



A Report for Phylloplane Myco-diversity : Minireview

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Abstract:

Phylloplane, landing tribunes for mycobiota, is the surface of plant leaves represents complex terrestrial habitat, characterized by a variety of microorganisms. Majority of the phylloplane microbiota constitutes yeasts and filamentous fungi. Investigation of plant-phylofungi interdependence, is an approach offers a unique correlation to survive and thrive. Seasonal changes, host species-specific trichomes, hairs, glands and other laminar surface attributes provides unrevealed milieu of complex interactions with fungi. Phylloplane mycodiversity, is a knot of many complex interactions that need to untie, and is essential to understand. It comprises variety of niches and encloses package of plant growth and survival, also affect different components of ecosystem in the relative food chain. Phylloplane studies are in demand for the future of Phylloplane mycodiversity to isolate them and to evaluate their intimate relationship.

Keywords: Phylloplane fungi, Mycodiversity, Seasonal changes, Colonization

Introduction:

Biodiversity is the variation of life forms within a given ecosystem, and often used as a measure of the health of biological systems. Fungi, being fascinating and vital components of nearly all ecosystems, interferes human health and economy in a various ways. Mycobiota and its diversity is necessary to investigate for collecting or monitoring the data as far as plant and human lives are concerned. Standardized methods for documenting mycodiversity and their distribution needs improvisation.⁽¹⁾

Plants host diverse species composition of fungal microorganisms; some are beneficial and hence promote their growth, while others being plant pathogens, may develop the disease or even kill the host plant. Rests of the colonists, forms majority on the host plants, plays no significant role on plant growth or function. An aerial surface of plant is a vital habitat for many micro-organisms, including bacteria, fungi and yeasts. Phyllosphere is a term, refers to the total aerial plant parts, especially three dimensional aspects of leaves.⁽²⁾ Phylloplane on the other hand, best defined as mycota growing on the surface of leaves. Phylloplane exhibits a complex terrestrial habitat, and Phylloplane fungi represented by residents and casuals. Residents can multiply on the surface of healthy leaves with no symptoms on host. Whereas, casuals land on the leaf surface but cannot grow.⁽³⁾

Phylloplane, being very rich in nutrients, plays a needy role for the colonization of various fungal species. Phylloplane fungal diversity is also influenced by host leaf epidermal attributes including its size, stomatal index, density of hairs and trichomes and architecture of leaf surface; as well as host plant surroundings such as temperature, humidity, light intensity, presence of air pollutants.^(4,5)

Mycodiversity of the epiphytic microbial population exhibits a large range of fluctuations, that may depend on nature, age and physiology of host plant and their parts, seasonal changes, and diverse leaf surface habitat in terms of their morphological and physicochemical properties.^(6,7) Phyllosphere, inhabited by many nonpathogenic fungi, moreover depends on nutrients exuded from laminar surface and on air depositions as well.⁽⁸⁾ Major proportion of mycodiversity in and on the leaf routes from fungal propagules, fallen accidentally onto the leaf blade by air dispersal. Furthermore, host species-specific trichomes, hairs, glands and other laminar surface attributes provide a largely unrevealed milieu of complex interactions with fungi.⁽⁹⁾

Majority of the phylloplane microbiota constitutes yeasts and filamentous fungi. Filamentous fungi from the phylloplane are diverse in nature like parasites, saprophytes, endophytes or epiphytes.^(10,11,12) Epiphytic fungi, without causing damage, exist on the leaf surfaces. While photosynthetic potential may get reduced, reported in some research.^(13,14) Existence of positive or negative complex interactions between epiphytes and pathogenic microorganisms, may result in beneficial or harmful relationships. In contrast to phytopathogens, few phylloplane fungi protect host leaves against infections.^(15,16,17)

Phylloplane mycodiversity is a knot of many complex interactions that need to be untied, and is essential to understand. It comprises a variety of niches and encloses a package of plant growth and survival, also affecting different components of ecosystem in the relative food chain. Phylloplane studies are in demand for the future of Plant-Mycodiversity interdependence.

Isolation Methods:

Healthy leaf samples of selected plants were collected from predefined sites, immediately put into sterile plastic bags, and brought to the laboratory for further processing. Different techniques were used and standardized by researchers depending on the target mycoflora. Isolation of leaf mycoflora techniques include Leaf imprinting⁽²⁾, Leaf washings and serial dilution methods, Agar plate method (Leaf discs), Moist chamber methods^(11,18,19,20); out of which agar plate (leaf disc) method is more commonly used.

In leaf disc method, washing leaves several times with sterile or double distilled water, followed by cutting them with the help of cork borer or scalpel, into equal segments (about 1 cm² each). Three - Five segments placed on the nutrient media specially made for fungal isolations. Each plate of the agar medium supplemented with desired antibacterial antibiotics, say Chloramphenicol (0.01%).

Different nutrient media used like, PDA, Czapek-Dox, Rose Bengal Agar. Later, plates incubated at 28°C for a week. Fungal colonies observed for their identification and processed for maintenance of pure cultures. Depending on their growth, at least one isolate of each macro-morphological type picked for purification. Identification of fungi done by standard methods.

Phylloplane as a landing tribune for mycobiota reservoir

Leaves of plant with spores and hyphae of fungi forms interkingdom runway, subject to the impact of range of abiotic factors and morpho-physiological changes in the aerial surfaces of plants.⁽¹⁴⁾ Leaves provide a variety of positive or negative modulators to regulate the fungal colonization.

Population dynamics on leaves in time and space area function of immigration, emigration, growth and death.⁽²¹⁾ Phylloplane fungi considered to be potential sources of Bio-Control Agents (BCAs), as are capable to colonize on the target plant.⁽¹⁶⁾

Phylloplane mycodiversity intimately depends on several host attributes such as, nature, age and polarity of leaves, and epidermal architects and appendages as well. Yeasts, and filamentous fungi represents lower densities in juvenile and fresh, tender leaves and higher in mature leaf stage. Furthermore, imprinting leaves mostly resulted in higher densities and diversities axially, but comparisons between abaxial and adaxial imprints are not always significant.⁽¹¹⁾

Fungal spore existence on leaf surface and their colonization involves series of complex interactions, including leaf topology as an important factor. Cell wall acts as initial physico-chemical barrier^(22,23); Cuticle, being hydrophobic, infers resistance to plants and inhibits fungal growth^(24,25); Simple Non-glandular Trichomes, traps the fungal spores and do not allow them to reach the plane of lamina; Glandular trichomes are very complex, sometimes inhibits fungal colonization by secreting antimicrobial compounds, and in many cases, it facilitates the growth and penetration of fungal spore and hyphae, hence become preferred sites of colonization.^(26,27) Trichomes, absorb ergot alkaloids from the epibiotic fungal species of the genus *Periglandula*, hence play dual and key function in a metabolic dialogue between fungus and host plant.⁽²⁸⁾

Seasonal Changes and Phyllo-Fungal Diversity

Diversities and variation in number and species composition of Phylloplane fungi have correlation with seasonal changes and shifts in temperature,⁽²²⁾ spatial locations,⁽¹⁶⁾ climate trends,^(11,23,24,25) leaf ages and habits,^(8, 23, 24) vegetative, flowering and/or harvesting phases.^(11,14) So, climatic and biotic factors appeared to be vital factors contributing to seasonal changes in the succession of interior colonizers.^(22,29) Frequency of phyllo-fungal species diverse significantly in wet and dry seasons, viz; low colonization in dry season and greater in wet. Dispersal of fungal spores enhanced due to greater rainfall in the wet seasons.^(23, 24)

Number of isolates obtained from the paddy field in wet seasons (dominated by *Epicoccum* sp.) found to be four times more than in winter (dominated by *Fusarium* sp.).⁽¹⁶⁾ Seasonal trends in phyllo-fungal diversities represented elevation from winter to spring, followed by a slight decrease in summer and a rise during autumn. Yeast species compositions peaked during summer and declined to a lowest level in spring. In a broader concept, yeasts diversities found to be lower than filamentous fungi, which further were lower than bacteria.⁽¹¹⁾ Fungal population observed significantly higher during flowering stage followed by vegetative and harvesting phase.⁽¹⁴⁾ Phylloplane mycodiversity represented elevation in winter (flowering phase), followed by a decline toward the end of the season in Mango. Preferably, flowering phase regarded as most active period of phylloplane communities, as liberation of large amounts of pollen propagules, serve as rich, exogenous source of nutrients for colonizers.⁽¹¹⁾

Phylloplane densities in number and species composition would be more numerous on the evergreens, with respect to the leaves longevity; on the contrary, overall densities of filamentous fungi for the autumn samplings of the deciduous trees, were statistically higher than those for the evergreens. In few specialized plants, populations of mycobiota become established on the phylloplane of deciduous trees in a relatively short period (from spring to autumn), also leaf

turnover, nutrient availability, or other intrinsic factors may affect the population levels on the evergreens.⁽³⁰⁾ Availability of nutrients, which is dependent on leaf age and growing conditions, can be of relevance for the diversities of the yeast communities as well.^(8,31, 32)

Phylloplane Mycodiversity :

Fungal diversity represented by 1.5 Million species⁽³³⁾, biogeographic distributions, levels of endemism, and host specificity are important aspects to consider for developing estimates of global fungal diversity.⁽³⁴⁾ Mycodiversity on aerial leaf surfaces on leafy vegetables, wild plants, and medicinal herbs shows huge variation in species compositions. Phylloplane studied extensively on wide range of plants, including herbs, shrubs and trees.

Filamentous fungal, and Yeast communities, on juvenile and mature leaves were more diverse than on foliole leaves (leaflets). Dominant member of the yeast population obtained from *Fagus*^(30,35), *Mango*⁽¹¹⁾, *Quercus* and *Acer* species⁽¹⁰⁾ was *Aureobasidium pullulans*, followed by *Sporobolomyces roseus*.⁽¹¹⁾ Basidiomycetous yeasts found to be more dominant than that of Ascomycetous, as confirmed on Maize and Sugarcane phylloplane.^(36, 37)

Diversities in range of filamentous fungi isolates was obtained from different species, as highest isolates from *Quercus robur* (20%) followed by moderate values from *Q. pubescens*, *Q. rubra*, *Q. petraea*, *Acer platanoides*, and *A. pseudoplatanus* (12 - 17%), *Acer campestre* had the lowest number of isolates (9%).⁽¹⁰⁾

Epicoccum sp. were the most prevalent fungi (34%), subsequently common members include *Cladosporium* and *Pestalotiopsis* (26% and 17%, respectively), and few were obtained less frequently like *Fusarium*, *Macrophoma*, *Volutella*, *Penicillium* and *Trichoderma*, isolated from Rice phylloplane.^(16, 38) Predominantly colonizing species, obtained from one most economically important crop, Wheat represented *Cladosporium*, *Aspergillus*, *Alternaria*, *Epicoccum*,⁽³⁹⁾ along with few most common phylloplane genera as *Stemphilum*, *Penicillium*, *Ulocladium*, *Nigrospora*, and *Torula*.⁽⁴⁰⁾

Filamentous fungi isolated from mango leaves comprised mainly hyphomycetes, as on other perennials (*Acer*, *Ilex*, *Malus* species) and annuals (Sugarbeet leaves). *Cladosporium cladosporioides* and *Alternaria alternata* were most prevalent filamentous fungi^(2,9) found in different sampling times, and has been reported for several plant species (*Pinus*, *Halimolobos*, *Barley*, *Canberry*, *Mangifera*, *Sugarbeet*, *Ocimum*, *Phyllanthus*, *Azadirachta*, *Tinospora*). The dominance and persistence of *Aspergillus*, *Cladosporium* and *Alternaria* is attributed to their excellent adaptation on leaf surfaces.^(3,11,18,19) Microbial isolates, *Rhodotorula sp.*, *Saccharomyces sp.*, and *Aspergillus sp.* were more frequent and found uniformly on *Okra* leaves.^(12,14) In *Fagus* plant, common inhabitants of the phylloplane, such as *Cladosporium sphaerospermum*, *Trichoderma harzianum*, *Penicillium brevicompactum*, were also found to occur with high frequencies.^(30, 35)

Phylloplane fungi were isolated from the medicinal plants viz, *Ocimum sanctum*, *Phyllanthus amarus*, *Azadirachta indica* by dilution plating technique, as *Aspergillus flavus*, *Penicillium expansum*, *Fusarium semitectum*, *Fusarium oxysporum* in the phylloplane of *Ocimum sanctum*; *Scopulariopsis sp.* from the phylloplane of *Phyllanthus amarus*; and *Penicillium janthinellum*, *Aspergillus fumigatus*, *Curvularia lunata* and *Fusarium moniliforme* were isolated from *Azadirachta indica*⁽³⁾

Most reports of phylloplane fungi, validates marked dominance of anamorphic fungi, mostly of ascomycetous affinity, only occasional colonies of basidiomycetous and zygomycetous microfungi have been observed.^(18,30,33,34,38)

Conclusions :

Fungi represents broad range from, exclusively- ectophytic occurrence on the leaf, partly- internal and external growth, to exclusively- endophytic life. Phylloplane myco-diversity emphasize on the internal fungal colonizers, without developing any external symptoms. Microbes colonizing the inner plant tissues have beneficial traits, which may contribute to produce a large variety of bioactive compounds that might play a role in biocontrol and plant growth promotion. As discussed in this mini-review, different plant species may represent few new fungal isolates, and many already reported common phylloplane fungi.

Further studies on the isolation and identification of fungi, as well as investigating and documenting their complex ecological roles on aerial leaf surfaces helps to reveal hidden information in partially opened book. Fungi isolated from medicinal and crop plants habitually express novel and important biological activities. Documenting the species composition of phylloplane myco-diversity existing over the world and comparing this information may contribute in understanding the complex interaction and correlation between microbes and many other dependant factors.

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