# Role of Actinomycete-Mediated Nanosystem in Agriculture

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**Abstract**

Actinobacteria are a group of microorganisms sharing the common behaviour of both bacteria and fungi known to play a multifunctional role in agricultural production systems. The major functions include the production of a wide array of growth-promoting compounds and metabolites including antibiotics that provide the host plants to withstand both biotic and abiotic stress conditions. Consequently, actinobacteria are often employed as a biocontrol agent (BCA) against dreadful plant pathogens. Further, actinobacteria colonized host plants and elute growth-promoting substances that assist in favouring stimulated growth of plants even under harsh environmental conditions such as nutrient deficiencies, drought, salinity and heavy metal contaminated soils. Several actinobacteria are involved in the nutrient solubilization and mobilization particularly phosphates and iron besides facilitating as helper bacteria in mycorrhizal symbiosis and biological nitrogen fixation. These groups of organisms also are responsible for the production of a volatile compound called "geosmin" which often referred as a biological indicator of soil fertility. Recently, large volume of research reports suggest that actinobacteria are capable of producing metal oxide nanoparticles that can be exploited in the green synthesis of nanomaterials and utilized in biological systems. Overall, the multifunctionality of actinobacteria makes this group of microorganisms very unique, and their potentials are yet to be exploited. This book chapter highlights the

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potential role of actinobacteria in growth promotion, biocontrol, alleviation of abiotic stresses and biosynthesis of metal oxide nanoparticles.

## 15.1 Introduction

Agricultural productivity has been hindered by extensive and indiscriminate use of chemical fertilizers and pesticides. Such activity has created concerns over the fertility of soil and environmental health. The use of plant growth-promoting and disease-suppressing beneficial microbes for improving the crop productivity has been considered as a viable alternative to chemical route. Actinobacteria are known to produce several plant growth-promoting substances and suppress plant diseases by secreting several compounds such as secondary metabolites and hence are considered to be important candidates for improving the agricultural productivity (Golinska et al. 2015). Further, these groups of organisms are known to improve the soil fertility through the rapid decomposition of crop residues (Abdulla 2007). Such processes are quite important to enhance the availability of nutrients without associated environmental hazard. Actinobacteria were detected even in root nodules of woody plants in forests indicating their role in biological N fixation. Baker et al. (1979) have reported first time that the actinobacteria are associated with the root nodules of *Elaeagnus umbellata* (Elaeagnaceae) and *Alnus viridis* ssp. *crispa* (Betulaceae). In addition, actinobacteria are involved in the solubilization of P. *Streptomyces galbus* inoculation to crop plants resulted in increased availability of P (Sahu et al. 2007). The multiple benefits of actinobacteria towards plant growth promotion (PGP), biocontrol agent (BCA) activity and soil fertility improvement for crop growth and productivity can be explored in order to gain insights
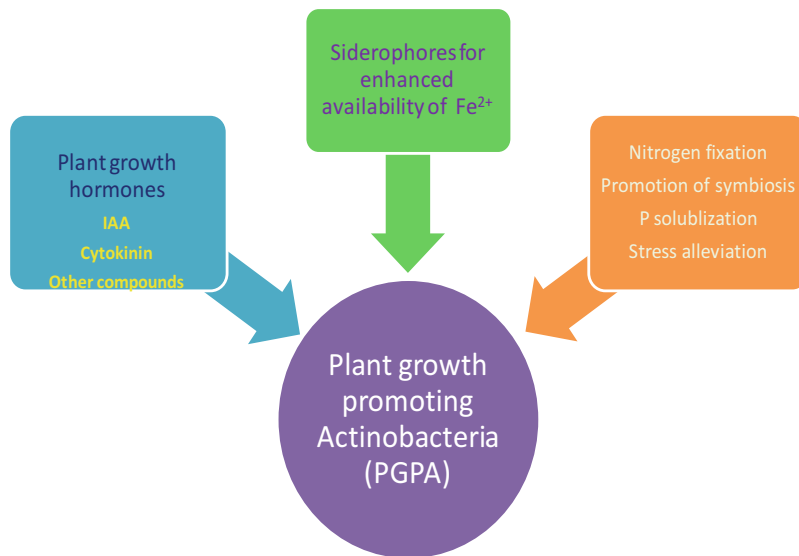
into the mechansims associated their roles. Recently, several reports have shown that actinobacteria can be exploited in the green synthesis of metal oxide nanoparticles which may be used for improving the productivity of crops (Sadhasivam et al. 2010; Sivalingam et al. 2012; Krishnakumar and Bai (2015)). This book chapter highlights the multifunctional roles of actinobacteria in soil fertility and crop management systems.

## 15.2 Ecology and Distribution of Actinobacteria

Actinobacteria are a diverse group of Gram-positive, spore-forming, anaerobic free-living saprophytic bacteria mostly associated with plant roots and soil. Molecular and phylogenetic analyses based on 16s rDNA revealed that actinobacteria phylum is one of the largest taxonomic units among 18 major lineages of bacteria and their DNA is constituted to have more than 70 % G+C content (Ventura et al. 2007). Due to their typical unicellular and filamentous morphology, their survival in the soil or any hostile environment becomes long-lasting. It was widely thought that actinobacteria are only soil inhabitants; however, genomic studies revealed that they are present in both freshwater and extreme environments such as thermal hot springs and Antarctic caves (Bentley et al. 2004). Actinobacteria plays an important role in the decomposition of organic matter and formation of humus; many plant-associated bacteria secrete plant growth regulators such as indole-3-acetic acid (IAA), cytokinin and other

**Fig. 15.1** Plant growth-promoting properties of actinobacteria

Siderophores for enhanced availability of $Fe^{2+}$

Plant growth hormones
IAA
Cytokinin
Other compounds

Nitrogen fixation
Promotion of symbiosis
P solublization
Stress alleviation

Plant growth promoting Actinobacteria (PGPA)

compounds like pteridic acids (Palaniyandi et al. 2013b). Root-associated actinobacteria help the plant by sequestering iron and enhancing the availability of iron near the root region by producing siderophores. *Frankia*, an endophytic actinobacteria associated with roots of forest plants, are important nitrogen fixers. The secondary metabolites produced by genera *Streptomyces* spp. are effective BCA in suppressing plant pathogens and thereby indirectly promoting the symbiosis between beneficial microbes and plants. Besides this, actinobacteria are known to dissolute nutrients such as P from its metallic complexes. More importantly, actinobacteria alleviate plant stresses by reducing the ethylene level in the root by secreting 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme (Hamedi and Mohammadipanah 2015).

## 15.3 Plant Growth-Promoting Actinobacteria (PGPA)

The first conclusive evidence on plant growth promotion by the inoculation of beneficial microorganisms onto the seeds was reported by Kloeppler and Schorth (Bloemberg and Lugtenberg 2001). A diagrammatic illustration of plant growth-promoting traits of actinobacteria is presented in Fig. 15.1. Actinobacteria promote primarily plant growth by stimulating hormones, improved availability of iron, nitrogen fixation and symbiosis, P solubilization and stress alleviation.

### 15.3.1 Production of Plant Growth Hormones

Actinobacteria facilitate the production of plant hormones such as IAA and cytokinin that are closely associated with plant growth (Ghosh et al. 2011). These hormones in the rhizosphere enhance plant growth by stimulating lateral root development, root hairs and release of sugars. These physiological effects secreted on the plants have multi-beneficial role, for instance, the sugars released in the root region act as a nutrient source for beneficial microbes which colonize the root and create scarcity of nutrients to pathogens and eventually suppress the disease occurrence (Boukaew et al. 2013).

### 15.3.2 Indole Acetic Acid Production

Several actinobacteria are known to produce IAA in considerable quantities (Marschner 1995;

Unyayar et al. 2001; Ghosh et al. 2011). Production of IAA in *Streptomyces* is tryptophan dependent, and it follows the route of indole acetamide (Lin and Xu 2013). *Streptomyces filipinensis* no. 26 isolate promoted the growth of tomato grown under greenhouse conditions by stimulating the root and shoot length and produced IAA at a concentration of 77.43 μg/100 g of dry weights on the roots (Khamna et al. 2009). A significant quantity of IAA (52.3 μg.ml$^{-1}$) was secreted by *Streptomyces* sp. isolated from the rhizosphere region of medicinal plants (Khamna et al. 2009). Maximal IAA secretion of 143 μg.ml$^{-1}$ was also observed for *Streptomyces* sp. isolated from the rhizosphere region of medicinal plants (Manulis et al. 1994). Similarly, many actinobacteria are known to produce IAA and reported to increase plant shoot and root lengths. Although above-reported cultures are known to produce only IAA, an interesting fact of three actinobacterial species, namely, *Streptomyces olivaceoviridis*, *S. rimosus* and *S. rochei* cultures, was that they produced all three growth hormones, viz. auxins, gibberellins and cytokinin-like substances, and enhanced the growth of wheat plants (Aldesuquy et al. 1998). Similarly, an interesting correlation between IAA production and growth promotion was established. In the study of screening functional and genetic diversity of activitobacteria were studied in yam rhizosphere soil and found that out of 29 isolates screened, 28 had produced IAA and 11 stimulated the growth of *Arabidopsis* in vitro; the reason for the lack of positive correlation for the rest of isolates was explained as due to the inhibitory effect of phytotoxins on IAA and additional factors requirement for effective functioning of IAA (Palaniyandi et al. 2013b).

### 15.3.3 Cytokinin and Other Plant Growth Substance Production

A very few reports are in support of cytokinin-producing actinobacteria that are lesser in numbers in comparison to IAA producers. Cytokinin-producing isolates are rarely found in species such as *Streptomyces turgidiscabies* and

*Rhodococcus fascians*, but they are pathogenic and produce leafy galls on tobacco leaves (Joshi and Loria 2007). The endophytic actinobacterium *Streptomyces hygroscopicus* was reported to produce pteridic acids A and B with auxin-like activity that enhanced the formation of adventitious roots in hypocotyls of kidney beans (Ortíz-Castro et al. 2008). Similarly, root-promoting hormonelike substances were observed on tissue culture seedlings of rhododendron by an endophytic *Streptomyces* sp. (Joshi and Loria 2007).

## 15.4 Soil Fertility

Actinobacteria are known to produce "geosmin" which is responsible for the soil flavour or earthy odour after the rain. Geosmin is a volatile compound produced by *Streptomyces* and released when these microorganisms die. The biosynthesis of geosmin by a bifunctional *Streptomyces coelicolor* enzyme was unveiled (Jiang et al. 2006, 2007). A single enzyme, geosmin synthase, converts farnesyl diphosphate to geosmin in a two-step reaction. Geosmin is a bicyclic alcohol ($C_{12}H_{22}O$) often used as the biological fertility of soil. The soil with intense "geosmin" is likely to be more fertile than the soil with less. The human nose is capable of detecting geosmin up to five parts per trillion. In addition to the indicator of soil fertility, actinobacteria can also be involved in the biogeocycling of nutrients particularly nitrogen, phosphorous and iron.

### 15.4.1 Iron Chelation

Iron is an important mineral for the growth of plants and metabolism of microorganisms which presents as an insoluble form ($Fe^{3+}$) in the soil. Plants and microbes can readily take up iron in the soluble form of $Fe^{2+}$ by reduction of iron from $Fe^{3+}$ to $Fe^{2+}$ which enhances the bioavailability of iron for plants and microbes. Direct reduction of iron was reported in some actinobacterial strain; *Arthrobacter maltophilia*

inoculation on common bean promoted the growth by reducing the iron in alkaline soil. Another mechanism by which plants can take up iron is by siderophores. Actinobacteria in the rhizosphere produce siderophores which chelates the iron in the $Fe^{3+}$ form, and the plants such as oats assimilate iron via siderophore. It has been reported that both hydroxamate, catechol-type siderophores and mixed-type siderophores are secreted in root-colonizing rhizobacteria (Valencia-Cantero et al. 2007). Some actinobacteria deprive iron from the rhizosphere by sequestration, and as a result the high iron affinity pathogens can't access it, and hence the population can be controlled (Crowley et al. 1991).

It has been observed that siderophore production not only chelates iron but also reduced the nickel stress in plants. It has also been observed that there were increased concentrations of N, P, Fe, and Mg in the wheat shoots when the siderophore-producing actinobacteria were inoculated in the soil (Khamna et al. 2009; Palaniyandi et al. 2011). An interesting positive correlation of iron uptake and phytoremediation of cadmium (Cd) was observed for *Streptomyces tendae* wherein the hydroxamate type of siderophore produced by the actinobacteria not only promoted the growth of sunflower but also enhanced the uptake of Cd by the plants (Dhungana et al. 2004). The results showed that actinobacteria can also be used for decontamination of metals such as Cd in soil.

## 15.4.2 Phosphorous Solubilization

Phosphorus deficiencies in soils are wide spectrum due to the fact that the major portion of P is an unavailable form in organic complexes. In general, the available form of P is present in very low concentration (less than $1 \text{ mg kg}^{-1}$) as a result of the formation of metal complexes with Fe, Al and Si (Hamdali et al. 2008a). Phosphate solubilization is most common among actinobacteria such as *Streptomyces*, *Micrococcus*, *Micromonospora*, *Kitasatospora* and *Thermobifida*. Rock phosphate-solubilizing actinobacteria were reported to promote the

growth of wheat plants in vitro as well as in vivo (Hamdali et al. 2008b). P-solubilizing actinobacterial strains are also shown to suppress damping off caused by *Pythium ultimum* and promote the growth of wheat in a P-deficient soil. Such a dual benefit by PGPA is advantageous in increasing the crop production (Oliveira et al. 2009).

The primary mechanism of P solubilization by PGPA is due to the production of organic acid and acidification of rhizosphere thereby solubilization of unavailable to available form of P (Palaniyandi et al. 2011). Further, phosphorus availability enhancement is attributed to the chelation of cations such as $Fe^{+2}$, $Al^{+3}$ or $Ca^{+2}$, which form insoluble phosphates and thereby help in the solubilization of insoluble phosphate. Actinobacteria can hydrolyze phytate (which constitutes up to 60 % of soil organic phosphorus) by secreting phosphatases such as phytases and acidic/alkaline phosphatases (Palaniyandi et al. 2013a).

## 15.4.3 Atmospheric Nitrogen Fixation

Incorporation of gaseous N into amino acids in plants is referred as nitrogen fixation. Most extensively studied nitrogen fixation by actinobacteria is *Frankia*, which lives in symbiotic association with dicotyledons. Almost 24 genera belonging to 8 families are infected with symbiosis and are called actinorhizal plants and form nitrogen-fixing root nodules in their roots (Yamaura et al. 2010). Apart from the most commonly studied *Frankia* N fixation, a thermophilic actinobacteria *Streptomyces thermoautotrophicus* isolated from charcoal pile at 65 °C can fix atmospheric nitrogen. The enzyme nitrogenase in *S. thermoautotrophicus* is not sensitive to $O_2$, and it utilizes $N_2$ as a sole source of nitrogen (Gadkari et al. 1992) which is unique in biological nitrogen fixation. In addition to these bacteria, nitrogen-fixing capacity was also reported from the family *Thermomonosporaceae* and *Micromonosporaceae*. These bacteria were isolated from surface sterilized roots of *Casuarina equisetifolia* (Valdés et al. 2005).

## 15.4.4 Promotion of Symbiosis Between Nitrogen-Fixing Bacteria and PGPA

Many PGPA are known to influence symbiosis between nitrogen-fixing bacteria and other microorganisms. *Streptomyces lydicus* WYEC108 enhanced root nodulation in pea after inoculating with *Rhizobium* sp. It colonizes within the surface cell layers of the nodules that lead to an increase in nodule size and vigour of bacteriods by the enhancement of nodular assimilation of iron and other nutrients. On the contrary, *Streptomyces kanamyceticus* showed a negative effect through inhibiting the nodule formation by *Bradyrhizobium japonicum* (Valdés et al. 2005). However, when they co-inoculated *S. kanamyceticus* with antibiotic-resistant *B. japonicum*, it resulted in a positive effect through an increase in root nodule size and number. This observation indicates that antimicrobial substances produced by *Streptomyces* masked its capacity to facilitate nodulation (Valdés et al. 2005).

Actinobacteria such as *Streptomyces*, *Micromonospora* and *Actinoplanes* were able to influence root nodule formation by *Frankia* sp. in *Discaria trinervis* (Solans 2007; Solans et al. 2009). Another study reports that strains belonging to these genera of actinobacteria are able to influence root nodule formation by *Sinorhizobium meliloti* strain 2011 on *Medicago sativa* (Glick 2005). It was noted that co-inoculation of *Frankia* with pure mycelia from the actinobacterial strains did not promote root nodulation (Solans 2007). However, root nodulation was promoted by co-inoculation of the culture filtrates with *Frankia* sp., suggesting the presence of nodule-promoting substances in their culture filtrate (Solans 2007). In addition to promotion of nitrogen-fixing symbioses, actinobacteria were also reported to promote symbiosis between plants and mycorrhiza (Frey-Klett et al. 2007).

## 15.5 Stress Alleviation

### 15.5.1 Abiotic Stress

Plant productivity is often limited by abiotic stresses such as drought, salinity, nutrient stress and heavy metal contamination. These stresses include the production of ethylene in plants which negatively modulate plant growth (Glick 2005). PGPA are renowned for their growth-enhancing effects on several plants by various mechanisms. One such mechanism is the production of ACC deaminase that converts ACC, the precursor of ethylene in plants, into ammonia and α- ketobutyrate, thereby lowering stress ethylene level and enhancing plant growth (Glick 2005). Halotolerant non-*Streptomyces* actinobacteria such as *Micrococcus yunnanensis*, *Corynebacterium variabile* and *Arthrobacter nicotianae* were reported to exhibit ACC deaminase activity (Siddiqui et al. 2010). These strains were able to significantly promote the growth of canola plants under salt stress conditions (Siddiqui et al. 2010). Endophytic *Arthrobacter* sp. EZB4, isolated from pepper plants, possessing ACC deaminase activity significantly reduced the expression of osmotic stress-inducible genes such as CaACCO and CaLTPI (Sziderics et al. 2007). The involvement of ACC deaminase in plant growth promotion by a *Streptomyces filipinensis* no. 15 strain was demonstrated (Sziderics et al. 2007). Inoculation of tomato plants with *S. filipinensis* no. 15 significantly reduced the levels of ACC in roots and shoots and promoted the growth of the plants (Sziderics et al. 2007). ACC deaminase activity was also reported from *Rhodococcus* sp., and plants associated with the strain contained low endogenous ACC level and low stress ethylene accumulation (Francis et al. 2010). Recent studies on the actinobacterial functional diversity from yam rhizosphere revealed the ACC deaminase activity in 6 of the 29 actinobacterial strains tested. All the six ACC deaminase-containing strains belonged

to the genus *Streptomyces* (Palaniyandi et al. 2013a), which showed that the distribution of ACC deaminase activity may not be a common trait among actinobacteria. A novel type of drought stress tolerance induced by the colonization of an endophytic actinobacteria was also reported (Golinska et al. 2015), where inoculation of tissue-cultured seedlings of mountain laurel with endophytic *Streptomyces padanus* AOK-30 resulted in the accumulation of callose in the plant cell wall, which resulted in enhanced drought tolerance of the seedlings.

### 15.5.2 Biotic Stresses

Actinobacteria are one of the important antagonistic microbes known to secrete antibiotic compounds and suppress the growth of the pathogens, thereby competing for nutrients. In addition, actinobacteria exhibit several mechanisms (Fig. 15.2) such as the production of antibiotics in the rhizosphere region suppressing the disease-causing fungi and help in effective colonization of rhizosphere. Volatile antibiotics such as methyl vinyl ketone produced by actinobacteria change the morphology of several pathogenic fungi and kill them, by secretion of cell wall-degrading enzymes such as chitinase and glucanase which cause degradation of cell wall of pathogenic fungi and inhibit the growth. Several *Streptomyces* and non-*Streptomyces* are reported to parasitize pathogenic fungi by hyperparasitism which is believed to be a mechanism of pathogen control as well as competition and induction of host resistance.

**Fig. 15.2** Mechanisms of plant disease suppression adopted by actinobacteria
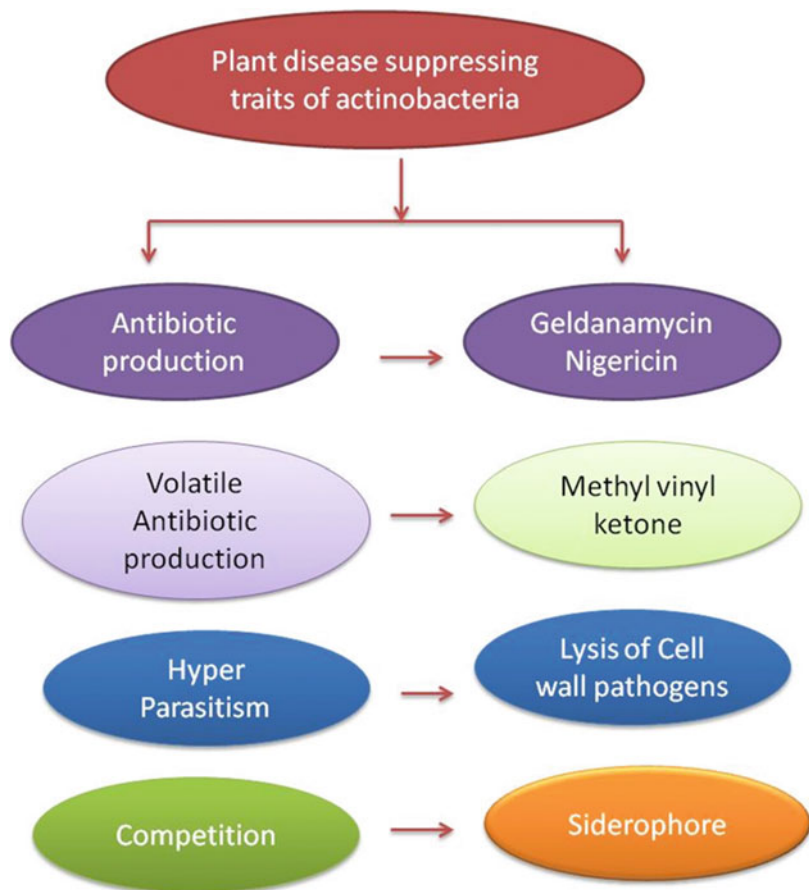
### 15.5.3 Antibiotic Production by Antagonistic Microbes

Actinobacteria are abundant antibiotic producers; 45 % of the antibiotics currently in use are produced by them. Approximately 10,000 compounds with diverse functional groups are produced by actinobacteria. Numerous studies have been reported to suppress plant diseases by actinobacteria. The first known antibiotics for control of plant disease were cycloheximide and streptomycin obtained from *Streptomyces griseus* (Trejo-Estrada et al. 1998). Similarly, geldanamycin-producing *Streptomyces* are viewed as promising BCA of several plant diseases (Samac et al. 2003). Multiple antibiotics are produced by actinobacteria which control a diverse group of pathogenic fungi; one of the strain *Streptomyces violaceusniger* YCED9 has been reported to produce nigericin, guanidylfungin A-like compound and geldanamycin, which can inhibit *Pythium*, *Fusarium* and *Phytophthora*. Similarly, azalomycin, an antibiotic compound produced by *Streptomyces malaysiensis* MJM1968, when treated in the soil as cultural filtrate resulted in the control of more than 80 % decrease in fungal population after 14 days of treatment. Azalomycin was stable over a broad range of pH and temperature and exhibited antifungal activity on *Fusarium oxysporum*, *Rhizoctonia solani*, *Cladosporium cladosporioides*, *Fusarium chlamydosporum*, *Colletotrichum gloeosporioides*, *Alternaria mali* and *Pestalotia* spp. Such type of antibiotics can be used as a broad-spectrum soil fungicide alternative to the use of chemical fungicides such as methyl bromide and metalaxyl.

### 15.5.4 Volatile Antibiotic Production

*Streptomyces* have been reported to produce volatile antifungal substances and were studied for their biocontrol efficacy on plant diseases (Herrington et al. 1987). Such volatile substances could cause several morphological abnormalities on target fungi such as *Aspergillus giganteus*, *Fusarium oxysporum*, *Penicillium viridicatum*, *Trichoderma viride* and *Zygorhynchus vuilleminii*. Methyl vinyl ketone, a volatile substance from *Streptomyces griseoruber*, inhibited spore germination in *C. cladosporioides*. Similarly, volatile compounds produced from *Streptomyces albidoflavus* TD-1 suppressed the growth of storage disease-causing fungi such as *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium citrinum* under in vitro condition (Herrington et al. 1987). GC-MS analysis revealed 27 different compounds, among which dimethyl disulfide was proved to have inhibitory activity towards *F. moniliforme* under in vitro conditions. A more detailed study was done with *Streptomyces philanthi* RM-1-138, which inhibited the growth of *R. solani* PTRRC-9, *Pyricularia grisea* PTRRC-18 and *Bipolaris oryzae* PTRRC-36. In this study, volatiles were collected on the 7th and 14th day of the incubation; volatiles collected on the 14th day had 36 compounds (in contrast with 17 compounds form 7th day) and had stronger inhibitory activity on the pathogens tested (Boukaew et al. 2013). The volatile substances were able to reduce sheath blight disease of rice caused by *R. solani* PTRRC-9 by damaging its cell wall. Volatiles from *Streptomyces globisporus* JK-1 inhibited mycelial growth, spore germination and aspersorium formation by *Botrytis cinerea* on tomato fruits and provided control over postharvest grey mould (Li et al. 2012). Volatiles from *S. globisporus* JK-1 were also inhibitory towards *Penicillium italicum* and suppressed infection of *Citrus microcarpa*. Another study showed that volatiles from *Streptomyces platensis* F-1 were able to reduce the incidence and/or the severity of leaf blight/seedling blight of rice caused by *R. solani*, leaf blight of oilseed rape caused by *Sclerotinia sclerotiorum* and fruit rot of strawberry caused by *B. cinerea*. A volatile substance from *Streptomyces* spp. was also reported to have antibacterial activity on *Bacillus subtilis* (Li et al. 2012). Volatiles produced by *Streptomyces* spp. have great potential in agriculture as biofumigants alternative to chemical fumigants such as methyl bromide, 1, 3-dichloropropane, and chloropicrin.

## 15.5.5 Induction of Host Resistance

There are two types of non-specific defence exhibited by plants that offer resistance to a broad spectrum of pathogens, namely, induced systemic resistance (ISR) and systemic acquired resistance (SAR). The type of resistance induced by rhizobacteria is called ISR, and the one induced by pathogen and salicylic acid (SA) is called SAR. Actinobacteria that are endophytic to wheat have been reported to induce defence pathways in *Arabidopsis*. These endophytic actinobacteria induced a low level of SAR and jasmonic acid/ethylene (JA/ET) gene expression. However, upon pathogen challenge, endophyte-treated plants showed high level of gene expression compared with non-treated controls. In contrast to the common understanding that pathogens induce SAR pathways, it is reported that the endophytic actinobacteria were able to induce both the SAR and JA/ET pathways (Tu 1988). Induction of JA/ET pathway resulted in resistance to the bacterial pathogen *Erwinia carotovora* subsp. *carotovora* and induction of SAR pathway resulted in resistance to the fungal pathogen *F. oxysporum*. In addition, it was also reported that the culture filtrate of an endophytic *Micromonospora* sp. strain EN43 induced SAR pathway when grown in minimal medium and induced JA/ET pathway when grown in complex medium. Similarly, endophytic *Streptomyces* sp. strain EN27 and defence-compromised mutants of *Arabidopsis* showed that resistance to *E. carotovora* ssp. *carotovora* occurred via an NPR1-independent pathway and required SA and not the JA/ET pathway. In contrast, resistance to *F. oxysporum* mediated by *Streptomyces* sp. EN27 is NPR1-dependent, required SA and is JA/ET independent. Treatment of the culture broth increased the activities of peroxidase, phenylalanine ammonia-lyase and β-1,3-glucanase in cucumber leaves, and the levels of chlorophyll and soluble sugars were also found to be increased (Schuhegger et al. 2006). Furthermore, actinobacteria were reported to occur in high abundance in the endophytic compartments of *Arabidopsis*, and among the actinobacteria,

*Streptomyces* were selectively enriched, which suggest that actinobacteria are effective colonizers of endophytic compartments overcoming the host defence system. This phenomenon also showed that there must be a selective advantage for both the endophytic colonizer and the host plant.

## 15.5.6 Hyperparasitism

Several fungi and bacteria exhibit hyperparasitism on other pathogenic fungi, in which they feed on the pathogenic microbes. *S. griseus* was reported to parasitize *Colletotrichum lindemuthianum* and showed growth not only on its hyphae surface (Tu 1988) but also showed internal parasitism of host hyphae, which resulted in the formation of several blebs. As a result, cell walls of the parasitized *C. lindemuthianum* hyphae degenerated having a sponge-like texture and holes (Palaniyandi et al. 2013b). Similarly, *Streptomyces griseoviridis* K61 (main component of the biofungicide Mycostop) had shown mycoparasitism on several fungal pathogens by penetrating the mycelial wall with little disintegration of the hyphae in case of *Pythium* spp., *R. solani* and *F. oxysporum*. *S. griseoviridis* was most effective against conidia of *Alternaria*, which were heavily colonized and destroyed.

## 15.6  Nanosystems in Agriculture

Over the past decades, many technological innovations have led to profound changes in the agricultural sector. Nanotechnology is one such innovation which refers to the controlled use of a matter at nanoscale, where its unique phenomenon enables novel applications. It can influence current agricultural practices through the improvement of inputs for crop productivity such as nanonutrients, nanopesticides, nanofungicides and nanoherbicides (Subramanian and Tarfdar 2011). Among the agricultural inputs, nanofertilizers are quite promising in promoting the growth, nutrition and productivity of crops besides regulated release of nutrients and improved

nutrient use efficiencies under controlled environmental conditions (Yuvaraj and Subramanian (2014) Subramanian et al. 2015). Further, nano-agrochemicals are mostly nano-reformulations of existing pesticides and fungicides that are known to have long-lasting plant protection effects while minimizing the requirement of chemicals (Sekhon et al. 2014). On the other hand, the synthesized nanoparticles through a metal reduction process also have similar impact. Attractiveness of such synthesized metal nanoparticles/nanostructure materials has aroused from their unique chemical, electronic, optical and photoelectrochemical properties (Peto et al. 2002).

### 15.6.1 Biological Synthesis of Nanoparticles Using Actinomycetes

Many methods are available for the synthesis of metal nanoparticles. Basically, there are two approaches used to synthesize nanoparticles, namely, top-down and bottom-up approaches. Size reduction of particles from macro- or micro- to nanosized particles is done by mere high-energy ball milling to achieve nano-dimension in the range of 1–100 nm. On the other hand, chemical method exploits bottom-up approach wherein nanoparticles are synthesized by atom-by-atom manipulation. Similarly, microbes and plants serve as a manufacturing factory of nanoparticles through bottom-up approach. The physical methods include attrition and pyrolysis, and the chemical methods employ a range of reducing and stabilizing agents. But both of the methods have their own disadvantages such as heterogeneity in dimension and low yield in physical methods and contamination by precursor chemicals, toxic solvents and hazardous by-products in chemical methods (Wang et al. 2007). This necessitates the development of safe, reliable, high-yielding and eco-friendly methods for nanoparticle preparation, and hence "green nanomaterials" have become the major objective of research in nanotechnology. Biological resources including plants and microbes (bacteria, fungi, yeasts and algae) can aid in nanoparticles synthesis. Among them, the microbe-mediated biosynthesis of nanomaterials has recently been recognized as a promising source for mining nanomaterials and an emerging viable alternative tool for chemical and physical methods (Kathiresan et al. 2009).

Microbe-mediated nanoparticles done through either whole cells or their culture supernatants are composite materials consisting of both inorganic component and a special organic matrix comprising of proteins, lipids and/or polysaccharides. This supports for unique chemical and physical properties than the conventionally produced nanoparticles and of other microorganisms even when they are incubated in the same experimental conditions (Lengke et al. 2007; Mohanpuria et al. 2008). The initial studies related to microbe-based nanoparticle synthesis begin with the bacterial domain by Klaus et al. (1999) who observed a single crystalline silver-based particle of well-defined compositions and shapes synthesized by *Pseudomonas stutzeri* AG259 isolated from silver mine. From there, much interest in inorganic material formation by microorganisms in various scientific fields has aroused (Sarikaya 1999; Mandal et al. 2006). This was followed by other bacterial genus such as *Bacillus*, *Pseudomonas*, *Escherichia*, *Klebsiella* and *Enterobacter* (Shahverdi et al. 2007a; Kalimuthu et al. 2008; Saifuddin et al. 2009; Shivaji et al. 2011). Meanwhile, the fungal domain has also proved its ability as a potential biological source mainly due to its large amounts of enzymes secreting potential and was demonstrated in *Fusarium* and *Penicillium*. (Mukherjee et al. 2001; Kowshik et al. 2003; Kathiresan et al. 2009).

Actinobacteria are also facilitating in the synthesis of nanoparticles. In comparison to bacteria and fungi, actinomycetes are known to secrete much higher amounts of enzymes, proteins, small molecules with reducing properties and secondary metabolites, and thereby it significantly enhances the biosynthesis of metal nanoparticles. In addition, as a prokaryote, actinomycetes can be easily subjected to genetic manipulations which help in the future to achieve better control over size and polydispersity of the

nanoparticles (Tsibakhashvili et al. 2011). It is observed that the time required for the completion of nanoparticle synthesis using both bacteria (Klaus et al. 1999) and fungi (Mukherjee et al. 2002) ranges between 24 and 120 h, whereas actinomycetes can be achieve in 24 h of incubation (Sadhasivam et al. 2010). Moreover, it is observed that they can produce nanoparticles in unique shapes, a key factor for biological activity (Pal et al. 2007). Actinomycetes allow the generation of rare geometrical forms such as nanotriangles and nanoprisms. Recently, Verma et al. (2013) have exploited endophytic actinobacteria *Saccharomonospora* sp. isolated from surface sterilized root tissues of *Azadirachta indica*, for the synthesis of prismatic gold nanotriangles. It was evidenced that proteins of 42 and 50 kD were involved in biosynthesis as well as in stabilization of the nanoparticles. On the other hand, Balagurunathan et al. (2011) have obtained spherical and rod-shaped gold nanoparticles using *Streptomyces viridogens* HM10. Despite the fact that the exact mechanism for this shape-oriented synthesis is not clear so far, the possibility of achieving nanoparticle shape control in actinobacterial system is exciting. Usha et al. (2010) had attempted to synthesize ZnO nanoparticles using *Streptomyces* sp. that can be used for developing antimicrobial fabrics.

Actinomycete-mediated metal nanoparticle synthesis including silver and gold was demonstrated in *Streptomyces* sp. BDUKAS10, an isolate of mangrove sediment (Sivalingam et al. 2012), *Streptomyces hygroscopicus* isolated near the Pacific shore region (Sadhasivam et al. 2010), *Streptomyces* sp. LK3 isolated from marine sediments (Karthik et al. 2014), *Streptomyces glaucus* 71 MD isolated from a soybean rhizosphere (Tsibakhashvili et al. 2011), *Streptomyces* sp. (09 PBT 005) 09 PBT 005 isolated from sugarcane rhizosphere soil (Saravanakumar et al. 2014) and an antagonistic *Streptomyces* sp.-SBU3 isolated from terrestrial red garden soil from groundnut (Krishnakumar and Bai 2015). An alkalotolerant actinomycete *Rhodococcus* sp. and extremophilic actinomycete (Ahmad et al. 2003a), *Thermomonospora* sp. (Ahmad et al. 2003b),

proved the efficacy of actinomycetes of extreme environments in synthesizing nanoparticles. From our understanding on the above reports, the actinomycetes from rhizospheric region demonstrated for nanoparticle synthesis might be a plant growth-promoting actinomycete. The report of Fernando et al. (2013) supports this by the synthesis of gold nanoparticles through the mediation of PGP bacteria isolated from Philippine soils. Further studies of potential PGP actinomycetes will bring new avenues in nanomaterial synthesis.

## 15.6.2 Nanomaterials as Crop Protection Tools

The nanomaterials have significant use as microbicidal and pesticidal agents and in fields of catalysis, microelectronics and biomolecular detection (Liong et al. 2009; Christopher et al. 2011). The biocidal property may be contributed by their interaction with enzymes, proteins or DNA so as to inhibit cell proliferation. Still detailed information on its mechanism of activity is lacking. Smaller size and high surface area to volume ratio are the key features enhancing these properties (Shahverdi et al. 2007b). Though the antibacterial activity was demonstrated on numerous pathogens, most of them are clinically relevant pathogens, and only a few demonstrations were done on phytopathogens and that too in vitro. One such report of Krishnakumar and Bai (2015) documented the antagonistic activity of silver nanoparticles synthesized through *Streptomyces* sp.-SBU3 isolated from terrestrial red garden soil from groundnut against the phytopathogens such as *Xanthomonas* sp., *Agrobacterium* sp., *Bacillus campestris*, *Erwinia amylovora* and *Pseudomonas campestris*. However, several reports are available for chemically synthesized nanoparticles such as polymer-based copper nanocomposite against plant pathogenic fungi (Cioffi et al. 2004); silica-silver nanoparticles against *Botrytis cinerea*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Magnaporthe grisea* and *Pythium ultimum* (Park et al. 2006);

and silver nanoparticles against the fungi *Raffaelea* sp., *Bipolaris sorokiniana* and *M. grisea* (Kim et al. 2009; Jo et al. 2009). Similarly, insecticidal properties were also demonstrated majorly on chemically synthesized nanoparticles, viz. polyethylene glycol-coated nanoparticles loaded with garlic essential oil against adult *Tribolium castaneum* (Yang et al. 2009); nanoparticles of silver, aluminium oxide, zinc oxide and titanium dioxide against rice weevil and *Sitophilus oryzae* (Goswami et al. 2010); nanostructured alumina against *S. oryzae* and *Rhyzopertha dominica* (Teodoro et al. 2010); and silver nanoparticles loaded with leaf extract of *Euphorbia hirta* against the first to fourth instar larvae and pupae of *Helicoverpa armigera* (Durga Devi et al. 2014). It is understood that nanoparticles can serve at many directions as crop protection agents by its biocidal properties. Exploration of microbe-mediated nanoparticles especially actinomycete-mediated process is still in its research and developmental stages; further exploration will pave a way for reducing chemical inputs in agriculture.

## 15.7 Conclusion and Future Perspectives

The literature review has unequivocally demonstrated that the actinobacteria possess multifunctions such as plant growth-promoting traits and disease-suppressing activity besides maintenance of soil fertility that eventually result in improving the agricultural productivity. Despite the fact that the major part of the research review brought out is from laboratory studies, extensive field studies are needed to gain insights into the mechanisms associated with plant growth promotion and biocontrol of pathogens. Further, limited information is available on monitoring PGPA on environment, their population dynamics, metabolic activity and spatial distribution in the ecosystems. Bioluminescence gene transformation approach can be used to monitor both in laboratory as well as field level survival. Actinobacteria on nodulation and

nitrogen fixation in legume plants lead us to conclude that the nitrogen-fixing function within legume nodules may be facilitated by *Streptomyces*. Actinobacteria in biogeocycling of Fe and P require in-depth studies to exploit them for the sustainable soil fertility management. It is noteworthy that actinobacteria have a capability of synthesizing high-quality stable nanoparticles that need to be extensively studied in the near future. Pulses production system continues to be a challenge to agricultural scientists for the past four decades, and utilization of multifunctional organisms like actinobacteria along with symbiotic rhizobia will provide solution for multifaceted unresolved problems by a single inoculation. The progress in identification and diversity of PGPA along with their colonization ability and mechanism of action will facilitate their application as a component in the management of sustainable agricultural production system. The literatures data on nanosystems strongly suggests that actinobacteria are potential microbial systems to develop nanoparticles which can be utilized for agri-food systems in the years to come.

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