Doi: http://doi.org/10.17584/rcch.2019v13i3.10503

# The phyllosphere microbiome and its potential application in horticultural crops. A review

# El microbioma de la filosfera y su aplicación potencial en la horticultura. Una breve revisión

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**Phyllosphere, host, plant surface.**

Photo: S.E. Barrera

## ABSTRACT

Microorganisms are essential for life on Earth. They are found in different environments and conditions, such as pH, temperature, pressure, and humidity, etc. In natural and agricultural ecosystems, nutrient cycling and plant protection are important roles played by microorganisms associated with plant species. However, the mechanisms to colonize those environments are not fully understood. This mini-review describes bacterial communities associated with the phyllosphere and an agricultural approach for potential applications. In the context of foodborne illnesses and losses in agricultural production, important issues have arisen because of pathogen attacks. On the other hand, the use of beneficial microorganisms in agriculture is an alternative for improving plant growth, health and production. In this sense, growth promoting bacteria and biocontrol agents isolated from the phyllosphere of several plant species have been less exploited than those from the soil or rhizosphere. However, the treatment of some plant diseases, reduction in pathogen incidence and nitrogen fixation in natural and agricultural systems are successful examples. In the context of food safety, a better understanding of how the indigenous phyllosphere microbiota enable plants to protect themselves against pathogens and to acquire nutrients is expected to prove its importance in the agricultural field. Microbial sources can be managed to reduce the use of chemical products and could be used as an alternative of agronomical applications for improving agroecosystem productivity.

**Additional key words:** epiphytic community; plant health and growth; ecosystem productivity; biocontrol.

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## RESUMEN

Los microorganismos son esenciales para la vida en la tierra. Ellos se encuentran colonizando diferentes ambientes y en diferentes condiciones de pH, temperatura, humedad, etc. En ecosistemas naturales y agrícolas, el ciclado de nutrientes y la protección de la planta, son funciones importantes desempeñadas por los microorganismos asociados a las especies vegetales. Sin embargo, los mecanismos para colonizar esos ambientes no son completamente entendidos. En esta corta revisión se describen las comunidades bacterianas asociadas a la filosfera, con un enfoque agrícola de sus aplicaciones potenciales en esta área, relacionadas con nutrición y control biológico. En el contexto de alimentos contaminados y pérdidas en la producción agrícola, han surgido graves problemas debido al ataque de patógenos. Por otra parte, es claro que el uso de microorganismos benéficos en la agricultura es considerado como alternativa para mejorar el crecimiento, la producción y la salud de la planta. En este sentido, bacterias promotoras de crecimiento vegetal y agentes de biocontrol, aislados de la filosfera de diferentes especies vegetales han sido menos explotados que los microorganismos de la rizosfera. No obstante, el tratamiento de enfermedades, reducción de la incidencia de patógenos y la fijación de nitrógeno, en sistemas naturales y agrícolas, son ejemplos exitosos. En el contexto de seguridad alimentaria, se espera entender mejor cómo la microbiota nativa de la filosfera ayuda a la planta a protegerse contra patógenos y a la adquisición de nutrientes, para demostrar su importancia en el área agrícola. Esto indica que, fuentes microbianas pueden ser usadas para reducir el uso de productos químicos y aplicarlas como una alternativa agronómica para mejorar la productividad de los agroecosistemas.



**Palabras clave adicionales:** comunidad epifítica; crecimiento y salud vegetal; productividad del ecosistema; biocontrol.

Received for publication: 08-09-2018 Accepted for publication: 29-11-2019


## INTRODUCTION

Microorganisms are found in various environments, such as water, air, soil, plant surfaces, animals, food, the human body and buildings (Prussin and Marr, 2015; Rosenberg and Zilber-Rosenberg, 2016). Some of them live in symbiosis with plants or animals, while others have a free-living lifestyle (Dutta and Paul, 2012). Symbiotic associations between plants and soil microorganisms such as arbuscular mycorrhizal fungi (Igiehon and Babalola, 2017), nitrogen-bacteria in legumes (Mus *et al.,* 2016) or the water fern *Azolla* and the cyanobacterium *Anabaena azollae* (Bhuvaneshwari and Singh, 2015) have been amply studied because of the positive outcomes of these associations in natural and agricultural systems. However, microorganisms associated with aerial plant surfaces could have positive effects, as do soil microorganisms, and could be used as sources of inoculum (Andreote *et al.,* 2014).

The phyllosphere is the aerial portion of plants, mainly the leaf surface, which is an environment widely inhabited by bacteria that form biofilms or larger aggregates (Lindow and Brandl, 2003; Baldoto and Olivares, 2008). Biofilm formations, extracellular polymeric substances (EPS) and enzyme production protect the epiphytic microbial community from a stressful environment (Remus-Emsermann and Vorholt, 2014; Müller *et al.,* 2016a). Despite the phyllosphere being low in nutrients, plants release an adequate concentration to support large microbial communities (Mercier and Lindow, 2000), and microbial communities develop mechanisms to acquire nutrients (Delmmote *et al.,* 2009; Bulgarelli *et al.,* 2013).

A core bacterial microbiome composed of Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes phyla has been found in different plant species, in both forests and agricultural ecosystems (Redford *et al.,* 2010; Vorholt, 2012; Rastogi *et al.,* 2012; Bulgarelli *et al.,* 2013; Kembel *et al.,* 2014; Lambais *et al.,* 2014; Laforest-Lapoint *et al.,* 2016, Müller *et al.,* 2016b). However, the bacterial abundance depends on several factors, such as plant species, geographical distance and environmental conditions (Remus-Emsermann and Vorholt, 2014; Copeland *et al.,* 2015; Laforest-Lapoint *et al.,* 2016). This suggests that some small bacterial groups are highly efficient at colonizing and surviving in the phyllosphere (Griffin and Carson, 2015). At the same time, bacterial communities share a core of proteins on different plant hosts, suggesting similar mechanisms for adaptation and survival on different plant species (Remus-Emsermann and Vorholt, 2014; Lambais *et al.,* 2017).

In the context of food safety, a better understanding of how the natural microbiota enables plants to protect themselves against pathogens and/or to acquire nutrients will be valuable in agricultural production (Rastogi *et al.,* 2013). This suggests that microbial sources can be managed as an alternative of agronomical applications to improve the productivity of the agricultural ecosystem (Peñuelas and Terradas, 2014). Likewise, agroecosystems are subject to intense chemical management; therefore, the microbial diversity associated with leaf surfaces can have variations across space, time, season and environmental conditions (Rastogi *et al.,* 2013).

Nowadays, we have to deal with difficult challenges, such as the concern for foodborne illnesses and agricultural production losses from pathogen attacks. In Colombia, a few studies have been done to identify microbial communities associated with the phyllosphere (Toloza and Lizarazo, 2014). These studies have focused mainly on the characterization of pathogenic microorganisms that inhabit the phyllosphere (Restrepo *et al.,* 2000; Marín *et al*., 2003), meanwhile other studies have focused on biological control (Salazar *et al.,* 2006; Medina *et al.,* 2009; Cruz-Martín *et al.,* 2016) and taxonomical profiles of bacterial communities (Ruíz-Pérez *et al.,* 2016). Although there is increasing evidence that beneficial bacteria may stimulate plant growth and health (Vogel *et al*., 2016), microbial community dynamics at the community level and their interactions with the plant host are still unknown (Schlechter *et al*., 2019). This mini-review provides an agricultural approach on the potential applications of microbial communities associated with the phyllosphere in horticulture crops through microbial bioprospecting. First, phyllospere generalities are stated, such as habitat for bacteria and fungi. Second, abiotic and biotic factors affecting the microbial community associated with the phyllosphere are also described. Subsequently, references are made about potential applications in agriculture, focusing on nutrition and biological control. Patent processes found in the Patentscope database from studies on the phyllosphere microbiome are shown. This information shows examples of bioprospecting bacteria with biotechnological potential. Finally, several examples of studies carried out in Colombia on horticultural plants are presented. The information was accessed with keywords such as: phyllosphere, microbiome, agriculture, bioprospecting, biotechnology, microbial communities associated with the phyllosphere, in Google Scholar and Scopus.

## THE PHYLLOSPHERE IS A MICROBIAL HABITAT

The phyllosphere is the portion found in upper and lower leaf surfaces (Fig. 1), covering an area of $640 \cdot 10^8$ $km^2$ on Earth (Lindow and Brandl, 2003; Peñuelas and Terradas, 2014). As well as bacteria, this environment is colonized by other microorganisms such as archaea, filamentous fungi, lichens, bryophytes, yeast and protozoa, all living in limited water and nutrients conditions (Vorholt, 2012; Rastogi *et al*., 2013; Müller and Ruppel, 2014).

The leaf surface is an oligotrophic environment that obligates microorganisms to compete for nutrients and space (Delmotte *et al*., 2009; Bringel and Couée, 2015). Carter *et al*. (2012) suggested that competition for nutrients, essentially carbon and nitrogen, is the first mechanism of interaction among microorganisms that colonize a phyllosphere. Leaf nitrogen content is correlated with the phyllosphere community structure in several plants (Kembel *et al.,* 2014; Kembel and Mueller, 2014; Laforest-Lapoint *et al.,* 2016), while carbohydrates produced by photosynthesis are exudated on the leaf surface along with methanol, volatile organic compounds (Vacher *et al.,* 2016), amino acids, organic acids, inorganic compounds, and various salts (Mercier and Lindow, 2000). Leaf chemical composition and morphology affect the distribution of microorganisms across leaves (Remus-Emsermann and Vorhold*,* 2014). For instance, the leaf cuticle is a substrate composed of polymeric and soluble lipids that is difficult to metabolize by microorganisms, but could be *involved* in phyllosphere colonization (Morris, 2002). Chemical, structural and physiological properties related to a spectrum running from quick to slow return on investments of nutrients and dry mass in leaves, "The leaf economics spectrum" (Wright *et al.,* 2004) could explain the variation in microbial community structures, according to acquisitive or retentive resource strategy (Friesen *et al.,* 2011).

Undoubtedly, bacteria are the most abundant microbial group in the phyllosphere followed by fungi (Lindow and Brandl, 2003). The density of bacteria is from $10^6$ to $10^7$ cells per square centimeter of leaf tissue (Vorholt, 2012; Rastogi *et al.,* 2013). Despite bacteria playing essential roles in nutrient cycling (Vacher *et al.,* 2016), plant protection against pollutants and pesticides (Müller and Ruppel, 2014) and improving plant development (Delmotte *et al.,* 2009), little is known about bacterial diversity and

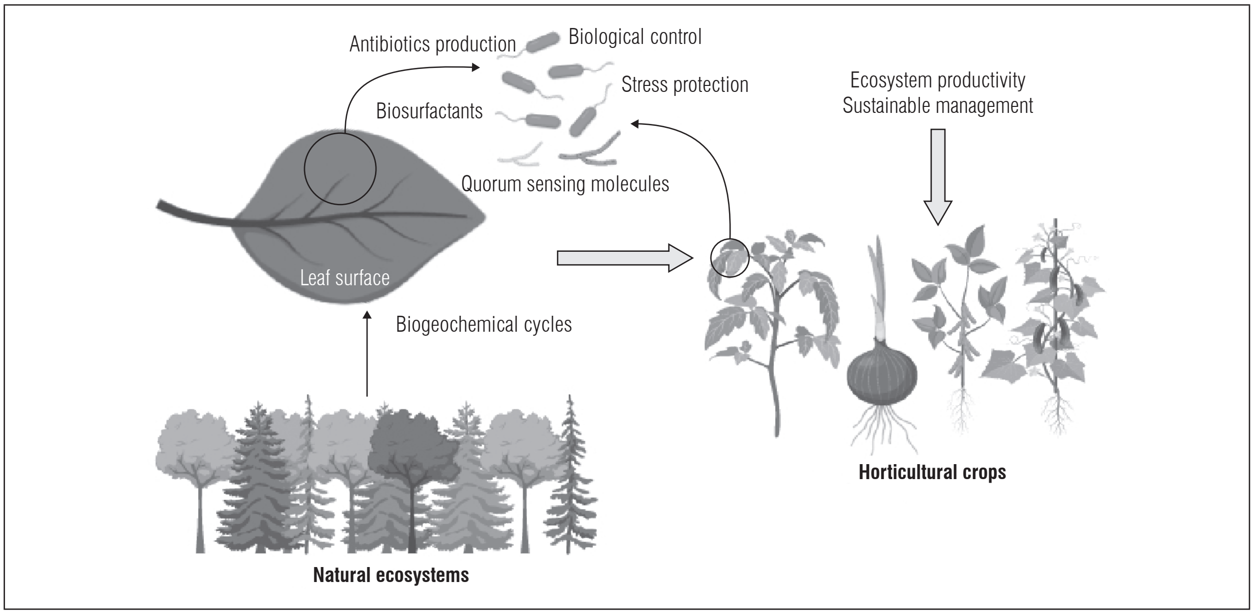


**Figure 1. Scheme of the leaf surface colonized by several bacteria and fungi genera detected in the phyllosphere, along with ecological functions and applications.**

biogeography in this environment since most of the bacteria detected in the phyllosphere have not yet been described (Lambais *et al.,* 2014). Filamentous fungi and yeast communities associated with the phyllosphere are also important components involved in nutrient cycling (Vacher *et al.,* 2016) and plant protection (Arnold *et al.,* 2003). Jumpponen and Jones (2009) suggested that plant exudates are decomposed by epiphytic fungi; however, the fungi colonization in phyllosphere has not been well characterized (Kembel and Mueller, 2014).

The assemblage of microbial communities on the phyllosphere of distinct plant species could be modulated by the interaction of several environmental factors (Andreote *et al.,* 2014). Similarly, interaction between microorganisms and their hosts drives the microbiota assembly (Müller *et al.,* 2016a). On the other hand, colonizing microorganisms of the phyllosphere not only can come from the air but also can come from an early recruitment on seeds, soil or other plants (Knief *et al.,* 2010; Copeland *et al.,* 2015; Lemanceau *et al.,* 2017).

The microbial community in the phyllosphere presents high richness (Kim *et al.,* 2012; Kembel *et al.,* 2014) but low diversity when compared with the rhizosphere (Delmotte *et al.,* 2009; Knief *et al.,* 2012). The classes Alphaproteobacteria and Gammaproteoacteria are the most abundant groups detected in several plant species (Fürnkranz *et al.*, 2008; Kim *et al.,* 2012; Lambais *et al.,* 2006, 2014, 2017). In tropical rainforests, *Sphingomonas* (Alphaproteobacteria) and *Pseudomonas* (Gammaproteobacteria) have been reported as the dominant bacterial genera (Lambais *et al.,* 2006, 2014; Bodenhausen *et al.,* 2013). In several crops such as rice, bean, cucumber, soybean, lettuce maize clover and *Arabidopsis*, *Sphingomonas, Methylobacterium* (Alphaproteobacteria) and *Pseudomonas* (Gammaproteobacteria) are the most abundant genera (Delmotte *et al.,* 2009; Knief *et al.,* 2012; Rastogi *et al.,* 2013; Müller *et al.,* 2016b). The dominance of *Sphingomonas* spp. and *Methylobacterium* spp. can be explained by the presence of carbon resources that they consume (Remus-Emsermann and Vorholt, 2014), while *Pseudomonas* spp. can be explained by its mobility towards nutrient sites on the leaf, with significant advantages over immobile bacteria (Vorholt, 2012). The phototrophic lifestyle, as part of their metabolic requirements, offers bacteria an ecological advantage to survive in the phyllosphere. For instance, the presence of anoxygenic phototrophic bacteria and rhodopsins in clover, *Arabidopsis* and Tamarisk phyllosphere (Atamna-Ismaeel *et al.,* 2012a, 2012b; Finkel *et al.,* 2016).

With respect to fungi phyllosphere community, it has been linked to pathogens, saprotrophs and lichenized fungi (Jumpponen and Jones, 2009). Filamentous fungi and yeast, such as Ascomycota and Basidiomycota, are reported as dominant groups in the phyllosphere (Cordier, 2012). For example, *Hypocrea, Aureobasidium* and *Cryptococcus* genera were reported as dominant in the tomato phyllosphere (Ottesen *et al.,* 2013).

## FACTORS AFFECTING BACTERIAL COMMUNITY IN PHYLLOSPHERE

The phyllosphere is a hostile and complex environment influenced by abiotic and biotic factors, including plant metabolism, UV radiation (Vorholt 2012), temperature (Delmotte *et al.,* 2009), carbohydrate levels (Hunter *et al.,* 2010), elevated $CO_2$ (Ren *et al.,* 2014), plant traits, seasonal leaf changes, position (Copeland *et al.,* 2015; Laforest-Lapoint *et al.,* 2016) and the chemistry of waxy cuticle covering the leaf (Bodenhausen *et al.,* 2014; Remus-Emsermann and Vorholt, 2014). However, how these factors affect the microbial communities in the phyllosphere is not fully understood.

Temperature is greatly variable on leaf surface over time and space (Chelle, 2005), even in the same plant or leaf (Vacher *et al.,* 2016). Changes in phyllosphere communities are related to rainfall events (Copeland *et al.,* 2015). Water films cover leaf surfaces, causing chemical reactions between water and compounds or molecules deposited on the leaves (Vacher *et al.,* 2016), which modify the water pH and affect the nutrient availability for microorganisms (Morris, 2002).

Leaves are exposed to large amounts of sunlight, causing DNA-damage (Remus-Emsermann and Vorholt, 2014); therefore, adaptation to and protection from stressful conditions from UV radiation are related to the detection of DNA protection proteins (Delmotte *et al.,* 2009). Furthermore, microbial communities in the phyllosphere can be highly variable depending on the season (Jackson and Denney, 2011; Williams *et al.,* 2013), while other plants have similar bacterial communities all year round (Redford and Fierer, 2009). Changes in plant metabolism caused by abiotic factors indirect affect the phyllosphere microbiome as well (Turner *et al.,* 2013).

Plant genotype may also drive the assembling of bacterial communities in the phyllosphere (Redford *et al*., 2010; Rastogi *et al*., 2013). Possible mechanisms used by plants to assemble their microbial community have not yet been elucidated, but interaction plant genotype-microbial community (Kim *et al.,* 2012) and identifications of essential genes in the plant and the microorganisms are important factors to manipulate the leaf microbiota and to improve plant protection (Müller and Ruppel, 2014). The selection of different bacterial consortia by plants (Lambais *et al.,* 2006) occurs by compounds exudated on the leaf surfaces (Yadav *et al*., 2011).

For instance, Beattie and Lindow (1999) reported indol-acetic acid and extracellular polysaccharides as compounds produced by phyllosphere bacteria to modify the environment, improving nutrient availability and increasing bacterial community survival on the leaf surface.

Changes in the composition of bacterial communities associated with the phyllosphere might occur because of geographical distance (Bokulich *et al.,* 2014). Redford *et al*. (2010) reported on bacterial communities that were highly similar in plants phylogenetically, suggesting evolutive history between plants and bacteria. However, different plant species from a single location can assemble bacterial communities that are highly similar, as influenced by local conditions (Whipps *et al.,* 2008; Finkel *et al.,* 2011). Furthermore, differences have also been observed in bacterial communities associated with individuals of the same plant species but geographically distant, which indicates that differences could arise from distinct climatic conditions or different leaf traits (Redford and Fierer, 2009; Finkel *et al.,* 2012). The composition and abundance of the microbial community associated with leaf surfaces are not enough to understand the driving factors affecting the phyllosphere microbiome because of leaf surface heterogeneity (Remus-Emsermann and Schlechter, 2018). Therefore, spatial scale analysis could be used to explain how ecological microbial interactions could contribute to the identification of key organisms associated with plant health and function (Berry and Widder, 2014; Poudel *et al.,* 2016).

## POTENTIAL AGRICULTURAL APPLICATIONS

Communication (volatile organic compounds), protection (antibiosis), nutrient cycling (N, C), and plant growth (phytohormones) are essential ecosystem services provided by the phyllosphere microbial communities (Tab. 1) (Morris, 2002; Vacher *et al.,* 2016). Manipulating the foliar microbiome to reduce the use of synthetic pesticides and inorganic fertilizers is a beneficial activity that promotes plant growth and health (Adesemoye and Kloepper, 2009).

**Table 1. Ecosystem functions of microorganisms in the phyllosphere.**

| Microbial function | Plant benefits | Ecosystem service | Source |
|---|---|---|---|
| Nitrogen fixation | Foliar nitrogen content<br>Plant growth | Productivity, nutrient acquisition | Fürnkranz *et al.* (2008) |
| Phytohormones production | Plant growth | Biomass production | Almethyeb *et al*. (2013) |
| Pathogen suppression | Protection, plant health | Productivity | Wei *et al.* (2016) |
| Antimicrobial activity | Protection, plant health | Productivity | Bulgarelli *et al.* (2013) |
| Induced systemic resistance | Protection, plant health | Productivity | Bulgarelli *et al.* (2013) |
| Phylloremediation | Detoxification | Atmospheric depollution | Bringel and Couée (2015) |
| Probiotic agents | Reduction chemical products | Sustainable agriculture | Berlec (2012) |

## Possible nutritional inputs through the phyllosphere

Microbial communities mediate the nutrient exchanges occurring between the phyllosphere and the atmospheric interface (Abril *et al.,* 2005). In tropical forests and agriculture or silviculture systems, foliar diazotrophs contribute to nitrogen fixation from the atmosphere (Abril *et al.,* 2005; Fürnkranz *et al.,* 2008). Proteogenomic analyses of several phyllosphere microbial communities have revealed species that assimilate simple carbohydrates, amino acids and ammonium exudated by plants (Turner *et al.,* 2013). On the other hand, ammonifiers and cellulose-degrading bacteria has been reported in the phyllosphere of woody plants (Abril *et al.,* 2005), and nitrifiers were found in soybean leaves (Arias *et al.,* 1999). A complex metabolic feedback between plants and phyllosphere communities may be occurring (Bringel and Couée, 2015) when the enzymatic activity of the microorganisms in the phyllosphere act as plant metabolites (Huang *et al.,* 2014).

Nitrogen fixation is one of the most studied functions of the foliar microbiota (Abril *et al.,* 2005; Daza *et al*., 2015). *Klebsiella* spp. and *Beijerinckia* spp. are common free-living nitrogen-fixing bacteria found in phyllosphere microbial communities (Morris, 2002). Furthermore, *Beijerinckia* spp. strains have increased rice yield, when compared between a *Beijerinckia* spp.-inoculated field and one with conventional fertilizers (Morris, 2002). Similarly, *Enterobacter radicincitans* has been isolated from the phyllosphere of wheat (Ruppel *et al.,* 2006) and promoted plant growth through nitrogen fixation and phytohormone production when inoculated in the soil (Almethyeb *et al*., 2013). On the other hand, *Klebsiella* spp. and various cyanobacteria were found in the phyllosphere of plants from a tropical forest in Costa Rica, related to high $N_2$ fixation rates (Fürnkranz *et al.,* 2008).

With respect to Actinobacteria class, *Arthrobacter* has been found to inhabit leaf surfaces (Rastogi *et al.,* 2012). This bacteria genus can degrade aromatic hydrocarbons and pesticides (Scheublin and Leveau, 2013) and presents a high resistance to desiccation (Labeda *et al.,* 1976). Thus, it could be a good choice for decreasing contamination by pesticides application (Turnbull *et al.,* 2001).

## Biological control

The use of biological control agents isolated from phyllosphere has been less exploited than those isolated from the soil or roots. However, there are biocontrol agents that have been successful in the treatment of some diseases associated with the phyllosphere (Fernando *et al.,* 2007).

Interactions between phyllosphere bacteria could trigger changes in leaf transcriptome, suggesting molecular recognition by plants (Lemanceau *et al.,* 2017). In this respect, the presence of the pathogen *Xanthomonas campestri* pv. *vitians* in lettuce is positively correlated to the presence of *Alkanindiges,* also reported in lettuce phyllosphere (Hunter *et al.,* 2010), and negatively correlated with *Bacillus, Erwinia* and *Pantoea,* which act as antagonists (Rastogi *et al.,* 2012). *Pseudomonas syringae* is an important pathogen found in the phyllosphere and can be controlled with *Sphingomonas melonis* because of the expression of pathogenesis-related proteins and antimicrobial proteins (Innerebner *et al.,* 2011). Meanwhile, *Pseudomonas fluorescens* A506 reduces fire blight disease in pear and suppresses *Erwinia amylovora* growth in the phyllosphere through competition for nutrients and space (Wilson and Lindow, 1993).

Furthermore, antagonistic activity from bacteria can reduce fungal pathogenicity in the phyllosphere (Griffin and Carson, 2015). For example, *Pseudomonas* spp. and *Bacillus* spp. can produce compounds, inducing systemic resistance responses in several plant species (Vorholt, 2012) and dramatically reducing fungal infection (Ceballos *et al.,* 2012). *Bacillus* spp. are the most used biological control agents in agriculture. They have a broad spectrum of antagonistic activity (Huang *et al.,* 2012), and several strains have been used as biocontrol agents in cacao (Melnick *et al.,* 2008; Villamil *et al.,* 2015), sugar beet (Collins *et al.,* 2003), citrus (Huang *et al.,* 2012), strawberry (Wei *et al.,* 2016), cotton, rice and amaranth leaves (Wang *et al.,* 2014). The ability to form endospore, produce secondary metabolites, proteins (Zhang *et al.,* 2008), and antibiotics (Raaijmakers *et al.,* 2002) and induce systemic resistance (Lahlali *et al.,* 2013) has made *Bacillius* a widely used biocontrol agent for phyllosphere pathogens (Wei *et al.,* 2016).

Recently, the use of native microorganisms as biocontrol agents has attracted special interest because of their special attributes (Kumar and Gopal, 2015; Cruz-Martín *et al*., 2016). They are adapted and established to local abiotic conditions or hosts and play a protecting role in the host plant against foreign pathogen microorganisms (Kumar and Gopal, 2015). For instance, representatives of the Bacillaceae family isolated from *Mussa* spp. phyllosphere showed antifungical activity against black Sigatoka disease (*Mycosphaerella fijiensis*) (Poveda *et al.,* 2010; Cruz-Martín *et al.,* 2016). Also, in *Mussa* spp., Salazar *et al*. (2006) found chitinolytic and glucanolytic activity against *M. fijiensis* by native bacteria.

On the other hand, changes in nutrient allocation towards leaf surfaces can manipulate the phyllosphere microbial community as a protection mechanism against pathogens (Manching *et al.,* 2014). In crops, specific bacterial communities colonizing the phyllosphere have an important role protecting plants against pathogens (Williams *et al.,* 2013; Manching *et al.,* 2014). For instance, rice (Ren *et al.,* 2014) and lettuce (Williams *et al.,* 2013) seem to benefit from the Enterobacteriaceae group, increasing biomass production through pathogen suppression (Pusey *et al.,* 2011) and nitrogen fixation (Feng *et al.,* 2003).

Several studies on the phyllosphere microbiome have revealed a large number of novelties, which could be important for maintaining agriculture sustainability (Gupta and Bhargava, 2018), and more institutions are using their resources for the search for new products based on microorganisms. Patent processes found in the Patentscope database attached to WIPO-World Intellectual Property confirm that products based on microorganisms isolated from the phyllosphere can be used in agriculture.

a) Use of lactic acid bacteria associated with the phyllosphere as biocontrol agents to reduce the growth of pathogenic bacteria (McGarvey *et al.,* 2017),
b) Microbial consortium for agricultural use and formulation (Suárez, *et al.,* 2019),
c) *Enterobacter* sp. 3bh19 for preventing downy mildew. Application in cucumber phyllosphere (Luo *et al.,* 2017),
d) Bacteria isolated from the phyllosphere promoting plant growth (Bai *et al.,* 2014),
e) Novel *Methylobacterium* sp. CBMB 27 having an effect of promoting plant growth (Sa *et al.,* 2012),
f) Bacteria degrading pyrethroid insecticide and method for preparing fungicide (Bai *et al.,* 2008b),
h) Screening method for thermophilic bacteria isolated from the phyllosphere (Bai *et al.,* 2008a).

## STUDIES OF PHYLLOSPHERE COMMUNITIES IN COLOMBIA

Pathogenic microorganisms inhabit the phyllosphere of two important crops in Colombia: *Xanthomonas axonopodis* pv. *Manihotis* and *M. fijiensis,* are causal agents of bacterial blight in cassava (*Manihot esculenta*) (Restrepo *et al.,* 2000) and black sigatoka in banana (*Musa* spp.) (Marín *et al*., 2003), respectively.

On the other hand, several examples of biological controls are known in Colombia. In Cundinamarca, *Candida kunwinensis* and *Rhodotorula colostri* were isolated from blackberry crops (*Rubus glaucus*), which presented antagonist activity against *Botritys cinerea* (Medina *et al.,* 2009). In another study in Uraba, bacterial strains with antagonist activity against *M. fijiensis* were isolated from leaves of *Mussa* spp. They were identified as *Bacillus subtibilis* and *B. amyloliquefaciens* and decreased *M. fijiensis* infection by more than 90% (Ceballos *et al.,* 2012).

Taxonomical profiles of the phyllosphere in crops such as the cape gooseberry (*Physalis peruviana*) have been identified. Leaf surfaces of cape gooseberry crops in Boyacá showed a high abundance of bacteria *Pseudomonas* spp. and *Enterobacter* spp., followed the yeast *Rhodotorula* and fungi *Capnodium, Cladosporium* and *Penicillium* (Toloza and Lizarazo, 2014). Furthermore, in natural ecosystems such as Andean high-mountain, the phyllosphere microbial community associated with *Espeletia* plants in the Los Nevados Natural National Park was accessed to compare important microbial contributions to geobiological processes, as well as the potential in terms of bioprospecting for microbial processes such as bioremediation, nutrient acquisition, and antimicrobial compound production (Ruíz-Pérez *et al.,* 2016).

## CONCLUSIONS AND FUTURE PERSPECTIVES

Most of the studies on phyllosphere have focused on plant protection or antagonistic activity. A few have characterized nitrogen-fixing communities or plant growth-promoting bacteria in natural or agricultural systems. The phyllosphere is an environment that can harbor microorganisms linked to ecosystem functions, especially those involved with carbon and nitrogen cycles that are closely related to plant nutrition. Research on foliar nutrition by microorganisms could expand our understanding of microbial mechanisms as a strategy to be applied in agriculture and used in the inoculation of soil microorganisms.

Characterizing the taxonomic and functional profiles of the microbial community, in addition to evaluating ecological interactions, identifies core groups and functions that can occur in different plant species in natural environments. In turn, the information obtained from studies on natural environments allows these profiles to be explored in plant species of horticultural interest in order to increase plant productivity.

Biotechnology offers great potential for applications of beneficial microorganisms in agriculture in order to increase plant productivity. Furthermore, it will help in understanding how the microbial community in the phyllosphere affects plant growth and health in agroecosystems. Bioprospecting the use of microorganisms associated with plants, in this case, the microbial community associated with the phyllosphere, is important as an alternative for fertilization and protection in horticultural crops (Fig. 2).

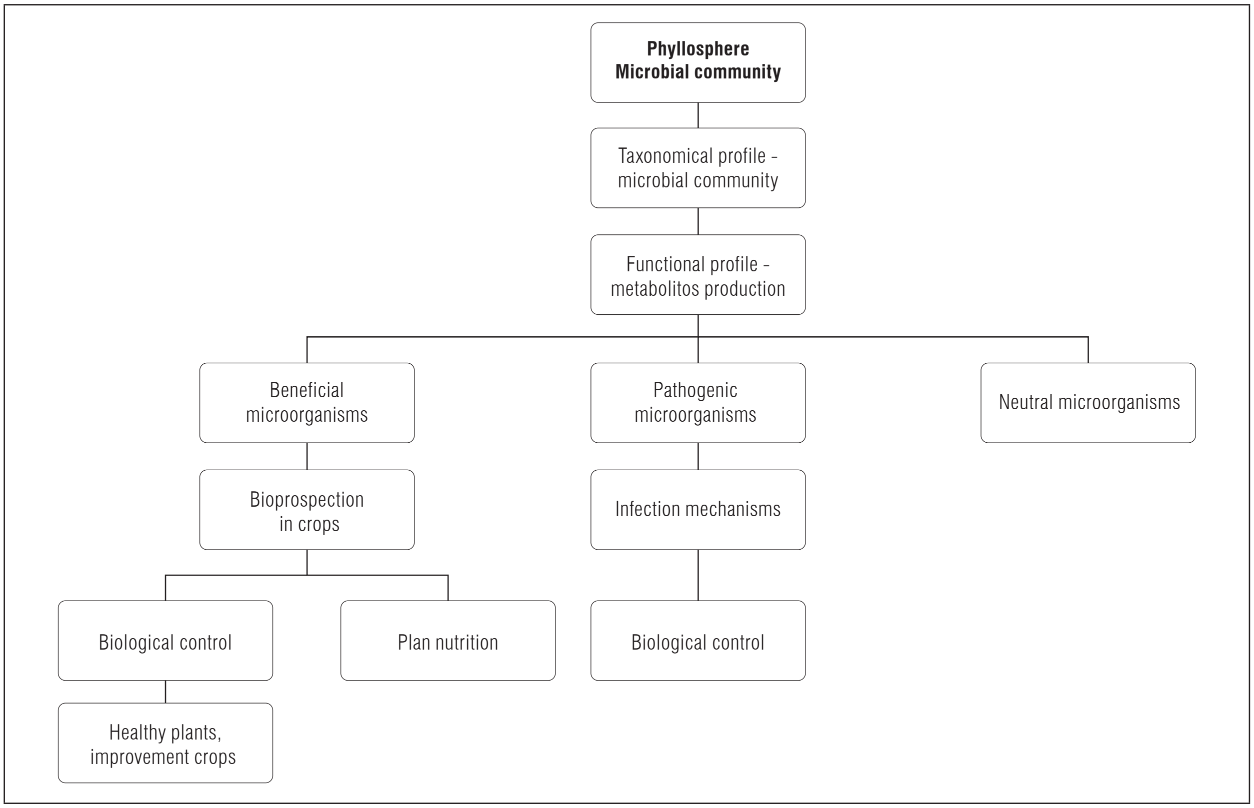


**Figure 2. Fluxogram showing the more important characteristics in phyllosphere studies.**

**Conflict of interest:** this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that that puts at risk the validity of the presented results.

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