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ENVIRONMENTAL MICROBIOLOGY - REVIEW

The unexplored bacterial lifestyle on leaf surface

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Abstract
Social interactions impact microbial communities and these relationships are mediated by small molecules. The chemical ecology of bacteria on the phylloplane environment is still little explored. The harsh environmental conditions found on leaf surface require high metabolic performances of the bacteria in order to survive. That is interesting both for scientific fields of prospecting natural molecules and for the ecological studies. Important queries about the bacterial lifestyle on leaf surface remain not fully comprehended. Does the hostility of the environment increase the populations' cellular altruism by the production of molecules, which can benefit the whole community? Or does the reverse occur and the production of molecules related to competition between species is increased? Does the phylogenetic distance between the bacterial populations influence the chemical profile during social interactions? Do phylogenetically related bacteria tend to cooperate more than the distant ones? The phylloplane contains high levels of yet uncultivated microorganisms, and understanding the molecular basis of the social networks on this habitat is crucial to gain new insights on the ecology of the mysterious community members due to interspecies molecular dependence. Here, we review and discuss what is known about bacterial social interactions and their chemical lifestyle on leaf surface.

Keywords Leaf surface · Microbial community · Social interaction · Chemical ecology

Introduction

In the social context, ecological relationships are shaped by the behaviour of an organism in response to an interaction with another organism, which is strongly influenced by the environmental conditions in which they are found [1]. Microorganisms in a community are linked in a social network that can vary regarding strength and type, and this dynamic affects the ecology and evolution of species [1]. So, a fundamental question in the ecological studies is how different organisms live together in nature [2]. Over the years, microbial interactions were surveyed by different approaches like by the direct interactions between two cultivable microorganisms [3], or between mixed populations in a microbial consortia [4, 5], and also by using computational models [6] and game theories [7].

The ecological social studies argue when organisms should cooperate or when they should be selfish when interacting with other organisms [8]. Why should an individual cell carry out a costly cooperative behaviour for the benefit of all the community? [8, 9]. This answer is more complex than the simple perspective that cooperation can increase the populations' fitness, mainly because individuals die and reproduce way faster than populations [8]. Because cooperation among individuals affects natural selection, understanding the

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evolutionary origins and maintenance of cooperation is a primary theme in biological research [10].

In the last years, the bacterial community members of the extreme phylloplane habitat were broadly studied by using next-generation sequencing [11–13], but a considerable part of the microorganisms that thrives on phylloplane still remains uncultivated in commonly used media and culture conditions compared with other natural environments [13–16]. That is especially true when considering the number of plant species in the world, estimated to be 374,000 [17], and only a portion of these plants had their epiphytic bacterial community studied [18]. The phylloplane of tropical forest trees remains largely unknown despite the rainforests being regarded as the climax of biodiversity [13, 19]. Only the Brazilian Atlantic forest can harbour between 2 and 13 million undescribed epiphytic bacterial species [13, 16]. Thus, identity and social interactions, as well as the metabolic potential of epiphytic organisms, are not fully understood [20], and this dynamic environment can reveal enormous genetic and metabolic microbial diversity [13, 21].

Few studies paid attention to the chemical potential of the epiphytic bacteria [16, 18, 21–24] and to their social interactions [21, 25, 26]; and most of those investigations focused on interactions with the intent to control plant diseases [27, 28], or frost injury [29] or within bacteria–host interactions [30, 31]. Much less understood are the non-pathogenic microorganisms that inhabit the phylloplane and their chemical potential [16, 20, 21]. Even reviews about interactions among microorganisms paid little or no attention to the chemical potential of the epiphytic bacterial populations [32].

The power of small molecules in the microbial world is great [33], and the most important challenge for the ecological studies on the phylloplane habitat is to understand the metabolic networks between epiphytic individuals and the types of interactions that structure the communities. Here, we review and discuss the recent studies about the chemical ecology of the epiphytic bacteria, which may help unveil the chemical lifestyle on leaf surface.

Bacterial assembly on the phylloplane habitat

Healthy plants in nature live in association with a multitude of microorganisms of several microbial types, such as bacteria, archaea, fungi, and oomycetes, collectively called the plant microbiota [34]. The phyllosphere comprises the areal part of plants while the phylloplane is the surface of the leaves and the microorganisms that thrive on this environment are called epiphytes [11].

Many microorganisms can be associated with the phylloplane as transients and residents, but the environmental conditions select few groups that persist as true epiphytic populations [35]. Bacteria are the dominant microorganisms on the phylloplane [11, 12, 36] and, until now, the most identified bacterial groups are from the phyla Proteobacteria, Bacteroidetes, and Actinobacteria; and among the classes, Alphaproteobacteria and Gammaproteobacteria are dominant [11, 12, 37].

To define a source for bacterial assemblages on the phylloplane is difficult because microbiota members can originate from rainwater, plant dispersal vectors [38], aerosols, animals [39], amd soil, and even by upward migration from the roots [34]. The most colonized spaces on the leaves by bacteria are the grooves, trichomes, vein cells [25], and regions near the stomata [40]. Site [26], plant species [40], soluble carbohydrates, calcium, phenolic compounds [41], and the plant genotype [42] are also important determinants of bacterial community composition on the phylloplane.

Knowledge about the mechanisms and compounds involved in interactions between microorganisms from the plant microbiome are essential for practical use in biological control programs and in the biotechnological aspects for natural molecule prospection [15]. Previous studies showed that many epiphytic bacteria establish benign commensal associations with contributions to the health of the ecosystem and the host plant [28, 43].

Epiphytic bacteria present potential to be used as bioinoculants for sustainable cultivation and biological control [44, 45]; they are metabolically capable of degrading phenols and then could potentially contribute to the natural attenuation of organic air pollutants [46]. They are capable of fixing atmospheric nitrogen, thus providing significant nitrogen input into ecosystems [47], and microbial interactions on the phylloplane can increase plant performance under herbivore biotic stresses [28].

The characteristics of the phyllosphere environment are good indicators that its inhabitants might have some metabolic features that could be applied in a biotechnological framework. For instance, they could be great agents of biological control [48]; however, little is known about their full biotechnological potential. Also, epiphytic microorganisms should be more investigated regarding their potential to the production of exopolysaccharides [49], of antibiotics [50], and pigmentation [51]. These characteristics are important adaptations for the epiphytic lifestyle since they are related to attachment/biofilm [9], communication/amensalism [50], and ultraviolet resistance (respectively) [51].

The phylloplane as a harsh habitat for microbial life

Extreme conditions are in the eye of the beholder and harsh environments are those that make metabolism difficult to function [52]; and as life is governed by organic chemistry, such chemistry must be allowed to operate [52]. The low and heterogenic levels of nutrient and moisture combined with the incidence of high levels of ultraviolet radiation and the oxygen exposure make the atmosphere a severe environmental aspect for microbial life [53] and that cause enormous stresses to microorganism's survival [54]. Besides that the aerobic metabolism is far more efficient than the anaerobic [33], the exploitation of oxygen metabolism has its costs; thus, all aerobic organisms can be considered extremophiles [52]. Reactive oxygen is a threat and oxidative damage resulting from the reduced forms of molecular oxygen, especially the hydroxyl radical, is extremely serious. Oxidative damage has been implicated in an array of health problems in humans and has a range of consequences in nature [52]. It is known that reactive forms of the oxygen can be toxic at elevated concentrations for a variety of cell types [55].

Leaves are the dominant aerial plant structure, with an estimated global area greater than the land surface [11, 12]; and because they have a relatively brief lifespan [12], the phylloplane ecosystem is highly dynamic and the microorganisms that colonize this habitat are exposed to cyclic and noncyclic environmental variables such as atmosphere exposure, atmospheric pollutants [56], wind and rain [14], low or fluctuating water availability, desiccation [57], ice [29], a scarce and heterogenic nutrient condition [37, 58], and the presence of antimicrobial secondary metabolites of plant [30, 59], and in dynamic coastal ecosystems like mangroves they are also exposed to salinity [60].

Leaves are the main photosynthetic organs of plants; therefore, their conformation and positioning allow optimal capture of solar energy [61, 62]. The ultraviolet radiation on phylloplane can reach temperatures of 40–55 °C under intense sunlight [63]. This direct exposition to ultraviolet radiations influences the diversity of epiphytic communities with increase in the UV-tolerant groups or a decrease in the non-tolerant ones [56, 62, 64]. Pigmentation and DNA repair are the two most well-known mechanisms for UV resistance [64].

The nutrients available on phylloplane are composed of sugars [37, 65], amino acids, organic acids, alcohols [59], mineral trace elements, vitamins, hormones [14], and chloromethane gas [66], as well as antimicrobial compounds [12, 14, 41]. These molecules can originate from the plant itself [59] and also from soil particles, dust, solutes in rainwater, dead microorganisms, bird and insect excrement, and pollen [39]. But the phylloplane cannot be described as a nutrient-rich environment because all these compounds can be easily removed from leaves either by leaching or other environmental actions such as fog and dew [14]. Epiphytic bacteria are mainly found in aggregates [12] and they are capable of growing on low nutrient concentrations but they preferentially grow on high-nutrient conditions [57].

Because of all these dynamic and harsh conditions on the phylloplane habitat (Fig. 1), epiphytic bacteria present mechanisms to mitigate the environmental adversities by means of the syntheses of proteins to deal with environmental stresses [20, 57, 67]; by the production of biosurfactants that benefit the bacteria by both attracting moisture and facilitating access to nutrients [68]; and by the production of pigments that confer UV tolerance and give the bacteria the ability to maintain their population sizes [62, 69].

The unexplored face of the chemical interactions among epiphytic populations of bacteria

The capacity to perceive neighbouring cells and answer environmental stimuli is contained in the bacterial genome [6, 70]. This ability is important when microorganisms are found in their natural habitats, which requires a huge number of genes that act as signalization systems and help them interpret the environmental conditions and the presence of competing species [70]. The soluble and volatile secondary metabolites are mainly mediators of antagonistic and synergistic relationships between microorganisms [5, 71, 72]. The molecules produced by one species can serve as nutrients or cause damage to others [33]. But not every molecule that affects the behaviour can be considered a true signal from the social aspect; for that, they must have consequences in the fitness of the sender and of the receiver [10].

Besides their importance in microbial interactions, biochemically diverse compounds have a wealth of different bioactivities, many of which have been exploited as drugs in human and veterinary medicine [21]. But the production of siderophores [73], quorum sensing-related molecules [74], quorum quenching enzymes [75], peptides [21], exopolysaccharide production [76], biofilms' formation [77], and antibiotic production [78] are some of the various ways in which bacteria can interact by means of the secondary metabolism. Antibiotics are the most known examples, and they form only a part, perhaps a smaller part, of the possible bioactive metabolites of microorganisms; they represent only the top of the iceberg [72].

The social interactions in the bacterial communities can alter the production of secondary metabolites [5]. The environmental aspect is the main trigger of cooperation and competition among species [32] and most of the secondary metabolites are silent under laboratory conditions [79]. It is the phylloplane environment that determines the morphological and primary metabolic properties of the epiphytic communities [21, 80], and these microorganisms have various lifestyles and modes of interactions [81]. In the harsh conditions of the phylloplane, the movements of bacteria are restricted and they only perceive signals such as sugar, amino acids, and volatiles that diffuse in the surrounding environment [82].

Competition for space and nutrient resources, production of antibiotics, and interference with cell signalling systems in

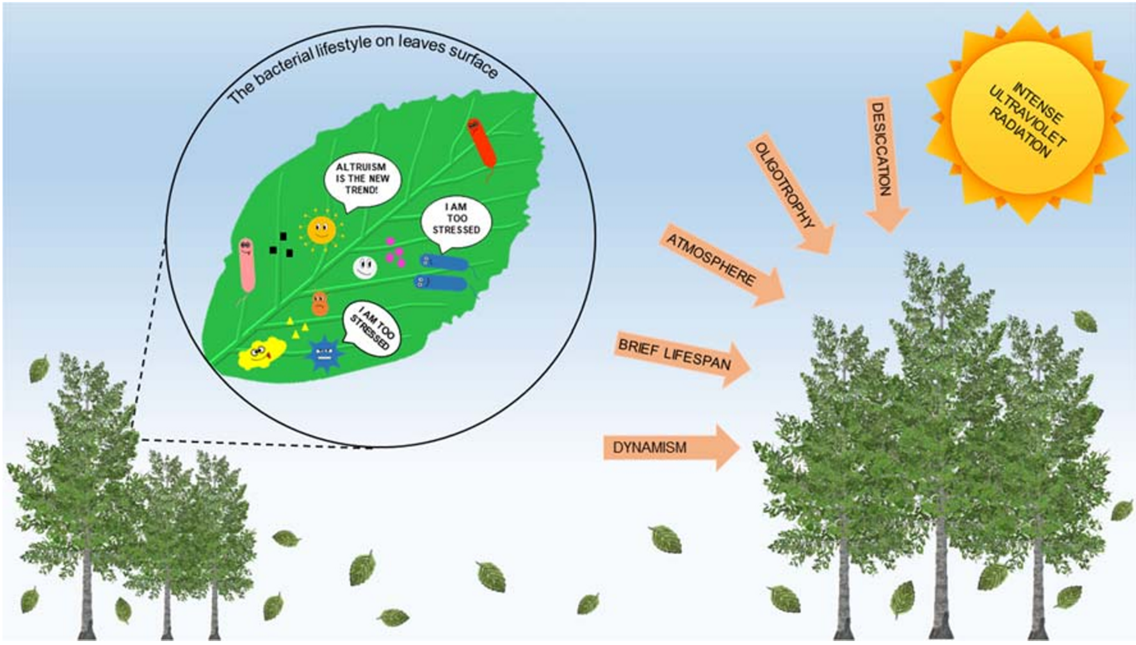

Fig. 1 The dynamic and harsh phylloplane habitat

microbial communities are the main mechanisms by which epiphytic bacteria interact [21, 23, 83]. A study that evaluated competitiveness of diverse *Methylobacterium* strains on the phylloplane of *Arabidopsis thaliana* showed that epiphytic bacteria are actively interacting during growth in mixed cultures, and that they have distinct metabolites strategies to explore the nutrients in the milieu, which enable them to compete successfully with each other and coexist [57]. From a biological perspective, this harsh environment of the phylloplane as a poor-nutrient condition might have selected for highly competitive species engaged in chemical warfare [21], but it also could be a great place to favour cooperative strategies and altruistic behaviours.

Epiphytic communities are important for the metabolic function of plants [16, 80, 81] and can help plant health [84]. Also, the plant immune system responds to epiphytic bacterial molecules and shapes their response according to the mixture of molecules present [85]. And also, some epiphytic isolates have the expression of gallate decarboxylase that presents antifungal activity [80]. Epiphytic bacteria are capable of detoxifying secondary metabolites of plant origins and the resulting molecules can present allelochemical roles against another phylloplane competing species [22]. And besides presenting antifungal activity [86], epiphytic bacteria also present proteolytic activity [87] and siderophore production [88]. The complex phylloplane environment requires unique adaptations for microbial survival, and that impacts their interactions with each other and also with their hosts [89]. The production of proteins related with methanol utilization and stress responses was most prominent on the phylloplane than in normally medium culture conditions [67].

A study that searched antimicrobial activity among epiphytic bacteria from four different plant species showed that 26% of the strains had good antimicrobial activities against one or more tested pathogen [18]. In a bioprospecting study with isolates from phylloplane and rhizosphere, the greater number of antagonistic bacteria against the phytopathogen *Rhizoctonia solani* was found on the phylloplane [54]. But, in general, in this same experiment, epiphytic bacteria produced fewer antimicrobial compounds than organisms from rhizosphere and the authors concluded that this could be due to the enormous stresses that they suffer in these harsh environmental conditions. In fact, a study with 224 strains of epiphytic bacteria from *Arabidopsis* leaf microbiome showed that among over 50,000 combinations of pairings interactions only 1.4% were inhibitory [21], which may suggest that the phylloplane habitat may induce more cooperation from epiphytic populations to survive than antagonism.

The discovery of bacterial communication by means of diffusible signal molecules known as quorum sensing [74, 90, 91] revolutionized the way scientists see bacterial populations (for a review of this theme, see [92]). Although not being required for all cooperative interactions, communication among neighbouring individuals is considered a fundamental mechanism to coordinate cooperative strategies [10]. Epiphytic bacterial populations live in aggregates on leaf surfaces [11, 16], and then the phenomenon of quorum sensing which affects the multicellular behaviour in a community gains importance [93]. In a study with bacteria isolated from the phylloplane of wheat heads, about 33% of the strains showed the production of quorum sensing-related molecules [94]. These quorum sensing molecules may affect polysaccharide production, and both polysaccharides and quorum sensing molecules may be involved in the survival and growth of bacteria on leaf surface [81].

The process that disturbs quorum sensing is named quorum quenching, which often involves enzymes [33, 75]. This is a natural mechanim by which quorum sensing producers recycle or clear their own signals or as a competitive action of quorum quenching organisms against quorum sensing producers [75]. A study with epiphytic bacteria from tobacco leaves showed that 14% of the isolated species presented production of quorum quenching molecules, with higher values on the phylloplane than in soil and rhizosphere [83]. And these values can be even higher when considering the large amount of yet uncultivated bacteria existing on leaf surface [13, 16, 83]. The authors concluded that quorum quenching could be a strategy for bacteria to survive on the phylloplane where they can acquire the nutrients via signal interference degrading quorum sensing molecules as an energy source.

In a metaproteomic study of the phylloplane of four plant species from Atlantic forest in Brazil, a total of 4413 peptide mass spectra did not have significant matches in the chemical databases, and those molecules may represent proteins from yet unknown microorganisms [16]. The most abundant proteins found in this study were from the glycolytic pathway, anaerobic carbohydrate metabolism, solute transport, protein metabolism, cell motility, stress and antioxidant responses, nitrogen metabolism, and iron homeostasis. In this work, the authors concluded that the protein profiles of microorganisms from the phylloplane may depend on the plant taxon and of the environmental conditions; and that epiphytic bacteria sampled from phylogenetically divergent hosts with similar functional niches have resembling core proteins necessary for survival, growth, and maintenance of biofilms on leaf surface [16].

A robust and recent study of binary interactions with more than 200 bacteria isolated from the phylloplane of *Arabidopsis thaliana* showed that 196 strains (88%) engaged in inhibitory interactions and that epiphytic bacteria tend to inhibit distinct phylogenetic groups rather than closely related strains [21]. The most frequent molecules ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by this synthetic community are of the families bacteriocins, lanthipeptides, lassopeptides, microviridins, linaridins, thiopeptides, thiopeptide-linaridin hybrids, and lantipeptide-proteusin hybrids. The results of the chemical ecology from this study indicated a broad structural diversity of ribosomally encoded peptides from epiphytic bacteria [21].

Conclusions and perspectives

The molecular strategies to survive in harsh environmental conditions are not fully comprehended, but it is known that microorganisms from severe habitats have developed interesting biomolecules and biochemical pathways for biotechnological purposes [95].

The scientific works mentioned above showed how metabolically rich the bacterial populations from phylloplane are, and that this habitat represents a promising and unique source for the isolation and discovery of bacterial natural products with a large and distinct biosynthetic repertoire, with unprecedented scaffolds [21]. Thus, the investigations of the chemical ecology on the little explored phylloplane environment have the potential to contribute with researches to both fields of social ecology and in the bioprospecting of compounds [21].

Although the molecular approach has increased the knowledge about the diversity of the microorganisms that thrive on the phylloplane in the last years [12], little is known about the chemical ecology of the epiphytic bacterial communities and much more on phylogenetic and metabolic diversity still needs to be discovered [13, 16]. Important aspects of microbial communities' ecology and structure cannot be inferred by genomic techniques alone. It is of great importance to have a holistic view of how microbial populations interact directly or indirectly, instead of considering the study of isolated groups [96].

Recent advances in metabolomics technologies imaging mass spectrometry, secondary ion mass spectrometry, stable isotope probing, nanospray desorption electrospray ionization (NanoDESI), Global Natural Products Social (GNPS) molecular networking project, and many other chemometric approaches have been helping scientists visualize the chemical world of microorganisms even directly from environmental samples [97–101].

Therefore, scientists around the world should look to the phylloplane environment as a great model for the exploration of the social interactions and the chemical ecology among epiphytic bacterial populations to gain insights into the social behaviours of the already cultured organisms and also possibly to improve the knowledge into the ecology of the mysterious community members of this habitat that we still do not know.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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