

# The therapy of diseases using Phylloplane bacteria

6065 WORDS / 3000-6000 WORDS

**ABSTRACT** Pathogens and pests account for 20 % to 30 % of global crop yield losses. To maintain a sufficient and constant production, we rely on chemical crop protection and growth-promoting goods like pesticides, herbicides, and fertilizers. Many of these chemicals compounds endanger human health and the environment. Driving a demand for safer alternatives. A viable alternative is the use of microbial-based treatments to protect crops from disease. Although the word "biocontrol" includes eukaryotic biocontrol agents such as yeasts, fungi, beneficial insects, and other nonmicrobial pests, this review focuses on Phyllospheric bacteria. We employ this interpretation of a phyllospheric bacteria because it not only focuses on antagonizing the pathogen. However, it also focuses on boosting plant health. Furthermore, this definition enables for comparisons between probiotic and biocontrol research. We will look at how bacteria target microbial infections. The phyllosphere or above-ground surface of plants is a complex environment in which microorganisms and the host plant interact intensively to form unique, yet dynamic, communities. Microbial communities live on both the plant's external surfaces (epiphytes) and inside regions (endophytes), and they play a key role in disease resistance. Pathogens frequently go through an epiphytic phase before penetrating a plant cell or the apoplast. Unless otherwise stated, this review focuses on biocontrol agent applications to the leaves.

Key words: biocontrol, phyllosphere, plant immune system, induced systemic resistance, antipathogenic mechanisms, plant pathogens, beneficial microbes.

## THE PHYLLOSHERE MICROBIAL HABITAT

The phyllosphere, which includes aboveground plant components like leaves, stems, buds, flowers, and fruits, is a fleeting and external habitat. It involves symbiotic relationships between different microbial communities that, through their interactions, support plant growth, development, and disease control. They are found on wetted aerial plant parts and living on their organic exudates. **REFERENCE???**

### Factors affecting the structure of the phyllosphere microbial community

1. The following are the primary ways that the phyllosphere microbial community's structure is impacted by climate: The relative importance of different ecological processes on the formation of the phyllosphere microbial community can vary depending on the climate.
2. Temperature and precipitation are two climate variables that can alter the physicochemical characteristics of soil and leaves, which in turn can alter the composition of the microbial community in the phyllosphere. Another significant source of phyllospheric bacteria is precipitation. For example, tomato.
3. The phyllosphere microbial community may have dust as a major reservoir, and dust is influenced by meteorological factors including wind speed and precipitation. For instance, sugarcane
4. The historical period's climate change altered the vegetation's spatial distribution pattern. [1]

### Phyllospheric bacteria habitation

Common phyllospheric bacteria have unique adaptations made for this setting. species of *Methylobacterium*, for example, have evolved to low-nutrient environments by breaking down single-carbon molecules like methanol [2] [3]. The ability of the *Sphingomonas* sp. to metabolise a variety of carbon sources helps them survive in environments with little nutrition [4]. The species of *Pseudomonas* use flagellar motion to go to more advantageous areas and generate the biosurfactant syringafactin to increase the availability of water on leaf surfaces [5] and to improve plant hydration, bacteria use effectors to direct water from cells into the apoplast.

**Comentado [AEFH1]:** 5. Minireview papers:

Minireviews are brief historical perspectives or summaries of developments in fast-moving areas covered within the scope of the journal. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of the journal. The goal of the Minireviews is to provide a concise summary of a particular field in a manner understandable to all readers. We advise a length of 3000-6000 words, (including 30-70 references plus 2-3 figures and/or tables (if required)).

**Comentado [AEFH2]:** WRONG!!!! ABSTRACT

THIS ABSTRACT DOES NOT COMPLY WITH THE JOURNAL'S GUIDELINES.

THE AUTHORS DID NOT DEFINE THE JUSTIFICATION OR OBJECTIVES OF THE RESEARCH.

WHAT IS THE METHODOLOGY USED??

WHICH INSTITUTION IS RESPONSIBLE FOR THE RESEARCH.

IN WHAT PERIOD WAS THIS RESEARCH EVALUATED???

RESULTS AND CONCLUSION???

THIS ABSTRACT IS ACTUALLY MORE INFORMATION ABOUT THE TYPE OF CONTROL

**Comentado [AEFH3]:** HERE SHOULD BE AN ITEM THAT WOULD BE THE INTRODUCTION. AT THE END OF THE INTRODUCTION THE JUSTIFICATION OF THE RESEARCH OR ITS OBJECTIVE SHOULD BE DEFINED. THEN IT IS POSSIBLE TO DEFINE IN MATERIALS AND METHODS, THE LOCATION OF THE RESEARCH, THE COUNTRY, THE INSTITUTION THAT PARTICIPATED, THE PERIOD ASSESSED AND THE METHODOLOGY THAT WAS USED.

**Comentado [AEFH4]:** The text, excluding the abstract, if required, can be divided into numbered sections with brief headings. Starting from introduction with section 1, subsections should be numbered (for example 2.1 (then 2.1.1, 2.1.2, 2.2, etc.), up to three levels.

**Comentado [AEFH5]:** THIS REFERENCE IS ONLY DEFINED FOR THIS ITEM AND NOT FOR ALL THESE ITEMS

**Comentado [AEFH6]:** REFERENCE OK.

Microbial interactions can be direct or indirect in biocontrol techniques. There are direct microbial interactions between the biocontrol agent and the disease. Indirect microbial interactions involve the biocontrol agent and the host plant, bolstering the plant's health, including its resistance to diseases. This symbiotic relationship contributes to overall plant vitality and disease management. **REFERENCEN???**

## **DIRECT INTERACTIONS OF MICROBES**

### **Metabolites of Antibiotics and Binary Inhibitory Reactions**

The initial crucial stage in identifying phyllospheric bacteria involves assessing their antagonistic effects, a method now expanding in scope. For instance, over 200 *Arabidopsis thaliana* leaf isolates are being screened for binary inhibitory interactions, as well as for discovering new antagonistic strains and intriguing metabolites [6]. Most of these strains (88%) were involved in inhibitory interactions, which primarily occurred between different phylogenetic groups rather than within the same family or genus. Previously, the antibiotic activity of strains (*Brevibacillus brevis* and *Botrytis cinerea*) was noted in Chinese cabbage phylogeny. The genus *Pseudomonas* is commonly abundant in plants, with several plant pathogens, including the model pathogen *Pseudomonas syringae* pv. tomato DC3000, belonging to this genus. *Pseudomonas* strains have been found to directly inhibit the growth of several pathogens, including *P. syringae* and *B. cinerea*, in both laboratory and field environments. The biosynthesis processes in *Pseudomonas* spp. are controlled by the *phz* gene cluster. Phenazine, a product of this biosynthesis, inhibits the growth of numerous pathogenic fungi like *B. cinerea* and *Fusarium oxysporum*. The antifungal action of phenazine is multifaceted, involving disruption of fungal histone acetylation and contributing to the development of bacterial biofilms on fungal hyphae, which further diminishes fungal pathogenicity. These findings highlight the intricate mechanisms underlying microbial interactions and their potential applications in disease management strategies [7]. In soil, biofilm formation on fungal mycelium is commonly observed. In addition to phenazine, various other potential compounds have been identified in *Pseudomonas* spp., such as siderophores, 4-hydroxy-2-alkylquinolines, volatile substances like cyanide and other volatile organic compounds, as well as cyclic lipopeptides (non-ribosomal peptides) and rhamnolipids (glycolipids) produced through a three-step biosynthetic process involving the enzyme *rhlABC*.

Less common inhabitants in plants but commonly applied in commercial products are *Bacillus* sp. [9]. *Bacillus* isolated from plants often engage in inhibitory interactions with competing microorganisms, supported by their ability to produce resistant endospores, which improves their formulation and prolongs their shelf life. *Bacillus subtilis* and *B. amyloliquefaciens* stand out as the most extensively studied biological control agents within this genus. *B. subtilis* exhibits inhibitory effects against various pathogens, including fungi like *F. graminearum*, *B. cinerea*, *Alternaria* sp. as well as bacterial pathogens such as *Xanthomonas campestris* and *Pectobacterium carotovorum*. The genus *Pantoea* includes both plant pathogens and potent biocontrol agents against various pathogens, with *B. cinerea*, *Xanthomonas campestris* and *Erwinia amylovora* being the most extensively studied targets [10]. Several antibiotics, such as pantocin, herbicidin and phenazine, have been identified to inhibit *E. amylovora*.

### **Hydrolytic Enzymes**

Fungal cell wall made up of chitin was hydrolysed by chitinase enzyme. The production of chitinases and other cell divider degrading proteins, including p-1.3-glucanase, may be a common plant defence mechanism [11]. Chitinases are also produced by the microorganisms, which play a crucial role as a biocontrol mechanism in

#### **Comentado [AEFH7]: Binomial name**

*Arabidopsis thaliana* (L.) Heynh.

For biological nomenclature, the conventions of the International Code of Botanical Nomenclature, the International Code of Nomenclature of Bacteria, and the International Code of Zoological Nomenclature should be followed.

Scientific names of all biological creatures (crops, plants, insects, birds, mammals, etc.) should be mentioned in parentheses at first use of their English term.

#### **Comentado [AEFH8]: Binomial name**

*Brevibacillus brevis* (Migula 1900) Shida et al. 1996

#### **Comentado [AEFH9]: Binomial name**

*Botrytis cinerea* Pers. (1794)

#### **Comentado [AEFH10]: Binomial name**

*Pseudomonas syringae* Van Hall, 1904

#### **Comentado [AEFH11]: Binomial name**

*Fusarium oxysporum* Schlecht. emend. Snyder & Hansen

ALL SCIENTIFIC NAME MUST BE WRITTEN IN ITS COMPLETE BINOMIAL FORM FIRST IN THE TEXT. THEN IT CAN BE WRITTEN IN ITS ABBREVIATED FORM. IT IS AN INTERNATIONAL STANDARD THAT REQUIRES THE WRITING OF THE NAME WITH THE AUTHOR'S IDENTIFICATION, AS THEY ARE THE AUTHOR OF THIS DISCOVERY. WRITING WITHOUT THIS CONDITION IMPLIES NOT RECOGNITION OF THE RESEARCHER'S AUTHORSHIP. THESE SCIENTIFIC NAMES, AS WELL AS THE ABBREVIATIONS OF THE RESEARCHERS, ARE REGISTERED AND FOR THIS REASON THERE ARE INTERNATIONAL CODES TO GIVE SCIENTIFIC NAMES TO PLANTS, FUNGI, BACTERIA, ETC.

the phyllosphere. Their significance within the phyllosphere underscores the potential assistance provided by microbial chitinase activity in the phyllosphere. For instance, the *B. subtilis* 19 strain has been shown to protect against *Botrytis cinerea* of strawberry plants in field conditions by producing extracellular chitinase and protease. Recently, certain *Lactobacilli* have been observed to prevent the growth of fungal hyphae in laboratory conditions by releasing proteins that have dual functions like, they can act as chitinases or peptidoglycan hydrolases. While *Lactobacilli* are uncommon on leaf surfaces (phyllosphere) and typically face challenges in surviving there, they flourish within *Origanum vulgare* plants, where they are predominant. This presence has been linked to reduced symptoms of leaf spot disease in cucumber plants, potentially caused by *Pseudomonas syringae* pv. lachrymans. Apart from these biocontrol agents themselves producing enzymes that break down fungal cell walls, microbes can also stimulate the host plant to produce its own chitinases, triggering a common defense mechanism in plants. This mechanism inhibits pathogens by stimulating a defensive reaction in the host.

### Quorum Sensing and Quorum Quenching

Quorum sensing systems are mechanisms through which bacteria alter their actions upon reaching specific thresholds of signalling molecules. In the phyllosphere, these molecules regulate behaviours crucial for bacterial survival on leaf surfaces, including biofilm formation, adhesion, motility and the production of enzymes that break down cell walls. Pathogenic bacteria utilize quorum sensing to gauge their population size and adjust behaviours upon entering the plant's interior, such as the apoplast or plant cells [12] [13]. Gram-negative bacteria frequently employ N-acyl-homoserine lactone (AHL) as a signalling molecule, which is synthesized by AHL synthase (luxI) and detected by a transcriptional regulator (luxR). Notably, AHL molecules can also trigger responses within the host plant.

Gram-positive bacteria diverge from the AHL system, instead relying on smfl peptides processed post-translationally or diffusible signalling elements. For example, lantipeptides nisin and subtilin produced by *Bacillus subtilis*, which also play a role in quorum sensing. *Lactococcus lactis* can thrive in plants and possesses biocontrol attributes. The dual-function peptides nisin and subtilin's role in biocontrol against phytopathogens remains uninvestigated. Non-pathogenic bacteria share signaling molecules with pathogens, potentially impacting disease progression or inhibition. Symbiotic *P. agglomerans* strains, utilizing a shared quorum sensing system employing AHL signaling molecules, exhibit biocontrol activity against *P. tomato* in tomato plants. However, whether quorum sensing contributes to this activity remains uncertain [14]. Besides communicating through the production of similar signaling molecules, bacteria can also reduce each other's signals, known as quorum quenching. This process regulates the virulence of *Xanthomonas* spp. and *Xylella fastidiosa* independently of AHL and quorum sensing mechanisms [15].

Quorum-depleting strains were found to rely on the carAB genes, responsible for carbamoyl phosphate synthesis, a precursor to pyrimidine and arginine, essential for the rapid degradation of the diffusible signalling factor. An evaluation of 109 isolates from potato plants examined their ability to break down different types of long and short-chain AHLs, essential signalling molecules used by gram-negative pathogens to control their virulence [16]. A comparable method was used to analyze isolates from fig, saffron and pomegranate plants and their rhizospheres for their ability to degrade AHL 3-oxo-C6-HSL. The study found that quorum quenching was prevalent among the tested isolates, with *Pseudomonas* spp. most frequently exhibiting this trait. *Pseudomonas* isolates showing strong quorum quenching activity also exhibited effective biocontrol against *Pectobacterium carotovorum* subsp. *carotovorum*, both in laboratory conditions and on potato tubers.

### Nutrient and Space Competition Among Microorganisms

The abundance of bacterial communities in the vegetation layer is constrained by the limited availability of carbon on the leaf surface [18]. Hence, carbon competition likely shapes community composition significantly. Studies in microcosms show that "invaders," such as introduced biological control agents, possess metabolic characteristics similar to those of resident species, which excel in competing for resources in environments with limited availability. While, in environments with abundant resources, species that grow rapidly tend to gain a competitive edge. Sucrose, fructose, and glucose are the primary carbohydrates on leaf surfaces. Their profiles undergo noticeable changes upon colonization by *Sphingomonas melonis* or the pathogen *Pseudomonas syringae* pv. tomato on plant leaves, although to a lesser extent by methylotrophs [19].

Bacteria on plant surfaces use various methods to exploit available carbon sources. Methylotrophs, such as methylotrophs, focus on breaking down single-carbon compounds like methane and methanol, which decreases their dependence on plant sugars. Methylotrophs also regulate methanol release, a byproduct of plant growth, promoting plant growth via hormone production. The *mxhF* gene, crucial for methanol oxidation, is highly conserved among methylotrophs and serves as a reliable marker for methylotrophy screening, highlighting its importance as an adaptive trait in plant-associated bacteria. Methylotrophs are not particularly effective at preventing pathogens through nutrient competition. However, methylotrophs might use other biocontrol strategies, such as producing antimicrobial metabolites [20] or indirectly enhancing plant immunity [21].

Another adaptive strategy involves the capacity to utilize a diverse array of carbon sources. The existence of various TonB receptors within the plant proteome suggests that resident species may possess the capacity to break down a diverse range of carbon compounds [3]. TonB receptors are crucial in Gram-negative bacteria, as they assist in the transport of carbohydrates, siderophores, and vitamin B12. While bacteria express a diverse array of TonB receptors across different taxonomic lineages, they collectively enable the metabolism of various carbohydrates. In *Xanthomonas* spp., increased expression of TonB receptors seems to improve their survival in plant environments by enhancing their ability to compete for resources [22]. Additionally, an analysis of the protein ecology in the biosphere of *Arabidopsis*, clover and soybean showed a notable presence and variety of TonB receptors linked to *Sphingomonas* spp. The higher abundance of TonB receptors is thought to give *Sphingomonas* spp. an advantage over other Gram-negative bacteria in coping with environments under carbon stress. This abundance of TonB receptors likely contributes to their success on the vegetation layer, explaining their relative dominance.

The niche overlap index (NOI) is used to evaluate the similarity in carbon source utilization between two bacterial strains. It is calculated by comparing the number of carbon sources both strains use to the total number of carbon sources used by one of the strains [23]. Research has shown that the NOI for epiphytic bacteria like *Pseudomonas fluorescens*, *P. agglomerans*, *Stenotrophomonas maltophilia* and *Methylobacterium organophilum* negatively correlates with their ability to coexist with the pathogen *P. syringae* on pea (*Phaseolus vulgaris*) plants. Another study identified a link between the NOI of 36 non-pathogenic plant bacteria and their effectiveness in preventing disease caused by *P. syringae* pv. tomato.

These investigations validate the notion that "antagonism resulting from competition between two strains escalates in tandem with the extent of overlap in their ecological niches." [24]. Hence, certain biological control agents prove more efficacious when employed preventatively rather than as a remedy. The disease resistance capabilities of both natural plant populations and simpler synthetic plant populations, comprising 12 different

bacterial strains, were evaluated against *Pseudomonas syringae* pv. tomato. Both natural and synthetic microbial communities have shown the capability to protect plants from diseases [25]. All synthetic communities were grown on KB medium before being applied to plants, a medium that supports the growth of *P. syringae*. This environment might promote the selection of metabolically similar strains, potentially increasing antagonism through competition for nutrients. Consequently, nutrient competition may be more prominent in synthetic communities than in diverse natural ones, where other mechanisms of action might be more prevalent. **Siderophores**

Beyond carbon sources, iron frequently acts as the limiting factor in microbial communities within the plant layer. Microorganisms secrete siderophores to capture and ferry iron into cells. The production of siderophores is crucial for the epiphytic success of *P. syringae* pv. *syringae*, a strain that shows effectiveness in biologically controlling the pathogen *P. syringae* pv. *Glycinea* [26]. Interestingly, when introduced into wounded leaves, the production of commensal siderophores did not affect the population size of either the commensal or the pathogen. This indicates that iron is not a limiting factor in damaged plant cells. As a result, siderophore production does not serve as a major biological control mechanism against *P. syringae* pv. *glycinea*, since the pathogen quickly penetrates living tissues. Nevertheless, siderophore production is an important adaptive trait for the biocontrol agent *P. syringae* pv. *syringae*. Ten days after inoculation, the population of a siderophore-deficient mutant was significantly smaller by two orders of magnitude compared to the wild type. Additionally, siderophores have been documented to play a role in inducing systemic resistance (ISR) in various systems [27]. It remains a possibility that the injury prompted the induction of induced systemic resistance (ISR) via pathways regulated by host Jasmonic Acid (JA) and ethylene. Wounding eliminates the necessity for supplemental siderophore, thereby activating ISR and this particular strain exhibits no biocontrol efficacy on wounded plants.

Siderophores potentially serve additional roles beyond iron acquisition, including the transport of ferrous metals, sequestration of toxic metals, signal transduction, defense against the oxidative stress and also antibiotic activity. This method involves attaching a bactericidal "warhead" to a siderophore, which is then taken up by the antagonistic bacteria. For example, the siderophore enantio-pyochelin, produced by *Pseudomonas protegens* CS1, which was isolated from lemon peel cells, showed antagonistic activity both in vitro and against the pathogen *Xanthomonas citri* subsp. *citri* in lemon peel cells [28]. The addition of iron and ascorbic acid indicated that the observed effects were not due to iron competition but were instead caused by oxidative stress resulting from reactive oxygen species (ROS) produced by pyochelin, which is the mechanism of action. Antibacterial activity was noted, as ascorbic acid can reduce the antibacterial effects of ROS, while iron supplementation had little impact.

The presence of iron and ascorbic acid suggested that the observed effect was not due to iron competition but was instead caused by oxidative stress resulting from reactive oxygen species (ROS) generated by pyochelin, which is the underlying mechanism. Antibacterial effectiveness was noted. Significantly, ascorbic acid can alleviate the antibacterial effects of ROS, whereas iron supplementation exerts minimal influence [29]. The siderophores pyoverdine and enantio-pyochelin, produced by *P. protegens*, provide resistance to the mycotoxin fusaric acid. This toxin, produced by pathogenic *Fusarium* fungi, is harmful to plants and bacteria, though its mechanisms of action are not yet fully understood. Furthermore, mutations in the *iucA* and *iutA* genes, which are involved in siderophore biosynthesis and receptor functions respectively, lead to reduced surface motility in the xylem-dwelling pathogen *Pantoea stewartii* and lower virulence in sweet corn. This underscores the role of

**Comentado [AEFH12]:** Binomial name  
*Pseudomonas syringae* Van Hall, 1904

siderophores in regulating motility and highlights their adaptive importance [30]. However, this mechanism has not yet been characterized as an adaptive strategy for plant biocontrol agents.

#### **INDIRECT INTERACTIONS**

In addition to direct interactions, phyllospheric bacteria can indirectly impede pathogens by altering the plant's immune responses or hormone balances. These microbe-plant interactions, fostering plant protection against pathogen invasion, are explored herein as indirect interactions.

Plants have developed a sophisticated immune system to thwart potential infections by detecting intruders and deploying suitable defense mechanisms. Conversely, pathogens constantly adapt to avoid detection or disrupt defense mechanisms. This constant cycle of action and counteraction is known as the "zigzag pattern" [31]. The host plant identifies bacterial-associated molecular patterns (MAMPs) through specific pattern recognition receptors (PRRs), triggering pattern-activated immunity. One well-studied MAMP is flagellin, especially the flg22 epitope, which is detected by the FLS2 PRR. Other MAMPs include lipopolysaccharides from gram-negative bacteria and glycans containing N-acetylglucosamine, such as peptidoglycan from gram-positive bacteria, fungal chitin, and elements from rhizobacterial modulation. Additionally, beneficial bacteria like *Bacillus* and *Pseudomonas* spp. release volatile compounds that may activate the plant's immune system, although the exact receptors involved have not yet been identified. The immune response may escalate in strength when augmented by supplementary virulence factors, such as tissue injury or phytohormone modulation.

Bacteria can evade the initial defense response by modifying MAMPs or secreting effectors into the host cell cytoplasm to interfere with immune signaling. In response, plants have developed additional recognition mechanisms called effector-triggered immunity. Gram-negative bacteria use a type III secretion system to deliver molecules into the cytoplasm that inhibit the immune response. When this secretion system is disrupted, such as by mutations in the *hrp* gene which is essential for its function resulting in hypersensitivity reactions in plants, the severity of disease symptoms typically decreases [32]. Mutations of this kind are rendered ineffective against pattern-activated immunity and are incapable of infecting host tissues. Typically, these mutations remain in the apoplast without causing damage and may even help protect the host from infection by virulent pathogens. When mutants and virulent pathogens are introduced together, they tend to invade separate cells or niches. While type III secretion and effector systems are mostly associated with pathogens, some beneficial microbes also interact with the plant's immune system through effectors. For example, rhizobacteria use type I and type VI secretion systems to induce nodulation in host plants. Recently, gene clusters related to the type II secretion system have been identified in the rhizosphere of beneficial *Pseudomonas* spp. [33]. These gene clusters are similar to the type III secretion systems found in other beneficial bacteria but differ from the secretion systems present in the pathogenic *P. syringae*.

Further research is needed to determine how common type III secretion systems are among other beneficial bacteria living in plant cells, to differentiate between pathogen-associated effectors and those that help control infections, and to clarify the roles of these systems in biological interactions. Additionally, N-acyl-homoserine lactones (AHLs), a often overlooked group of molecules that affect the host immune system, should be given more consideration in this context [35]. W H E R E [34]? ?

AHL regulates the activities of gram-negative pathogens, offering the host plant a chance to either respond to or disrupt this quorum-sensing signal. Indeed, exposing roots to AHL or AHL-producing bacteria has been shown to activate plant defense genes and induce systemic resistance through salicylic acid (SA) signalling. In Arabidopsis,



AHL-induced **defense** responses include stomatal closure, which is a common initial defense mechanism that restricts pathogen entry into the apoplast. Additionally, plants may either degrade AHLs or transport them through the vascular system to remove them from the bacteria that produce them, actions similar to quorum suppression. AHL detection in Arabidopsis involves the G protein-coupled receptor encoded by AtGPA1. However, more research is needed to identify AHL receptors in other plant species [35].

### Plant Hormones

Key plant hormones in this context encompass salicylic acid, jasmonic acid and ethylene, with SA and JA regarded as antagonistic. Typically, JA and ethylene participate in **defense** responses from necrotrophic pathogens (which feed on dead host cells) or following injury, whereas SA primarily orchestrates **defense** responses against biotrophic pathogens [36]. Experiments involving the application of bacterially derived rhamnolipids onto Arabidopsis leaves via spray revealed that SA occupies a pivotal position in disease resistance mediated by rhamnolipids [37]. Bacteria in the **rhizosphere** can directly influence plant hormone levels through the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which breaks down the ethylene precursor ACC. This enzyme is found in plant growth-promoting rhizosphere bacteria such as *Azospirillum*, *Rhizobium* and *Pseudomonas* spp., as well as in **rhizosphere** bacteria like specific *Methylobacteria* spp. and *Rhodococcus fascians*.

The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase reduces ethylene levels, which in turn suppresses plant **defense** mechanisms and promotes the establishment of symbiotic microorganisms.

Additionally, ACC deaminase supports plant growth by improving resilience to environmental stresses such as drought, flooding, salt, or pathogen pressure. Ethylene has been directly linked to the regulation of plant community composition, with ethylene-insensitive plant mutants supporting different plant communities. Moreover, increasing evidence suggests that ACC plays a direct role in regulating both plant development and **defense** responses [38]. *Methylobacteria* genus members promote plant growth through auxin and cytokinin production. This symbiotic interaction benefits the symbiont's growth by metabolizing methanol released during plant growth [3]. Cytokinin derived from *Methylobacteria* are thought to confer resistance to drought and salt stress in the **host** [39]. The type of cytokinin and the presence of the *miaA* gene suggest that cytokinin production in methyl bacteria is probably associated with tRNA. Additionally, there is evidence of biological control activity; for example, treating peanut seeds with *Methylobacterium* spp. enhanced protection against pathogens like *Aspergillus niger* and *Sclerotium rolfsii* [21]. This implies that *Methylobacteria* spp. interact with the host plant's defense systems, providing protection against *A. niger* and *S. rolfsii*. *Rhodococcus fascians* is known for its ability to influence plant hormone levels. Both pathogenic and non-pathogenic strains of *R. fascians* are capable of producing TAA and cytokinin, while also reducing ethylene levels [40].

In pathogenic bacteria, genes that control auxin and cytokinin production are transferred via plasmids. The production of IAA increases when external tryptophan, which is the precursor of IAA, is present. Interestingly, when pathogenic or non-pathogenic *R. fascians* are introduced into plants, the host plant's metabolism is altered, leading to an accumulation of tryptophan that stimulates the bacteria's IAA production. On the other hand, cytokinin production by *R. fascians* enhances auxin production in the plant, and elevated auxin levels significantly contribute to the development of disease symptoms [41]. Non-pathogenic variants of this strain lack the plasmid that carries the virulence genes. The main pathogenic factor on the plasmid is the production of various methylated cytokinin, which are resistant to degradation by cytokinin oxidase. These cytokinin mimic plant cytokinin, leading

to increased auxin production in the plants and subsequently promoting the onset of disease symptoms [42]. As a result, cytokinin and auxin production by pathogenic *R. fascians* is harmful and contributes to disease progression. In contrast, cytokinin and auxin production by non-pathogenic *R. fascians* and other beneficial symbiotic species, like the previously mentioned *methylobacterium*, is considered advantageous because it promotes plant growth.

In summary, the ability to regulate auxin levels by both augmenting auxin production and breaking down excess auxin in the field allows the symbiotic bacteria to optimize its ecological niche. Increased auxin production promotes host growth while deterring other pathogens. Conversely, when auxin is overproduced, bacterial breakdown mechanisms prevent pathogenic invasion. The interaction between auxin and ethylene, along with bacterial degradation of ACC, helps prevent excessive immune responses and aging induced by ethylene. Moreover, cytokinin serves as a reservoir for sugars and other metabolites. Therefore, microbial cytokinin production likely functions as a metabolite sink, benefiting both the symbiont (as a carbon source) and the host by delaying aging and prolonging photosynthetic activities. This intricate interplay between microbial-produced hormones and host responses underscores the complex and mutually beneficial relationship between plants and their symbiotic bacteria in the phyllosphere. [REFERENCE] ???

#### Induced Systemic Responses

Once the plant detects the presence of bacteria, it initiates a specific immune response mediated by plant hormones, as previously explained. In this scenario, beneficial bacteria can activate the plant's defence mechanisms upon recognition, thus inducing immunity against pathogens throughout the entire plant [43]. This process is known as induced systemic resistance (ISR). Additionally, pathogens can trigger a systemic response that protects other parts of the plant, a mechanism called systemic acquired resistance (SAR). For example, *S. melonis* Frl induces the production of camalexin in host plants [19]. The pathogen *P. syringae* pv. tomato also

produces camalexin, though in larger amounts. Camalexin, an indole alkaloid derived from tryptophan, is a common defence response in Arabidopsis and other cruciferous plants. Due to its lipophilic nature, camalexin effectively combats various bacteria and fungi by disrupting membrane integrity, including by binding to phospholipids. Therefore, the camalexin production stimulated in Arabidopsis by *S. melonis* Frl is considered the main mechanism driving the plant defense observed from this symbiont.

The host immune system not only defends against bacterial and fungal pathogens but can also protect against viral diseases. In three years of field trials using foliar applications of *Bacillus amyloliquefaciens* 5B6, there was a consistent reduction in cucumber mosaic virus accumulation, as measured by qPCR. Changes in gene expression within host plants suggest that the activation of salicylic acid (SA) and ethylene signalling pathways is crucial for developing disease resistance. Interestingly, genes regulated by biological control agents coincide with those regulated during defence response induced by certain viruses, including cucumber mosaic virus.

The *Paenibacillus polymyxa* AC-1 [44] strain effectively inhibits the growth of plant pathogens such as *P. syringae* pv. tomato DC3000 and *P. syringae* pv. tabaci under laboratory conditions. Additionally, the cell-free supernatants from *P. polymyxa* AC-1 also show inhibitory effects on these pathogens, suggesting that the antibacterial metabolites released by the bacteria act as direct antagonists. When axenic Arabidopsis plants' root tips are inoculated with a bacterial suspension of *P. polymyxa* AC-1, it triggers a defense response dependent on

salicylic acid (SA) and jasmonic acid (JA). Interestingly, this root inoculation results in *P. polymyxa* AC-1 colonizing the leaf endosphere of Arabidopsis. Colonization levels in the leaf endosphere were ten times higher



in *Arabidopsis* mutants with reduced JA sensitivity, while they were ten times lower in mutants lacking the isoprenoid plant hormone abscisic acid, compared to wild-type plants.

In JA-deficient plants, the colonization of the leaf endosphere by *P. polymyxa* AC-1 can even induce disease symptoms. This suggests that JA plays a role in limiting the harmful internal growth of AC-1, highlighting the crucial role of the plant defense system in controlling the microbial load and preventing the invasion of symbiotic bacteria. The way bacteria are recognized by the host and trigger an immune response is similar for both non-pathogenic and pathogenic strains. However, non-pathogenic strains lack additional virulence factors, leading to a milder defense response from the host. Many plant bacteria can activate the immune system, thereby providing resistance against a range of different pathogens. At times, these bacteria share close genetic relatedness with pathogenic strains or may even act as opportunistic pathogens (e.g., *Pseudomonas*, *R. fascians*, *P. polymyxa*). In such instances, the distinction between ISR and SAR becomes less discernible [45]. WHERE!!!

## CONCLUSION AND FUTURE ASPECTS

In the phyllosphere, a critical battle unfolds between destructive pathogens and beneficial bacteria, holding promise for sustainable crop protection. These beneficial microbes employ diverse tactics to combat pathogens, including direct inhibition, communication disruption, and secretion of antibacterial compounds. Moreover, they can stimulate the plant's immune system and regulate hormone levels, indirectly impeding pathogen growth. Research into these mechanisms involves both in vitro experiments and field trials to validate biological control agent efficacy. This review integrates these approaches, shedding light on phyllospheric biological control and adaptation mechanisms. Key areas for further exploration include discovering new antibacterial peptides, understanding the role of the type III secretion system, and investigating the impact of bacterial-produced plant hormones. Integration of biological control agents into resident microbial communities is essential, with advanced techniques like metagenomics offering insights into complex plant-microbe interactions. These biocontrol communities host diverse factors that synergistically suppress disease and thrive in the phyllosphere. By providing sustainable alternatives to pesticides, biocontrol agents play a pivotal role in safeguarding agricultural production.

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WHERE REFERENCE [45].????????????????

REFERENCE IN TEXT 45 ... REFERENCE IN LIST: 44.????

41 REFERENCE 2000-2019: MEDIA = 2012 (12 YEARS OLD).

3 REFERENCE : 1987-1994.