Plant Pathobiome

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Contributor: Mohamed Mannaa

Although there is an inconsistency in the definition of pathobiome in the literature, it could simply be defined as the set of organisms (i.e., complex eukaryotic, microbial, and viral communities) within the plant's biotic environment, which interact with the host to deteriorate its health status. The recent advances in the multi-omics studies facilitated the understanding of the plant holobiont as an ecological unit with the associated living species. The plant microbiota serves various essential and beneficial roles, while pathogenic microbes can damage the plant tissues through transient blooming under specific conditions. The one pathogen–one disease hypothesis is becoming insufficient to describe the disease process in many cases, particularly when complex organismic communities are involved. Here, we cover the steady transition of plant pathology from the one pathogen–one disease hypothesis to the emerging pathobiome paradigm and review previous reports on model plant diseases in which more than one pathogen or co-operative interactions amongst pathogenic microbes are implicated

Keywords: plant ; plant pathobiome ; plant-microbe-interaction

1. Introduction

The concept of pathobiome originally emerged from research on the human microbiome, which was found to be essential for sustaining human health. The dysbiosis of such a balanced, diverse, and rich community structure is always linked to an unhealthy status, exposing the gut to pathogenic infections and other metabolic disorders. Accordingly, the term pathobiome was coined to describe the overall disease-related microbial community[1]. The concept of pathobiome has also been used to represent the complex pathogenic organisms affecting animals and plants[2]. In general, a healthy plant is closely associated with a stable and diverse community of organisms, representing the biotic factors that support plant growth and serve important functions for the host—which are together described as the symbiome. A collective shift of this ecologically stable symbiome to the pathobiome involves a compositional transition, leading to the disruption of normal growth such that the plants cannot perform functions to the best of their genetic potential[3]. Although there is an inconsistency in the definition of pathobiome in the literature, it could simply be defined as the set of organisms (i.e., complex eukaryotic, microbial, and viral communities) within the plant's biotic environment, which interact with the host to deteriorate its health status[2].

Although the concept of pathobiome is relatively new to plant pathology and have originated following the recent advancements in the multi-omics approaches, several early studies have investigated the occurrence of diseases with multi-species of causal agents and mixed infections which were referred as "disease complex"[4][5]. Several examples from previous studies where a complex of bacterial or fungal species are involved in the disease process, such as the tomato pith necrosis, broccoli soft rot and young grapevine decline where more than one bacterial or fungal species are in synergistic interaction and implicated in the disease development[6][7][8][9].

Recent plant pathological studies have demonstrated that the one pathogen—one disease hypothesis based on the fundamental Koch's postulates is insufficient to describe the disease process in a more holistic and realistic way, particularly when complex communities of organisms are involved[10]. Even when a single pathogenic agent is implicated, other accompanying organisms are likely to mitigate or enhance the pathogenic effects and should thus be considered a part of the disease process[11][12]. Previous studies have reported diverse co-operative interactions amongst pathogenic microbes, which result in the promotion of growth of the involved pathogenic agents and consequent increase in the disease severity.

2. Co-Infections by and Interactions Amongst Pathogenic Agents

In a previous study, a co-operative interaction between the seed-borne pathogenic rice bacterium *Burkholderia glumae*, the causative agent of panicle and seedling blight, and the airborne pathogenic fungus *Fusarium graminearum* was observed; as such, both pathogens positively affected the dispersal and survival of each other, as well as promoted

disease progression[12]. The study demonstrated that both organisms have co-evolved to adapt and maximise the benefits of such a co-existence on rice as a host, even though one is seed-borne and the other is airborne. *Fusarium graminearum* produced abundant spores and toxins and was resistant to toxoflavin, the key virulence factor with antifungal activity produced by *Burkholderia glumae*. The colonisation and competitive ability of *Fusarium graminearum* were also promoted by *Burkholderia glumae*, as toxoflavin produced by the bacterium suppressed other competitor fungi in favour of the allied attacker *Fusarium graminearum*. Meanwhile, *Burkholderia glumae* could physically attach to the *Fusarium graminearum* conidia, which offered protection from UV-induced damage and facilitated spread through aerial dispersal[12]. Previous studies have also reported the beneficial interaction and close association between *Burkholderia* sp. And other fungi, as well as the ability of the bacterium to establish a close association with the fungus to utilise fungal-secreted metabolites for its own benefit[13].

Another example of the evolution of this unique tripartite (bacteria–fungi–plant) system for making an allied effort to cause disease is the bacterial endosymbionts of plant pathogenic fungi[14]. For over two decades, the zygomycete fungus *Rhizopus microsporus* was thought to be the sole causative agent of rice seedling blight and producer of the major virulence factor rhizotoxin, which effectively binds to the β-tubulin of eukaryotic cells and inhibits mitosis in the roots of rice seedlings[15][16]. Intriguingly, rhizotoxin was then confirmed to be biosynthesised by a bacterium residing within the fungal cytosol, proving that it is in fact not a fungal metabolite[17]. The fungal obligate endosymbiont *Mycetohabitans rhizoxinica* (formerly known as *Burkholderia rhizoxinica*) was isolated from the fungus, and the rhizotoxin biosynthesis gene cluster was characterised, confirming its inevitable role in causing the disease and establishing the bacterium as another etiological agent along with its host fungus *Rhizopus microsporus*[16][17]. The host fungus metabolism and vegetative reproduction were strictly dependent on the endosymbiotic bacterium, which also provided chemical weapons, and in turn, received shelter and nutrients from the fungus[18]. Moreover, the endosymbiont-free fungus, treated with antibiotics, was unable to sporulate. This dependence ensures a strong, unbreakable alliance and continued co-existence of both organisms as a phytopathogenic unit[18][19].

Plant pathogens could also co-operate by suppressing plant innate immunity, paving the way for subsequent secondary infections by other pathogens to which the plants are naturally resistant, and plants would not be infected by them unless the plant defence is initially broken by the first striker. The biotrophic oomycete *Albugo candida* causes broad-spectrum suppression of defence in wild and domesticated crucifer hosts (i.e., *Arabidopsis thaliana* and *Brassica juncea*), resulting in enhanced susceptibility to infection by several pathogens causing downy or powdery mildew[20]. In a comprehensive study investigating these suppressive mechanisms, *Albugo* spp. were found to suppress plant defence in *Arabidopsis thaliana* through physiological changes in resistance-related tryptophan-derived secondary metabolite biosynthesis and salicylic acid-mediated plant defence to facilitate infection and complete colonisation of *Phytophthora infestans*[21].

Plant pathogens sharing the host plants may also interact in an antagonistic way and compete for colonisation of the affected tissues. Three genetically related pathogenic *Burkholderia* species, namely, *Burkholderia glumae*, *Burkholderia gladioli*, and *Burkholderia plantarii*, causing rice seedling and panicle blight have been reported to co-exist on rice plants, and their interactions have been previously studied. *Burkholderia gladioli* exerted a strong antagonistic activity against both *Burkholderia glumae* and *Burkholderia plantarii*, as demonstrated by in vitro experiments and *in planta* assays[22]. Consistent with these results, the antagonistic activity of *Burkholderia gladioli* against other bacterial species, including the related rice pathogenic *Burkholderia* species, was reported in several other studies[23][24][25]. Hence, pathogenic organisms do not always interact in a co-operative manner to promote the disease and can also interact in an antagonistic manner. Examples of the different types of interactions among plant pathogenic fungal and bacterial species are summarised in Table 1.

Table 1. Examples of the different types of interactions among plant pathogenic species.

Interacting Species	Host Plant and Disease	Type of Interaction
Burkholderia glumae ↔ *Fusarium graminearum*	Rice panicle and seedling blight—*Fusarium* head blight	Bacteria-fungi co-operative interaction
Mycetohabitans rhizoxinica ↔ *Rhizopus microsporus*	Rice seedling blight	Bacteria-fungi endosymbiotic mutualism

Albugo candida ↔ *Phytophthora infestans*	Crucifers, downy or powdery mildew and *Phytophthora* blight	Fungi-fungi co-operative interaction
Burkholderia gladioli ↔ *Burkholderia glumae* and *Burkholderia plantarii*	Rice panicle and seedling blight	Bacteria-bacteria antagonism

In this light, understanding the pathobiome requires a deeper knowledge of the types of organisms involved, the influence they have on one another, the survival and transmission of the pathogens, and the biotic and abiotic factors that may affect the pathobiome and pathogenesis[26]. These points represent a research challenge for ongoing and future studies. Nonetheless, a transition is evident from the classic disease triangle comprising the pathogen, host, and environment to a more realistic disease pyramid in which pathogens within their community are considered as the pathobiome, the host is supported by its symbiome, and time is added as the fourth dimension representing dynamics of the other factors (Figure 1).

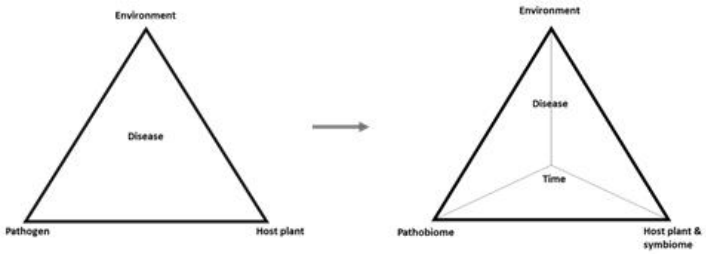

Figure 1. Plant pathology is currently witnessing a steady transition from the classical three-dimensional disease triangle to a more holistic view to explain plant disease through a four-dimensional disease pyramid, including time as the fourth dimension, representing the dynamics of the factors involved, and the plants are supported by the symbiome (beneficial organisms), reflecting a paradigm shift from the one pathogen–one disease hypothesis to the pathobiome concept.

3. Development and Assembly of the Pathobiome

Microorganisms co-evolve within the context of their community as a composite of many species within the boundaries of ecological factors, which shape their microenvironment and control the direction of their evolution[27]. This concept was established early in microbial ecology, as the famous and frequently cited quote of Baas Becking L., stating that ‘Everything is everywhere, but, the environment selects’, which summarises the environment-dependent mechanisms of community assembly[28]. This is also true for pathogenic microbes, which are affected by the biotic and abiotic factors of their host plants, vectors, and surrounding microbes, all of which exert selective pressures. Such selective pressures drive the development of the pathobiome and evolution of adaptive mechanisms of pathogens to overcome the host defence; to enable dispersal inside and outside the host; and to facilitate vector adaptability, antagonism, and mutualism with the surrounding microbes[26].

An example of the co-evolution of mutualistic adaptative mechanisms is the bacterial endosymbionts of plant pathogenic fungi. The endosymbiont *Mycetohabitans rhizoxinica* undergoes genomic alteration by rearrangement and deletion of genomic information for adaptation to diverse habitats, and a large part of its horizontally acquired coding region is related to the biosynthesis of the virulence factor rhizoxin and harbours other genes involved in metabolic adaptation to intercellular life[19][29]. This interaction shifts from initial parasitism, where the bacterium is infectious to the fungus, to mutualism, where both benefit and successfully cause the disease[30]. The strong mutualism is maintained by endosymbiont-dependent host reproduction as a kind of treaty between the two partners, in which the bacterium provides a chemical weapon, rhizotoxin, to the fungus and the fungus, in turn, provides a powerful dispersal tool for the bacterium via its spores[19][30].

Evolutionary processes, such as gene flow and mutations are responsible for the emergence of novel genetic variants, representing the pathogenic agents equipped with weapons for survival and virulence to facilitate host colonisation and pathogenicity. These virulence-related weapons, such as different types of secretion systems and the ability to produce phytotoxins, virulence-related enzymes, and exopolysaccharides, differentiate the pathobiome from other commensal or symbiotic microbes[31]. Even within the same group of bacteria, the presence or absence of genetic material responsible for pathogenicity can differentiate pathogenic from beneficial plant-associated members. The bacterial group *Burkholderia sensu lato* is a powerful example in which genetically close bacterial species could be pathogenic or beneficial based on the presence of evolutionarily driven changes in their genetic material, such as the presence of pathogenicity-related

genomic islands in the phytopathogenic members[29]. These genomic islands are foreign DNA regions that can be horizontally transferred—an evolutionary mechanism amongst bacteria—and integrated into pathogenic variants, endowing them with several specific accessory functions[32]. In addition, the diseased tissues with altered characteristics are ideal for colonisation by specific microbes associated with the disease. Although such microbes do not initiate pathogenesis, they can facilitate the development of symptoms of even a pre-existing condition[33]. These mechanisms shape the microbial and genetic composition along with the functional capacity of the microbial communities, including the pathobiome assemblage[26].

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