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MICROBIOME INTERACTIONS ON PHYLLOSHERE: ITS IMPACT ON PLANT HEALTH

Email

¹Harish J*, ²Ruchira Bagepai and ¹Bhargavi G

harishbpl5021@gmail.com

¹Department of Plant Pathology, RLBCAU, Jhansi, U.P, India

² Indian Institute of Technology, Guwahati, Assam, India

The Plant Microbiome is the sum of the genomic contributions made by the various microbial communities that inhabit the surface and internal tissues of plant parts. Members of these microbial communities interact with one another and with the plant, and there is growing evidence that the microbial community may promote plant growth and aid in pathogen defence. As a result, it is critical to comprehend the mechanisms that influence the composition and structure of microbes. Plants have evolved a sophisticated innate immune system comprised of membrane-localized receptors (PRRs) and intracellular receptors (NLRs) that detect elicitors and activate immune responses. The plant's innate immunity is stimulated by microbiota, which confers resistance against various pathogens (ISR). Aside from these, the microbiome suppresses pathogens through hyperparasitism, the secretion of antimicrobial compounds, and competition for resources such as nutrients or space, which ultimately reduces pathogen growth. Understanding tritropic interactions even better for the development of plant probiotics and the identification of potential agents for more eco-friendly disease combat.

Joshua Lederberg coined the term "microbiome" to describe an "ecological community of commensal microorganisms, symbionts, or pathogens that literally occupy a space everywhere," and plants' microbiome can be defined as "the sum total of the genomic contribution made by the diverse microbial communities that inhabit the surface and internal tissues of the plant parts" (Fernando et al., 2014). Plants are home to a wide variety of microorganisms. Members of these microbial communities interact with one another and with the plant, and there is growing evidence that the microbial community may promote plant growth, aid in pathogen defence, and even aid in environmental remediation. As a result, it is critical to better understand the mechanisms that influence the composition and structure of

microbial communities, as well as the role that the host may play in the recruitment and control of its microbiome in order to combat diseases in a more environmentally friendly manner. Phytobiomes live in various parts of plants, including the Rhizosphere, Endosphere, and Phyllosphere.

1. Rhizosphere Microbiome

The rhizosphere is defined as the soil region influenced by roots. The microbial community that lives in this niche differs from that found in the bulk soil; against pathogens, the plant can defend more specifically with the help of the rhizosphere microbiome. The term 'suppressive rhizosphere' refers to a microbial community that has evolved in the rhizosphere and is capable of limiting pathogen development even in the presence of the host plant. *Pseudomonas fluorescens*, *Bradyrhizobium japonicum*, *Rhizobium leguminosarum*, *Bacillus cereus*, *Bacillus amyloliquefaciens*, *Burkholderia cenocepacia* are the most common pathogen-fighting microorganisms found in the rhizosphere.

2. Phyllosphere Microbiome

The plant microbiome is made up of organisms that colonise the phyllosphere, which is the external area of aerial plant tissues. Although this term can refer to any external surface of a plant, it is most commonly used to describe the leaf surface. The phyllosphere's microbial communities play critical roles in plant development processes by protecting plants from invading pathogens and biosynthesizing phytohormones. Fungi (filamentous and yeasts), bacteria, algae, protozoa, and nematodes make up the phyllosphere community. The bacterial community is the most abundant, with between 10^5 and 10^7 cells per cm^2 .

3. Endosphere Microbiome

Endophytes are microorganisms (bacteria, fungi, or actinomycetes) that live in plant tissues in a symbiotic relationship. Endophytes can synthesise bioactive compounds that plants use to defend themselves against pathogens. These bioactive compounds include alkaloids, terpenoids, flavonoids, and steroids.

Phytobiome Recruitment

There are numerous potential sources of new microbial strains and species to join phytobiome communities. The importance of each of these routes is likely to shift over the course of a plant's life cycle (Baltrus *et al.*, 2017).

- Vertical transmission from plant to seed • Invasion of seed endosphere by root endophytes and vice versa
- Invasion of the seed endosphere from an environmental source
 - Invasion of the roots from an environmental source
- Colonization of the leaves from root associated microbes
- Colonization of the leaves from an environmental source
- Cross-species colonisation
- Insect vectoring

Composition of Plant Microbiome

Plant microbiomes above and below ground are being compared. Microbial communities in the phyllosphere have a low species diversity and a high rate of change. Rhizosphere microbial communities are far more complex than phyllosphere communities, and they are remarkably consistent from sample to sample (Lemanceau *et al.*, 2017).

Evolution of Plant and Associated Microbiome

The microbiome in plants evolves through processes such as natural selection, diversification, dispersion, and drift, which result in a functional trait of microorganisms that increases plant fitness against pathogens. Natural selection is the most common mode of evolution among these (Lemanceau *et al.*, 2017).

Plant-microbiome Interaction

The plant microbiome refers to the interaction between host plants and the entire microbiome (both pathogen and beneficial microbes). Plant-pathogen interaction causes physiological changes in the plant system, i.e., plant innate immunity in resistance condition; otherwise, the plant becomes diseased. Plant-beneficial microbe interactions result in the development of induced systemic resistance as well as improved plant health via hormonal regulation (Kusari *et al.*, 2012).

Plant innate Immunity

Plants are invaded by a variety of pathogens, only a few of which cause disease. Others' attacks are countered by the plants' sophisticated immune system. Microbial-associated molecular-patterns-triggered immunity (MTI) and effector-triggered immunity (ETI) are the two broad categories of the plant immune system (ETI).

Molecular pattern Triggered Immunity (MTI)

MTI is a generalised plant defence that is also known as horizontal resistance. Plants are governed by several genes that code for protein, which becomes receptors known as PRRs. Usually multigenic and contribute to plant immunity in a minor way via PRR signalling or MTI. Hundreds of thousands of MTI events are happening on the plant surface that is similar to innate immunity exhibited by animals. Plant divert its metabolic energy towards this event. Usually this form of immunity is long lasting.

Effector Triggered Immunity (ETI)

Plants are controlled by one or a few genes that code for proteins that become receptors, which are referred to as R-genes. Typically monogenic, they play a significant role in plant immunity via R-gene signalling. On the plant's interior, there are very few R-gene triggered events. This is similar to the adaptive immunity displayed by animals. This event directs the plant's metabolic energy. Usually this form of immunity is short lived and leads to R-gene breakdown.

Effector Triggered Susceptibility (ETS)

MTI suppression is enabled by "specialised molecules," which can be a pathogen or race specific. These molecules are known as pathogen effectors or effectors. The pathogen that is successful is the one that renders MTI ineffective. The primary distinction between microbes and pathogens is that pathogens suppress MTI while microbes do not. MTI cannot be suppressed by a general microbe. The suppression of MTI aids the pathogen's population establishment within the plants. The phenomenon is known as colonisation, and it is also known as tangible "plant disease."

$[(MTI - ETS) + ETI]$ is the ultimate amplitude of disease resistance or susceptibility.

Steps Involved in Molecular Pattern Interaction With Plants

Chemically pure MAMPs/PAMPs (microbe or pathogen associated molecular pattern) can reach all cells and activate their receptors simultaneously when applied to suspension-cultured plant cells or cells in thin segments of plant tissue.

Very Early Responses (1–5 Minutes)

i. Ion fluxes

Alkalinization of the growth medium due to changes in ion fluxes across the plasma membrane is one of the earliest and most easily recordable physiological responses to MAMPs and PAMPs in plant cell cultures, beginning after a lag phase of 0.5–2 min.

These changes include an increase in H⁺ and Ca²⁺ influx, as well as a concomitant efflux of K⁺; an efflux of anions, particularly nitrate, has also been observed. Ion fluxes cause membrane depolarization. MAMPs are known to stimulate Ca²⁺ influx from the apoplast, resulting in a rapid increase in cytoplasmic Ca²⁺ concentrations, which may act as a second messenger to promote the opening of other membrane channels or to activate calcium-dependent protein kinases (Pieterse *et al.*, 2009).

ii. Oxidative burst

The oxidative burst is another very early response to MAMPs, with a lag phase of 2.0MIN. Reactive oxygen species can act as antibiotic agents directly, as has been demonstrated in macrophages, or they can contribute indirectly to defence by causing cell wall cross-linking; additionally, reactive oxygen species can act as secondary stress signals, inducing a variety of defence responses. Several studies have found that MAMP causes the reactive oxygen species nitric oxide (NO), a well-known second messenger in animals, to be produced..

ISR and SAR responses

Systemically acquired resistance (SAR), induced by the exposure of root or foliar tissues to abiotic or biotic elicitors, is dependent on salicylic acid and associated with the accumulation of pathogenesis-related (PR) proteins. Plants use pattern-recognition receptors to recognize conserved microbial signatures. SAR is a systemic defense network in plants that is triggered by exposing the plant to some virulent, avirulent, and nonpathogenic microbes.

Induced systemic resistance is caused by exposing roots to PGPR, is dependent on ethylene and jasmonic acid, and is not associated with PR protein accumulation.

Rhizobacteria can mediate ISR responses, which have been shown to be effective against necrotrophic pathogens and insect herbivores that are vulnerable to JA/ET defences.

Microbiota-mediated Extension of The Plant Immune System

The stimulation of plant innate immunity by microbiota has been extensively described in order to confer resistance against various microbial leaf pathogens (a phenomenon referred to as priming or induced systemic resistance [ISR]). ISR has been well described in *Arabidopsis thaliana*, and the mechanisms that control its onset appear to be conserved across organisms. The transcription factor MYB72, in particular, is important in the regulation of ISR triggered by the bacterium *Pseudomonas simiae*. MYB72 is also involved in the response of *A. thaliana* to iron deficiency, implying a direct link between nutrient stress and immunity. ISR may occur because plants have evolved to use microbial molecules as developmental signals for the maturation of their immune systems, implying that early contact with microbe-derived molecules is required for plant survival in natural soils (Vannier *et al.*, 2019).

Mechanism of Pathogen Control by Beneficial Microbes

1. Competition

The majority of biocontrol agents are rapidly growing and compete with pathogens for space, organic nutrients, and minerals. E.g., Fe has a low water solubility and is a limiting factor for both pathogens and microbes. Plants and microbes both obtain Fe by producing Siderophore, which are Fe binding compounds. *Pseudomonas fluorescens*, which produces Pseudobactins, aids in the control of the soft rot pathogen.

2. Antagonism

Antagonism mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances is known as antibiosis.

a. Antibiotics

Antibiotics are organic compounds with low molecular weight that are produced by microbes. Antibiotics are harmful to the growth or metabolic activities of other microorganisms at low concentrations. *Gliocladium virens*, for example, produces gliotoxin, which caused *Rhizoctonia solani* to die on potato tubers. *Trichoderma viride* colonisation of

pea seeds resulted in the accumulation of a significant amount of the antibiotic viridin in the seeds

b. Bacteriocins

These are antibiotic-like compounds with bactericidal activity that are closely related to bacteriocin producers. E.g. Crown gall (caused by *Agrobacterium tumefaciens*) is controlled by the related *Agrobacterium radiobacter* strain K 84 through the production of bacteriocin, Agrocin K84.

c. Volatile compounds

Antibiosis mediated by volatile compounds has been produced by *Enterobacter cloacae*. The volatile fraction responsible for inhibition was identified as ammonia.

3. Hyperparasitism

Hyperparasitism is the direct parasitism or lysis and death of a pathogen by another microorganism while the pathogen is in the parasitic phase. *Cladosporium cladosporioides*, for example, on *Puccinia striiformis* f. sp. tritici.

Rational Design of SynComs with Predictable Pathogen Biocontrol Activities

SynComs are small consortia of microorganisms. It will observed function and structure of the microbiome in natural. It may also increase stability through synergistic interactions between their members. This is based on microbial ecology and genetics of predictable traits. The role of each microbial member can be investigated & factors governing community assembly. SynComs could confer more efficient plant protection than individual strains (Vannier *et al.*, 2019).

Case Studies

1. The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen

The study's main goal was to test the hypothesis that phyllosphere microbes, including epiphytes and endophytes, contribute to *Arabidopsis thaliana* resistance to *Botrytis cinerea*, with a focus on the cuticle. Three different types of *Arabidopsis* plants (wild type, Bdg mutant, and Lacs2.3 cuticle mutants) were inoculated with *Botrytis cinerea* and allowed to develop disease. Based on the severity of the disease, microbes from the phyllosphere were

collected to identify the microbe involved in controlling botrytis. To determine the role of phyllosphere microbes in *A. thaliana* resistance to pathogens, leaf washes of three *Arabidopsis* forms were sprayed on both sterile and non-sterile plants, but leaf washes from cuticle mutant *Bdg* provided good resistance against pathogen. Resistance of sterile *Arabidopsis thaliana* Col-0 and cuticle mutants to *Botrytis cinerea* after treatment with microbes extracted from nonsterile plants' phyllospheres. Individual microbial strains extracted from the phyllosphere of the *bdg* mutant have an effect on *Arabidopsis thaliana* Col-0 plant resistance to *Botrytis cinerea*.

Summary

The role of the phyllosphere microbiome in plant resistance is investigated in the cuticle mutants *bdg* (BODYGUARD) or *lacs2.3* (LONG CHAIN FATTY ACID SYNTHASE 2) that are highly resistant to the fungus *Botrytis cinerea*. The presence of microbes on the plant surface contributes to resistance to *B. cinerea*. When inoculated under sterile conditions, *bdg* plants became as susceptible as wild-type (WT) plants, while *lacs2.3* mutants retained their resistance. Adding washes of its phyllosphere microbiome could restore *bdg* mutant resistance, whereas *lacs2.3* resistance is due to endogenous mechanisms. When WT plants were compared to cuticle mutants, the phyllosphere microbiome showed distinct populations. *Pseudomonas* spp isolated from the *bdg* microbiome provided resistance to *B. cinerea* on *Arabidopsis thaliana* as well as apple fruits.

2. Endophytic bacteria enhancing growth and disease resistance of potato (*solanum tuberosum* L.)

The primary goal was to investigate the impact of endophytic strains *Pseudomonas* spp IMBG294 and *Methylobacterium* spp IMBG290 on plant growth and inducible defences.

Potato plants were inoculated with the endophytes *Pseudomonas* sp IMBG294 and *Methylobacterium* spp IMBG290, and three weeks later they were infected with the pathogen *Pectobacterium atrosepticum* to study the development of disease resistance genes and enzymes. Endophytes were tested for their ability to induce disease resistance in potatoes against the soft rot disease caused by *P. atrosepticum*.

Summary

Potato shoot growth is encouraged. *Pseudomonas* sp. improved potato resistance to soft rot disease. The size of the bacterial population used to induce disease resistance by *Methylobacterium* sp. was inversely proportional to the size of the bacterial population used. Some endophytes are capable of activating both basal and inducible plant defence systems.

Conclusion

Plant developmental and evolutionary dynamics are shaped by the phytobiome, which can protect hosts from pathogen infection and produce plant growth hormones. Phytobiome is a low-cost, environmentally friendly tool for managing plant diseases. More research is needed to better understand the tritropic interactions for the development of plant probiotics and the identification of potential agents for more eco-friendly disease combating.

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