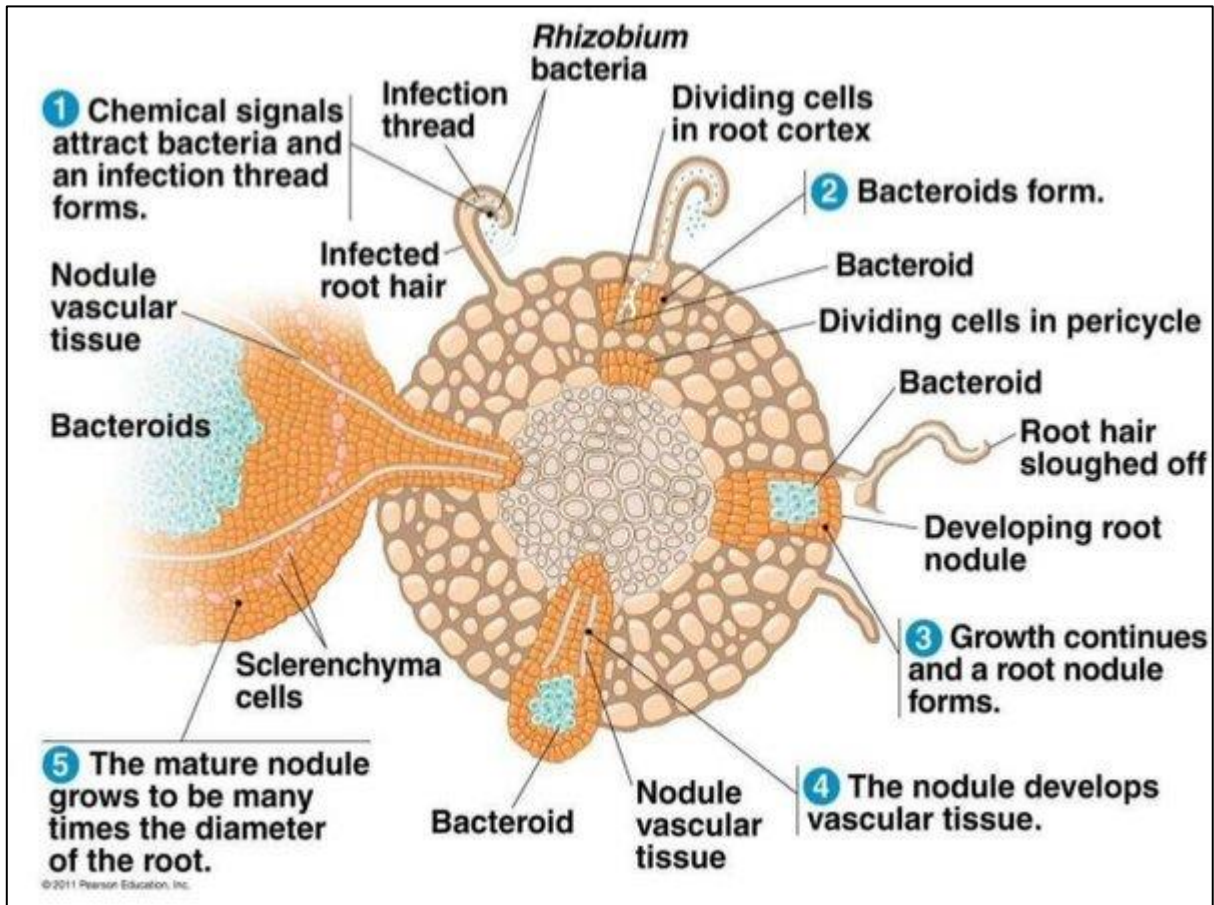


Figure 3. Plant-Rhizobia interaction. Dividing cells in root cortex and pericycle leads to the nodule meristem formation.

1.1.2. in other families

The fixation of atmospheric nitrogen has also been observed in some plant species not belonging to the Fabaceae. *Alnus* (Betulaceae), *Dryas* (Rosaceae), *Myrica* (Myrtaceae) and *Casuarina* (Casuarinaceae) are examples of plants with root nodules in association with soil actinomycetes of the genus *Frankia*.

Frankia is a filamentous, Gram-positive, free-state nitrogen-binding bacteria first described in the late 19th century. Unlike Rhizobia that induce nodules on plants of a single family, *Frankia* interacts with 8 different plant families. The stages of infection are generally similar to those described in legumes. *Frankia* also induce the curvature of root hairs that constitute the site of their penetration.

Other associations between plants and microorganisms in which nitrogen fixation occurs, have been observed in *Cycas* (Cycadophytes), *Gunnera* (Rosaceae) and *Azolla* (an aquatic fern native to America) in association with atmospheric nitrogen-fixing cyanobacteria. In this symbiosis, nodules are not formed, and nitrogen fixation is therefore not the result of a symbiotic action. Cyanobacteria lives as endophyte inside the plant tissues and can be positioned in different plant parts, remember that cyanobacteria are able to fix nitrogen in the free state.

- **Symbiotic nitrogen fixation: challenges and perspectives**

One of the challenges of understanding the symbiotic nitrogen fixation process is to be able to transfer this property to plants unable to develop nodules, and especially cereals, which are strategic products for all countries. The comparative study of the infection mechanism of plants by *Frankia*, with that of legumes, will highlight the common or specific mechanisms developed during these nitrogen-fixing endosymbioses. This comparison should lead to innovative strategies that will enable cereals to achieve biological nitrogen fixation. If this objective has long been considered very complex to achieve, the knowledge generated on the perception of symbiotic signals and their transduction, and the development of synthetic biology, open the way in the medium term to applications with the aim of creating so-called “N-self-fertilizing” crops capable of performing autonomous nitrogen fixation to avoid the need for chemical fertilizers.

1.2. Mycorrhiza: an amazing association (Figure 4)

Mycorrhiza are another example of positive interactions between plants and microorganisms. This symbiotic association between the roots of higher plants and fungi is widespread in nature, it is known in 83% of Dicotyledons, 79% of Monocotyledons and in all Gymnosperms. Through this association, exchanges between the two partners are established: the fungus promotes the hydromineral nutrition of the plant by increasing its absorption surface (thanks to the network of hyphae it forms around the roots) while the plant feeds the carbonaceous metabolism of the fungus unable to perform photosynthesis. Mycorrhiza can influence plant community composition, enhance competition, and create synergism through the sharing of resources

between species. The ability of mycorrhiza fungi to accumulate heavy metals and radionuclides make them possible candidates for restoration and remediation of polluted environments.

According to the anatomical relationship between the two partners, morphology and physiology, the following types of mycorrhiza are distinguished:

- **Ectomycorrhizae:** very common between Basidiomycetes and temperate trees. Some Ascomycetes (5%) also participate in some of these associations. The exchange of signals still poorly known allow the installation of the fungus around the roots. They are characterized by an interwoven **mantle of hyphae** (fungal sheath) around the roots, hyphae that penetrate the root intercellular space of the cortex to form a network of fungal mycelium, the **Hartig net**, which surrounds the cortex cells and increases the surface area at the fungus–root interface, and an extensive network of external hyphae. However, the hyphae of the Hartig net remain intercellular, they do not penetrate the host cell walls. Nutrient transfer across the fungus–plant interface has to occur through the cell walls and membranes of both partners.
- **Endomycorrhizae:** are largely dominant affecting about 80% of current plants. The fungi form structures within the cortical cells and also grow intercellularly. Hyphae pass through the walls of plant cells and come into contact with the plasma membrane without ever crossing it. The contact zone between hyphae and plant plasma membrane can be increased by branching fungal hyphae and the formation of specific structures called **arbuscules** (hyphal coils) with a short lifespan (about one week) but they are renewed quickly. The most common case is that of **vesicular-arbuscular mycorrhiza** involving fungi of the Glomeromycota phylum previously included in the Zygomycota phylum; They belong mainly to four genera, *Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis*. The exchange of specific signals are also at the origin of the appearance of this association, flavonoids and root exudates allow the installation of this symbiosis. Other types of endomycorrhiza exist such as **peloton mycorrhiza**, where the hyphae coming into contact with the plant cell membranes form **pelotes** (clusters of hyphae) increasing the contact surface and thus exchange with the plant cell, found in *Erica* genus and Ascomycetes or orchids and Basidiomycetes.
- A third type of mycorrhizae is ectendomycorrhizae. They have been observed in *Picea* and *Pinus*, mainly on juvenile trees and often in disturbed soils or nurseries. Ectendomycorrhizae have a thin hyphal or hyaline mantle that does not cover the end of the root, and a Hartig net that can extend to the inner cortex, but they also have intracellular penetrations (pelotons) like those of Endomycorrhizae.

Studies have shown that mycorrhiza intensively and significantly promote plant growth and form a highly developed underground contact network between different plants allowing the passage of substances from one plant to another.

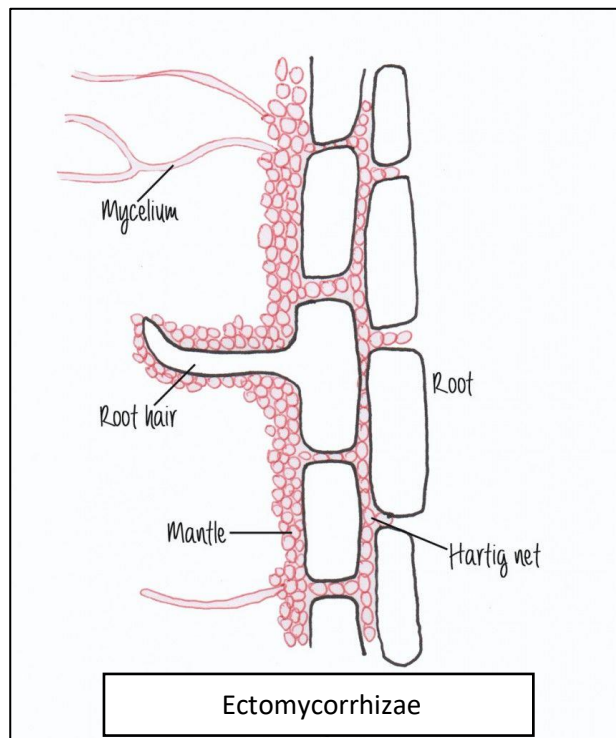
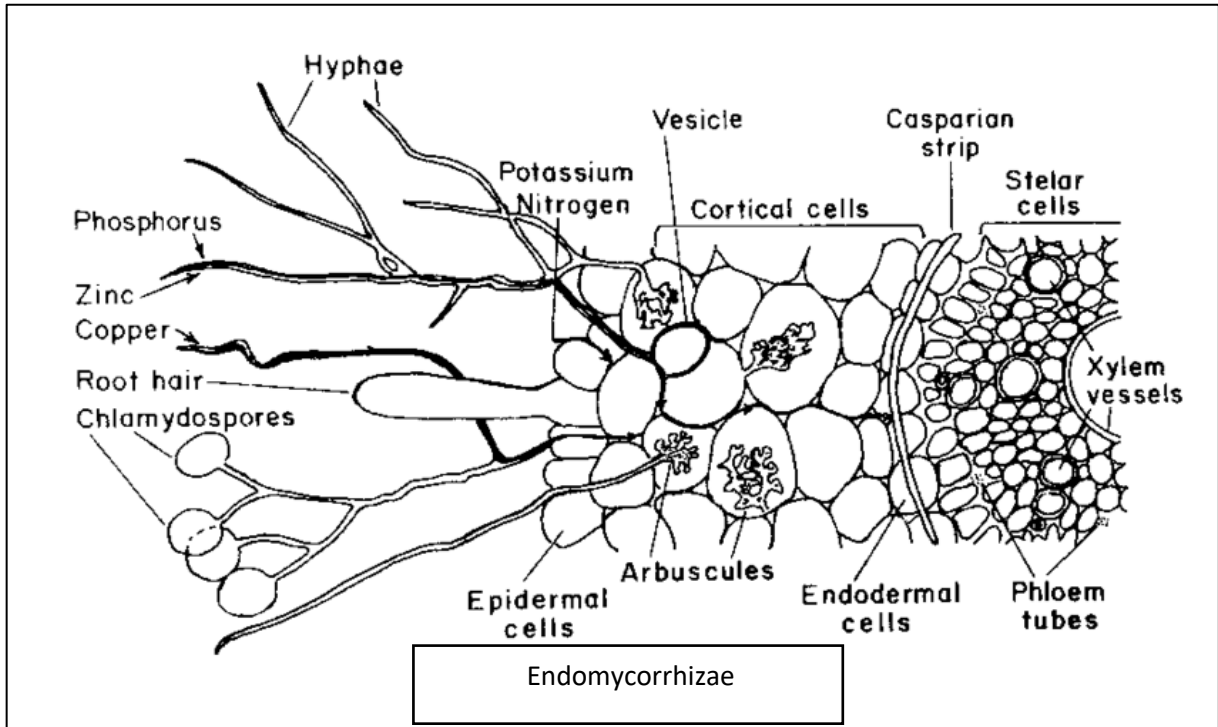


Figure 4. Endomycorrhizae and Ectomycorrhizae anatomy.

2. Associative symbioses: PGPR (Plant Growth Promoting Rhizobacteria)

2.1. Diversity of PGPR communities

Plant Growth Promoting Rhizobacteria (PGPR) are a group of non-pathogenic bacteria that enhances plant growth and yield via various substances as well as biofertilizers. Given the negative environmental impact of artificial fertilizers and their increasing costs, the use of beneficial soil microorganisms such as PGPR has increased in the last decades.

The rhizospheric soil contains diverse types of PGPR communities, which exhibit beneficial effects on crop productivity. Several research investigations are conducted on the understanding of the diversity, dynamics and importance of soil PGPR. Some common examples of PGPR genera exhibiting plant growth promoting activity are: *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholdaria*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Mycobacterium*, *Mesorhizobium*, *Pseudomonas*, etc.

PGPR are known to improve plant growth in many ways. Below, are the most important mechanisms of PGPR.

2.2. PGPR as antagonists

Several PGPR can be considered as natural antagonist of several pathogen microorganisms such as fungi, bacteria and viruses. The mechanisms followed in this case are varied, including:

- Competition for nutrients;
- Synthesis of antifungal metabolites such as antibiotics and fungal wall degrading enzymes or toxins that block fungal growth. *Pseudomonas fluorescens* is one of the best studied bacteria for its antagonist against phytopathogens. Banana bunchy top virus (BBTV) is one of the deadly virus which severely affects the yield of banana (*Musa* spp.) crop. It has been demonstrated that application of *P. fluorescens* strain significantly reduced the BBTV incidence in hill banana under greenhouse and field conditions. **Table 2** presents some PGPR that can provide phytosanitary action to plants, if inoculated in the soil, against certain diseases.

Table 2. Antagonistic PGPR

PGPR	Diseases resistance
<i>Bacillus subtilis</i>	CMV in cucumber
<i>Bacillus cereus</i>	Foliar diseases of tomato

<i>Bacillus pumilus</i>	Bacterial wilt disease in cucumber (<i>Cucumis sativus</i>), Blue mold disease of tobacco (<i>Nicotiana</i>)
<i>Pseudomonas fluorescens</i>	Sheath blight disease and leaf folder insect in rice (<i>Oryza sativa</i>), Reduce the Banana Bunchy Top Virus (BBTV) incidence, Saline resistance in groundnut (<i>Arachis hypogea</i>)

2.3. PGPR as inducers of resistance

Some experiments have shown that *Pseudomonas aeruginosa* colonizing the rhizosphere of the bean (*Phaseolus vulgaris*) confers resistance to this species against *Botrytis cinerea* (a phytopathogenic fungus). The research revealed that the bacteria (*P. aeruginosa*) produces three types of siderophores including salicylic acid, the latter seems to play the role of elicitor inducing the activation of resistance mechanisms in the plant.

2.4. PGPR and phytohormone production

Several species of PGPR can produce a variety of hormones similar to those naturally produced by plants. These hormones can be absorbed by plants and thus promote their growth. The improvement of various agronomic yields due to PGPR has been reported due to the production of these growth-stimulating phytohormones (**Table 3**) such as indole-3-acetic acid (IAA), gibberellic acid (GA3), zeatin, ethylene and abscisic acid (ABA).

Recent studies confirm that the treatment of seeds or cuttings with non-pathogenic bacteria, such as *Agrobacterium*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Alkaligenes*, etc. induce root formation in some plants due to promoting substances produced by bacteria. Although the mechanisms are not fully understood, root induction by PGPR is the accepted result of phytohormones such as auxins.

Table 3. Some PGPR and their phytohormones.

Phytohormones	PGPR
Indole-3-acetic acid (IAA)	- <i>Acetobacter diazotrophicus</i> and <i>Herbaspirillum seropedicae</i>
Zeatin et ethylene	- <i>Azospirillum</i> sp.
Gibberellic acid (GA3)	- <i>Azospirillum lipoferum</i>
Abscisic acid (ABA)	- <i>Azospirillum brasilense</i>

2.5. PGPR as biocontrol agents

All of the mechanisms and positive actions cited above have encouraged the use of PGPR as a biological control agent to reduce the impact of chemicals on health and the environment in general. PGPR that indirectly improve plant growth through the suppression of plant pathogens do so through various mechanisms, including:

- The ability to produce siderophores (as discussed above) that chelate iron, making it inaccessible to pathogens.
- The ability to synthesize antifungal metabolites such as antibiotics, fungal cell wall lysis enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens.
- The ability to successfully compete with pathogens for specific nutrients or root niches; and the ability to induce systemic resistance.

Thanks to their countless positive actions, PGPR are used in agriculture in different operations, on the other hand, their study continues to try to discover other species and other benefits.

3. Soil resistant to diseases

3.1. Definition

These soils are also known by the name of suppressive soils because of their ability to suppress phytopathogens. Examples of these soils have been reported in different parts of the world; an example of such suppressive soils is the *Fusarium*-suppressive soil in the Chateaufort District of the Rhone Valley in France. Soil that suppresses crop disease due to the specific structure of its microbial communities is known as disease-suppressive soil. Suppressive soil is an attractive method of biocontrol, because it has the potential to be sustainable over many seasons under favorable conditions.

The addition of 1% of suppressive soil to another nonsuppressive soil can make it suppressive but lasts a limited time. Suppressive soils are used in planting holes for seedlings, which gives them protection from phytopathogens. The ability of these soils to suppress phytopathogens is due to the presence of certain microorganisms acting in different ways: some are effective competitors, such as *Pseudomonas putida* that captures Fe⁺, or some strains of fluorescent *Pseudomonas* spp. that produce antibiotics (e.g. phenazine-1-carboxylic acid-2,4-diacetylphloroglucinol involved in root disease suppression), and others are natural parasites of phytopathogens inducing their death. In Hawaii, papaya seedlings are made in planting holes filled with soil resistant to *Phytophthora palmivora*, with age, the adult papaya naturally resists root rot caused by *P. palmivora*. Suppressive soils therefore contain a beneficial microflora that acts against phytopathogens.

Suppressive soil is divided into two categories, general and specific.

3.2. Types

- **General soil suppression**

General suppressive soils are those that have a high total microbial biomass, resulting in low levels of protection against multiple pathogens. This strategy is dependent on the quality and quantity of soil organic matter (composts, green manures, and cover crops) that provides supplemental nutrients to enhance populations of beneficial microbes intended to antagonize associated crop pathogens primarily by occupying plant infection sites.

- **Specific soil suppression**

In contrast to a general suppressive soil, specific suppressive soil is one that has a high concentration of one or more specific microbial species and results in high levels of protection

against specific pathogens. Take-all disease of wheat, caused by *Gaeumannomyces graminis* var. *tritici*, is one of the most studied root diseases and its control by specific soil suppression is used as a model system for biocontrol research. This disease is mainly controlled by biological and cultural strategies as there is no resistant cultivar nor effective fungicide available. The suppressive soil is developed by continuous monocropping up to 5–7 years. The suppression is due to soil enhancement of *Pseudomonas fluorescens* that produces the antibiotics, phenazine carboxylic acid and 2,4-diacetylphloroglucinol.

Another example of specific soil suppression involves control of *Fusarium* wilt, a crop disease caused by *F. oxysporum*. Here, an abiotic factor such as soil pH affects the microbial community and results in enhancement of nonpathogenic *Fusarium* spp., which induce host systemic resistance and outcompete the pathogen for access to nutrients and infection sites. This disease-suppressive soil was also found to contain a high density of *Pseudomonas fluorescens*, which induces host defense and releases siderophores (to decrease iron availability to other organisms). As previously mentioned, both *Fusarium* sp. and *Pseudomonas* act cooperatively rather than individually.

4. The application possibilities of beneficial microorganisms

4.1. Biological control

4.1.1. Definition

The concept of biological control was known for a long time but it was completely neglected in favor of the use of phytosanitary chemicals. It is only with the knowledge of the negative effects of these products on health and environment in general that we began to rethink old methods such as biological control.

Biological control is the use of living organisms or their substances to prevent or reduce damage from pests and plant pathogens.

Mode of action of microorganisms used as biological control agents

The protection conferred by a biological control agent relies on certain mechanisms of action, the most important are cited below.

4.1.2. Mode of action of biological control agents

- **Competition for Nutrients**

Comes into play when the same element is consumed by several plant pathogens. To be an effective competitor, an antagonist must be able to use nutrients in low concentrations in the environment quickly and effectively. For example, competition for Fe³⁺ is involved in the suppression of *Fusarium* head blight (caused by *Fusarium oxysporum*) by certain strains of *Pseudomonas fluorescens*. *Pseudomonas fluorescens* is able to capture iron through the siderophores it produces.

- **Antibiosis**

Several microorganisms are able to synthesize antibiotics with a very pronounced effect on the growth or reproduction of plant pathogens. Some authors defined antibiosis as the reversible or irreversible suppression of a live organism by various specific or nonspecific metabolites which have been produced by a PGPR. There are large numbers of bacterial biocontrol agents that produce antibiotics and other secondary metabolites that appear to be important for the control of different fungal pathogens. Two well-known examples are the production of phenazine-carboxylic acid by *Pseudomonas fluorescens* strain 2-79, which has been demonstrated to be involved in the biocontrol activity of this strain against take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*; and the production of pyrrolnitrin by *Burkholderia cepacia* which acts positively on some edaphic phytopathogens. However, the use of agents

acting by antibiosis has certain risks, especially with the growing problem of resistance of pathogenic microorganisms to antibiotics.

- **Parasitism**

This involves using as a biological control agent a microorganism capable of parasitizing another phytopathogenic microorganism. The best studied agent for parasitism is *Trichoderma* spp. This fungus secretes hydrolytic enzymes (Chitin degrading enzymes) capable of degrading the cell walls of several pathogenic microorganisms such as bacteria and fungi, it is also able to inhibit conidial germination and hyphae elongation of several fungal species. *Trichoderma* mycelium hyperparasitized *Fusarium graminearum* by clinging, twining, inter-penetration, and other mechanisms, which causes deformation of the mycelium of *F. graminearum* which eventually disappear.

Trichoderma can parasitize about 18 genera of *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Peronospora*. They directly invade or wound the mycelium of the pathogen, causing the deformation, shortening, narrowing of the protoplasm, and the rupture of the cell wall.

- **Decrease in pathogen aggressiveness**

The most studied case is the sweet chestnut blight agent of *Cryphonectria parasitica* in chestnut (*Castanea* sp.). At its first detection, this fungus caused considerable damage to chestnut trees, and then in 1950, in Italy, hypovirulent strains that caused less damage than the first were detected. These hypovirulent strains were able to transform virulent strains into hypovirulent strains. Studies have shown that a bi-catenary RNA molecule was responsible for this transformation and that it was transmitted to other strains during reproduction. This is how the hypovirulent strain gradually spread naturally, to heal the trees of the forests, while those cultivated, received a treatment (direct inoculation on the trees) based on hypovirulent strains. This case has paved the way for the use of less aggressive strains (these strains are either created in the laboratory or taken from nature) to protect plants against aggressive and dangerous strains in an operation called **premunitio** resembling vaccination in humans.

- **Induction of plant resistance**

Experiments have shown that the *Pseudomonas aeruginosa* that colonizes the rhizosphere of the bean (*Phaseolus vulgaris*) confers resistance to this species against *Botrytis cinerea* (a phytopathogenic fungus). The research revealed that the bacteria (*P. aeruginosa*) produces

three types of siderophores including salicylic acid, the latter seems to play the role of elicitor inducing the activation of resistance mechanisms in the plant.

4.1.3. Risks associated with the use of microorganisms as biological control agents

Microorganisms are enormous but largely untapped natural resources for biological control of pests and diseases. There are two primary reasons for their underemployment for pest or disease control: the technical difficulties of using microorganisms for biological control, owing to a lack of fundamental information on them and their ecology, and the costs of product development and regulatory approvals required for each strain, formulation, and use.

Four potential adverse effects are identified as safety issues (hazards) associated with the use of microorganisms for the biological control of plant pests and diseases. These are:

- displacement of nontarget microorganisms,
- allergenicity to humans and other animals,
- toxigenicity to nontarget organisms,
- And pathogenicity to nontarget organisms.

The probability of occurrence of a particular adverse nontarget effect of a microbial biocontrol agent may be a function of geographic origin or a specific trait genetically added or modified, but the safety issues are the still the same, including whether the microorganism intended for pest or disease control is indigenous, nonindigenous (imported and released), or genetically modified by traditional or recombinant DNA (rDNA) technology.

4.2. Biofertilization

4.2.1. Definition

Biofertilization is the use of biofertilizers which can be defined as biological products containing living microorganisms that, when applied to seed, plant surfaces, or soil, promote growth by several mechanisms such as increasing the supply of nutrients, increasing root biomass or root area and increasing nutrient uptake capacity of the plant. Biofertilizers are viable microorganisms in contrast with chemical fertilizers that are not the nutrients source but help the plants to access the nutrients in rhizospheric zone.

Many microorganisms are used typically as biofertilizers, including nitrogen-fixing Cyanobacteria (*Anabaena*), *Rhizobium*, *Azotobacter*, arbuscular mycorrhiza (AM fungi), and *Pseudomonas* spp.

There are many advantages of biofertilizers such as their low-cost and renewability. In laboratory, these beneficial soil microbial strains can be packed and cultured in suitable carrier.

A carrier is a material (for example, wheat bran, paddy, straw compost, rock phosphate pellet, soil, charcoal, seed, rice bran, talc, clay, vermiculite, lignite powder, peat, or a mixture of these materials) that provides better shelf life for biofertilizer formulation. Biofertilizers have lately become more useful because of their enormous benefits, including preserving soil quality as well as reducing emissions by the utilization of chemicals in agriculture. Various forms of industrial biofertilizer formulations are currently.

4.2.2. How do biofertilizers work?

Numerous microorganisms as well as their connection with crop plants are exploited for biofertilizers production. They can have several functions :

- Increase nutrient availability in soil,
- Nitrogen fixation,
- Solubilization of phosphate, zinc, iron, potassium,
- Secretion of plant growth regulating substances (hormones and vitamins),
- Besides increasing the uptake of nutrients to plants, biofertilizers also perform other plant physiological tasks, such as augmenting water uptake and the photosynthetic rate of plants.
- The biofertilizers also act as bio-controller of diseases as they exhibit antagonistic properties against soil-borne multiple plant pathogens such as *Rhizoctonia* root rot, chill wilt, *Pythium* root rot.

Free-living PGPR have shown promise as biofertilizers. Many studies and reviews have reported plant growth promotion, increased yield, solubilization of P (phosphorus) or K (potassium), uptake of N (nitrogen) and some other elements through inoculation with PGPR. In addition, studies have shown that inoculation with PGPR enhances root growth, leading to a root system with large surface area and increased number of root hairs.

4.3. Phytostimulation

4.3.1. Definition

Phytostimulation is the process of enhancement in the microbial activity by some signaling molecules secreted by the plant root part in the rhizospheric sites of the soil for the sequestration of the soil contaminants.

Plants can release a vast repertoire of compounds with signaling or nutritional roles for bacteria. As part of their metabolism, they can produce alkaloids, organic acids, and aminolipid volatiles.

The major proportion of the microbiome resides at the rhizosphere, the soil zone in tight contact with roots and concomitantly, its composition and richness is chemically influenced by sugars, amino acids, organic acids and mucilage secreted by roots, which largely depend upon plant species, age and growth conditions. Roots play the essential functions of soil exploration, acquisition of minerals, water uptake and support the stem and leaves.

4.4. Phytoremediation

4.4.1. Definition

Phytoremediation is defined as the use of higher plants and their associated microbes for the cost-effective, environmentally friendly rehabilitation of soil and groundwater contaminated by toxic metals and organic compounds. The plant growth promoting rhizobacteria have an important role in this process and some examples will be developed below.

4.4.2. Diversity and mechanisms of microorganisms used as phytoremediators

A very diverse community of microorganisms can be used in phytoremediation, including PGPB. Phytoremediation assisted by plant growth promoting bacteria (PGPB) has been highly touted for cleaning up toxic metals from soil. PGPB include rhizospheric bacteria, endophytic bacteria and the bacteria that facilitate phytoremediation by other means.

Pseudomonas putida, a PGPR species, has been extensively used in bioremediation because of this capability to metabolize hydrocarbons.

Phosphate-solubilizing microorganisms such as *Pseudomonas*, *Enterobacter*, *Klebsiella*, etc., have proven effective in bioremediation of metals and influencing phytoremediation by phytoextraction or by phytostabilization in metal-contaminated soil. Various phosphate-solubilization mechanisms like organic acid production, H⁺ excretion, siderophore production, and exopolymeric substance production contribute in bioremediation; some of which are presented in **Table 4**.

Table 4. Mechanisms of phosphate-solubilizing bacteria (PSB) that aid in phytoremediation of metal contaminated soil.

Metal chelated	PSB	Host plant	Phosphate-solubilization mechanism
Ni	<i>Rhodococcus globerulus</i> X80619 <i>Rhodococcus erythropolis</i> X79289	<i>Alyssum serpyllifolium</i>	N.R. (=not reported)
Cu	<i>Pseudomonas</i> sp.	<i>Maize and Sunflower</i>	Organic acid, Siderophore
Zn	<i>Rahnella</i> sp.	<i>Brassica napus</i>	Organic acid, Siderophore
Cr	<i>Pseudomonas aeruginosa</i>	<i>Cider arietinum</i>	Organic acid, EPS
Co, Pb, Zn	<i>Enterobacter ludwigii</i>	<i>Helianthus annuus</i>	Organic acids
Cd, Pb, Zn	<i>Enterobacter</i> sp., <i>Klebsiella</i> sp.	<i>Brassica napus</i>	Organic acids
Cd	<i>Rahnella</i> sp.	<i>Amaranthus</i> sp.	Siderophores, organic acids
Ni, Zn, Fe	<i>Psychrobacter</i> sp., <i>Pseudomonas</i> sp.	<i>Brassica juncea, Ricinus communis</i>	Organic acids, Siderophore
As Pb	<i>Trichoderma</i> sp.	<i>Helianthus annuus</i>	Siderophore, phosphatase
Cu	<i>Paenibacillus polymyxa</i>	<i>Wedelia trilobata</i>	N.R.

5. Microbial inoculants used in agriculture

Several microorganisms as we have seen have very interesting beneficial activities and have therefore been used in different agronomic operations and in different ways. Microbial inoculants are products that contain beneficial microorganisms such as bacteria (*Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Stenotrophomonas*, and *Streptomyces*), fungi (*Ampelomyces*, *Coniothyrium*, and *Trichoderma*), and mycorrhizae. Below, we give an overview of the various uses of microbial inoculants (**Table 5**).

5.1. Some examples of uses of microbial inoculants in agriculture

- PGPR acting by various mechanisms are used as seed coating agent because they have the ability to stimulate rhizogenesis and act against plant pathogens. In general, the application of microbial inoculants through seed treatments is widespread and more significant than soil application. Therefore, carriers with good adhesion capacity to seeds coupled with high-efficiency microbial inoculants should be developed for seed coating.
- Common method used for rhizobia inoculants is to inoculate legume seed with peat slurry just before sowing.
- The P deficiency of the soil can be corrected by inoculating seeds with PSB (Phosphate-Solubilizing Microorganisms) along with phosphate fertilizers.
- The fungal spores of edible species are used to improve the production of these species including truffles that are becoming scarce in nature following climate change and deforestation. Inoculation of these spores in soil (Rhizosphere) may also improve the growth of woody species in the vicinity of which inoculation has been made because they are mycorrhizae.
- In Hawaii, papaya seedlings are made in planting holes filled with soil resistant to *Phytophthora palmivora*.

Table 5. Some inoculums available on the market for seeds

Active agent	Target plant
<i>Sinorhizobium meliloti</i> , <i>Azospirillum brasilense</i> , <i>Bacillus megaterium</i> , <i>Glomus intraradices</i>	<i>Medicago sativa</i>

<i>Bradyrhizobium lupini</i>	<i>Lupinus</i> sp.
<i>Bradyrhizobium japonicum</i>	<i>Glycine max</i>
<i>Bradyrhizobium japonicum</i> - souche G49	<i>Glycine max</i>

5.2. Techniques used for inocula production

Producing ectomycorrhizal fungi is relatively easy and can be produced by fermentation using suitable media. The same is true for producing inocula of various *Trichoderma* species. Inocula for most bacteria used in plant growth formulations is usually prepared by growing the organisms as aseptic liquid cultures, harvested, and the bacterial suspension is diluted to give a desired concentration of viable bacteria/ml (usually $\geq 10^8$ /ml). When multiple organisms are used in a given formulation, each organism is grown individually and then mixed together to give a formulation with the right amount of viable bacteria per ml. Preparation of inocula for arbuscular mycorrhiza Fungi (AMF) is quite involved because of their obligatory requirement for plant tissue. Commercial AMF inoculants are produced mainly by growing host plants in controlled greenhouse conditions, with an inoculant containing different fungal structures (spores, hyphal fragments, and mycorrhizal root residues) from the roots of plants used for propagation such as sorghum, maize, and onion. Substrates such as sand, soil, or other materials such as zeolite and perlite are used to mass-produce AM fungal inoculum in pots, bags, or beds. The inoculum is diluted as needed prior to marketing using a carrier such as peat, clay, and fly ash.

A number of commercial inocula are sold as lyophilized products that can be reconstituted with different liquid (or solid carriers). The advantage with this is that the viability can be maintained for months or years but a large-scale preparation is more involved, careful handling is required, and the product may be relatively expensive.

Liquid or powder-type inoculants can be used to coat the seeds, for root dipping at the time of transplantation of seedlings, or apply directly into the furrow (or seed beds) or as a foliar spray.

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