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REVIEW

Biofilms and nanoparticles: applications in agriculture

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Abstract

A profound need to explore eco-friendly methods to practice sustainable agriculture leads to the research and exploration of plant growth-promoting rhizobacteria (PGPRs). Biofilms are assemblages of microbial communities within a self-secreted exopolymeric matrix, adhering to different biotic and abiotic surfaces and performing a variety of desired and undesired functions. Biofilm formation by PGPRs is governed by effective root colonization of the host plant in providing plant growth promotion and stress management. Biofilms can also provide a suitable environment for the synthesis and entrapment of nanoparticles. Together, nanoparticles and PGPRs may contribute towards biocontrol and crop management. This review discusses the significance of biofilms in agriculture and their confluence with different types of nanoparticles for plant protection and improved crop production.

Introduction

Microorganisms can interchangeably thrive either independently or in groups, depending upon the prevailing conditions. The mode of living in groups gives rise to microbial communities called biofilms. Biofilms are highly structured, surface-attached communities of cells encased within a self-produced extracellular polymeric matrix, the EPS (Flemming et al. 2016; Koo et al. 2017). The distinguishing features between biofilms and their planktonic counterparts include high cell density, surface attachment ability, and increased resistance to physical, chemical, and metabolic stresses. Apart from this, cells in a biofilm coordinate with each other via certain biological and physicochemical signaling molecules and locally cooperate like multicellular organisms (Ono et al. 2014).

Biofilm formation enables the bacterial community to live cooperatively in a sheltered environment, acquire nutrients, enhance fitness by acquiring defense mechanisms or living close to other bacteria inherently possessing these mechanisms, and inhabit niches that can require a critical cell density (Ercan and Demirci 2015). Biofilms offer opportunities for horizontal gene transfer (Qiu et al. 2018), allow the production of extracellular metabolites or exo-enzymes in effective concentrations above threshold levels and may improve the efficiency of processes that are otherwise ineffective at single-cell level (Feng et al. 2016). The EPS of a biofilm hosts several functional groups like the carboxyl, phosphoric, amine, and hydroxyl groups which create a reducing environment and greatly assist in the reduction of metal ions leading to the formation of nanoparticles (Khan et al. 2013). These nanoparticles are of different sizes and shapes and possess unique properties which can be targeted towards specific functions.

Biofilm formation

Enough evidence is available on the composition, structure, and process of biofilm formation (Abee et al. 2011; Hobley et al. 2015; Desmond et al. 2018). Briefly, the microbial cells initially attach to an abiotic or biotic surface through weak van der Waals forces. This leads to an irreversible attachment. The process of colonization begins with the attachment of incoming microbial cells on diverse sites along with cell division of the already adhered cells. This leads to the development and maturity of biofilm wherein the cells and other constituents of the biofilm (mostly, extrapolymeric substances (EPS)) rearrange into a characteristic shape and structure. Mature biofilms are a complex structure of dormant and actively growing microbial cells along with their excretory products and channels. With the subsequent

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depletion of nutrients, cells start disintegrating and the biofilm starts dissipating off from the surface it is attached to. The cycle of biofilm formation is illustrated in Fig. 1.

Biofilms can be formed either as free-floating aggregates or on a variety of surfaces. Nature of the surface dictates the structure of a biofilm that could either be well defined (on abiotic surfaces) or multicellular irregular aggregates (on biotic surfaces) (Kragh et al. 2016; Bjarnsholt et al. 2018). The strength of adhesion on a surface depends on the hydrophobic and hydrophilic properties of surface layers of bacterial cells and the biotic/abiotic surfaces. Microorganisms such as *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *Sinorhizobium meliloti*, *Rhizobium leguminosarum*, and *E. coli* can form biofilms when grown under specific conditions on biotic surfaces such as plant, animal, or human tissue (Perez and Patel 2015; Niederdorfer et al. 2016; Artini et al. 2017).

With plant tissues, biofilm-forming bacteria can interact by employing a pathogenic, symbiotic, or commensal relationship. Factors that influence the structure of their biofilms are water, nutrient availability, and surface properties of the plant tissue. These biofilms may be formed as unorganized structures on different plant parts such as the rhizosphere or phyllosphere (Yaron and Römling 2014). Danhorn and Fuqua (2007) reviewed the association of plants with biofilm-forming bacteria and reported that biofilms can have a different structure and composition on different plant surfaces. Biofilms formed in the rhizosphere are influenced by root exudates and those formed on the phyllosphere are in the form of large aggregates in areas rich in nutrients and moisture. Vascular pathogens and endophytes can form biofilms not only on the plant surface but also inside the plant tissue. These biofilms lead to the initiation and spread of disease within the plant. However, in the rhizosphere, biofilms result in binding of soil particles (Flemming et al. 2016). EPS component of the biofilm acts as a storehouse of excessive carbon and may also form complexes with heavy metals, hence limiting the bioavailability and noxiousness of heavy metal ions. Rinaudi and Giordano (2010) have discussed the mechanisms involved in biofilm formation on plant roots and the function and survival of rhizobial communities. Wang et al. (2017) demonstrated that nitrogen-fixing bacteria within an EPS encapsulated biofilm can convert carbon sources to exopolysaccharides and allow embedded bacteria to carry on the process of nitrogen fixation under aerobic conditions.

Application potential of biofilms

Biofilms can be both beneficial and harmful and find widespread applications in diverse fields such as health (Lindsay and von Holy 2006), industry (Li et al. 2007), agriculture (Ünal Turhan et al. 2019), and bio-nanotechnology (Ng et al. 2015). Their roles range from treatment of wastewater, remediation of contaminated soils, microbial leaching,

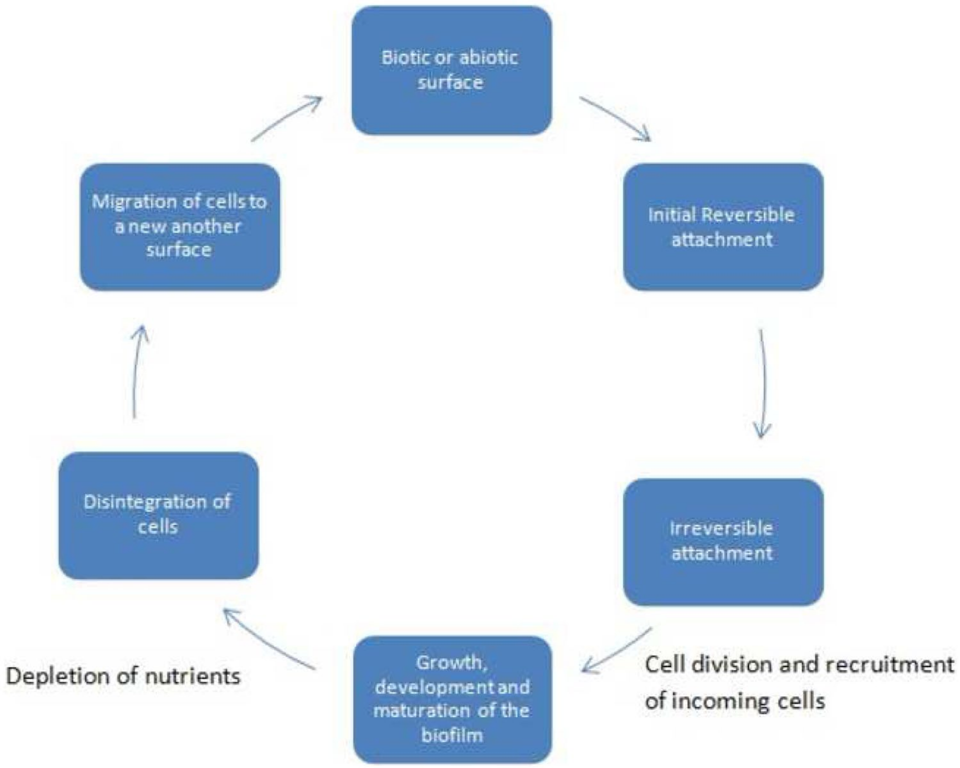

Fig. 1 The cycle of biofilm formation

biofouling, biocorrosion, product contamination, persistent infection, to synthesis of nanomaterials. Biological wastewater treatments may be carried out using bacterial biofilms in fixed-film processes, membrane bioreactors (MBRs), and activated sludge processes (Sheng et al. 2010; More et al. 2014; Salama et al. 2015). This review documents the coordinated role of biofilms and nanotechnology in the field of agriculture.

Biofilms in agriculture

Agriculture forms an important economic sector worldwide, and it is always an ongoing effort to bring improvements in the way farming is done to attain wholesome benefits. The focus is not only on increased crop productivity but also the maintenance of soil health and fertility. In this direction, the multi-faceted promotion of plant growth by application of plant growth-promoting rhizobacteria (PGPRs) has been well recognized (Gouda et al. 2018). Microorganisms are the most abundant of all living systems and play a crucial role in ecological maintenance of the natural environment (Velmourougane et al. 2017). PGPRs have been extensively used as biofertilizers (Katiyar et al. 2016), biocontrol agents (Babu et al. 2015), and bioremediators (Etesami and Maheshwari 2018). They have also been known to stimulate plant growth by providing phytohormones, ammonia, enzymes, and other secondary metabolites of economic importance (Gouda et al. 2018). A diverse range of microorganisms falls under the umbrella of PGPRs and colonizes different plants. These include *Azospirillum*, *Azotobacter, Gluconacetobacter*, *Bacillus*, *Paenibacillus*, *Rhizobium*, *Pseudomonas*, *Pantoea*, and *Herbaspirillum* to name a few.

Application of PGPRs has largely involved the use of suspended cultures, and role of biofilms formed by these microorganisms has been considerably under-investigated. Bacteria ensconce in and around the roots of a plant and form associations with roots in a way that they beneficially survive. Biofilms help maintain cell density for long enough duration to initiate hostile or favorable responses when interacting with the plant. PGPRs exhibit their effects on plants via direct or indirect mechanisms, that is, they limit the role of phytopathogenic microorganisms, up-regulate the availability of macro and micronutrients from the environment and can be used as a low-cost and environment-friendly technology for the alleviation of plant stresses (Pathania et al. 2020b). PGPR biofilms can act as anti-pathogenic, besides providing sustained benefits of beneficial substances excreted by the microorganisms. Environmental stresses such as salinity, drought, flooding, and pathogens can also be mediated by using PGPR biofilms (Rekadwad and Khobragade 2017).

Pseudomonas, *Rhizobium*, *Bacillus*, and other PGPRs are capable of forming biofilms on roots and associated structures and influence plant growth and development. These microorganisms express characters such as motility, exopolysaccharide production, competitive colonization, and biocontrol against pathogenic organisms. Bais (2004) reported that biofilms formed by *Bacillus subtilis* were responsible for biocontrol of *Pseudomonas syringae* infecting *Arabidopsis* roots. Their results strongly supported the role of surfactin, a non-ribosomally synthesized antibacterial compound, towards biocontrol in conjunction with biofilm formation. Guo et al. (2004) stated that *Serratia* sp. and *Bacillus* sp. colonize the root rhizosphere of tomato plant and act as biocontrol agents against tomato wilt caused by *Ralstonia solanacearum*. Watt et al. (2006) studied field-grown wheat roots for natively associated bacteria and found biofilm formation that extended 2–30 μm from the root surface. Fujishige et al. (2006) upon their observations of biofilm formation by *Sinorhizobium meliloti* and *Rhizobium leguminosarum* suggested that both overproduction and underproduction of exopolysaccharides and lack of flagella in fast-growing *Sinorhizobium meliloti* leads to reduced biofilm phenotypes and a change in the nodulation ability on host plants alfalfa and white sweet clover. This study reflected the role of exopolysaccharides and the presence of flagella for establishment of rhizobial biofilms in the rhizosphere of host plant. Zhang et al. (2013) reported that colonization and biofilm formation by *Bacillus* strains in the banana and cucumber rhizospheres is driven by root exudates. Yaryura et al. (2008) reported similar results for the colonization of soybean seeds. Singh and Siddiqui (2014) reported that biofilm formation by *Bacillus subtilis*, *Aspergillus awamori*, and *Pseudomonas fluorescens* in the root rhizosphere of plants infected by *Meloidogyne javanica*, *Ralstonia solanacearum*, and *Xanthomonas campestris* pv. *vesicatoria* causing the wilt–leaf spot disease complex of tomato resulted in reduced galling, nematode multiplication, wilt, and leaf spot disease indices and positively influenced plant growth and chlorophyll level. Mallick et al. (2018) reported that arsenic contamination in crops causes many health hazards. Rhizo-inoculation and subsequent biofilm formation by *Kocuria flava* and *Bacillus vietnamensis* influenced the growth of rice seedlings positively and decreased the uptake and accumulation of arsenic by plants. Patel and Minocheherhomji (2018) have cited the importance of biofilms in maintaining moisture levels, its function as a biocontrol agent, and maintenance of osmotic pressure under saline stress. Kasim et al. (2016) reported the plant growth-promoting activity and positive effect of biofilm formation in amelioration of salt stress in the root rhizosphere of barley crops resulting in improved growth parameters.

Seneviratne et al. (2011) reported that use of biofilm-based biofertilizers combined with reduced quantities of chemical fertilizers resulted in a 20% increase in soil organic carbon content and a 40% decrease in leaf transpiration thereby supporting plant growth and aiding the process of rhizo-remediation. Balasundararajan and Dananjeyan (2019) demonstrated that various bacteria that can produce biofilms through AHL-mediated quorum sensing are present in the rice rhizosphere and a careful selection of PGPRs among them can lead to development of biofilm-forming inoculants. Ricci et al. (2019) reported an enhance in root and shoot dry weight, leaf area, root length, and plant height of tomato plants on inoculation of biofilm inoculants of *Bacillus* and *Pseudomonas*. *Paenibacillus* biofilms are more competitive over other PGPRs. *P. polymyxa* was reported to produce a plant growth-promoting compound similar to IAA and isopentenyladenine (cytokinin-like compound) that positively affected the growth in *Agropyron cristatum* (Holl 1988). The commercial scale-up of *Paenibacillus* is a more user-friendly process because of its spore-forming ability, thereby making it a favorable bio-inoculant (Chauhan et al. 2015). Application of various PGPRs and their subsequent impact on different plants is also listed in Table 1.

Many of the PGPR strains are inadvertently capable of biofilm formation. More focus on this ability of PGPRs can greatly assist in combating the persistent problem of lowered cell numbers while applying bioinoculants at the field scale. This can eventually contribute to augment the output of sustainable agricultural practices.

Potential role of nanoparticle-entrapment by biofilms in biocontrol

Nanomaterials are increasingly finding applications in various fields of health, industry, and environment (Vijayaraghvan and Ashokkumar 2017). In agriculture, applications of nanomaterials include efficient, slow-releasing plant growth and protection products such as fertilizers, pesticides, seed cover treatments, improved pathogen detection systems, and improved delivery systems. Nanotechnology in agriculture makes possible the advancements in farm mechanization practices and is directed towards promoting economic growth and safeguarding the environment.

One potential benefit of nanoparticles is their use for control of pathogenic microorganisms that pose a major threat to crop productivity. With an increasing demand for organic agriculture, the use of different agrochemicals for disease suppression is discouraged. Biological control of pathogens is an effective alternative which also provides the benefit of soil sustainability. Biocontrol agents are self-replicating and therefore limit the need for repeated application. The problem of resistance to these agents of disease control is also not encountered. Several PGPRs are capable of preventing various plant and postharvest diseases and offer defense against pathogens. Besides, several mechanisms such as the production of antibiotics (Ali et al. 2015), siderophores (Sabet and Mortazaeinezhad 2018), enzymes and other secondary metabolites, production of phytoalexins (Jeandet et al. 2014), and induced systemic resistance offer biocontrol (Pangesti et al. 2016). However, the rhizospheric cell count of inoculated PGPRs decreases variedly resulting in less pronounced effects of their beneficial properties. Effective biofilm formation in the rhizosphere and rhizoplane can assist to maintain a high population of cells for prolonged time durations and with sustained biocontrol advantages. Overall, the complex interaction between bacteria that exhibit biocontrol ability along with other plant growth promotion characteristics, plants, and pathogens results in varying degrees of disease suppression, plant growth, and soil health. Nanoparticles can play a significant role in improving the levels of biocontrol.

Microbial synthesis of nanoparticles is eco-friendly and can be carried out both intracellularly and extracellularly at ambient temperature and pressure conditions (Vetchinkina et al. 2018). In a biofilm, high biomass concentration, larger surface area, and the reducing environment of EPS result in more efficient production of nanoparticles. Kang et al. (2014) reported the reduction Ag^+ to AgNPs by the polysaccharide component of EPS of *E. coli* biofilms. Lin et al. (2014) and Singh et al. (2015) reported that c-type cytochromes, peptides, periplasmic enzymes like nitrate reductase, and reducing cofactors are responsible for AgNP synthesis. Cao et al. (2011) detected the role of two c-type cytochromes in EPS from *Shewanella* sp. in the extracellular electron transfer process for nanoparticle synthesis. Li et al. (2016) reported the presence of cytochrome c in the EPS of three electro-active bacteria and its role in the production of silver nanoparticles.

Entrapment of nanoparticles in polymeric matrices helps to impart biocompatibility, have controlled release, and reduce toxicity levels. Since EPS is a naturally occurring polymeric matrix, it can be used to embed nanoparticles to impart them a biological identity. This improves the uptake and retention of nanoparticles by cells. The presence of EPS leads to the concentration, partitioning, and transformation of nanoparticles. Several groups have suggested that biofilms can act as binding matrices for trapping nanoparticles due to the presence of EPS (Flemming and Wingender 2010; Nevius et al. 2012; Kroll et al. 2014). Also, the size and charge of the nanoparticle and variable density of the biofilm control the diffusion of nanoparticles across the biofilm. This has been studied on several nanoparticles such as dextrans, fluorescent microspheres, AgNPs in situ in a biofilm composed of *Pseudomonas fluorescens* (Peulen and Wilkinson 2011).

Nanoparticle-entrapped biofilms can be used to provide enhanced biocontrol ability against pathogenic strains. For

Table 1 Impact of plant growth-promoting rhizobacteria on different plants

PGPR	Beneficial trait	Response in host plant	Host plant	Reference
Bacillus subtilis	Biofilm formation	Biocontrol	Mouse-ear cress	Rudrappa and Bais 2007
Bacillus amyloliquefaciens	Chemotaxis Growth Biofilm formation	Profuse colonization of seeds and roots	Soybean	Yaryura et al. 2008
Trichoderma–Bacillus *Trichoderma–Pseudomonas*	Biofilm formation Antifungal activity Ammonia excretion Indole acetic acid production Siderophore production	High germination of cotton seeds	in vitro	Triveni et al. 2013
Trichoderma–Azotobacter	Biofilm formation High nitrogenase activity High ACC deaminase activity	-	in vitro	Triveni et al. 2013
Bacillus subtilis	Biofilm formation Matrix production	Biocontrol	Tomato	Chen et al. 2013
Bacillus thuringiensis *Bacillus cereus* *Bacillus megaterium* *Bacillus pumilus* *Paenibacillus polymyxa*	Biofilm formation ACC deaminase activity High phosphate solubilization High salt tolerance	Drought tolerance Higher photosynthetic activity Higher biomass production	Wheat	Timmusk et al. 2014
Bacillus amyloliquefaciens	Biofilm formation Chemotaxis	Profuse root colonization	Banana	Yuan et al. 2015
Ochrobactrum intermedium	Indole acetic acid production Siderophore production ACC deaminase activity High temperature tolerance High salt tolerance Increased phosphatidylcholine biosynthesis	Increased shoot length Increased root length Higher dry weight	Groundnut	Paulucci et al. 2015
Bacillus amyloliquefaciens	Biofilm formation	Salt stress tolerance	Barley	Kasim et al. 2016
Pseudomonas anguilliseptica	Biofilm formation Exopolysaccharides production	Salt stress tolerance	Faba bean	Mohammed, 2018
Kocuria flava *Bacillus vietnamensis*	High salt tolerance Arsenic resistance Biofilm formation	Growth promotion Decreased uptake and accumulation of arsenic in plants	Rice	Mallick et al. 2018
Aeromonas sp.	Biofilm formation Cadmium resistance	Removal of cadmium Increased root length Increased shoot height	Vetiver grass	Itusha et al. 2019
Bacillus amyloliquefaciens	Biofilm formation	Drought tolerance	Tomato	Wang et al. 2019
Pseudomonas fluorescens *Bacillus licheniformis*	Indole acetic acid production Siderophore production Ammonia Excretion Phosphate solubilization Biofilm formation Production of EPS and alginate Swarming motility	Improved vegetative growth Increased chlorophyll content Increased transpiration rate Increased stomatal conductance Increased internal CO_2 concentration Higher net photosynthetic rate Higher leaf water potential	Wheat	Ansari and Ahmad 2019
Aeromonas hydrophila *A. enteropelongenes* *Aeromonas veronii* *Enterobacter* sp. *Klebsiella pneumoniae* *Kosakonia cowanii* *Providentia rettigeri* *Sphingomonas aquatilis* *Pseudomonas sihuiensis*	Biofilm formation Production of *N*-acyl homoserine lactone as the QS signal	Profuse root colonization	Rice	Balasundararajan and Dananjeyan 2019

Table 1 (continued)

PGPR	Beneficial trait	Response in host plant	Host plant	Reference
Bacillus licheniformis Pseudomonas plecoglossicida	Biofilm formation Phosphate solubilization Indole acetic acid production ACC-deaminase activity Oxidative stress reduction by upregulation of antioxidant enzymes (catalase, superoxide dismutase and guaiacol peroxidase)	Halotolerance	Sunflower	Ahmad et al. 2020
Pseudomonas fluorescens Pseudomonas putida *Bacillus safensis*	Biofilm formation Lead tolerance	Enhanced growth and yield Improved antioxidant profile Increased lead uptake in roots and shoots Decreased lead uptake in seeds	Rapeseed Clover	Shah et al. 2020
Bacillus sp.	Biofilm formation Salt tolerance Drought tolerance	Improved plant growth Profuse root colonization	Tomato	Pathania et al. 2020a

this purpose, several nanoparticles have been studied. These include nanoparticles of silver (Mahawar et al. 2020), titanium oxide (Palmqvist et al. 2015), carbon (Raliya et al. 2013), zinc oxide (Sabir et al. 2014), and silica (Rangaraj et al. 2014). Nanosilver is the most studied nanoparticle for biological systems with strong antimicrobial effects. It kills unicellular microorganisms by inactivating enzymes having metabolic functions. Duran et al. (2005) stated the extracellular synthesis of AgNPs by *Fusarium oxysporium* and mentioned its potential as an antibacterial agent. However, there are limited studies on the use of nanosilver for controlling plant diseases (Lamsal et al. 2011). Prasad et al. (2012) treated peanut seeds with different concentrations of ZnO nanoparticles and reported improved seed germination, seedling vigor, and plant growth. Dimkpa et al. (2013) addressed the potential of ZnO nanoparticles and biocontrol bacterium, *Pseudomonas chlororaphis* O6 in *Fusarium* control strategies. They found ZnO NPs to be inhibitory to fungal growth compared to the micro-sized particles of ZnO. The ZnO NPs also did not prevent metabolites of *Pseudomonas chlororaphis* O6 from inhibiting *Fusarium* growth. Their findings of pathogen growth control suggest the use of ZnO NP-based formulations in addition to the existing strategies of improving crop health. Rangaraj et al. (2014) studied the impact of nano-silica and *Pseudomonas* sp. to enhance the biocontrol activity against maize pathogens. Treatment with nano-biocomposites led to higher phenol content and hardness in leaves inducing silica accumulation and thereby initiating more effective physical barriers for crop protection. Vishwakarma et al. (2019) suggested the role of silicon, PGPR, and indole acetic acid (IAA) in reversing stress induced by AgNP in *Brassica juncea.* They found that silicon in combination with AgNPs enhanced toxicity towards *B. juncea* whereas PGPR and IAA reduced toxicity and promoted plant growth. Combined treatments of AgNP, Si, PGPR/IAA, however, reduced AgNPs induced stress and enhanced plant growth. Nitric oxide (NO) is known to play a crucial role in plant growth and defense. Pereira et al. (2015) investigated the NO release kinetics from the alginate/chitosan nanoparticles containing GSNO (S-nitrosoglutathione) or GSH (glutathione) and observed sustained and controlled NO release from nanoparticles containing GSNO. Plant assays showed no significant inhibitory effects on the development of *Zea mays* and *Glycine* sp. They concluded that alginate/chitosan nanoparticles could be effectively used as controlled release systems applied by the foliar route.

Nandini et al. (2017) synthesized *Trichoderma*-mediated selenium nanoparticles (SeNPs) and studied the control of downy mildew disease in pearl millet. The SeNPs were found to suppress growth, sporulation, and zoospore viability of *Sclerospora graminicola* and the biological activities were inversely proportional to the size of SeNPs. Under greenhouse conditions also they observed that a combined application of SeNPs and *T. asperellum* enhanced early plant growth and suppressed disease incidence. Abdelmoteleb et al. (2017) characterized silver nanoparticles from *Prosopis glandulosa* leaf extract and reported their biocontrol activity against *Acinetobacter calcoaceticus* and *Bacillus cereus*. Byczyńska (2017) reported the beneficial effects of nano-silver on horticultural crops and concluded that nano-silver may be used in horticultural practice as a potential plant growth regulator. Applications of nano-silver increased plant height, leaf number, plant biomass, seed germination, root length, increased content of chlorophylls, carotenoids, and flavonoids to name a few. Hassan et al. (2018) used the biomass filtrate of endophytic actinomycete *Streptomyces capillispiralis* for the rapid and eco-friendly synthesis of copper nanoparticles. These biosynthesized copper nanoparticles were able to exhibit biocontrol activity against infectious microorganisms, phytopathogenic fungi, and health-threatening insects. Pour et al. (2019) studied micropropagation in pistachio by the nanoencapsulation of plant growth-promoting rhizobacteria and their metabolites in silica nanoparticles and carbon nanotubes and obtained a significant improvement in root and shoot dynamics by the application of nanoencapsulated *Pseudomonas fluorescens* and *Bacillus subtilis* strains. Nawaz and Bano (2019) evaluated the role of *Pseudomonas putida* and AgNPs on two varieties of cucumber plants and concluded that the PGPR along with AgNPs can increase the antioxidant and defense enzyme activities of the plant to enable it to withstand different types of stresses. Mahawar et al. (2020) determined the biocontrol efficacy of a cyanobacterium *Calothrix elenkinii*, AgNPs, and the complex and found that foliar application of the complex significantly reduced the infestation by *A. alternata* in tomato plants along with significant increases in leaf chlorophyll, carotenoid content, and polyphenol oxidase activity.

The interaction between the introduced microorganism and root exudates is quite complex and different NPs can affect microbial and plant health. Bonebrake et al. (2018) studied the correlation of artificial root exudates with biofilm formation and response to nanoparticles. A root-mimetic hollow fiber membrane (HFM) was constructed and biofilms of two microbial isolates, a *Bacillus* endophyte, and a *Pseudomonas* root surface colonizer, were examined. Whereas the *Bacillus* isolate sparsely colonized the HFM, the pseudomonad formed sturdy biofilms that were minimally affected by ZnO NPs. However, when CuO NPs were added before the biofilm had maturated, the biofilm formation was greatly reduced. Timmusk et al. (2018) used nano-titania as agents of interaction between plants and PGPR. They used a formulation of harsh environment PGPR strains and monitored its effect on wheat under conditions of biotic and abiotic stress (salt, drought, and pathogen). They report that nano-titania can attach stably to plant roots and induce a positive interaction between the plant and PGPR colonization. Their study emphasizes the importance of natural soil nanoparticles for PGPR applications. The beneficial role of various nanoparticles cited in the field of agriculture is also listed in Table 2.

There are also reported some cases where nanoparticles have been observed to cause biofilm degradation (Table 3). These have been reported in the biofilms of *Pseudomonas*, *E. coli*, *Staphylococcus*, *Candida*, and *Helicobacter*. The degradation effects have been brought about by the nanoparticles of silver, titanium, iron, copper, and zinc, to name

Table 2 Beneficial role of various nanoparticles in the field of agriculture

Nanoparticles	Benefits	References
Carbon nanotubes	Delivery of agrochemicals targeting the host plants	Raliya et al. 2013
Mn/ZnS quantum dots	Live imaging in plant root systems	Das et al. 2015
Gold nanorods	Transportation of 2,4-D leading to better plant growth	Nima et al. 2014
Gold nanoparticles	Improvement of agricultural products and biosensing Concentration-dependent antimicrobial activity against *Escherichia coli*, *Aeromonas hydrophila*, and *Klebsiella pneumoniae*	Kandasamy and Prema 2015 Vidotti et al. 2011 Aziz et al. 2016
Chitosan/tripolyphosphate Chitosan-saponin Chitosan-Copper nanoparticles	Antifungal activity	Saharan et al. 2013
Alginate/chitosan nanoparticles Alginate/chitosan nanoparticles containing gibberellic acid	Controlled release of agrochemicals Seed treatment	Silva et al. 2011 Pereira et al. 2019
Zinc oxide nanoparticles	Inhibition of *S. aureus* Useful in photocatalysis Nanofertilizer	Liu et al. 2009 Ong et al. 2018 Dimpka et al. 2020
Magnesium oxide nanoparticles	Antibacterial activity against *Ralstonia solanacearum*	Lin et al. 2018
Copper oxide nanoparticles	Enhanced seedling growth Improved plant growth	Pelegrino et al. 2020
Ceramic nanoparticles	Catalysis, photocatalysis, photodegradation of dyes and imaging	Thomas et al. 2015
Lipid-based nanoparticles	Drug delivery	Puri et al. 2009
Silver nanoparticles	Antibacterial properties	Duran et al. 2005
Silicon nanoparticles	Biocontrol activity against maize pathogens	Rangaraj et al. 2014
Copper nanoparticles	Growth inhibition of *Phytophthora cinnamomi* and *Pseudomonas syringae*	Banik and Pérez-de-Luque 2017
Selenium nanoparticles	Suppression of growth, sporulation and zoospore viability of *Sclerospora graminicola*	Nandini et al. 2017

Table 3 Role of various nanoparticles in biofilm degradation

Nanoparticles	Organisms affected	References
Zinc nanoparticles	*S. aureus*, *S. epidermidis*, *S. pyogenes*, *B. subtilis*, *E. coli*	Jones et al. 2008
Gold nanoparticles	*E. coli*, *Enterococcus* sp., *S. aureus*, *Candida albicans*	Roe et al. 2008
Titanium nanoparticles	*S. epidermidis*	Tang et al. 2013
Iron nanoparticles	*S. epidermidis*, *E. faecalis*	Webster 2009 Chifiriuc et al. 2013
Lipid polymer nanoparticles	*H. pylori*	Cai et al. 2015
Ciprofloxacin-loaded poly (lactic-co-glycolic acid) nanoparticles	*P. aeruginosa*	Baelo et al. 2015
Silicon dioxide capsule nanoparticles	*E. coli*, *P. aeruginosa*, *S. aureus*	Duncan et al. 2015
Copper nanoparticles	*P. aeruginosa*	LewisOscar et al. 2015
Ferumoxytol nanoparticles	*S. mutans*	Liu et al. 2018
Chitosan nanoparticles-cellobiose dehydrogenase-deoxyribonuclease I	*C. albicans* *S. aureus*	Tan et al. 2020
Zinc oxide nanoparticles	Methicillin-resistant *S. aureus*	Banerjee et al. 2020

a few. However, Banik and Pérez-de-Luque (2017) reported that CuNPs along with non-nano-copper such as copper oxychloride (CoC) recorded growth inhibition of the oomycete *Phytophthora cinnamomi*, *Pseudomonas syringae* and inhibition of mycelial growth and sporulation of *A. alternata*. These CuNPs were not significantly biocidal against the beneficial *Rhizobium* spp. and *Trichoderma harzianum*. The beneficially interactive effect of CuNPs and CoC in inhibiting *P. cinnamomi* offers an avenue for developing fungicidal formulations for effective management of oomycetes.

Therefore, the still-nascent field of nanomaterial-based applications in agriculture involves the permeation of biotechnology, particularly the potential of PGPRs, with nanotechnology and aims to change traditional agriculture to precision agriculture. Nanoparticles can target specific phytopathogens and offer new ways of biocontrol. The nanosensor devices can assist in the early detection of disease incidence. Management strategies can be aimed at the application of stable formulations of nano fertilizers and nano pesticides, giving a boost to crop protection and high production.

Future prospects and conclusion

A major challenge in the application of PGPRs is the stable colonization of applied strains in the rhizosphere. In this regard, use of biofilm-based biofertilizers can be a very effective strategy. Naturally occurring biofilms mostly employ single species inoculations. However, multispecies biofilms are capable of producing new polysaccharides with unique compositions along with several bioactive substances of higher agricultural importance as compared to single-species biofilms. In future, these biofilms can be developed into sprays for herbigation to reduce biotic stress in crop plants. Gene expression and signalling in these multi-species biofilms also need to be explored to discover the chemical and physical aspects of these biofilms to appropriately use them for rhizosphere colonization and biocontrol potential.

Along with this, nanotechnology has the potential to offer many improvements in the agricultural sector. Use of biosensors and metagenomic tools has improved the understanding of microbial activity that contributes towards sustainable agriculture. Further, incorporation of nanomaterials in biosensors can enable enhanced detection of key microbial players and needs to be explored. There is also a requirement to extensively analyse the interactions between nanoparticles and bacterial cell surfaces.

Although the contributions of nanoparticles can be huge, long-term risk assessment on microorganisms of ecological importance is necessary under field conditions. There is also a need to gain more understanding regarding the influence of nanoparticles in soil, effect of different soil types and soil characteristics on transformations and mobility of nanoparticles, activity and interaction of nanoparticles in the presence of other soil components like oxides and hydroxides of metals, enzymes, and organic components.

In conclusion, biofilms offer several advantages in plant microbe interaction and can positively impact soil fertility, provide plant protection, and improve crop production. A confluence of microbial biofilms with nanotechnology can potentially provide suitable environment for the development of smart agricultural technologies. However, it would be prudent to establish standards for the engineering, application and disposal of nanoparticles in order to avoid long term ramifications especially in the application of PGPRs.

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