



# The Therapy of Diseases Using Phylloplane Bacteria

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## Authors' contributions

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## 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 chemical 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

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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.

**Keywords:** *Biocontrol; phyllosphere; plant immune system; induced systemic resistance; antipathogenic mechanisms; plant pathogens; beneficial microbes.*

## 1. THE PHYLLOSHERE MICROBIAL HABITAT

“The phyllosphere is inhabited by a complex and dynamic community. The composition of this community depends on which microbes reach the phyllosphere in addition to abiotic factors such as climate, season and surrounding land use, and biotic factors such as leaf characteristics and host plant species. Microbes arrive on the phyllosphere rather stochastically *via* the air, soil, rain or insects. However, only selected taxa successfully colonize the phyllosphere” (Maignien et al., 2014). Frequently occurring genera in phyllosphere communities are *Methylobacterium*, *Sphingomonas*, and *Pseudomonas*.

“These common phyllosphere microbes have particular adjustment variables to the phyllosphere. For illustration, *Methylobacterium* spp. have adjusted to the low-nutrient environment by metabolizing single-carbon compounds such as methanol” (Kutschera, 2007). “*Sphingomonas* spp. adapt with the shortage of supplements by being able to metabolize a wide run of carbon sources” (Delmotte et al., 2009). “*Pseudomonas* spp. utilize flagellar motility to reach more favorable destinations, synthesize the biosurfactant syringafactin to extend the water accessibility on leaf surfaces” (Hernandez and Lindow, 2019) and utilize effectors to spill water from the cells into the apoplast. Biocontrol variables can be related to coordinate or circuitous microbial intuitive. Coordinate intuitive happen between the pathogen and the biocontrol specialist. Roundabout intelligent are the intelligent between the biocontrol specialist and the have plant which moves forward the plant's wellness, like its resistance to the illness.

## 2. DIRECT INTERACTIONS

### 2.1 Antibiotic Metabolites and Binary Inhibitory Interactions

“The first important step in identifying phyllospheric bacteria is screening for antagonistic activities. Such screening is increasingly being applied on a larger scale. For example, screening of over 200 *Arabidopsis thaliana* leaf isolates for binary inhibitory interactions, novel antagonistic strains, and interesting metabolites” (Helfrich et al., 2018). “Most of these strains (88%) were involved in such inhibitory interactions. Most repression also occurred between different phylogenetic groups rather than within the same family or genus. Previously, the antibiotic activity of *Brevibacillus brevis* strains against *Botrytis cinerea* was observed in the phylogeny of Chinese cabbage. The genus *Pseudomonas* is commonly found in plants in relatively high numbers. Additionally, several plant pathogens belonging to this genus, such as the model plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Pseudomonas* strains have been observed to directly inhibit the growth of a variety of pathogens (such as *P. syringae* and *B. cinerea*) in laboratory and field experiments. Biosynthesis in *Pseudomonas* spp. encoded by the *phz* gene cluster. Phenazine inhibits the growth of many different pathogenic fungi, such as *B. cinerea* and *Fusarium oxysporum*. The mechanism of antifungal action of phenazine is diverse. For example, phenazine inhibits mycelial growth of *Fusarium graminearum* by interfering with fungal histone acetylation and participating in the formation of bacterial biofilms on fungal hyphae, further reducing pathogenicity” (Chen et al., 2018). Biofilm formation on fungal mycelium is a common feature in soil. In addition to phenazine, many other potential metabolites have been identified in *Pseudomonas* spp. such

as siderophores, 4-hydroxy-2-alkylquinolines (Yasmin et al., 2017), volatile compounds such as cyanide and other volatile organic compounds and cyclic lipopeptides (non-ribosomal peptides) and rhamnolipids (synthesized glycolipids following a three-step biosynthetic pathway that includes the enzyme rhlABC).

“Less common inhabitants in plants but commonly used in commercial products are *Bacillus* spp.” (Ongena and Jacques, 2008) “Bacilli isolated from plants often engage in inhibitory interactions with other competing microorganisms, and their ability to form resistant endospores facilitates formulation and shelf life of them. *Bacillus subtilis* and *Bacillus amyloliquefaciens* are by far the two most described biological control agents in this genus. *B. subtilis* inhibits a variety of pathogens, both fungal and bacterial, such as *F. graminearum*, *B. cinerea*, *Alternaria* spp., *X. campestris* and *P. carotovorum*. The genus *Pantoea* contains a number of plant pathogens, as well as biocontrol agents that are effective against a variety of pathogens such as *B. cinerea*, *X. campestris* and *E. amylovora* being the most studied” (Walterson and Stavrinides, 2015). Several antibiotics, such as pantocin, herbicolin and phenazine, have been identified to inhibit *E. amylovora*.

## 2.2 Hydrolytic Enzymes

“Generation of chitinases, as well as other cell divider debasing proteins, such as  $\beta$ -1,3-glucanase, could be a common defense component of plants” (Boller, 1993). Microbes also create chitinases, which are an imperative biocontrol mechanism within the rhizosphere. Their significance within the rhizosphere shows the potential of assist considering the microbial chitinase movement on the phyllosphere. It has been demonstrated that *B. subtilis* J9 strain secures strawberry plants against *B. cinerea* in field conditions which this strain produces extracellular chitinase and protease. As of late, we watched that certain lactobacilli can repress hyphae arrangement of parasites *in vitro* by creating bifunctional proteins with chitinase or peptidoglycan hydrolase action. Lactobacilli are not normal phyllosphere occupants, and regularly have a low survival rate. Be that as it may, they have been appeared to rule the endosphere of *Origanum vulgare* plants and have been connected adversely with infection indications of leaf spot on cucumber plants, probably caused by *P. syringae* pv. *lachrymans*. Another to the generation of hydrolytic proteins by the biocontrol

specialists themselves, organisms can actuate the generation of chitinases within the have plant, a common defense response in plants. Inhibition of a pathogen by triggering a defense reaction in the host is further discussed in section “Indirect Interactions.”

## 2.3 Quorum Sensing and Quenching

“Quorum sensing systems are systems in which bacteria modify their behavior when certain concentrate on thresholds of signaling molecules are exceeded. In the phytosphere, signaling molecules regulate behaviors that allow bacteria to survive on leaf surfaces, such as biofilm development, adhesion, motility, and production of wall-degrading enzymes. Pathogenic bacteria use quorum sensing to measure their population size and adjust when they enter the apoplast or plant cell” (Pfeilmeier et al., 2016, Leach et al., 2017). Gram-negative bacteria commonly use N-acyl-homoserine lactone (AHL) as a signal molecule, which is synthesized by AHL synthase (*luxI*) and detected by a transcriptional regulator (*luxR*). Interestingly, AHL molecules can also trigger responses in the host plant, which will be discussed in more detail in the “Indirect Interactions” section.

“Gram-positive bacteria do not use the AHL system but typically use small post-translationally processed peptides as signal molecules or diffusible signaling elements (see later in this section). *B. subtilis* produce the antibiotic lantipeptides nisin and subtilin, respectively, which are also involved in quorum sensing. *Lactis* can persist in plants and even has biocontrol properties. However, the involvement of the bifunctional peptides nisin and subtilin in phytoplankton biocontrol has not been described. Interestingly, non-pathogenic bacteria use the same signaling molecules as pathogens and can therefore contribute to disease development or inhibition, depending on how they intervene. A shared quorum sensing system using AHL signaling molecules has been observed among symbiotic *P. agglomerans* strains showed biocontrol activity against the pathogen *P. tomato* in tomato plants, but it is currently unknown whether quorum sensing is involved” (Hosni et al., 2011). “In addition to cross-communication by producing similar signaling molecules, bacteria can attenuate each other’s signals, also known as quorum quenching. This signal is involved in regulating the virulence of *Xanthomonas* spp. and *Xylella fastidiosa* in an AHL- and quorum-

sensing-independent manner” (Newman et al., 2008).

“In quorum-depleting strains, the *carAB* genes, involved in the synthesis of carbamoyl phosphate, a precursor to pyrimidine and arginine, were determined to be required for the rapid degradation of this diffusible signaling factor. Screened 109 potato plant isolates for their ability to degrade multiple short-chain and long-chain AHLs, as gram-negative pathogens use AHLs as signal molecules to regulate their virulence” (Morohoshi et al., 2009). “The same method was used to screen plant and rhizosphere isolates of saffron, fig, and pomegranate for AHL 3-oxo-C6-HSL degradation. They concluded that quorum quenching was a common feature among the isolates tested and was most commonly observed in *Pseudomonas* spp. These *Pseudomonas* isolates with strong quorum quenching activity also showed biocontrol activity against *P. carotovorum* subsp. *carotovorum* in vitro and on potato tubers” (Alymanesh et al., 2016).

## 2.4 Competition for Nutrients and Space

“The size of bacterial communities in the vegetation layer is limited by low carbon availability on the leaf surface” (Mercier and Lindow, 2000). “Therefore, carbon competition is likely to play an important role in community structure. Microcosm experiments show that “invaders”, such as introduced biological control agents, have similar metabolisms to resident species, which are strong competitors in low-resource environments, while fast-growing species have an advantage when resource availability is high. The main carbohydrates found on the leaf surface are sucrose, fructose and glucose. These sugars are specifically altered after *Sphingomonas melonis* or the pathogen *P. syringae* pv. *tomato* colonizes epiphytic leaves, but only to a lesser extent by methylobacteria” (Ryffel et al., 2016).

Phyllosphere bacteria have developed different strategies to utilize all available carbon sources. Methylobacteria, such as methylobacteria, specialize in using single carbon compounds, such as methane and methanol. Therefore, they do not depend much on the sugars available in plants. Methylobacteria even regulate the release of methanol, which is released as plant cells grow, promoting plant growth through the production of plant hormones. The *mxoF* gene,

which contains the active site of the methanol oxidation complex, was found to be highly conserved among methylobacteria and is a suitable probe for methylobacteria screening. Therefore, methylobacteria is an important adaptive factor for some bacteria in plants. However, methylobacteria are not capable of inhibiting pathogens by competing for nutrients. Methylobacteria can possess other biocontrol mechanisms such as antimicrobial metabolites (Kwak et al., 2014) or indirect mechanisms by triggering plant immunity (see further in section “Plant Immunity”) (Madhaiyan et al., 2006).

“Another adaptation strategy is the ability to harvest a variety of carbon sources. The presence of multiple types of TonB receptors in the plant proteome is thought to be an indication that resident species can metabolize a variety of carbon compounds” (Delmotte et al., 2009). “Indeed, TonB receptors are involved in the transport of carbohydrates, siderophores and vitamin B12 in Gram-negative bacteria. Bacteria express a variety of TonB receptors, but belong to different taxonomic lineages, sharing the ability to metabolize a variety of carbohydrates. Over expression of the TonB receptor in *Xanthomonas* spp. seems to facilitate their survival in the vegetation layer by making them competitive scavengers” (Blanvillain et al., 2007). Furthermore, community biosphere protein ecology of Arabidopsis, clover, and soybean assigned a high proportion and diversity of TonB receptors to *Sphingomonas* species. The high abundance of TonB receptors is thought to allow *Sphingomonas* spp. are more effective than other gram-negative bacteria in resisting carbon-stressed environments and explain their success on the vegetation layer in terms of their relative abundance.

“The niche overlap index (NOI) is a metric that can be used to quantify the similarity in carbon source characteristics of two bacteria. Calculate NOI as the number of carbon sources used by both strains as a proportion of the total number of carbon sources used by one strain” (Wilson and Lindow, 1994). They demonstrated that the NOI of the epiphytic bacteria *Pseudomonas fluorescens*, *P. agglomerans*, *Stenotrophomonas maltophilia* and *Methylobacter organophilum* was negatively correlated with their ability to coexist with the pathogen *P. syringae* on pea (*Phaseolus vulgaris*) plants. In another study, the NOI of 36 non-pathogenic plant bacteria was correlated with their ability to prevent disease caused by *P. syringae* pv. *tomato*.

These studies confirm that “antagonism due to competition between one strain and other increases proportionally to the overlap of their ecological niches” (Lindow, 1987). “Therefore, some biological control agents are more effective as a preventative measure than as a treatment. The disease resistance effects of natural plant populations and simpler synthetic plant populations (including 12 bacterial strains) were tested against *P. syringae* pv. *tomato*. Both natural and synthetic communities protect plants from diseases” (Berg and Koskella, 2018). All synthetic communities were grown on KB medium before application to plants. It is the ideal substrate for *P. Syringae* for development. This may lead to selection for metabolically similar strains, thereby increasing antagonism due to nutritional competition. Therefore, competition for nutrients may play a larger role in synthetic communities than in more diverse natural communities, where alternative forms of activity may prevail.

## 2.5 Siderophores

“In addition to carbon sources, iron is often the limiting element in the microbial communities of the plant layer. Siderophores are secreted by microorganisms to bind and transport iron into cells. Siderophore production is essential for the epigenetic capacity of *P. syringae* pv. *syringae* 22d/93, a strain with biological control activity against the pathogen *P. syringae* pv. *Glycinea*” (Wensing et al., 2010). “Interestingly, when inoculated into injured leaves, commensal siderophore production did not affect its own population size or that of the pathogen. This indicates that iron is not a limiting factor in damaged plant cells. Therefore, the production of siderophores is not an important biological control mechanism for *P. syringae* pv. *glycinea*, as the pathogen quickly penetrates living tissues. However, siderophore production is an important adaptive factor for the biocontrol agent *P. syringae* pv. *syringae* 22d/93, 10 days after inoculation, the population size of the siderophore-negative mutant was 2 orders of magnitude smaller than that of the wild type. Furthermore, the role of siderophores in the induction of systemic resistance (ISR) (see section “Induced systemic reactions”) has been reported in several systems” (Bakker et al., 2007). It cannot be ruled out that the injury triggered the ISR, through pathways mediated by host jasmonic acid (JA) and ethylene (see “Plant Hormones” section). Wounding abolishes the need for additional siderophore, activating ISR,

and this strain has no biocontrol activity on wounded plants.

Siderophores may have alternative functions beyond iron scavenging, such as ferrous metal transport, toxic metal sequestration, signal transduction, protection against oxidative stress, and antibiotic activity. The second occurs by attaching a bactericidal “warhead” to a siderophore, which is then taken up by the antagonistic bacteria. The siderophore enantio-pyochelin, produced by *Pseudomonas protegens* CS1, isolated from lemon peel cells, showed antagonistic activity in vitro and in lemon peel cells against the pathogen *Xanthomonas citri* subsp. *Citri* (Michavila et al., 2017). The addition of iron and ascorbic acid indicated that it was not due to iron competition but rather due to oxidative stress, caused by the formation of reactive oxygen species (ROS) from pyochelin, which is the mechanism of action. Antibacterial activity was observed. Indeed, ascorbic acid can counteract the antibacterial activity of ROS while iron supplementation has practically no effect.

On the other hand, experiments with *P. Orientalis* F9 and a mutant lacking the ability to produce the siderophore pyoverdine (see also “Antibiotic metabolites”) show that the mutant is still capable of antagonizing *E. amylovora* and the three tubular pathogens *P. syringae* test, as well as *E. amylovora* in a detached flower test and *P. ultimum* in a soil microcosm test. This indicates that pyoverdine does not play a role in the biological control mechanism of *P. Orientalis* F9. Another function of siderophores on plant cells (Ruiz et al., 2015). “The siderophores pyoverdine and enantio-pyochelin, synthesized by *P. protegens*, are responsible for its resistance to the mycotoxin fusaric acid. Fusaric acid is produced by pathogenic fungi of the genus *Fusarium* and is toxic to plants and bacteria by mechanisms that are not yet fully understood. Finally, mutations in the *iucA* and *iutA* genes, responsible for the biosynthesis of siderophores and receptors, respectively, lead to loss of surface motility of the xylem-dwelling pathogen *Pantoea stewartii* and reduced virulence. in sweet corn. This indicates that siderophores also play an adaptive role by regulating motility” (Burbank et al., 2015). However, this mechanism has not yet been characterized as an adaptive strategy for plant biocontrol agents.

## 3. INDIRECT INTERACTIONS

Next to direct interactions, Phyllospheric bacteria can inhibit pathogens indirectly, by

modulating the plant's immune system or hormone levels. Microbe-plant interactions that protect the plant against pathogen infection are discussed here as indirect interactions.

Plants have evolved a complex immune system to prevent infection by recognizing potential invaders and responding with appropriate defense responses. In contrast, pathogens continuously evolve to evade recognition or interfere with defense responses. This action and counterattack are part of the "zigzag pattern" (Jones and Dangl, 2006). The host plant recognizes bacteria-associated molecular patterns (MAMPs) through specific pattern recognition receptors (PRRs), leading to pattern-activated immunity. One of the best studied MAMPs is flagellin, more precisely the flg22 epitope, recognized by the FLS2 PRR. Other MAMPs are lipopolysaccharides from gram-negative bacteria and glycans containing N-acetylglucosamine, such as bacterial peptidoglycan, which is more commonly present in gram-positive bacteria, fungal chitin, or rhizobacterial nodulation elements. Additionally, volatile compounds released by beneficial bacteria such as *Bacillus* and *Pseudomonas* spp. may activate the plant's immune system, but the receptors have not yet been identified. The immune response can become more robust in the presence of additional virulence factors, such as tissue damage or phytohormone regulation.

Bacteria can overcome this first line of defense by modifying MAMP or secreting effectors into the host cell cytoplasm to interfere with activated immune signaling. Therefore, plants have evolved additional mechanisms to detect these microbial agents: effector-triggered immunity. Gram-negative bacteria use a type III secretion system to deliver molecules acts on the cytoplasm to inhibit the immune system. When such a secretion system is inactivated (through mutations in the *hrp* gene, which is required for the functioning of the type III secretion system and causes hypersensitivity reactions in plants) in pathogens, disease symptoms will occur. decrease (Hanemian et al., 2013). Such mutations are then unable to overcome patterned-activated immunity and cannot infect host tissues. These mutations typically reside in the apoplast without causing damage and may even protect the host against invasion by rabies pathogens. The mutants and rabies pathogens

invade distinct cells/niches when co-inoculated. Type III secretion and effector systems have mainly been described in pathogens. However, some rewards also interact more actively with the plant's immune response through effectors. For example, rhizobacteria secrete effectors that use type III and type VI secretion systems to induce nodulation in host plants. Recently, type III secretion system gene clusters in the rhizosphere were beneficial to *Pseudomonas* spp. (Stringlis et al., 2019). These gene clusters are very similar to the type III secretion systems of other beneficial bacteria, but differ from the secretion system of the phytopathogenic *P. syringae*.

"However, further research is needed to determine the presence of type III secretion systems in other beneficial bacteria of plant cells, to identify pathogen-associated effectors versus control agents. biology and determine the roles of these systems. interact. An often-overlooked group of molecules that influence the host immune system are N-acyl-homoserine lactones (AHL)" (Schenk and Schikora ,2015) "AHL regulates the behavior of gram-negative pathogens. The host plant may benefit from the ability to respond to or interfere with this quorum sensing signal. Indeed, exposure of roots to AHL or AHL-producing bacteria has been shown to trigger the upregulation of plant defense genes and induce systemic resistance through salicylic acid signaling (SA). One of the defense responses induced by AHL in Arabidopsis is stomatal closure, a common first defense response that limits pathogen entry into the apoplast. Additionally, plants respond by degrading AHLs or transporting AHLs through the vascular system to remove them from the bacteria that produced them. Both actions can be described as equivalent to quorum suppression. AHL sensing in Arabidopsis is mediated by the G protein-coupled receptor encoded by AtGPA1. However, more research is needed to identify AHL receptors in other plants" (Lebeer et al., 2010).

### 3.1 Plant Hormones

"Recognition of beneficial or pathogenic microbial attacks, as described above, leads to the activation of signaling hormones in plants, homologous to immunoregulatory cytokines in cells. people and animals. Related plant hormones include SA, JA and ethylene, of which SA and JA are considered antagonists. JA and ethylene are normally involved in defense

responses against necrotic (feeding on dead host cells) pathogens, or after injury, while SA is involved in defense responses against biogenic pathogens” (Glazebrook, 2005). “Spray experiments with bacterially produced rhamnolipids on Arabidopsis leaves showed that SA plays a central role in rhamnolipid-mediated disease resistance” (Sanchez et al., 2012) Bacteria in the phytosphere can directly regulate plant hormone concentrations through the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase that degrades the ethylene precursor ACC. It has been detected in plant growth-promoting rhizosphere bacteria such as *Azospirillum*, *Rhizobium* and *Pseudomonas* spp., as well as in phyllosphere bacteria, such as some *Methylobacteria* spp. and *Rhodococcus fascians*.

“The activity of 1-Aminocyclopropane-1-carboxylate deaminase reduces ethylene concentrations, thereby reducing plant defense responses and thereby facilitating symbiotic microorganisms. ACC deaminase also promotes plant growth, as plants become more resilient to environmental stresses such as drought, flood, salt stress or pathogen pressure. Direct evidence for a role for ethylene in regulating plant community composition, as ethylene-insensitive plant mutants harbor a different plant community. In addition, there is increasing evidence for a direct role of ACC in regulating plant development and defense responses” (Tsang et al., 2011). “Members of the genus *Methylobacteria* enhance plant growth by producing auxin and cytokinin. The interaction with the host is beneficial for the growth of the symbiont because it metabolizes methanol released during plant growth” (Kutschera, 2007). Cytokinins derived from *Methylobacteria* are thought to confer resistance to drought and salt stress in the host (Jorge et al., 2019). “The type of cytokinin present and the presence of the *miaA* gene suggest that methyl bacterial cytokinin production occurs simply via tRNA. In addition, biological control activity has been observed, for example in the inoculation of peanut seeds with *Methylobacteria* spp. increased protection against pathogens *Aspergillus niger* and *Sclerotium rolfsii*” (Madhaiyan et al., 2006). “This indicates that *Methylobacteria* spp. interacts with the host plant's defense system, providing protection against *A. niger* and *S. rolfsii*. The bacteria known to regulate plant hormone levels is *Rhodococcus fascians*. Pathogenic and non-pathogenic *R. fascians* are both capable of

producing IAA and cytokinin and reducing ethylene concentrations” (Francis et al., 2016).

“In pathogenic bacteria, genes encoding auxin and cytokinin production are transmitted via plasmids” (Jameson, 2000). “IAA production was higher in the presence of exogenous tryptophan, the IAA precursor. Interestingly, when inoculated into pathogenic or non-pathogenic *R. fascians* plants, the host plant's metabolism changes and accumulates more tryptophan, ultimately stimulating bacterial IAA production. On the other hand, cytokinin production by *R. fascians* increases auxin production in the plant. Increased auxin levels play an important role in the development of disease symptoms” (Stes et al., 2012). “Non-pathogenic derivatives of this strain lack the plasmid containing the virulence genes. The main pathogenic factor on the plasmid is the production of variable methylated cytokinins, which are not degraded by cytokinin oxidase activity, mimicking plant cytokinins causing an increase in auxin production in plants and leading to the development of disease symptoms” (Radhika et al., 2015). Therefore, cytokinin and auxin production in pathogenic *R. fascians* is detrimental and contributes to disease development. In contrast, cytokinin and auxin production in non-pathogenic *R. fascians*, as well as other symbiotic species (e.g., *Methylobacteria*, described above), has been described as a beneficial trait because it promotes plant growth.

In summary, we can assume that through the ability to control the steady state of auxin by generating additional auxin on the one hand and through the breakdown of auxin when overproduction of auxin occurs in the field. On the other hand, more pathogens invade the host. The symbiont can ultimately optimize its ecological niche by enhancing the growth of its host and by excluding other invaders. The interference between auxin and ethylene, as well as bacterial degradation of ACC, can prevent the occurrence of ethylene-induced excessive immune responses and aging. Additionally, it is commonly known that cytokinin acts as a reservoir for sugars and other metabolites. Therefore, we consider it plausible that microbial cytokinin production functions as a sink of metabolites to benefit the symbiont (carbon source) as well as the host by reducing aging and thus prolongation of photosynthetic activities.

**Table 1. Overview of several known mechanisms by which phyllosphere microbes can inhibit pathogen growth**

<b>In vitro screening</b>	<b>In silico screening</b>	<b>Compound</b>	<b>Mechanism/specific activity</b>	<b>Identified in</b>
<b>Siderophores:</b> Plate assay with indicator for detection of siderophores (Chrome azurol S assay)	Screening for siderophore gene clusters, using antiSMASH	Siderophores	Primary function is iron chelation. Siderophores can also have antibacterial properties through the production of ROS and play a role in motility on the phyllosphere	<i>Pseudomonas protegens</i> CS1
<b>Hydrolytic enzymes:</b> Zymogram, or specific colorimetric assays	chiA, chiB, chiC or other genes encoding for glycosyl hydrolases from family 18 or 19 in the CAZy database	Hydrolytic enzymes: e.g., chitinase	Hydrolyses fungal cell wall	<i>Bacillus subtilis</i>
<b>Antibiotic metabolites:</b> Binary inhibitory interactions, purification and identification of compounds in supernatant	Screening for biosynthetic gene clusters using the antiSMASH tool	Antimicrobial secondary metabolites		
	srfAC, srfAD fenF, mycABC ppsABCDE	. Lipopeptide, surfactin . Lipopeptide, iturin . Phenazine	. Triggers biofilm formation . Interferes with lipid layers . Interferes with histone acetylation and biofilm formation	<i>Bacillus</i> spp. <i>Bacillus</i> spp. <i>Pseudomonas</i> spp., <i>Pantoea</i> spp.
<b>Quorum quenching and sensing:</b> Screening of bacteria interfering with the transcription of a reporter gene, induced by the signalling molecule of interest	nis gene cluster (nisin), spa gene cluster (subtilin), luxI and luxR (AHLs)	Signalling molecules. Some gr- bacteria use bacteriocins (nisin and subtilin) that also have a signalling function	Quorum sensing	Nisin in <i>Lactococcus lactis</i> and subtilin in <i>Bacillus subtilis</i>
<b>Competition for nutrients and space:</b> Carbon source profiling and calculation of NOI	Genes related in carbohydrate metabolism (e.g., glycosyl hydrolases), or transport (e.g., TonB receptors), using the CAZy database	Enzymes ensuring flexible carbohydrate metabolism, e.g., high diversity of TonB receptors	Increased competitiveness in a carbon limited environment	<i>Sphingomonas</i> spp.
<b>Induced systemic response:</b> Transcriptomics of the host plant	Creation of a MAMP database, compare between beneficial and pathogenic microbes	MAMPs that trigger an immune response, that increases protection against pathogens	Detection results in immune response	<i>Sphingomonas melonis</i> fr1

### 3.2 Induced Systemic Responses

“Once the plant detects the bacteria and its presence is signaled through plant hormones, as described above, a specific immune response is triggered in the plant. Beneficial bacteria can activate the plant defense system when recognized and induce immunity against pathogens throughout the plant body” (Pieterse et al., 2014). “This phenomenon is called induced system resistance (ISR). Additionally, pathogens can induce a systemic response that then protects other parts of the plant, a phenomenon known as systemic acquired resistance (SAR). The term ISR is often used when activated by rhizosphere bacteria. *S. melonis* Fr1 induces camalexin production in host plants” (Ryffel et al., 2016). The pathogen *P. syringae* pv. *tomato* also produce camalexin but at higher concentrations. The production of camalexin, an indole alkaloid derived from tryptophan, is a typical defense response of Arabidopsis and other cruciferous plants. Due to its lipophilic nature, camalexin is effective against a variety of bacteria and fungi by interfering with membrane integrity (e.g. by binding to phospholipids). Therefore, camalexin production by Arabidopsis, triggered by *S. melonis* Fr1, is considered to be the originating mechanism of the plant defense observed by this symbiont.

The host immune system not only targets bacterial or fungal pathogens but can also protect against viral diseases. Three-year field trials of foliar application of *Bacillus amyloliquefascians* 5B6 showed consistently reduced accumulation of cucumber mosaic virus as measured by qPCR. The observed changes in gene expression in host plants suggest that activation of the SA and ethylene signaling pathways play a key role in acquired disease resistance. Here too, the genes regulated by biological control agents are the same as those regulated in defense responses induced by some viruses, including cucumber mosaic virus.

Another intriguing observation was made on the plant growth-promoting rhizosphere bacterium known as *Paenibacillus polymyxa* AC-1 (Hong et al., 2016). This strain inhibits the growth of the plant pathogen *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *tabaci* in *in vitro* environment. Cell-free supernatants of *P. polymyxa* AC-1 also inhibited these pathogens, suggesting that the antibacterial metabolites excreted by the antagonist act as direct antagonists (see “Antibacterial metabolites”). Inoculation of the

root tip of axenic Arabidopsis plants with a bacterial suspension of *P. polymyxa* AC-1 resulted in an SA- and JA-dependent defense response. Interestingly, inoculation of this axenic root resulted in the colonization of the endosperm of Arabidopsis leaves by *P. polymyxa* AC-1. Leaf endospheric colonization was 10-fold higher in Arabidopsis mutants with reduced sensitivity to JA and 10-fold lower in mutants deficient in abscisic acid, an isoprenoid plant hormone, compared to man-made plants. savage.

Colonization of the leaf endosphere by *P. polymyxa* AC1 in JA-deficient plants even causes disease symptoms in the plant. This indicates that JA has a negative effect on the detrimental endogenous growth of AC-1. This demonstrates that the plant defense system plays an important role in regulating the total microbial load and preventing symbiotic bacterial invasion. The mechanisms by which bacteria are detected by the host and subsequently trigger the host immune response are similar in 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 have the ability to activate the immune system and thereby confer resistance to phylogenetically distinct pathogens. Sometimes these bacteria are very closely related to pathogenic strains or may even be opportunistic pathogens (e.g., *Pseudomonas*, *R. fascians*, *P. polymyxa*). In this case, the difference between ISR and SAR becomes less clear.

### 4. CONCLUSION AND FUTURE ASPECTS

Among them are pathogens that cause disease in host plants and reduce agricultural productivity, but also beneficial bacteria that could be the key to environmentally friendly solutions to protect crops against diseases. These beneficial bacteria can directly inhibit the growth of pathogens by competing for nutrients and space, hindering their communication, and secreting antibacterial or parasitic metabolites or enzymes. On the other hand, beneficial bacteria can activate the plant's immune response and regulate plant hormone levels, thereby indirectly inhibiting the growth of pathogens. Knowledge of these mechanisms is typically obtained through *in vitro* experiments using sterile or gnotobiotic plants, while the effectiveness of biological control agents must be confirmed through field testing. In this review, we have linked these two

types of research and provided insight into the biological control and adaptive mechanisms that play a role in the phyllosphere. Several mechanisms still need to be confirmed, for example the characterization of new antibacterial peptides, the role of the type III secretion system, the biological control of small peptides involved in quorum sensing, and the impact of plant hormones produced by bacteria on the host immune system. Finally, the biological control agent must integrate into the resident microbial community. Techniques that can help us understand the mechanisms that play a role in complex communities in plant cells are metagenome, meta-transcriptome, metaproteome and metabolome analysis. Biocontrol communities possess many complementary biological control and adaptation factors, cooperating to suppress disease and survive in the phyllosphere. Biocontrol agents and communities can provide effective and sustainable alternatives to conventional pesticides, essential to protecting our agricultural production.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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