



Review Article

## Microbiome Interactions on Phyllosphere: It's Impact on Plant Health

Harish J\* and Bhargavi G

### Abstract

The Plant Microbiome can be described 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. The members of these microbial communities interact among each other and with the plant, and there is increasing evidence to suggest that the microbial community may promote plant growth, facilitate pathogen defense. Therefore, it is important to understand the mechanisms that influence the composition and structure of microbes. Plants have evolved a complex innate immune system comprising Membrane-Localized Receptors (PRRs) and Intracellular Receptors (NLRs) that detect the elicitors and activate immune against pathogens. Some commensals also able to activate PRR-triggered immunity through conserved nature of Microbe Associated Molecular Patterns (MAMPs). Microbiota stimulates the plant innate immunity which confers resistance against various pathogens (ISR). Apart from these, microbiome suppresses the pathogens via hyper parasitism, secretion of antimicrobial compounds and competition for the resources like nutrients or space which ultimately mitigate pathogen growth. Phyllosphere microbiome in plant resistance in the cuticle mutants *bdg* (BODYGUARD) or *lacs 2.3* (LONG CHAIN FATTY ACID SYNTHASE 2) that are strongly resistant to the fungal pathogen *Botrytis cinerea* and also reported that phyllosphere microbiome showed distinct populations in Wild Type (WT) plants compared to cuticle mutants. Endophytic bacteria to promote growth and resistance of potato plants towards infection by the necrotroph *Pectobacterium atrosepticum*, both tested strains (*Pseudomonas* and *Methylobacterium*) promoted growth of potato shoot but only the *Pseudomonas sp.* Increased potato resistance towards soft rot. Understanding the tritropic interactions even better for Development of plant probiotics and for identification of potential agents for combating diseases more eco-friendly.

**Keywords:** Microbiome; Pathogen; Competition; Antagonism

### Introduction

The word 'microbiome' was first used by Joshua Lederberg as the "ecological community of commensal microorganisms, symbionts or pathogens, that literally occupy a space everywhere" and in the same way plants Microbiome can be described 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" [1].

Plants serve as host to numerous microorganisms. The members

of these microbial communities interact among each other and with the plant and there is increasing evidence to suggest that the microbial community may promote plant growth, facilitate pathogen defence and even assist in environmental remediation. Therefore, it is important to better understand the mechanisms that influence the composition and structure of microbial communities, and what role the host may play in the recruitment and control of its microbiome for combating diseases more eco-friendly. Phyto biomes occupies different parts of the plants namely on Rhizosphere, Endosphere and Phyllosphere.

### Rhizosphere microbiome

The rhizosphere is defined as the soil region under the influence of the roots. The microbial community residing in this niche is structured differentially from that found in the bulk soil, against pathogens plant can defend more specifically with the help of rhizosphere microbiome. The term 'suppressive rhizosphere' refers to the microbial community that is selected in the rhizosphere and is able to limit the development of pathogens, even in the presence of the host plant. Most commonly found microorganism against the pathogen in rhizosphere are *Pseudomonas fluorescens*, *Bradyrhizobium japonicum*, *Rhizobium leguminosarum*, *Bacillus cereus*, *Bacillus amyloliquefaciens*, *Burkholderia cenocepacia*, *Streptomyces filamentosus*, *Trichoderma viride*, *Trichoderma harzianum* and *Paecilomyces lilacinus*.

### Phyllosphere microbiome

Plant microbiome is made of organisms colonizing the external area of aerial plant tissues called phyllosphere. Although this term can be used for any external surface of plants, it is commonly applied when describing the leaf surface.

The microbial communities found in the phyllosphere have essential roles in processes related to plant development by protecting plants against invading pathogens and biosynthesizing phytohormones. The phyllosphere community is composed of fungi (filamentous and yeasts), bacteria, algae, protozoa and nematodes. The most abundant group amongst these is the bacterial community of about  $10^5$  and  $10^7$  cells per  $cm^2$ .

### Endosphere microbiome

Endophytes are microorganisms (bacteria or fungi or actinomycetes) that dwell within robust plant tissues by having a symbiotic association. Endophytes are capable of synthesizing bioactive compounds that are used by plants for defence against pathogens, those bioactive compounds are alkaloids, terpenoids, flavonoids and steroids.

### Phytobiome recruitment

There are numerous inputs for new microbial strains and species to join phytobiome communities. The influence of each these routes will likely change over the course of an individual plant's life cycle [2]. It includes

- Vertical transmission from plants to seed
- Invasion of seed endosphere from root endophytes and vice versa

\*Corresponding author: Harish J, Department of Plant Pathology, RLBCAU, Jhansi, India, Tel: +91 8660799656; E-mail: harishbpl5021@gmail.com

Received: October 05, 2021 Accepted: October 22, 2021 Published: October 29, 2021

- Invasion of seed endosphere from environmental source
- Invasion of roots from environmental source
- Leaves colonization from root associated microbes
- Leaves colonization from environmental source
- Cross-species colonization
- Insect vectoring

### Composition of plant microbiome

Comparing above ground and below ground plant microbiomes. Phyllosphere microbial communities have relatively low species diversity with high rate of change. Rhizosphere microbial communities are significantly more complex than phyllosphere communities and remarkably consistent from sample to sample [3].

### Evolution of plant and associated microbiome

The microbiome in the plants evolve through the processes like natural selection, diversification, dispersion and drift, these create a functional trait of microorganisms which increases the fitness of the plants against the pathogens. Among these natural selection is the predominant way of evolution [3].

### Plant-microbiome interaction

Plant microbiome includes the interaction between the host plants and entire microbiome (both pathogen and beneficial microbes). Interaction between the plant and pathogen leads to physiological changes in the plant system i.e., Plant innate immunity in resistance condition, otherwise plant will be diseased. Interaction between plant and beneficial microbes lead to the development of induced systemic resistance and also enhances the plant health through hormonal regulation [4].

### Plant innate immunity

Plants are invaded by an array of pathogens of which only a few succeed in causing disease. The attack by others is countered by a sophisticated immune system possessed by the plants. The plant immune system is broadly divided into microbial-associated Molecular-Patterns-Triggered Immunity (MTI) and Effector-Triggered Immunity (ETI).

### Molecular Pattern Triggered Immunity (MTI)

MTI is nothing but the generalized plant defense popularly termed as Horizontal resistance. Governed by several genes in the plants that codes for protein which becomes receptors termed as PRRs. Usually multigenic and contribute in a small way for plant immunity through PRR signaling or MTI. Several thousand 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)

Governed by one or very few genes in the plants that codes for protein which becomes receptors termed as R-genes. Usually Monogenic and contribute in a big way for plant immunity through R-gene signaling. Very few R-gene triggered events are happening on the plant interior. That is similar to adaptive immunity exhibited by animals.

Plant divert its metabolic energy towards this event.

Usually this form of immunity is short lived and leads to R-gene breakdown.

### Effector Triggered Susceptibility (ETS)

MTI suppression is enabled by "specialized molecules" or pathogen or even race specific molecules. These molecules are called as effectors or pathogen effectors. Successful pathogen is the one that makes MTI ineffective. The major difference between microbe and pathogens is that the one which suppress MTI is pathogen while the one which does not is a microbe. A general microbe can't suppress MTI. The suppression of MTI helps the pathogen to establish its population within the plants. The phenomenon is called as colonization which is also termed as tangible "plant disease".

The ultimate amplitude of disease resistance or susceptibility is proportional to [(MTI- ETS) + ETI].

### Steps involved in molecular pattern interaction with plants

When chemically pure MAMPs/PAMPs (microbe or pathogen associated molecular pattern) are applied to suspension-cultured plant cells or cells in thin segments of plant tissue, they can reach all cells and trigger their receptors simultaneously.

### Very Early Responses (1-5 Minutes)

#### Ion fluxes

Among the earliest and most easily recordable physiological responses to MAMPs and PAMPs in plant cell cultures, starting after a lag phase of 0.5-2 min, is an alkalization of the growth medium due to changes of ion fluxes across the plasma membrane.

These changes include increased influx of H<sup>+</sup> and Ca<sup>2+</sup> and a concomitant efflux of K<sup>+</sup> an efflux of anions, in particular of nitrate, has also been observed. The ion fluxes lead to membrane depolarization. MAMPs are known to stimulate an influx of Ca<sup>2+</sup> from the apoplast and cause a rapid increase in cytoplasmic Ca<sup>2+</sup> concentrations, which might serve as second messenger to promote the opening of other membrane channels or to activate calcium-dependent protein kinases.

#### Oxidative burst

Another very early response to MAMPs, with a lag phase of 2.0 MIN is the oxidative burst. Reactive oxygen species can act as antibiotic agents directly, as well established in macrophages, or they may contribute indirectly to defense by causing cell wall cross-linking; in addition, reactive oxygen species may act as secondary stress signals to induce various defense responses. Several studies report a MAMP-induced production of the reactive oxygen species Nitric Oxide (NO), a well-known second messenger in animals.

### 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, a virulent, and nonpathogenic microbes.

Induced systemic resistance, induced by the exposure of roots to PGPR and is dependent on ethylene and jasmonic acid and is not associated with the accumulation of PR proteins. ISR responses can be mediated by rhizo bacteria which has shown to be effective against necro trophic pathogens and insect herbivores that are sensitive to JA/ET defences.

### Microbiota-mediated extension of the plant immune system

Microbiota-mediated stimulation of plant innate immunity has been extensively described 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 identified mechanisms controlling its onset appear to be conserved for different organisms. Particularly, the transcription factor MYB72 plays a key role in the regulation of ISR triggered by the bacterium *Pseudomonas simiae*. MYB72 is also involved in *A. thaliana*'s response to iron deficiency, suggesting a direct interplay between nutrient stress and immunity. ISR may occur because plants have evolved to use microbial molecules as developmental signals for plant immune system maturation, implying that early contact with microbe-derived molecules is needed for plant survival in natural soils [5].

### Plant Growth Promoting Rhizobacteria (PGPR)

Rhizosphere bacteria that favourably affect plant growth and yield of commercially important crops are designated as plant growth promoting rhizobacteria. The growth promoting ability of PGPR is due to their ability to produce phytohormones, Siderophores, Hydrogen Cyanide (HCN), chitinases, volatile compounds or antibiotics which will reduce infection of host through phyto-pathogenic micro-organisms. Many bacterial species, viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, etc., are usually used for the management of plant pathogenic microbes. *Bacillus* has ecological advantages as it produces endospores that are tolerant to extreme environmental conditions. *Pseudomonas fluorescens* have been extensively used to manage soil borne plant pathogenic fungi due to their ability to use many carbon sources that exude from the roots and to compete with microflora by the production of antibiotics, HCN and Siderophores that suppress plant root pathogens.

### Mechanism of pathogen control by beneficial microbes

#### Competition

Most of the bio control agents are fast growing and they compete with pathogen for space, organic nutrients and minerals. E.g. Fe has low solubility in water and is limiting for both pathogen and microbe. Both plants and microbe obtain Fe by the production of Fe binding compounds known as Siderophore. *Pseudomonas fluorescens* known to produce Pseudo bactins helps in control of soft rot pathogen.

#### 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.

#### Antibiotics

Antibiotics are generally considered to be organic compounds of low molecular weight produced by microbes. At low concentrations, antibiotics are deleterious to the growth or metabolic activities of

other micro-organisms. E.g. *Gliocladium virens* produces gliotoxin that was responsible for the death of *Rhizoctonia solani* on potato tubers. Colonization of pea seeds by *Trichoderma viride* resulted in the accumulation of significant amount of the antibiotic viridin in the seeds.

#### Bacteriocins

These are antibiotic like compounds with bactericidal specificity closely related to the bacteriocin producer. E.g. The control of crown gall (caused by *Agrobacterium tumefaciens*) by the related *Agrobacterium radiobacter* strain K 84 is by the production of bacteriocin, Agrocin K 84.

#### Volatile compounds

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

#### Hyperparasitism

Direct parasitism or lysis and death of the pathogen by another micro-organism when the pathogen is in parasitic phase is known as hyperparasitism. E.g. *Cladosporium cladosporioides* 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. Syn Coms could confer more efficient plant protection than individual strains [5].

### Case studies

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

The main objective of the study was to test the hypothesis that the phyllosphere microbes including epiphytes and endophytes contribute to the resistance of *Arabidopsis thaliana* to *Botrytis cinerea* with a special emphasis on the cuticle. Three different form of *Arabidopsis* plants (wild type, Bdg mutant and Lacs 2.3 cuticle mutants) were inoculated with *Botrytis cinerea* and allowed for the development of disease, based on the level of disease severity, microbes from the phyllosphere were collected to identify the microbe which is involved controlling botrytis [6].

In order to determine the implication of phyllosphere microbes in the resistance of *A. thaliana* to pathogens leaf wash of three forms of *Arabidopsis* were sprayed on both sterile and non sterile plants, but leaf wash from cuticle mutant Bdg provide good resistance against pathogen. Resistance of sterile *Arabidopsis thaliana* Col-0 and cuticle mutants against *Botrytis cinerea* after treatment with the microbes of their respective phyllosphere extracted from nonsterile plants. Effect of individual microbial strains extracted from the phyllosphere of bdg mutant on the resistance of *Arabidopsis thaliana* Col-0 plants to *Botrytis cinerea*.

## Summary

The importance of the phyllosphere microbiome in plant resistance in the cuticle mutants *bdg* (BODYGUARD) or *lacs 2.3* (LONG CHAIN FATTY ACID SYNTHASE 2) that are strongly resistant to the fungal pathogen *Botrytis cinerea* is studied. Microbes present on the plant surface contribute to the resistance to *B. cinerea*. When inoculated under sterile conditions *bdg* became as susceptible as Wild-Type (WT) plants whereas *lacs 2.3* mutants retained the resistance. Adding washes of its phyllosphere microbiome could restore the resistance of *bdg* mutants, whereas the resistance of *lacs 2.3* results from endogenous mechanisms. The phyllosphere microbiome showed distinct populations in WT plants compared to cuticle mutants. One species identified as *Pseudomonas spp* isolated from the microbiome of *bdg* provided resistance to *B. cinerea* on *Arabidopsis thaliana* as well as on apple fruits [7].

## Endophytic bacteria enhancing growth and disease resistance of potato (*Solanum tuberosum L.*)

The main objective was to study the effect of endophytic strains *Pseudomonas spp* IMBG294 and *Methylobacterium spp* IMBG290 on plant growth and inducible defences.

Potato plants were inoculated with endophytes *Pseudomonas sp* IMBG294 and *Methylobacterium sp* IMBG290, three weeks after inoculation potato plants were infected with pathogen *Pectobacterium atrosepticum* to examine the development of disease resistance genes and enzymes. The capacity of endophytes to induce disease resistance was tested on potato against soft rot disease caused by *P. atrosepticum*.

Promotion of growth of potato shoots. *Pseudomonas sp.* increased potato resistance towards the soft rot disease. Induction of disease resistance by the *Methylobacterium sp.* was inversely proportional to the size of bacterial population used. Some endophytes have the potential to activate both basal and inducible plant defense systems [8,9].

## Conclusion

Phyto-biome shape plant developmental and evolutionary dynamics can protect their hosts against pathogen infection and produce plant growth-promoting hormones. Phyto biome used as cheap and less environmental damaging tool for management of plant diseases. Further study is needed to Understanding the tri-tropic interactions even better for Development of plant probiotics and for Identification of potential agents for combating diseases more eco-friendly.

## References

1. Fernando D, Gumiere T, Durrer A (2014) Exploring interactions of plant microbiomes. *Sci Agric* 71: 528-539.
2. Baltrus DA, McCann HC, Guttman DS (2017) Evolution, genomics and epidemiology of *Pseudomonas syringae*: challenges in bacterial molecular plant pathology. *Molecular plant pathology* 18: 152-168.
3. Lemanceau P, Blouin M, Muller D, Moenne-loccoz Y (2017) Let the core microbiota be functional. *Trends Plant Sci* 22: 583-595.
4. Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry & biology* 19: 792-798.
5. Vannier N, Agler M, Hacquard, S (2019) Microbiota-mediated disease resistance in plants. *New Phytol* 15: 100-124.
6. Armengol G, Filella I, Llusia J, Penuelas J (2016) Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. *Trends Plant Sci* 21: 854-860.
7. Jones P, Garcia BJ, Furches A, Tuskan GA, Jacobson D (2019) Plant host-associated mechanisms for microbial selection. *Front. Plant Sci* 10: 862-868.
8. Pavlo A, Leonid O, Natalia K, Maria PA (2011) Endophytic bacteria enhancing growth and disease resistance of potato (*Solanum tuberosum L.*). *Biol Control* 56: 43-49.
9. Ritpitakphong U, Falquet L, Vimoltust A, Metraux JP, Lharidon, F (2016) The microbiome of the leaf surface of *Arabidopsis* protects against a fungal pathogen. *New Phytol* 210: 1033-1043.