

REVIEW ARTICLE

Microbial biofilms in nature: unlocking their potential for agricultural applications

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Summary

Soil environments are dynamic and the plant rhizosphere harbours a phenomenal diversity of micro-organisms which exchange signals and beneficial nutrients. Bipartite beneficial or symbiotic interactions with host roots, such as mycorrhizae and various bacteria, are relatively well characterized. In addition, a tripartite interaction also exists between plant roots, arbuscular mycorrhizal fungi (AMF) and associated bacteria. Bacterial biofilms exist as a sheet of bacterial cells in association with AMF structures, embedded within a self-produced exopolysaccharide matrix. Such biofilms may play important functional roles within these tripartite interactions. However, the details about such interactions in the rhizosphere and their relevant functional relationships have not been elucidated. This review explores the current understanding of naturally occurring microbial biofilms, and their interaction with biotic surfaces, especially AMF. The possible roles played by bacterial biofilms and the potential for their application for a more productive and sustainable agriculture is discussed in this review.

Introduction

The rhizosphere is a hub of microbial interactions that influence plant functioning. The rhizosphere encompasses the endorhizosphere, ectorhizosphere and the rhizoplane layers each of which are colonized by various groups of micro-organisms (Hassani *et al.* 2018). Plant roots influence the surrounding soil through rhizodeposition of carbohydrate-rich mucilage, sloughed cells and root exudates. The secreted materials contain sugars, fatty acids, amino acids, phytohormones, vitamins and antimicrobial compounds which assist the plant in nutrient uptake and safeguarding the roots against pathogenic attack (Dhawi 2016). Bacteria attach to both abiotic surfaces (such as glass, metal and Teflon) and biotic surfaces (like plants, animals and other microbes) and can exist either as single cells, aggregates and biofilms. Biofilm is defined as a well-organized structure formed by a bacterial community assemblage that is enclosed in a self-produced matrix in which bacterial cells communicate

(Armbruster and Parsek 2018). Biofilms can be mono or multi-layered with single or multiple species of bacteria within the matrix associated with a particular surface. Bacterial biofilms have been studied in detail because they have characteristics that have enabled their use in a variety of applications ranging from water purification (Sehar and Naz 2016), bioremediation (Edwards and Kjellerup 2013) and agriculture (Velmourougane *et al.* 2017). Biofilms have also been reported to break down different compounds containing complex nutrients such as nitrogen and phosphorous (Ikuma *et al.* 2013). They are able to trap pathogens from contaminated water prior to its release into the environment or utilization for agriculture purposes (Sehar and Naz 2016). There have been many investigations into biofilm associations with biotic surfaces and this has provided impetus for the use of bacterial biofilms in agriculture (Singh and Chauhan 2017).

Bacterial biofilms are found in diverse niches within agro-ecosystems and can successfully compete with other micro-organisms present on the plant root. Bacteria and

fungi also cohabitate and share common micro-niches in the soil. This coexistence not only provides benefit to the micro-organism's metabolic activities but also contribute towards plant and ecosystem health (Deveau *et al.* 2018).

Bacterial biofilms attached to fungal surfaces, known as fungal–bacterial biofilms, have been shown to enhance nutrient uptake, plant growth and tolerance against environment stress compared to mono or mixed cultures without biofilm formation (Hettiarachchi *et al.* 2014; Hassani *et al.* 2018). Such naturally existing biofilms, associated with plant roots not only improve crop production but also protect the host plant under different environmental stresses. Understanding of such agriculturally important cross-kingdom biofilms in greater details will provide more insights for their implications on plant protection, bioremediation and improving plant nutrition and soil quality. For the plant, the microbial community present in the rhizosphere and their interactions as community/biofilm assemblages is likely to significantly influence the plant metabolic processes.

There is a requirement to explore in greater detail the particular role of association, the mechanisms involved and importance which could unlock the application of such bacterial biofilms in sustainable agriculture and enhancement of crop productivity. This review highlights the natural microbial biofilms found associated in symbiosis with plant roots and their potential applications.

Rhizosphere components

Micro-organisms associated with plants are well-regulated and have significant mutualistic effects on plants, as well as on the microbial community (Backer *et al.* 2018). The rhizosphere is colonized by plant-beneficial bacteria which can form biofilms and are involved in important processes such as plant development, nutrient cycling, biological control of plant pathogens, resistance against and bioremediation of contaminated soils (Shaikh *et al.* 2018). Understanding of the functional relevance of rhizosphere biofilms is still limited and warrants further efforts using metagenomics approaches to elucidate their role(s) for specific soil applications (Schmeisser *et al.* 2017).

Another major microbial symbiont group in the plant microbiome are the arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF), which help in improvement of fitness of plants in almost all ecosystems alongside improving soil quality along with plant growth (Chen *et al.* 2018; Guennoc *et al.* 2018). Interestingly, these fungi have been confirmed to be associated with several bacterial species that exist on the surface of mycorrhizal structures such as spores or reside inside them, sometimes both (Torres-Cortés *et al.* 2015). Mycorrhiza-

associated bacteria are involved in root development; enhance root and AMF fungal recognition process, stimulate sporulation, mycelial establishment and growth, protection against antagonistic substances and promote traits such as phosphate solubilization, nitrogen fixation, antibacterial or antifungal properties (Wagner *et al.* 2019). These bacteria can influence AMF (through bipartite interaction) by altering the fungal structures or by providing citric and maleic acid as a carbon source while playing a major role in bacteria–mycorrhiza interaction and signalling (Kannan *et al.* 2011). However, such bacteria are non-specific, as they do not depend on the type of mycorrhizal symbiosis (Table 1).

In natural environments, there are multiple microbial communities found in association with plant roots and this three-way plant–fungi–bacterial biofilm symbiotic association has likely existed since the arrival of vascular plants. A variety of signalling communication strategies such as quorum sensing and trophic-mediated communication are utilized by microbial communities in nature to influence plants and it has become increasingly evident that an understanding of these is critical in developing future strategies for improving agricultural productivity (Lemanceau *et al.* 2017; Mhlango *et al.* 2018). The use of new technologies such as next-generation sequencing and metagenomics approaches allows researchers to perform a mechanistic analysis of the microbial communities associated with plants, which helps to explain their physiological potential. Such vital information aids to predict the

Table 1 Key beneficial bacteria associated with arbuscular mycorrhizal fungi

Bacterial strain	Mycorrhizal species	Effect on AMF	Reference
<i>Bacillus megatarium</i> , <i>Bacillus</i> sp.	<i>Gigaspora margarita</i>	Enhances mycorrhization	Budi <i>et al.</i> (2012)
<i>Rhizobium tropici</i>	<i>Glomus intraradices</i>	Increase in hyphae, vesicles, arbuscules	Tajini <i>et al.</i> (2011)
<i>Pseudomonas fluorescens</i>	<i>Glomus mosseae</i>	Promotes saprophytic growth and fungal root colonization	Pivato <i>et al.</i> (2009)
<i>Paenibacillus validus</i>	<i>Glomus intraradices</i>	Increases fungal growth	Hildebrandt <i>et al.</i> (2005)
<i>Bacillus pabuli</i>	<i>Glomus clarum</i>	Increases fungal growth, spore germination and AM fungal root colonization	Xavier and Germida (2003)

The table does not contain the entire list of beneficial bacterial strains. For additional information, readers may refer to Frey-Klett *et al.* (2007) and Selvakumar *et al.* (2012).

microbial processes and interactions in the rhizosphere region (Knief 2014). While the cross-talk between plants and their naturally associated micro-organisms has been studied in depth for symbiosis, and pathogenesis but the extent of the impact of microbial biofilms on plant growth, health and disease needs to be explored (Altaf and Ahmad 2016).

Biofilms in nature and their matrix

Traditionally, bacterial cells were identified and characterized as free-living cells but under different environmental conditions they exist as biofilms (Omar *et al.* 2017). The formation and dispersal of biofilm are very well described and known to involve the following steps: initial attachment, intercellular attachment, desorption, biofilm maturation and biofilm dispersion (Achinas *et al.* 2019). Biofilm structures are complex, consisting of high cell densities of 10^8 – 10^{11} cells per gram wet weight and the matrix accounts for over 90% dry weight while the micro-organism dry weight contributes <10% (Flemming *et al.* 2016). Bacterial biofilms can be classified on the basis of their diversity, ranging from simple to complex, tower to flat, clustered film to featureless and possess various physiological properties (Koo *et al.* 2017). The properties of biofilm communities are different compared to the free-living bacterial cells due to the presence of self-produced matrix (Berlanga and Guerrer 2016).

Biofilm matrix: structural components and their role

Extracellular polymeric matrix (EPS) is formed during the attachment stage of biofilm formation providing stability to the micro-organism during the interaction in the biofilm (Jamal *et al.* 2018). The EPS comprises of organic matter, exopolysaccharides, proteins, extracellular DNA, nucleic acid, enzyme, lipids, membrane vesicles and bacterial refractory compounds (Jamal *et al.* 2018). Each structural element involved in the bacterial biofilm matrix formation has a specific function and role in biofilm formation and its stability (Fong and Yildiz 2015). Exopolysaccharides are very important for the formation and organization of microbial biofilm. Hence, polysaccharides are categorized as aggregative (adhesion of cells on the surface), protective (diffusion barrier where it protects the cells from antibiotic penetration) and architectural (biofilm formation regulation and structure) (Limoli *et al.* 2015).

One of the best studied biofilm forming organisms is *Pseudomonas aeruginosa* which produces a biofilm consisting of three polysaccharides: Pel, alginate and Psl. Structures for both alginate and Psl have been determined. Alginate is a high molecular weight acidic

polysaccharide consisting of non-repeating subunits of O-acetylated D-mannuronic acid and its C5 epimer L-guluronic acid, whereas Psl polysaccharide is composed of a repeating pentamer containing D-mannose, L-rhamnose and D-glucose. Although the structure of Pel has not been determined, but it has been proposed to have a glucose-rich polysaccharide that is distinct from cellulose. These regulatory systems are involved in providing architecture to the matrix (Franklin *et al.* 2011). Alginates have recently been implicated in biofilm formation in the PGPB *Pseudomonas* sp. where it was identified to be involved in metal biosorption and deletion of the *alg8* gene (coding for a sub-unit of alginate polymerase) led to a drastic reduction in exopolysaccharide production by the organism (Upadhyay *et al.* 2017).

Biofilm-associated proteins have also been identified to induce the formation of biofilm even in the presence of exopolysaccharides in *Staphylococcus aureus* (Shukla and Rao 2017). Apart from polysaccharides, another significant element is extracellular DNA which is actively secreted by the bacteria. The major function of eDNA in surface-associated microcolonies is to ensure bacterial attachment, stabilization, aggregation and maturation and it has been shown that eDNA is also required in the early formation of biofilm in *P. aeruginosa* (Chang 2018). Formation of biofilm on any surface under suitable environment initiates with the interplay of individual planktonic bacterial cells. The third crucial element in biofilm matrix is the pili, lectins, flagella, fimbriae and sugar-binding proteins. These surface proteins, flagella and pili are known to take part in the initial attachment of bacterial cells to the surface and migration in some cases which help cells in colonizing the attachment surface (Fong and Yildiz 2015). Matrix protein either enables biofilm matrix reorganization or degradation and dispersal as proteins have enzymatic properties towards other components of matrix (e.g. DNases-degrading eDNA). EPS is composed of 97% water which helps the matrix to remain hydrated for a longer duration and this protects the bacterial cells against desiccation (Flemming *et al.* 2016).

Importance of extracellular polymeric matrix

Bacterial cells living in biofilms have various advantages which include defence against stress environment such as osmotic shock, UV radiation, change in pH, exposure to antimicrobial agents, dessication and exposure to antimicrobial agents (Koo and Yamada 2016). The matrix acts a hydrated barrier between the bacterial cells and outer environment, thereby helping in accumulation of inorganic and inorganic compounds, guards against dessication, and penetration of toxic elements into the biofilm (Flemming 2016).

Hence, EPS is responsible for the maintenance and formation of biofilms where they stabilize the cells, protect them against hydrodynamic shift forces and offer an opportunity for exchange of genetic information (Ates 2015). Although biofilms are not required by the bacteria for their survival, they enhance the chances of propagation, survival and metabolism under adverse conditions. Biofilms are adaptive and cells within them often display significant coordination, cooperation and communication.

Biofilm types

In the environment, microbes not only compete for resources but also for space (Stubbenieck *et al.* 2016). After surface attachment, bacterial communities form an organized structure which varies from simple to mushroom-like structures. The different morphology of bacterial community depends on various parameters such as carbon sources, flow velocity and oxygen gradient encountered (Duvernoy *et al.* 2018). Initially, single bacteria adhere to the surface and are converted to microcolonies after few cycles of division. Microcolony morphogenesis involves mechanical coupling between cell elongation forces, cell rearrangements, adhesion, friction and steric interaction. At the beginning, bacterial cells proliferate within a single layer and microcolonies can quickly change into biofilm structures at the liquid–solid interface (Berk *et al.* 2012) even though they remain as a monolayer on agarose and glass (Su *et al.* 2012).

Biofilms in plant-associated habitats

The rhizosphere is influenced by the plant roots and its exudates in the soil and they are colonized by bacteria via migration of bacteria from bulk soil to the rhizoplane. The association and successful establishment of rhizobacteria to plant roots is dependent on the cell adherence and formation of microcolonies and many beneficial interactions have been studied between plant root surfaces and micro-organisms (*Pseudomonas*, *Burkholderia*, *Bacillus* and *Paenibacillus*) (Vardharajula *et al.* 2011; Jung *et al.* 2018) (Table 2). Formation of biofilm on plant roots may also be initiated by signals such as nutrient and water availability and bacteria–bacteria interactions and can be formed under stressed conditions (Zúñiga *et al.* 2017). The characteristics of the root surface vary along length of the roots and biofilm formation is influenced by the exudates and nutrients released by the roots at different sites. The zone of cell division and root cap are prominent sites for bacterial colonization compared to the less colonized mature root zone and hairs (Timusk *et al.* 2005).

Root biofilms in nature

Arabidopsis thaliana roots present a complex microbial consortia where bacteria were seen to form biofilm that had a positive impact on plant productivity and growth (Hassani *et al.* 2018). The root exudate's chemical compositions have direct effect on the microbial community in the rhizosphere. For instance, citric acid from cucumber root exudates attracted *Bacillus subtilis* N11 and fumaric acid from banana attracted *B. amyloliquefaciens* SQR9 and promoted biofilm production (Mhlongo *et al.* 2018). Colonization, attachment and formation of *B. subtilis* biofilm on *A. thaliana* roots were observed to be dependent in the presence of the microbial TasA protein and exopolysaccharide. It was also shown in the study that plant polysaccharides such as xylan, pectin and arabinogalactan were stimulated at the initiation stage of biofilm formation (Poole 2017).

Root biofilms under controlled conditions

In polycyclic aromatic hydrocarbons contaminated agricultural soil, robust biofilms were formed by phenanthrene-degrading bacteria, *Pseudomonas* sp. with the root surface of three rice varieties: *Liaojing401*, *Koshihikari* and *Zhenzhuhong*. The biofilm formation at different time intervals was confirmed using scanning electron and confocal laser scanning microscopy. The EPS formed was constituted with proteins, carbohydrates, lipids and nucleic acids but the content varied with the species of rice. The study concluded that the formation of biofilm with the rice roots played an important role in degradation of phenanthrene which contaminated agriculture soil (Zhou and Gao 2019). Co-inoculation of plant-growth-promoting rhizobacteria, *Pseudomonas putida* and *Bacillus amyloliquefaciens* resulted in the formation of biofilms with the root surface which significantly showed enhancement in plant-growth-promoting attributes and plant growth under drought conditions (Kumar *et al.* 2016). An *in-vitro* study has shown that the higher concentration of malic acid in tomato root exudates can stimulate biofilm formation as well as matrix gene expression (Vlamakis *et al.* 2013). Biofilm formed by *B. subtilis* on the *Arabidopsis* roots has been found to provide signals for the synthesis of EPS matrix (Beauregard *et al.* 2013). *Bacillus subtilis* is commonly found associated with plant roots and it also protects the plants from infection in soil. *Bacillus subtilis* biofilm formation was demonstrated and confirmed with *Arabidopsis thaliana* roots under *in-vitro* condition (Beauregard *et al.* 2013). The formation of biofilm by *Paenibacillus polymyxa* on *A. thaliana* roots was observed immediately after colonization under both gnotobiotic

Table 2 Beneficial bacterial biofilm associated with plant roots

Bacterial strain	Experimental host plant	Functional traits	Observed under natural/controlled conditions	Reference
<i>Proteus</i> sp., <i>Pseudomonas</i> sp., <i>Ensifer meliloti</i> (metal-polluted soil)	Alfalfa	Remediation of metal-contaminated soils	Controlled conditions	Raklami et al. (2019)
<i>Pseudomonas</i> sp. (sewage sludge)	Rice	Bioremediation of phenanthrene	Controlled conditions	Zhou and Gao (2019)
<i>Pseudomonas entomophila</i> (rhizoplane of wheat)	Wheat	Tolerance to abiotic stress	Controlled conditions	Ansari and Ahmad (2018)
<i>Bacillus subtilis</i> (rhizoplane)	Arabidopsis	Biocontrol	Controlled conditions	Dwivedi et al. (2017)
<i>Pseudomonas putida</i> (roots of chickpea) and <i>Bacillus amyloliquefaciens</i> (alkaline soil)	Chickpea	Synergistic growth enhances the plant-growth-promoting attributes	Natural and Controlled conditions	Kumar et al. (2016)
<i>Azospirillum brasilense</i> (rhizosphere of sorghum)	Sorghum	Enhance NO and indole-3-acetic acid	Controlled conditions	Koul et al. (2015)
<i>Azospirillum brasilense</i> (rhizosphere)	Wheat	Nitrogen fixation	Natural conditions	Souza et al. (2014)
<i>Bacillus subtilis</i> (Rhizosphere)	Arabidopsis	Biocontrol	Controlled conditions	Beauregard et al. (2013)
<i>Bacillus amyloliquefaciens</i> , <i>Bacillus polymyxa</i>	Tomato	Biocontrol	Controlled conditions	Nihorimbere et al. (2012)
<i>Microbacterium</i> sp. (sugarcane stem)	Sugarcane	Nitrogen fixation	Controlled conditions	Lin et al. (2012)
<i>Pseudomonas putida</i> (rhizosphere of chickpea)	Plant source unknown	Plant-growth-promoting activities	Controlled conditions	Srivastava et al. (2008)
<i>Paenibacillus polymyxa</i> (peanut rhizosphere)	Peanut	Biocontrol against crown rot disease	Natural conditions	Haggag and Timmusk (2008)
<i>Bacillus subtilis</i> (commercial strain)	Arabidopsis	Biocontrols	Controlled conditions	Bais et al. (2006)
<i>Paenibacillus polymyxa</i> (rhizosphere of wheat)	Arabidopsis	Protection against abiotic and pathogen stress	Controlled conditions	Timmusk et al. (2005)
<i>Pseudomonas aeruginosa</i> (human pathogenic strains)	Arabidopsis and sweet basil	Antimicrobial activity	Controlled conditions	Walker et al. (2004)
<i>Bacillus cereus</i> (commercial strain)	Arabidopsis	Biocontrol	Controlled conditions	Bais et al. (2004)

and soil systems. The inoculated natural isolate showed the colonization in the form of microcolonies with the primary root preferably within the elongation and differentiation zone as well as in the intracellular spaces of the root through fluorescence microscopy (Timmusk et al. 2005).

Mycorrhizal–bacterial biofilms: cross-kingdom tripartite associations

Plants are commonly associated with AMF in the natural ecosystem in a mutualistic relationship. In this symbiotic association, mycorrhiza consumes approximately 20% photosynthates released by plants and in-return provides water and nutrients to the host plant (Wipf et al. 2019). Since these organisms are found near the root region and help enhance the absorption of nutrients while promoting

plant development and growth they are used as biofertilizers (Alori et al. 2017).

The hyphosphere of AMF may be a preferred microhabitat for microbes and many bacteria can colonize the fungal hyphal surface to varying degrees and form biofilms (Frey-Klett et al. 2011). This is likely due to the hyphosphere being a rich nutrient feeding zone but also because they can serve as a ‘highway’ to attain distant nutrients by migrating along the hyphal film or growing hyphal tips in biofilms (Guennoc et al. 2018). The formation of biofilm on AMF not only provides bacterial nutrients but also may confer advantages to the bacteria by providing them a safe niche away from bulk predators and stresses.

The external mycorrhizal hyphae interact with bacteria present in the soil directly or indirectly by modifying the physiology of the plant host and root exudation pattern.

There is very high specificity in such ‘tripartite’ associations between the plant colonizing AMF and some bacterial species as different strains respond variably in the presence of certain mycorrhiza (Ghignone *et al.* 2012). Different bacterial strains can interact with diverse fungal structures such as spores and hyphae and these associations can be maintained in the colonized plant root as biofilm, as depicted in Fig. 1. These bacteria can exist with the fungi either on the surface of the spore or hyphae in the form of biofilm or within the cytoplasm of the spore as endobacteria (Taktek *et al.* 2017). The fungal–bacterial association can be selective to both the type of fungal species and the region of colonization (Agnolucci *et al.* 2019). Bacteria can colonize the fungal surface to explore the source of nutrients and reach previously inaccessible nutrient sites. They can move easily along the fungal hyphae using flagella (Warmink *et al.* 2011).

There are several reports where AMF spore and spore walls associated culturable bacteria has been isolated and identified with diverse isolates (Lecomte *et al.* 2011; Battini *et al.* 2017). Apart from spore-associated bacteria, mycorrhiza also host endobacteria in their cytoplasm (Bonfante and Desirò 2017). Till date, two types of endobacteria have been identified with AMF: *Candidatus Glomeribacter gigasporarum* and Mollicutes-related endobacteria. Therefore, single or multiple bacterial populations can exist inside the AMF cytoplasm as their host (Desirò *et al.* 2016). Studies have characterized the isolated mycorrhizosphere bacteria for their functional traits, to have a better understand about the individual bacterial strain or consortia for plant development and growth. Most of the mycorrhiza-associated bacteria enhanced the availability of nutrients such as nitrogen and phosphorous, produced phytohormones, siderophores and indole acetic acid as well as provided resistance against fungal pathogens (Agnolucci *et al.* 2015). Moreover, mycorrhizosphere bacteria are reported to improve spore germination, hyphal extension and boost mycorrhizal activity (Agnolucci *et al.* 2019). Bacteria associated with mycorrhiza (EMF or AMF) have the ability to form biofilm with the mycorrhizal structures. Much attention has been given to *Pseudomonas fluorescens* forming biofilm like structures on *Laccaria* (Deveau and Labbé 2017; Guennoc *et al.* 2018). There are few reports on biofilm formation with AMF (Table 3). With *Gigaspora margarita*, *P. fluorescens* WCS 365 showed greater efficiency of biofilm formation on the hyphae in comparison with strain *Pseudomonas* sp. MI14 which displayed lower efficiency in forming biofilm (Bianciotto *et al.* 1996). From extremely polluted land (petroleum hydrocarbons), a wide variety of AMF were identified and were found associated with different bacterial OTUs of *Sphingomonas* sp., *Pseudomonas* sp., *Massilia* sp. and *Methylobacterium* sp. The

bacteria associated with AMF formed biofilms with the AMF propagules (spores and vesicles; Iffis *et al.* 2014). A carrot root *P. fluorescens* was found to form biofilm on the extraradical mycelia of the arbuscular mycorrhizal fungus, *Glomus intraradices* (Bianciotto *et al.* 2001).

Both bacteria and AMF stimulate plant growth by having direct interactions with the host roots (tripartite interactions; Fig. 2) and there have been many studies on the synergistic effects of mycorrhizal fungi and bacteria on plant health (Revillini *et al.* 2016). Recent studies have shown synergistic positive effects of mycorrhizal fungi and bacteria on plants. The bacterial biofilms present in the maize mycorrhizosphere solubilized phosphorous and enhanced host growth (Magallon-Servín *et al.* 2019). Biofilm formed by *Bacillus* sp., *Bacillus thuringiensis* and *Paenibacillus rhizospherae* strains on spore surfaces of *G. margarita* in the maize mycorrhizosphere. The activities of isolated bacteria were screened against soil-borne plant pathogens as well as phosphorus solubilization, ethylene production, nitrogenase activity (Cruz and Ishii 2012). Strains of *Rhizobium miluonense* and *Burkholderia anthina* were observed to strongly attach to the surface of *Rhizoglyphus irregulare* and solubilize phosphate (Taktek *et al.* 2017).

Most of the recognized bacteria with mycorrhizal fungi are culturable bacteria but still further investigation is required to identify the non-culturable bacteria associated with the fungi to obtain a full picture of novel strains associated with fungi and their specific roles. At this stage, there are no reports to explain how the bacteria are able to colonize the spores of AMF. A significant amount of work remains to decipher the molecular interactions between such associations and the plants, the genes that trigger AMF associated bacteria to form biofilm and their pathways which are involved in this entire process. Developing an understanding of systems and interactions between the two partners must be conducted with selected bacterial and AMF species and their plant hosts. Such studies would not only help in better understanding of the biology of biofilm–root or bacterial–biofilm interactions but could also contribute significantly to understanding the functional biology of the rhizosphere. The functional relevance of the biofilm under different environment conditions, associated with mycorrhiza–plant symbiosis, can be explored with combination of traditional cultural-dependant approaches and genome meta-genome analysis.

Biofilm functions relevant for agriculture biotechnology

Biofilms formed in the soil rhizosphere are a hotspot for interaction between inter-kingdom associations which

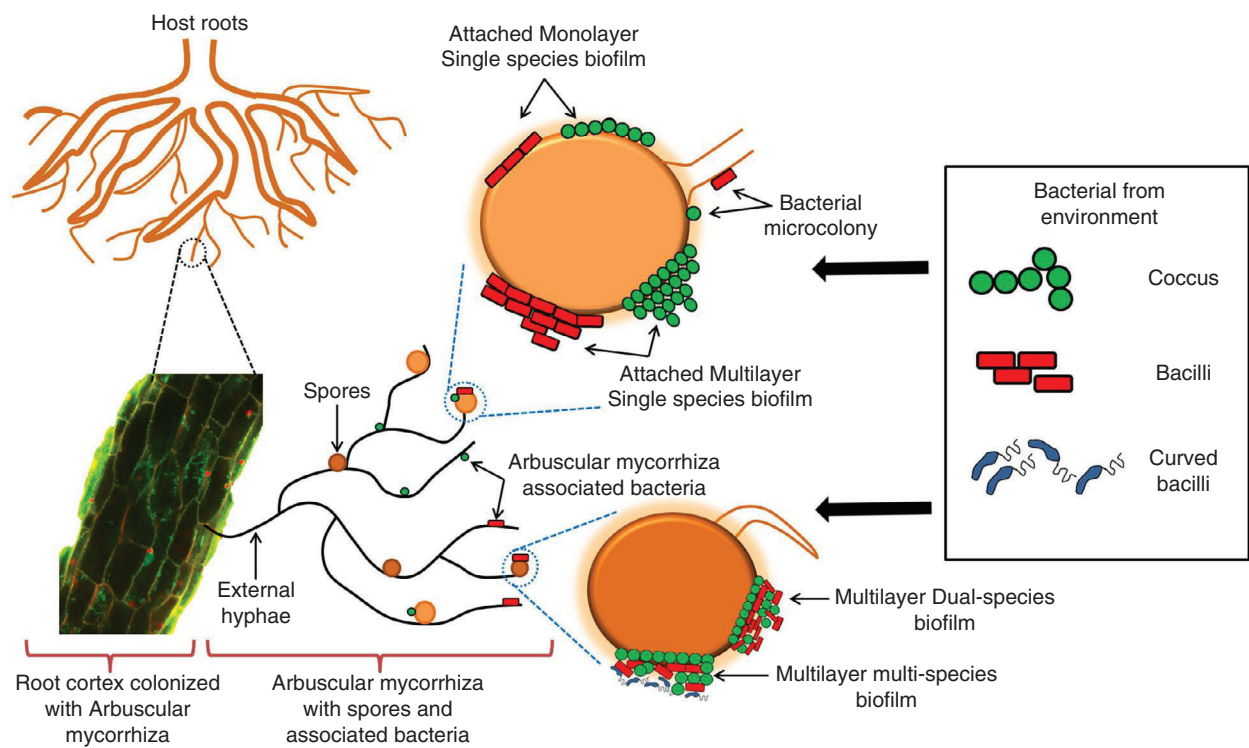


Figure 1 A simplified diagrammatic representation of different types of biofilm formed on the surface of arbuscular mycorrhizal fungi (AMF) spores and hyphae. Once the plants roots are colonized with the beneficial AMF, spores and hyphae start to grow in the soil, where bacterial community gets associated with them. The bacteria can either form monolayer or multi-layered biofilm on the surface consisting of single or multiple species of bacteria.

Table 3 Beneficial bacterial biofilms associated with mycorrhizal fungi

Bacterial strain	Host mycorrhizal species	Effect on plant	Observed under natural/controlled conditions	Reference
<i>Burkholderia anthina</i>	<i>Rhizoglomus irregulare</i> (AMF)	Phosphate solubilization	Controlled Conditions	Taktek <i>et al.</i> (2017)
<i>Pseudomonas</i> sp.	<i>Rhizophagus irregularis</i> (AMF)	Phosphorous solubilization	Controlled conditions	Ordoñez <i>et al.</i> (2016)
<i>Bacillus</i> sp., <i>Bacillus thuringiensis</i> and <i>Paenibacillus rhizosphaerae</i>	<i>Gigaspora margarita</i> (AMF)	Increases hyphal growth, ethylene production, nitrogenase activity, phosphate solubilization, growth inhibitor of fungal pathogens	Controlled conditions	Cruz and Ishii (2012)
<i>Pseudomonas fluorescens</i>	<i>Laccaria bicolor</i> (EMF)	–	Controlled conditions	Guennoc <i>et al.</i> (2018)
<i>Pseudomonas fluorescens</i>	<i>Laccaria bicolor</i> (EMF)	Promote growth of the host	Controlled conditions	Noirot-Gros <i>et al.</i> (2018)

also shape the microbial assemblages (Hassani *et al.* 2018). Focusing on the agriculture sector, biofertilizer biofilms can have significant potential benefits (Fig. 3). Such innovative eco-friendly products have the potential to overcome the inadequacies of conventional chemical-based fertilizers.

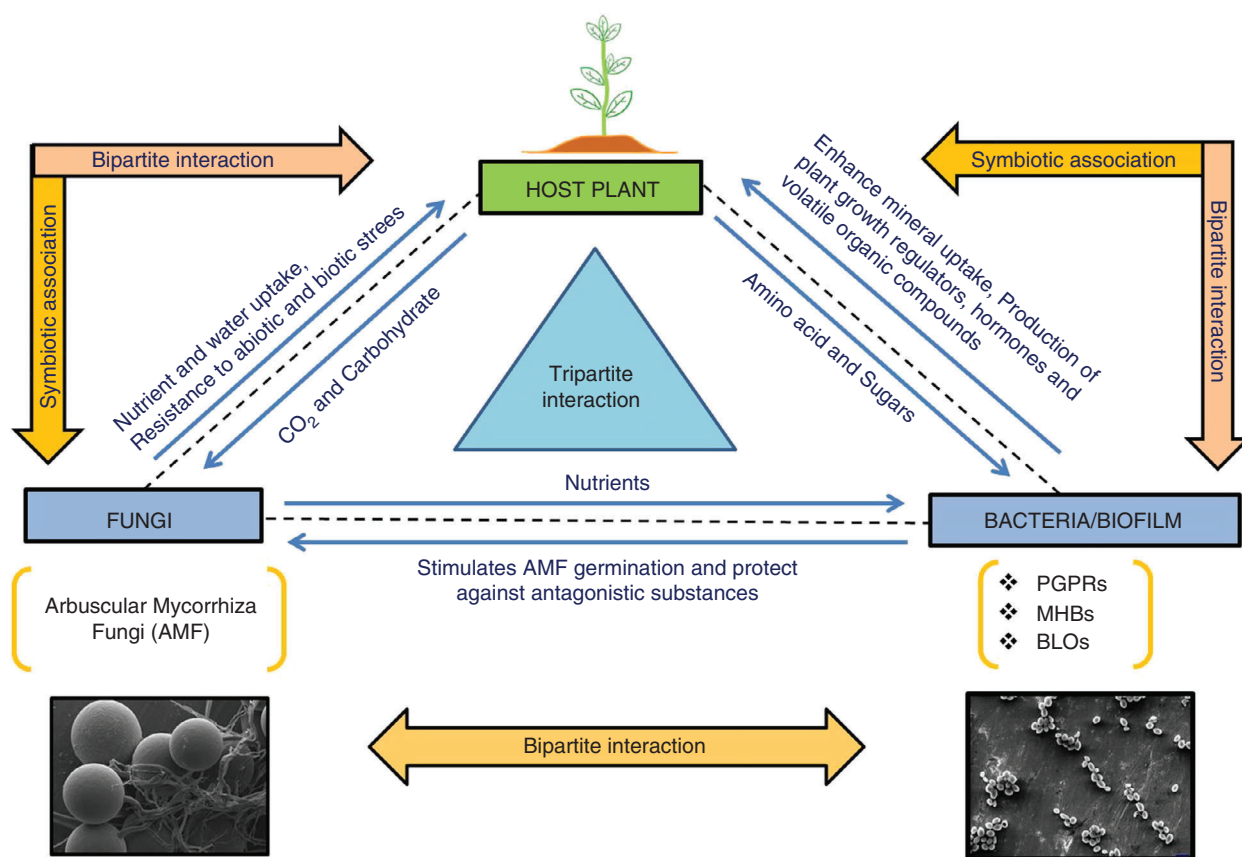


Figure 2 General overview of tripartite interaction involved between host plant, arbuscular mycorrhizal fungi and bacteria or bacterial biofilm present in the soil/plant rhizosphere. Examples of the multi-directional exchanges of goods and services between the three partners have been shown here. Tripartite interactions can be both positive and negative, though only beneficial goods or services are listed here (arrows). Also, the beneficial bipartite interaction between plant–mycorrhiza and bacteria, bacteria–plant and mycorrhiza have been depicted.

Such products may guard the plant not only against various environment and soil-borne diseases but also against abiotic stresses such as salinity, drought, inorganic and organic pollutants, potentially increasing crop productivity (Malusá *et al.* 2012). Biofilms in the rhizosphere may also be helpful in improving water stability along with microbial biomass, which can encourage the root exudation response under stress (Kasim *et al.* 2016). Even biodegradation of organic pollutants and heavy metals might be achieved in soil through the presence of biofilm as such structures can easily survive against harsh environments due to the presence of matrix (Gkorezis *et al.* 2016). They can be applied as beneficial bacterial biofilm inoculum or as mixed inoculum directly to the plants in the form of sprays on the aerial parts or as inocula in the soil to promote plant development and growth (Hettiarachchi *et al.* 2014).

Metabolic cooperation in the presence of fungi is important in the creation of new bacterial niches in the soil due to the utilization of fungal exudates for bacterial attachment to its surface (Deveau *et al.* 2018). Metabolic

cooperation between bacteria and fungi offers greater protection to the biofilm's constituent microbes, particularly for the bacterial species, and are likely to be less susceptible to abiotic stresses than in monocultures. The plant–microbe interactions taking place in mycorrhized plants can further help to increase mycorrhization and directly influence the diversity of microbial population in its surrounding (Gui *et al.* 2017). Thus, there not only plant growth benefits as a consequence of such interactions but also the soil quality improves with an increase in the nutrient cycling (Velmourougane *et al.* 2017). Increased focus must also be on multiple-species biofilms as they may produce polysaccharides and bioactive compounds with greater positive impacts on plant growth and soil health.

Future perspectives

The knowledge of fungal–bacterial interactions and their roles is increasing; however, there is still a need of

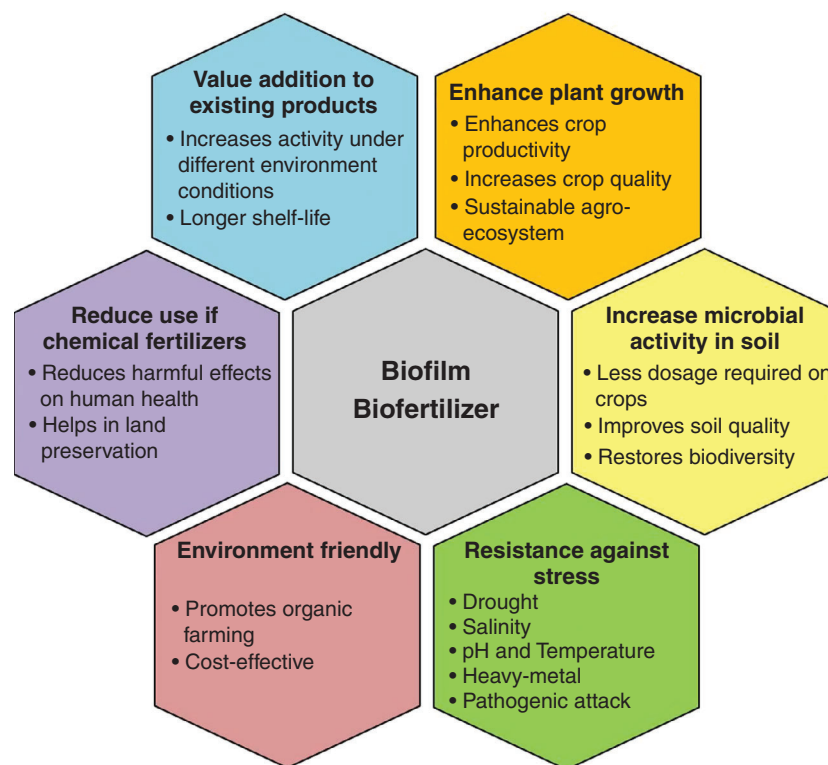


Figure 3 Snapshot of the benefits and applications of the biofilm biofertilizer.

advancing the molecular and systems understanding of ecological niches offered by fungi to the bacteria. Despite reports on the tripartite interaction between fungi colonized plants and bacteria, the actual detailed mechanism behind these associations needs to be understood in greater detail. To better understand the tripartite association in rhizosphere biology and the specific role of biofilm, more investigation must be conducted using some culture-independent approaches such as metagenomics, to understand the regulatory mechanisms, genetic and ecological role of the organism involved in biofilm communities. Also, ‘omics’ technologies can be used to provide insight of the various molecules involved in the tripartite association in soil rhizosphere. Metatranscriptomics, metabolomics and metaproteomics will provide insights into localized gene expression and metabolic profiling of beneficial biofilms.

Co-migration of bacteria with fungal hyphae will also be an interesting area of research. An increase in the availability of bacterial and fungal genome sequences can be utilized to examine the evolutionary link of fungi-associated bacteria and also the horizontal gene transfer between the members of this tripartite association. Naturally existing biofilms can be attenuated as biofertilizer biofilms based next-generation products since they truly resonate with natural ecosystems. While this approach

has the potential in enhancing the yield of the crop production along with soil quality and fertility, application of such biofilm biofertilizer has remained limited worldwide due to the establishment failure of these microbial inocula in the rhizosphere. The biofertilizer biofilms are also likely to be more successful as applied products if they include indigenous microbes present in the rhizosphere as they will be able to maintain site specificity traits effectively.

More studies are required to attain reproducible results of biofilm-associated organisms under laboratory and field conditions. A better understanding of the interaction between plant and biofilm will help to improve the efficiency of the fungal–bacterial biofilms and biofertilizer biofilms in the field. The major challenge in the research of biofertilizer biofilms lies in the identification and selection of potential strains with essential properties to lead to novel products for future sustainable agriculture and efficient agricultural practices.

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Conflict of Interest

The authors declare there are no potential conflicts of interest regarding this review.

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