Gopalakrishnan Subramaniam
Sathya Arumugam
Vijayabharathi Rajendran *Editors*

Plant Growth Promoting Actinobacteria

A New Avenue for Enhancing the Productivity and Soil Fertility of Grain Legumes

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We dedicate this book to Dr Om Prakash Rupela (1948–2015), Ex Principal Scientist (Microbiology), ICRISAT Patancheru, Telangana, India, who mentored and encouraged us to work toward biological options for agriculture.

Foreword

The year 2016 is a special one for pulses. The United Nations announced 2016 as the "International Year of Pulses (2016 IYOP)" in order to emphasize the need to focus on pulses as critical components for global food and nutritional security and to create awareness and understanding of the challenges faced in pulse farming and value chains. Pulses, or grain legume crops, are often referred to as "poor man's meat," as they offer a cost-effective alternative to animal proteins. Besides protein, their richness in micronutrients and other vital elements make pulses critical entities in food and feed value chains around the world.

As with all crops, pulse production is hindered by biotic and abiotic constraints, including pest and pathogen attacks, infertile soils, and climate variability and change. Improved cultivars and management practices are continuing and required outputs from research to ensure that crops are productive and profitable and their grains provide nutritious and healthy food. Production practices must also address the risks associated with the use of pesticides and fertilizers and must explore alternate options, especially biological resources, for enhancing the production of pulses.

In the context of biological options, plant growth-promoting (PGP) bacteria, actinobacteria in particular, are well known for their usefulness in crop production and protection and in maintaining soil health. Actinobacteria are commonly found in soil, compost, fresh and marine water, and decomposing organic materials, and they produce secondary metabolites of agricultural importance. Such metabolites hold fungicidal, bactericidal, insecticidal, and plant growth-promoting traits and can fill the need for biological agents. Exploration of such potential PGP actinobacteria offers the prospect of alternative chemical crop protection agents and so improved environmental health and sustainability.

I commend the editors, Gopalakrishnan Subramaniam, Sathya Arumugam, and Vijayabharathi Rajendran of the book *Plant Growth-Promoting Actinobacteria: A New Avenue for Enhancing the Productivity and Soil Fertility of Grain Legumes*. They have the expertise in basic research, crop production, and plant protection with reference to the use of PGP actinomycetes from the laboratory to field levels. This book contains 19 chapters reporting on the combination of grain legumes with actinomycetes, with details starting from the diversity of actinomycetes

through the commercialization of PGP actinomycetes and their metabolites. Each chapter is stand alone and contributes to the field.

This book is a strong contribution to supporting pulses in food and feed systems globally. It critically assesses the data and offers study on practical aspects of field applications. I strongly endorse this book as it makes a lasting contribution to the field of plant growth-promoting actinomycetes and for its contribution to sustainable agriculture around the globe.

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January 21, 2016

Peter Carberry

Preface

Grain legumes are the abundantly used plant protein source mainly in developing countries of South East Asia, Africa, and Latin America. They are a cost-effective option for animal proteins including fish, meat, and dairy products and hence attained the name "poor man's meat." Besides the protein, their richness in micronutrients and other vital elements made them essential entities in food and feed. Their unique association with rhizobia contributes 65 % of nitrogen necessity in agriculture through the process of symbiotic nitrogen fixation. Their better adaption as intercrop with cereals or tuber crops helps in income generation and livelihood resilience. Cultivation of grain legumes benefits small holder families since they are the primary cultivators of these crops, especially the women since their participation in value chain paves a way for combating nutritional deficiencies and improving the well-being of their children.

Constraints related to the production of legumes are pest and pathogen attacks, unstable yield, poor adaptation, and climate changes. Besides this, the increasing per capita consumption of grain legumes by low-income and developing countries made a gap between grain legume supply and demand. In case of chickpea, groundnut, and pigeonpea, the current shortfall of 7 million tons of supply in low-income food-deficit countries is projected to increase by almost 50 % by 2020, if the same production system continues. The productivity of grain legumes is stagnant for the last two to three decades in spite of using the best breeding and molecular techniques. Further, the increasing costs associated with the improved cultivars and negative effects associated with pesticides and fertilizer use necessitate alternate options. The United Nations also emphasized the need for focusing on grain legumes by announcing year 2016 as the International Year of Pulses (2016 IYOP), in order to create awareness and understanding of the challenges faced by pulse farmers.

Rhizospheric soil, inhabited and influenced by the plant roots, is usually rich in nutrients when compared to the bulk soil, due to the accumulation of numerous amino acids, fatty acids, nucleotides, organic acids, phenols, plant growth regulators/promoters, putrescine, sterols, sugars, and vitamins released from the roots by exudation, secretion, and deposition. This results in enrichment of microorganisms (10–100-folds than the bulk soil) such as bacteria, fungus, algae, and protozoa, among which bacteria influence plant

growth in a most significant manner. Such rhizobacteria present in various proximity to the roots as (1) bacteria living in soil near the roots (rhizosphere), (2) bacteria colonizing the root surface (rhizoplane), (3) bacteria residing in root tissue (endophytes), inhabiting spaces between cortical cells, and (4) bacteria living inside cells in specialized root structures, or nodules, which include two groups – the legume-associated rhizobia and the woody plant-associated *Frankia* sp. Such microbes which promote plant growth are referred as plant growth-promoting (PGP) bacteria. This includes the genera *Bacillus*, *Pseudomonas*, *Erwinia*, *Caulobacter*, *Serratia*, *Arthrobacter*, *Micrococcus*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Hyphomicrobium*, *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium Allorhizobium*, and *Streptomyces*. Besides rhizospheric organisms, microbes present in vermicompost, vermiwash, and earthworm, in specific earthworm gut, nephridia, and alimentary canal, contribute to the beneficial properties of vermicompost and vermiwash in enhancing soil health, plant growth, and hence agricultural productivity. Reports for the diversity of bacteria, fungi, and actinomycetes in vermicompost and earthworm and also for the enhanced plant growth by vermicompost application are available. Actinomycetes, a group of Gram-positive bacteria, are found commonly in soil, compost, fresh, and marine water, and decomposed organic materials and produce secondary metabolites of commercial interest. They also play a role in plant growth and protection and considered as an emerging group of PGP microbes.

PGP actinomycetes directly influence crop growth in several ways: (i) production of plant growth hormones including auxin (indole-3-acetic acid: IAA), cytokinins, gibberellins, and abscisic acid; (ii) nitrogen fixation; and (iii) solubilization of phosphorous, zinc, iron, and potassium and hence increased nutrient availability. In spite of fulfilling these basic nutritional needs, they also promote crop growth indirectly by producing ACC deaminase, a stress relieving enzyme, and by providing protection against pathogen and pest attacks by (i) production of antibiotics such as 2,4-diacetylphloroglucinol, kanosamine, phenazine-1-carboxylic acid, pyoluteorin, neomycin A, pyrrolnitrin, pyocyanin, and viscosinamide; (ii) secretion of siderophores enabling iron uptake, depriving the fungal pathogens in the vicinity; (iii) production of low molecular weight metabolites such as hydrocyanic acid (HCN) which inhibits electron transport and hence disruption of energy supply to the cells; (iv) production of lytic enzymes such as chitinase, β-1,3-glucanase, protease, and lipase which lyse the pathogenic fungal and bacterial cell walls; (v) successfully competing for nutrients against phytopathogens and thereby occupying the colonizing site on root surface and other plant parts; and (vi) induction of systemic resistance in plants by any of the metabolites mentioned above or by inducting the production of phenyl alanine lyase, antioxidant enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase, catalase, lipoxygenase, and ascorbate peroxidase and also phytoalexins and phenolic compounds in plant cells. Many of the PGP actinomycetes were reported to use any of these mechanisms to execute their PGP effect on crops.

Besides the biocontrol mechanisms described above, the use of PGP actinomycetes is reported to trigger the resistance of plants against plant pathogens, referred as induced systemic resistance (ISR). In this process, a signal is generated involving jasmonate or ethylene pathway, thus inducing the host plant's defense response. Various microbes including actinomycetes are reported to induce ISR in plants by producing bio-stimulatory agents. Even individual cellular components had been shown to induce ISR, viz., lipopolysaccharides, flagella, cyclic lipopeptides, homoserine lactones, acetoin, and butanediol.

Apart from the free living microbes, endophytic microbes including bacteria, fungi, and actinomycetes colonize the internal tissue of host plants without causing any damage to the colonized plant. Endophytic microbes also have beneficial PGP traits. Endophytic actinomycetes are reported to produce abundant amounts of bioactive compounds against an array of phytopathogens and also induce systemic resistance, which makes them a suitable platform for biocontrol explorations.

Pathogenic microbes are one of the major threats for legume production and also ecosystem stability, because a single crop is affected by multiple pathogens and vice versa. Many phytopathogens have broad host range and hence affect multiple crops. For example, chickpea is affected by multiple pathogens including (i) bacteria, *Xanthomonas campestris* (bacterial blight); (ii) fungi, *Ascochyta rabiei* (*Ascochyta* blight), *Botrytis cinerea* (*Botrytis* gray mold), *Alternaria alternata* (*Alternaria* blight), *Sclerotinia sclerotiorum* (*Sclerotinia* stem rot), *Fusarium oxysporum*(*Fusarium* wilt), *F. solani* (black root rot), *Sclerotium rolfsii* (collar rot), *Rhizoctonia solani* (wet root rot); and (iii) virus, stunt (leaf roll virus), narrow leaf (yellow mosaic virus), and necrosis (necrotic yellows virus). Pathogens such as *Ascochyta* and *Botrytis* have the host range of around 50 and 200 plant species, respectively. To overcome this multi-pathogen attacks, farmers are in a situation to increase the use of chemical inputs, which further leads to pathogen resistance against the agents and other nontarget environmental impacts. Use of PGP actinomycetes which imposes the above said mechanisms, including indirect mechanisms and ISR for pathogen control, is of great importance in grain legumes.

Insect pests are another major constraint to legume production, and about 40 % of the insecticides were targeted toward Lepidoptera insects, the major contributor for crop loss at both field and store level. As per the survey of European Plant Protection Organization, *Helicoverpa armigera*, a lepidopteran insect, has been widespread in Asia, Africa, and Oceania. In India, *H. armigera* commonly destroys over half the yield of pulse crops like pigeon pea and chickpea, which leads to a loss of US $300 million per annum. Chemical pesticides play a vital role in enhanced crop protection. So the need for developing new pesticides with safe, sustainable, and economic control measures mainly relies on natural products such as plant/microbial compounds. Among them, compounds from microorganisms constitute an infinite pool for novel metabolites/compounds, since they are ubiquitous in nature and highly diverse than the higher order of organisms. Among the microbial compounds, metabolites of actinomycetes have agriculturally favorable traits as fungicidal, bactericidal, insecticidal traits. These

metabolites are blasticidin-S, natamycin, streptomycin, validamycin, avermectins, and spinosad which fill the void for the need of biological control agents. Among the actinomycetes, *Streptomyces* are the major producer of secondary metabolites with unique structure and mode of action with major options for biocontrol. Approximately 17 % of biologically active secondary metabolites (7,600 out of 43,000) have been characterized from streptomycetes. Exploration of such potential PGP actinomycetes will give great relief for chemical crop protection agents and indirectly promotes environmental health and sustainability.

Abiotic stresses, such as drought, extremes of temperature, soil salinity, acidity, alkalinity, and heavy metals, also cause severe yield loss in grain legumes. The response of legumes to various stresses depends on the host plant. The best option for developing stress-tolerant crops with minimized production costs and environmental hazards can be the use of PGP microbes as stress relievers. PGP actinomycetes have some key tolerance mechanism/pathways to execute their beneficial PGP traits, even under stress conditions, and also to reduce stress.

Mineral malnutrition, which affects ~2 million people around world, is deceptive and hidden, increasing vulnerability to illness and infections especially in children and women. Biofortification of staple foods has a targeted activity toward rural communities. This strategy can be achieved through agronomic practices, conventional breeding, and genetic engineering, and each has their own pros and cons. The sustainability of such grain fortification with higher seed mineral concentration is soil health dependent, especially on the availability of minerals in the rhizosphere. As already noted, microorganisms, the invisible engineers in improving soil health by solubilizing trace elements and by driving various biogeochemical cycles of soil, have the ability to serve as a key solution for this complex issue. Increasing mineral density with the use of such PGP microbes especially actinomycetes in the legumes is in its infancy, and the success will serve as low-cost supply of protein and minerals and substantially reduce chemical fertilizer inputs. Utilization of such fortified grain legumes cultivated by small holder families will help in combating nutritional deficiencies and in improving the well-being of their children.

Many of the anthropogenic activities lead to shrinkage of healthy agricultural crop land. This increasing demand for lands forced the farmers to use contaminated sites for crop cultivation. The practice of phytoremediation is suggested for lands contaminated with heavy metals, as it helps to preserve natural physical and biological properties of soil and legume plant. Many of the PGP actinomycetes were reported not only to enhance plant growth but also to alleviate the stress caused by heavy metals in various crops including legumes.

Soil health is the major driving factor for sustainable agriculture. Microorganisms are an essential and integral part of living soil, influencing various biogeochemical cycles on major nutrients such as carbon, nitrogen, sulfur, phosphorous, and other minerals and playing superior role in maintaining soil health than any other biological component of soil. They also have the capacity to suppress soil-borne pathogens and indirectly help in agricultural productivity. Besides all the above-mentioned known

mechanisms, several other unknown mechanisms may also involve in PGP traits of microbes. Genomics has emerged as a powerful tool to identify functionally important genomic elements. Comparison between PGP actinomycetes will reveal previously unknown common traits related to plant growth promotion and also the genetic basis of diversity and adaptation. The availability of whole genome sequence of chickpea, pigeon pea, and peanut will be helpful in understanding the molecular mechanisms between PGP actinomycetes on legumes.

The inconsistency of beneficial results of microbial use, when single microbe was used in the field application, brought an emphasis on co-inoculation of microbes. Certain specific co-inoculation causes synergy by functioning as helper bacteria to improve the performance of the other bacteria. Therefore, in such co-inoculations, the combination of two or multiple PGP bacteria can be used. However, synergistic activity between the selected microbes and the plant host has to be selected after extensive careful evaluations. A range of PGP microbes including actinomycetes were reported not only to have the potential to enhance legume plant growth and grain yield but also control important plant pathogens. The development of a PGP microbe needs several steps starting with isolation of a pure culture and screening of its PGP or antagonistic traits by means of different efficacy bioassays performed in vitro, in vivo, or in trials under greenhouse and/or field conditions. In order to maximize the potential of an efficient PGP microbe, it is essential to optimize mass multiplication protocols that promote product quality and quantity and a product formulation that enhances bioactivity, preserves shelf-life, and aids product delivery. Selection of formulation is very crucial as it can determine the success or failure of a PGP microbe. A good carrier material should be able to deliver the right number of viable cells in good physiological conditions, should be easy to use, and economically affordable by the farmers. Several carrier materials have been used in formulation that include peat, talc, charcoal, cellulose powder, farm yard manure, vermicompost and compost, lignite, bagasse, and press mud. Each formulation has its advantages and disadvantages. The formulation of PGP actinomycetes with suitable carrier materials followed by their efficacy testing under field conditions will bring better inoculants for legume farmers.

This book is proposed to give focused information and views on PGP actinomycetes, an emerging group in the field of microbial inoculants, in combination with grain legumes in a detailed layout in the context of enhanced soil fertility, control of insect pests and diseases, induced systemic resistance, grain yield, biofortification of the seeds for nutrition, and phytoremediation of the contaminated soils. This book will bring the information from ground level to the current situation and helps for better pulse farming in the future.

Patancheru, Hyderabad, Telangana, India Gopalakrishnan Subramaniam
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Contents

Direct Plant Growth-Promoting Ability of Actinobacteria in Grain Legumes

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Salam Nimaichand, Asem Mipeshwaree Devi, and Wen-Jun Li

Abstract

Grain legumes are important crops especially in developing countries for their high nutrient values. In a country like India where many people are vegetarian, they are a source of dietary protein. In addition to their food values, they are also a source of livestock fodder. They also can be used as biofertilizer due to their ability to fix nitrogen thereby making them the ideal crops for use in crop rotation. The role of bacteria including *Bacillus* and *Pseudomonas* in plant growth promotion is well established in various crops including grain legumes. While actinobacteria have not been fully explored for potential application in sustainable agriculture, their ubiquitous presence and capability for producing various plant growth-promoting traits make them an ideal candidate for use as biofertilizer for plants. The current chapter discusses the various direct plant growth-promoting abilities of actinobacteria with special reference to grain legumes.

Keywords

PGP actinobacteria • Siderophore • Biological Nitrogen Fixation • Phosphate solubilization • IAA production • ACC deaminase

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1.1 Introduction

Crops require nutrients and other essential cofactors for their growth and production. Normally, these requirements are made available through nature by the biological process of nutrient cycles. However the slow natural processes of food production and the high demand of a burgeoning human population result in a large gap between production and requirements. To seal the gap, yearning for improvement in crop production is essential, either with the use of improved crop varieties or use of artificial means for enhancement of nutrients in the soil. With the advancement in genetic engineering and technology, these requirements were met by the use of genetically modified (GM) crops along with synthetic chemical fertilizers, which has somehow resulted in filling the gaps between supply and demand. However one is neither very sure of the resulting effects of the use of GM crops nor is aware of the effect of agrochemicals in the health of soil. Besides, bioaccumulations of recalcitrant in soil have also been reported from long-term uses of chemical fertilizers, thus constituting a threat for the environment (Gunnell et al. 2007; Leach and Mumford 2008).

Therefore a cleaner and greener approach toward the improvement of crop production is essential and will require the use of naturally available plant growth regulators or producers of such regulators. One such example is the use of plant growth-promoting rhizobacteria (PGPR), a term coined by Kloepper and coworkers (Kloepper and Schroth 1978) for the group of heterogenous bacteria found in the rhizosphere, in root surfaces, and in association with roots. PGPRs have been reported to provide nutrients required for plant growth, induce their growth by production of hormones, supply trace elements, and also induce systemic resistance against phytopathogens (Glick 1995). The most extensively reported PGPR till date belongs to the phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, of which the most commonly studied were the gram-positive *Bacillus* spp. and gram-negative *Pseudomonas* spp. (Tilak et al. 2005; Podile and Kishore 2006; Jog et al. 2012). The plant growth-promoting traits of actinobacteria despite its ubiquitous existence are rarely reported (Doumbou et al. 2002; Al-Aksar 2012; Sadeghi et al. 2012). These microorganisms have been recognized as prolific producers of several secondary metabolites (Goodfellow and Williams 1983). Many actinobacteria produce spores which can spread and resist environmental stress (Chater 1993) and can therefore be promising as biocontrol agents. Thus, actinobacteria are among the most promising biocontrol PGPR agents for future agriculture (Doumbou et al. 2002; Franco-Correa et al. 2010).

1.2 Plant Growth-Promoting (PGP) Traits

The PGP activities of microorganisms can be observed from two different aspects: direct and indirect means. Direct plant growth promotion requires production or supplementation of essential factors required for the growth of the plant. Among these factors, biological nitrogen fixation (BNF) and solubilization of phosphates are important as these processes convert the most readily but unutilizable nitrogen and phosphorus sources, viz., dinitrogen gas and mineral phosphate, into more easily accessible forms (Boddey et al. 1995; Gyaneshwar et al. 2002; Caravaca et al. 2005; Meunchang et al. 2006).

Nitrogen, a key element of nucleic acids and proteins, is a limiting factor essential for plant growth. Despite constituting 78 % of the atmosphere, dinitrogen gas is not directly accessible to plants and therefore requires to be converted to soluble form for its uptake (Lam et al. 1996; Santi et al. 2013). Majority of the nitrogen required by plants is fixed through BNF as ammonium and nitrates using the nitrogenase enzyme system (Kim and Rees 1994). In agricultural practice, additional nitrogen is supplied to complement the limited availability of nitrogen from BNF through application of synthetic

nitrogen fertilizers (Westhoff 2009). As these synthetic fertilizers are not completely absorbed by plants, they often lead to soil and groundwater contamination. The resulting effects are health hazards and compromised agricultural sustainability. Therefore a more agronomical method for sustainable agriculture will be the effective utilization of BNF.

Phosphorus is available in abundance in soils both in inorganic and organic forms (Khan et al. 2014). If one is to utilize these resources, it could fulfill the need of farmers around the world for at least this century (Khan et al. 2009). However soluble phosphorus forms, which the plant can uptake, account for only 0.1 % of the total soil phosphate and thereby making it the second most essential element for plants after nitrogen (Brady and Weil 2002; Wang et al. 2009). Similar to nitrogen, the immediate need of phosphorus is supplied by chemical fertilizers. The preparation of chemical fertilizers is not only costly but its values are wasted as majority of applied fertilizers are fixed to the soil into its insoluble forms just after application (Reddy et al. 2002; Khan et al. 2014). Microorganisms solubilizing insoluble phosphates have been reported several decades earlier (Pikovskaya 1948). Since then, several researches have been done to investigate the phosphate-solubilizing capabilities of several microorganisms including bacteria, fungi, and actinobacteria (Glick 1995; Rodriquez and Fraga 1999; Reddy et al. 2002; Rodriguez et al. 2006; Hamdali et al. 2008a; Jog et al. 2014). It is economically more feasible to utilize microorganisms capable of solubilizing these abundant phosphorus in soil as biofertilizers rather than using the chemical fertilizers.

Another important direct PGP factor is the availability of trace elements such as iron, zinc, etc., which are essential for various biochemical pathways. Iron in soil is trapped by many microorganisms through the production of siderophores, thereby making it available to plants (Tokala et al. 2002; Nassar et al. 2003). Siderophores, though initially seem to be confined to microbes (Neilands 1981, 1995), are now reported to be produced by plants as well (Romheld and Marschner 1986; Dell'mour et al. 2012). They are classified into four major groups based on the chemical nature of the chelating ligands, catecholates, hydroxamates, hydroxypyridonates, and aminocarboxylates (Stintzi et al. 2000), though few siderophores have been reported to mixed functional groups (Hider and Kong 2010). They differ in their redox potential and reactivity and hence in the stability of their complex formed with iron. The various roles of siderophores are resided by the stability of the complex. Catecholate siderophores, due to their strong affinity for iron complex, are most likely involved in bioleaching of iron from mineral ores (Kraemer 2004; Rogers and Bennett 2004). Siderophores also play an important role in indirect plant growth promotion by inhibiting phytopathogens through starvation of essential iron (Ahmed and Holmstrom 2014). Plant growth regulators such as phytohormones are also produced by few microorganisms which directly influenced the growth of the plants (Pattern and Glick 2002; Hayat et al. 2010). These hormones, including auxins (indole-3-acetic acid), gibberellins, cytokinins, abscisic acid, and ethylene, induce the growth of plants.

Among the indirect PGP mechanisms, PGPRs act as biocontrol agents against various plant pathogens through production of antimicrobial compounds and extracellular enzymes (Doumbou et al. 2002; Glick 2012). Actinobacteria especially the genus *Streptomyces* have been the major producer for bioactive metabolites (Alexander 1977) and have exhibited immense biocontrol action against a range of phytopathogens (Wang et al. 2013). They account for nearly 60 % of the production of agriculturally important antibiotics (Ilic et al. 2007). *Streptomyces griseoviridis* has been reported to be antagonistic to a variety of plant pathogens including *Fusarium* and *Rhizoctonia* spp. (Tahvonen 1982; Tahvonen and Lahdenpera 1988). *Streptomyces vinaceusdrappus* has been reported to have antagonistic activity against four important rice fungal pathogens, viz., *Bipolaris oryzae*, *Pyricularia oryzae*, *Fusarium oxysporum*, and *Curvularia oryzae* (Ningthoujam et al. 2009). *Streptomyces*

hundungensis sp. nov. has also been reported to have antifungal activities (Nimaichand et al. 2013). These antifungal activities may be influenced by both diffusible and volatile antifungal metabolites. Volatile compound-producing *Streptomyces philanthi* inhibited the mycelial growth of rice fungal pathogens such as *Rhizoctonia solani*, *Pyricularia grisea*, *B. oryzae*, and *Fusarium fujikuroi* (Boukaew et al. 2013). Inorganic volatile compounds such as ammonia have also been reported to inhibit fungal growth (Howell et al. 1988; Brimecombe et al. 2001). Accumulation of ammonia in soil can raise the pH to about 9–9.5, thus suppressing the growth of certain fungi. It may also upset the microbial community and inhibit fungal spore germination (Martin 1982). Chitinase- and β-1,3-glucanase-producing *Streptomyces* sp. 385 lyses the fungal cell walls of *F. oxysporum* and significantly suppresses the disease incidence of *Fusarium* wilt disease in cucumber plants (Singh et al. 1999). *Streptomyces* sp. 9p, producing chitinase, β-1,3-glucanase, lipase, and protease, exhibited antagonistic activity against *R. solani*, *Colletotrichum gloeosporioides*, *Alternaria brassicae*, and *Phytophthora capsici* (Srividya et al. 2012). In addition, interactions of the plants with the microorganisms result in production of systemic resistance among the plants against disease-causing agents (Phi et al. 2010).

1.3 Mechanism of Direct Plant Growth Promotion by Actinobacteria

1.3.1 Nitrogen Fixation

BNF has been well documented for symbiotic relation between the α-proteobacterium *Rhizobium* and leguminous plants (Schultze and Kondorosi 1998; Desbrosses and Stougaard 2011). The gram-negative rhizobia infect and establish an endosymbiotic relationship with the roots of leguminous plants resulting in the formation of nodules (Franche et al. 2009). Unlike rhizobia, the actinobacterium *Frankia* is more versatile in its role as nitrogen fixer. This may be attributed to its highly diverse ecological niches. They fix nitrogen in non-legumes under both symbiotic and free-living aerobic conditions (Benson and Silverster 1993). *Frankia* infect the root cells of actinorhizal plants (mostly non-leguminous woody shrubs and trees) through either one of the two mechanisms: intracellular root-hair infection or intercellular root invasion (Wall and Berry 2008). In the earlier mechanism, the hyphae of *Frankia* strains penetrate the root hair which triggers the formation of prenodules. The infecting hyphae after infecting the prenodule stimulate nitrogen fixation. In this case, the formation of nodule primordia and finally the mature nodules occurs from pericycle cells and not from actively dividing prenodule cells as in legumes. In the second mechanism, *Frankia* hyphae progressively penetrate the middle lamella between adjacent cells and finally infect the primordium cells (for details, refer to Wall and Berry 2008; Franche et al. 2009; Franche and Bogusz 2011).

The process of BNF requires a large amount of energy in the form of ATP. This energy requirement is largely made up by the endosymbiotic interaction between *Frankia* and actinorhizal plants where the former fixed nitrogen for plants in exchange of C-sources from plants. During this interaction, *Frankia* hyphae are differentiated into vesicles which are surrounded by a hopanoid lipid coat (Harriots et al. 1991; Berry et al. 1993; Dobritsa et al. 2001). Nitrogen fixation occurs in this vesicle in a reaction catalyzed by the oxygen-labile nitrogenase enzyme complex (Kim and Rees 1994; Franche et al. 2009). The enzyme complex comprises of two components, dinitrogenase reductase (provides reducing equivalent) and dinitrogenase (reduces N_2 to NH_3 by utilizing the reducing equivalent). This special endosymbiotic establishment thereby provides an added advantage in that it prevents the nitrogenase from O_2-induced deactivation. Majority of the fixed nitrogen is attributed to this mode of interaction due to the favorable environment.

Molecular studies have established the fact that many actinobacteria can occur as

endophytes in various leguminous and non-leguminous plants without forming nodules. Endophytic actinobacteria which have been shown to exhibit a nitrogen-fixing ability include species of *Arthrobacter*, *Agromyces*, *Corynebacterium*, *Mycobacterium*, *Micromonospora*, *Propionibacterium*, and *Streptomyces* (Sellstedt and Richau 2013). These actinobacteria have been demonstrated for their nitrogen-fixing capability by their ability to grow on nitrogen-free medium, acetylene reduction activity, and ^{15}N isotope dilution analysis in addition to identification of *nif* genes via PCR amplification (Gtari et al. 2012). Table 1.1 describes a list of non-*Frankia* actinobacteria having nitrogen-fixing capability under experimental conditions.

Free-living rhizospheric bacteria also account for some of nitrogen fixed through the process known as "associative nitrogen fixation." Among the *Frankia*, a small group of noninfective strains have been isolated (Kucho et al. 2010) but their mode of interaction with the host plant is not completely understood. In addition, many actinobacteria have been isolated from the rhizosphere of grain legumes (Franco-Correa et al. 2010; Garcia et al. 2010). They often exhibited cell-wall-degrading enzyme activities such as chitinase, glucanase, pectinase, etc., thereby helping the rhizobacteria to establish colonies around the root surface through degradation of the plant cell walls (Reinholdt-Hurek et al. 1993; 2006; Kovtunovych et al. 1999; Adriano-Anaya et al. 2005). Another factor which helps the rhizobacteria to colonize the rhizosphere is the plant root exudates (Santi et al. 2013). The root exudates modify the chemical and physical properties of the soil and thus regulate the structure of soil microbial community in the immediate vicinity of the root surface (Dakora and Phillips 2002). However a complete

Table 1.1 List of non-*Frankia* actinobacteria with probable nitrogen-fixing capabilities

Strain	Nitrogen fixation model	References
Mycobacterium flavum 301	Growth on N_2-free medium	Fedorov and Kalininskaya (1961)
	Acetylene reduction	
Mycobacterium spp.	Acetylene reduction	Rao (1973)
Propionibacteria		Baranova and Gogotov (1974)
Corynebacterium autotrophicum GZ 29	Acetylene reduction	Berndt et al. (1978)
	Presence of nitrogenase	
Arthrobacter fluorescens	Acetylene reduction	Cacciari et al. (1979)
Streptomyces spp.	Acetylene reduction	Ding et al. (1981)
Streptomyces spp.	Acetylene reduction	Knapp and Jurtshuk (1998)
Microbacterium spp.	Acetylene reduction	Ruppel (1989)
Mycobacterium spp.		
Streptomyces thermoautotrophicus UBT1	Formation of H_2	Gadkari et al. (1992)
	Incorporation of N_2 in cell	Ribbe et al. (1997)
Corynebacterium sp. AN1	Acetylene reduction	Giri and Pati (2004)
Pseudonocardia dioxanivorans CBI190	Growth on 1,4-dioxane	Mahendra and Alvarez-Cohen (2005)
Micromonospora spp.	Growth on N_2-free medium	Valdes et al. (2005)
	Acetylene reduction	
Thermomonospora spp.	N_2 isotope dilution method	
	Presence of *nif*H gene	
Agromyces sp. ORS 1437	Growth on N_2-free medium	Zakhia et al. (2006)
Microbacterium spp.	Presence of *nif*H gene	
Mycobacterium sp. ORS 1481	Growth on N_2-free medium	
Ornithinicoccus sp. STM 379		
Streptomyces spp.	Growth on N_2-free medium	Pankratove and Dedysh (2009)
	Acetylene reduction	
Streptomyces sp. P4	Ureide method	Soe et al. (2012)

mechanism of the pathway involved in the nitrogen fixation by soil actinobacteria still needs to be established.

1.3.2 Phosphate Solubilization

Phosphate-solubilizing microorganism (PSM) has been a major contributing factor toward an eco-friendly approach of plant growth promotion. But the major problem with PSM is that its application is limited by the nature of soil and therefore its study can be considered to be in its infant stage (Sharma et al. 2013). To understand the mechanisms of phosphate solubilization, it is important to understand the forms in which they are present in the soil and how these can be made available to plants by microorganisms. The major form of inorganic phosphate present in the soil is mineral phosphate. Depending on the pH of the soil, the phosphate either exists as tricalcium phosphate, aluminum phosphate, or iron phosphate. Under in vitro conditions, these phosphates could be solubilized by means of small-molecular-weight organic acids (Maliha et al. 2004; Pradhan and Shukla 2005). Organic acids function in acidification of the soil. Soil acidification had significant effect in the release of monovalent phosphate anion from mineral phosphate by H^+ substitution for cation bound to phosphate (Goldstein 1994; Omar 1998; Mullen 2005). Microorganisms have been demonstrated to adopt this mechanism by releasing organic acids through oxidation of organic carbon sources (Bajpai and Rao 1971; Illmer and Schinner 1992; Khan et al. 2007; Yi et al. 2008). However actinobacteria are rarely reported for their role in organic acid production despite the fact that they are the major sources of many microbial bioactive metabolites (Rozycki and Strzelczyk 1986; Jog et al. 2014). The major organic acids reportedly produced by actinobacteria are citric acid, gluconic acid, lactic acid, malic acid, and oxalic acid (Chen et al. 2006; Yi et al. 2008; Jog et al. 2014).

A different mechanism is adopted by microorganisms for solubilizing (mineralizing) the organic phosphorus, the major form of soil phosphate (Tarafdar and Claassen 1988; Richardson 2001; Rodriguez et al. 2006). These organophosphates occur in forms including inositol phosphates, phytin, sugar phosphates, nucleotides, phosphoproteins, phosphonates, and phospholipids. These organic phosphorus compounds are used as substrate to release soluble inorganic phosphates in a reaction mediated by enzymes especially phosphatases and phytases released by the soil microbes (Yadav and Tarafdar 2007; Maougal et al. 2014). Among these enzymes, the most important one is the nonspecific acid phosphatase which reacts on the phosphoester or phosphoanhydride linkage present in the organic compound (Nannipieri et al. 2011). The microbial acid phosphatase activity is often confused with the phosphatase produced by plant roots (Richardson et al. 2009a, b). However it has been reported that phosphatases of microbial origin have more affinity toward organophosphate compounds than those of plant origin (Tarafdar et al. 2001). A similar phosphatase enzyme known as alkaline phosphatase is released by microbes in alkaline and neutral soil. Phytate is another source of organic phosphate in the soil. It is also the major stored form of phosphorus in plant seed and pollen (Richardson 1994). But its utility as source of soluble phosphate in plants through internal degradation of phytate is very limited. This shortcoming is overcome through microorganism-mediated phytate degradation, thereby playing an important role in enhancing the availability of soluble phosphorus in soil for plant growth (Richardson and Simpson 2011). Therefore the preparation of proper phosphate-solubilizing microbial bioinoculant for a particular soil type will require preliminary understanding of the soil and microbial activity.

1.3.3 Iron Acquisition

Siderophores can be of both microbial and plant origin, and therefore microbes and plants can simultaneously trap iron present in the soil. The exact mechanism how the microbial siderophores help in plant growth is not

completely understood, but under the condition of low iron availability, plant growth promotion is assumed to involve either one of the two mechanisms:

1. Microbial siderophores with high redox potential transfer their ferrous iron to a plant's transport system in the apoplast of plant roots (Leong and Neilands 1976; Crowly et al. 1991; Mengel 1995; Kosegarten et al. 1999). The exact mechanism is not completely established. But the process seems to involve binding of the iron-siderophore complex to the protein receptor causing a conformational change in the receptor. This change pumped the complex through the receptor into the periplasmic space. On release of the complex, the receptor protein returns to its original conformation (Stintzi et al. 2000). The ultimate result is a rise in the iron concentrations in the roots.
2. Iron chelation by microbial siderophores from soil depends on its concentration, stability constant, pH, and redox potential and does a ligand exchange with phytosiderophores (Masalha et al. 2000; Stintzi et al. 2000; Crowley 2006). During this process, the iron-free phytosiderophore is initially bound to the receptor protein. The microbial iron-siderophore complex then binds to the receptor, where the iron exchange between the two siderophores occurs. The formation of iron-phytosiderophore complex initiates a conformational change on the receptor and which thereby enters the cytoplasm. Finally the microbial siderophore is released and the receptor returns to the original conformation.

1.4 Production of Phytohormones

Plant produces hormones to regulate its growth and metabolism throughout its entire lifetime (Stamm and Kumar 2010) and in response to a number of nonlethal stresses from the environment (Davies 2004). Each hormone has multiple effects, depending on its site of action, the developmental stage of the plant and the concentration of the hormones. The regulatory mechanisms of the different phytohormones have been perceived through a series of signaling cascades involving various genes (Teale et al. 2006; To and Kieber 2008; Schwechheimer and Willige 2009; Yoo et al. 2009). Under growth-limiting environmental conditions, these responses often result in negative effect to the plant growth. In addition, excessive release of phytohormones in the soils is deleterious in nature, leading to effects like wilting of plants. The most common one can be seen from the production of ethylene in response to stress condition in the plants. Under in vitro conditions, bacteria including actinobacteria producing phytohormones are found to modulate the hormonal levels required for plant growth and thereby their responses to outer environmental stress (Glick et al. 2007). However, the exact mechanism through which these bacteria play a regulatory role in plant metabolism is still not properly understood. It was seen that some phytopathogens especially *Agrobacterium*, through production of plant hormones, seem to seize plant cells for nutrient production essential for their growth (Camilleri and Jouanin 1991). Despite the contrasting report, bacteria especially PGPR exert their beneficial effect to plants through the production of phytohormones (Bloemberg and Lugtenberg 2001). The most important among them is the release of bacterial indole-3-acetic acid (IAA) in the soil which enhanced lateral and adventitious root development leading to improved mineral and nutrient uptake (Duca et al. 2014). In plant roots, endogenous IAA may be suboptimal or optimal for growth. Continuous release of bacterial IAA in small quantity therefore provides the plant with necessary level of hormone (Glick 2012). During the process, the plant also provides the bacteria with nutrient through excretion of exudates (Ahmed and Hasnain 2010).

Another plant hormone with tremendous biological activity and simplest structure is ethylene. This hormone is involved in promoting

root initiation, inhibiting root elongation, promoting fruit ripening, promoting flower wilting, stimulating seed germination, promoting leaf abscission, and inhibiting nodule formation (Abeles et al. 1992). In addition, ethylene acts as a stress-responsive hormone, which protects the plant against external abiotic and biotic stresses including extremes of temperatures, drought, and presence of phytopathogens (Abeles et al. 1992). The production of "stress ethylene" however results in the retarded development of plant. PGP bacteria respond to this stress condition and promote plant growth by the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick 2005). This enzyme converts ACC, the immediate precursor for stress ethylene, into ammonia and α-ketobutyrate. The immediate effect of inoculation of ACC deaminase-producing bacteria is the enhancement of plant root elongation and promotion of shoot growth (Shaharoona et al. 2006; Onofre-Lemus et al. 2009).

1.5 Direct PGP Ability of Actinobacteria in Grain Legumes

Actinobacteria have been widely reported for their potential as biocontrol agents but have been rarely reported for their use as agricultural bioinoculant, especially with reference to grain legumes. Grain legumes have been known to fix their own nitrogen, through association of *Rhizobium* in the nodule. The major nitrogen-fixing actinobacterium *Frankia* has not yet been reported to occupy this nodulation system. However, in certain cases, non-*Frankia* actinobacteria have been reported of their ability to fix nitrogen (Gadkari et al. 1992; Gregor et al. 2003). There are also reports that many actinobacteria have the capacity to reduce acetylene, of which some are positive for the amplification of *nif*H gene (Table 1.1). Despite the absence of experimental proof, the genus *Micromonospora* has been reported to be in close association with *Frankia* in some nodules (Carro et al. 2013) thereby indicating that this genus might be playing a supplementary role in nitrogen-fixing activity of *Frankia*.

During the last few decades, the interest in PGPR for their role in productivity of agriculture and stability of soil has increased tremendously. The study is slowly shifting its focus on PGP actinobacteria over other PGP bacteria due to their relative abundance in the soil and their capacity to produce antimicrobial metabolites. They, especially the genus *Streptomyces*, have the capability for producing spores and occur in filamentous state (Duca et al. 2014). Moreover, it has been reported that a large part of their genome (~5–10 %) is devoted to the secondary metabolite production. These help them to sustain even under extreme conditions where plants tend to respond to stress by producing ethylene.

A list of actinobacteria with PGP traits is given in Table 1.2. Majority of these PGP actinobacteria have been studied *in planta* with cereals but lesser in grain legumes. Mishra et al. (1987) have shown the effect of culture filtrates of *Micromonospora* and *Streptomyces* in growth promotion of soybean. Gopalakrishnan et al. (2015a, b) demonstrated the PGP ability of *Streptomyces* in chickpea. An ACC deaminase-producing *Rhodococcus* strain was able to promote the growth of garden pea (Belimov et al. 2001). Gregor et al. (2003) have shown that a *Streptomyces* sp. isolated from the rhizosphere of soybean was able to fix nitrogen and thereby induce the growth of the plant. *Streptomyces* have been reported to produce a trihydroxamate siderophore known as desferrioxamine (Meiwes et al. 1990; Imbert et al. 1995; Yamanaka et al. 2005). In addition, they also produce enterobactin, coelichelin, and griseobactin (Challis and Ravel 2000; Fiedler et al. 2001; Lautru et al. 2005; Patzer and Braun 2010; Lee et al. 2012). The siderophore heterobactin was initially reported to be only produced by *Rhodococcus* and *Nocardia* (Carrano et al. 2001; Mukai et al. 2009) which was later shown to be produced by *Streptomyces* as well (Lee et al. 2012).

Table 1.2 Actinobacteria and their PGP traits

Strain	PGP traits	Host plant for plant growth promotion	Reference
Actinoplanes spp.		Soybean (*Glycine max* L.)[a]	Filonow and Lockwood (1985)
Micromonospora spp.		Soybean[a]	Mishra et al. (1987)
		Cucumber (*Cucumis sativus*)	
		Sorghum (*Sorghum bicolor*)	
		Corn (*Zea mays*)	
Streptomyces hygroscopicus strain 576		Soybean[a]	Mishra et al. (1987)
Thermomonospora sp.		Soybean[a]	Mishra et al. (1987)
		Cucumber	
Streptomyces thermoautotrophicus	Nitrogen fixation		Gadkari et al. (1992)
Micromonospora endolithica	Phosphate solubilization	Carrot (*Daucus carota*)	El-Tarabily et al. (1997)
Streptomyces spp.	IAA production	Wheat (*Triticum aestivum*)	Aldesuquy et al. (1998)
Rhodococcus sp. Fp2.	ACC deaminase activity	Garden pea (*Pisum sativum*)[a]	Belimov et al. (2001)
Streptomyces spp.	Nitrogen fixation	Soybean [a]	Gregor et al. (2003)
Streptomyces griseoluteus WT	IAA production	Bean (*Phaseolus vulgaris* L.)[a]	Nassar et al. (2003)
Rhodococcus sp.	IAA production	*Brassica juncea*	Belimov et al. (2005)
	Siderophore production		
Mycobacterium sp.	IAA production	Orchid	Tsavkelova et al. (2005)
Rhodococcus sp.			
Arthrobacter sp. strain EZB4	ACC deaminase activity	Pepper (*Capsicum annuum* L.)	Sziderics et al. (2007)
Microbacterium sp. 44	IAA production	Pea[a]	Egambardieva (2008)
Streptomyces spp.	Phosphate solubilization	Wheat	Hamdali et al. (2008b)
	IAA production		
Kitasatospora sp.	IAA production		Shrivastava et al. (2008)
Streptomyces acidiscabies E13	Siderophore production	Cowpea (*Vigna unguiculata* L.)[a]	Dimpka et al. (2008)
Kitasatospora spp.	Phosphate solubilization	Wheat	Oliveira et al. (2009)
Streptomyces spp.	Nitrogen fixation	*Trifolium repens* L.[a]	Franco-Correaa et al. (2010)
	Phosphate solubilization		
Streptomyces spp.	IAA production	*Alnus glutinosa*, *Casuarina glauca*	Ghodhbane-Grari et al. (2010)
		Elaeagnus angustifolia	
Brevibacterium epidermidis RS15	Nitrogen fixation	Canola	Siddikee et al. (2010)
	IAA production		
Micrococcus yunnanensis RS222	ACC deaminase activity		
Streptomyces spp.	IAA, siderophore production	Tomato (*Solanum lycopersicum*)	Verma et al. (2011)
Streptomyces spp.	IAA, siderophore production, phosphate solubilization	Wheat	Jog et al. (2012)

(continued)

Table 1.2 (continued)

Strain	PGP traits	Host plant for plant growth promotion	Reference
Streptomyces sp. GMKU 3100	Siderophore production	Rice (*Oryza sativa* L.)	Rungin et al. (2012)
		Mung bean (*Vigna radiata* L.)[a]	
Streptomyces sp. C	IAA production	Wheat	Sadeghi et al. (2012)
	Phosphate solubilization		
Streptomyces spp.	IAA production	Sorghum	Gopalakrishnan et al. (2013)
		Rice	
Microbispora spp.	IAA production	Mandarin (*Citrus reticulata* L.)	Shutsrirung et al. (2013)
Micromonospora spp.			
Nocardia spp.			
Nocardiopsis spp.			
Spirillospora spp.			
Streptomyces spp.			
Streptomyces griseoflavus P4	Nitrogen fixation	Soybean (*Glycine max*)[a]	Soe et al. (2012), Soe and Yamakawa (2013)
Streptomyces sp. VSMGT1014	IAA production	Rice (*Oryza sativa*)	Harikrishnan et al. (2014)
Streptomyces spp.	IAA, siderophore production	Wheat	Jog et al. (2014)
	Phosphate solubilization		

[a]Grain legumes

1.6 Concluding Remarks

Genetic engineering of PGP actinobacteria will play a major role in the coming year for utilization in agriculture, particularly in grain legumes. Being ubiquitous in nature, these organisms are well adapted to colonize different environmental ecosystems including endosymbiotic relationships with plants or as free-living organisms in soil. If these organisms are engineered for production of multiple PGP traits, the financial constraint in the studies of PGP activity over every single plant with different conditions can be restricted, thereby increasing its utility on various biological systems.

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Indirect Plant Growth Promotion in Grain Legumes: Role of *Actinobacteria*

2

Simi Jacob and Hari Kishan Sudini

Abstract

Grain legumes (beans, pulses, and oilseeds) are protein-rich crops and to a larger extent diversify farming systems. These crops are often confronted by a number of biotic and abiotic stresses in the natural environment resulting in a significant reduction in their productivity. Management options employed to counter such stresses include cultural and agronomical practices apart from the use of chemicals. Among others, biological control using beneficial microbes is environmentally safe and sustainable solution to minimize the deleterious effects of biotic and abiotic stresses in grain legumes. Microbes are known to exhibit a number of mechanisms conferring resistance to plants. Many such useful organisms, termed plant growth-promoting microbes (PGPM), have been studied extensively for their role in agriculture. Among the microbes studied, plant growth-promoting *Actinobacteria* (PGPA) have been gaining popularity. These microbes are a group of important free-living, spore-forming organisms exploited for their role in producing many agriculturally important substances. These microbes have shown the ability to both suppress pathogen inoculums employing one or more mechanisms of antagonism (hyperparasitism and the production of lytic enzymes, antibiotics, and siderophores) and also resist abiotic stresses (drought, salinity, heavy metals, heat, etc.) by lowering the levels of ethylene by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase. It is not only important to identify such microbes but also to

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extrapolate these findings and achieve similar results under field conditions. This chapter focuses on the common mechanisms reported for *Actinobacteria* majorly streptomycetes and to a lesser extent by non-streptomycetes in protecting the crop plants particularly grain legumes. We believe it also helps to encourage further investigations especially with the lesser explored non-streptomycetes.

Keywords
Plant growth promotion • Actinomycetes • Grain legumes • Indirect mechanism • Biocontrol

2.1 Introduction

Grain legumes (beans, pulses, and oilseeds) belonging to the family *Fabaceae* are a class of leguminous plants predominantly cultivated for their edible seeds. These usually provide affordable protein (up to 52 %) and energy-rich foods which form a major component of the human diet. They can be aptly termed as "the poor man's meat" and rich man's health food. Apart from their higher protein content, grain legumes have considerably good amounts of vitamins, minerals, and micronutrients such as iron in addition to carbohydrates. Grain legumes also considered cash crops and provide income for smallholder farmers in several countries of Asia and sub-Saharan Africa. Legumes have the innate ability to fix atmospheric nitrogen into the soil (through rhizobacteria-root interaction), thus improving fertility and reducing the application of inorganic fertilizer. This ability has been harnessed by intercropping legumes with cereal crops with reported increase in yield and reducing farmer's vulnerability to crop failures (Luscher et al. 2011; Nyfeler et al. 2011).

There are more than 40 species and many varieties of grain legumes cultivated globally for their seeds predominantly used for food and feed purpose. Some of the important crops in this category are chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), pea (*Pisum sativum*), soybean (*Glycine max*), peanut (*Arachis hypogaea*), common bean (*Phaseolus vulgaris*), cluster bean (*Cyamopsis tetragonoloba*), hyacinth bean (*Dolichos lablab*), lentil (*Lens culinaris*), horse gram (*Dolichos uniflorus*), and green gram (*Phaseolus aureus*). Though the demand for food legumes has been increasing rapidly owing to urbanization and increase in population, production has been growing more slowly especially in the developing countries (Gowda et al. 2009). There are a number of constraints to grain legume production broadly classified as biotic and abiotic factors, which militate against growth and reduce the yield of these crops (Rao et al. 2010). Biotic stresses such as insect pests and diseases (bacterial, viral, and fungal) and abiotic stresses such as drought, salinity, temperature, water logging, and low P are key factors which induce a disruption in plant metabolism ultimately causing a reduction in the overall productivity (Heil and Bostock 2002; Bolton 2009).

Environmental stress factors have the most impact on crop production in cultivated field. Moreover, crops affected with more than one kind of stress exhibit an even larger effect as observed in the case of drought-exposed common beans which resulted in higher damage when infected by *Macrophomina phaseolina* (Suleman et al. 2001). With the application of synthetic chemical-based pesticides, the devastation caused by insect pests and diseases has been kept under check to a greater extent, but their injudicious use has attracted widespread environmental concern and advanced the need for finding alternative strategies for sustainable agriculture and environmental protection. Resistance

of pests to chemicals is another major concern to find better, sustainable, and environmentally acceptable solutions. In this direction, a viable option put forward by scientists is the use of beneficial microbes as growth promoters and disease control agents.

In general, plant biosphere (rhizosphere, spermosphere, and phylloplane) is inhabited by microbes belonging to endophytic and epiphytic nature. This native microbial community confers a shield from harmful pathogens and protects the crop plants in the natural conditions (Tapadar and Jha 2013). Plant roots are major attractants of microbes due to the presence of exudates such as organic acids, amino acids, sugars, vitamins, enzymes, purines/nucleosides, inorganic ions, gases, phytosiderophores, phenolics, and flavonoids (Dakora and Phillips 2002) which boost the growth of microbes such as mycorrhizae, rhizobia, and plant growth-promoting rhizobacteria (PGPR) (Badri and Vivanko 2009). Since the first report of Kloepper and Schroth (1978), rhizospheric microbes have got considerable attention for their diverse activities. Under natural conditions, the type of microbes dominating rhizospheric and endophytic compartments of a crop plant determines its health (Nihorimbere et al. 2011). These microbial agents promote growth and development of crop plants. A prominent mode of growth promotion used by these agents is to keep in check the external inhibitors that hamper the well-being of plants, thereby increasing the yield. Beneficial microbes do so either directly by supplying resources for proper growth or indirectly by manipulating the factors acting against plant growth (Glick 2012).

Plant scientists are now increasingly recognizing the role of rhizospheric microbes in plant growth enhancement and overall well-being of the plant (Vessey 2003). Microbes isolated and used for plant growth promotion (PGP) majorly include bacteria and fungi. Among them, *Actinobacteria* has been gaining popularity owing to its vast array of bioactive compounds that have the potential to positively affect plant growth (Martinez-Noel et al. 2001; Xiao et al. 2002; Lehr et al. 2008). *Actinobacteria* are a group of gram-positive aerobic, saprophytic bacteria (Crawford et al. 1993) with high G+C content (Bouizgarne 2013). They are morphologically similar to fungi in forming mycelia. *Actinobacteria* are well-studied group for their bioactive metabolites and are known to be one of the major producers of antibiotics and other compounds (Berdy 2012). Approximately 80 % of the known microbial metabolites are produced by *Streptomyces*, a major group belonging to *Actinobacteria* (Berdy 2012).

2.2 Indirect Growth Promotion

2.2.1 Overview

Indirect growth promotion refers to the use of plant growth-promoting agents for controlling and minimizing the deleterious effects of external factors to improve the overall health and fitness of the plant. Such microbes are referred to as biological control agents (BCAs). Generally, indirect growth promotion can be classified as PGP and non-PGP action depending on their ability to enhance growth of plant apart from suppressing external stress factors (Bouizgarne 2013). Several researchers have reviewed the use of microbes for disease suppression and growth promotion (Glick 2012; Bouizgarne 2013; Palaniyandi et al. 2013a; Dey et al. 2014). This chapter mainly focuses on the use of *Actinobacteria* and their reported indirect growth-promoting mechanisms in few important grain legumes.

2.2.2 Bio-mechanisms

Antagonism is the property exhibited by one living organism by which it creates a hostile environment for another organism. Mechanisms employed by free-living rhizospheric and endophytic *Actinobacteria* for antagonism include competition, production of external inhibitory substances, and hyperparasitism. There has been a substantial advancement in deciphering the underlying molecular, physiological, and

morphological mechanisms pertaining to bacteria-mediated biotic stress tolerance (Van Loon et al. 1998). Additionally, microbes depicting different modes of action have a higher success rate in suppressing disease, and such microbes make good candidates as BCA (Palaniyandi et al. 2013a).

2.2.2.1 Competition

Soil plays host to a plethora of microorganisms that maintain its structure and integrity. There exists a large pool of potential competitors, and a variety of mechanisms can be responsible for the dominance of certain populations (Hibbing et al. 2010). Monod, through his experiments using limiting nutrients, demonstrated that nutritional availability plays a crucial role in microbial competition (Monod 1949, 1950). Plant root exudates play a major role in determining the specific community of microbes living in its vicinity. The presence of phenolic and flavonoid compounds influences plant symbiosis with beneficial rhizobacteria (Palaniyandi et al. 2013a), and other substances such as sugars, amino acids, vitamins, and organic acids serve as vital nutrients for microbes (Dakora and Phillips 2002). The production of siderophores (Sontag et al. 2006; Macagnan et al. 2008), lytic enzymes (Potgieter and Alexander 1996), volatile compounds (Wan et al. 2008), and antibiotics (Agbessi et al. 2003) is one of the mechanisms exerted by microbes. Antagonism through competition for available nutrients is one form of mechanism used to outlive pathogenic microbes (Siddikee et al. 2010) and suppression of disease (Palaniyandi et al. 2013a).

2.2.2.2 Siderophores

Iron, an essential element for the growth of both plants and microbes, is present in lower concentration in the rhizosphere (Pal and Gardener 2006). Siderophores are low-molecular weight, high iron affinity compounds secreted by microbes. They are able to chelate Fe^{3+} molecules, and these ferric complexes are taken up by bacteria using specialized receptors (Crosa 1989) and also make them available for plants. The production of siderophores is induced even under lower iron concentrations (Bouizgarne 2013). Two major classes of siderophores, classified based on their functional group, are catechols and hydroxamate (Lee et al. 2012a, b). A mix of carboxylate–hydroxamate group of siderophores also reported (Hider and Kong 2010; Raymond and Dertz 2004). Reports on the production of different types of siderophores belonging to either of these classes have been established. Some of the types of microbial siderophores are enterobactin, heterobactin (Lee et al. 2012a), ferrioxamine, and ferrichrome (Muller and Raymond 1984). Numerous strains of *Streptomyces* spp. have been reported as siderophore producers. Examples include *S. pilosus* (Muller et al. 1984, Muller and Raymond 1984) *S. lydicus* (Tokala et al. 2002), and *S. violaceusniger* (Buyer et al. 1989).

Biological control strategy of siderophore-producing microbes is a two-way process. It works by acquiring iron, thereby putting up competition for other pathogenic microbes in the vicinity, and also by supplying it to the plant which can directly be used for their own growth (Glick 2012). The production of siderophores by beneficial microbes is perceived as a means of biological control of phytopathogens which especially is an effective method in controlling fungal pathogens which produce low-affinity siderophores (Schippers et al. 1987). Microorganisms which can produce siderophores with highest affinity for iron efficiently colonize the rhizosphere, and those with low-affinity siderophores are eliminated (Kloepper et al. 1980). Several reports indicate the involvement of siderophores from *Actinobacteria* in pathogen suppression (Sontag et al. 2006; Macagnan et al. 2008). Studies by Barona-Gómez et al. (2006) revealed the production of multiple siderophores by certain *Streptomyces* spp. Moreover, it was also reported that siderophores produced by one group of *Actinobacteria* could also be utilized by other groups, thereby promoting its growth (Yamanaka et al. 2005; D'Onofrio et al. 2010).

2.2.2.3 External Inhibitory Substances

Microorganisms are capable of secreting a wide variety of extracellular compounds depending on the substrate availability. Most of these compounds serve as inhibitory factors for pathogenic organisms. These compounds inhibit pathogenic microbes either by "static" or "cidal" activity. Cell wall-degrading lytic enzymes and metabolites (Gopalakrishnan et al. 2014) are the common types of inhibitory compounds synthesized by antagonistic microbes. *Actinobacteria* antagonize disease-causing microbes by producing one or more of these compounds.

2.2.2.4 Cell Wall-Degrading Enzymes

The production of bacterial or fungal cell wall-degrading enzymes is a way of targeting plant pathogens. They disrupt the cell wall components which results in cell lysis. Actinobacteria are reported to produce different kinds of lytic enzymes such as chitinases (Gupta et al. 1995; Mahadevan and Crawford 1996), glucanases (Damude et al. 1993; Thomas and Crawford 1998; Trejo-Estrada et al. 1998), peroxidases (Fodil et al. 2011), and proteases (Dunne et al. 1997). The general role of these enzymes is to decompose organic residues providing carbon nutrition (Pal and Gardener 2006). A detailed study on the biology of extracellular compounds from *Streptomyces* spp. was established by Chater et al. (2010). Cell wall of most fungal pathogens consists of polymers like chitin, glucan, cellulose, proteins, and lipids (Garcia 1968). *Actinobacteria* capable of excreting lytic enzymes as one mechanism would make a good choice as biocontrol agents.

2.2.2.5 Protection by Metabolites

Antibiotics are a major class of secondary metabolites produced by microorganisms that are poisonous to the growth of other pathogenic microbes even at lower concentrations. The discovery of antibiotics is considered as a major milestone in medical history. Microorganisms are excellent sources of antibiotics and among them *Actinobacteria* form a major share of producers. According to Liu et al. (2012), about 45 % of the antibiotics currently in use come from *Actinobacteria*. So far, about 33,500 bioactive metabolites have been reported of which *Actinobacteria* constitute about 13,700 compounds (Berdy 2012). The metabolites belong to chemically diverse class of compounds such as macrolides, polyether antibiotics, cyclopolylactones, anthracyclines, aminoglycosides, streptothricins, and quinoxaline peptides. Non-*Streptomyces Actinobacteria* mainly produce glycopeptides and orthosomycins (Berdy 2012). Antibiotic-producing microbes have a competitive edge over nonproducers as it increases their chances of survival. This property has been exploited and studied by several researchers for the biological control of plant pathogens and disease suppression Crawford et al. 1993; Chamberlain and Crawford 1999; Agbessi et al. 2003; Khamna et al. 2009; Meschke et al. 2012).

A study by Beausejour et al. (2001) using mutants defective in the production of certain antibiotic could not control disease as opposed to nonmutants of the strain. Commercial formulations with the antibiotic or microbes as an active ingredient are marketed for plant disease control. Actinovate® and Actino-Iron® by *Streptomyces lydicus* WYEC 108 (Crawford et al. 2005), Mycostop® by *Streptomyces griseoviridis* K61 (Figueiredo et al. 2010), and Arzent™ by four different strains of *S. hygroscopicus* are some of the examples of commercial products (Hamby and Crawford 2000). Besides this, cycloheximide from *Streptomyces griseus*, kasugamycin from *Streptomyces kasugaensis*, blasticidin S from *Streptomyces griseochromogenes*, and Rhizovit from *Streptomyces rimosus* are some of the antibiotic compound of *Actinomycete* origin.

The secondary metabolites of *Actinobacteria*, namely, tetranectin, avermectins, faerifungin, macrotetrolides, and flavonoids, produced were found to be toxic to many insects. Avermectins are compounds produced by a novel species *Streptomyces avermitilis* isolated from soil. Initially, it was observed as an effective antihelminthic compound (Burg et al. 1979), but later it was found to be a potent insecticide, acaricide, and nematicide (Putter et al. 1981).

Spinosyn is a large family of unprecedented compounds isolated from two species of *Saccharopolyspora spinosa*. The fermentation of *S. spinosa* produces several metabolites that are called spinosyn A and spinosyn D. They have a novel molecular structure, and their mode of action is by affecting nicotinic acetylcholine receptors at the postsynaptic cells. They are very selective toward target insects such as lepidoptera and diptera and show very low specificity against many beneficial insect predators and nontarget species (Thompson et al. 2000; Salgado and Sparks 2005). The efficiency of spinosad depends on the type of species and their stage of development, exposure time, and method of administration. The significant advantage of spinosad includes less toxicity toward mammals, avians, and aquatic organisms compared to other insecticides, thus making it safer to use (Thompson and Sparks 2002).

2.2.2.6 Volatile Substances

Apart from the production of antibiotics, some biocontrol agents are also known to produce volatile compounds as tools for pathogen inhibition. Common volatile compounds are hydrocyanic acid (HCN), certain acids, alcohols, ketones, aldehydes, and sulfides (Bouizgarne 2013). Reports on the production of HCN by beneficial microbes in order to minimize the deleterious effect of pathogenic fungi are available (Ahmad et al. 2008). Défago et al. (1990) suggested that HCN production works by inducing resistance in plants. Actinobacteria as producers of volatile compounds for plant disease control were reported by Moore-Landecker and Stotzky (1973), Wang et al. (2013), and Boukaew et al. (2013).

2.2.2.7 Hyperparasitism

In hyperparasitism, BCAs directly attack and parasitize disease-causing fungi and kill them. This phenomenon is exhibited by a range of bacteria and fungi in which they feed on pathogenic microbes. Four kinds of hyperparasites can be found: obligate bacterial pathogens, hypoviruses, facultative parasites, and predators (Pal and Gardener 2006). Hyperparasites are reported to penetrate fungal hyphae forming branches and coagulating its cytoplasm and ultimately degrading the hyphae (Upadhyay and Rai 1987). Several reports on hyperparasitism by *Actinobacteria* on a range of fungi are available (Tu 1988; Tapio and Pohto-Lahdenpera 1991; Yuan and Crawford 1995). Though parasitism is demonstrated in many BCA, it is not reported as the sole mechanism of pathogen control (Palaniyandi et al. 2013a). Apart from *Streptomyces* spp., hyperparasitism is also reported and extensively reviewed in non-*Streptomyces* spp. Enzymes and antibiotics produced by the antagonistic microbes can parasitize fungal hyphae susceptible to parasitization (El-Tarabily and Sivasithamparam 2006).

2.2.2.8 Induced Systemic Resistance

Resistance is one of the best tools for management of plant pathogens and pests. Induced resistance is a form of defense mechanism in plant activity that is elicited by the interaction with an external factor. This factor can be chemical or biological in origin. Two types of nonspecific defense systems are reported in plants: beneficial microbe-induced systemic resistance (ISR) and pathogen-induced systemic acquired resistance (SAR) (Schuhegger et al. 2006). In both the types, protection is conferred systemically even in the nonexposed parts of the plant (Kuc 1982). In ISR, plants are primed by beneficial microbes providing protection from a broad spectrum of pest and pathogen attack (Alstrom 1991; Van Peer et al. 1991; Wei et al. 1991; Walters et al. 2013). As opposed to SAR, ISR does not involve the accumulation of pathogenesis-related (PR) proteins. This was first demonstrated in radish by Hoffland et al. (1995). ISR is regulated by components such as jasmonic acid, salicylic acid, and ethylene (De Meyer et al. 1999; Thomma et al. 2001; Audenaert et al. 2002; Verhagen et al. 2004).

2.3 Biotic Stress Management

2.3.1 Overview

The role of *Actinobacteria* in disease control was recorded more than a century ago. Greig-Smith (1917) recorded the competence of *Streptomyces* to suppress certain soil microbes. Since then, many researches forayed into the field of natural disease control by tapping the vast potential of *Actinobacteria*. Apart from *Streptomyces* sp., non-streptomycete *Actinobacteria* both endophytic and rhizosphere competent have shown properties of disease suppression and growth promotion employing any of the above-mentioned mechanisms like antibiosis, hyperparasitism, and lytic enzymes (El- Tarabily et al. 1996, 1997; El-Tarabily and Sivasithamparam 2006).

2.3.2 Pathogen Control

To achieve global food security, one important area to focus is curbing the menace of yield limiting factors which reduce both quantity and quality of the crops (Atkinson and Urwin 2012). Among these factors, the level of destruction caused by disease-causing pathogens is notable. Irish famine (1845) and Bengal famine (1943) are some of the examples of plant disease induced yield losses causing a massive impact on human life and crippling economy. Several pathogens are reported to hamper the production of grain legumes. Important diseases of grain legumes include sterility mosaic, *Fusarium* wilt and *Phytophthora* blight of pigeon pea and *Botrytis* gray mold, *Ascochyta* blight, and *Fusarium* wilt of chickpea.

Biological control of plant pathogens is a sustainable solution of disease management. Tu (1988) reported the parasitization of *Colletotrichum lindemuthianum* by the soil *Actinobacteria S. griseus*. It was noted that the pathogen produced appersorium-like swellings on the surface of hyphae. Internal formation of bulbs resulting in the degeneration of pathogen hyphae was also observed. Hyperparasitism of fungal pathogens as a mechanism was also observed in *Streptomyces cyaneofuscatus* ZY-153, *Streptomyces kanamyceticus* B-49, *Streptomyces rochei* X-4, *Streptomyces flavotricini* Z-13 (Xue et al. 2013), and *Streptomyces phaeopurpureus* ExPro138 (Palaniyandi et al. 2013b). The production of antibiotics is a characteristic feature of this group of bacteria which can be exploited for disease control. An antibiotic geldanamycin from *S. hygroscopicus* pv. *geldonus* was reported to suppress the growth of *Rhizoctonia solani* by Rothrock and Gottlieb (1984). *Streptomyces* spp. were reported for direct growth enhancement and antibiotic production to control root rot caused by *Phytophthora sojae* in soybean. Population densities of pathogen were effectively reduced under controlled conditions with naturally infested soil (Xiao et al. 2002).

The production of cell wall-degrading enzymes has been reported as a biocontrol mechanism by certain *Actinobacteria*. *S. cavourensis* SY224 reported to produce chitinase and glucanase against *Colletotrichum gloeosporioides* (Lee et al. 2012b). Glucanase production by *S. violaceusniger* XL-2 (Shekhar et al. 2006) and by some *Streptomyces* spp. was reported to suppress the growth of phytopathogenic fungi (Valois et al. 1996). Spore germination of *Fusarium udum* was inhibited by the proteases of *Streptomyces* sp. A6 (Singh and Chhatpar 2011). Biological control of *Fusarium* spp. was also reported by some non-streptomycete *Actinobacteria*. *Nocardiopsis dassonvillei* inhibited the growth of *F. oxysporum* f. sp. *albedinis* (Sabaou et al. 1983), and *Micromonospora globosa* controlled populations of *F. udum* (Upadhyay and Rai 1987) through mycoparasitism. Field studies using *Streptomyces* sp. P4 isolate effectively reduced the incidence of powdery mildew of *Pisum sativum* (Sangamee et al. 2009). Foliar spray using spore suspensions of the antagonistic isolates inhibited the formation of appersoria (Sangmanee et al. 2009). The production of siderophores as a means of pathogen control was established in *Streptomyces albovinaceus*, *S. griseus*, and

S. virginiae (Macagnan et al. 2008). Siderophore-producing *Streptomyces philanthi* RM-1-138 effectively controlled populations of *Rhizoctonia solani*, *Pyricularia grisea*, *Bipolaris oryzae*, and *Fusarium fujikuroi* (Boukaew et al. 2013).

Pathogen control and disease suppression by antibiotic production is another important mechanism studied in *Actinobacteria*. The use of metabolites having a broad spectrum activity is a crucial step in the management of plant diseases. *Streptomyces malaysiensis* MJM1968 reported to produce azalomycin (Jinhua et al. 2010) effectively reduced the inhibitory effects of phytopathogenic *Fusarium oxysporum*, *Rhizoctonia solani*, *Cladosporium cladosporioides*, *Fusarium chlamydosporum*, *Colletotrichum gloeosporioides*, *Alternaria mali*, and *Pestalotia* spp. (Jinhua et al. 2010). Wang et al. (2013) reported that *Streptomyces alboflavus* TD-1 curbed the growth of economically important fungi *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger*, and *Penicillium citrinum* in vitro. *Streptomyces galbus* R-5 an endophytic microbe was reported to produce actinomycin X2 and fungichromin (Hasegawa et al. 2006). Two or more of these mechanisms usually act in combination with each other to tackle the pathogen completely. Several reports are available on the successful use of *Actinobacteria* for disease control in different plant species (Neeno-Eckwall et al. 2001; Conn et al. 2008; El-Tarabily et al. 2009; Palaniyandi et al. 2013b).

2.3.3 Insect Pest Control

Insect pests along with other pathogenic organisms are reported to cause yield losses of about 20–40 % in economically important food crops (Zhou 2001). There are about 70,000 different insect species known to damage food crops. Major pests of the cultivated crops are belonging to the order *Lepidoptera* (Pimental 2009). *Helicoverpa armigera* and *Spodoptera litura* are important pests of this group causing significant losses to crops such as groundnut, pigeon pea, chickpea, soybean, cowpea, chili, tobacco, caster, and okra (Armes et al. 1996). These insects pose a major threat to chickpea productivity with annual losses of up to 40 % besides the losses due to *Maruca* (another important pod borer) and bruchids, an important storage pest (Rao et al. 2010). The devastating potential of these pests is currently managed using insecticides. Reports on the emergence of resistance make them more notorious to control and necessitate for finding cleaner and greener alternatives which is important.

Many biological management options are studied which includes the use of bacteria, fungi, and viruses (Deshpande 1999). The insecticidal activity of *Actinobacteria* against some major pests was studied by Gopalakrishnan et al. (2011), Arasu et al. (2013), Vijayabharathi et al. (2014), and Sathya et al. (2015). Extracellular metabolites from three strains of *Streptomyces*, *S. griseoplanus*, *S. bacillaris*, and *S. albolongus*, were shown to be effective broad spectrum entomopathogens showing activity against *Helicoverpa armigera*, *S. litura*, and *Chilo partellus*. The tested biocontrol strains produced insecticidal metabolites that clearly inhibited the growth of these pests under in vitro as well as in vivo conditions (Vijayabharathi et al. 2014).

2.4 Abiotic Stress Management

Abiotic stresses such as drought, waterlogging, heat, salinity, metal toxicity, and nutrient deficiency also cause productivity loss (Shao et al. 2008). These abiotic stressors threaten to decrease the total arable land, which, coupled with the burgeoning population, contaminate agricultural sustainability (Shahbaz and Ashraf 2013). Drought and heat stress challenge chickpea cultivation from realizing its full potential especially in the semiarid region (Rao et al. 2010). Likewise, the production of pigeon pea is significantly restrained by drought, water logging, and salinity. Abiotic stresses have higher economic impact on pigeon pea production than biotic stresses (Ryan 1995). These

factors are mostly overcome by the selection of tolerant varieties through breeding and genetic engineering (Rai et al. 2011). Plant responses to such stress conditions involve molecular, cellular, and physiological changes (Rejeb et al. 2014). A known mechanism by which plants overcome stress in natural environment is the production of phytohormones which promotes plant growth. Specifically, plants exposed to water or nutrient stress conditions exhibit certain physiological changes in their root structure which involves the production of growth-related hormones (Potters et al. 2007). Auxins are phytohormones which promote cell elongation at low concentrations (Martin and Elliott 1984). Exploiting microorganisms' ability to withstand harsh environmental conditions and production of certain enzymes could impart microbe-assisted abiotic stress tolerance in plants (Shrivastava and Kumar 2015). This approach promises a cheaper and sustainable solution. Though many studies regarding the use of bacteria in abiotic stress tolerance are found, the specific mechanisms underlying these are still elusive (Roman et al. 1995; O'Donnell et al. 1996; Penninckx et al. 1998).

Plants respond to any form of stress by producing the stress hormone ethylene. The production of ethylene is significantly increased under stress conditions having deleterious effect on plants by speeding up cell senescence (Abeles et al. 1992; Woltering and Van Doorn 1988; Nayani et al. 1998; Ali et al. 2012). Certain microbes are capable of regulating ethylene biosynthesis with the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme metabolizes ACC (a precursor to ethylene) into α-ketobutyrate and ammonia. Lately, this property of beneficial microbes has been exploited by researchers for plant growth and development under abiotic stress conditions (Arshad et al. 2008; Bal et al. 2013; Chookietwattana and Maneewan 2012). ACC deaminase positive strains work by decreasing the levels of ethylene which promotes root growth (Glick et al. 1998; Burd et al. 2000; Belimov et al. 2007, 2009; Long et al. 2008). This was experimentally shown in a study using endophytic *Actinobacteria* producing ACC deaminase. Treatment of mung bean plants with PGPA resulted in the enhanced root and shoot growth along with increased chlorophyll content. These physiological changes conferred the bean plants with the ability to better tolerate high saline (100 mM NaCl) and waterlogging conditions compared to uninoculated and ACC deaminase-deficient mutants (Jaemsaeng et al. 2013).

Several *Actinobacteria* have been reported to produce indoleacetic acid (IAA), a type of auxin (Lin and Xu 2013; Manulis et al. 1994; Kaur et al. 2013). The production of IAA by beneficial microbes has been reported to stimulate growth of plants in the face of abiotic stressors. Microbes use tryptophan exudates by plants as a substrate and release IAA which is taken up by plants. Gopalakrishnan et al. (2014) showed that rice plants upon inoculation with PGP *Actinobacteria* recorded an increase in root growth and root hairs. Research done by Sadeghi et al. (2012) on *Streptomyces* isolates having the ability to produce auxins and siderophores under saline soil conditions showed a significant increase in the biomass of the plant. Their study showed an increase in the concentration of N, P, Fe, and Mn in wheat shoots grown in normal and saline soil. Enhanced root growth promotes increase in root surface area which aids in nutrient uptake and water acquisition (Dimkpa et al. 2009). Some strains of *Actinobacteria* have been reported to stimulate root nodule formation by *Rhizobia* (Tokala et al. 2002). Colonization of *S. lydicus* WYEC108 with pea plants has shown an increase in size and vigor of the nodules which in turn assimilates soil nutrients (Tokala et al. 2002). Nodule formation by *Frankia* spp. was also influenced by many species of *Actinobacteria* including *Streptomyces*, *Actinoplanes*, and *Micromonospora* (Solans 2007). Apart from direct involvement of *Actinobacteria* in the promotion of nodule formation, culture filtrates have also been reported to enhance nodulation (Solans 2007).

Phosphorous is an essential nutrient for plants and deficiency directly affects crop productivity. Soil phosphorus is generally present as insoluble

metal complexes which decrease the amount of available phosphorus for plants (Hamdali et al. 2008). Beneficial microbes have the ability to solubilize these complexes releasing free phosphates (Rodríguez and Fraga 1999). Many species from *Actinobacteria* have been reported to solubilize phosphorous (Hamdali et al. 2008; Oliveira et al. 2009; Franco-Correaa et al. 2010). Particularly, greenhouse studies by (El-Tarabily et al. 2008) using phosphate-solubilizing isolate *Micromonospora endolithica* showed an increase in the growth of root and shoot of bean plants (*Phaseolus vulgaris* L.) compared to nonphosphate-solubilizing *M. olivasterospora*. The positive isolates made phosphorous accessible to the bean plants (El-Tarabily et al. 2008). Ability of microbes to solubilize essential elements and make them available for plant use along with stimulation of nodulation can be perceived as an important characteristic to overcome stress related to nutrient deficiency.

2.5 Conclusion

Scientifically proven role of *Actinobacteria* in crop improvement assures a promising future in the field of biological control and indirect growth promotion. The ability of *Actinobacteria* to produce a wide range of antibiotics is regarded as an important tool in disease suppression. Discovery of spinosyn and reports of its nonpersistence in natural environment (Kirst 2010) gave the much needed boost to antibiotic-mediated pathogen suppression. However, it is now clear that beneficial microbes don't just rely on one single mode of action to suppress pathogens. Mechanisms like the competition for nutrients and antibiosis and production of lytic enzymes can all act together for achieving better results. Though considerable research has been conducted with the use of *Actinobacteria* as disease control agents, most of these studies have been restricted to greenhouse level focusing on an immediate response. It is necessary to conduct more field level evaluations. For this, it is important to have a better understanding of the interaction between the beneficial organism and the plant, indigenous microbes, pathogen, and environment (Raja et al. 2006). Additionally, it also important to focus on the different factors detrimental for proper functioning of the introduced microbes in the field soil in order to provide optimum conditions (Bouizgarne 2013). Beneficial microbes capable of conferring cross protection against both biotic and abiotic factors are preferable (Dimkpa et al. 2009). A boost in the production achieved through external stress control increases both quality and quantity of grain legumes making it available to resource poor farmers. It is a common practice by farmers to grow grain legumes as intercrops, relay crops, and end-of-season crops in underutilized habitats enabling them to reap more food from less land. However, before commercializing *Actinobacteria* or actinobacterial products for the development of sustainable agricultural solution, it is important to educate the farming community and general public the benefits of microorganisms without which all the research will remain in the laboratories (Glick 2012).

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Enhancing Soil Health and Plant Growth Promotion by Actinomycetes

3

R. Jog, G. Nareshkumar, and S. Rajkumar

Abstract

In recent times, numerous concrete efforts have been made by global scientific community for maintenance and judicious utilization of certain non-renewable natural resources like metal ores, fossil fuels and to an extent groundwater. However, soil, an important non-renewable asset, has received little attention and demands more awareness and exploration by researchers worldwide. Soil regeneration through chemical and biological processes of rock weathering takes several thousand years; thus, soil is classified as a vital, finite and non-renewable source. Soil health, thus, becomes a critical factor for humans, animals, plants and all natural ecosystems. Soil health deterioration, increased by industrialization and indiscriminate use of chemical fertilizers, has become a major environmental concern with high precedence. Public awareness to these problems has shifted approach to alternative strategies like using plant growth-promoting rhizobacteria (PGPR), also popular as bio-fertilizers, for achieving cleaner, safer and cost-effective increase in agricultural productivity. Amongst several bacteria reported as PGPR, actinomycete is one of the most promising options due to properties like nutrient cycling, antibiosis, rhizosphere competence and beneficial plant growth-promoting (PGP) traits. In this chapter, we intend to discuss about how actinomycetes are crucial as PGPR in maintaining soil health, fertility and agricultural productivity and investigate underlying PGPR

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mechanisms. We shall also briefly enlist few successful PGP actinomycetes, challenges associated and future implications to increase soil fertility.

Keywords
Actinomycetes • PGPR mechanism • Phosphate solubilization • Antibiosis • Soil health • Soil enzymes • Field trials

3.1 Soil Health and Degradation

Soil, an integral part of earth's biosphere, is crucial in managing nutritional requirements and environmental quality and affects the difference between survival and extinction for most terrestrial life forms. Earlier, the term soil quality was used to assess soil parameters, which typically meant soil with good agricultural productivity without substantial degradation over several years of farming. However, productivity is only a fraction of roles that soil plays and does not include interactions with surrounding environment and its implications on the health of associated animals and humans. Hence, soil health, an inclusive term, is defined as "The continued capacity of soil to function as a vital living system within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environment, and maintain plant, animal and human health" (Doran and Safley 1997). Primary component dominating soil is solid phase consisting of various sized particles surrounded by water and gases, which significantly differ with time and space. A unique balance that is established between these components by systemic continuous exchange of molecules and ions through several biochemical processes is vital for maintenance of soil health (Nielsen and Winding 2002).

Human-induced soil degradation is the biggest threat for soil health and subsequent agricultural productivity. Chemical degradation by nutrient depletion, salinization and acidification has emerged as an issue of serious concern with agricultural mismanagement (58 %) and deforestation (28 %) as the main factors. Nutrient depletion occurs by application of lower than required amount of chemical fertilizers or more often due to non-absorption by plants due to nutrient leaching. Although nutrient leaching does occur in natural vegetation, it is significantly increased by agricultural activities especially in soils with high water infiltration and lower nutrient retention capacity. Chemical fertilizers further compound the problem, for example, nitrate. Nitrates, an immobile ion in negatively charged topsoil layer, are produced in large quantities due to nitrification of ammonia added as fertilizer, thus causing serious problems. Similarly, phosphates in fertilizers are precipitated by soil cations like calcium, aluminium, iron and manganese, leading to its immobilization and non-availability to plants (Khan 2014). Nutrients leached from soil contaminate groundwater and open reservoirs surrounding agricultural landscape resulting in eutrophication and ecosystem destabilization. Lower level of oxygen in waterbodies reduces drinking water availability, limits number of aquatic species and proliferates unwanted species releasing foul odour and toxic compounds. It is amid these global concerns that beneficial microorganisms growing in the root zone, also referred to as plant growth-promoting rhizobacteria (PGPR), have emerged as an effective, safe, cheap and environment-friendly alternative for chemical fertilizers.

3.2 Microorganisms Associated with Plant Rhizosphere

Rhizosphere is defined as the area (soil) surrounding roots (approximately 1–10 cm) characterized by greater microbial activity as compared to bulk soil (Hiltner 1904). It is well known that concentration of bacteria in

rhizosphere (rhizobacteria) is 10–1000 times higher than that in bulk soil. The root exudates comprising 5–21 % of carbon fixed by plants contain various organic compounds, which serve as a rich nutrient source for bacteria resulting in an ecological hotspot (Marschner 1995). Rhizobacteria colonization is a highly selective and systematic process resultant of a complex plant-microbe interaction and acts as a driving force for recycling soil nutrients and consequently enhancing soil fertility. In addition to nutrient cycling, several rhizobacteria also have capability to promote plant growth either directly by facilitating nutrient acquisition (nitrogen, phosphate and essential minerals) and modulating plant hormones or indirectly by decreasing the inhibitory effects of various pathogens as biocontrol agents, thus making them an attractive alternative for chemical fertilizers in agricultural practice (Glick 2012). PGPR strains broadly are spread amongst varied taxa including Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria (Tilak et al. 2005). Actinobacteria (also referred to as actinomycetes) are abundantly distributed with average 10^4–10^6 propagules or spores (CFU) g^{-1} soil in cropped fields (Mareckova and Kopecky 2012).

3.3 Actinomycete Rhizosphere Diversity and Its Role as PGPR

Actinomycetes, Gram-positive filamentous bacteria, thrive in rhizosphere and colonize plant tissues after dormant sporulation to survive in agricultural soils for long period. Actinomycetes have been studied for their PGP and biocontrol activities for improving agricultural yields (Shaharokhi et al. 2005). Few actinomycetes are also reported as endophytes, colonizing plant tissues of various crops including grain legumes, wheat, maize and several medicinal plants. Actinomycetes are considered to be one of the important rhizobacterial communities and play a crucial role in maintaining rhizosphere ecology and soil nutrient cycling (Benizri et al. 2005). Actinomycetes have been reported in abundance from diverse plant rhizosphere (potato, maize, winter rye, medicinal plants, tomato, groundnut and grain legumes) by either culturable or molecular methods (Heuer et al. 1997; Tan et al. 2006; Shaharokhi et al. 2005; Khamna et al. 2009). Whilst *Streptomyces* is the predominant genus, numerous other genera of common and/or rare actinobacterial strains have been reported from rhizosphere of edible crops (cereals and legumes), cash crops and medicinal plants as summarized in Table 3.1.

Rhizospheric actinomycetes influence plant growth directly by plant growth-promoting (PGP) activities including phosphate solubilization, indole acetic acid (IAA) and siderophore and hydrolytic enzyme production and indirectly by exerting pathogen control through chitinase and antimicrobial secondary metabolite production. Moreover, they also secrete an array of soil enzymes to help maintain soil fertility and nutrient cycling.

3.3.1 Actinomycetes and Phosphate Solubilization

Phosphorous (P) is the second most important plant nutrient after nitrogen; however, 95–99 % of it remains in insoluble form, thus unavailable to plants (Corona et al. 1996). Chemical fertilizers applied to overcome P deficiency readily precipitate upon application (Pundarikakshudu 1989); hence, to counterbalance, excess phosphate fertilizers are added that leads to eutrophication (Correll 1998). Rare, but extremely high P-solubilizing actinomycetes have been reported by many researchers, using buffered tricalcium phosphate (TCP) as well as rock phosphate (RP) medium. Some of them, including *Arthrobacter*, (RP 519 mg l^{-1}), *Streptomyces* mhcr0816 (TCP 1916 mg l^{-1}, RP 990 mg l^{-1}) and *Streptomyces* sp. (RP 250 mg l^{-1}), are comparable to highly cited *Bacillus* (TCP 957 mg l^{-1}) or *Pseudomonas* (TCP 1500 mg l^{-1}) strains under similar conditions (Chen et al. 2006; Mehta et al. 2010; Hamdali et al. 2012; Jog et al. 2014). Organic acid secretion results in acidification of the microbial cells and its surroundings, thus leading to proton substitution of Ca^{+2} and solubilizing mineral phosphate (Rodríguez and Fraga 1999).

Table 3.1 Actinomycete diversity in rhizosphere

Plant	Actinomycete genera	References
Medicinal plants	*Streptomyces*, *Actinomadura*, *Microbispora*, *Micromonospora*, *Nocardia*, *Nonomuraea*	Khamna et al. (2009)
Oryza sativa (rice)	*Mycobacterium*, *Frankia*, *Streptomyces*, *Micromonospora*, *Actinoplanes*	Tian et al. (2007)
Lycopersicon esculentum (tomato)	*Streptomyces*, *Nocardia*	Cao et al. (2004)
Gossypium herbaceum (cotton)	*Streptomyces*	Hassanin et al. (2007)
Hevea brasiliensis (rubber)	*Streptomyces*, *Nocardia*, *Nonomuraea*, *Micromonospora*, *Saccharopolyspora*, *Verrucosispora*	Poomthongdee et al. (2015)
Rhizophora mangle (mangrove)	*Micromonospora*, *Streptosporangia*, *Thermomonospora*	Ara et al. (2013)
Triticum aestivum (wheat)	*Streptomyces*	Jog et al. (2012)
Lupinus angustifolius (blue lupin)	*Micromonospora*	Trujillo et al. (2010)
Medicago sativa (alfalfa)	*Micromonospora*	Hidalgo-Martinez et al. (2014)

Mineral phosphate solubilization via gluconic acid production by bacteria, such as *Pseudomonas* spp., *Erwinia* spp. and *Burkholderia* spp., is highly reported (Rodríguez and Fraga 1999). Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid, produced by *Rhizobium* spp. and *Bacillus* spp. (Duff and Webley 1954). Organic acids such as glycolic acid, oxalic acid, malonic acid, citric acid and propionic acid have also been reported amongst phosphate solubilizers (Chen et al. 2006). Actinomycetes are known to produce organic acids such as pyruvate, lactate, α-ketoglutarate, succinate, malate and oxalate in varying concentrations (Rozycki and Strzelczyk 1986) that solubilize mineral phosphate in rhizosphere of diverse crops, thus improving plant growth (Postma et al. 2010; Jog et al. 2012).

3.3.1.1 Mechanism of Organic Acid Production

Microbial metabolism in soil is regulated by several biotic and abiotic factors including carbon source availability. Metabolism and physiology of phosphate-solubilizing microorganisms (PSMs) is also one of the important factors that can regulate their mineral phosphate solubilization (MPS) phenotype, as the carbon source availability and metabolism of carbon source will decide the organic acid to be produced. As an example, several Gram-negative bacteria oxidize peripheral glucose via pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) to produce gluconic acid which on further oxidation is converted to 2-ketogluconic acid. However, except few *Streptomyces* strains, actinomycetes lack PQQ cluster and subsequent dehydratase enzyme comprising Entner-Doudoroff oxidation pathway.

Glucose uptake is facilitated by glucose permease belonging to major facilitator superfamily (MFS) encoded by two independent glucose-induced gene loci (Wezel et al. 2005). Embden-Meyerhof-Parnas (EMP) Pathway (glycolysis) and subsequent tricarboxylic acid (TCA) cycle form central sugar metabolism pathway for actinomycetes involved in energy and secondary metabolite precursor production. *Streptomyces* sp. producing pyruvic acid and 2-oxoglutaric acid in the presence of glucose as sole carbon source has been reported (Madden et al. 1996). Jog et al. (2014) reported the involvement of glyoxylate bypass in high phosphate-solubilizing *Streptomyces* sp. for production of malic acid (Fig. 3.1). Surprisingly, they found that malate production profile was not significantly influenced by type of hexose sugar available or glucose concentration in medium (50 or

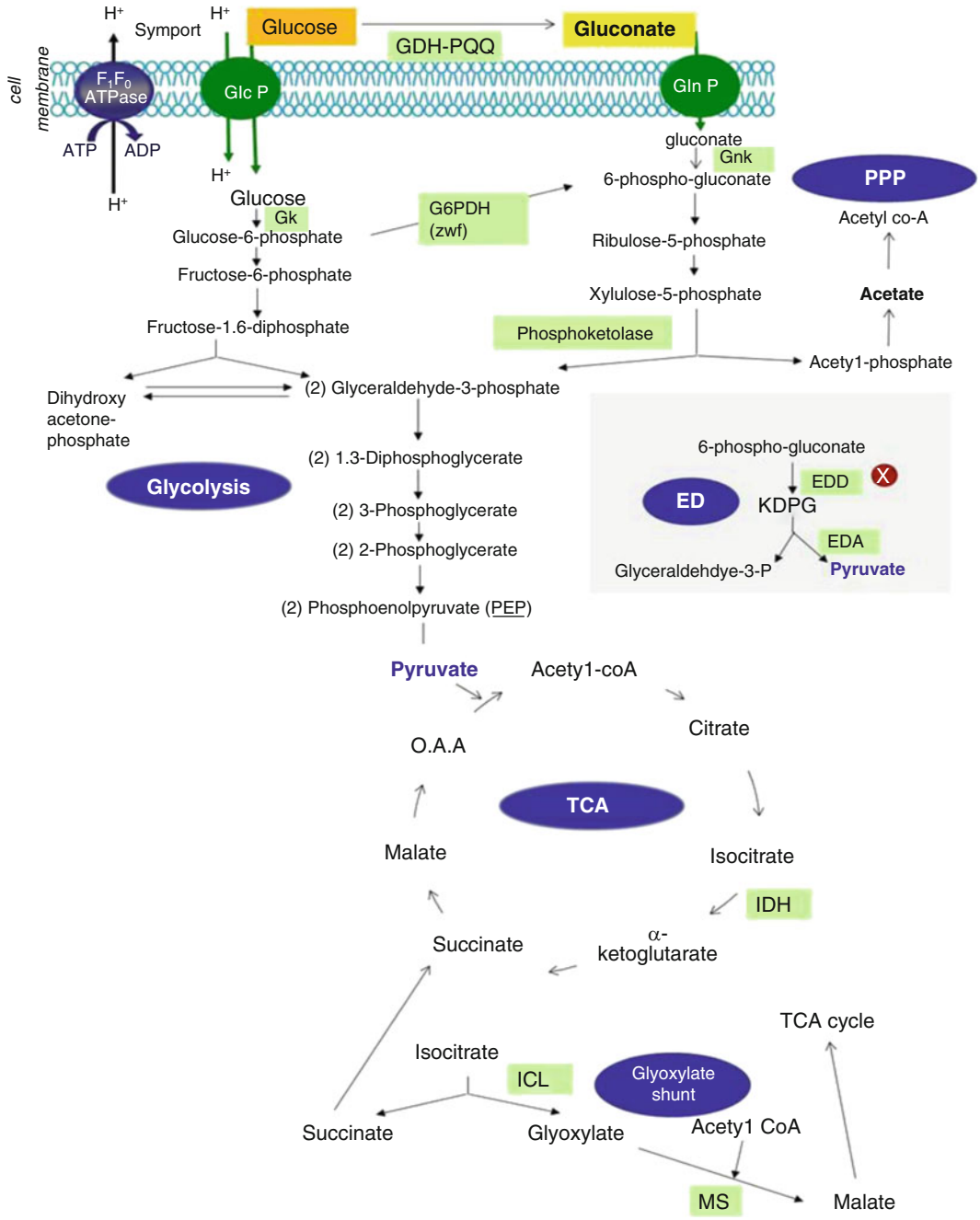

Fig. 3.1 Overview of central and peripheral carbon metabolism pathways in actinomycetes. *GDH* glucose dehydrogenase, *GK* glucokinase, *Gnk* gluconate dehydrogenase, *G6PDH* glucose-6-phosphate dehydrogenase, *EDD* 2-keto-3-deoxy-6-phosphogluconate dehydratase, *EDA* 2-keto-3-deoxy-6-phosphogluconate aldolase, *IDH* isocitrate dehydrogenase, *ICL* isocitrate lyase, *MS* malate synthase, *PQQ* pyrroloquinoline quinone, *OAA* oxaloacetate, *KDPG* 2-keto-3-deoxy-6-phosphogluconate

100 mM). Actinobacteria usually utilize glucose by EMP or pentose phosphate pathway (PPP) followed by TCA cycle (Hodgson 2000). Key enzymes that regulate TCA metabolic flux are citrate synthase and isocitrate dehydrogenase (IDH). It has been reported in enteric bacteria that IDH activity is regulated by IDH kinase/phosphatase, thus acting as a switch between TCA and glyoxylate bypass (Nimmo and Nimmo 1984). In their study, inhibition of IDH activity and gene expression was not observed, and IDH activity was inherently higher in mhcr0816. However, significant increase in isocitrate lyase (ICL) and malate synthase (MS) activity and expression during late stationary phase correlated over the production of malate in mhcr0816 which was not observed in non-malate-producing *Streptomyces* mhce0811. A tenfold increase in MS activity and expression in *Streptomyces* mhcr0816 was noted compared to control (mhce0811) when grown on glucose, suggested the presence of malate synthase isoforms that may have been active during glyoxylate phase. The malate synthase isoform followed glyoxylate shunt when grown on medium lacking C2 (acetate) compounds as sole carbon source. Similar isoforms have also been reported in cephalosporin-producing *Streptomyces clavuligerus* (Chan and Sim 1998).

Alternatively, high malate synthase and isocitrate lyase expression and activity in malate producer (*Streptomyces* mhcr0816) may be due to inactivation of a non-specific repressor protein, which probably binds upstream of both (ICL and MS) genes. Under specific metabolic conditions during late stationary phase, this repressor is somehow removed/inactivated, resulting in high level of gene expression and subsequent enzyme activity. The excess malate thus produced could be secreted out of the cell probably via secondary transporter SAV1515 (Ikeda et al. 2003) which has a 1.5-kb nucleotide sequence annotated in whole genome sequence of *Streptomyces avermitilis* (Omura et al. 2001). SAV1515 is a secondary membrane transporter of auxin efflux carrier (AEC) family assigned for IAA secretion.

3.3.2 Actinomycetes and IAA Production

Microbial synthesis of the phytohormone auxin (IAA) from L-tryptophan precursor is reported in over 80 % of rhizobacteria isolated from various crops (Patten and Glick 1996). Bacterial IAA affects plant cell division, extension and differentiation; stimulates seed and tuber germination; increases rate of xylem and root development; controls process of vegetative growth; initiates root formation; intervenes response to light, gravity and florescence; and affects photosynthesis, pigment formation, biosynthesis of secondary metabolites and resistance to stressful conditions. IAA synthesis by actinomycetes is a widely reported beneficial plant-microbe interaction that promotes plant growth and yield (Aldesuquy et al. 1998). Manulis et al. (1994) described the production of the plant hormone (IAA) and the pathway for its synthesis in *Streptomyces* spp. using GC-MS and HPLC and reported that IAA induces rapid cell division, enlargement and extension of plant tissues. Moreover, it has been hypothesized that IAA, other than enhancing plant growth, may act as an inducer for sporulation and secondary metabolite production in actinomycetes (Matsukawa et al. 2007). In soil, addition of organic fertilizers increases tryptophan as it is abundantly found in organic wastes after transformation by aerobic or anaerobic bacteria (Kravchenko et al. 2004).

Actinomycetes usually produce IAA in moderate range of (0.2–15 mg l^{-1}) (Narayana et al. 2009; Nimnoi et al. 2010); however, significantly high IAA production comparable to standard IAA-producing PGPRs has also been reported. Jog et al. (2014) reported IAA production (136 mg l^{-1}) by *Streptomyces* mhcr0816 that was comparable to reported values of standard strains – PGP *Rhizobium* sp. (142 mg l^{-1}) (Ghosh et al. 2013) and PGP *Bacillus* sp. (55 mg l^{-1}) (Yasmin et al. 2009) whilst slightly lower to excellent IAA producer *Pseudomonas fluorescens* CHA0 (195 mg l^{-1}) (Beyeler et al. 1999).

3.3.3 Actinomycetes and Siderophore Production

Iron is a vital nutrient for almost all forms of life including plants and soil microorganisms (Neilands 1995). In the aerobic environment, iron occurs principally as Fe^{3+} in insoluble hydroxides and oxyhydroxides form, thus making it generally inaccessible to both plants and microorganisms (Rajkumar et al. 2010). Siderophores are low molecular weight high-affinity iron chelators produced by many microorganisms, including actinomycetes, to scavenge ferric iron forming ferric-siderophore complexes which are shuttled back into the cells via active transport mechanisms (Matzanke et al. 1989). *Streptomyces* species are known to produce hydroxamate-type siderophores, which inhibit the growth of phytopathogens by limiting iron in rhizosphere (Khamna et al. 2009). Plants may also utilize microbial siderophores as an iron source (Wang et al. 1993). Researchers have reported high siderophore production by *Streptomyces* sp., *Pseudonocardia* sp. and *Arthrobacter* sp. (hydroxamate type, 39 mg l^{-1}) (Nimnoi et al. 2010; Emmanuel et al. 2012).

3.3.4 Actinomycetes and Antibiosis

As producers of antimicrobial secondary metabolites, actinomycetes especially *Streptomyces* play a crucial role by inhibiting soilborne plant pathogens in rhizosphere. The antagonistic potential of *Streptomyces* isolated from plant rhizosphere soils to pathogenic fungi, through production of antifungal compounds, has been reported. Crawford et al. (1993) reported actinomycetes from *Taraxacum officinale* rhizosphere active against *Pythium ultimum*, whilst Ouhdouch et al. (2001) found *Streptomyces* spp. from medicinal plant rhizosphere soils active against *Candida albicans* and *C. tropicalis*. Thangapandian et al. (2007) reported *Streptomyces* isolates with antipathogenic activity and strains of rhizospheric *Streptomyces* and *Micromonospora* produced antifungal metabolites and strongly inhibited *Botrytis cinerea* (Loqman et al. 2009). Apart from antibiotic activity, secondary metabolites also act as quorum-sensing molecules that coordinate with morphological development (aerial hyphae development and sporulation) in surface grown cultures. Consequently, different secondary metabolic gene clusters are likely to respond to distinct environmental and physiological indications and stresses mediated by an array of signal transduction systems (Bibb 2005).

Over thousand secondary metabolites, primarily possessing antimicrobial activity, have been discovered from actinomycetes till date. There are several reports that explore antimicrobial potential of actinomycete species and demonstrate effective pathogen control in laboratory or controlled environmental conditions. Actinobacteria produce a variety of antibiotics possessing polyketides, β-lactams and peptide moiety in addition to an array of other secondary metabolites that have antifungal activity (Behal 2000). Considering the diversity of secondary metabolites secreted by actinobacteria, it is vital to identify them as antimicrobial agents and characterize responsible antimicrobial metabolites that inhibit plant pathogens. Jog et al. (2014) reported the production of low molecular weight antifungal metabolites from *Streptomyces* mhcr0817; GC-MS analysis and ^{1}H-NMR spectra predicted it to be a mixture of isoforms of pyrrolo ring derivatives (phenylmethyl and methylpropyl) (Fig. 3.2). Similar pyrrolo compounds with methylpropyl and phenylmethyl derivatives from *Gillisia* sp. (Flavobacteria) and *Vibrio* sp. have been implicated in antimicrobial activities (Dash et al. 2009; Pandey et al. 2010).

3.4 Actinomycetes and Soil Enzymes

Soil enzymes play a crucial biochemical role in the overall process of organic matter decomposition in the soil system. They transform several vital processes that sustain and propagate microorganisms in soils and stabilize soil structure, decompose organic wastes and enhance

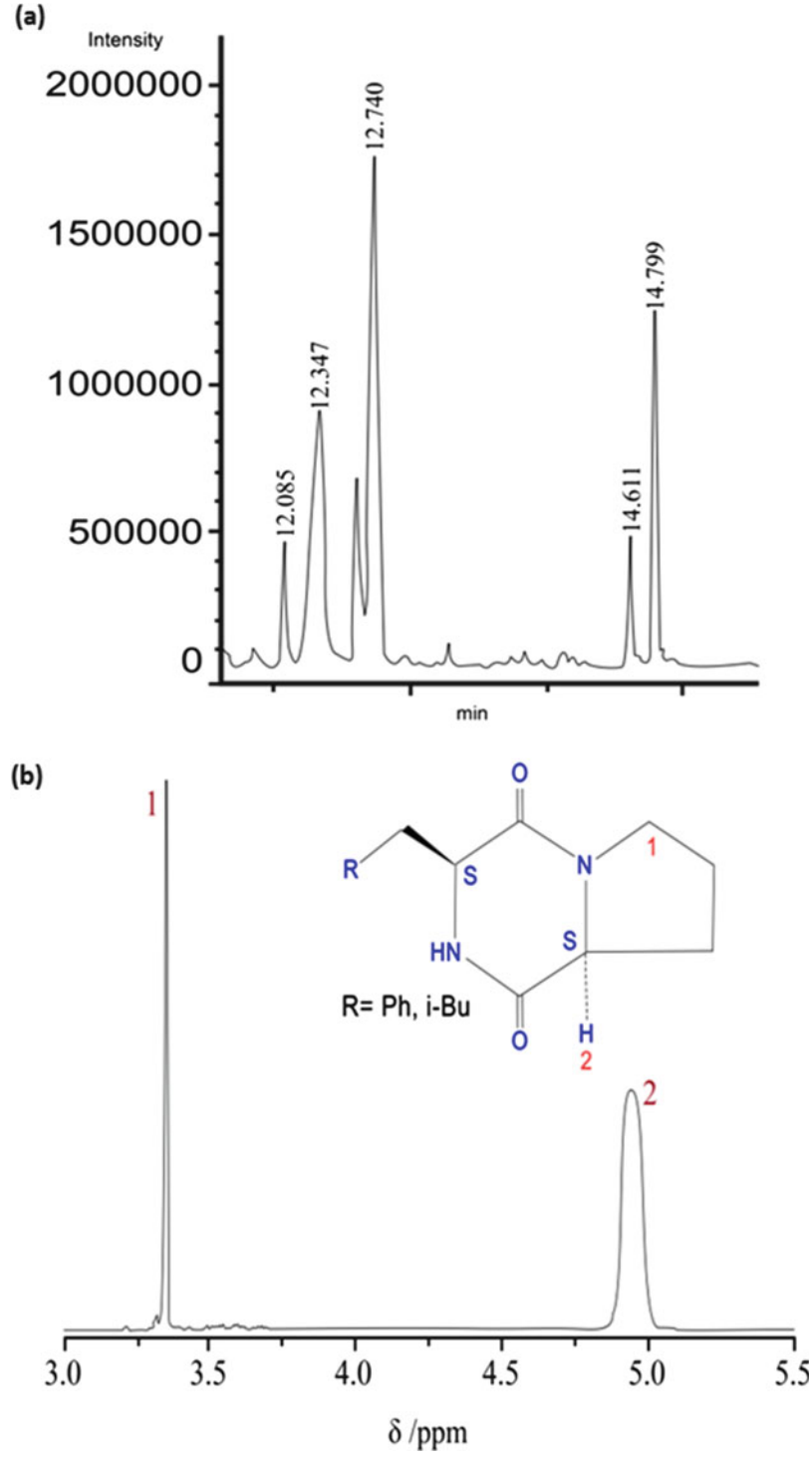

Fig. 3.2 The antifungal secondary metabolite in extract of *Streptomyces* mhcr0817 (**a**) GC-MS profile. (**b**) ^{1}H-NMR spectra

organic matter formation and nutrient cycling. Soil enzymes are constantly synthesized, accumulated, inactivated and/or decomposed in nature, thus forming an integral part of agriculture especially in nutrients cycling. Various soil types contain a unique group of enzymes that determines its metabolic cycling chain (McLaren 1975) which, in turn, depend on its physico-chemical and biological properties. These enzymes may include amylase, arylsulphatases, β-glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease and urease that are secreted by various microorganisms and plants (Makoi and Ndakidemi 2008). These soil enzymes are the fertility markers (e.g. cellulase, invertase, C-cycling; protease, chitinase, N-cycling; phytase, P-cycling) and are indicators of soil ecology, health and fertility (Sinsabaugh et al. 1991).

Actinomycetes have been known to secrete a wide array of hydrolytic enzymes in natural conditions as a dominant member of saprophytic community. Actinomycetes are primary decomposers of dead organic matter, especially lignocellulosic wastes. They have remarkable ability to produce cellulase, xylanase, lignin peroxidase and chitinase enzyme cocktail that can initiate plant biomass degradation, thus converting it into simpler form for secondary decomposers. Ultimately, complex nutrients are converted into simplest mineral forms that act as natural fertilizers promoting plant health. Jog et al. (2012) demonstrated soil enzyme production potential by actinomycetes in nature-like conditions (solid-state fermentation) using soil extract and agro-waste substrates. Table 3.2 represents soil enzyme production potential by actinomycetes.

3.5 Application of Actinomycetes as PGPR Agent

To develop commercially viable bio-inoculant PGPR strains, it is imperative to identify challenges faced by microbes in field conditions. Although depending on environmental and soil conditions, each field has a unique set of challenges; generally, PGPRs face issues like competition, washouts and rhizosphere inadaptability. Actinomycetes have distinct advantage over other PGPR strains due to strong antimicrobial activity, rhizosphere colonization (filamentous structure), excellent survival efficiency (sporulation), high PGP activity and nutrient cycling capability. Actinomycete inoculation significantly improves plant health, nutrient uptake, disease resistance and development as indicated in Table 3.3.

3.6 Conclusion, Challenges and Future Implications

The use of rhizo-microflora as bio-fertilizers and biocontrol agents has become an essential constituent of modern agricultural practice with an

Table 3.2 Soil enzyme production by actinomycetes

Enzymes	Actinomycete genera	References
Cellulase	*Streptomyces*	Bui (2014)
Xylanase	*Streptomyces*	Ninawe et al. (2006)
Lignin peroxidase	*Streptomyces*	Ramchandra et al. (1988)
Chitinase	*Streptomyces*	Brzezinska et al. (2013)
Pectinase	*Amycolata* sp.	Bruhlmann et al. (1994)
Lipase	*Streptomyces*	Cardenas et al. (2001)
Protease	*Actinopolyspora*	Suthindhiran et al. (2014)
Keratinase	*Microbispora*	Gushterova et al. (2005)
Amylase	*Streptomyces*	Kafilzadeh and Dehdari (2015)
Invertase	*Streptomyces*	Kaur and Sharma (2005)
Phytase	*Streptomyces*	Nasrabadi et al. (2012)

Table 3.3 Plant growth promotion by actinomycete inoculation

Actinomycetes	Inoculant plant	Parameter/conditions	References
Streptomyces	Cucumber	Biocontrol, greenhouse	Costa et al. (2013)
Streptomyces	Clover	Nutrient uptake, pot experiment	Franco-Correa et al. (2010)
Micromonospora	Alfalfa	Nodulation, sterile condition	Solans et al. (2008)
Streptomyces	Rice, chickpea	Nutrient, growth; field condition	Gopalakrishnan et al. (2014, 2015)
Streptomyces	Pea	Nodulation, field condition	Tokala et al. (2002)
Streptomyces	Maize	Biocontrol, greenhouse	Bressan (2003)
Streptomyces	Mung bean	Growth, pot experiment	Rungin et al. (2012)
Streptomyces	Wheat	Nutrient, growth; pot experiment	Jog et al. (2014)
Nocardia	Soybean	Nutrient, growth; pot experiment	Nimnoi et al. (2014)

enormous potential to dominate agri-markets in the coming decade. Actinomycetes, as filamentous spore-forming bacteria with superior antipathogen and nutrient cycling activity, are amongst the most promising PGPR that can increase overall soil health and boost agricultural productivity. However, several unconquered problems need to be addressed to reproduce results from controlled laboratory environment to large-scale field trials and commercial marketing. Although actinomycetes can sporulate and have high survival capability, novel formulation methods are needed for increasing shelf life and transportation. Moreover, further extensive studies of complex actinomycete-rhizosphere environment and mechanisms of PGP action are needed. The use of molecular tools and genetic engineering to enhance colonization, add more PGP traits to already beneficial actinomycete strain and increase compatibility with specific crops can be perused. Furthermore, symbiotic association of actinomycetes with other PGPR should also be explored to develop highly effective and efficient bio-inoculant system viable across different soil types and environmental conditions.

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Recent Advancement in the Development of Biopesticides by Actinomycetes for the Control of Insect Pests

4

Karnam Venkatachalapathy Hariprasad

Abstract

Use, misuse, and abuse of synthetic pesticides have led to pesticide residue problems, environmental pollution, and disturbances in ecological balance by way of causing mortality to natural enemies. These problems forced scientists to look for newer avenues of managing the insect pest such as integrated pest management (IPM). Out of all the methods advocated in IPM, use of 'green chemistry' insecticides particularly from microorganism are of significant importance as they are ubiquitous in nature. Actinomycetes and their bioproducts are treasures of valuable products to mankind. In this chapter, actinomycetes producing products of insecticidal properties, their distribution, isolation, mode of action, and application of modern technologies such as quantitative structure–activity relationships (QSAR) and gene sequencing for enhancing the insecticidal properties have been reviewed briefly.

Keywords

Actinomycetes • *Streptomyces* • Spinosyns • Spinetoram • QSAR • GABA chitinase

4.1 Introduction

The world's population has already crossed seven billion, and policy makers across countries are faced with a daunting task of increasing the food production to meet the demand of ever growing population from the world's limited crop land (Zhang et al. 2006; Zhang 2008). Since the dawn of modern agriculture, cultivated crops are being inundated with both abiotic and biotic constraints. These biotic and abiotic constrains have been hindering the ability of cultivated crops in realizing their full yield potentials. Among the biotic constraints, approximately 9000 species of insects and mites, 50,000 species of plant pathogens, and 8000 species of weeds damage cultivated crops. Among these,

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insect pests cause an estimated 14 % of loss, plant pathogens cause a 13 % loss, and weeds a 13 % loss (Zhang et al. 2011; Pimentel 2009). Pesticides are and have been a major tool with which these biotic constraints have been kept under check. It has been estimated that approximately one-third of the world agricultural products are produced by using pesticides (Liu et al. 2002). Without pesticide application, global loss of fruits, vegetables, and cereals from pest injury would reach 78 %, 54 %, and 32 %, respectively (Cai 2008).

4.2 Pesticides/Insecticides and Their Role in Insect Pest Management

Synthetic pesticides have quick response in managing insect pest, phytopathogens, and weeds. However, its application has several potential harmful effects on the environment as well as on the personnel exposed to these pesticides. Other important ill effects of the use of synthetic chemicals are their deleterious effects on natural enemies particularly parasitoids and predators, thus disturbing the ecological balance leading to pest resurgence (Jiang and Ma 2000). Among the biggest potential drawbacks of synthetic pesticides are their long shelf life and residual toxicity. Insecticides such as DDT and HCH have been a predominate component in the process of biomagnification, and traces of them were found even in mother's milk.

4.3 Microbial Pesticides/ Insecticides

Since the publication of *Silent Spring* by Rachel Carson in 1962, several attempts have been made to find out an alternate way of managing the insect pest menace with more environmentally friendly approaches such as IPM. IPM integrates different insect pest management strategies, such as biological control, host plant resistance, use of semiochemicals, etc., to bring the insect pest population below economic threshold levels (ETL). Of all these strategies, the one that gained a real momentum, in the recent years, is synthesis and use of "green chemistry" or biopesticides or pesticides from naturally available microorganisms (Copping and Duke 2007). One of the biggest advantages in the use of products from microorganism is they are easily biodegradable and can be easily broken down to nontoxic compounds and hence are benign to the environment. Biopesticides include metabolic products from microorganisms such as bacteria (including actinomycetes), fungi, and their secondary metabolites (Zhu et al. 2002; Zhang and Pang 2009). Besides being biodegradable, the active ingredients of these biopesticides can also be easily manipulated with modern biotechnological approaches to improve their efficiency. Being biodegradable, they are less harmful to natural enemies and development resistance problems become negligible (Yang 2001). As these are naturally available, human exposure toxicity will also be at minimum level.

4.4 Bioactive Compounds from Microorganisms

Recent years have seen a very good progress in isolation of bioactive compounds from microorganism. Several bioactive compounds have been isolated from various microorganisms such as bacteria and fungus (Ratnakumari et al. 2014). Of the various microorganisms used for isolation of bioactive compounds, actinomycetes contributed more than 65 % of it. These developments became possible owing to technological developments in the field of:

- Ease with isolation and culturing of microorganism – progress in fermentation techniques
- Development of sophisticated screening methods for microorganisms and their bioactive compounds (Hayakawa and Nonomura 1987; Arifuzzaman et al. 2010; Baskaran et al. 2011; Dhananjeyan et al. 2010)
- Increased understanding of interaction among plants–microorganisms–insects (tritrophic interactions)

- New techniques for detection of bioactive compounds from microorganisms
- Use of modern genetic tools for breeding mutants with desirable bioactivity
- Developments in the field of pesticide science (Tanaka and Omura 1993)

4.5 Actinomycetes

Of all the microorganisms tested, bacteria particularly belonging to actinomycetes have proved to be highly potent in their biological activities against other potential biological entities such as insect pest and diseases. Actinomycetes are a group of gram-positive bacteria belonging to the phylum Actinobacteria, class Actinobacteria, order Actinomycetales (Lechevalier and Lechevalier 1981), and family Actinomycetaceae. The members of this group form mycelia and asexual spores on artificial media (Balagurunathan and Radhakrishnan 2010) and are genetically highly diversified. Actinomycetes thrive naturally in the soil, are saprophytic in nature, and help in decomposition of soil organic matter constituting mainly starch, chitin, cellulose, and others. The smell of the soil after the rainfall (wet earth odor) is due to a volatile compound called "geosmin" produced as by-product of soil actinomycetes (Wilkins 1996). The DNA of actinomycetes are rich in molecular guanine and cytosine content (Lo et al. 2002). The members of actinobacteria comprise the most prolific source of bioactive compounds such as aminoglycoside, streptomycin, actinomycin, and tetracycline (Barrios-Gonzalez et al. 2005; El-khawaga and Megahed 2012). The bioactive compounds produced have diverse functions such as antibacterials (Usha et al. 2011; Mahajan 2012), antifungals (Gupte et al. 2002; Reddy et al. 2011), antivirals, antithrombotics, immunomodifiers, antitumors, and enzyme inhibitors, and in agriculture, they are included in insecticides, herbicides, and growth-promoting substances for plants and animals (Bressman 2003).

Actinomycetes, especially *Streptomyces* sp., has been a source for many bioactive compounds useful to mankind. About 65 % of known insecticides and herbicides developed in the recent years originate from *Streptomyces* spp. (Tanaka and Omura 1993). Actinomycetes have been exploited commercially on industrial scale owing to the following salient features. Over 10,000 (Berdy 2005) bioactive compounds have been isolated from members of class Actinobacteria and the numbers are still increasing. Members of this group are genetically highly diversified. The members are also diversified in terms of bioactive compounds they produce. For example, *S. hygroscopicus* produces 286 different kinds of bioactive compounds. The bioactivity of the compounds produced by actinomycetes has a great diversity of activities such as antibiotic, antifungal, antiviral, insecticidal (Bream et al. 2001; Huamei et al. 2008), and herbicidal activities including some compounds such as munumbicin, showing activity against human diseases such as TB, anthrax, and malaria (Balagurunathan and Radhakrishnan 2010).

4.5.1 Distribution

Actinomycetes are ubiquitously present in nature ranging from terrestrial and aquatic habitats to habitats with extremes of high temperature, pH, and salinity. Actinomycetes are unique in their habitat requirements. For example, *Streptomyces* sp. prefers soils rich in compost, *Micromonospora* sp. prefers aquatic habitat, *Dactylosporangium* prefers soils with more leaf litter, and species of *Salinispora* and *Verrucosispora* prefer deep sea beds (Baltz 2007), but are also found greatly in terrestrial soils (Ghanem et al. 2000; Zheng et al. 2000; Fiedler et al. 2005; Maldonado et al. 2009).

A single gram of rich agriculture soil may contain 10^6 *Streptomyces* colony forming units and 10^4–10^5 *Micromonospora*. These bacteria are also present in close association with plants as endophytes. Members belonging to genera *Streptomyces*, *Micromonospora*, and *Nocardia* have been isolated from healthy crop plants which are endophytes (Balagurunathan and Radhakrishnan 2007). If one looks at the distribution of different genera of actinomycetes,

Streptomyces contribute up to 95 % of total actinomycetes isolated, followed by *Nocardia* (2 %) and *Micromonospora* (1 %). The rest includes *Thermomonospora*, *Actinoplanes*, *Microbispora*, and others (Balagurunathan and Radhakrishnan 2010; Arifuzzaman et al. 2010).

4.5.2 Isolation and Identification of Actinomycetes

Before isolation, pretreatment of samples to remove unwanted bacteria and fungi is very crucial to facilitate a required potent culture of actinomycetes. Dry heating the soil samples at 55 °C for 10 min and supplementing the isolation media with antibacterial agents such as nalidixic acid (100 mg/ml) and any antifungal agent (20 mg/ml) facilitate isolation of actinomycetes (Balagurunathan and Radhakrishnan 2007) and avoid the growth and multiplication of other soil microorganisms. Several researchers have used various media and various methods for isolation and identification of actinomycetes which are briefed in Table 4.1.

4.5.3 Insecticidal Compounds from Actinomycetes

4.5.3.1 Avermectin

The discovery of avermectin, a group of macrocyclic lactones from the fermentation by-products of *Streptomyces avermitilis* (Burg et al. 1979), and its potent antithelmintic and antiparasitic characters have revolutionized the field of veterinary medicine by introducing chemicals that are toxic to external and internal parasites of farm animals. These toxins were termed as "endectocide." Avermectin consists of four major components, viz., A_1a, A_2a, B_1a, and B_2a, and four homologous minor components, viz., A_1b, A_2b, B_1b, and B_2b. Avermectin generally contains a mixture of 80 % of *a* and 20 % of *b* components. "A" series has methoxy group attached at C_5 position of cyclohexene moiety, and "B" series has a hydroxyl group at C_5 position. The *a* component has a secondary butyl substitution at C_{25} and *b* component has an isopropyl group at C_{25} position (Lasota and Dybas 1991). Besides having antithelmintic characters, avermectin also has high affinity to bind to the muscular neurons of insect species mostly by acting as agonists for γ-aminobutyric acid (GABA)-gated chloride channels leading to paralysis and death of the treated insects (Mellin et al. 1983; Albrecht and Sherman 1987; Deng and Casida 1992; Rohrer et al. 1995; Bloomquist 2001).

4.5.3.2 Abamectin

This was first the commercial avermectin product isolated from a soil actinomycete *S. avermitilis*. It contains 80 % avermectin B1a and 20 % avermectin B1b (Fischer and Mrozik 1989) (Fig. 4.1) and was toxic to phytophagous mites and insects (Lasota and Dybas 1991) with limited systemic and translaminar action.

Abamectin acts on GABA receptor in the peripheral nervous system (Fisher 1993). GABA is a major inhibitory neurotransmitter in insects. Abamectin binds to the GABA-gated chloride channel. This channel, when activated, normally blocks neurotransmission, thus preventing excessive stimulation of the nervous system. The binding of abamectin to GABA channel results in an increased flow of chloride ions into the cell, with consequent hyperpolarization and elimination of signal transduction which results in an inhibition of neurotransmission leading to mortality (Jansson and Dybas 1996). Abamectin breaks down in the presence of UV light, oxidatively and photooxidatively. The half life of abamectin in soil in the presence of sunlight is 21 h. Natural enemies and pollinators are highly sensitive to exposure to abamectin either by contact or by ingestion. However, because of rapid photodegradation, exposure of them after 24–48 h of treatment has not produced any harmful effects.

4.5.3.3 Emamectin (Emamectin Benzoate)

This is a mixture of emamectin B1a and emamectin B1b (Fig. 4.2) and is a derivative from avermectin having an insecticide and acaricidal properties.

Table 4.1 Various media and methods used for isolation and identification of actinomycetes

Isolation methods	Source	Identification method	Reference
Dispersion and differential centrifugation (DDC)	Soil	Chemotaxonomy, numerical taxonomy, and molecular systematics	Semedo et al. (2001)
Starch-casein agar			
Glycerol-asparagine-tyrosine agar			
Humic acid vitamin agar media	Termite guts	Chemotaxonomy and isolation and comparison of nucleotide sequence using Genetyx version 5.0	Khucharoenphaisan et al. (2012)
Starch-casein agar	Soil	Physiological characterization, chemotaxonomic characterization, and DNA-DNA homology study	Dhanasekaran et al. 2010)
Glycerol-asparagine agar			
Ultrasonication/ dilution method	Soil	Phylogenic analysis based on 16S rRNA	Danheng et al. (2008)
Oat mealmeat agar			
Glycerol-asparagine agar			
Modified starch-casein agar			
Humic acid vitamin agar	Soil	Chemotaxonomic analysis, probabilistic identification, and DNA-DNA hybridization test	Hayakawa et al. (2004)
Soil extract agar	Soil	Probabilistic identification of bacteria for windows (PIBW) based on morphological, physiological, and biochemical features	Saxena et al. (2013)
Soil dilution phase method	Soil	Morphology was identified by slide culture method	
Yeast-malt dextrose agar medium			
Humic acid vitamin	Soil	Phylogenic analysis based on 16S rRNA and use of GenBank/EMBL/DDBJ database with the BLAST program (http://www.ncbi.nlm.nih.gov/) to determine relative phylogenetic positions	Kamil et al. (2014)
Tryptone-yeast glucose extract			
Glucose-yeast extract agar			
Oatmeal agar			
Starch nitrate agar	Soil	Cultural, morphological, and physiological characters were identified based on International *Streptomyces* Project (ISP)	El-Khawaga and Megahed (2012)
		Phylogenic analysis based on 16S rRNA and use of GenBank/EMBL/DDBJ database with the BLAST program (http://www.ncbi.nlm.nih.gov/) to determine relative phylogenetic positions	
Starch-casein agar	Soil	Based on morphological, microscopic, biochemical, and staining characters	Shukla et al. (2015)
Glycerol casein KNO_3 agar	Saline soil	Based on morphological, biochemical, physiological, and cultural characters and by 16S rRNA gene sequencing	Anwar et al. (2014)
Actinomycetes isolation agar			
Starch-casein agar	Soils	Based on phenotypic, microscopic, biochemical, and staining characters	Prashith Kekuda et al. (2010)
Starch-casein nitrate agar	Soil	Morphological and physiological characters were identified based on International *Streptomyces* Project (ISP)	Kaur and Manhar (2013)
		Phylogenic analysis based on 16S rRNA	

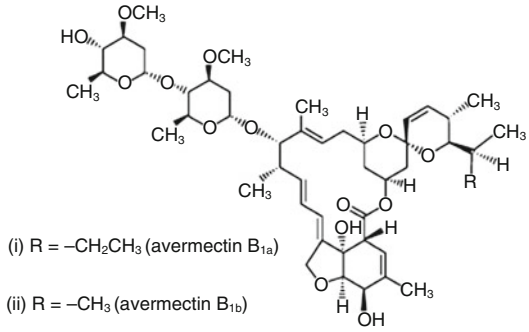

Fig. 4.1 Structure of abamectin (Copping and Duke 2007)

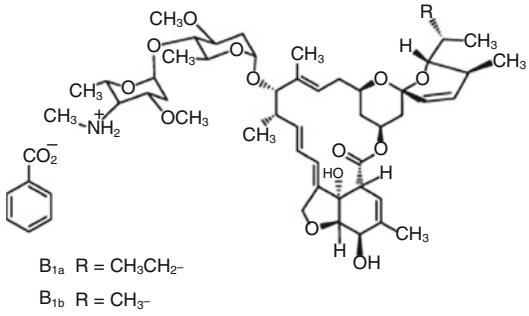

Fig. 4.2 Structure of emamectin benzoate (Copping and Duke 2007)

The target of emamectin benzoate is also the GABA receptor in the peripheral nervous system and has similar action of abamectin. Emamectin benzoate has contact and stomach action, with limited plant systemic activity, but with a good translaminar movement. Emamectin benzoate works specifically against lepidopteran and other insects such as *Spodoptera littoralis* and *Helicoverpa armigera* and the western flower thrips *Frankliniella occidentalis* (Cox et al. 1995; Ishaaya et al. 2002). This compound irreversibly paralyzes treated lepidopteran insects, preventing subsequent crop damage. The insects stop feeding within hours of ingestion and die 2–4 days after treatment.

4.5.3.4 Milbemycin (Also Known as Milbemectin)

This is a fermentation product isolated from a soil actinomycete *S. hygroscopicus* subsp. *aureolacrimosus* (Takiguchi et al. 1980; Herbert

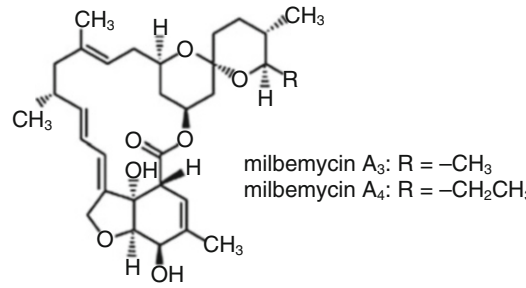

Fig. 4.3 Structure of milbemycin (Copping and Duke 2007)

2010) having insecticidal and acaricidal properties. It is a mixture of milbemycin A3 and milbemycin A4 in the ratio 3:7. Milbemycin acts on a wide range of mite pests such as the two-spotted spider mites *Tetranychus urticae*, *Tetranychus cinnabarinus*, and the citrus red mite *Panonychus citri* (Fig. 4.3).

4.5.3.5 Polynactins

These (Fig. 4.4) are secondary metabolites from the actinomycete *Streptomyces aureus* Waksman and Henrici isolate S-3466 and are mixture of tetranactin, trinactin, and dinactin (Ando et al. 1971). Polynactins are used to control spider mites, such as carmine spider mite *T. cinnabarinus* (Boisduval), two-spotted mite *T. urticae* (Koch), and European red mite *Panonychus ulmi* (Koch) in orchard fruit trees. Polynactins are very effective at controlling spider mites under wet conditions. The mode of action is thought to be through a leakage of basic cations (such as potassium ions) through the lipid layer of the membrane in the mitochondrion. Water is considered to be an essential component of this toxic effect by either assisting penetration or accelerating ion leakage (Ando et al. 1974).

4.5.3.6 Spinosyns

During the process of fermentation, *Saccharopolyspora spinosa* produces compounds called "spinosyns" that exhibit biological activity. The structure of spinosyn comprises a 12-member macrolide tetracycle to which two deoxy sugars are attached, viz., a neutral saccharide substitute

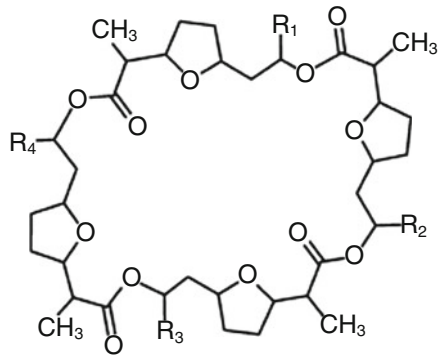

Fig. 4.4 Structure of polynactin (Copping and Duke 2007)

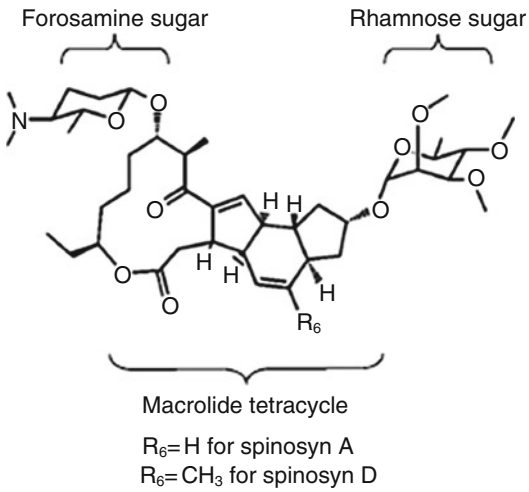

Fig. 4.5 Structure of spinosad (Salgado and Sparks 2010)

(2,3,4-tri-O-methyl-α-L-rhamnosyl) on the C-9 hydroxyl group and an amino sugar moiety (β D-forosaminyl) on the C-17 hydroxyl group (Fig. 4.5). Both of these two deoxysugars are needed for the insecticidal bioactivity (Salgado and Sparks 2010). The spinosyns produced from the parent strain included spinosyns A–H and J. Out of all these spinosyns extracted, spinosyn A (primary) and spinosyn D (secondary) are produced in higher quantities and together in the ratio of 85–15 and have been named spinosad and were introduced in the market in 1997 as insecticide.

Spinosyns show both rapid contact and ingestion activity in insects. Several studies Salgado (1998), Nauen et al. (1999), and Watson (2001) suggested that these insecticidal compounds alter both nicotinic as well as GABA receptors. Spinosyn interacts with both γ-aminobutyric acid receptors (antagonistic) and nicotinic acetylcholine receptors (activators), and the mode of action is distinct from other insecticides (Watson 2001). Spinosad effectively controls insects belonging to the order Lepidoptera, Diptera, Thysanoptera and to some extent other members of class Insecta (Legocki et al. 2010). Spinosyns are more active as insecticide compared to organophosphates and carbamates (Crouse and Spark 1998; Sparks et al. 2001) and have very low mammalian and avian toxicity. Several other species of *Saccharopolyspora*, such as *S. pogona* (NRRL30141), have also been identified producing more than 30 structurally spinosyn-related compounds (Hahn et al. 2006; Lewer 2009). One distinctive feature of these new compounds from *S. pogona* is the presence of 2-butenyl at C-21- instead of ethyl group as in spinosyn and is termed as butenyl-spinosyns (or pogonins). 2-Butenyl analogs of spinosyns A (1) and D (2) are shown in Fig. 4.6. The bioactivity of these compounds is under investigations and expected to yield unprecedented results.

4.5.4 Spinosoids: New Mutant Strains of Spinosyns

Occurrence of very low quantities of spinosad from the fermentation products of *S. spinosa* has led to development of new fermentation technology and development of new strains that could increase the titer quantity and bioactive properties. Mutant strains developed subsequently (called as spinosoids Crouse et al. 2001) have lead to isolation of spinosyns L, M, N, Q, R, S, and T and spinosyns K, O, P, U, V, W, and Y (Legocki et al. 2010). Most of mutant strains developed

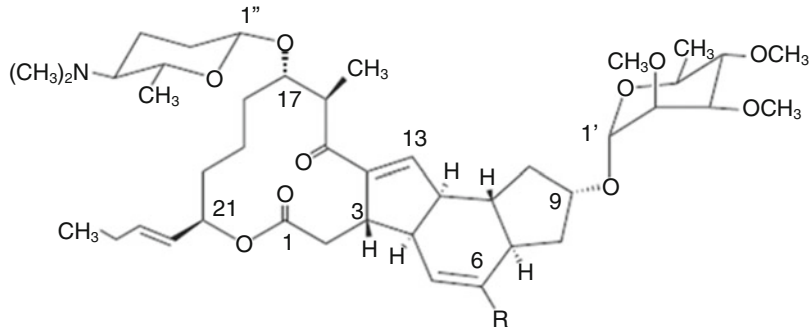

Fig 4.6 Structure of pogonins (Kirst 2010)

were based on synthetic modification of spinosyn structures. The structural differences between spinosyns depend on the degree of forosamine *N*-methylation, the presence or absence of *O*-methylated groups in rhamnose, and the presence or absence of the methyl group(s) in the positions C6, C16, and C21 of their tetracyclic ring system (Sparks et al. 2007). QSAR in the form of artificial neural network (ANN), which aids in studying the structure–activity relationship, was used to synthesize spinosads having more potent insecticidal properties. By this procedure, some spinosoids with greater activity than spinosad against lepidopteran species were obtained. Crouse et al. (2001) reported spinosoids with more activity than spinosyn A (LC50 0.31 ppm) against neonate larvae of *Heliothis virescens*, especially in the case of the 2,3,4-tri-O-ethyl-L-rhamnopyranosyl (No.2 in Fig. 4.7) and 3-O-ethyl-2,4- di-O-methyl-L-rhamnopyranosyl (No. 3 in Fig. 4.7) (name as spinetoram) (Fig. 4.8) derivatives.

Spinetoram was found not only effective on managing the insect pest of field crops but also on adult coleopteran insect pest of stored grains such as the rice weevil, *Sitophilus oryzae*; the lesser grain borer, *Rhyzopertha dominica*; the larger grain borer, *Prostephanus truncatus*; the confused flour beetle, *Tribolium confusum*; the granary weevil, *Sitophilus granarius*; and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (Thomas et al. 2012). Though effective in managing these stored grain insects, the authors are of the opinion that the efficacy varied with host species, concentration of the chemical, and period of exposure. Several new insecticides with new molecular structures have been designed based on QSAR and has been reviewed by Speck-planche et al. (2011).

4.5.5 Spinosyns and Gene Sequencing

Besides synthetic modification of the spinosyn structure, efforts were also made in sequencing genes involved in spinosyn biosynthesis. Cloning and analysis of spinosyn biosynthetic gene clusters in *S. spinosa* revealed that aglycone of spinosyn A is assembled through type I polyketide synthase pathway (PKS) and the two sugar molecules (bioactive) are subsequently added to the aglycone molecule. The PKS genes for assembling the polyketide chain were identified. Several genes, viz., *spn*F, *spn*J, *spn*L, or *spn*M, have been identified to be associated with polyketide chain assembling (Kirst 2010).

Sequencing the genes involved in spinosyn biosynthesis (Waldron et al. 2000, 2001; Madduri et al. 2001) has allowed the production of novel spinosyns through biotransformation and modification of the biosynthetic pathways. A genetically engineered strain of *Saccharopolyspora erythraea* expressing the *spn*P glycosyltransferase gene was used to produce biotransformed spinosyns, wherein the β-D-forosamine moiety was replaced by α-L-mycarose at the C17 position (Gaisser et al. 2002). Modifications were also done to

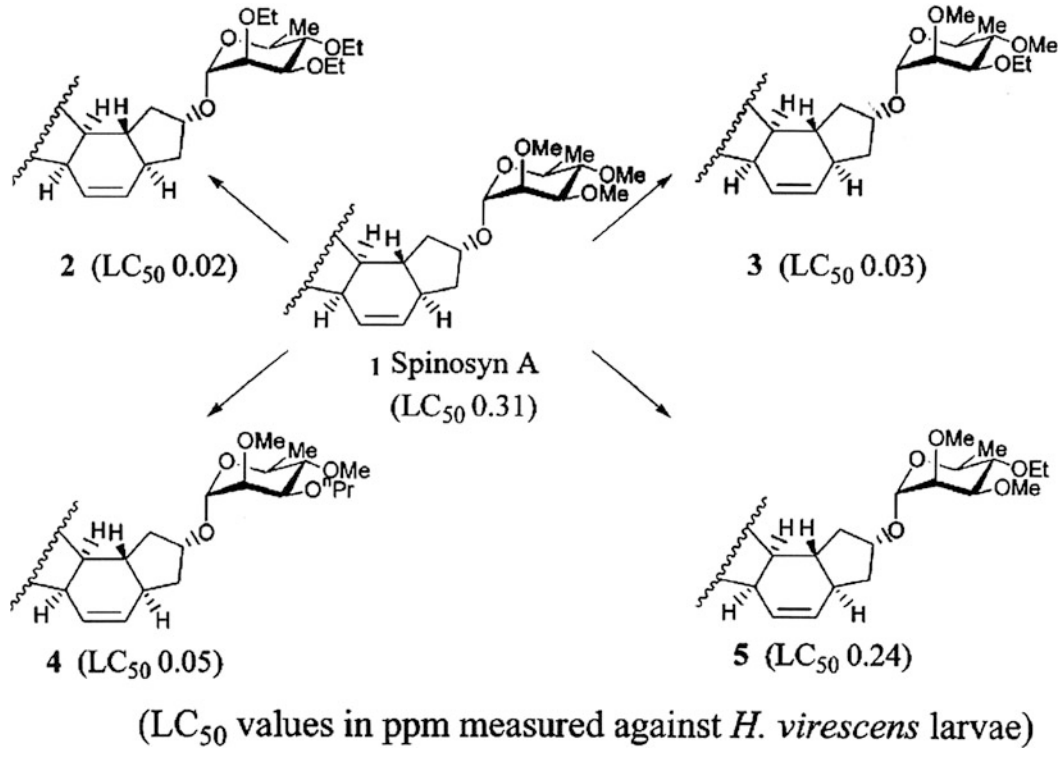

Fig. 4.7 Spinosyn A and their analogues (López et al. 2005)

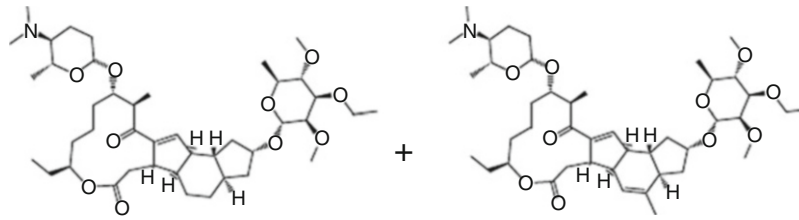

Fig. 4.8 Structure of spinetoram (Speck-Planche et al. 2011)

the basic tetracycle with substitutions at C21 and other positions of the tetracycle (Burns et al. 2003; Martin 2003). Martin (2003) has reported replacement of ethyl group at C21 with an n-propyl group that resulted in a unique spinosyn as active as spinosyn A.

4.5.6 Persistence of Spinosyns

It has been reported that spinosyn does not have a relatively long period of persistence in many parts of the environment (Thompson et al. 2002a; Salgado and Sparks 2005). Being a

product of naturally occurring microorganism, these compounds are degraded through facile degradation by soil microorganism in the presence of light to aqueous products which further is decomposed to organic matter in the absence of light (Thompson et al. 2002b). Spinosyns also found to undergo facile degradation in mammals and fowl (Salgado and Sparks 2005). Its lesser life span either in the soils or in the biosystems has made these products unique than the other products.

4.5.7 Genetic Mapping and Sequencing of Actinomycetes (*Streptomyces* spp.)

Chromosome genetic mapping of *Streptomyces coelicolor* A3(2) has been developed by David Hopwood in 1999. It was found that genes for biosynthesis of secondary metabolites (such as actinorhodin genes) were assembled in clusters and these clusters could be transferred to other strains. It was believed earlier that *S. coelicolor* produces only four compounds—actinorhodin, undecylprodigiosin, methylenomycin, and calcium-dependent antibiotic (CDA) (Rebets et al. 2014). However, when the actual genome sequence of the strain *S. coelicolor* A3(2) was obtained, 22 secondary metabolite biosynthesis clusters were recognized (Bentley et al. 2002). When the genome of *S. avermitilis* was sequenced, it was found that the there were about 38 secondary metabolite gene clusters (Ikeda et al. 2013; Omura et al. 2001). Some of these genes were nonfunctional or silent under standard laboratory conditions. Transcriptome analysis showed that majority of these genes is transcribed at very low levels. DNA microarrays of *S. coelicolor* showed transcription of 12 out of 22 secondary metabolism gene clusters (Yague et al. 2013), out of which seven were considered earlier as cryptic or silent. It was also found that during the exponential growth of *S. coelicolor*, RNA sequencing showed transcription of 7800 individual genes including 22 gene clusters involved in secondary metabolism (Gatewood et al. 2012). The proteomics revealed the presence of enzymes from three gene clusters with unknown products (Hesketh et al. 2002; Jayapal et al. 2008).

It seems obvious from above that the majority of secondary metabolism gene clusters in streptomycetes are not silent but expressed at a very low level under laboratory conditions. Often the transcription of the gene clusters under these conditions is not sufficient to produce detectable amounts of secondary metabolites. Several approaches have been used to boost secondary metabolism gene expression in order to reveal novel compounds.

4.6 Rare Actinomycetes

Besides the well-known actinomycetes, i.e., *Streptomyces* and *Micromonospora*, several other actinomycetes were also being isolated and evaluated to find bioactive compounds. Quinomycin, a secondary metabolite of *Streptomyces* sp. KN-0647, isolated from a forest soil sample of Dali Cangshan mountain, Yunnan Province, China, exhibited growth inhibition on *Spodoptera exigua*, *Dendrolimus punctatus*, *Plutella xylostella*, *Aphis glycines*, and *Culex pipiens* (Liu et al. 2008). Vijayabharathi et al. (2014) demonstrated three strains of *Streptomyces*, isolated from herbal vermicompost, having insecticidal against *H. armigera*, *S. litura*, and *Chilo partellus*. Sathya et al. (2016) reported a compound, diketopiperazine, cyclo(Trp-Phe), from *Streptomyces griseoplanus* SAI-25 that showed insecticidal activity against cotton bollworm, *Helicoverpa armigera*. Marine actinomycetes such as *Streptomyces* sp. 173 was found to have strong insecticidal activity against both brine shrimp and *H. armigera*, similar to that of avermectin B1 (Xiong et al. 2004). Omura et al. (1982, 1989) discovered setamycin, a 16-membered macrolide (Otoguro et al. 1988) from a rare actinomycete *Kitasatospora*. *Streptomyces bikiniensis* A11 isolated from desert soils of Egypt by

El-Khawag and Megahed (2012) showed insecticidal action against second instar larvae of *Spodoptera littoralis* (Boisd.).

4.7 Actinomycetes and Chitinase Enzymes

In addition to production of biologically active antibiotic, insecticidal, and herbicidal compounds, actinomycetes also produce several enzymes with potent biological activities, viz., chitinase (*Streptomyces viridificans*), cellulases (*Thermomonospora* spp.), peptidases, proteases (*Nocardia* spp.), xylanases (*Microbispora* spp.), ligninases (*Nocardia autotrophica*), amylases (*Thermomonospora curvata*), sugar isomerases (*Actinoplanes missouriensis*), pectinase, hemicellulase, and keratinase (Solans and Vobis 2003). Of all the enzymes actinomycetes produce, chitinase is important from the insect management point of view.

Chitin, an important ingredient of insect cuticle, gives rigidity and shape to the insect and also helps in prevention of moisture loss from the insect integument. Chitinase enzyme can form a very important tool in the management of insect pest by actively digesting the insect cuticle, causing ruptures in insect skin, leading to moisture loss and mortality (Reguera and Leschine 2001). Actinomycetes belonging to the genus *Streptomyces* and *Actinoplanes* were reported to produce chitinase enzyme and was used for the biological control of insects (Gadelhak et al. 2005; Gopalakrishnan et al. 2014).

Chitinase genes were identified in *S. coelicolor* and *S. griseus* (Itoh et al. 2003; Williamson et al. 2000; Saito et al. 2003) and were found responsible for the production chitinase enzymes. Chitinase enzyme isolated from actinomycetes has demonstrated to be an effective biological control agent of insects (Reguera and Leschine 2001) and plant pathogenic fungi (El-Tarabily et al. 2000; El-Tarabily 2003). Species of Actinomycetes, such as *Actinoplanes philippinensis*, *Actinoplanes missouriensis*, and *Streptomyces clavuligerus*, isolated from soils of the United Arab Emirates showed to affect the pupation of *Drosophila melanogaster*, most probably by affecting the chitin production.

4.8 Conclusions

Actinomycetes and their by-products have been an excellent source for isolation and extraction of potent compounds having insecticidal properties called "green chemicals." The future of these "green chemicals" is very promising since 99 % of the bacteria and 95 % of the fungi have not been cultivated in the laboratory and can still become a repository of bioproducts of use to mankind (Kaeberlein et al. 2002). Researchers are now working on extracting bacterial DNA from soil, marine, and other habitats, cloning them and expressing them into other host bacterium and screening the library for new bioactive compounds. These consistent efforts may lead to discovery of new species of microorganisms that may contain a hidden treasure of new compounds that may revolutionize the field of microbiology and agriculture. Efforts were also put into sequencing of the genome of actinomycete species such as *S. spinosa* (Pan et al. 2011) and genes responsible for the production of bioactive compounds. With the modern tools of gene transfer, these genes encoding bioactive compounds could also be used for the development of transgenics.

However, being an inherent character of any living organism, insects will always try and probably eventually will develop resistance to these new synthetic green chemicals and or genes encoding resistance to insects (Horowitz et al. 2002; 2004; 2007; Tabashnik et al. 2013). Hence, a particular attention should be given to the development of various insecticide resistance management (IRM) strategies that helps in slowing down the built up of resistance in insects and also in advocating and popularizing these IRM strategies among the personnel working in

the field of crop protection, policy makers, and the end users, i.e., farmers.

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Actinomycetes Bio-inoculants: A Modern Prospectus for Plant Disease Management

5

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Abstract

Plant disease management by using natural resources receives considerable awareness all over the world, because it is eco-friendly in nature. Among them, microbes especially actinomycetes have received widespread attention due to its ability to produce biologically active compounds. These compounds have been extensively exploited against different kinds of pathogens such as fungus, bacteria, pest, and insects. Actinomycetes are gram-positive saprophytic bacteria and ubiquitous in nature. Numerous strains of actinomycetes have been extensively utilized to manage plant diseases. Actinomycetes are a reservoir of several bioactive compounds and industrially important enzymes. It is widely distributed in the agro-environment, particularly in the plant rhizosphere, and influences plant growth in a significant manner. This chapter provides a comprehensive overview of diversity and application of actinomycetes as bio-inoculums against plant pathogens. It also discusses the essential mechanisms and explores the future prospect in order to enhance formulation technology and application practices to acquire full advantage of this group of organism for modern agriculture system.

Keywords

Actinomycetes • Bio-inoculant • Mycoparasitism • Bioactive compound • Disease management

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5.1 Introduction

Conventional agriculture practices play a significant role in meeting the food demands of a mounting population over the globe, which has also led to an escalating reliance on chemical fertilizers and pesticides (Bhardwaj et al. 2014). Due to this fast growth of population, industrialization, and urbanization, the biggest challenge for the scientific community is environmental pollution, and it becomes a potential threat to the human community. The situation can be credited to the continuous decline in agricultural land area that reduces crop productivity simultaneously. Moreover, decrease in agricultural productivity can be attributed to a variety of reasons, but pests and pathogens play a momentous position for crop losses. Crop yield losses due to the pathogenic infection have been ranged between 20 and 40 % over the world (Savary et al. 2012). Plant pathogens not only reduce the crop yield but also damage the quality of food by producing toxins. Crop losses due to pests and pathogens in a changing environment are still constant, and regular use of pesticides creates major problems and risks against human health and the environment. Complete degradation of chemical pesticide in soil takes a long time, and several pesticides may leave residues in or on treated fruits, vegetables, and grains in addition to soil even if they are used according to the manufacturer's instructions (EEA 2005). Due to increased resistance of pathogens, a number of chemical pesticides are not so effective or need overdoses for the significant result, which requires a high-cost investment as compared to expected income of farmers. Modern biotechnology contributes to sustainable agricultural productivity for poor and/or small-scale farmers in different developing countries (OECD 2009). Genetically modified and hybrid varieties have ability to reduce the dominance of pathogen and pests (Gould 2003). However, climate change and/or human health consequences arising from the introduction of genetically engineered or transgenic plants need to take care of human and environmental health. Improved crop management systems based upon genetically modified cultivars, high-yielding cultivars, chemical fertilization, use of synthetic pesticides for pest control, and proper irrigation were trademarks of the green revolution, and these strategies allowed the world food production to double in the past decades. But, during these times, diverse ecosystems have been substituted by simple agroecosystems in many regions which are more susceptible to pest and pathogen attack (Oerke and Dehne 2004). Fungicides and chemicals can control crop diseases to a certain level; however, it is expensive, and with the concern of human health and the environment, utilization of microorganisms as biological control agents is the best alternative. Plant beneficial microbes are abundant in the soil nearby the plant roots (rhizosphere) and within the healthy plant tissue (endophytic). Recently, the use of microbe-based biopesticides for sustainable agriculture has increased tremendously around the world. Bio-fertilizer and biopesticides are mostly comprised of beneficial bacteria and fungi including the arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR). Bio-inoculants have the potential to maintain the soil environment by recycling all kinds of micro- and macronutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of the plant growth-promoting substances, antibiotic synthesis, and biodegradation of soil organic matter (Bhardwaj et al. 2014). Several kinds of PGPR play a significant role in plant disease management via nutrient competition, mycoparasitism, hydrolytic enzyme production, induced systemic resistance, acquired systemic resistance, antimicrobial compound production, and plant defense regulation (Bhattacharyya and Jha 2012). The global efforts to explore the natural source for the plant disease management have progressed drastically. Among all kinds of bacteria and fungi, filamentous gram-positive bacteria (actinomycetes) play the most significant role in plant disease management. Most of the

actinomycetes are capable to produce secondary metabolite such as antibiotics and antifungal compounds, especially those belonging to the genus *Streptomyces*, and appear to be superior candidates to find new approaches for crop protection (Behal 2000). Therefore, this chapter describes the potential applications of actinomycetes bio-inoculants in the modern agriculture system and their essential mechanisms.

5.2 Actinomycetes as Bio-inoculants

Actinomycetes are ubiquitous in nature and found in soils, compost, freshwater basin, foodstuffs, and the atmosphere. These organisms exist and grow most profusely in different depths of soil and compost and in temperate and tropical regions all over the world (Elamvazhuthi and Subramanian 2013). Actinomycetes belong to the order *Actinomycetales*, a gram-positive bacteria illustrated by a high genomic G + C content (74 mol %) (Fox and Stackebrandt 1987; Goodfellow et al. 1992). Actinomycete species are distinguished as saprophytic bacteria that decompose organic matter, particularly biopolymers such as lignocellulose, starch, and chitin in soil and water (Crawford et al. 1993). Several actinomycetes have typical biological features such as a mycelia growth and sporulation. They also hold the ability to biosynthesize a wide variety of antimicrobial compounds as secondary metabolites including agro-active compounds (Doumbou et al. 2001; Behal 2000; Tanaka and Omura 1993). Since the discovery of streptomycin, actinomycete has received valuable interest and has resulted in the detection of diverse novel bioactive compounds of marketable value. A large number of actinomycetes have been isolated, characterized, and screened for their ability to produce commercially important compounds from different terrestrials (Malviya et al. 2009, 2014; Gopalakrishnan et al. 2011). Among all, *Streptomyces* spp. are well known as a major source of bioactive natural products, which are mostly used in agrochemicals and pharmaceuticals. *Streptomyces* produce about 75 % of commercially useful antibiotics (Berdy 2005). Moreover, numerous species of the genus *Streptomyces* have established attention due to their capability to produce a variety of secondary metabolites and bioactive compounds, including antibiotics and industrially important extracellular enzymes (Chater et al. 2010). Antifungal metabolite and extracellular hydrolytic enzyme production by different species of *Streptomyces* has been well explored by several researchers, under the major area of plant disease management (Joo 2005; Prapagdee et al. 2008; Gopalakrishnan et al. 2011; Elamvazhuthi and Subramanian 2013). Many reports have illustrated the in vitro and in vivo antifungal potential of the actinomycetes (Table 5.1). Their modes of action include via enzymes such as cellulase, hemicellulase, chitinase, amylase, and glucanase (Yuan and Crawford 1995), antagonism with pathogens (Malviya et al. 2009), production of antibiotic (Igarashi 2004), parasitism of hyphae (El-Tarabily and Sivasithamparam 2006), and siderophore production (Khamna et al. 2009). A number of S*treptomyces* spp. are well known as antifungal biocontrol agents (Yuan and Crawford 1995) that inhibit numerous plant pathogenic fungi like *Phytophthora capsici* (Joo 2005), *Fusarium oxysporum* f. sp. *cubense* (Cao 2005), *Fusarium oxysporum* f. sp. *ciceri* (Gopalakrishnan et al. 2011), *Sclerotium rolfsii* (Errakhi et al. 2007), *Alternaria alternata* and *Phomopsis archeri* (Malviya et al. 2009), and *Rhizoctonia solani* (Patil et al. 2011). All actinomycetes strain has possibly inherent potential for producing antimicrobial metabolites (Bentley et al. 2002; Elamvazhuthi and Subramanian 2013). Actinomycetes are used as plant growth-promoting agents, biocontrol tools, biopesticide agents, and antifungal compounds and as a source of agro-active compounds (Sharma 2014). Plant growth promotion potential of *Streptomyces* was reported on bean (Nassar et al. 2003), tomato (El–Tarabily 2008), wheat (Sadeghi et al. 2009), and sorghum, rice, and chickpea (Gopalakrishnan et al. 2013, 2014, 2015). Actinomycetes produce many antibiotics including amphotericin, nystatin, chloramphenicol, gentamicin, erythromycin, vancomycin, tetracycline, novobiocin, and neomycin. Urauchimycins a member of antimycin class utilized as antifungal antibiotic against fungal pathogens and it act by hinders the electron flow

Table 5.1 List of antagonistic actinomycetes and their disease-suppressing activity against plant pathogens

Actinomycetes	Plant	Disease	Pathogen	References
Streptoverticillium rimofaciens B-98891	Barley	Powdery mildew	*Erysiphe graminis* f. sp. *hordei*	Iwasa et al. (1978)
Streptomyces viridodiasticus	Basal	Basal drop	*Sclerotinia minor*	El-Tarabily et al. (2000)
Actinomadura roseola Ao108	Pepper	Blight	*Phytophthora capsici*	Kim et al. (2000)
S. violaceusniger G10	Banana	Wilt	*Fusarium oxysporum* f. sp. *cubense* race 4	Getha and Vikineswary (2002)
Streptomyces sp. KH-614	Rice	Blast	*Pyricularia oryzae*	Rhee (2003)
Streptomyces sp. AP77	Porphyra	Red rot	*Pythium porphyrae*	Woo and Kamei (2003)
Streptomyces sp. S30	Tomato	Damping-off	*Rhizoctonia solani*	Cao et al. (2004)
S. halstedii	Red peppers	Blight	*P. capsici*	Joo (2005)
Streptomyces spp. 47W08, 47W10	Pepper	Blight	*P. capsici*	Liang et al. (2005)
S. violaceusniger XL-2	–	Wood rot	*Phanerochaete chrysosporium*, *Postia placenta*, *Coriolus versicolor*, and *Gloeophyllum trabeum*	Shekhar et al. (2006)
S. ambofaciens S2	Red chilli fruits	Anthracnose	*C. gloeosporioides*	Heng et al. (2006)
Streptomyces sp.	Sugar beet	Damping-off	*Sclerotium rolfsii*	Errakhi et al. (2007)
S. hygroscopicus		Anthracnose and leaf blight	*Colletotrichum gloeosporioides* and *S. rolfsii*	Prapagdee et al. (2008)
Streptomyces sp.	Sunflower	Head and stem rot	*Sclerotinia sclerotiorum*	Baniasadi et al. (2009)
Streptomyces sp	Sweet pea	Powdery mildew	*Oidium* sp.	Sangmanee et al. (2009)
S. vinaceusdrappus	Rice	Blast	*Curvularia oryzae*, *Pyricularia oryzae*, *Bipolaris oryzae*, and *Fusarium oxysporum*	Ningthoujam et al. (2009)
Streptomyces sp. RO3	Lemon fruit	Green mold and sour rot	*Penicillium digitatum* and *Geotrichum candidum*	Maldonado et al. (2010)
S. spororaveus RDS28	–	Root rot, collar or root rot, stalk rot, leaf spots, and gray mold rot or botrytis blight	*R. solani*, *Fusarium solani*, *Fusarium verticillioides*, *Alternaria alternata*, and *Botrytis cinerea*	Al-Askar et al. (2011)
S. toxytricini vh6	*Tomato*	Root rot	*R. solani*	Patil et al. (2011)
Streptomyces spp.	Sugar beet	Root rot	*R. solani* and *Phytophthora drechsleri*	Karimi et al. (2012)
Streptomyces spp.	Chilli	Root rot, blight, and fruit rot	*Alternaria brassiceae*, *Colletotrichum gloeosporioides*, *R. solani*, and *Phytophthora capsici*	Srividya et al. (2012)
Streptomyces spp.	Chilli	Wilt	*F. oxysporum* f. sp. *capsici*	Saengnak et al. (2013)

(continued)

Table 5.1 (continued)

Actinomycetes	Plant	Disease	Pathogen	References
Streptomyces spp.	Ginger	Rhizome rot	*F. oxysporum* f. sp. *zingiberi*	Manasa et al. (2013)
Streptomyces sp. CBE	Groundnut	Stem rot	*S. rolfsii*	Adhilakshmi et al. (2014)
Streptomyces spp.	Tomato	Damping-off	*R. solani*	Goudjal et al. (2014)
Streptomyces spp.	Tobacco	Brown spot	*Alternaria* spp.	Gao et al. (2014)
S. aurantiogriseus VSMGT1014	Rice	Sheath blight	*R. solani*	Harikrishnan et al. (2014)
S. felleus YJ1	Oilseed rape	Stem rot	*S. sclerotiorum*	Cheng et al. (2014)
S. vinaceusdrappus S5MW2	Tomato	Root rot	*R. solani*	Yandigeri et al. (2015)

in the mitochondrial respiratory chain (Sharma 2014).

5.2.1 Agro-active Metabolites and Antibiosis

Microbes work as a reservoir of agro-active metabolites, for the past several years (Doumbou et al. 2001; Ratna Kumari et al. 2014; Sharma 2014). It has been estimated that approximately two-thirds of the thousands of naturally occurring antibiotics have been recovered from actinomycetes (Miyadoh 1993). It is positively recognized that the proportion of all the actinomycetes that can be isolated from soil and other natural substrates have the capacity of producing antibiosis compounds (Waksman et al. 2010) such as volatiles, toxins, and antibiotics (Fravel 1988). It is a mechanism of biological control of plant disease that has been assessed in several systems (Crawford et al. 1993; Chamberlain and Crawford 1999; Patil et al. 2011). Systematic screening of antagonistic actinomycetes from soil (Raytapadar and Paul 2001; Nanjwade et al. 2010) has been carried out for the production of antibiotics. Poosarla et al. (2013) have identified an actinomycete from marine sediments of Andaman Islands with strong inhibitory activity against bacteria *Streptococcus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus vulgaris* and fungi *Aspergillus niger*, *Candida albicans*, *Penicillium*, *Mucor*, and *Rhizopus*. Similarly, many actinomycetes have been found to be effective against a wide range of bacterial strains (Oskay 2009; Arifuzzaman et al. 2010; Cwala et al. 2011). *Streptomyces padanus*, recovered from the soil collected in Jiangxi Province, China, produced actinomycin X2, fungichromin, and a new polyene macrolide antibiotic which showed good antifungal activity (Xiong et al. 2013). Antimycins have been identified from *Streptomyces* isolated from the integument of attine ants (Seipke et al. 2012). *Streptomyces olivaceiscleroticus* AZ-SH514 and *Streptomyces antibioticus* AZ-Z710 produced antifungal compounds 4′ phenyl-1-napthyl-phenyl acetamide and mycangimycin (Atta 2009; Atta et al. 2010).

5.2.2 Mycoparasitism/Hydrolytic Enzymes

The role of enzymes in biocontrol is often considered by different mechanisms, parasitism and antibiosis in particular. Cell wall-degrading enzymes such as chitinase, β-1,3-glucanase, protease, and cellulase are important for mycoparasitism and antifungal activities (Haggag and Mohamed 2007). Actinomycetes are known to produce chitinase, β-1,3-glucanase, pectinase, xylanase, cellulase, amylase, protease, and lipase. Actinomycetes originated from agricultural soil have been producers of proteases,

amylases, CMCase, xylanase, pectinase, and chitinase activities (Sonia et al. 2011). Ten actinobacteria isolated from sediment samples of Kodiyakarai coast, the Bay of Bengal, India, exhibited multiple enzyme activity including amylase, cellulase, and protease (Manivasagan et al. 2010). Chitinase and glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, for example, cell walls of *Fusarium oxysporum*, *Sclerotinia minor*, *S. rolfsii*, and *Aspergillus* (Singh et al. 1999; El-Tarabily et al. 2000; Hassan et al. 2011). Thirteen actinomycete strains were found to produce β-1,3-, β-1,4-, and β-1,6-glucanases, and these enzymes hydrolyze glucans from *Phytophthora* cell walls and cause lysis (Valois et al. 1996). Pattanapipitpaisal and Kamlandharn (2012) isolated 283 different chitinolytic actinomycete strains from rhizosphere-associated soils, from Ubon Ratchathani and Sisaket Province of Thailand, out of which 13 isolates have remarkably inhibited the growth of the fungus. Chitinases are group of the hydrolytic enzymes that catalyze depolymerization of chitin. After cellulose, chitin is the second most abundant organic compound in nature and is found to be rich in fungal cell walls. Among actinomycetes, species of the genus *Streptomyces* are well known for the production of chitinase, and hence the potential application of chitinase for biocontrol of fungal phytopathogens is promising (Gomes et al. 2000; Kim et al. 2003; Mukherjee and Sen 2006). The chitinase-producing strains could be used directly in biocontrol or indirectly by using purified proteins or through gene manipulation (Doumbou et al. 2001; Manivasagan et al. 2010; Sonia et al. 2011).

5.2.3 Root Colonizer and Plant Defense Activation

Roots operate a multitude of functions in plants including anchorage, nutrient and water acquisition, and production of exudates for plant development. The root–soil interface, or rhizosphere, is the reservoir of all the biological and chemical reactions within the soil matrix. Rhizosphere contains all kinds of microbes (beneficial and deleterious) with complex interactions (Raaijmakers et al. 2009; Compant et al. 2010; Glick 2012). Deleterious microbes compete for nutrients with plant in rhizosphere and cause diseases, while PGPR support their host by nutrient mobilization and growth stimulation and protect the plant from biotic and abiotic stresses (Compant et al. 2010; AeronA et al. 2011; Smith and Smith 2011; Yandigeri et al. 2012; Solanki et al. 2013). PGPR are well known to regulate the plant health by controlling plant pathogens or via direct enhancement of plant development by providing nutrient. Literatures indicate that actinomycetes are playing an important role in the rhizosphere (Doumbou et al. 2001), where they may influence plant growth and protect plant roots against pathogen invasion by root (Lechevalier 1988). Root colonization is an essential character for the biocontrol agents against the pathogens, and higher colonization of biocontrol agents should reduce disease incidence (Doumbou et al. 2001). *Streptomyces* spp. 47W08 and 47W10 were used as protective agents against *Phytophthora capsici* in capsicum (Liang et al. 2005). Biocontrol bacteria have activated the plant defense system by producing peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and superoxide dismutase (SOD) against pathogen invasion (Kim et al. 2007). POD, PPO, PAL, and SOD are strongly associated with plant disease resistance. POD catalyzes the lignin formation, enhances the thickness of plant cell wall to prevent pathogen invasion, and also balances the active oxygen metabolism (Joseph et al. 1998). PPO oxidizes the phenols to quinone materials that have inhibitory effect on pathogen and also is involved in lignin synthesis (Wang et al. 2005). PAL is a rate-limiting enzyme that contributes in synthesis of phytoalexin, lignans, and phenolic compounds and promotes plant systemic resistance (Wang and Zhu 2002). SOD is an endogenous active oxygen scavenger in plants coupled with lignin synthesis (Dou et al. 2010). Host–microbe interaction is a very complex

system. Lehr et al. (2008) have reported a complex interaction of *Streptomyces* spp. GB 4-2 with Norway spruce and *Heterobasidion abietinum*. GB 4-2 has promoted not only phytopathogenic fungus growth but also induced plant defense responses. Host responses indicate that GB 4-2 induced both local and systemic defense responses in Norway spruce (Lehr et al. 2008). *Streptomyces griseoviridis* is a superior model for colonization of plant rhizosphere by actinomycetes. *S. griseoviridis* is an antagonistic bacterium which has been isolated from light-colored *Sphagnum* peat (Tahvonen 1982) and is successful as biocontrol agent against the diseases such as damping-off of brassicas, fusarium wilt of carnation, and root rot of cucumber (Tahvonen and Lahdenpera 1988). Kortemaa et al. (1994) have reported active root colonization of *S. griseoviridis* on turnip rape and carrot with higher colonization frequencies in turnip rape than carrot root. It concludes that colonization frequencies depend on host and environment factors. Curl and Truelove (1986) have reported that different plant species produces various types and quantities of root exudates, which positively affect the root colonization (Weller 1988). The efficiency of *S. griseoviridis* bio-inoculum for seed dressing of barley and spring wheat against foot rot disease was investigated by Tahvonen et al. (1994) who observed higher yields in wheat than barley. Similarly, Cheng et al. (2014) observed colonization by *Streptomyces felleus* YJ1 against *Sclerotinia sclerotiorum* in oilseed rape and SOD, POD, PPO, and PAL activities.

5.3 Application of Actinomycetes Bio-inoculants

5.3.1 Against Fungal Plant Pathogens

Fungal plant pathogen causes serious damage in quantity and quality food production. The plant pathogens are controlled by chemical treatment; however, these chemicals also pose a negative impact on the environment and human health. Hence, microbe-based technology gained the attention to reduce the use of chemicals as they serve for both biocontrol and plant growth promotion (Igarashi 2004; Khamna et al. 2009; Yandigeri et al. 2012, 2015). Golinska et al. (2015) have reported endophytic *Streptomyces* in enhancing the plant growth by nutrient mobilization and secondary metabolite production. Isono et al. (1965) discovered polyoxins A and B as new antifungal antibiotics from *Streptomyces cacaoi* var. *asoensis*. Iwasa et al. (1978) reported mildiomycin, a new antifungal compound isolated from *Streptoverticillium rimofaciens* B-98891, active against powdery mildew of barley. Chandra (1979) studied the mode of antifungal action of tetraene derived from *Streptomyces* sp. Rothrock and Gottlieb (1984) evaluated biocontrol activity of geldanamycin, a new antifungal agent from *S. hygroscopicus* var. *geldanus* and *S. griseus*, against *Rhizoctonia* root rot of pea. Tanaka et al. (1987) assessed globopeptin, a new antifungal antibiotic, and its in vitro antifungal activities against fungal pathogens. Novel antifungal antibiotics, phosmidosine, and their structure were studied and reported by Philips and Mc Closkey (1990). Matsuyama (1991) reported AC-1, an antifungal compound from *Streptomyces* sp. AB-88. Mand Nair et al. (1994) identified biocontrol application of gopalamicin against wheat powdery mildew, grape downy mildew, and rice blast pathogens. Tubercidin, a new antifungal compound reported by Kook and Kim (1995), was very effective against *Phytophthora capsici* blight in *Capsicum annuum*. Marten et al. (2001) reported the fungicidal activity of RhizovitR isolated from *Streptomyces rimosus* against *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, *Alternaria brassicicola*, and *Botrytis* sp. *Streptomyces violaceusniger* strain YCED-9, an antifungal biocontrol agent, produces three antimicrobial compounds (guanidyl fungin A, nigericin, and geldanamycin) against *Pythium* and *Phytophthora* spp. (Trejo-Estrada et al. 1998). Oligomycins A and C are macrolide antibiotics produced by *Streptomyces diastaticus* and

exhibit a strong activity against *Aspergillus niger*, *A. alternata*, *Botrytis cinerea*, and *Phytophthora capsici* (Yang et al. 2010). Brief description of bioactive compounds produced by actinomycetes on various plant pathogens is given in Table 5.2. Besides production of antibiotic molecules, commercial bio-inoculants containing actinomycetes as active ingredients are also utilized for plant disease management. Cells of *Streptomyces griseoviridis* (Mycostop®) are used for the control of fusarium wilt of carnation and root rot disease of cucumber, and it has been used in greenhouse production to protect flowers from pathogens (White et al. 1990). Actinovate®, a biocontrol formulation of *S. lydicus* registered from AgBio in the United States of America, has been suggested for a wide range of environments ranging from greenhouses to field conditions. *S. lydicus* WYEC 108 (MicroPlus®) has been reported to possess disease suppression against powdery mildew and several root decay fungi.

5.3.2 Against Bacterial Plant Pathogens

Actinomycetes produce a broad spectrum of antimicrobial compounds, and these compounds are also useful for controlling bacterial diseases in different plants. Baz et al. (2012) reported 65–94 % reduction in the symptoms of disease severity caused by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum*, causal agents of potato soft rot, by *Streptomyces* sp. strain OE7. Abdallah et al. (2013) studied biocontrol activity of actinomycete strains *Burkholderia cepacia* and *S. coelicolor* HHFA2 from Egyptian soils against onion bacterial rot diseases caused by *Erwinia carotovora* subsp. *carotovora* and observed significant reduction of disease incidence and enhancement of photosynthetic pigments. Hwang et al. (2001) explored the antimicrobial activity of phenylacetic acid and sodium phenylacetate isolated from *Streptomyces humidus* against the fungal and bacterial pathogens; both metabolites show inhibitory effect against *Saccharomyces cerevisiae* and *Pseudomonas syringae* pv. *syringae*. Lee et al. (2005) also reported multiple antimicrobial activity of 4-phenyl-3-butenoic acid against pathogenic fungus and bacteria in in vitro testing. *Streptomyces* sp. strain JJ45 showed antibiotic activity against the plant pathogenic bacteria *Xanthomonas campestris* pv. *campestris* and inhibitory compound identified as alpha-l-sorbofuranose (3-->2)-beta-D-altrofuranose (Kang et al. 2009). Donghua et al. (2013) identified an antibacterial metabolite aloesaponarin II isolated from *Streptomyces termitum* ATC-2 that possessed strong antimicrobial activity against *Xanthomonas oryzae* pv. *oryzae* which causes bacterial blight in rice. Muangham et al. (2014) assessed a melanogenic actinomycete *Streptomyces bungoensis* TY68-3 for its ability to restrain the growth of *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola*. Mingma et al. (2014) reported inhibitory effect of *Streptomyces* sp. strain RM 365 against soybean pathogen *Xanthomonas campestris* pv. *glycines*.

5.3.3 Against Insect Pests

Many actinomycetes are utilized as pesticide, and at present, microbial insecticides are the main components of the biopesticide industry (Xiong et al. 2004). Biopesticides fall into three main groups: plant-incorporated protectants (PIPs), biochemical pesticides, and microbial pesticides. A microbial pesticide contains microorganisms as the active ingredient. Although each microbial active ingredient is comparatively specific for its target pest, it can also control various pests (Mazid et al. 2011; Ratna Kumari et al. 2014). Many reports are available for actinomycete's insecticidal activity including the boll weevil (Purcell et al. 1993), cotton leafworm, *Spodoptera littoralis* (Bream et al. 2001), *Culex quinquefasciatus* (Sundarapandian et al. 2002), housefly, *Musca domestica* (Hussain et al. 2002), *Drosophila melanogaster* (Gadelhak et al. 2005),

Table 5.2 List of antifungal metabolites of actinomycetes assessed against different pathogens under in vitro and in vivo conditions

Metabolite	Source organism	Pathogen/disease	References
Polyoxins A and B	*Streptomyces cacaoi* var. *asoensis*	*Alternaria kikuchiana*, *Cochliobolus miyabeans*, *Pellicularia filamentosa* f. *sasakii*, *Pyricularia oryzae*	Isono et al. (1965)
Mildiomycin	*Streptoverticillium rimofaciens* B-98891	*Rhodotorula rubra*	Iwasa et al. (1978)
Tetraene	*Streptomyces* sp. A-7	*Helminthosporium oryzae*, *Curvularia lunata*	Chandra (1979)
Geldanamycin	*S. hygroscopicus* var. *geldanus*, *S. griseus*	*R. solani*	Rothrock and Gottlieb (1984)
Globopeptin	*Streptomyces* sp. MA-23	*Mucor racemosus*, *Pyricularia oryzae*, *B. cinerea*, and *A. kikuchiana*	Tanaka et al. (1987)
Phosmidosine	*Streptomyces* sp. RK-16	*B. cinerea*	Philips and Mc Closkey (1990)
AC-1	*Streptomyces* sp. AB-88 M	*P. oryzae*, *B. cinerea*, *Helminthosporium maydis*, *H. oryzae*, and *Fusarium roseum* f. sp. *cerealis*	Matsuyama (1991)
Gopalamicin	Actinomycetes MSU-625 and MSU-616	Wheat powdery mildew, grape downy mildew, and rice blast pathogens	Nair et al. (1994)
Tubercidin	*Streptomyces violaceusniger*	*P. capsici*, *Magnaporthe grisea*, and *Colletotrichum gloeosporioides*	Kook and Kim (1995)
Manumycin	*Streptomyces flaveus* strain A-11	*P. capsici*, *M. grisea*, *Cladosporium cucumerinum*, and *Alternaria mali*	Hwang et al. (1996)
Streptimidone	*Micromonospora coerulea* strain Ao58	*P. capsici*, *M. grisea*, and *B. cinerea*	Kim et al. (1999)
Daunomycin	*Actinomadura roseola* Ao108	*P. capsici and R. solani*, *Phytophthora*	Kim et al. (2000)
Bafilomycins B1 and C1	*S. halstedii* K122	*Aspergillus fumigatus*, *Mucor hiemalis*, *Penicillium roqueforti*, and *Paecilomyces variotii*	Frandberg et al. (2000)

(continued)

Table 5.2 (continued)

Metabolite	Source organism	Pathogen/disease	References
Phenylacetic acid and sodium phenylacetate	*Streptomyces humidus* S5-55	*Pythium ultimum*, *P. capsici*, *R. solani*, *Saccharomyces cerevisiae*, and *Pseudomonas syringae* pv. syringae	Hwang et al. (2001)
RhizovitR	*Streptomyces rimosus*	*Pythium* spp., *Phytophthora* spp., *R. solani*, *Alternaria brassicicola*, and *Botrytis* sp.	Marten et al. (2001)
Fungichromin	*Streptomyces padanus* strain PMS-702	*R. solani*	Shih et al. (2003)
4-Phenyl-3-butenoic acid	*Streptomyces koyangensis* strain VK-A60	*Colletotrichum orbiculare*, *M. grisea*, *Pythium ultimum*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *Ralstonia solanacearum*	Lee et al. (2005)
Antimycin A17	*Streptomyces* sp. GAAS7310	*Curvularia lunata*, *Rhizopus nigricans*, and *Colletotrichum nigrum*	Chen et al. (2005)
Neopeptins	*Streptomyces* sp. KNF2047	*A. mali*, *B. cinerea*, *C. cucumerinum*, *Colletotrichum lagenarium*, *Didymella bryoniae*, and *M. grisea*	Kim et al. (2007)
Natamycin	*Streptomyces lydicus* strain A01	*F. oxysporum*, *B. cinerea*, *Monilinia laxa*	Lu et al. (2008)
5-Hydroxyl-5-methyl-2-hexenoic acid	*Actinoplanes* sp. HBDN08	*B. cinerea*, *C. cucumerinum*, and *Corynespora cassiicola*	Zhang et al. (2010)
Oligomycins A and C	*Streptomyces diastaticus*	*Aspergillus niger*, *Alternaria alternata*, *B. cinerea*, and *P. capsici*	Yang et al. (2010)
Strevertenes	*Streptomyces psammoticus* KP1404	*A. mali*, *Aspergillus oryzae*, *Cylindrocarpon destructans*, *Colletotrichum orbiculare*, *F. oxysporum* f. sp. *lycopersici*, and *S. sclerotiorum*	Kim et al. (2011)

(continued)

Table 5.2 (continued)

Metabolite	Source organism	Pathogen/disease	References
Filipin III	*Streptomyces miharaensis* KPE62302H	*A. mali*, *A. niger*, *C. gloeosporioides*, *C. orbiculare*, *C. destructans*, *Diaporthe citri*, and *F. oxysporum*	Kim et al. (2012)
Resistomycin and tetracenomycin D	*Streptomyces canus* BYB02	*M. grisea*	Zhang et al. (2013)
Antifungalmycin 702	*Streptomyces padanus* JAU4234	*M. grisea*	Xiong et al. (2013)
1H-Pyrrole-2-carboxylic acid (PCA)	*Streptomyces griseus* H7602	*P. capsici*	Nguyen et al. (2015)
Bafilomycins B1 and C1	S*treptomyces cavourensis* NA4	*Fusarium* spp., *R. solani*, and *B. cinerea*	Pan et al. (2015)

Helicoverpa armigera (Xiong et al. 2004; Osman et al. 2007; Vijayabharathi et al. 2014), larvae of *Aedes aegypti* (Kekuda et al. 2010), *Anopheles* mosquito larvae (Dhanasekaran et al. 2010), and *Culex pipiens* (El–Khawagh et al. 2011). Bream et al. (2001) observed mortality of secondary metabolites of some actinomycete isolates including *Streptomyces* and *Streptoverticillium* on last instar larvae and pupae of the cotton leafworm *Spodoptera littoralis*. Osman et al. (2007) observed that cells of *Streptomyces* isolates were more active against cotton leafworm than culture filtrate. It shows that insecticidal activity present in both the actinomycete cell and cell filtrate could be utilized against insect pest. A macrotetrolide antibiotic identified from the acetone extract of *Streptomyces aureus* exhibited significant insecticidal activity against *Callosobruchus chinensis* (Oishi et al. 1970). The active compound quinomycin A extracted from ethyl acetate extract of *Streptomyces* sp. KN-0647 exhibited significant growth inhibition on the pathogenic insects *Aphis glycines*, *Culex pipiens*, *Dendrolimus punctatus*, *Plutella xylostella*, and *Spodoptera exigua* (Liu et al. 2008). Xiong et al. (2004) identified a strong insecticidal activity against both brine shrimp and *H. armigera* by avermectin B1 extracted from *Streptomyces* sp. 173. A new member of the tartrolone series of macrodiolides, tartrolone C, an insecticidal compound, was isolated from a *Streptomyces* sp. (Lewer et al. 2003). Kekuda et al. (2010) evaluated the larvicidal effect of two *Streptomyces* species isolated from the soil of Agumbe, Karnataka, India, against second instar larvae of *Aedes aegypti*. Selvakumar et al. (2011) reported entomopathogenic properties of *Brevibacterium frigoritolerans* against *Anomala dimidiata* and *Holotrichia longipennis*, and grub mortality occurred between the second and fifth weeks after inoculation under in vitro conditions. Sathya et al. (2016) reported a compound, diketopiperazine, cyclo(Trp-Phe), from *Streptomyces griseoplanus* SAI-25 that showed insecticidal activity against cotton bollworm, *Helicoverpa armigera*. Brief description of inhibitory compound of insect is given in Table 5.3.

5.4 Advantages and Disadvantages of Actinomycetes Bio-inoculants

Several reports have discussed regarding the advantages and disadvantages of PGPR as bio-inoculants (Saharan and Nehra 2011; Trabelsi and Mhamdi 2013), and actinomycetes

Table 5.3 List of actinomycetes active against the insect pests and their inhibitory compounds

Actinomycetes	Insect pests	Inhibitory compound	References
Streptomyces aureus	*Callosobruchus chinensis*	Macrotetrolide antibiotic	Oishi et al. (1970)
Streptomyces sp.	Boll weevil	Protein	Purcell et al. (1993)
Streptomyces and *Streptoverticillium* spp.	*Spodoptera littoralis*	Secondary metabolite	Bream et al. (2001)
Streptomyces 98-1	*Culex quinquefasciatus*	Extracellular metabolites	Sundarapandian et al. (2002)
Streptomyces sp. 173	Brine shrimp and *Helicoverpa armigera*	Crude extract	Xiong et al. (2004)
Streptomyces spp.	*S. littoralis*	Cell protein	Osman et al. (2007)
Streptomyces sp. KN-0647	*Spodoptera exigua*, *Dendrolimus punctatus*, *Plutella xylostella*, *Aphis glycines*, and *Culex pipiens*	Quinomycin A	Liu et al. (2008)
Streptomyces sp.	*Aedes aegypti*	Butanol extract	Kekuda et al. (2010)
S. microflavus neau3	Adult mites and *Caenorhabditis elegans*	Macrocyclic lactone (1), isolated from fermented broth	Wang et al. (2011)
Brevibacterium frigoritolerans	*Anomala dimidiata* and *Holotrichia longipennis*	Bacterial cells	Selvakumar et al. (2011)
S. bikiniensis A11	*S. littoralis*	Aminoglycoside antibiotic	El-Khawaga and Megahed (2012)
Streptomyces sp. LC50	*A. aegypti* and brine shrimp	Crude extract	Kekuda et al. (2012)
Streptomyces sp.	*Sitophilus oryzae*	Crude extract	Rishikesh et al. (2013)
Streptomyces sp. AP-123	*H. armigera* and *Spodoptera litura*	Polyketide metabolite	Arasu et al. (2013)
S. hydrogenans DH16	*S. litura*	Secondary metabolites in the fermentation broth	Kaur and Manhas (2013)
Streptomyces sp. AIAH-10	*S. oryzae*	Ethyl acetate extracts	Haque et al. (2014)
S. griseoplanus SAI-25, *S. bacillaris* CAI-155, *S. albolongus* BCA-698	*H. armigera*, *S. litura*, and *Chilo partellus*	Extracellular metabolites	Vijayabharathi et al. (2014)
S. griseolus	*Fasciola gigantica*	Proteases	El-Gammal et al. (2014)

bio-inoculants are also one of them. Actinomycetes bio-inoculants and metabolites are naturally occurring substances that control pathogen and pests by nontoxic mechanisms. The beneficial effects of actinomycetes and their metabolites have been well assessed in the past; therefore, in recent times the agro-active antibiotics of actinomycetes are taking commercial importance in the market. Some actinomycetes are pathogenic in nature, so that regulatory regimes of most countries have actinomycetes inoculants banned in past time. Recently, considering the potential of the actinomycetes and their frequency and dominance in the agro-environment, it would be judicious to promote actinomycetes inoculants,

after inclusive biosafety evaluation. The actinomycetes bio-inoculants advantages and disadvantage are described below.

Advantages

- It is naturally less harmful and eco-friendly.
- It affects only specific pathogen or, in some cases, a few target organisms.
- It decomposes quickly, thereby resulting in lower exposures and largely avoiding the pollution problems.
- It supports the colonization of mycorrhizae.
- It balances the soil nutrient cycle and contributes to the residual pool of organic N and P, reducing N leaching loss and P fixation, and also supplies micronutrients to the plant to improve the metabolic activities.
- It provides food and supports the growth of beneficial insect, pest, and earthworms.
- They augment the plant defense and vice versa soil immunity to restrain the unwanted plant diseases, soil-borne diseases, and parasites.
- They normalize the plant metabolism against the biotic and abiotic stresses.

Disadvantages

- Proliferation rate is slow than other bacterial inoculants.
- Preparation and application is moderately different and susceptible to environmental factors.
- Success rate is not identical like chemical fertilizer.
- For storage, lower temperature is needed for longtime use.

5.5 Conclusion and Future Perspectives

The universal efforts in the exploration of natural products for the crop protection market have progressed significantly. Actinomycetes appear to be a good candidate to find new approaches to control plant diseases as actinomycetes bio-inoculants are competent against different kinds of pathogens including fungi, bacteria, insect, and pest. Bio-inoculants become a leading choice and are able to compete with conventional practices, i.e., chemical fertilizers and pesticides, due to their environment-friendly features. While having slow mode of action, bio-inoculants hold status in grower's choice (particularly organic farmers), due to their significant role as natural scavenger, which helps to secure healthy environment and human health. A number of actinomycetes bio-inoculants performed well under in vitro or controlled environment; however, only few plant pathogens have been controlled effectively by actinomycetes bio-inoculants under field conditions due to numerous factors. Therefore, for commercial use of actinomycetes, the consistency of their performance must be enhanced. Also, the potential actinomycetes need to be evaluated under different field conditions (multilocation trials) because disease management is the culmination of complex interactions between the host, pathogen, antagonist, and environment. Actinomycetes produce metabolites, chemicals, and enzymes and rely on the emission for destruction of phytopathogens. Important discoveries pertaining to the genomics sequence of rhizospheric bacteria provide a variety of insights into the organism's lifestyle in plant–microbe–-pathogen interaction. Further, developments and discovery of novel bioactive compounds from actinomycetes would give superior insights into induction of increased disease resistance. Some important fields need to be explored, like plant colonization and pathogen antagonism by molecular approaches. In light of sustainable and long-term use of actinomycetes bio-inoculants in agro-industry, several concerns may require the following issues: An extensive research system for screening multifunctional and reliable strains, which can be utilized in different rhizosphere zones. Exploring the plant–microbe interactions by different molecular tools and trying to make it more advantageous. Monitoring the bio-inoculants for their survival and dispersal in treated soil for assured performance and efficacy.

Harmonized and attentive use of actinomycetes bio-inoculants for better production quality and application. Soil microbiome and proteome study will also give insight into the beneficial or lethal effect on soil biota.

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Managing Pests and Diseases of Grain Legumes with Secondary Metabolites from Actinomycetes

6

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Abstract

Protection of agricultural crops against fungal pathogens, pests, insects, and weeds by the application of chemical pesticides was slowdown in many countries because of the disadvantage of the products toward consumer's health and environmental protection. Therefore, alternative approaches for protecting the crops from various pests and diseases and production of pesticides from microbial route have been attracted. Actinomycetes are the potential sources of novel metabolites, enzymes, and other chemicals with various biological applications. Among them, the applications of actinomycetes toward the protection of soil fertility and controlling the crop diseases are gaining increasing attention by the scientific community. A large number of novel compounds and enzymes with antifungal and insecticidal properties from actinomycetes have been isolated and characterized from various geographic regions around the world. In this chapter, metabolites and enzymes from actinomycetes with antifungal, insecticidal, and commonly available pesticide in the world market are discussed. The products derived from actinomycetes may also serve as key models for the crop protection and soil fertility with respect to the environmental protection.

Keywords

Actinomycetes • Crop protection • Metabolites • Enzymes

6.1 Introduction

Cultivation of economically important agricultural plants and its yield was seriously suppressed by the infections caused by fungal pathogens and insects. The occurrence of spoilage fungus and insects in the agriculture products creates serious

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problems and was responsible for >30 % of spoilage (Schnurer and Magnusson 2005). Spoilage fungus and insects are the main factors responsible for the economic losses in agricultural products (Torkar and Vengust 2008) (Table 6.1). Especially legumes and grains such as chickpea, cowpea, pigeon pea, wheat, and other cereals suffer significant losses to insect pests, particularly *Helicoverpa armigera*. Besides this, certain fungal pathogens present in these agricultural foods synthesize mycotoxins which are harmful to consumer's health (Pitt 2000; Gopalakrishnan et al. 2012). To avoid fungal spoilage, numerous chemical preservatives, fungicides, and insecticides produced by chemical methods are used on a wide range of crops (cereals, grains, etc.) to prevent diseases in conventional farming (Ivic 2010). Continuous use of fungicides and pesticides in enhancing the yields of agricultural products has led to many social problems related to accumulation of nondegradable chemical compounds, emergence of resistant strains, development of pesticide resistance in pests, pest resurgence, destruction of beneficial insects and natural enemies, secondary pest outbreak, human health problems, and environmental pollution (Fox et al. 2007). Therefore, alternative methods that were considered to reduce the use of toxic chemicals in the cultivation places have been a focus. Fungus and pest management in agricultural crops is an essential task for preventing yield loss and ensuring adequate food supply to people. Therefore, integrated pest management (IPM) has emerged as an eco-friendly and economic alternative to conventional use of chemical pesticides for controlling plant diseases in agricultural fields. In the search for useful and eco-friendly alternative chemicals, attention has been focused on the molecules recovered from living organisms including plants, animals, and microorganisms (Harborne and Baxter 1993; Ahn et al. 1998). Antifungal agents from natural sources represent uniquely to solve/control fungal phytopathogens and are more environmental friendly (Bevan et al. 1995). Among the microorganisms, actinomycetes are the biggest source for bioactive compounds and produce a wide variety of chemical molecules with interesting applications (Procopio et al. 2009). They are the source for the production of about more than 50 % of discovered bioactive compounds, including antifungal and pesticidal agents (Berdy 2005). The molecules obtained from actinomycetes are often target specific, biodegradable, and potentially suitable for use in IPM programs; they could lead to the development of new classes of safer controlling agents (Park et al. 2002; Mansour et al. 2004). Due to their potential to alter the fungal and insect's physiological mechanisms involved in nutrition, reproduction, metamorphosis, and behavior, the compounds recovered from actinomycetes are considered to be eco-friendly alternatives to control agricultural pathogens and pests (Sharma et al. 1992; Koul et al. 2000). Though several molecules isolated from plants and novel molecules synthesized by chemical routes have been reported as antifungal, insecticides, antifeedants, repellents, and growth regulators, there is a wide scope for the discovery of novel compounds from the actinomycetes, because the novel species that belongs to actinomycete family still possesses many untapped species with new molecules. This chapter contains the secondary metabolites derived from actinomycetes for the management of diseases and pests of grain legumes.

6.2 Actinomycetes

Actinomycetes are Gram-positive, filamentous, aerobic, spore-forming, and multicellular soil bacteria belong to the order *Actinomycetales* (Balachandran et al. 2015; Velayudam and Murugan 2015). All members of this order are characterized by high G+C content (>50 %) in their genomic DNA (Ventura et al. 2007). The name actinomycetes is derived from Greek word "aktino" meaning ray and "mykes" meaning mushroom/fungus, owing to the formation of its filamentous and sporulating colonies. Thus they are documented as a transition group between primitive bacteria and fungi. They occur in the terrestrial and aquatic environments and play a prevailing role in natural bio-geochemical cycles.

Table 6.1 Major diseases of pulses and its causative agents

Pulse	Disease	Causative agents
Pigeon pea (*Cajanus cajan L.*)	Wilt	*Fusarium oxysporum*
	Leaf spot	*Cercospora indica*
	Sterility mosaic	Pigeon pea sterility Mosaic virus
	Powdery mildew	*Leveillula taurica*
	Bacterial leaf spot	*Xanthomonas campestris*
	Canker	*Diplodia Cajani*
Bengal Gram (*Cicer arietinum L.*)	Rust	*Uromyces ciceris – arietini*
	Blight	*Ascochyta rabiei*
	Dry root rot	*Macrophomina phaseolina*
	Powdery mildew	*Erysiphe polygoni*
	Cercospora leaf spot	*Cercospora* spp.
	Bacterial leaf spot and blight	*Xanthomonas campestris phaseoli*
	Wilt	*Fusarium oxysporum*
	Anthracnose	*Glomerella lindemuthiana shear*
Black gram (*Phaseolus mungo* var. *radiatus L.*)	Dry root rot	*Macrophomina phaseolina*
	Leaf spot	*Cercospora canescens*
	Anthracnose	*Glomerella lindemuthiana* shear
	Powdery mildew	*Erysiphe polygoni*
	Rust	*Uromyces phaseoli typica*
	Mosaic	*Myzus persicae*
	Leaf crinkle	*Bemisia tabaci*
Green gram (*Phaseolus aureus Roxb.*)	Dry root rot	*Macrophomina phaseolina*
	Rust	*Uromyces phaseoli typica*
	Leaf spot	*Cercospora canescens*
	Powdery mildew	*Erysiphe polygoni*
	Yellow mosaic	*Bemisia tabaci*
	Anthracnose	*Glomerella lindemuthiana* shear
Cluster bean (*Cyamopsis tetragonoloba L.*)	Bacterial leaf spot	*Co-xanthomonas campestris*
	Powdery mildew	*Leveillula taurica*
	Blight	*Alternaria cyamopsis rang*
	Dry root rot	*Macrophomina phaseolina*
Lentil (*Lens esculentus* Moench)	Wilt	*Fusarium oxysporum*
	Rust	*Uromyces fabae*
	Dry root rot	*Macrophomina phaseolina*
Horse gram (*Dolichos biflorus L.*)	Root rot	*Pellicularia koleroga*
	Die back	*Colletotrichum capsici*
	Anthracnose	*Glomerella lindemuthiana*
	Dry root rot	*Macrophomina phaseolina*
	Rust	*Uromyces phaseoli typica arth*
Soybean (*Glycine Max* Merr.)	Rust	*Phakopsora pachyrhizi*
	Frog eye leaf spot	*Cercospora sojina*
	Bacterial blight	*Xanthomonas campestris*
Pea (*Pisum sativum L.*)	Rust	*Uromyces fabae*
	Downey mildew	*Peronospora pisi*

(continued)

Table 6.1 (continued)

Pulse	Disease	Causative agents
Cowpea (*Vigna sinensis* Endl.)	Dry root rot	*Macrophomina phaseolina*
	Powdery mildew	*Erysiphe polygoni*
	Anthracnose	*Glomerella lindemuthiana*
	Rust	*Uromyces phaseoli typica arth*
	Bacterial blight	*Xanthomonas campestis*
	Mosaic	*Cowpea mosaic virus*
	Root rot	*Pellicularia koleroga*

Source: Chapman and Carter (1976), Jalali and Chand (1992), and FAOSTAT (2014)

However, terrestrial actinomycetes are ubiquitous and are considered to select the soil constituents such as humus, litter, dung, and even rock surfaces.

6.3 Microbial Fungicides

6.3.1 Antifungal Compounds of Actinomycetes Against Plant Pathogenic Fungi

Plants are exposed to serious fungal infections such as rusts, smuts, and rots which lead to crop losses. Biological control offers an attractive alternate to synthetic fungicides (Sharma and Parihar 2010). Intake of mycotoxin-infested grains leads to critical infections in human such as hallucination and gangrene. Such infections usually happen after eating grains contaminated with fungi, for example, *Ergot* poisoning is caused by *Claviceps purpurea* (Dhanasekaran et al. 2012). Therefore, controlling fungal phytopathogens and their toxins represents an important challenge to the researchers interested in the field of agriculture to avoid crop damage and human infection.

6.3.2 Biological Control

Success of microbial metabolites in plant protection begins with the use of streptomycin for the control of fire blight of apple and pear caused by *Erwinia amylovora* (Beer et al. 1984; Vanneste et al. 1992). Similarly, a chemical compound Terramycin produced by *Streptomyces* sp. is routinely used for the control of fire blight disease in grains. Doumbou et al. (2001) reviewed the literature on the biological control of fungal plant pathogens and plant growth promotion by *Streptomyces* sp. Many reports claimed that *Streptomyces* sp. have both in vitro and in vivo potentials against plant pathogens. Axelrood et al. (1996) demonstrated the antifungal activity of *Streptomyces* strains against plant pathogenic fungi such as *Fusarium*, *Cylindrocarpon*, and *Pythium* in the field conditions. Yuan and Crawford (1995) reported that *Streptomyces lydicus* showed both strong in vitro antifungal activity and inhibition of *Pythium* root rot in pot tests with pea and cotton seeds. Reddi and Rao (1971) controlled *Pythium* damping off in tomatoes and *Fusarium* wilt of cotton with *Streptomyces ambofaciens*. *Streptomyces* strains isolated from Moroccan rhizosphere soils showed a potential for controlling root rot on sugar beet and used in integrated control against diverse soil-borne plant pathogens (Errakhi et al. 2009). The lactone and ketone carbonyl functional group compounds derived from *Streptomyces* sp. showed promising activity against the blast and sheath blight diseases causing *Pyricularia oryzae* and *Rhizoctonia solani*, respectively (Prabavathy et al. 2006). Chamberlain and Crawford (1999) studied in vitro and in vivo antagonism of turfgrass fungal pathogens by *Streptomyces hygroscopicus*. Crawford (1997) patented the use of *S. hygroscopicus* to control plant pathogens in the USA (patent number: 5,527,526). Suh (1998) patented *Streptomyces* sp. was active against *R. solani* and *Phytophthora capsici*. The product of *Streptomyces griseoviridis* against *Fusarium* spp. and other soil pathogens is available in the market as Mycostop (Wearing 2003).

Table 6.2 Antifungal compounds against plant pathogenic fungi produced by actinomycetes

Name of the fungicide	Source of the fungicide	Reference
Polyoxin B and D	*Streptomyces cacaoi*	Endo and Misato (1969)
Natamycin	*S. lydicus*	Lu et al. (2008)
Indole-3-acetic acid	*Streptomyces* CMU-PA101	Khamna et al. 2009
β-1,3-glucanase	*Streptomyces aureofaciens*	Haggag et al. (2011)
2,3- dihydroxybenzoic acid	*Micromonospora* sp. M39	Ismet et al. (2004)
Phenylacetic acid		
Cervinomycin A1 and A2		
Resistoflavin	*Streptomyces chibaensis* AUBN1/7	Gorajana et al. (2007)
Tetracenomycin D	*Streptomyces* sp. B8005	Kock et al. (2005)
Neopeptine A	*Streptomyces* sp. KNF2047	Kim et al. (2007)
Malayamycin	*Streptomyces malaysiensis*	Li et al. (2008)
2-Furancarboxaldehyde	*Streptomyces cavourensis*	Lee et al. (2012)

The treatment of crop yields through chemical substances including benzimidazoles, dicarboximide, anilinopyrimidine, and demethylation inhibitors (DMI) is a common method and extensively used in modern agriculture to prevent the infection of the plants by fungi; however, chemical fungicides may cause environmental pollution and pose serious threat to the health of human and animals. On other hand, pathogenic fungi may easily acquire resistance against frequently used fungicides (Shimizu et al. 2000; Yang et al. 2008). A number of antifungal agents produced by actinomycetes are used as control of plant pathogenic fungi (Table 6.2). Kasugamycin, an antifungal compound discovered in 1965 (Umezawa et al. 1965) produced by *Streptomyces kasugaensis*, was developed to control plant pathogenic fungi *Magnaporthe grisea* that cause rice blast disease (Yoshii et al. 2012) (Fig. 6.1a). Mildiomycin (Fig. 6.1b), an antimycotic agent produced by *Streptoverticillium rimofaciens*, was discovered in 1978 (Iwasa et al. 1978). It exhibited a potent activity against several plant pathogenic fungi by inhibiting protein synthesis (Feduchi et al. 1985). Soil actinomycete *S. hygroscopicus* produces validamycin as fungicide (Fig. 6.1c). It was detected by Takeda in 1968 during screening extracts of streptomycetes for activity against rice sheath blight disease (Doumbou et al. 2001). Validamycin is extensively used for the control of sheath blight disease of rice plants caused by *R. solani* (He et al. 2003). Jinggangmycin, a fungicide similar to validamycin in terms of chemical structure and activity against rice sheath blight, is also produced by *S. hygroscopicus* (Jian et al. 2006) (Fig. 6.1d).

During the last decade, actinomycetes were the subject of interest of researchers in discovering new/novel antifungal drugs as biocontrol agents to control fungal plant infections (Kathiresan et al. 2005; Khamna et al. 2009). A number of antifungal substances have been discovered and were applied in the field of agriculture, for example, antimycin A_{17} (Fig. 6.1e) is a novel fungicide agent obtained from strain *Streptomyces* sp.; GAAS7310, isolated from soil sample in China, revealed potent antifungal activity against plant pathogenic fungi such as *F. oxysporum*, *Alternaria solani*, *Curvularia lunata*, and *Colletotrichum nigrum* (Chen et al. 2005). Antimycin A inhibits NADH oxidase and succinate oxidase and blocks the mitochondrial electron transport between cytochrome b and c (Huang et al. 2005; Han et al. 2008). Indole-3-acetic acid and siderophore that have been derived from *Streptomyces* CMU-PA101 and *Streptomyces* CMU-SK126 isolated from *Curcuma mangga* rhizosphere soil showed promising activity against fungal phytopathogens including *Sclerotium rolfsii*, *F. oxysporum*, *Alternaria brassicicola*, *Penicillium digitatum*, and *Colletotrichum gloeosporioides* (Khamna et al. 2009).

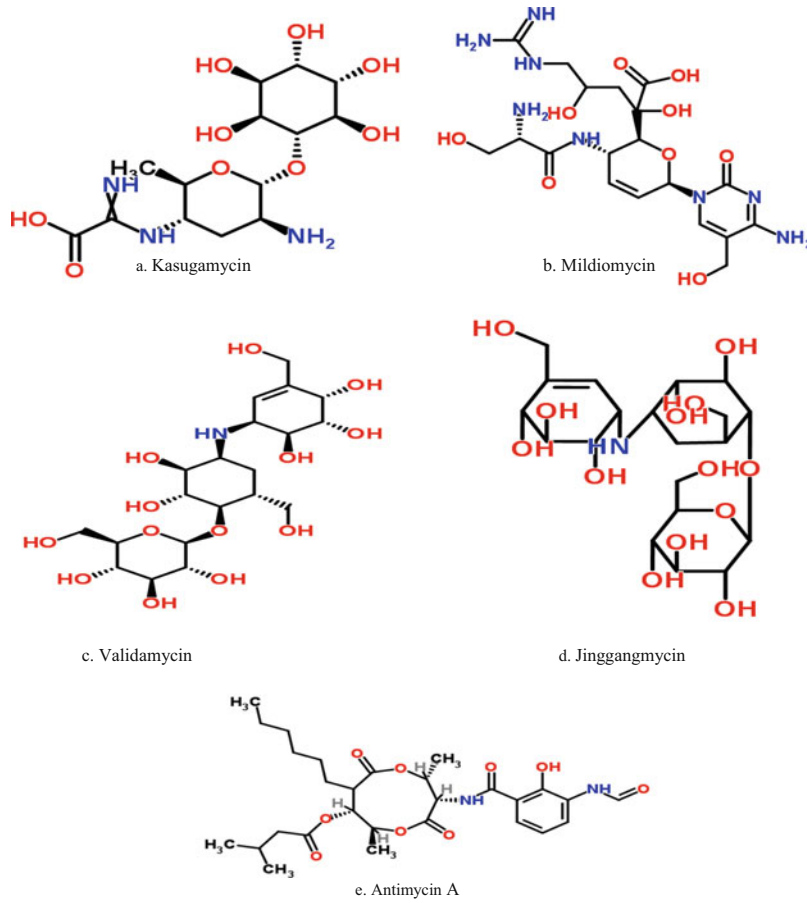

Fig. 6.1 Chemical structures of fungicides derived from actinomycetes used in control of fungal plant infections. (**a**) Kasugamycin, (**b**) Mildiomycin, (**c**) Validamycin, (**d**) Jinggangmycin, (**e**) Antimycin A (Umezawa et al. 1965; Iwasa et al. 1978; Feduchi et al. 1985; Chen et al. 2005; Jian et al. 2006)

Interestingly, endophytic *Streptomyces* showed antimycotic activity against *P. oryzae* and *R. solani*, the main causative agents for sheath blight diseases of rice (Prabavathy et al. 2006). Natamycin obtained from *S. lydicus*, *Streptomyces chattanovgensis*, *Streptomyces natalensis*, *and Streptomyces gilvosporeus* possesses strong inhibitory activity against plant pathogenic fungi such as *Monilinia laxa*, *Botrytis cinerea*, and *F. oxysporum* (Lu et al. 2008).

6.3.3 Biocontrol of Plant Pathogenic Fungi Using the Extracellular Enzymes of Actinomycetes

The hydrolytic enzymes such as chitinase, glucanase, cellulase, protease, amylase, phospholipase, etc. produced by actinomycetes are capable of controlling the plant diseases by degrading the fungal cell wall, cell membrane proteins, and cell membrane and decreasing the activity of extracellular virulence factors (Ramesh and Mathivanan 2009). *Streptomyces* is extensively studied for these cell wall-degrading extracellular enzymes and their involvement in growth and biocontrol (Chater et al. 2010). Generally antagonistic activity of *Streptomyces* spp. to fungal pathogens is related to the production of antifungal compounds and extracellular hydrolytic enzymes like β-1,3-glucanase and chitinase (Table 6.3). These two hydrolytic enzymes are considered to be important in the lysis of fungal cell walls. Recent reports claimed that chitinase and β-1,3-glucanase recovered from *S. hygroscopicus* and *Streptomyces aureofaciens* showed significant antimycotic activity against

Table 6.3 Antagonistic actinomycetes suppressing plant pathogens by the production of hydrolytic enzymes

Diseases	Pathogen	Antagonistic strain	Hydrolytic enzymes	References
Root rot of lupine	*P. cinnamomi*	*M. carbonacea*	Cellulase	El-Tarabily (2003)
Root rot of turfgrass	*P. infestans*	*S. violaceusniger* strain YCED-9	Glucanase	Trejo-Estrada et al. (1998a, b)
Root rot of wheat	*P. infestans*	*S. olivaceoviridis*	Amylase	Aldesuquy et al. (1998)
Lupin root rot	*P. tabacinum*	*A. missouriensis*	Chitinase	El-Tarabily (2003)
Lupin root rot	*F. oxysporum*	*S. halstedii* AJ-7	Chitinase	Joo (2005)
Wood rot	*P. chrysosporium*	*S. violaceusniger* XL-2	Endo-chitinase	Shekhar et al. (2006)
Wood rot	*P. placenta*	*S. violaceusniger* XL-2	Endo-chitinase	Shekhar et al. (2006)
Wood rot	*C. versicolor*	*S. violaceusniger* XL-2	Endo-chitinase	Shekhar et al. (2006)
Wood rot	*G. trabeum*	*S. violaceusniger* XL-2	Endo-chitinase	Shekhar et al. (2006)
Damping off	*P. aphanidermatum*	*A. campanulatus*	β-glucanase	El-Tarabily et al. (2009)
Crown rot	*P. aphanidermatum*	*M. chalcea*	β-glucanase	El-Tarabily et al. (2009)
Damping off chickpea	*P. aphanidermatum*	*S. rubrolavendulae* S4	Cellulase	Loliam et al. (2013)
Damping off chickpea	*F. oxysporum*	*Streptomyces sp.*	Pectinase	Ashokvardhan et al. (2014)
Lupin root rot	*R. solani*	*S. vinaceusdrappus*	Chitinase	Yandigeri et al. (2015)

plant pathogenic fungi including *Alternaria alternata*, *Phytophthora parasitica*, *R. solani*, *Aspergillus flavus*, *Aspergillus niger*, *F. oxysporum*, and *Glomerella cingulata* (Prapagdee et al. 2008). Similarly, biocontrol of anthracnose in pepper has been monitored to the production of chitinase and glucanase by *S. cavourensis* SY224 (Lee et al. 2012). Chitinase-producing endophytic *Streptomyces* are viewed as potential biocontrol agents (Quecine et al. 2008), and a chitinase-producing *S. violaceusniger* XL-2 was able to suppress wood rotting fungi (Shekhar et al. 2006). Glucanolytic actinomycetes reduced root rot in raspberry seedlings caused by *Phytophthora fragariae* (Valois et al. 1996). Glucanase-producing *Streptomyces spiralis* was reported to protect seedlings and mature plants of cucumber from *Pythium aphanidermatum* (El-Tarabily et al. 2009). Involvement of protease in antifungal activity was also demonstrated in a *Streptomyces* sp. strain A6 with inhibitory activity toward *Fusarium udum* (Singh and Chhatpar 2011). The cellulase-producing strain, *Micromonospora carbonacea*, was used for the control of *Phytophthora cinnamomi* (El-Tarabily and Hardy 1997). A wealth of information is available on the antifungal activity of chitinolytic *Streptomyces* and other actinomycetes (Froes et al. 2012), since they are among the chief chitinolytic microorganisms present in the soil habitats. Singh et al. (1999) used a chitinolytic *Streptomyces* sp. for the suppression of cucumber wilt caused by *F. oxysporum*. Actinomycetes efficiently inhibit many soil plant pathogenic fungi by degrading their chitinous cell walls (Xiao et al. 2002). Actinomycetes from diverse soils are attractive source of natural metabolites and various hydrolytic enzymes, which draw increasing attention as prospective for managing phytopathogens.

6.4 Microbial Insecticides

The use of nontoxic microbial pesticide to prevent pest problems and protect wildlife from their harmful effects is attractive. There are many nontoxic microbial pesticides commercially available. Microbial pesticides are having biological actions and are toxic only to specific pests; hence, their appropriateness for use in organic farming is interesting. Various microbial pesticides prepared in different brand names and

Table 6.4 Different microbial insecticidal products available in the world

Country	Product name	Source of production	Targeted insect
Africa	Agree	*Bacillus thuringiensis* subspecies *aizawai* and *kurstaki*	Lepidoptera larvae
	DiPel	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Florbac WG	*Bacillus thuringiensis* subsp. *kurstaki* H7	Lepidoptera larvae
	Bb Plus	*Beauveria bassiana*	Thrips, weevils, whiteflies
	Green Muscle	*Metarhizium anisopliae* subspecies *acridum* IMI 330 189	Locust
	Agriphage	*Pseudomonas resinovorans* bacteriophage	Insect pest control
	Bio-Nematon	*Paecilomyces lilacinus*	Nematodes
	Ditera	*Myrothecium verrucaria*	Nematodes
	Biolep	*Bacillus thuringiensis* subsp. *kurstaki*	African bollworm
	Halt	*Bacillus thuringiensis* subsp. *kurstaki*	*S. exigua*
	Thuricide	*Bacillus thuringiensis* subsp. *kurstaki*	Diamond black moth
	Delfin	*Bacillus thuringiensis* subsp. *kurstaki* SA11	Diamond black moth
	Botanigard	*Beauveria bassiana* GHA	Sucking insect pest
	Bio – Nematon	*Paecilomyces lilacinus*	Root knot nematode
India	Tacibio	*Bacillus thuringiensis* subspecies *israelensis*	Lepidopteran pests
	Bio-Dart	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Myco-Jaal	*Beauveria bassiana*	Diamondback moth
	Biosoft	*Beauveria bassiana*	Thrips
	ATEC Beauveria	*Beauveria bassiana*	Grasshoppers
	Larvo-Guard	*Beauveria bassiana*	Whiteflies
	Biogrubex	*Beauveria bassiana*	Aphids
	Biorin	*Beauveria bassiana*	Codling moth
	Biowonder	*Beauveria bassiana*	Grasshoppers
	Meta-Guard	*Metarhizium anisopliae*	Coleoptera
	Biomet	*Metarhizium anisopliae*	Leafhoppers
	Biomagic	*Metarhizium anisopliae*	Beetles
	Sun Agro Meta	*Metarhizium anisopliae*	Termites
	Bio-Magic	*Metarhizium anisopliae*	Grubs
	Nemato-Guard	*Paecilomyces fumosoroseus*	Whitefly
	Yorker	*Paecilomyces lilacinus*	Whitefly
	Vert-Guard	*Verticillium lecanii*	Homopteran pests
	Helicide	*H. armigera* nucleopolyhedrosis virus	*Helicoverpa armigera*
	Spodi-cide	*S. litura* nucleopolyhedrosis virus	*Spodoptera litura*
South Korea	Salchungtan	*Bacillus thuringiensis* subsp. *aizawai*	Lepidopteran pests
	Tobagi	*Bacillus thuringiensis* subspecies *aizawai*	Lepidopteran pests
	Solbichae	*Bacillus thuringiensis* subspecies *aizawai*	Lepidopteran pests
	Biocan	*Bacillus thuringiensis* subspecies *aizawai*	Lepidopteran pests
	Imperial	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Biobit	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Shuricide	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Bigule	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Biobit	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Youngil BT	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidopteran pests
	Ceremoni	*Beauveria bassiana*	Greenhouse whitefly
	Bangsili	*Paecilomyces fumosoroseus*	Two spotted spider mite
	Ddangumi	*Monacrosporium thaumasium*	Root knot nematode

(continued)

Table 6.4 (continued)

Country	Product name	Source of production	Targeted insect
Europian Union	Turex	*Bacillus thuringiensis* subspecies *aizawai* GC-91	Lepidoptera pests
	Dipel WP	*Bacillus thuringiensis* subspecies *kurstaki* HD-1	Lepidoptera pests
	Novodor	*Bacillus thuringiensis* subspecies tenebrionis NB 176	Coleoptera pests
	Naturalis L	*Beauveria bassiana* ATCC 74040	Whiteflies
	Mycotal	*Lecanicillium muscarium* (Ve6) (former *Verticillium lecanii*)	Thrips
	Nofly	*Paecilomyces fumosoroseus* Fe9901	Whiteflies
	BioTepp	*Cydia pomonella granulosis* virus	Codling moth
	Spod-X GH	*S. littoralis* nucleopolyhedrosis virus	Spodoptera exigua
	Curbit	Zucchini Yellow Mosaic Virus	Yellow mosaic virus
United Kingdom	Mycotal	*Verticillium lecanii*	Scale insects
	Naturalis L	*Beauveria bassiana* ATC74040	Whitefly
	DiPel	*Bacillus thuringiensis* subspecies *kurstaki*	Lepidoptera pests
	Cyd-X Extra	*Cydia pomonella granulosis* virus	Codling moth
Russia	Boverin	*Beauveria bassiana*	Insect pests
	Lepidocid	*Bacillus thuringensis* subspecies *kurstaki*	Larvae
	Bitoxibacillin	*Bacillus thuringensis*	Colorado potato beetle
	Nemabact	Entomopathogenic nematode	Cabbage fly
Ukraine	Gaupsin	*Pseudomonas aureofaciens*	Larvae of harmful insects
	Bactophil	*Micrococcus roseus*	Seed germination diseases
	Bactophil	*Streptomyces albus*	Seed germination diseases
	Albobacteryn	*Achromobacter album*	Sprouting inhibition
	Dendrobacillin	*Bacillus thuringiensis*	Flying insects
	Turingin	*Bacillus thuringiensis*	Web mites
	Bitoxibacillin	*Bacillus thuringiensis* subsp. *thuringiensis*	Various harmful insects
	Actofit	*Streptomyces avermitilis*	Complex of phytophatogens
	Astur	*Streptomyces avermitilis*	Colorado potato beetle
	Pecilomin	*Paecilomyces* spp.	Larvae of various insects
	Boverin	*Beauveria bassiana*	Various insects
	Nematophagin	*Arthrobotrys* spp.	Nematodes
Argentina	Agro Roca	*Cydia pomonella granulosis* virus	*Cydia pomonella*
	Biagro BT	*Bacillus thuringiensis* subspecies *israelensis*	Black flies
Brazil	Bactur	*Bacillus thuringiensis*	Lepidopteran pests
	Xentari	*Bacillus thuringiensis*	Lepidopteran pests
	Bac-Control	*Bacillus thuringiensis*	Lepidopteran pests
	Dipel	*Bacillus thuringiensis*	Lepidopteran pests
	Metarril E9	*Metarhizium anisopliae*	Hemiptera
	Boveril PL 63	*Beauveria bassiana*	Coleoptera
	Protégé	*Anticarsia gemmatalis* nucleopolyhedrosis virus	*Anticarsia gemmatalis*
	Baculovirus Nitral	*Anticarsia gemmatalis* nucleopolyhedrosis virus	Lepidopterans

(continued)

Table 6.4 (continued)

Country	Product name	Source of production	Targeted insect
Cuba	Thurisav 1	*Bacillus thuringiensis* LBT-1	Lepidopteran larvae
	Thurisav 26 (LBT-26)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-26	*Spodoptera* spp.
	Thurisav 26 (LBT-24)	*Bacillus thuringiensis* subsp. *kurstaki* LBT-24	*P. xylostella*
	Thurisav 26 (LBT-26)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-26	*Heliothis* spp.
	Thurisav 26 (LBT-24)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-24	*Spodoptera frugiperda*
	Thurisav 26 (LBT-26)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-26	*Diaphania* spp.
	Thurisav 26 (LBT-24)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-24	*Erinnyis alope*
	Thurisav 26 (LBT-26)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-26	*Heliothis* spp.
	Thurisav 26 (LBT-24)	*Bacillus thuringiensis* subspecies *kurstaki* LBT-24	*Phyllocnistis citrella*
	Bibisav 2	*Beauveria bassiana* MB-1	*Atta insularis*
	Bibisav 2	*Beauveria bassiana* MB-1	*Attamyces bromatificus*
	Bibisav 2	*Beauveria bassiana* MB-1	*Acromyrmex octospinosus*
	Metasav 11	*Metarhizium anisopliae* LBM-11	*Lissorhoptrus brevirostris*
	Metasav 11	*Metarhizium anisopliae* LBM-11	*Tagosodes oryzicola*
	Metasav 11	*Metarhizium anisopliae* LBM-11	*Spodoptera* spp.
	Metasav 11	*Metarhizium anisopliae* LBM-11	*Thrips palmi*
	Metasav 11	*Metarhizium anisopliae* LBM-11	*Pachnaeus litus*
	Vertisav 57	*Verticillium lecanii* Y-57	*Frankliniella* spp.
	HeberNem-L	*Tsukamurella paurometabola*	Plant parasitic nematodes
	HeberNem-S	*Tsukamurella paurometabola* C-924	Plant parasitic nematodes
	KlamiC	*Pochonia chlamydosporia* subsp. *catenulate*	Soil nematodes
Canada	VectoBac	*Bacillus sphaericus* serotype H5a5b	Nuisance fly
	Teknar HP-D	*Bacillus thuringiensis* subsp. *israelensis* SA3A	Blackfly
	Bioprotec	*Bacillus thuringiensis* subsp. *kurstaki* HD-1	Brown spanworm
	Foray	*Bacillus thuringiensis* subsp. *israelensis* SA3A	Diamondback moth
	Dipel	*Bacillus thuringiensis* subsp. *kurstaki* HD-1	Elm spanworm
	Teknar HP-D	*Bacillus thuringiensis* subsp. *israelensis* SA3A	Hemlock looper
	Foray	*Bacillus thuringiensis* subsp. *israelensis* SA3A	Spring cankerworm
	Foray	*Bacillus thuringiensis* subsp. *israelensis* SA3A	Western spruce budworm
	Thuricide 48 LV	*Bacillus thuringiensis* subsp. *kurstaki* SA-12	Fall webworm
	Novodor	*Bacillus thuringiensis* subsp. *tenebrionis* HB 176	Elm leaf beetle
	Botanigard	*Beauveria bassiana* GHA	Whitefly
	Nemasys G	*Heterorhabditis bacteriophora*	*Hepialus lupulinus*
	Nematode HB	*Heterorhabditis bacteriophora*	*Welch chafer*
	B-Green	*Heterorhabditis bacteriophora*	*Hepialus lupulinus*
	Larvanem	*Heterorhabditis bacteriophora*	Larvae of curculionids

(continued)

Table 6.4 (continued)

Country	Product name	Source of production	Targeted insect
	Traunem	*Steinernema feltiae*	Sod webworm
	Nolo Bait	*Nosema locustae*	Grasshopper
	Lecontvirus	*Neodiprion lecontei* nucleopolyhedrosis virus	Red-headed pine sawfly
	Virtuss	*Orgyia pseudotsugata* nucleopolyhedrosis virus	Douglas fir tussock moth,
	TM Biocontrol 1	*Orgyia pseudotsugata* nucleopolyhedrosis virus	White-marked tussock moth
United States	BMP	*Bacillus thuringiensis* subsp. *israelensis*	Blackflies
	Gnatrol	*Bacillus thuringiensis* subsp. israelensis EG2215	Flies
	Thuricide	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Dipel	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Foray	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Deliver	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Wilbur-Ellis BT 320	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Javelin WG	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Britz BT	*Bacillus thuringiensis Diaphania* spp.	Lepidopteran larvae
	Hi-Yield Worm Spray	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Bonide	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Security Dipel Dust	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran larvae
	Condor	*Bacillus thuringiensis* subsp. *kurstaki* EG2348	Lepidopteran larvae
	BMP123	*Bacillus thuringiensis* subsp. *kurstaki* BMP 123	Lepidopteran larvae
	Crymax	*Bacillus thuringiensis* subsp. *kurstaki* BMP 123	Lepidopteran larvae
	Naturalis L	*Beauveria bassiana* ATCC 74040	Various insects
	Botanigard 22WP	*Beauveria bassiana* GHA	Various insects
	PFR-97	*Paecilomyces fumosoroseus apopka* 97	Whitefly
	CLV-LC	*Anagrapha falcifera* nucleopolyhedrosis virus	Lepidopteran larvae
	Gypchek	Gypsy moth nucleopolyhedrosis virus	Gypsy moth
	Virosoft	*Mamestra configurata* nucleopolyhedrosis virus (107308)	Bertha armyworm
	Bull Run	*Saccharomyces cerevisiae*	Fly attractant
	Spod-X	*S. exigua* nucleopolyhedrosis virus	Beet armyworm
Australlia	Bacchus	*Bacillus thuringiensis* subsp. *aizawai*	Lepidoptera larvae
	XenTari	*Bacillus thuringiensis* subsp. *aizawai*	Lepidoptera larvae
	Caterpillar Killer	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Delfin	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Costar	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Full-Bac WDG	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	DiPel	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Biocrystal	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Full-Bac WDG	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidoptera larvae
	Green Guard	*Metarhizium anisopliae* subsp. acridum	Locusts and grasshoppers
	Heliocide	*Helicoverpa armigera* nucleopolyhedrosis virus	*Helicoverpa* spp.
	Vivus Max	*Helicoverpa armigera* nucleopolyhedrosis virus	*Helicoverpa* spp.
	Vivus Gold	*Helicoverpa armigera* nucleopolyhedrosis virus	*Helicoverpa* spp.
	Gemstar	*Helicoverpa zea* nucleopolyhedrosis virus	*Helicoverpa* spp.

(continued)

Table 6.4 (continued)

Country	Product name	Source of production	Targeted insect
New Zealand	Biocrystal *kurstaki*	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran pests
	Xentari	*Bacillus thuringiensis* subsp. *aizawai*	Lepidopteran pests
	Bactur	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran pests
	Delfin	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran pests
	Organic no caterpillars	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran pests
	Foray 48B	*Bacillus thuringiensis* subsp. *kurstaki*	Lepidopteran pests
	Beaublast	*Beauveria bassiana*	Whiteflies
	Vertiblast	*Beauveria bassiana*	Whiteflies
	Nemasys	*Steinernema feltiae*	Fungus gnats
	Virex	*Cydia pomonella granulosis* virus	Codling moth
	CYD-X	*Cydia pomonella granulosis* virus	Codling moth
	Madex	*Cydia pomonella granulosis* virus	Codling moth

The information's were collected from Kabaluk et al. (2010)

their activity against certain insects and pests are tabulated (Table 6.4). Till today majority of the microbial pesticides were prepared and reported using various strains of *Bacillus thuringiensis* (Leonard and Julius 2000). Whereas, in the last two decades, novel molecules identified from actinomycetes have gained significant importance in the development of new biocontrol agents. The actinomycetes, especially *Streptomyces* sp., were widely studied for the use of insect control with the potential identification of new bioactive compounds (Harman 2000). Nevertheless, the exploitation of actinomycetes as a source for the biocontrol agents is still at an early stage; despite that, numerous novel therapeutic and pharmacologically important compounds were isolated during the last few years. The secondary metabolites obtained from *Streptomyces* and *Streptoverticillium* species exhibited significant activity against the Egyptian cotton leaf worm *Spodoptera littoralis* (Bream et al. 2001). Similarly the other promising metabolites such as dianemycin, avermectin, spinosad, doramectin, milbemycin, prasinons, and nanchangmycin recovered from the fermented broth of the *Streptomyces* species were actively suppressed the spreading of the pest insects (Montesinos 2003; Omura 2008). A polyketide metabolite (Fig. 6.2) produced by marine *Streptomyces* sp. AP-123 revealed significant antifeedant, larvicidal, and growth

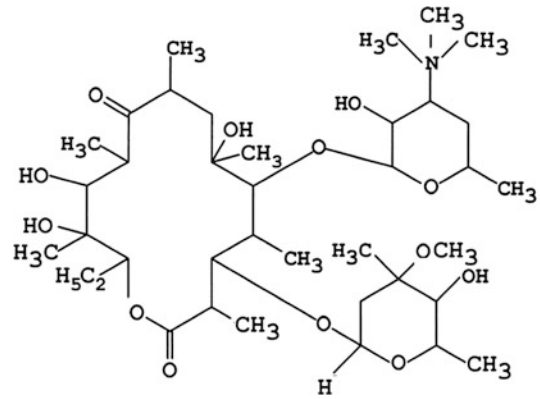

Fig. 6.2 Structure of polyketide antifeedant metabolite isolated from *Streptomyces* sp. AP-123 (Arasu et al. 2013)

inhibitory bioactivities against polyphagous pests such as *Helicoverpa armigera* and *Spodoptera litura* (Arasu et al. 2013). The novel metabolite extracted from *Streptomyces hydrogenans* DH16 exhibited insecticidal and growth inhibitory potential against the major pest in India (Kaur et al. 2014). The extracellular metabolites of the soil actinomycetes control the spreading of cotton leaf worm *S. littoralis* (Bream et al. 2001). Interestingly, marine actinomycetes were reported to contain significant activity against the several insects. To support this, Xiong et al. (2004) characterized avermectin B1 from marine *Streptomyces* sp.173 with activity against *H. armigera*. Also, actinomycetes isolated

from marine sponge revealed the positive activity toward *Spodoptera exigua*, *Plutella xylostella*, *Heliothis zea*, and aphids (Xiong et al. 2005). Avermectin produced from the soil actinomycete *Streptomyces avermitilis* exhibited insecticidal activity by blocking the GABA- and glutamate-gated chloride channels of insect (White et al. 1998), whereas the emergence of Lepidoptera was arrested by a novel molecules spinosad excreted from the soil actinomycetes (Hainzl et al. 1998; Tomlin 2001). On the other hand, quinine-related compounds, namely, quinomycin A recovered from the fermented broth of the terrestrial *Streptomyces sp*. KN-0647 documented activity against *P. xylostella*, *S. exigua*, *Aphis glycines*, *and Dendrolimus punctatus* (Liu et al. 2008). *Streptomyces* sp. and *Streptoverticillium* sp. were found to be the most potent actinomycetes affecting the biological and physiological criteria of the insect species (Takahash et al. 1989).

6.5 Advantage of Using Actinomycetes in the Insect Pest Management

The insecticides and pesticides obtained from actinomycetes are good alternatives to chemical pesticides. They are target specific, economic, eco-friendly, and biodegradable in nature. The application of actinomycete metabolites protects the beneficial microbes of the crops such as micorrhizae and symbiotic rhizobacteria and enhances the soil fertility. Actinomycetes exist in common compost mainly involved in the degradation of organic materials and enhance the nutrient quality, and its application not only promotes plant growth, as it contains beneficial microorganisms that help the plants to mobilize nutrients (Perner et al. 2006; Gopalakrishnan et al. 2011; Yin et al. 2011). Since actinomycetes have antimicrobial activity and other extracellular enzyme production capabilities, there is less chance of insect attack to the crops. Therefore, the major cost for the pesticides for the cultivation crops is reduced.

6.6 Conclusions and Future Prospects

Soil, marine samples, and halophilic regions represent a good source for the isolation of unexplored actinomycetes with special biocontrol properties. Such potential actinomycetes would be highly adapted to the crop cultivation area. Despite the huge demand of synthetic molecules with effective insecticidal and pesticidal properties, novel methods and technologies for discovering novel natural products from actinomycetes from unexplored regions should be studied. The antifungal and insecticidal metabolites producing novel actinomycetes are good bugs for enhancing the soil fertility thereby cultivation, and continuous bioprocessing using low-cost-contributing media components would be beneficial to the farmers. It will be interesting to identify the mechanism of the insecticidal properties of novel actinomycete enzymes, which may lead to significant novel biotechnological applications. Additionally, the stability of products in harsh conditions such as stress, alkalinity, acidity, multiple metal ions, or organic solvents should be investigated. In conclusion, actinomycetes will be a useful host for the production of antifungal, insecticidal compounds, and pesticides in bulk with low cost.

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Role of Secondary Metabolites of Actinomycetes in Crop Protection

7

N. Aggarwal, S.K. Thind, and S. Sharma

Abstract

Diseases and insect pests are the major hurdle in enhancing the production of agricultural crops. The frequent use of synthetic pesticides has led to the development of pesticide-resistant pathogens and insect pests, environmental pollution, negative effects on natural enemies, human health hazards, and pollution of underground water, thereby causing ecological imbalance. The use of bacteria having antimicrobial properties has become one of the most attractive options for enhancing the sustainability of agricultural production due to their ecofriendliness, low production cost, and reduced use of nonrenewable resources. Among them, actinomycetes are the good alternative for the management of insect pests and diseases. These are the most economically and biotechnologically valuable prokaryotes. These represent a high proportion of the soil microbial biomass and have the capacity to produce wide variety of secondary metabolites. Several strains of actinomycetes have been acknowledged as prolific producer of valuable bioactive metabolites as antibacterial, antifungal, antibiotic, antiparasitic, insecticide, and herbicide. However, only a few microbial compounds are applicable at the field level or presently commercialized. Here, the authors have provided an overview of the uses and importance of actinomycetes and their secondary metabolites with special emphasis on managing insect pests and diseases of cultivated crops.

Keywords

Actinomycetes • Biocontrol • Insect pests • Plant diseases • Secondary metabolites • Streptomyces

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7.1 Introduction

Diseases and insect pests are the major hurdle in enhancing the agriculture production. The frequent use of synthetic pesticides in the last decade has led to the development of pesticide-resistant pathogens and insect pests, adverse effects on natural enemies, and environmental pollution which leads to ecological imbalance (Doumbou et al. 2001). Actinomycetes are prokaryotic microbes being exploited as biocontrol agents for the management of insect pests and diseases of plants. They are well known for the production of primary and secondary metabolites having antibiotic activities against a variety of pathogens (Copping and Duke 2007; Blunt et al. 2009; Lei et al. 2013; Sharma 2014; Zahaed 2014). Besides inhibiting soilborne plant pathogens, actinomycetes also promote plant growth by producing plant growth hormones such as indole-3-acetic acid (IAA) and siderophores (Wraight and Roberts 1987; Lucas et al. 2009; Gopalakrishnan et al. 2015). Among the actinomycetes, *Streptomyces* are effective in controlling plant pathogens and mobilizing and acquiring the nutrients (Postma et al. 2003; Gopalakrishnan et al. 2011; Jalilian et al. 2012) which are aided by several metabolites and hydrolytic enzymes such as cellulase, amylase, lipase, xylanases, collagenase, protease, chitinase, and ligase (Crawford et al. 1993; Goudjal et al. 2014). Among the known antibiotics, more than 60 % are produced by *Streptomyces* spp. which are largely being exploited for production of antibiotics and agrochemicals (Franklin et al. 1989; Bérdy 2005). Therefore, actinomycetes are the good alternative for the management of insect pest and diseases, and many reports are well documented to explore its potential (Copping and Duke 2007; Blunt et al. 2009; Costa et al. 2013; Sharma 2014; Zahaed 2014). The use of actinomycetes having antimicrobial properties has become one of the most attractive options for enhancing the sustainability of agricultural production due to their ecofriendliness, low production cost, and reduced use of nonrenewable resources. The authors have provided an overview of the use of actinomycetes in managing insect pests and diseases of cultivated crops.

7.2 Development of Actinomycetes as Bio-agrochemicals

In modern agriculture, application of agrochemicals is still an invaluable and effective method to control plant diseases and insect pests. The use of microbe-based biocontrol agents, particularly actinobacteria, as substitute for agrochemicals, has been gaining momentum in agricultural production. Actinobacteria are prevalent in soil, water, humus, litter, dung, deep sea, arctic ice, rock, and as endophytes in plants, human, and animals either friendly or hostile way (Lechevalier 1988; Matsukuma et al. 1994; Okazaki et al. 1995; Sharma 2014). Today, marine actinomycetes are being exploited having unique secondary metabolic and physiological capabilities (Mayer et al. 2007; Williams 2009). Endophytic microbes colonize the plant tissues to get nutrition, and in return, they confer fitness to the host plants by producing certain functional secondary metabolite and by giving physiological and environmental advantages to their host plants (Gholami et al. 2009; Adesemoye et al. 2010; Luo et al. 2010; Qiu et al. 2012). Many actinomycete-based biocontrol agents are commercially available, and they are given in detail in Table 7.1. It is observed that *Streptomyces* has been greatly exploited for the production of antibiotics, fungicides, bactericides, herbicides, insecticides, and acaricides. Generally, they are applied to target crops in the form of culture filtrate, spore suspension, wettable powder, emulsifiable concentrate, and wettable granules.

Table 7.1 Actinomycete's compounds used commercially as crop protection agents

Biocontrol metabolite	Actinomycetes	Formulation available	Commercial product	Target disease/insect pest	Mode of action
Bio-fungicides/bactericides					
Blasticidin S	*Streptomyces griseochromogenes*	DP, EC, and WP	Bla-S	Rice blast (*Pyricularia oryzae*)	It inhibits protein biosynthesis by binding to the 50S ribosome in prokaryotes, leading to the inhibition of peptidyl transfer and protein chain elongation
Kasugamycin	*Streptomyces kasugaensis*	WP, DP, GR, and EC	Kasugamycin, Kasumin, Kasu-rab-valida-sumi	Leaf spot in sugar beet and celery (*Cercospora* spp.) and scab in pears and apples (*Venturia* spp.), soybean root rot (*Phytophthora sojae*)	It inhibits protein biosynthesis by interfering with the binding of aminoacyl-tRNA to both the mRNA-30S and the mRNA-70S ribosomal subunit complexes, preventing amino acids into proteins incorporation
Streptomycin	*Streptomyces griseus*	WP	Paushak, Cuprimicin 17, AAstrepto, Agrimycin 17 and AS-50, Dustret, Cuprimic 100 and 500	Bacterial rots, canker, and other bacterial diseases, *Xanthomonas oryzae*, *Xanthomonas citri*, and *Pseudomonas tabaci* of pome fruit, stone fruit, citrus, olives, vegetables, potatoes, tobacco, cotton, and ornamentals	It inhibits protein biosynthesis by binding to the 30S ribosomal subunit and causing a misreading of the genetic code in protein biosynthesis
Oxytetracycline	*Streptomyces rimosus*	SP	Mycoshield, Cuprimic 100 and 500, Phytomycin, Mycoject	Fire blight (*Erwinia amylovora*) and diseases caused by *Pseudomonas* and *Xanthomonas* sp. and mycoplasma like organisms	It is an inhibitor of protein biosynthesis in bacteria and binds to the 30S and 50S bacterial ribosomal subunits and inhibits the binding of aminoacyl-tRNA and the termination factors RF1 and RF2 to the A site of the bacterial ribosome

(continued)

Table 7.1 (continued)

Biocontrol metabolite	Actinomycetes	Formulation available	Commercial product	Target disease/insect pest	Mode of action
Validamycin	*Streptomyces hygroscopicus*	DP, SL, DS	Validacin, Valimun, Dantotsupadanvalida, Mycin Hustler, Valida, Sheathmar	*Rhizoctonia solani* and other *Rhizoctonia* in rice, potatoes, vegetables, strawberries, tobacco, ginger, cotton, rice, sugar beet, etc.	It causes abnormal branching of the tips of the pathogen followed by a cessation of further development and has a potent inhibitory activity against trehalase in *R. solani* AG-1, without any significant effects on other glycohydrolytic enzymes
Polyoxins	*Streptomyces cacaoi* var. *asoensis*	WP, EC, SG, and paste	Polyoxorim (Endorse, Polyoxin Z and Stopit)	Plant pathogenic fungi, viz., *Sphaerotheca* spp. and other powdery mildews, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Corynespora melonis*, *Cochliobolus miyabeanus*, *Alternaria alternata* and other species in vines, apples, pears, vegetables, and ornamentals. Rice sheath blight (*R. solani*), apple, pear canker, and *Helminthosporium* in rice	It inhibits cell wall biosynthesis and causes abnormal germ tube swelling of spores and hyphal tips, rendering fungus nonpathogenic
			Polyoxin AL and Z, Polybelin		
Mildiomycin	*Streptoverticillium rimofaciens*	WP	Mildiomycin	Powdery mildews (*Erysiphe* spp., *Uncinula necator*, *Podosphaera* spp., and *Sphaerotheca* spp.) in ornamentals	It inhibits protein biosynthesis in fungi by blocking peptidyl transferase
Natamycin	*Streptomyces natalensis* and *Streptomyces chattanoogensis*	WP	Delvolan	Basal rots on daffodils and ornamentals caused by *Fusarium oxysporum*	Precise mode of action is not known
Actinovate	*Streptomyces lydicus* WYEC 108	WP	Actinovate	Soilborne diseases, viz., *Pythium*, *Fusarium*, *Phytophthora*, *Rhizoctonia*, and *Verticillium*. Foliar diseases like powdery and downy mildew, *Botrytis* and *Alternaria*, *Postia*, *Geotrichum*, and *Sclerotinia*	It colonizes the pathogen and grow around the structure of the plants and also forms synergistic relationship with plant by secreting beneficial and protective by-products

Mycostop	*Streptomyces* K61	WP	Mycostop	Damping-off caused by *Alternaria* and *R. solani* and *Fusarium*, *Phytophthora*, and *Pythium* wilt and root diseases	It deprives pathogenic fungi of space and nourishment by colonizing plant roots and also acts as a hyperparasite, disrupting cell walls of pathogens. It kills the pathogen by producing metabolites
Bio-insecticides					
Abamectin/avermectins (mixture of avermectin B_{1a} and avermectin B_{1b})	*Streptomyces avermitilis*	EC	Agri-Meck Avid, Clinch, Dynamec, Vertimec, Abacide, Abamex, Vapcomic, Vibamec, Agromec, Belpromec, Vamectin 1.8 EC and Vivid	Mites, leaf miners, suckers, beetles, fire ants, and other insects in ornamentals, cotton, citrus, pome and nut fruit, vegetables (potatoes)	It acts as the γ-aminobutyric acid (GABA) receptor in the peripheral nervous system
Emamectin benzoate (synthesized from abamectin)	*S. avermitilis*	EC and WG	Proclaim , Affirm, Shot-One, Arise and Denim	Caterpillars (Lepidoptera) It is used in vegetables, maize (corn), tea, cotton, peanuts, and soybeans	Emamectin affects the nervous system by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis
Spinosad	*Saccharopolyspora spinosa*	SC	Tracer, Spinoace, Entrust, Conserve, Entrust, Success, SpinTor, GF-120, Justice, Laser, Naturalyte	Caterpillars, leaf miners, thrips and foliage beetles. It is used in cotton, vegetables, fruits, turf, vines, and ornamentals	It interacts with GABA receptors and nicotinic acetylcholine receptors, eventually leading to the disruption of neuronal activity and consequent insect paralysis and death
Polynactins	*Streptomyces aureus*	EC	Mitecidin	Spider mites (*Tetranychus cinnabarinus*), two-spotted mite (*Tetranychus urticae*), European red mite (*Panonychus ulmi*) in orchard fruit trees	It causes leakage of basic cations (such as potassium ions) through the lipid layer of the membrane in the mitochondrion
Milbemycin (a mixture of milbemycin A_3 and milbemycin A_4)	*S. hygroscopicus* subsp. *aureolacrimosus*	EC and WP	Milbeknock, Koromite, and Matsuguard	Citrus red mites, Kanzawa spider mites, and leaf miners in citrus, tea, eggplant	This enhances GABA binding, resulting in an increased flow of chloride ions into the cell, with consequent hyperpolarization and elimination of signal transduction, resulting in an inhibition of neurotransmission

Modified form Copping and Menn (2000), Copping and Duke (2007), Saxena (2014)
DP dispersible powder, *EC* emulsifiable concentrate, *WP* wettable powder, *GR* granule, *SP* water-soluble powder, *SL* soluble concentrate, *DS* powder for dry seed treatment, *SG* water-soluble granules, *WG* water-dispersible granules

7.3 Biocontrol of Soilborne Plant Diseases

Biocontrol of plant diseases is though slow in action, it can be long lasting and harmless to living beings. Weller (1988) demonstrated that microorganisms that colonize the rhizosphere are ideal for the use of biological control agents against soilborne diseases. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and secondary spread on the root (Getha and Vikineswar 2002). Table 7.2 illustrates a list of actinomycetes documented to control soilborne diseases.

Kunoh (2002) produced disease-resistant tissue-cultured seedlings of *Rhododendron* using endophytic *Streptomyces* R5 against soilborne pathogens such as *Phytophthora*, *Pythium*, and *Rhizoctonia* and airborne pathogens such as *Colletotrichum* and *Corynespora*. This strain found to be insensitive to some of the fungicides and can be exploited for integrated pest management (IPM) programs. The most useful commercial products developed from *Streptomyces* strains are Actinovate (Natural Industries Inc.) and Mycostop (Verdera Oy). Actinovate is prepared from the spores of *S. lydicus* WYEC108, whereas Mycostop is prepared from spores of *S. griseoviridis* strain K61 and was isolated from the natural environmental soil of England and Finland, respectively (Crawford et al. 1993; Lahdenperä 1987). Actinovate is widely used for the control of soilborne diseases, viz., *Pythium*, *Fusarium*, *Phytophthora*, *Rhizoctonia*, *and Verticillium*, and Mycostop for damping-off caused by *Alternaria* and *R. solani*, *Fusarium*, *Phytophthora*, and *Pythium* wilt and root diseases.

Mohandas et al. (2013) reported five actinomycete strains, viz., *S. fradiae*, *S. avermitilis*, *S. cinnamonensis*, *S. canus*, and *Leifsonia poae* from *Glomus mosseae* spores in the rhizosphere of guava orchards possessing strong antifungal activity against *F. oxysporum* and *A. solani* and also promoted plant growth. *S. avermitilis* produced higher quantity of growth hormone, whereas *S. cinnamonensis* and *L. poae* exhibited highest activity of phosphate solubilization, siderophore production, and chitin-degrading activity. These mycorrhizae associated actinomycetes as bio-inoculant have initiated the possibilities of developing commercial formulation for growth promotion and disease control of various crops.

Costa et al. (2013) isolated 40 strains of *Streptomyces* from healthy maize plants in which isolate 16R3B was able to reduce 71 % damping-off incidence in cucumber (*Cucumis sativus*). Application of potential native strain *Streptomyces plicatus*, isolated from the soil, with horse dung compost exhibited inhibition against *Phytophthora infestans* and *Sclerotium rolfsii* (Khushboo et al. 2014).

7.3.1 Biocontrol of Plant Diseases by Production of Secondary Metabolites

Streptomyces spp. produce secondary metabolites, antibiotics, and lytic enzymes, which have been used extensively as potential biological control agents against fungal phytopathogens such as *P. ultimum* (Crawford et al. 1993), *F. oxysporum* (El-Shanshoury et al. 1996), *S. homeocarpa* (Trejo-Estrada et al. 1998a), and *Phytophthora fragariae* (Valois et al. 1996). The production of most antibiotics is species specific, and these secondary metabolites are important for *Streptomyces* species in order to compete with the other microorganisms that come in contact, even within same genre (Rothrock and Gottlieb 1984; Hwang et al. 1994; Raatikainen et al. 1994; Procopio et al. 2012). *S. violaceusniger* YCED9 exhibits biocontrol activity against a variety of fungal pathogens by producing antifungal antibiotics, viz., nigericin, geldanamycin, and a complex of polyenes including guanidylfungin A (Trejo-Estrada et al. 1998b) and also by fungal cell wall-hydrolyzing enzymes, chitinase and β-1,3-glucanase, and lignocellulolytic enzymes

Table 7.2 Various isolates of actinomycetes used for the control of plant diseases

Actinomycetes	Name of isolate/strain	Target pathogen/disease	References
Streptomyces sp.	CACIS-1.16CA	*Curvularia* sp., *Aspergillus niger*, *Helminthosporium* sp., *Fusarium* sp. *Alternaria* sp., *Phytophthora capsici*, *Colletotrichum* sp., and *Rhizoctonia* sp.	Zahaed (2014)
S. griseus	–	*Fusarium* wilt in tomato	Anitha and Rabeeth (2009)
Streptomyces sp.	–	Silver scurf of potato (*Helminthosporium solani*)	Elson (1997)
Streptomyces rochei	–	Pepper root rot (*P. capsici*)	Ezziyyani et al. (2007)
Streptomyces sp.	–	Maize seed fungi *Aspergillus* spp.	Bressan (2003)
S. lydicus	WYEC108	Foliar and root fungal diseases	Crawford et al. (1993) and Lahdenpera (1987)
Streptomyces griseoviridis	K61	Root rot and wilt pathogenic fungi	
Streptomyces sp.	YCED9 and WYEC108	Lettuce damping-off (*Pythium ultimum*) *Sclerotinia homeocarpa*, *R. solani*	Crawford et al. (1993) and Crawford (1996)
S. lydicus	WYEC108	Seed and root rot (*P. ultimum*)	Yuan and Crawford (1995)
Streptomyces violaceusniger	g10	Banana wilt (*F. oxysporum* f. sp. *cubense*)	Getha et al. (2005)
S. violaceusniger	YCED9	*R. solani* and crown-foliar disease of turfgrass (*S. homeocarpa*)	Trejo-Estrada et al. (1998a, b)
Streptomyces sp.	–	Cucurbit anthracnose (*C. orbiculare*)	Kim and Chung (2004)
Streptomyces sp.	A 1022	Anthracnose of pepper and cherry tomato (*Colletotrichum gloeosporioides*)	Kim et al. (2014)
Streptomyces halstedii	K122	*Aspergillus fumigatus*, *Mucor hiemalis*, *Penicillium roqueforti*, and *Paecilomyces variotii*	Frandberg et al. (2000)
S. cacaoi	182-2	Brown spot of tobacco (*A. alternata*)	Gao et al. (2012)
Streptomyces sp.	MT 17	Wood-rotting fungi	Nagpure et al. (2014)
Streptomyces lavendulae	HHFA1	Onion bacterial rot (*Erwinia carotovora* subsp. *carotovora* and *Burkholderia cepacia*)	Abdallah et al. (2013)
Streptomyces coelicolor	HHFA2		
Streptomyces	S01, S02, S03, S04, S05, S06, S07, S08, S09, S10, S11, S12, S13, S14, and S15	*Rhizopus nigricans*, *A. niger*, *F. oxysporum*, *Helminthosporium gramineum*, and *Spodoptera littoralis*	Osman et al. (2007)
Streptomyces spp.	5406	Cotton soilborne plant pathogens	Valois et al. (1996)
S. griseoviridis and *S. lydicus*	K61 and WYEC108	Root rot and wilt (*Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., and *Phytophthora* spp.)	Mahadevan and Crawford (1997)
S. griseus	H7602	*P. capsici*	Nguyen et al. (2015)
Streptomyces albidoflavus	–	*A. solani*, *A. alternate*, *Colletotrichum gloeosporioides*, *F. oxysporum*, *F. solani*, *R. solani*, and *Botrytis cinerea*	Haggag et al. (2014)

(Crawford et al. 1993; Chamberlain 1997). Singh et al. (1999) reported the control of *Fusarium* wilt of cucumber caused by *F. oxysporum* f. sp. *cucumerinum* and by *Paenibacillus* sp. and *Streptomyces* sp. They observed higher degree of protection in a zeolite-based, chitosan-amended formulation. Cheah et al. (2000) tested the effectiveness of *Trichoderma* and *Streptomyces* spp. in suppressing clubroot of brassicas (*Plasmodiophora brassicae*) in glasshouse and field conditions. Lei et al. (2013) isolated about 712 actinomycetes from the rhizosphere soil and found that *Streptomyces cyaneofuscatus* ZY-153, *Streptomyces kanamyceticus* B-49, *S. rochei* X-4, and *Streptomyces flavotricini* Z-13 were effective in vitro for the biocontrol of cotton wilt (*Verticillium dahliae*). Under greenhouse conditions, they conferred biocontrol efficacy of 18.7–65.8 % which might be due to production of cell wall-degrading enzymes. They also produced siderophores and IAA in vitro, and X-4 is found to significantly increase the cotton growth in greenhouse and field studies. Bhai (2014) reported the bio-efficacy of actinomycetes against black pepper and ginger pathogens, *Phytophthora*, *F. oxysporum*, *C. gloeosporioides*, *S. rolfsii*, *Pythium myriotylum*, and *Ralstonia solanacearum*. Gopalakrishnan et al. (2015) reported that CAI-17, CAI-68, and CAI-78 strains of *Streptomyces* sp. were found effective for the control of charcoal rot disease of sorghum and enhance plant growth and crop productivity.

Searching of novel antimicrobial secondary metabolites from marine actinomycetes is gaining momentum in recent years (Lange and Lopez 1996; Ramesh and Mathivanan 2009; Prabavathy et al. 2009). About 137 different isolates of marine actinomycetes exhibited antibacterial activity against human bacterial pathogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus* spp., and plant pathogenic fungi *R. solani* and *F. oxysporum* (Krishnaraj and Mathivanan 2011). The metabolites produced by various actinomycetes include a microcline antibiotic brasilinolide A from *Nocardia brasiliensis* active against *A. niger*, polyene antibiotic from *Streptomyces* sp. active against *B. cinerea*, oligomycin A from *Streptomyces libani* active against pathogenic fungi, and isochainin from an actinomycete strain Ap1 inhibitory toward *F. oxysporum* f. sp. *albedenis* and *V. dahliae* (Sharma 2014; Saxena 2014).

Various researchers reviewed the literature on the biological control of soilborne fungal plant pathogens and plant growth promotion by actinomycetes, especially *Streptomyces* spp. (Lechevalier 1988; Doumbou et al. 2001; El-Tarabily and Sivasithamparam 2006). However, El-Tarabily and Sivasithamparam (2006) isolated a variety of non-streptomycete actinomycetes (NSA) by selective methods, and these endophytic strains have shown potential to suppress soilborne fungal pathogens by antibiosis, hyperparasitism, and production of cell wall-degrading enzymes and promote plant growth. These NSA have shown their rhizosphere competence and adaptation for an endophytic life in root cortices.

7.3.2 Biocontrol of Plant Diseases in Combination with Organic Fertilizer and Growth Promoter

Enhancement of plant growth by the antagonists is helpful for the host plant to produce compensatory roots to mask the impact of root diseases (Cross 1982; Williams and Wellington 1982; Williams et al. 1984). Actinomycetes are well known for the control of common scab of potato. Wild oat (*Avena strigosa*, bristle oat), when used as green manure, reduced the severity of potato common scab indicating that wild oat cultivation increases the populations of microorganisms (Liu et al. 1995; Sakuma et al. 2002; Konagai et al. 2005; Hiltunen et al. 2009). Kobayashi et al. (2012) isolated fungi, actinomycetes, and bacteria from soil and plant samples of potato field in which wild oat was pre-cultivated and assessed them for their suppressive effect on the severity of potato scab (*Streptomyces turgidiscabies*). One of the isolates, WoRs-501, was found most effective in inhibiting in vitro mycelial growth of *S. turgidiscabies* up to 78–94 %. Besides, this application of wild oat as a green manure reduces brown stem rot of adzuki bean (Kondo 2001), *Verticillium* wilt of

tomato (Konagai et al. 2005), and *Verticillium* black spot of Japanese radish (Komatsu et al. 2003). The wild oat application changed the indigenous soil microflora, which showed synergistic effect with antagonistic microbes in disease control (Sakuma et al. 2002; Konagai et al. 2005). Suppressive strains of *Streptomyces* such as *Streptomyces melanosporofaciens* EF-76 (Beauséjour et al. 2003), *S. griseoviridis* K61 (Hiltunen et al. 2009), *Streptomyces diastatochromogenes* PonSSII, and nonpathogenic *Streptomyces scabies* PonR (Liu et al. 1995) have been evaluated for control of potato scab (*S. scabies* and *S. turgidiscabies*). In addition to wild oat, common buckwheat (*Fagopyrum esculentum*) and canola (*Brassica napus*) crops have also been suggested for the antibiotic activity of antagonistic *Streptomycetes* against *S. scabies* (Wiggins and Kinkel 2005). Hence, it is suggested that the disease-suppressive soils can be brought to cultivation by application of a green manure (Kondo 2001; Komatsu et al. 2003; Shiga and Suzuki 2005).

Liu et al. (2013) reported that combination of either of the two biocontrol agents, *S. rochei* (L-9) and *B. brevis* (L-25), together with organic fertilizers is effective in the control of tobacco bacterial wilt by affecting soil microbial structure. The nutrient carrier like crop residues, farmyard manure (FYM), and composts provides nutrients to the microbes, thereby increasing the antagonist's viability and making them more competitive in rhizosphere soil and on plant roots (Boehm and Hoitink 1992; Boehm et al. 1993). The application of bioorganic fertilizers to control soilborne diseases in tomato (Wei et al. 2011), banana (Zhang et al. 2011), watermelon (Ling et al. 2010), sweet melon (Zhao et al. 2011), cucumber (Zhang et al. 2008; Yang et al. 2011), cotton (Lang et al. 2012), and tobacco (Ren et al. 2012) is becoming more popular in China.

7.4 Biocontrol of Foliar Diseases

The potential use of actinomycetes for the management of soil and plant health has been well demonstrated. Smith (1957) isolated actinomycetes (*Micromonospora* sp.) from apparently healthy tomato which showed a strong inhibitory effect to *F. oxysporum* f. sp. *lycopersici*. The management of foliar diseases with an effective endophytic was first reported by Shimizu et al. (2001). Among the ten actinomycete strains from *Rhododendron* plants, *Streptomyces galbus* MBR-5 showed significant antagonistic activity against *Phytophthora cinnamomi* and *Pestalotiopsis sydowiana* and also aided protection for tissue-cultured seedlings against *P. sydowiana*. Similarly, Meguro et al. (2004) reported management of *P. sydowiana* in tissue-cultured seedlings of mountain laurel with *Streptomyces padanus* AOK-30. Shimizu et al. (2009) isolated 43 endophytic actinomycete strains (MBCu series) from cucumber (*Cucumis sativus*) and 135 (MBPu series) from pumpkin (*Cucurbita moschata*) for the control of cucumber anthracnose caused by *Colletotrichum orbiculare*. Six strains (MBCu-32, MBCu-36, MBCu-42, MBCu-45, MBCu-56, and MBPu-75) significantly reduced the number and size of the lesions on cotyledons, in which strain MBCu-56 was the best candidate.

Ara et al. (2012) identified four potential strains among the 80 isolated antagonist against brown rot of mango (*Pestalotiopsis mangiferae*) with the disease inhibition rate of 70–89 %. The *Streptomyces aureofaciens* CMUAc130 strain isolated from root tissue of *Zingiber officinale* was antagonistic to *Colletotrichum musae* and *F. oxysporum* (Taechowisan et al. 2005). *S. aureofaciens* application protects the mango against postharvest anthracnose caused by *Colletotrichum gloeosporioides* (Haggag and Abdall 2011).

7.5 Actinomycetes Vis-à-vis Pesticides

During the application of agrochemicals in the soil to combat soilborne diseases, they alter the natural population, development, and function of different actinomycetes community, thereby enhancing their efficiency. In general, the degree of tolerance of actinomycetes to pesticides is the application of fungicides>herbicides >insecticides>bactericides. The synergistic

effect of actinomycetes with pesticides has been reviewed by various researchers (Agnihotri 1973; Das and Mukherje 2000; Shetty and Magu 2000; Pampullah et al. 2007; Madakka et al. 2011). The population of actinomycetes was significantly enhanced in the soil treated with profenofos, deltamethrin, thiram, and difenoconazole and combinations of profenofos + cypermethrin and deltamethrin + endosulfan (Madakka et al. 2011), dithiocarbamate metham (vapam) (Kreutzer 1963), aretan (Hofer 1958), benomyl (Fassen 1974; Hofer et al. 1971), and metaxyl (Shetty and Magu 2000). The population of actinomycetes communities also remained unchanged in filed application levels of pentachloronitrobenzene, dithane M-45, bavistin, topsin, emisan, dexon, nylon, nabam, and vapam (Agnihotri 1973; Balasubramanian et al. 1973; Corden and Young 1960; Ko and Fraley 1969; Thopate et al. 1990). Singh and Singh (2005) reported that application of diazinon to groundnut seed and soil treatments with imidacloprid and lindane increased populations of *Azotobacter* and fungi. Das and Mukherje (2000) reported that application of insecticides β-hexachlorocyclohexane, phorate, carbofuran, and fenvalerate significantly increased the population of bacteria, actinomycetes, and fungi in soil. The proportions of *Streptomyces* were highly increased due to the incorporation of insecticides, while those of *Nocardia* and *Micromonospora* were reduced.

Pugoshetty and Rangaswamy (1969) showed that pretreatment of cotton seedlings with Agrosan GN reduced the actinomycetes counts in the initial stage of plant growth but not in later stages. Similar observations were also recorded for captan (Agnihotri 1971), isoproturon (Nowak et al. 2004; Pampullah et al. 2007), ceresin M and dithane (Thompson 1967), and herbicide, butachlor (Min et al. 2001).

7.6 Insect Pest Management with Actinomycetes

In recent years, biopesticides have been gaining increased attention among those concerned with developing environment-friendly and safe integrated crop management, with compatible approaches and tactics for pest management (Copping and Menn 2000; Leonard and Julius 2000; Rimando and Duke 2006; Bale et al. 2008). Recently, microbial insecticides have attracted considerable attention because they are more specific, have low relative cost, and are more eco-friendly (Xie 1998; Castillo et al. 2000). Among the biological control agents derived from different microbes, actinobacteria especially *Streptomyces* spp. are one of the most important microbial resources which can provide potential new bioactive compounds for use as insect control agents (Oka et al. 2000). Several metabolites from genus *Streptomyces*, such as avermectin, emamectin, polynactins, milbemycin, and spinosad, have been established as potential protective agents against a variety of insect pests (Copping and Menn 2000). Reports on actinomycetes for the biological control against insects including *Spodoptera littoralis* (Bream et al. 2001), *Musca domestica* (Hussain et al. 2002), *Culex quinquefasciatus* (Sundarapandian et al. 2002), *Drosophila melanogaster* (Gadelhak et al. 2005), *Helicoverpa armigera* (Osman et al. 2007), *Anopheles* (Dhanasekaran et al. 2010), and *Culex pipiens* (El-khawaga and Megahed 2012) are available. The importance of actinomycetes and their secondary metabolites with an emphasis on field pests is reviewed in the below section.

7.6.1 Avermectins

The avermectins, a group of macrocyclic lactones, were isolated from *S. avermitilis* and act as agonists for GABA-gate chloride channels (Albrecht and Sherman 1987; Jansson and Dybas 1998; Bloomquist 2001). Crude fermentation product of *S. avermitilis* yielded a complex of eight closely related avermectins homologues in which avermectins B_1 (a and b) were the major components. Avermectin B_1 (abamectin) was developed with the combination of avermectin B_{1a} (80 %) and avermectin B_{1b} (20 %) (Fisher and Mrozik 1989). It is a broad-spectrum pesticide and is highly toxic to many arthropods, including spider mites, leaf miners, ants,

cockroaches, and some lepidopteran pest species (Dybas 1989; Lasota and Dybas 1991).

7.6.1.1 Abamectin

Abamectin is considered as a selective pesticide. It has several advantageous traits which include safe to human beings and environment and low toxicity to nontarget pests. It is used at very low doses and degrades rapidly when exposed to light (Wislocki et al. 1989). Rapid penetration of abamectin into leaves within few hours of application aids for its rapid action (Dybas 1989). The use of mineral oil or surfactants in combination with abamectin extends its foliar residual toxicity, especially to phytophagous mites, under greenhouse and field conditions (Wright et al. 1985; Dybas 1989). Abamectin is more harmful to the pests than to their parasitoids (Peinkowski and Mehring 1983; Chandler 1985). Trumble (1985) found that abamectin gave satisfactory control of *Liriomyza trifolii* and it had only minimal effect on adult and larval stages of six different parasitoid species that attack this leaf miner. Similarly, complete selectivity of abamectin to early stages of *Trichogramma demoraesi* in *Anagasta kuehniella* eggs has been reported (de Souza et al. 1987). Abamectin (Avid ™), used against mites and leaf miners, was found to spare some of the major parasites of leaf miners (Parella 1987) and some predacious mites (Hoy and Conley 1987). Fein et al. (1994) observed that after abamectin treatment, the surviving *Encarsia formosa* parasitoids were sufficient to improve control of the greenhouse whitefly, *Trialeurodes vaporariorum*, by host feeding and parasitism. Abamectin resulted in 15.6 % mortality in *Coccinella septempunctata* as compared to tebufenozide (19.6 %), cartap (16.7 %), and lambda-cyhalothrin (41.8 %) (Lui and Sengonca 2002). Abamectin did not have any adverse effect on the egg viability and larval development of *Chrysoperla externa* (Bueno and Freitas 2004). In potato fields in Java (Indonesia), applications of abamectin led to a reduction in leaf miner infestations but did not have any adverse effects on predators and parasitoids (Hidrayani et al. 2005). Abamectin seed treatment on cucumber reduced the penetration of *Meloidogyne incognita* juveniles within the roots (Becker et al. 2006). Abamectin (vertemic 1.8 % EC) as soil application proved its nematicidal activity by suppressing the root-knot nematode *Meloidogyne* spp. on different vegetables crops (Hamida et al. 2006; Khalil 2009). Birah et al. (2008) reported the efficiency of abamectin (8.1, 10.8, 14.5, 18.5, 22.5 g ai/ha) in suppressing bollworm incidence and increasing the yield of cotton. Abamectin expressed high efficacy in control of *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium confusum*, and *Tribolium castaneum* (Kavallieratos et al. 2009). In Bulgaria, the post-bloom applications of abamectin provide a significant control of summer populations of pear psylla, *Cacopsylla pyri*, and recommended for the integrated pest management programs in pear production (Arnaudov and Kutinkova 2009). Soudamini et al. (2010) reported that initial residues of abamectin persisted for 3 days on brinjal and reached below the quantifiable limit of 0.01 mg/kg on the fifth day. Andrić et al. (2011) reported high progeny reduction (≥95 %) of rice weevils in wheat treated with 1 and 2 mg/kg of abamectin. Abamectin and spinosad proved to be the efficient insecticides for the control of all developmental and adult stages of leaf miner fly, *Liriomyza sativae* in Iran (Saberfar et al. 2012). Santosa (2014) reported that different concentrations of abamectin had no resurgency effect against the first, second, and third generation of brownhopper, *Nilaparvata lugens*.

7.6.1.2 Emamectin Benzoate

It is a synthetic version of abamectin having broader insecticidal activity than abamectin. It comprises of two homologues emamectin B_{1a} and emamectin B_{1b} in a ratio 90:1. It stimulates the release of GABA and results in chloride ion flux in neuronal cells, leading to loss of cell function and the disruption of nerve impulses and irreversible paralysis (Jansson et al. 1997). Emamectin benzoate exhibits translaminar activity which helps in maintaining reservoir of the active substance, resulting in long-term residual

pest control through larval feeding (Ishaaya et al. 2002). It has low toxic effects to beneficial organisms due to fast degradation rates (Ishaaya et al. 2002; López et al. 2011). Emamectin benzoate is particularly effective against Lepidoptera as the LC_{90} values against a variety of lepidopterous pests range between 0.002 and 0.89 μg/ml (Dybas et al. 1989; Cox et al. 1995; Ishaaya et al. 2002), and this is followed by mites, leaf miners, and thrips (Dunbar et al. 1998). It is recommended for use in vegetables, corn, tea, cotton, peanuts, and soybeans (Takai et al. 2004). Most formulations of emamectin were found to be effective in controlling populations of lepidopterous pests on vegetables, including *Plutella xylostella* on cabbage, *Spodoptera eridania* on pepper, and *Trichoplusia ni* and *Spodoptera exigua* on celery (Jansson et al. 1997). Emamectin was 1720-, 884-, and 268-fold more potent to *Spodoptera eridania* than methomyl, thiodicarb, and fenvalerate, respectively, and 105- and 43-fold more toxic to *Helicoverpa zea*, and *H. virescens*, larvae than abamectin (Dybas and Babu 1988).

Sublethal concentrations of emamectin benzoate significantly reduced percent larval hatch of eggs and mating frequency in *H. armigera* (Vojoudi et al. 2011), *H. zea*, and larval survival to the pupal stage in female corn earworm (López et al. 2010). In a field experiment for the management of spotted pod borer, *Maruca vitrata*, in yardlong bean (*Vigna unguiculata* L. subsp. *sesquipedalis* Verdc.), a cultivated subspecies of cowpea, emamectin benzoate gave the highest net yield (18.5 mt/ha) of green pods (Regmi et al. 2014). It showed superior results in terms of shoot infestation in controlling brinjal shoot and fruit borer (Anil and Sharma 2010) and *Spodoptera litura* in castor (Shaila and Rao 2013). Anwar et al. (2015) recommended the use of emamectin benzoate for effective control of brinjal fruit borer, *Leucinodes orbonalis*, at Pakistan as it found to lower infestation (40.1 %) as compared to control plots (58.2 %). It is also effective against in controlling *Phenacoccus solenopsis* (Dhawan et al. 2008) and *Bactrocera zonata* (Badr El-Sabah et al. 2009).

Emamectin benzoate has a lower mammalian toxicity than abamectin; however it is active against beneficial insects and as such should not be sprayed during flowering (Fisher 1993). But Lasota and Dybas (1991) found that it is comparatively safer to most beneficial arthropods, parasitoids, and predators especially when exposure occurs beyond 1 day after application. Similarly, on broccoli emamectin hydrochloride displayed minimal adverse effects against hymenopterous parasitoids *Pteromalus puparum* and *Cotesia orobenae* (Kok et al. 1996). It is also found to be relatively safe to the adults of the rove beetle *Paederus alfieri*, green lacewing *Chrysoperla carnea*, and the two ladybird beetles *Scymnus* spp. and *Coccinella undecimpunctata* in cotton ecosystem at Egypt (Sechser et al. 2003). White et al. (1997) reported that emamectin benzoate provides ecological selectivity to a wide range of beneficial arthropods and is compatible with IPM programs.

7.6.2 Spinosyns

The spinosyns are a distinctive family of fermentation-derived insecticides having potent activity against a number of insects and at the same time having lower environmental effect. The discovery of spinosyns was associated with isolation of novel soil actinomycete, *S. spinosa* (Mertz and Yao 1990). Natural mixture of fermentation of *S. spinosa* contains spinosyn A as the major component and spinosyn D as the minor component (Kirst et al. 1992). Its mechanism of action is considered unique in comparison with other insecticides as it interacts with GABA receptors and nicotinic acetylcholine receptors, eventually leading to the disruption of neuronal activity and consequent insect paralysis and death (Orr et al. 2009).

7.6.2.1 Spinosad

Spinosad is the defined mixture of spinosyn A and spinosyn D, with former being the major component of the product. Spinosad is reported to be an effective pest control agent, particularly for control of Lepidoptera (Wanner et al. 2000;

Brickle et al. 2001) followed by Diptera, Thysanoptera, Coleoptera, and Orthoptera (Thompson and Sparks 2002). Globally, it has been applied to over 200 different crops to manage different insect pests. The commercial use of spinosad in conventional agriculture commenced with field applications of the Tracer® on cotton in 1997 against the caterpillars with resistance to pyrethroids or other broad-spectrum insecticides at that time (Bret et al. 1997). It rapidly gained wide acceptance being highly effective against pests like *P. xylostella*, *Helicoverpa* sp., *Heliothis* sp., *Pieris rapae*, *Hellula hydralis*, *Chrysodeixis* sp., *Crocidolomia pavonana*, and *Thrips tabaci* (Kharboutli et al. 1999; Johnson et al. 2000; Downard 2001; Wang et al. 2009). Several commercial products contain spinosad as their active ingredient. These products include Tracer® 45 SC for the control of lepidopteran pests on many field crops, SpinTor® 2 SC and Success™ 2.5 SC for broad-spectrum insect control on a variety of crops, Entrust™ for insect control on organic crops and fruitfly and fire ant bait traps, and Conserve® SC for insect control on turf and ornamental plants (Racke 2007). A reduction of 88 % in the incidence of tomato fruit borer *H. armigera* has also been reported using spinosad 45 % SC at 60 g a.i. ha^{-1} (Ghosal et al. 2012). In field studies, application of spinosad significantly suppressed *H. armigera* population and damage to locules, squares, bolls, and seed cotton (Nogia and Meghwal 2013).

Spinosad showed high efficacy in controlling all instar larvae of *Tuta absoluta* and *H. armigera* infesting tomato plants, giving an average mortality of 66.5 and 85.6 % respectively (Hanafy and El-Sayed 2013). Pineda et al. (2006) observed that spinosad exhibited 100 % larvicidal activity against *S. littoralis*. A comparison of field collected and susceptible strain of *S. littoralis* revealed that field strain was approximately 4.4-fold less sensitive to spinosad, suggesting potential importance of insecticide under filed conditions (Aydin and Gurkan 2006).

In addition to toxicity against lepidopteran pests, spinosad has also reported to exert significant effect on stored grain pests, beetles, and mites. The efficacy of spinosad against seven major stored grain insects, red flour beetle *T. castaneum*, rusty grain beetle *Cryptolestes ferrugineus*, lesser grain borer *R. dominica*, saw-toothed grain beetle *Oryzaephilus surinamensis*, rice weevil *S. oryzae*, maize weevil *Sitophilus zeamais*, and Indian meal moth *Plodia interpunctella* on corn in the laboratory at 1 mg/kg corn, revealed that the insecticide was very effective against six stored grain insects excluding *T. castaneum* adults, wherein it effectively suppressed progeny production and kernel damage (Huang and Subramanyam 2007). The insecticide has shown oral toxicity to the adults of crucifer flea beetle, *Phyllotreta cruciferae*, when beetles were exposed to treated canola cotyledons for 120 h (Elliott et al. 2007). Van Leeuwen et al. (2005) recorded that the residual toxicity of spinosad to female *T. urticae* was comparable to the toxicity level following application of acaricides like dicofol, bromopropylate, or fenbutatin oxide on tomatoes. Villanueva and Walgenbach (2006) demonstrated that spinosad affects larvae and adults of tetranychids, *T. urticae* and *P. ulmi*.

Spinosad degrades in the environment primarily through photodegradation and microbial degradation and converts into its natural compounds of carbon, hydrogen, oxygen, and nitrogen (Eger and Lindenberg 1998). In addition to quick degradation, low toxicity to mammals and birds and efficacy at lower doses make spinosad a choice for IPM programs in vegetables and ornamentals (Crouse and Sparks 1998; Pineda et al. 2004). Spinosad has been reported to be selective and relatively less toxic to a range of beneficial insects and natural enemies such as honey bee, *Apis mellifera*; whitefly parasitoid, *E. formosa*; minute pirate bug, *Orius insidiosus*; lady beetle, *Hippodamia convergens*; lacewings, *Chrysoperla rufilabris* and *C. carnea*; big-eyed bug, *Geocoris punctipes*; and predaceous mite, *Phytoseiulus persimilis* (Schoonover and Larson 1995; Williams et al. 2003). Laboratory and field studies demonstrating ovicidal and ova-larvicidal action of spinosad on freshly laid eggs of *Heliothis virescens* and *H. zea* showed natural parasitism of the eggs by *Trichogramma*

sp. comparable to that of untreated control (Peterson et al. 1996). However, there are a number of studies (Pietrantonio and Benedict 1999; Hill and Foster 2000; Tillman and Mulrooney 2000; Nowack et al. 2001) on moderately harmful or harmful effects of spinosad formulations on populations of hymenopteran parasitoids have been reported which necessitates the careful evaluation of this novel insecticide.

7.6.3 Milbemycin

Milbemycin (milbemectin) is an insecticidal and acaricidal product isolated from the fermentation broth of *S. hygroscopicus* subsp. *aureolacrimosus* (Takiguchi et al. 1980; Barrett et al. 1985). It is a mixture of milbemycin A3 and milbemycin A4 in the ratio 3:7. Milbemycin acts through stimulation of the release of GABA and binding to the receptor sites of inhibitory motor neurons leading to hyperpolarization and inhibition of neurotransmission (Clark et al. 1995). It has plant systemic activity, though in limited proportion, but it exhibits translaminar movement. It has been reported to manage a wide range of spider mites such as *T. urticae*; *T. cinnabarinus*; Kanzawa spider mite, *Tetranychus kanzawai*; citrus red mite, *Panonychus citri*; and pink citrus rust mite, *Aculops pelekassi*, and is also recommended for control of leaf miners in citrus, tea, eggplant, and ornamental plants (Mishima 1983). The commercial formulations of milbemycin include Matsuguard®, Koromite®, Mesa®, Milbeknock™, and Ultiflora™. It is used at 5.6–28 g a.i. ha^{-1} for the control of mites. An increased effectiveness of the spray mixture has been reported by the addition of paraffinic oils. In mammals, it is moderately toxic through oral route but has much less dermal toxicity. It has low persistence in the environment and is reported to be relatively safe to nontarget organisms.

7.6.4 Polynactins

These are the secondary metabolites of actinomycetes, *S. aureus*, and are a mixture of tetranactin, trinactin, and dinactin (Ando et al. 1971). The polynactins have been reported to be very effective against spider mites under high moisture conditions. These have been utilized for the management of spider mites, like *T. cinnabarinus* and *T. urticae*, and European red mite *P. ulmi* in fruit trees. It causes leakage of potassium ions from the lipid layer of the mitochondrion membrane and exhibits insecticidal activity. The penetration or acceleration of this ion leakage is considered to be assisted by water as an essential component for the toxic effect (Ando et al. 1974). The commercial formulations are available in combination with other acaricides under the trade names of Mitecidin C® (tetranactin + chlorfenson), Mitecidin® (polynactin + fenobucarb), and Mitedown® (polynactin + fenbutatin oxide). The polynactins are considered to be relatively nontoxic to mammals and beneficial insects, though high toxicity to fish has been reported.

7.7 Miscellaneous

Actinomycetes produce a wide range of active metabolites, and their exploration can provide an overwhelming reservoir of potentially active compounds. These have been isolated from diverse habitats like shallow costal sediments to the deepest sediments of the Mariana Trench and thus regarded as omnipresent (Bernan et al. 2004). Mishra et al. (1987) reported 27 predominantly active actinomycete strains with insecticidal and nematicidal traits. The secondary metabolites of *Streptomyces* strains inhibited growth of test insects, such as *S. exigua*, *Dendrolimus punctatus*, *P. xylostella*, *Aphis glycines*, and *Culex pipiens* (Huamei et al. 2008). In another study, aminoglycosidic antibiotic produced from *Streptomyces bikiniensis* was also effective against second instar larvae of *S. littoralis* (El Khagwa et al. 2012). Arasu et al. (2013) identified a novel polyketide metabolite with antifeedant, larvicidal, and growth inhibitory properties on *H. armigera* and *S. litura* from a marine actinomycete *Streptomyces* sp. AP-123. Anwar et al. (2014) also reported that the metabolites

of three actinomycete isolates exhibit 100 % mortality against third instar larvae of *T. castaneum*. Vijayabharathi et al. (2014) evaluated 111 microbes isolated from various herbal vermicomposts and organically cultivated fields against second instar *H. armigera*, *S. litura*, and *Chilo partellus*. Among these, three *Streptomyces* isolates, SAI-25 (*S. griseoplanus*), CAI-155 (*S. bacillaris*), and BCA-698 (*S. albolongus*), showed potential entomopathogenic activity against these insects. Kaur et al. (2014) also reported the anti-insect potential of ethyl acetate extract of *Streptomyces hydrogenans* DH16, a soil isolate to *S. litura*. It proved toxic to the larvae at higher concentrations, whereas lower concentrations significantly reduced its reproductive potential.

7.8 Conclusion

Extensive application of synthetic pesticides in agriculture and public health has lead to numerous undesirable effects. The environmental contamination, adverse impact on beneficial arthropods, and the human health by these toxic chemicals have prompted the development of alternative approaches for controlling pest populations. In the last few decades, awareness of health consciousness lead to organically produced food stuffs. Many novel and unique products have been discovered from the research and development on biological or ecological control methods. Owing to their high specificity, these novel pesticides of microbial origin are superior in safety for beneficial insects, humans, and animals. Since these new chemicals are mostly contact and stomach poisons, they are reported to be highly efficient in the field, and growers have a wide range of alternatives in the form of old and new chemicals; the best strategy would be to use effective compounds as one of the components of pest management strategy. However, the concrete research on coherent use of these biological products is needed to further explore their ability to make them functional and future resilient. Any nonchemical strategy or reduced insecticides for managing crop pests and diseases with biorational approaches in mind could be a welcome approach.

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Endophytic Actinobacteria: Nitrogen Fixation, Phytohormone Production, and Antibiosis

8

K. Swarnalakshmi, M. Senthilkumar, and B. Ramakrishnan

Abstract

Rhizospheric and endophytic microbial population plays a key role in sustaining plant production under either nutrient-limited or sufficient conditions. Endophytic microorganisms that are internalized in plants offer a competitive advantage over rhizospheric microorganisms in the growing environments. Among various endophytic microorganisms, the Gram-positive actinobacteria have gained considerable interests due to their secondary metabolites production. Besides *Frankia*, a large number of cultivable actinobacteria such as *Streptomyces* spp., *Nocardiopsis*, *Actinoplanes* spp., *Micromonospora*, *Microbispora*, and *Streptosporangium* show endophytic lifestyles in diverse plant species. Recent advances in molecular tools show the existence of "yet to be uncultured but viable organisms" within this group. The colonization of endophytic actinobacteria depends on plant species, soil types, and varied environmental factors. Their metabolic capabilities even make them an important source of plant hormones, antibiotics, and other bioactive molecules that are used in agriculture and pharmaceutical industries. In the legume plants, the nodule inhabiting actinobacteria such as *Micromonospora*, *Streptomyces* sp., *Nocardia alba*, *Nonomuraea rubra*, and *Actinomadura glauciflava* have probiotic effects with *Rhizobium*. In the present chapter,

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the potential role of the plant-associated actinobacteria in sustainable agriculture and their endophytic lifestyles has been reviewed.

Keywords
Actinobacteria • Endophytes • Mode of entry • Cultivation techniques • Plant growth promotion • Biocontrol • Synergism

8.1 Introduction

Plants harbor many microorganisms in and on their surfaces. The microorganisms within the plant tissues include endophytes. The term "endophyte" is derived from the Greek words of "endon" (within) and "phyte" (plant). Initially, this term was applied only to fungi (Carroll 1988). The endophytic fungi include three classes of symbionts: mycorrhizal fungi, the Class 1 endophytes (clavicipitaceous fastidious endophytes) that infect cool season grasses (Read 1999; Schardl et al. 2004), and the Class 2 endophytes (fungal symbionts) which infect both monocot and eudicot plants (Petrini 1996; Rodriguez et al. 2005). Later, Kado (1992) defined endophytes as bacteria which cohabit within the living plant tissues, with no apparent effect on the hosts. According to Reinhold-Hurek and Hurek (1998), bacteria that are isolated from the surface-disinfected tissues, with their concurrent microscopic visualization within the plant tissues, are termed as "true endophytes." These endophytes can also re-infect the disinfected seedlings. But those isolates that are not subjected to microscopic validation are called as "putative endophytes." While the obligate endophytes are vertically transmitted and complete their life cycle strictly within the host, the facultative endophytes can survive outside the host.

Endophytes are ubiquitous, residing latently or actively colonizing the tissues of most plant species. According to Sturz et al. (2000), there are not a single plant species devoid of endophytes. These bacteria have been isolated from both monocotyledonous and dicotyledonous plants, ranging from woody tree species such as oak (Brooks et al. 1994) and pear (Whitesides and Spotts 1991) to herbaceous crop plants such as sugar beets (Jacobs et al. 1985) and maize (Fischer et al. 1992; McInroy and Kloepper 1995; Gutierrez-Zamora and Martinez-Romero 2001). In the vegetatively propagated plants such as potatoes, the parent material (e.g., tubers) can be a source of endophytic bacteria that subsequently colonize the developing roots and shoots via vascular tissues. Among various endophytic microorganisms, there is a growing interest on the endophytic actinobacteria as they are the primary source of bioactive compounds with biotechnological significance. A large number of actinobacteria have been reported from a diverse range of plant species. Some of these are known to stimulate plant growth, fix nitrogen, and induce resistance to plant pathogens. The current review focuses on the ecology of endophytic actinobacteria, their colonization, and their potential contributions to plant growth and health.

8.2 Ecology of Endophytic Actinobacteria

8.2.1 Endophytic Entry into Plants

The endophytic bacteria belonging to *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* are capable of entering plants and could establish mutualistic associations (Hallmann et al. 1997; Azevedo et al. 2000). Quadt-Hallmann et al. (1997) and Coombs et al. (2004) reported the site and spread of endophytic colonization in various plant tissues, their mode of entry, and mechanisms that control their

colonization behavior. Though the lack of penetrating bacterial appendages and thick epidermal plant cells usually acts as barriers, the endophytes' entry into the hosts is similar to those by pathogens. The plant pathogens gain their access via germinating seed radicle, stomata, lenticels, and lesions in the lateral roots created by mechanical injury and those actively growing undifferentiated meristem cells. Likewise, the pathogenic actinobacterial endophyte (*Streptomyces scabies*) of potato enters via lenticels, stomata, or wound caused by mechanical injury (Adams and Lapwood 1978). The beneficial actinobacterial endophytes can colonize plants passively. These bacteria are recruited out of a large pool of soil or rhizospheric species and are best adapted to live inside plants. Plants contain variable amounts of cellulose, hemicellulose, and pectin in their cell walls that offer several advantages to prevent or overcome the pathogenic infections. The microbial interaction initiates all the processes necessary to encounter the complex sugars at first. The cytochemical studies along with transmission electron microscopy show that the endogluconase activity is critical to the bacterial invasion. Pectin hydrolysis mediated by microbial pectinase can further lead to the colonization of endophytic bacteria. Seed application of bacterial strains showed that they could colonize the root surface, grooves between the epidermal cells, and intercellular spaces of roots and cortical parenchyma cells. The endophytic bacteria make their way through invagination of root hair cells or the junction between root hair cells and epidermis or by enzymatic hydrolysis of cell wall polysaccharides. They also move through epidermis by passive plant uptake and spread to various tissues via intercellular spaces or conducting cells. These endophytic bacteria generally colonize the intercellular spaces, and they have been isolated from all plant compartments such as roots, stems, leaves, fruits, tubers, ovules, and even inside legume nodules (Posada and Vega 2005). The actinobacterial endophytic entry into host plants by infection of seed is also reported. Truyens et al. (2015) reported that seeds of 13 out of 30 plant species were actively colonized by 25 different genera of actinobacteria. They suggested that many seedlings were colonized prior to germination and seedling development. The seed-borne endophytes inherited from parents to progeny are gaining prime importance (Selosse and Schardl 2007); the host plants select them to gain resistance against pathogens. Even within fruits, the actinobacterial endophytic populations may arise by entry through flowers and then they can proliferate to other aboveground parts (Compant et al. 2011). Although the endophytes can invade plants inter- and intracellularly, there is no evidence for endosymbiosis in the living plant cells as in the case of the legume symbiosis (James and Olivares 1998; Reinhold-Hurek and Hurek 1998). They can spread systemically into shoots without causing any symptom of plant damage/injury (Hurek et al. 1994). Thus, a competent endophyte does not colonize locally but spread systemically throughout the entire plant (Dong et al. 2003; Zakria et al. 2007). Similar to pathogens, endophytic bacteria also trigger hypersensitive reactions which in turn can induce systemic resistance against pathogens (Harish et al. 2008) and/or insect pests. The plant-associated, obligate endophytic actinobacteria are critical to plant fitness under any given environment. More importantly, the nature of the host interactions of these endophytes is somewhere between the pathogenic and the endosymbiotic lifestyles (Miche et al. 2006). The acquired heritable traits by plants due to the microbial interactions can be explained by the hologenome concept; the hologenome is a collective term to describe the microbial genomes associated with the plant genome. Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization.

8.2.2 Endophytic Colonization Within Plants

The population densities of endophytic bacteria are extremely variable depending on the bacterial species, host genotypes, host developmental

stages, and environmental conditions (Tan et al. 2003). For example, bacterial colony-forming units (CFU) recovered from the alfalfa xylem tissue varied from 6.0×10^3 to 4.3×10^4 per g (Gagne et al. 1987), 1×10^2 to 11×10^3 per g in the cotton xylem tissue (Misaghi and Donndelinger 1990), 3.3×10^3 to 7.0×10^5 per g in the sugar beet tissue (Jacobs et al. 1985), and 0 to 1.6×10^4 per g in the potato tubers (De Boer and Copeman 1974). The actinobacterial endophytic population in *Ocimum sanctum* is about 10.4×10^5 per g (Karthikeyan et al. 2008). The plant-associated endophytic actinobacteria impart antimicrobial activities in several medicinal plants (Passari et al. 2015). The roots and other belowground tissues tend to yield the highest numbers of CFU of bacteria compared with the aboveground tissues (Rosenblueth and Martinez-Romero 2004); this clearly indicates the upward path of bacteria from roots to stem during the plant development (Gagne et al. 1987). The endophytic actinobacteria were isolated from a large diversity of plants. Among various endophytes, actinobacteria comprise of 20 % including 102 genera. The predominant endophytic genera include members of genus *Streptomyces*, *Microbacterium*, *Mycobacterium*, *Arthrobacter*, and *Curtobacterium*. These are known to produce diverse antimicrobial compounds such as coronamycins, alnumycin, munumbicins, kakadumycins, and goadsporin. The species of *Streptomyces*, *Microbispora*, *Micromonospora*, and *Nacardiodes* were found to inhabit the tissues of healthy wheat plants (Coombs et al. 2004).

8.2.3 Host Specificity

Many plants constitute vast and diverse niches for the endophytic organisms. The major ecological niches of these bacteria include soil, root nodules, plant surfaces, and interior tissues. The community structure of endophytes is shaped largely by the host genotype, the plant organs as well as physiological status of the plants (Reiter et al. 2002; Sessitsch et al. 2002; Rasche et al. 2006), soil types (Berg and Smalla 2009), and microbial population and diversity (Conn and Franco 2004b). But, in the genetically related maize hybrids, the endophytic population was found to be similar to that of species and genera (Liu et al. 2013). In soybean, the presence of different endophytic species depended on the plant genotype, age, and tissue sampled and also on the season of isolation (Kuklinsky-Sobral et al. 2004). Several actinobacterial endophytes are reported to possess many beneficial traits such as nitrogen fixation (Huss-Danell 1997), antibiosis (Trejo-Estrada et al. 1998; Horinouchi 2007; Quecine et al. 2008), and plant growth promotion (Tsavkelova et al. 2006; El-Tarabily 2008; Lin and Xu 2013). The actinobacterial genus *Frankia* is able to fix nitrogen both under free-living and symbiotic conditions. It can form root nodules in angiosperm plant and improve the nitrogen economy of the plant per se. In fact, the N-fixing potential of actinorhizal plants is comparable with that of rhizobial symbiosis (Hibbs and Cromack 1990). Recent reports suggest that endophytic actinobacteria may promote plant growth by the plant disease suppression (Quecine et al. 2008; Shi et al. 2010; Misk and Franko 2011). Tokala et al. (2002) reported that the preferential colonization of *Streptomyces lydicus* in the root nodules of pea even altered the host plant physiology. Compared to the extensive reports available on the legume–rhizobia symbiosis, few reports are available on the relationships between plants and actinobacterial endophytes. The process of host–endophyte signaling and colonization and the mechanisms leading to mutual benefit are poorly characterized for several other endophytic bacteria. It is not known whether plants benefit more from an endophyte compared with a rhizospheric bacterium or the bacterium gains more advantages by becoming endophytic than remaining as a rhizospheric organism. Evidence remains difficult to gather as to which population of microorganisms, endophytic or rhizospheric, contributes more to the plant growth.

8.3 Methodologies Used in Actinobacterial Endophytic Study

8.3.1 Culture-Dependent Approach

The endophytic *Streptomyces* is the commonly occurring actinobacterium isolated from several plant species (Tokala et al. 2002; Coombs and Franco 2003; Taechowisan et al. 2003; Coombs et al. 2004; Tian et al. 2004; Cao et al. 2005). The isolation frequency of this actinobacterium was 95.3 % over other actinobacterial genera suggesting that the media used for isolation can be selective (Williams and Wellington 1982). For the isolation of endophytic bacteria, the media formulation should mimic the plant ecosystem. For instance, the isolation of endophytes from Kallar grass necessitates the malate-rich media, largely to imitate the malic acid content of the plant in situ (Reinhold et al. 1986). Similarly, the sucrose-rich medium (10 %) is used for the isolation of endophytic *Acetobacter diazotrophicus* from sugarcane stems since high amounts of sucrose get accumulated in these stems (Cavalcante and Dobereiner 1988). Besides these rich media with specific compounds, the nutrient-poor media such as humic acid, vitamin B agar; tap water, yeast extract agar; and yeast extract, casein hydrolysate agar media are the most effective for isolating several endophytic actinobacteria (Coombs et al. 2004).

Surface sterilization and subsequent homogenization of plant tissues release endophytic bacteria. However, bacteria that are closely attached to crevices and embedded in mucilage can escape chemical surface sterilization; they may be considered as endophytes. This can be overcome by comparing the microbial population obtained from the plant surface after surface sterilization with suitable control. The presence of higher numbers of bacteria in the homogenized material than in control indicates that the population is largely from the interior plant tissues (Reinhold et al. 1986).

The endophytic lifestyle of bacterial isolates should be validated for their endorhizospheric competence by the microscopic studies (Hurek et al. 1994). What is more important is the scanning electron microscopy having better resolving power (>1 nm) combined with immunogold labeling. This is an absolute requirement for visualizing the endophytes. Otherwise, the plant cell organelle might be mistaken as "endophytes" (Quadt-Hallmann and Kloepper 1996). The colonization of *Frankia* in the nodules of *Casuarina* was demonstrated convincingly using scanning electron microscopy (Berg and McDowell 1987). Bacteria in intact plant tissues can be examined by confocal laser scanning microscopy combined with immunological technique, specific gene probes, or tagging with reporter genes. Under unsterilized condition which is the norm for plants growing in the natural conditions, a precise in situ identification of plant endophytic bacteria is very important. Otherwise, several uncultivable endophytic bacteria may be overlooked in the uninoculated controls. Abmus et al. (1997) used the in situ labeling of fluorescence antibodies with high specificity and the species-specific rRNA-targeted oligonucleotide probes for visualizing the endophytes.

The microscopic visualization of endophytic bacteria can be carried out using reporter genes such as β-galactosidase of *Escherichia coli* (Silhavy and Beckwith 1985), β-glucuronidase of *E. coli* (Jefferson et al. 1987), and the green fluorescent protein (*gfp*) (Chalfie et al. 1994) under the control of a constitutively expressed promoter. However, diffusible product released after cleavage of chromogenic substrates such as X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) or X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide) may not show the spatial colonization of bacteria which further necessitates microscopic validation. *Streptomyces* sp. strain EN27 tagged with *egfp* (enhanced GFP) gene showed their endophytic colonization in embryo, endosperm, and emerging radicle of wheat at an early stage of

growth. The gene (*egfp*) expression of this endophytic actinobacteria was confirmed by the confocal microscopic visualization (Coombs et al. 2004).

8.3.2 Culture-Independent Approach

The culture-independent techniques depend more on the nucleic acid-based approaches that reveal the structure of the microbial community. The analyses include a selection of rRNA genes and the whole microbial genomes. The phylogenetic and functional diversity of microbial community can be studied using approaches either for partial community analysis or whole community analysis. The partial community analysis includes the polymerase chain reaction (PCR)-based methods where the PCR amplification of a product from the community of DNA/RNA reflects a mixture of gene signatures from a targeted group of organisms present in a sample. In addition to rDNA, other conserved genes such as recombinase A (*RecA*), gyrase beta subunit (*gyrB*), and RNA polymerase beta subunit (*rpoB*) have been used to study the microbial community structures (Ghebremedhin et al. 2008). The amplified products of targeted genes can be analyzed by various genetic fingerprinting techniques such as amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TTGE), single strand conformation polymorphism (SSCP), length heterogeneity PCR (LH-PCR), and ribosomal intergenic spacer analysis (RISA) to generate microbial community profile on the basis of either sequence or length polymorphism. These techniques demonstrate the differences between microbial communities but fail to provide direct taxonomic identities.

Most information obtained currently on the endophytic bacterial diversity is by the culture-dependent approaches. But the microbial diversity accounted for by the conventional cultivation techniques is less than 1 % of the total bacterial species present. To overcome the limitations of culture-dependent approaches, the culture-independent molecular approaches based on 16S rRNA gene analysis such as PCR amplification of 16S rDNAs, ARDRA, DGGE, and T-RFLP techniques have been successfully used. The severe limitation of culture-independent techniques for the analysis of endophytic bacterial communities in the plant tissues is the presence of the organelle SSU rDNA (chloroplast 16S rDNA and mitochondrial 18S rDNA); these genes can be amplified by polymerase chain reaction with universal primers used for the endophytic SSU rDNA. Nevertheless, this interference caused by plant organelle genomes can be bypassed by the analysis of ribosomal intergenic spacers (Ikeda et al. 2007) and using primers that specifically amplify the SSU rDNA without amplifying the SSU rDNA of the plant plastids (Sun et al. 2008). The PCR-amplified 16S rDNA may be cloned and further analyzed by sequencing or T-RFLP in order to identify the endophytic bacteria. Such a strategy was used by Sessitsch et al. (2002) to analyze the endophytic actinobacterial populations using actinobacterium-biased primer pair (F243-R518GC) in potato cultivars. A similar approach (16S rRNA – T-RFLP) was also employed for the identification of actinobacterial association (*Streptomyces*, *Kitasatospora*, and *Mycobacterium*) in wheat (Conn and Franco 2004a). Using the 16S rRNA gene-cloning method, Tian et al. (2007) demonstrated the association of uncultivable actinobacteria such as *Mycobacterium*, *Streptomyces*, *Micromonospora*, *Actinoplanes*, *Frankia*, *Dactylosporangium*, *Amycolatopsis*, *Corynebacterium*, *Rhodococcus*, and uncultured *Actinobacterium* with root and shoot tissues of rice.

8.4 Application of Actinobacterial Endophytes in Agriculture

Actinobacterial endophytes can accelerate seedling emergence, promote plant establishment, and growth. The actinobacteria elicit plant growth promotion either directly by helping plants to acquire nutrients via fixation of

atmospheric nitrogen, producing phytohormones such as auxins, gibberellins, and cytokinins that can enhance various stages of plant growth, and synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase that lowers plant ethylene levels for modulating plant growth and development or indirectly by iron chelation, prevention of pathogenic infections via antifungal or antibacterial agents, out competition of pathogens for nutrients by siderophore production, and establishment of plant's systemic resistance.

8.4.1 Nitrogen Fixation

Nitrogen is an essential element for plant nutrition, and its deficiency is the major cause of low agricultural productivity worldwide. The molecular nitrogen (N_2) present in the atmosphere is metabolically unavailable to higher plants. Only the microorganisms can convert it into an available form; the atmospheric nitrogen is mostly reduced to the plant available form of ammonia during the biological nitrogen fixation process. The microorganisms that fix nitrogen are referred to as diazotrophs. They are capable of fixing nitrogen either under free-living conditions or in symbiotic association with higher plants. It is estimated that 100–175 million metric tons of nitrogen per year are fixed through biological nitrogen fixation (Burns and Hardy 1975) in which the symbiotic nitrogen fixation contributes 70 million metric tons of nitrogen annually (Brockwell et al. 1995) or 200–300 kg nitrogen per hectare (Peoples et al. 1995). While the symbiotic diazotrophs in legumes provide large amounts of nitrogen, making a significant impact on agriculture, the free-living diazotrophs also contribute albeit little fixed nitrogen. In the symbiotic relationships, two distant phylogenetic groups of bacteria interact with vascular plants: *Rhizobia* (α-proteobacteria) with leguminous plants of *Fabaceae* family and *Frankia* (actinobacteria) with eight angiosperm families, viz., Betulaceae, Casuarinaceae, Coriariaceae, Datiscaceae, Elaeagnaceae, Myricaceae, Rhamnaceae, and Rosaceae (Huss-Danell 1997). The actinorhizal symbiosis contributes about 15 % to the total biologically fixed nitrogen; this symbiosis is distributed in 200 plant species of 24 genera of 8 families that are perennial woody trees/shrubs except *Datisca*. The pure culture of *Frankia* was obtained in 1978 by Callahan et al. (1978), and his group from *Comptonia peregrina*. It is a slow growing Gram-positive bacterium, shows filamentous/hyphal growth, and produces nitrogen-fixing vesicles and reproductive spores (Lechevalier 1994). It enters either intracellularly or intercellularly in the root cortex. In the intracellular infection pathway, host signal molecules induce root hair curling, as the infection thread reaches toward root cortex, actinorhizal hyphae become encapsulated and form prenodule, a primitive symbiotic structure. Concurrently cell division is induced in pericycle, leading to the formation of true nodule primordium (Berry and Sunell 1990; Laplaze et al. 2000). In the intercellular invasion, the frankial hyphae enter in between the rhizodermis cells and grow through the cortical cells (Miller and Baker 1985; Wall and Berry 2008). In its response, the plant deposits extracellular material at the invasion site. Simultaneously, the cell division occurs at pericycle that leads to the development of nodule primordium (Wall 2000).

The N_2 fixation process is mediated by the oxygen-sensitive nitrogenase (coded by *nif* genes) enzyme complex present in bacteria. In the frankial symbiosis, hyphal tip differentiates into symbiotic vesicle in which nitrogenase enzyme is protected (Newcomb and Wood 1987). In contrast to that of rhizobia, the nitrogenase activity of free-living *Frankia* KB5 is about 60 nmol C_2H_2 mg protein^{-1} h^{-1} (Mattsson and Sellstedt 2000). Though *nif* sequence homology exists both in *Rhizobia* and *Frankia*, the *nod* genes are absent in the latter (Ceremonie et al. 1999). The molecular studies demonstrated the presence of *nif*H genes in other non-frankial actinobacteria such as *Slackia exigua*, *Rothia mucilaginosa*, and *Gordonibacter pamelaeae*; however, their nitrogen-fixing potential has yet to be estimated (Gtari et al. 2012). The free-living, diazotrophic Thermomonosporaceae and Micromonosporaceae can also colonize *Casuarina equisetifolia* (Valdes et al. 2005). The

presence of *Streptomyces* species can increase the frankial nodulation in *Discaria trinervis* (Tokala et al. 2002). Recently, *Micromonospora* in nodules is reported to influence the rhizobial symbiosis in alfalfa (Martinez-Hidalgo et al. 2014). There exists a synergistic effect of *Frankia* with mycorrhizal fungi (*Paxillus involutus*) for improved N and P nutrition in *Alnus* sp. (Roy et al. 2007). The endophytic inoculations to commercially important crops like rice, sugarcane, and wheat were found to reduce the N fertilizer inputs (Govindarajan et al. 2006, 2008). Even the use of endophytic N_2-fixing bacteria in combination with N fertilizer reduced the amount of fertilizer to be applied externally (Yanni et al. 1997). On the contrary, Baker et al. (1997) showed that the presence of inorganic nitrogen could inhibit both nodulation and nitrogen fixation.

8.4.2 Phytohormones

Many plant endophytic bacteria are able to produce phytohormones that regulate plant growth. They affect physiological and morphological processes of plants at very low concentrations (Arshad and Frankenberger 1998). These phytohormones change growth pattern and result in bigger and branched roots with greater surface area enabling the plants to access more nutrients from soils. Several actinobacterial genera are capable of producing auxins, cytokinins, gibberellins, abscisic acid, and ACC deaminase (Table 8.1). Auxin is an important plant hormone for the developmental activities such as organogenesis, tropic responses, and cellular responses such as cell expansion, division and differentiation, and gene regulation. IAA (indole-3-acetic acid) is the most important native auxin; plant growth stimulation is concentration dependent. Low concentrations of IAA stimulate, while higher concentrations inhibit plant growth. IAA production by various microorganisms including *Azospirillum*, *Agrobacterium* spp., *Pseudomonas* spp., and *Streptomyces* spp. increases the seedling root length, root hairs, root branching, and root surface areas (De-Bashan et al. 2008; Lin and Xu 2013). In addition to these bacterial species, the IAA-producing endophytic *Streptomyces atrovirens*, *Streptomyces olivaceoviridis*, *Streptomyces rimosus*, *Streptomyces rochei*, and *Streptomyces viridis* also improve seed germination, root elongation, and growth (El-Tarabily 2008; Khamna et al. 2010; Abd-alla et al. 2013). *Nocardiopsis*, an endophytic actinobacterium associated with mandarin recorded highest IAA production (222.75 ppm) (Shutsrirung et al. 2013). A variety of auxins such as IAA, indole-3-pyruvic acid, indole-3-butyric acid, and

Table 8.1 Production of phytohormones and activity of ACC deaminase by plant growth-promoting actinobacteria

Phytohormone/ ACC deaminase	Actinobacterial species	References
Auxin/IAA	*Actinomyces* spp., *Frankia* sp., *Micrococcus*, *Nocardia* sp., *Streptomyces* spp., *S. atrovirens*, *S. griseoviridis* K61, S. *lydicus* WYEC108, *S. olivaceoviridis*, *S. rimosus*, *S. rochei*, *S. viridis*	Kaunat (1969), Brown (1972), Wheeler et al. (1984), Mahadevan and Crawford (1997), Tokala et al.(2002), Tsavkelova et al. (2006), El-Tarabily (2008), Khamna et al.(2010), Verma et al. (2011), Abd-alla et al. (2013), and Lin and Xu (2013)
Gibberellin	*Actinomyces* spp., *Arthrobacter*, *Micrococcus*, *Nocardia* spp., *Streptomyces* spp.	Katznelson and Cole (1965), Kaunat (1969), Brown (1972), Merckx et al. (1987), and Tsavkelova et al. (2006)
Cytokinins	*Arthrobacter*, *Frankia* sp., *Leifsonia soli*, *Rhodococcus fascians*, *Streptomyces turgidiscabies*	Sang-Mo et al. (2014), Cacciari et al. (1980), Stevens and Berry (1988), Joshi and Loria (2007), and Pertry et al. (2009)
ACC deaminase	*Arthrobacter*, *Microbacterium azadirachtae* sp. nov., *Leifsonia soli* sp. nov., *Micrococcus* sp., *Rhodococcus* sp. R04, *Streptomyces* spp.	El-Tarabily (2008), Dastager et al. (2010), Madhaiyan et al. (2010a, b), and Nascimento et al. (2014)

indole-lactic acid are produced by endophytic bacteria via diverse IAA biosynthetic pathways. The beneficial bacteria such as *Bacillus amyloliquefaciens*, *Azospirillum*, and *Rhizobia* produce IAA via both the tryptophan (trp)-dependent pathway (indole-3-pyruvic acid-IPyA) and the trp-independent pathway. The molecular genetic studies showed the expression of *ipdc* (code for indole-3-pyruvic acid decarboxylases of IPyA pathway) in *Azospirillum lipoferum* FS and *Azospirillum brasilense* Sp7 (Yagi et al. 2001; Mallhotra and Srivastava 2008). But several pathogens also produce IAA through the indole-3-acetamide (IAM) pathway in which trp-2-monooxygenase (*iaa*M) converts tryptophan into IAM and further to IAA by IAM hydrolase (*iaa*H). Similar to some of these pathogens, the endophytic *Streptomyces violaceus*, *Streptomyces scabies*, *Streptomyces griseus*, *Streptomyces exfoliates*, *Streptomyces coelicolor*, and *Streptomyces lividans* synthesize IAA in the presence of tryptophan via IAM pathway, leading to enhanced plant growth (Lin and Xu 2013). Moreover, low level of IAA (20 ppm) is known to regulate the expression of rhodomycin biosynthetic genes such as *rdm*A, *rdm*B, *rdm*C, *rdm*D, and *rdm*E in *Streptomyces purpurascens* NBRC 13077 (Matsukawa et al. 2007). In addition to its effect on plant growth, IAA also triggers cell differentiation, hyphal elongation, and sporulation in *Streptomyces atroolivaceus* (Matsukawa et al. 2007). Likewise, the periodic acids A and B produced by endophytic *Streptomyces hygroscopicus* TP_A045 show auxin-like activity at 20 ppm and even induce root elongation in common bean (Igarashi et al. 2002).

Cytokinins and gibberellins are produced by several rhizospheric microorganisms (Gaudin et al. 1994). Cytokinins mediate signal exchange from roots to shoots under environmental stresses, promote cell division, cell enlargement, and increase root surface area through intense proliferation of adventitious and lateral roots (Jackson 1993). In soybean, the microbial production of cytokinins is found to enhance plant growth (Noel et al. 1996; Timmusk et al. 1999; Garcia de Salamone et al. 2001). They are also known to confer a plant-fitness advantage in both beneficial as well as pathogenic actinobacteria (Stevens and Berry 1988; Lichter et al. 1995). Gibberellins are another important phytohormone involved in modifying plant morphology by extension of stem tissues. The application of GA_3 was found to increase plant height, total biomass, and grain in common bean (Ngatia et al. 2004).

IAA production by endophytic bacteria can activate the ACC synthase for the production of ACC in higher plants (Glick et al. 1998). The conversion of ACC to ethylene by ACC oxidase consequently suppresses nodule formation and plant growth in many leguminous plants. Certain endophytic bacteria are capable of reducing ethylene level by producing ACC deaminase that cleaves ACC and promote plant growth by obviating the *in planta* ethylene inhibition. The genes encoding ACC deaminase (*acd*S) was first reported in *Cyberlindnera saturnus* and *Pseudomonas* sp. ACP (Honma and Shimomura 1978). The actinobacterial strains such as *Micrococcus*, *Corynebacterium*, *Arthrobacter*, *Rhodococcus*, and *Streptomyces* spp. with exemplified ACC deaminase activity were found to improve plant growth (Palaniyandi et al. 2013). The protein sequence analyses suggest that *Rhodococcus* sp. R04 shared 70–82 % of *acd*S gene identity with the true ACC deaminase (Nascimento et al. 2014). The presence of ACC deaminase genes in *Sinorhizobium meliloti* has been shown to improve nodule formation and occupancy (Ma et al. 2004). Although *Mesorhizobium* sp. does not exhibit ACC deaminase activity under free-living conditions, the gene expression of *acdS* under symbiotic conditions is demonstrated by Uchiumi and coworkers (2004). The mutation studies in *Rhizobium leguminosarum* bv. *viciae* and *Mesorhizobium loti* MAFF303099 for the ACC deaminase showed a reduction in the nodulation efficiency (Tittabutr et al. 2008).

8.4.3 Antibiosis

8.4.3.1 Antibiotics

Antibiosis is the major biocontrol mechanism of actinobacteria in nature. The diffusible compounds produced by actinobacteria inhibit the colonization of several rhizospheric pathogens. A wide spectrum of antibiotics including macrolide, benzoquinones, aminoglycosides, polyenes, and nucleosides produced by actinobacteria has been attributed to their versatile biological effects. The total number of microbial bioactive molecules is about 33,500 in which about 41 % (13,700 metabolites) is contributed by actinobacteria (Berdy 2012). About 11,500 actinobacterial metabolites exhibit antibiotic activity (Table 8.2); nearly 1800 metabolites show antibiosis against pathogenic fungi (Berdy 2005). Nevertheless, less than 1 % of these compounds are in practical use.

The most widely studied group of actinobacteria with respect to the production of antibiotics is of the *Streptomyces* spp. (Horinouchi 2007). Among several actinobacteria, *Streptomyces* sp. alone contributes about 10,400 molecules (76 %) and represents the largest group of antibiotic-producing actinobacteria (Berdy 2012). The members of this genus produce branched substrate hyphae as well as aerial hyphae. In the aerial hyphae, the secondary metabolites production induces the formation of arthrospores. IAA at low concentration regulates the cellular differentiation as well as the antibiotic production. Germicidin and hypnosin synthesized by *Streptomyces alboniger* inhibit spore germination (Challis 2008). Geldanamycin and elixophyllin producing

Table 8.2 Diversity of actinobacteria capable of producing antibiotics

Actinobacterial species	Antibiotic	References
Streptomyces sp., *S. alboniger*, *S. padanus*	Alnumycin, coronamycins, fungichromin, goadsporin, kakadumycins, pamamycin-607, rhodomycin	Shockman and Waksman (1951), Kondo et al. (1987), Bieber et al. (1998), Onaka et al. (2001), Castillo et al. (2003), Shih et al. (2003), and Ezra et al. (2004)
Actinoplanes teichomyceticus	Teichomycins, teicoplanin	Parenti et al. (1978) and Somma et al. (1984)
Actinoplanes friuliensis sp. *nov. II.*	Friulimicins	Vertesy et al. (2000)
Actinoplanes ianthinogenes N. sp.	Purpuromycin	Coronelli et al. (1974)
Actinoplanes	Lipiarmycin	Coronelli et al. (1975)
A. utahensis	Echinocandin	Boeck et al. (1989)
Actinomadura sp.	Cationomycin, chandrananimycins, oxanthromicin	Nakamura et al. (1981), Patel et al. (1984), and Maskey et al. (2003)
Actinomadura spiralis	Pyralomicins	Kawamura et al. (1995)
Microbispora sp.	Cochinmicins, glucosylquestiomycin	Igarashi et al. (1998) and Lam et al. (1992)
Microbispora aerata	Microbiaeratin	Ivanova et al. (2007)
Micromonospora l omaivitiensis	Lomaiviticins A and B	He et al. (2001)
Micromonospora inyoensis	Sisomicin	Reimann et al. (1974)
Micromonospora carbonacea	Everninomicin	Weinstein et al. (1964)
Micromonospora echinospora subsp. *armeniaca* subsp. *nov.*	Clostomicins	Omura et al. (1986)
Nocardiopsis	New thiopeptide antibiotic	Engelhardt et al.(2010)
Nocardia sp. *I.*	Nocathiacins	Li et al. (2003)
Nocardia mediterranei subsp. *kanglensis*	Chemomicin A	Sun et al. (2007)

Streptomyces hygroscopicus suppress *Rhizoctonia* root rot of pea (Rothrock and Gottlieb 1984). *Streptomyces violaceusniger* YCED-9 synthesizes polyketide antibiotics (nigericin and geldanamycin) and lytic enzymes, viz., chitinase and β-1, 3-glucanase. These actinobacterial metabolites are associated with direct inhibitory action against *Phytophthora infestans* and *Rhizoctonia solani*. The secretion of polyene-like compounds related to guanidyl-containing macrocyclic lactones by *Streptomyces* strain shows the anti-Fusarium activity (AFA) against *Fusarium oxysporium* (Trejo-Estrada et al. 1998).

An additional mechanism by which actinobacteria can reduce plant disease is mycoparasitism/hyperparasitism on fungal pathogens. Different actinobacteria, viz., *Actinoplanes* spp. (Arora 1986), *Nocardiopsis dassonvillei* (Sabaou et al. 1983), *Micromonospora globosa* (Upadhyay and Rai 1987), and *Streptomyces* spp. (Yuan and Crawford 1995) showed hyphal parasitism against various fungal pathogens. These antagonistic actinobacteria are capable of propagating even in the presence of resting oospores and parasitize the growing mycelium of pathogen during their active phase (Sutherland and Lockwood 1984; El-Tarabily et al. 1997). The digestion of fungal cell wall is accomplished by excreted enzymes including chitinase, glucanase, and peroxidase. Individually, all these enzymes display antifungal activity, but they often act synergistically with antibiotics (Lorito et al. 1994). The chitinolytic activity of endophytic *Streptomyces virididiasticus* and *Micromonospora carbonaceae* has been implicated in cell wall lysis of *Sclerotinia minor* (El-Tarabily et al. 2000). Similar to plants, the chitinase synthesis by *Streptomyces* sp. belongs to the family 19 of glycosyl hydrolases with molecular mass of $\leq$30,000 Da (Hoster et al. 2005) and makes them the potential biocontrol agents. The production of chitinase by *Streptomyces* species showed antagonistic potential against *Colletotrichum sublineolum*, *Guignardia citricarpa*, *R. solani*, *Fusarium oxysporum*, *Phytophthora parasitica*, and *Pythium* sp., but not oomycetes which have cellulose in their cell walls (Quecine et al. 2008). The hyphae of actinobacterial members belonging to Micromonosporaceae family including *Amorphosporangium auranticolor*, *Ampullariella regularis*, *Spirillospora albida*, *Actinoplanes*, and *Micromonospora* spp. coil around oospores and cause cytoplasmic disintegration (Sutherland and Papavizas 1991). The mixtures of the cellulose-producing *Micromonospora carbonacea* and antibiotic-producing *Streptomyces violascens* synergistically suppress the oomycete *Phytophthora cinnamomi* (El- Tarabily et al. 1996). The molecular genetic study showed that endo-1,3–βglucanase synthesized by *Streptomyces* sp. S27 could destroy the cell walls of *R. solani*, *F. oxysporum*, *Fusarium crookwellense*, and *Paecilomyces variotii* (Shi et al. 2010). This enzyme shares high sequence identity with bacterial endoglucanase with temperature optima at 65 °C (Shi et al. 2010). Misk and Franko (2011) suggested that *Streptomyces* sp., BSA25, and WRA1 could be an effective biocontrol agent against *Phytophthora* root rot; they also promoted the chickpea growth in coordination with *Mesorhizobium*. The rhizopheric actinomycete such as *Streptomyces rochei* and *S. rimosus* showed the antagonistic potential against *F. oxysporum* f.sp. *ciceri* (Bashar and Rai 1994). The antagonistic actinomycetes capable of delaying the onset of *Fusarium* wilt in chickpea under wilt sick plot was also demonstrated by Gopalakrishnan et al. (2011). The endophytic *Streptomyces aureofaciens* CMUAc130 could antagonize *Colletotrichum musae* (banana anthracnose) and *F. oxysporum* (wheat wilt) (Taechowisan et al. 2005). In addition to the pathogen control, the metabolites of *Streptomyces* sp. SANK 63997 could exhibit herbicidal activities (Okazaki 2006).

8.4.3.2 Siderophores

Iron is abundant in the Earth's crust but most of it is in the form of insoluble ferric hydroxide and thus unavailable to soil organisms and plants. Some of the endophytic bacteria are capable of sequestering iron from the soil solution and the organic iron complexes through siderophores, a

specific ferric iron (Fe^{3+}) carrier (Neilands and Nakamura 1991). Hence, they can mediate the nutritional competition for iron and inhibit the growth of plant pathogens under conditions of low iron availability (Kloepper et al. 1980). However, under acidic conditions (pH < 6), iron availability increases, and siderophores become less effective (Neilands and Nakamura 1991). The endophytic bacteria can produce different structural types of siderophores such as catecholates, hydroxamates, and citrate-based polycarboxylates (Raymond et al. 2003). The Ton-B-dependent outer membrane proteins are responsible for the specific uptake of ferric–siderophore complexes. The siderophores form complexes with iron (1:1); Fe^{3+} (insoluble) is reduced to Fe^{2+} (soluble) by esterase activity and then released into the cells. The energy required for translocation of the ferric–siderophore complex into the periplasmic spaces is driven by the proton motive force/ATPase activity. The siderophore production under the iron stress conditions provides rhizospheric competency to actinobacteria by the exclusion of pathogens due to iron starvation (Table 8.3).

Table 8.3 Types of siderophores produced by actinobacteria

Siderophore type	Actinobacterial species	References
Albachelin	*Amycolatopsis alba*	Kodani et al. (2015a)
Coelichelin	*S. coelicolor*	Challis and Ravel (2000)
Desferrioxamine (tris-hydroxamate siderophores)	*S. coelicolor*, *Streptomyces ambofaciens*	Barona-Gomez et al. (2006)
Enterobactin	*Streptomyces tendae*	Fiedler et al. (2001)
Erythrobactin	*Saccharopolyspora erythraea*	Oliveira et al. (2006)
Ferrioxamine, ferrichrysin, rhodotorulic acid (RA), and synthetic enantio-RA	*Streptomyces pilosus*	Muller et al. (1984)
Griseobactin	*Streptomyces* sp. ATCC 700974	Patzer and Braun (2010)
Peucechelin	*S. peucetius*	Kodani et al. (2015b)

Iron is also a key component of proteins such as nitrogenase, ferredoxins, cytochromes, and leghemoglobin in the *Rhizobium*–legume-rhizobia symbiosis (Ranjeet et al. 2002). The presence of metal-chelating symbiotic *Streptomyces* in pea root nodules suggests their possible role in iron assimilation for nodule growth and even in the bacteroides differentiation.

8.4.4 Biocontrol

8.4.4.1 Induced Systemic Resistance

The endophytic actinobacteria have the fungistatic potential against a wide range of soilborne fungal pathogens (Table 8.4). The production of several bioactive metabolites as well as the ability to colonize plants makes them successful as biocontrol agents. Besides, these endophytes can induce resistance in the plant system. Among several plant defense mechanisms, the induced systemic resistance (ISR) and systemic acquired resistance (SAR) are significant. The ISR mediated by rhizobacteria predisposes the plants to resist further attacks. On the contrary, the SAR is induced by pathogens, resulting in the activation of resistance mechanisms in other uninfected parts of plants. Generally, the ISR is mediated by signaling molecules such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET); they coordinate the defense responses by the induction of resistance (Pieterse et al. 1996). Jasmonic acid is produced through the octadecanoid pathway in which linolenic acid (18:3) is converted to JA, an important oxylipin molecule (Van Spronsen et al. 2003). The production of JA can be in response to the pathogen invasion in plants. This signaling molecule activates defense-related genes: defensins, thionines, and pectinase inhibitors (Hause et al. 2002). Besides, JA also plays an important role in the onset of senescence, root formation, and ethylene synthesis. The salicyclic acid induces genes that encode the pathogenesis-related proteins (PRs) (Uknes et al. 1992). These proteins have the antimicrobial activity (Kombrink and Somssich 1995). Several bacterial metabolites show direct inhibitory action

Table 8.4 Biocontrol potential of actinobacteria on different plant–pathogen systems

Actinobacterial species	Plant	Pathogen	References
Actinoplanes missouriensis	Soybean	*Phytophthora megasperma* f. sp. *glycinea*	Sutherland and Lockwood (1984)
Amorphosporangium auranticolor, *A. missouriensis*, *A. utahensis*, *Micromonospora* sp.	Soybean	*Phytophthora megasperma* f. sp. *gylcinea*	Filonow and Lockwood (1985)
Streptomyces sp.	Soybean	*Xanthomonas campestris* pv. *glycine*	Mingma et al. (2014)
Streptomyces sp. BSA25 and WRA1	Chickpea	*Phytophthora medicaginis*	Misk and Franko (2011)
Streptomyces sp.	Chickpea	*Fusarium oxysporum* f. sp. *ciceri*	Gopalakrishnan et al. (2011)
Micromonospora globosa	Pigeon pea	*Fusarium udum*	Upadhyay and Rai (1987)
Streptomyces sp.	Sorghum	*Macrophomina phaseolina*	Gopalakrishnan et al. (2014)
Streptomyces viridodiasticus, *Micromonospora carbonaceae*	Lettuce	*Sclerotinia minor*	El-Tarabily et al. (2000)
Streptomyces sp.	Cucumber	*F. oxysporum*	Singh et al. (1999)

against several *Rhizoctonia*, *Fusarium*, *Pythium*, *Phytophthora*, and *Colletotrichum* pathogens (Raaijmakers et al. 2009).

The use of actinobacteria as inducers of plant immunization against different pathogens has been demonstrated under field conditions (Lacava et al. 2007). The seed inoculation of endophytic actinobacteria induces disease resistance in wheat. The endophytic *Streptomyces* sp. was able to control take-all disease of wheat and potato scab under the field conditions (Coombs et al. 2004; Liu et al. 1996). Both actinomycin *X2* and fungichromin produced by the *Streptomyces galbus R-5* induce ISR and jasmonate-associated defense responses in rhododendron seedlings (Meguro et al. 2004). The endophytic *Streptomyces* sp. (EN27) and *Micromonospora* sp. (EN 43) are able to induce resistance in *Arabidopsis thaliana* by up-regulating genes involved in SAR. Even the inoculation of *Streptomyces* sp. EN28 activates jasmonate/ethylene pathways, whereas *Nocardioides albus* EN46 can induce SAR (PR-1 and PR-5) against *Erwinia carotovora* and *F. oxysporum* (Conn et al. 2008). They also observed that the culture filtrates of *Micromonospora* sp. (EN 43) are able to induce SAR and the jasmonate/ethylene pathway. The different bacterial determinants involved in triggering ISR include secondary metabolites and siderophores and also their colonization efficiency. The endophytic actinobacterial colonization in the tissue-culture plant imparts host resistance against many plant pathogens (Suzuki et al. 2005). In contrast to those plants inoculated with actinobacteria, the *Pseudomonas fluorescens*-treated plants trigger ISR by activating the genes encoding pathogenesis-related (PR) proteins and those associated with the phenylpropanoid metabolic pathway. The major-induced PR-proteins include chitinase, β-1, 3-glucanases, thaumatin-like proteins (TLP), and peroxidases. Besides, the phenylpropanoid pathway enzymes such as phenylalanine ammonia lyase (PAL), peroxidase (PO), and polyphenol oxidase (PPO) are induced during the developments of ISR.

8.5 Role of Nodule-Inhabiting Actinobacterial Endophytes

The symbiotic association between the endophytic, root-nodulating *Rhizobium* and legumes are well documented where the microsymbiont fixes nitrogen in exchange of carbon from the host plant. The members of endosymbiotic

Rhizobiaceae are remarkable bacteria and exhibit both a saprophytic as well as a symbiotic lifestyle; they can change from the free-living soil saprophytic *Rhizobium* to the symbiotic nitrogen-fixing nodule bacteroid. The *Rhizobia* can interact with other rhizospheric microorganisms as well as host–endophytic microorganisms. In addition to the colonization of roots, shoots, leaves, seeds and fruits, etc., the endophytic bacteria also colonize the nodules of legume plants. A great diversity of non-rhizobial nodule endophytic bacteria such as *Arthrobacter*, *Bacillus*, *Burkholderia*, *Dyella*, *Methylobacterium*, *Microbacterium*, *Staphylococcus*, and *Streptomyces* was isolated from wide range of legume root nodules (Tokala et al. 2002; Li et al. 2008; Muresu et al. 2008; Zhao et al. 2011; Dudeja et al. 2012). These nodule endophytes can coexist in the root nodules but may not induce nodulation (Wang et al. 2006). They are reported to possess many plant growth-promoting activities such as nitrogen fixation, P solubilization or iron chelation, and promote plant growth and may also suppress plant pathogens.

The presence of nodule-enhancing actinobacteria such as *Curtobacterium*, *Microbacterium*, *Micromonospora*, and *Streptomyces* inside the nodules of various crops has been reported (Martinez-Hidalgo et al. 2014). Co-inoculation with non-*Bradyrhizobium* endophytic bacteria and *Bradyrhizobium japonicum* increased the plant biomass in soybean under the nitrogen-free conditions (Bai et al. 2002). The synergistic interaction of *Rhizobium* with nodule endophytes can improve plant growth, nodulation, and yield in different legume crops (Sturz et al. 1997; Rajendran et al. 2008). The combined inoculation of endophytic *Streptomyces* spp. with *Rhizobia* was observed to exert positive effects on the growth of legumes. The successful colonization of introduced rhizobial strain needs to compete effectively with many native rhizospheric microorganisms. The slow growing nature of many rhizobia is considered to offer competitive disadvantage as the native species are numerous and can proliferate profusely. The members of other rhizobial genera can compete for the colonization or prevent the nodulation by the inoculants rhizobia. But, the co-inoculation of antibiotic-producing *S. griseus* with *Sinorhizobium meliloti* can improve the rhizobial competitiveness over other microflora, nodulation, and yield in alfalfa. Similar results were obtained with the combined inoculation of *S. griseus* with *B. japonicum* in soybean (Li and Alexander 1990). The dual inoculation of *B. japonicum* with each of non-*Streptomyces* actinobacteria such as *Nocardia alba*, *Nonomuraea rubra*, and *Actinomadura glauciflava* led to the enhancement of nitrogenase activity by 1.07–2.7-fold (Nimnoi et al. 2010). The application of *Bradyrhizobium yuanmingense* MAS34 with *Streptomyces grieseoflavus* P4 at low density level (10^5 CFU per g seed) increased the symbiotic potential and even the seed yield of soybean (Soe et al. 2013). Some of these nodule endophytes can gain the improved symbiotic potential with host plants by acquiring symbiotic genes through lateral gene transfer; they may even show the host preference like rhizobia (Taghavi et al. 2005). Although *Streptomyces* MM40, *Actinoplanes* ME3, and *Micromonospora* MM18 act as helper bacteria in the *Frankia–Discaria trinervis* symbiosis, these actinobacteria do not have any plant growth-promoting effects (Solans 2007). Recent studies show that co-inoculation of these actinobacteria induced nodulation of *S. meliloti* at high N (7 mM) which was otherwise inhibiting nodulation. This result suggests that the possible role of actinobacteria in the autoregulation of alfalfa is nodulation at high N (Solans et al. 2009). Though many species of *Streptomyces* are used for the disease suppression, some of them are inhibitory to the beneficial bacteria (Samac et al. 2003). The combined inoculation of endophytic *Streptomyces* sp. along with *Bradyrhizobium* was also not effective in soybean (Soe et al. 2012). Increased nodule occupancy and shoot N content using synergistically competent (antibiotic-resistant mutant) *B. japonicum* along with *Streptomyces kanamyceticus* in soybean emphasizes the need for careful selection of actinobacteria–rhizobial combination (Gregor et al. 2003). The co-inoculation of *Streptomyces* sp. BSA 25 along with *Mesorhizobium ciceri* WSM1666 was found to suppress *Phytopthora medicaginis* in chickpea. Nevertheless, *Streptomyces* sp. WRA1 could

manifest a fourfold increase in the shoot and about eightfold increase in root weight of chickpea. In the pea plants, the nodulation is influenced by *S. lydicus* WYEC 108 (Tokala et al. 2002). The basic mechanism involved in this synergistic activity is the alteration of the host secondary metabolism and/or the elimination of competition of *Rhizobium* with the deleterious microorganisms.

8.6 Conclusions

Microorganisms can form complex associations with plants ranging from mutualism to pathogenesis. Certain microorganisms are capable of colonizing the exterior surface (epiphytes) or interior tissues (endophytes) of the plants. Among various groups of endophytic microorganisms, the association of actinobacteria with plants has gained considerable interest in the recent times. These plant-associated actinobacteria can influence the plant health and productivity through many direct or indirect mechanisms. These actinobacteria can impart disease resistance in plants; the bioactive molecules produced by them have the potential even to modulate the plant metabolism. The growth and development stages of plant alter the dynamics of these endophytic actinobacteria over time and in space. From the seed to harvest stage, the host plants can select the endophyte for their beneficial associations from the microbial resources in soils, water, and the atmosphere. The key plant-fitness trait is due in large part to the plants' recruitment of endophytes. It is not known how vital and indispensible for the plant metabolism of these endophytic associations. Evidence suggests that endophytes are even vertically transmitted via seed in the stable host–endophytic interactions. Improved understanding of their origins, the exact roles of these endophytes on the development of plant disease resistance, and their contributions to the productivity of plants can help in selecting the agronomically significant host–endophyte combinations. Future research efforts are necessary to comprehend the ecological and evolutionary principles behind these associations, especially for translating this knowledge into practical applications.

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Role of Endophytic Actinomycetes in Crop Protection: Plant Growth Promotion and Biological Control

9

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Abstract

Endophytes are microorganisms that inhabit the interior of plant tissues without causing apparent disease in the host plant. The utilization of endophytic microorganisms for agricultural purposes has increased recently, especially in the biological control of insect-pest and plant disease and in plant growth promotion. Research has shown that many endophytic actinomycetes are beneficial to host plants with regard to the biological control of phytopathogens and plant growth promotion. Endophytic actinomycetes may promote plant growth by a combination of mechanisms, such as the solubilization of nutrients and the production of growth hormones and enzymes. Because actinomycetes are able to produce spores, a dissemination structure that offers resistance to many adverse conditions. These actinobacteria could be used for the formulation of novel bioinoculants composed of spores and/or mycelium. An understanding of the mechanisms enabling these endophytes to interact with host is important for realizing the potential of these microorganisms in agriculture production.

Keywords

Biocontrol • Endophytes • IAA • Phosphate solubilization • Siderophore

9.1 Introduction

The term endophyte is applied to microorganisms, frequently bacteria and fungi, which colonize the living plant tissues for all or part of their life cycle but cause no apparent infection or symptoms of disease (Azevedo et al. 2000; Saikkonen et al. 2004). Hallmann et al. (1997) described endophytic microorganisms as those microorganisms that from surface-sterilized plant parts are isolated from inner tissues of host plant with no symptoms of disease. In addition, Azevedo and Araújo (2007) have suggested that endophytes are all microorganisms, culturable or not, that inhabit the interior of plant tissues, cause no

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harm to the host, and do not produce external structures. Coined by de Bary (1866), the term endophyte was applied to describe the existence of microorganisms inside plant without negative effects on the host plant (Schulz and Boyle 2006), and almost all plants have been found to be colonized by one or more (Petrini et al. 1992). These microorganisms produce molecules that function as growth-promoting metabolites, insect-pest repellents, antimicrobials against plant pathogens, and protectants against stress (Rai et al. 2014). They also possess the potential to produce secondary metabolites of agricultural and biotechnological importance (Golinska et al. 2015). The utilization of endophytic microorganisms for agricultural purposes has increased recently, especially with regard to insect-pest and disease control and plant growth promotion. Endophytic microorganisms promote plant growth in many ways, by production of enzymes; hormones such as auxin (indole-3-acetic acid, IAA); antagonism against plant pathogens via the production of siderophores, chitinases, or antibiotics; and the solubilization of nutrients, such as phosphates (Lacava and Azevedo 2013, 2014).

9.2 Endophytic Actinomycetes

Currently, it is estimated that there are approximately $4-6 \times 10^{30}$ different prokaryotic cells exceeding by various orders of magnitude, all plant and animal (Whitman et al. 1998) as well as fungi diversity (Baker et al. 2009), yet most largely unexplored. The ability of bacteria to exploit new environments and respond to new selective pressures (Woese 1987) can be most explained by the acquisition of new genes via horizontal transfer during evolution (Davison 1999).

Streptomycetes, mycelial members of *Actinomycetales*, are among the most studied antibiotic-producing bacteria and the most developmentally complex organisms belonging to the domain *Eubacteria* (Chater and Losick 1997). These prokaryotic organisms grow, typically in soil, as branching threadlike hyphae to form a vegetative or substrate mycelium. Phylogenetically, *Streptomyces* belongs to actinobacteria, the class of gram-positive and morphologically diverse bacteria with DNA that has a comparatively high G+C content, approximately 69 ± 78 mol% (Korn-Wendisch and Kutzner 1992). In addition to the soil as a habitat (Hopwood 2007), the environments of some saprophytic *Actinomycetales* range from sediments of marine origin (Cruz-Hernandez et al. 2009) to endophytic niche (Ratti et al. 2008; Piza et al. 2015). This growth habit, combined with the activities of extracellular hydrolytic enzymes, helps these microbes gain access to the nutrients sequestered in the insoluble polymers of complex environments. These microorganisms are capable of degrading complex molecules as well as recalcitrant substances, especially cellulose, lignocellulose, xylan, and lignin, playing an important role in decomposition processes of soil organic matter (Ding et al. 2004).

In general, endophytic bacteria promote plant growth and yield, suppress phytopathogens, and may help to solubilize phosphate or contribute to nitrogen assimilation in plants (Rosenblueth and Martinez-Romero 2006). Some of the symbiotic functions attributed to associated microbes include nutrient acquisition, metabolic waste processing, and secondary metabolite production. Plants from the Brazilian tropical savannah constitute an excellent source in searches for endophytic microbes (Favoretto et al. 2008). Serrano et al. (2012) studied the effects of glucose and inoculum concentrations on the production of molecules by endophytic *Paenibacillus polymyxa* RNC-D isolated from *Prunus* in Brazilian tropical savannah tree and found a significant and positive effect on biomass formation. Bioactivity results were also affected by the two variables studied. The lowest minimum inhibitory concentration (MIC) value for *Escherichia coli* was obtained when the highest glucose and inoculum concentrations were used, whereas the MIC for *Staphylococcus aureus* was increased when the maximum glucose concentration was applied. In addition, surface tension was affected by the two variables and also by their interaction. The highest biomass formation

(4.11 g/l) and the lowest MIC for *E. coli* (15.6 μg/ml) were attained under the highest concentrations of glucose and inoculum, whereas surface tension reduction reached a maximum (20.0 mN/m) when using the lowest glucose and highest inoculum concentrations. However, such results can be improved by performing additional assays for the establishment of quadratic models, as suggested by an analysis of the experimental design.

Working with endophytic actinomycetes isolated from Brazilian tropical savannah trees in Sao Paulo state, Brazil, Ratti et al. (2008) showed that microorganisms isolated from *Cassia leptophylla* had no ability to inhibit *Staphylococcus*, while those isolated from the leaves of *Prunus* spp. did present antibacterial activity. The endophytic microorganisms isolated from these plants inhibited *Staphylococcus* when cultivated in peptone agar (PA) as well as in yeast extract agar (YA). When cultivated in YA, the inhibition of the second isolated microorganism (bottom morphology), cultivated at the same conditions, presented inhibition zones of 2.0 cm in diameter, and the same microorganism (yellow colony) inhibited *Staphylococcus*, with 2.0 cm and 1.4 cm when cultivated on PA and in YA, respectively (Fig. 9.1).

Serrano et al. (2010) reported the isolation of bioactivity from endophytic *Paenibacillus polymyxa*, most likely small molecules, against *S. aureus* and *E. coli*. The synergy or contingency in the activity of individual metabolites against biological competitors may, in some cases, be a powerful driving force in the evolution of multiple secondary metabolites (Challis and Hopwood 2003). These authors verified that this process can be illustrated by examples of coproduction of synergistically acting antibiotics and contingently acting siderophores, classes of secondary metabolites.

Endophytic properties of actinomycetes have been reported for *Streptomyces galilaeus*, *Microbispora amethystogenes*, *Micromonospora yulongensis*, *Streptomyces argenteolus*, *Streptomyces peucetius*, and *Nocardioides albus* (Coombs and Franco 2003). Such intimate associations between actinomycete strains and host are thought to greatly improve the efficiency of the transport of beneficial compounds from the microorganism to the host plant (Cao et al. 2004; Schrey et al. 2005; Firakova et al. 2007) and are extremely important to the success of the bioinoculants (Gyaneshwar et al. 2002; Khan and Zaidi 2007) and also for biocontrol of phytopathogens (Hamdali et al. 2008). Because

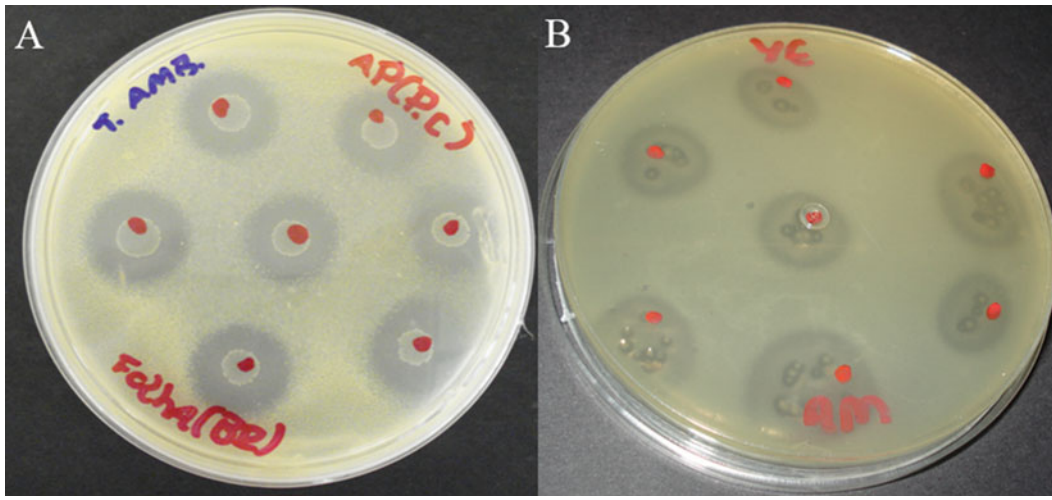

Fig. 9.1 The isolated microorganism from *Prunus* shows inhibition potential against *Staphylococcus coagulase*-positive strain when cultivated in PA (**a**) and YA (**b**) medium

actinomycetes are able to produce spores, a dissemination structure that offers resistance to many adverse conditions (Chater 1993), these actinomycetes are useful for the formulation of bioinoculant products composed of spores and/or mycelium (Thirup et al. 2001).

9.3 Plant Growth Promotion by Endophytic Actinomycetes

Agriculture is anti-ecological by nature, and profound biological modifications have occurred with the use of agrochemical, including chemical fertilizers, herbicides, and insecticides. Consequently, interest in the development of new mechanisms to achieve more sustainable agricultural practices has increased significantly in recent years (Azevedo et al. 2000). Endophytes have many key mechanisms and a particular species may utilize different strategies during the plant life cycle. The effects of plant growth promotion by endophytic microorganisms are well known to improve the plant height and number of tillers; biomass of shoots, stems, and roots; lignification of xylem vessels and wilting time; and increases in crop yield (Ahmad et al. 2008). Research has shown that many endophytic actinomycetes are also beneficial to host plants, including the biological control of phytopathogens (Cao et al. 2005; Misk and Franco 2011) and plant growth promotion (Qin et al. 2014). The solubilization of nutrients, like phosphorus, and production of growth hormones, specifically IAA, and siderophores, antibiotics, and enzymes are the different ways of endophytic actinomycetes to promote plant growth directly and indirectly (Qin et al. 2011, 2015; Rungin et al. 2012; Glick 2014).

9.3.1 Direct Plant Growth Promotion

Endophytic actinomycetes have been considering a source of metabolites that promote plant growth and reduce the plant disease caused by phytopathogenic fungi by biocontrol (Shimizu 2011). Several scientific investigations have demonstrated the plant growth-promoting activity and secretion of plant growth hormones by endophytic actinomycetes (Golinska et al. 2015). The ability of endophytes to stimulate plant growth has been attributed to direct and indirect mechanisms. The production of phytohormones and the solubilization of phosphate are among the direct mechanisms. Indirect mechanisms include the induction of host systemic resistance, production of siderophores, decreases in stress factors, and synthesis of antibiotics, as well as factors that are responsible for antagonism against phytopathogens (Quecine et al. 2014).

9.3.1.1 Production of IAA

The ability to synthesize phytohormones is found widely among plant-associated bacteria, and 80 % of the bacteria associated with plants are able to produce IAA (Cheryl and Glick 1996), the physiologically most active auxin in plants. IAA is known to stimulate both rapid (increases in elongation) and long-term (cell division and differentiation) responses in plants (Cleland 1990; Hagen 1990).

Actinomycetes are known for their ability to promote plant growth by producing IAA to help root development or by producing a siderophore to bind Fe3+ from the environment and help to improve nutrient uptake (Qin et al. 2011; Shimizu 2011; Gangwar et al. 2014). It has been observed that growth by various means via the secretion of plant growth regulators such as IAA, pteridic acids A and B, which has auxin-like activity (Igarashi et al. 2002) and promotes plant establishment under adverse conditions (Hasegawa et al. 2006). Within this context, Igarashi et al. (2002) purified pteridic acids A and B from the culture broth of the endophytic *Streptomyces hygroscopicus*. These metabolites accelerated the formation of adventitious roots in kidney bean hypocotyls at 1 nM, which was as effective as IAA. Additionally, El-Tarabily et al. (2009) reported that strains of endophytic actinomycetes, *Actinoplanes campanulatus*, *Micromonospora chalcea*, and *Streptomyces spiralis*, produce IAA and IPYA (indole-3-pyruvic acid) which significantly enhanced the growth of cucumber plants. Similarly, Nimnoi

et al. (2010) examined the productivity of IAA by actinomycetes isolated from eaglewood, and all of the endophytic isolates tested produced IAA.

Three medicinal plants, *Aloe vera*, *Mentha arvensis*, and *Ocimum sanctum*, were explored for endophytic actinomycete diversity and plant growth promotion (Gangwar et al. 2014), with actinomycetes being commonly recovered from roots (70 % of all isolates), followed by stems (17.5 %) and leaves (12.5 %). Genus *Streptomyces* ranked first (60 % of all isolates), followed by *Micromonospora* (25 %), *Actinopolyspora* (7.5 %), and *Saccharopolyspora* (7.5 %). Eighteen (45 %) out 40 isolates produced phytohormone IAA, with 14 of these belonging to *Streptomyces*. The range of IAA production was 9.0–38.8 μg/ml, the maximum amount produced by a strain of *Streptomyces griseorubroviolaceus*, whereas a *Streptomyces cinereus* isolate produced the smallest amount of IAA. Nimnoi et al. (2010) isolated endophytic actinomycetes from *Aquilaria crassna* and found that *Nocardia jiangxiensis* produced the highest yield of IAA, at 15.14 μg/ml, and *Actinomadura glauciflava* produced the lowest, at 9.85 μg/ml. IAA-producing actinomycetes such as *Actinomadura*, *Micromonospora*, and *Streptosporangium* have been reported to increase the dry weight of corn, cucumber, tomato, sorghum, and carrot (Mishra et al. 1987; El-Tarabily et al. 1997; Gangwar et al. 2014).

Endophytic actinobacterial diversity in the native herbaceous plant species of Korea was analyzed using a culture-based approach by Kim et al. (2012), and four strains were found to be prominent IAA producers: *Streptomyces* sp. DF09-05, *Streptomyces* sp. GB09-03, *Streptomyces* sp., and *Micrococcus* sp. HW05-10. These types of studies demonstrate that there is clear need to include plant growth-promoting endophytic actinomycetes in programs aimed at utilizing microorganisms to enhance plant productivity at the field scale.

9.3.1.2 Phosphate Solubilization

Phosphates applied to agricultural soils are rapidly immobilized, rendering them inaccessible to plants (Rodríguez and Fraga 1999). In this context, the ability of microorganisms to release metabolites such as organic acids; through their hydroxyl and carboxyl groups, these acids chelate cations bound to phosphate, converting it to a soluble form is fundamental to phosphate solubilization (van der Heijden et al. 2008; Lacava and Azevedo 2013).

Among soil microorganisms, phosphate-solubilizing bacteria (PSBs) play an important role in solubilizing P for plants and allowing the more efficient use of P fertilizers (Gyaneshwar et al. 1998). This plant growth-promoting rhizobacteria can colonize root surfaces, and some have been shown to also colonize endophytically (Naher et al. 2009). The association and colonization of PSBs on the surface of roots involves direct competition with other rhizosphere microorganisms; in contrast, the endophytic population of PSBs may have more beneficial effects on plants due to reduced competition. Within this context, the endophyte offers several advantages over rhizobacteria; for example, as the endophyte is more closely associated with the plant, greater effects may be present in complementary niches of the endophyte and its host. Furthermore, the host plant provides a ready-made environment, offering endophytic bacteria protection from biotic and abiotic stresses compared to rhizobacteria (Newman and Reynolds 2005). Additionally, phosphate solubilization is a common trait among bacteria (Forchetti et al. 2007; Puente et al. 2009; Palaniappan et al. 2010). Phosphate solubilization by endophytes is also of plant growth promotion because endophytic bacteria are compatible with host plants and able to colonize host plant tissues without being perceived as pathogens (Rosenblueth and Martinez-Romero 2006; Lacava and Azevedo 2013).

Hamdali et al. (2008) assessed the different plant growth-promoting abilities of eight rock

phosphate (RP)-solubilizing actinomycete isolates originating from Moroccan phosphate mines. Six of these strains were able to grow on root exudates of wheat plants as the sole nutrient source and efficiently released soluble phosphate from RP. Four of these strains showed endophytic properties. When these strains were grown in the presence of wheat plants in a synthetic minimum medium (SMM) containing insoluble RP as the unique phosphate source or in a soil experiment, the most active RP-solubilizing strains had the highest stimulatory effect on plant biomass production. The most efficient strain, *Streptomyces griseus* (BH7), stimulated aerial growth of the plant by more than 70 % in test tubes and more in RP soil compared to the non-inoculated control treatment.

A total of 35 endophytic actinomycete strains were isolated from the roots, stems, and leaves of healthy wheat plants and identified as *Streptomyces* sp., *Actinopolyspora* sp., *Nocardia* sp., *Saccharopolyspora* sp., *Pseudonocardia*, and *Micromonospora* sp. (Gangwar et al. 2012). Seventeen isolates able to solubilize phosphate on National Botanical Research Institute–bromophenol blue (NBRI-BPB) medium were further evaluated for the amount of phosphate solubilized, which ranged from 5 to 42 mg/100 ml, with *Streptomyces roseosporus* W24 solubilizing the maximum amount of phosphate. These results are in accordance with earlier reports (Hamdali et al. 2008) in which a high amount of phosphate-solubilizing activity by *Streptomyces cavourensis* (83.3 mg/100 ml) was observed, followed by *S. griseus* (58.9 mg/100 ml) and *Micromonospora aurantiaca* (39 mg/100 ml) (Gangwar et al. 2012). Microbial solubilization of mineral phosphate might be either due to the acidification of the external medium or to the production chelating substances that increase phosphate solubilization (Whitelow 1999). Hence, P-solubilizing actinomycetes play an important role in improving plant growth (Gangwar et al. 2012).

A total of 36 endophytic actinomycetes, identified as *Streptomyces* sp., *Micromonospora* sp., and *Microbispora* sp., were isolated by Gangwar et al. (2015) from the roots, stems, and leaves of *Emblica officinalis* Gaertn (gooseberry). Of the 36 isolates, 16 (44.4 %) were found to solubilize phosphate, as a clear zone around the colony was formed on Pikovskaya medium. The amount of phosphate solubilized by the isolates ranged from 0.014 ± 0.005 to 0.45 ± 0.004 mg/ml, with the maximum amount of solubilization by *S. cinereus* AR3 (0.45 ± 0.004 mg/ml) followed by *Streptomyces griseofuscus* strain AL4 and one *Micromonospora* isolate, AR15 (0.38 ± 0.004 mg/ml each). These results were also in accordance with Gangwar and Kataria (2013), who reported that actinomycetes from medicinal plants were able to solubilize phosphorus in the range of 0.02–0.68 mg/ml, with *Streptomyces albosporus* A4 solubilizing the maximum amount (16.5 mg/100 ml). Accordingly, it has been suggested that these actinomycetes could be used as phosphate solubilizers (El-Tarabily and Sivasithamparam 2006; Gangwar et al. 2015).

9.4 Indirect Plant Growth Promotion

The biological control ability by endophytic bacteria by indirect growth promotion has grown with considerable interest in terms of development of understanding of the mechanisms that these bacteria use for biocontrol and to use these bacteria with the potential to produce biopesticides rather than the use of agrochemicals. In fact, these objectives are largely complementary; that is, understanding the mechanisms employed by biocontrol bacteria should facilitate the successful use of these microbial strains in applications (Glick 2012; Lacava and Azevedo 2014).

9.4.1 Endophytic Actinomycetes in the Biocontrol of Plant Disease

Endophytic actinomycetes have been isolated from a wide variety of host plants, and the most

frequently isolated species belong to the genera *Microbispora*, *Nocardia*, *Micromonospora*, and *Streptomyces* (Taechowisan et al. 2003). *Streptomyces* are prolific producers of antimicrobial compounds, like antibiotics, and endophytic *Streptomycetes* are no exception (Seipke et al. 2012). Numerous endophytic *Streptomyces* strains inhibit growth of phytopathogens by antagonisms, and this antibiosis has been proposed to be one of the mechanisms by which endophytes suppress plant disease (Sardi et al. 1992; Coombs and Franco 2003; El-Tarabily 2003; Taechowisan et al. 2003; Rosenblueth and Martinez-Romero 2006; Franco et al. 2007).

As stated above, actinomycetes, particularly members of the genus *Streptomyces*, produce antifungal compounds and can protect a range of plant species from phytopathogens (Knauss 1976; Bolton 1978; Lechevalier 1989). However, activities other than antibiosis have also been described for the actinomycetes in the biological control of phytopathogens. These include mycoparasitism of the phytopathogenic fungi *Pythium coloratum* Vaartaja by *Actinoplanes* spp. and *Micromonospora* spp. (Coombs et al. 2004).

Coombs et al. (2004) isolated endophytic actinomycetes from healthy plants and assessed them for their ability to control fungal phytopathogens of cereal crops both in vitro and *in planta*. In this study, 38 strains belonging to the genera *Streptomyces*, *Microbispora*, *Micromonospora*, and *Nocardioides* were assayed for antifungal compound production in vitro against *Gaeumannomyces graminis* var. *tritici*, the causal agent of take-all disease in wheat, *Rhizoctonia solani*, and *Pythium* spp. Sixty-four percent of this group of endophytic actinomycetes exhibited antifungal activity in vitro. The active endophytes included a number of *Streptomyces*, as well as *Microbispora* and *Nocardioides* spp., and were also able to control the development of disease symptoms in plants exposed to *G. graminis* and *R. solani* in field soil. These authors indicated that due to their ability to colonize the internal tissues of the host plant, endophytic actinomycetes may provide an advantage as biological control agents for use in the field, a site where other controls have failed.

El-Tarabily et al. (2009) evaluated the potential of endophytic actinomycetes isolated from cucumber candidates for biocontrol by screening for the production of cell wall-degrading enzymes including β-1,3, β-1,4, and β-1,6-glucanases and for antagonism toward *Pythium aphanidermatum* in vitro. The most promising antagonistic isolates were screened for their ability to protect cucumber from disease caused by *P. aphanidermatum*, one of the most important soilborne diseases, under greenhouse conditions. In this study, the results showed that three endophytic actinomycetes belonging to *Actinoplanes campanulatus*, *Micromonospora chalcea*, and *Streptomyces spiralis* species possessed the ability to reduce the impact of *P. aphanidermatum* disease in both seedlings and mature plants. Thus, Costa et al. (2013) evaluated the biodiversity of maize endophytic actinomycetes potential to control the phytopathogenic fungus *P. aphanidermatum*. Forty endophytic strains were isolated from healthy maize plants from several regions of Sao Paulo State, Brazil. The identification of these strains showed that most belong to the *Streptomyces* genus. Two strains were selected based on inhibition in vitro and then evaluated for the biological control of *P. aphanidermatum* in *Cucumis sativa* L. under controlled conditions at greenhouse. The endophytic strain 16R3B was able to reduce up to 71 % of damping-off incidence, whereas the strain 14F1D/2 reduced the disease incidence by 36 %. The strain 16R3B has been suggested for use in greenhouse cucumber production and to be tested in field trials.

R. solani is one of the most important soilborne fungal pathogen and is found in both cultured and non-cultured soils (Coa et al. 2004). Damping-off of seedlings is the most common disease caused by *R. solani* (Moussa 2002), which has a wide host range and causes disease in a variety of crops, such as lawn grass (Parmeter et al. 1969), tomato (Coa et al. 2004), cucumber (Coa et al. 2004), and sugar beet (Sadeghi et al. 2006). Several studies have reported the strains of actinomycetes for the

biocontrol of *R. solani* damping-off (Moussa 2002; Coa et al. 2004; Chung et al. 2005; Sadeghi et al. 2006; Patil et al. 2010). Morphological and chemical studies showed that 29 endophytic actinomycete strains isolated from native plants of the Algerian Sahara belong to the *Streptomyces*. The endophytic strains were tested for their in vitro antagonisms to *R. solani*. The six endophytic strains exhibiting the greatest pathogen inhibitory capacities were subsequently tested for their in vivo control of *R. solani* in tomato seedlings. The results indicated that the severity of disease, damping-off of tomato seedlings, caused by *R. solani*, reduced.

Many works have reported positive findings using endophytic actinomycetes species to control different plant pathogens (Bérdy 2000; Berg et al. 2001; Zucchi et al. 2008, 2010). In vitro tests are the most common strategy to screen a new candidate with potential for biological control or antagonistic activity against phytopathogens (Kunoh 2002). Antagonism is usually the most suitable approaches for screening antibiotic-producing organisms for further strategy of biocontrol and development of commercial product (Pliego et al. 2011; Costa et al. 2013). However, other mechanisms should be considered, such as parasitism of hyphae (El-Tarabily and Sivasithamparam 2006), oospores or fungal sclerotia (Crawford et al. 1993), competition with pathogens (Kunoh 2002), siderophores (Khamna et al. 2009), herbicides (Hasegawa et al. 2006), and enzymes such as cellulase, hemicellulase, chitinase, and glucanase (Yuan and Crawford 1995). Quecine et al. (2008) evaluated chitinase production by endophytic actinomycetes and their potential in the control of phytopathogenic fungi. In this study, the correlation of chitinase production by endophytic strains and phytopathogen inhibition by scanning electron microscopy on *Colletotrichum sublineolum* cell walls was confirmed.

9.4.1.1 Antagonism Against Phytopathogenic Microorganisms via Siderophore Production

All living organisms require iron for growth and for the proper function of metabolic pathways that are crucial to their survival. Iron is a cofactor for many enzymes, and in microorganisms that grow under aerobic conditions, iron is responsible for the reduction of oxygen during the synthesis of ATP and plays an important role in many bacteria-plant interactions (Neilands 1995; Van Vliet et al. 1998). Siderophores are low-molecular-weight iron sequestration molecules with a high affinity for their substrate and are secreted by microorganisms in response to a low availability of Fe3+ in solution. Although siderophores have a high affinity for iron by definition, many can also form relatively stable complexes with copper, aluminum, molybdenum, and certain other elements (Benite et al. 2002). These compounds function outside the cell membrane, capturing iron molecules in solution and binding to particular receptors located in the membrane; the compounds are then absorbed, rendering the iron available for plant growth (Quecine et al. 2014). The production of siderophores by microorganisms is beneficial to plants because it can inhibit the growth of plant pathogens (Etchegaray et al. 2004; Siddiqui 2005). Siderophores can offer resistance mechanisms in the host plant (Schroth and Hancook 1995). Plant growth promotion, including prevention of the deleterious effects of phytopathogenic organisms (Sharma and Johri 2003), can be achieved through the production of siderophores (Hayat et al. 2010). Endophytic bacteria colonize an ecological niche similar to that of plant pathogens, especially vascular wilt pathogens, which might favor them as potential candidates for biocontrol and growth-promoting agents (Ramamoorthy et al. 2001).

In addition to iron chelation, siderophore production is a mechanism by which endophytic biocontrol agents suppress pathogens indirectly by stimulating the biosynthesis of other antimicrobial compounds by increasing the availability of minerals to the biocontrol agent (Duffy and Defago 1999; Persello-Cartieaux et al. 2003). Most researchers have adopted the premise that the production of siderophores is beneficial based on their biological control of plant pathogens and their positive effect on plant nutrition (Quecine et al. 2014). Within this context, Vendan

et al. (2010) suggested that siderophore production may be a common phenotype. Several recent studies have demonstrated that endophytic actinomycetes produce compounds such as IAA and siderophores in vitro (de Oliveira et al. 2010; Ghodhbane-Gtari et al. 2010; Nimnoi et al. 2010). For example, Nimnoi et al. (2010) examined siderophores by endophytic actinomycetes isolated from eaglewood. Eight of them were found to produce siderophores in culture broth (Shimizu 2011).

Streptomyces sp. strain S96, an endophytic actinomycete isolated from banana by Cao et al. (2005), is an example of the possible association of siderophore production with biocontrol activity. The treatment of roots with this endophytic actinomycete was effective at protecting banana plantlets from infection by *Fusarium oxysporum*. The authors assumed that siderophore production by the strain could reveal its biocontrol potential. However, further work using mutant strains lacking siderophore activity is necessary to provide more evidence (Shimizu 2011). In a recent study of the diversity of endophytic actinomycetes from wheat and their potential as plant growth-promoting and biocontrol agents, Gangwar et al. (2012) described 19 isolates that produced a catechol type of siderophore, ranging between 1.3 and 20.32 μg/ml. Additionally, a hydroxamate-type siderophore was produced by nine isolates in the range of 13.33–50.66 μg/ml. The maximum production of the catechol type of siderophore was observed in *S. roseosporus* W9 (20.32 μg/ml), which also displayed the greatest antagonistic activity against different pathogenic fungi. Also, Gangwar et al. (2015) reported that 13 isolates produced the catechol type of siderophores, and 15 isolates produced hydroxamate type of siderophores. *S. griseofuscus* AL5 produced the highest amount of catechol-type siderophore (55.6 ± 0.4 μg/ml), whereas the greatest production of the hydroxamate type was observed in *Streptomyces griseorubroviolaceus* AR3 (88.6 ± 0.7 μg/ml). Additionally, in this same study, endophytic isolates were found to be active against the phytopathogenic fungus.

9.4.2 Endophytic Actinomycetes in the Biocontrol of Insect Pests

Actinomycetes possess insecticidal activity against different insects, such as house fly (*Musca domestica*), mosquito (*Culex quinquefasciatus*), fruit fly (*Drosophila melanogaster*), and cotton leaf worm (*Spodoptera littoralis*), with varying larval mortality (Ghazal et al. 2001; Gadelhak et al. 2005; Osman et al. 2007; Dhanasekaran et al. 2010). Actinomycetes are also reported to play an important role in the biological control of various insects through production of insecticidal compounds and chitinase enzymes (Bream et al. 2001; Reguera and Leschine 2001; Hussain et al. 2002). However, there are few reports describing the insecticidal properties of endophytic strains against plant pests (Bream et al. 2001; Xiong et al. 2004; Osman et al. 2007). The actinomycetes can produce enzymes and antibiotics for biotechnological applications such as eco-friendly crop protection, among which the *Streptomyces* genus is particularly efficient in chitinolytic enzyme production (Bhattacharya et al. 2007; Quecine et al. 2008). There are a wide variety of chitinases studied, as well as their optimal temperature and pH for potential biocontrol of insect-pest (Kramer and Muthukrishnan 1997).

Quecine et al. (2011) reported the characterization of a chitinolytic extract produced by a *Streptomyces* sp., isolated from citrus plant as an endophytic actinobacteria. The extract produced by this strain was also tested against the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), an important insect-pest from cotton (Quecine et al. 2011). The extract was partially characterized and showed an optimum temperature of 66 °C and an optimum pH between 4 and 9 (80 % of relative activity). The chitinolytic extract was added to an artificial boll weevil diet, resulting in prolonged development from the egg stage to the adult stage, with the percentage of adults emerging being approximately 66 % less than on the diet (Quecine

et al. 2011). This study showed that the larval development of this major insect-pest that affects cotton production in the Americas could be inhibited by the presence of the chitinolytic extract (Martins et al. 2007, 2008). Although *A. grandis* is controlled with agrochemical agents, these pesticides are expensive and can bring ecological risk such as disruption of predator and parasitoid populations (Burton 2006; Wolkers et al. 2006).

9.5 Concluding Remarks

Endophytic microorganisms are believed to elicit plant growth in many ways: by helping plants to acquire nutrients, e.g., via nitrogen fixation, phosphate solubilization, and iron chelation, preventing infections via antifungal agents, outcompeting pathogens for nutrients by producing siderophores, establishing plant systemic resistance, and producing phytohormones. Although all of the approximately 300,000 plant species documented to date have been estimated to harbor one or more endophytes, few relationships between plants and endophytes have been studied in detail; legume-rhizobia symbiosis and associations between fungi and plant root (mycorrhizae) are exceptions (Lacava and Azevedo 2013). A number of endophytic actinomycetes inhabit the tissues of a wide variety of native and cultured crop plants. However, *in planta* microfloras are diverse, and complicated associations of endophytic actinomycetes with host plants and/or other endophytes remain poorly understood. Nevertheless, some are undoubtedly beneficial to the host plant: the endophytic presence of some actinomycetes may play an important role in plant development and health because of their role in nutrient assimilation and in secondary metabolite production (Shimizu 2011). Endophytic actinomycetes are natural resources that are effective and reliable for use in agriculture and are a sustainable biotechnology resource for securing and improving grain yields. This technology is being commercialized and opens a new paradigm that can also be applied to increase pasture production, horticulture, and floriculture (Franco 2010). Research in this field is clearly very promising, with significant economic and environmental impacts in the future (Quecine et al. 2014).

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Synergy of Actinomycete Co-inoculation 10

M. Solans, G. Vobis, L. Jozsa, and L.G. Wall

Abstract

Historically the symbioses between leguminous plants and rhizobia have attracted the attention of researchers due to the incidence of legumes for sustaining nutritional requirements to humans and animals. There have been large efforts to increase the ability to symbiotic N_2 fixing and productivity of legumes. New research is focusing on increasing the legume–rhizobia symbiosis with increased biological nitrogen fixation (BNF), growth, and productivity. The inoculation of legumes with rhizosphere bacteria has often been found to increase symbiotic properties, plant biomass, and yields under greenhouse or field conditions. The potential to enhance plant growth, nodulation, nitrogen fixation, productivity of legumes by plant growth-promoting rhizobacteria (PGPR), and *Rhizobium* co-inoculation does exist, although most of studies have been conducted with *Bacillus* spp., *Pseudomonas* spp., or other genera and few with actinomycetes. The latter, a group of actinobacteria widely distributed in terrestrial ecosystems contribute to soil nutrient cycling and live in association with plants and are considered as one of the most important communities in the rhizosphere. They have a great ability to synthesize a series of bioactive metabolites and potential within the agroecosystem, where they play important roles in disease suppression and plant growth promotion in cultivated plants. In this sense, the purpose of this chapter is to show the synergistic effect of actinomycete co-inoculation on N_2-fixing symbioses and their potential use in agriculture.

Keywords

Actinomycetes • Co-inoculation • Plant growth promotion • Helper bacteria • Symbiosis • Crop yields • Legumes

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10.1 Introduction

Ever since antiquity (Theophrastus 372–287 BC), there has been an interest in the issue of increasing plant growth and improving soil fertility (Tisdale and Nelson 1975). In 1888, Hellriegel and Wilfarth investigated the rhizosphere root colonization in grasses and legumes and suggested the ability of soil bacteria to convert atmospheric N_2 into plant usable forms. Later, Kloepper and Schroth (1978) introduced the term "rhizobacteria" to the soil bacterial community that competitively colonized plant roots and stimulated growth, thereby reducing the incidence of plant diseases. Then, they termed these beneficial rhizobacteria as plant growth-promoting rhizobacteria (PGPR). They are beneficial bacteria living in the rhizosphere and have positive effects on plant growth promotion and/or preventing infection by root (Kloepper and Schroth 1981; Bhattachryya and Jha 2012). Recently, Jeffries et al. (2003) reported the benefit of the interactions in the rhizosphere, especially arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility (Franco-Correa et al. 2010) in an eco-friendly way of agricultural practices (do Vale Barreto Figueiredo et al. 2010). Among the microbial groups, PGPR, arbuscular mycorrhiza (AM), cyanobacteria, and actinomycetes are rhizospheric microbes that synthesize various biomolecules that stimulate plant growth and/or prevent them of disease (Glick 1995; Schrey and Tarkka 2008). Barea et al. (2005) showed a microbial cooperation in the rhizosphere, involving interactions to affect plant fitness and soil quality. These beneficial microorganisms may include diverse natural populations that can be applied as inoculants to enhance the soil microbial diversity, plant growth, health, yield, and quality of soil. These microbial populations may consist of selected species of microorganisms including PGPR, N_2-fixing cyanobacteria, plant disease-suppressive bacteria and fungi, soil toxicant-degrading microbes, actinomycetes, and other useful microbes (Singh et al. 2011). Investigations have shown that the plant growth, nodulation by N-fixing bacteria (rhizobia and non-rhizobia), and mycorrhizal development are promoted by certain rhizobacteria (Dahsti et al. 1998; Bai et al. 2002a), including actinomycetes (Nadeem et al. 2013). Furthermore, it has reported that co-inoculation and coculture of microbes have better ability to fulfill the task in an efficient way than single-strain inoculation (Guetsky et al. 2002). The co-inoculation of *Rhizobium* with PGPR proved useful for promoting growth and increasing nodulation (Tilak et al. 2006). Legume growth and yield are increased by some soil bacteria such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, and actinomycetes, among others, when used in combination with rhizobia as co-inoculants (Mehboob et al. 2013; Nadeem et al. 2013).

Recently, research is focusing on increasing the legume–rhizobia symbiosis with increased biological nitrogen fixation (BNF), growth, and productivity. The inoculation of legumes with rhizosphere bacteria has often found to increase symbiotic properties, plant biomass, and yields under greenhouse or field conditions. The potential of PGPR for improving growth and yields of various crops has been extensively documented. Generally speaking, PGPR are able to improve plant growth by themselves as single inoculant while can add positive effect or induce synergism when co-inoculated with other beneficial bacteria. However, most of studies have been conducted with *Bacillus* spp., *Pseudomonas* spp., or other genera and few with actinomycetes. This group of actinobacteria is the most widely distributed as saprophytic soil inhabitants and is also considered as one of the important communities in rhizosphere microbiota, which has a high ability to produce various bioactive substances such as antibiotics, enzymes, hormones, and antifungal metabolites (Goodfellow and Cross 1974; Takana and Omura 1990; Tokala et al. 2002; Gregor et al. 2003; Solans et al. 2011; Bhattachryya and Jha 2012). The synergist effect of actinomycete co-inoculation on N-fixing symbioses and

their potential use in agriculture is discussed in this chapter.

10.2 Importance of Actinomycetes

Actinomycetes (phylum *Actinobacteria* in the sense of Goodfellow 2012) are a very important group present in the soil microbiota. In soil, actinobacteria represent a high proportion of the microbial biomass. Their populations are found at 10^6–10^9 bacteria g^{-1}, and they represent more than 30 % of total population of soil microorganisms. Actinobacteria represent a large fraction of microbial populations in the root systems and is well established that they are dominant fraction of the microbial community in soils of wild and agricultural plant species. Together with other phyla, they account for a large proportion in the rhizosphere of numerous plants (Bouizgarne and Ben Aouamar 2014). Actinomycetes represent a very heterogeneous group of Gram-positive bacteria, aerobic, frequently mycelium forming, with significant ecological functions in soil nutrient cycling (Ames et al. 1984; Nonomura 1989; Halder et al. 1991; Elliot and Lynch 1995; Franco-Correa et al. 2010; Bhattachryya and Jha 2012).

Within the soil microbiota, the actinomycetes are an important group, which produce over 70 % of the bioactive compounds that are used in the pharmaceutical industry, agriculture, and the environment (Bérdy 2005). The saprophytic actinomycetes are known as common bacteria that colonize the rhizoplane and rhizosphere and are involved in diverse physiological processes such as the decomposition of plant and animal material, as well as biological control of fungal plant diseases (Crawford et al. 1993; Vobis and Chaia 1998; Solans and Vobis 2003; Strap 2011; Selvakumar et al. 2014). They are also considered as important producers of bioactive secondary metabolites and extracellular enzymes (Goodfellow and Cross 1974; Takana and Omura 1990; Bérdy 2005). Actinomycetes are the most widely distributed group as saprophytic soil inhabitants (Takisawa et al. 1993). They are known to be common rhizoplane- and rhizosphere-colonizing bacteria (Solans and Vobis 2003; Frioni 2006), which have a high capacity to produce several types of extracellular enzymes to degrade complex macromolecules (McCarthy 1989), playing an important role in the decomposition of recalcitrant biopolymers of plant waste (McCarthy and Williams 1992). Actinomycetes not only degrade biopolymers but also starch, cellulose, hemicellulose, pectin, lignin, lignocellulose and keratin (Solans and Vobis 2003), and humus and chitin (Semèdo et al. 2001). At the end of the degradation processes, the products are again available in the soil (Vobis and Chaia 1998). Actinomycetes utilize a wide range of carbon sources, degrade complex polymers such as lignin, and possess advantageous characteristics of fungi, i.e., mycelial growth, production of spores resistant to drought, and production of enzymes (McCarthy and Williams 1992; April et al. 2000).

Historically, actinomycetes have been the origin of the largest number of a new antibiotic drug candidates and lead molecules with applications in many other therapeutic areas (Genilloud et al. 2011). They have long gained significance in the agro-environment due to their ability to produce a wide range of antibiotic molecules that suppress the growth and development of a wide range of soil plant pathogens, and some of them impede the growth of plant pathogenic organisms by the production of high levels of extracellular lytic enzymes such as the chitinase and the glucanase (Selvakumar et al. 2014). Members of the genus *Streptomyces* produce useful compounds, notably antibiotics, enzymes, enzyme inhibitors, and pharmacologically active agents (Bérdy 2005; Khamna et al. 2009; Zhao et al. 2011). Besides a wide metabolic versatility, they may represent an underexplored reservoir of novel species of potential interest in the discovery of new compounds useful for agricultural technology and pharmaceuticals industry (Qin et al. 2011). For these reasons, the actinomycetes are good candidates for application in agriculture where they could play an important role in plant health by acting as PGPR and as the main

microorganisms implicated in controlling the infection of roots by soil-borne pathogenic fungi and bacteria.

10.3 Synergism of Co-inoculation

Conventionally, selection of persistent, efficient, and competitive rhizobial strains is used to increase the nodulation and nitrogen fixation. However, the use of the best *Rhizobium* strains has no guarantee for maximum productivity of legumes (McLoughlin et al. 1985; Gupta et al. 1998); it prompts the need to investigate how one can increase the growth and effectiveness of rhizobia and the plant productivity (Mehboob et al. 2013). Therefore, the use of beneficial rhizosphere bacteria together with rhizobia has been found to be an alternative effective approach in the last years to get effective nodules and a successful BNF (Janisiewicz 1996; Pankaj et al. 2011; Mehboob et al. 2013). This results in eco-friendly improved growth and productivity of legumes (Requena et al. 1997).

In this sense, the new fashion in agriculture is the use of microbial consortiums of plant growth-promoting bacteria (PGPB), including rhizobia. Thus, these exogenous bacteria, introduced in agricultural systems, would act positively on plant growth (Castro-Sowinski et al. 2007; Morel et al. 2012). It is possible to enhance yield crop and suppress or decrease the use of chemical fertilizers and pesticides (Adesemoye et al. 2009; Vessey 2003; Singh et al. 2011) even in marginal soils (Gamalero et al. 2009) when the formulation contains different PGPB.

In co-inoculation, the co-inoculants interact synergistically or function as "helper" bacteria to improve the performance of other beneficial microorganisms (Mehboob et al. 2013). However, diverse studies have shown that the growth-promoting capacity of co-inoculants can be affected by various factors such as the inoculant's strain specificity, strain inherent potential and genotype, the cell density of applied inocula or optimal inoculation dose, strain's effectiveness, composition of root exudates of host plant, and temperature variation or interaction of applied inocula with rhizospheric microflora predominant in the particular crop (Sindhu et al. 2002; Mehboob et al. 2013). Furthermore, it has been reported by various authors that inoculation with beneficial microorganisms of the rhizosphere not only promotes nodulation and N uptake and consequently the yield in legumes and also improves the absorption of other important mineral as P (Zaidi et al. 2003). The combination of PGPR with *Rhizobium* species is a valuable, relevant, profitable, efficient, and environmentally friendly tool, which increases the reliability of inocula, increasing growth and productivity, under different conditions, as they have a wide range of mechanisms, without genetic manipulation. In the following sections, examples of the effects of actinomycete co-inoculations are described in different symbiotic systems.

10.4 Synergistic Effect of Actinomycetes in Symbioses

N_2 fixation is the first step for cycling N to the biosphere from the atmosphere, a key input of N for plant productivity (Vance 2001). The bacteria responsible belong to 14 genera of α-proteobacteria, including the best-known *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Azorhizobium*, collectively termed rhizobia, and 3 genera of β-proteobacteria (Weir 2012). These bacteria interact with legume roots leading to the formation of N_2-fixing nodules (Spaink et al. 1998; Sprents 2002). Other bacteria, actinobacteria of the genus *Frankia*, also form N_2-fixing root nodule in actinorhizal plants, which are of great ecological importance (Vessey et al. 2004).

The other major group of microbial plant symbionts is the fungi which form the mycorrhizal symbiosis with the roots of most plant species. Mycorrhizal symbioses improve plant fitness and soil quality through diverse ecological processes, benefiting growth and plant health (Barea et al. 2005). Because of these qualities, mycorrhizas have received great interest in agronomic systems to be incorporated into the field as

mycorrhizal fungi, native or introduced, can benefit various crops, such as cereals, legumes, vegetable crops, temperate fruit trees or shrubs, tropical plantation crops, ornamentals, and spices (Azcón-Aguilar and Barea 1997; Vestberg et al. 2002; Barea et al. 2005).

Several reports show the improvement of legume symbiosis and mycorrhizal symbiosis in dual inoculations with diverse PGPR (Barea et al. 2005; Schrey and Tarkka 2008), although there is less information on this subject with actinomycetes. However, it was observed that nitrogen-fixing symbioses between plants and actinobacteria can be promoted by actinomycetes (Tokala et al. 2002; Solans 2007; Solans and Vobis 2013). They are attractive because their secondary metabolites might be promising sources of novel antibiotics and growth regulators for other organisms (Matsukuma et al. 1994; Okazaki et al. 1995). Actinobacteria have been shown to have beneficial effects on the nodulation and plant growth of legumes and nonlegume plants, which will be presented in the following sections.

10.4.1 Mycorrhizal Symbiosis

Another ecologically important group in the soil is the arbuscular mycorrhizal fungi that form symbioses in most plants, which are influenced and influence other soil microorganisms, fulfilling a critical role in agricultural sustainability (Bagyaraj 1984; Jeffries et al. 2003; Barea et al. 2005; Rillig et al. 2006; Lehr et al. 2008; Franco-Correa et al. 2010). The symbiotic establishment of mycorrhizal fungi on plant roots is affected in various ways by other rhizospheric microorganisms and more especially by bacteria. Some of these bacteria, which consistently promote mycorrhizal development, lead to the concept of "mycorrhization helper bacteria" (MHB) (Garbaye 1994). To date, many bacterial strains have been reported to be able to promote either arbuscular or ectomycorrhizal symbiosis (Garbaye 1994; Barea et al. 2002, 2004; Duponnois 2006). The MHB strains which were identified belong to diverse bacterial groups and genera, such as *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Bacillus*, and *Streptomyces*, among other bacterial genera, and showed the role of MHB in the mycorrhizal symbiosis from different points of view (Frey-Klett et al. 2007). Schrey and Tarkka (2008) showed that the *Streptomyces* genus promotes the formation of symbioses between plant roots and microbes, and this is in part due to their direct positive influence on the symbiotic partner, expressed as, e.g., promotion of hyphal elongation of symbiotic fungi. Mycorrhiza formation is promoted by MHB (Garbaye 1994; Frey-Klett et al. 2007), including several actinomycete species: *Rhodococcus* sp. (Poole et al. 2001) and *Streptomyces* sp. (Abdel-Fattah and Mohamedin 2000; Schrey et al. 2005). Abdel-Fattah and Mohamedin (2000) showed that the roots of sorghum and arbuscules had higher mycorrhizal colonization were those plants grown in soil inoculated with *Streptomyces coelicolor* compared with untreated mycorrhizal plants. These results support the idea of the potential use of actinomycetes to increase the development of symbiosis. Franco-Correa et al. (2010), showed that co-inoculation of actinomycetes and *Glomus mosseae* produced synergic benefits on plant growth and P acquisition by *Streptomyces* spp. MCR9 and MCR24. The selected actinomycetes improved AM formation in clover plants. The reported experiments show that the target actinomycete strains are able to improve plant growth and nutrition and benefit root colonization by AM fungi. Co-inoculation with both types of microorganisms showed synergic effects at enhancing plant growth and nutrient acquisition. Here reported results support the use of actinomycetes as plant growth-promoting and mycorrhiza helper bacteria.

10.4.2 Actinorhizal Symbiosis

The term actinorhizal refers to the root nodule symbioses between the nitrogen-fixing actinomycete *Frankia* and about 220 angiosperm species of woody, nonlegume plants (Huss-Danell

1997). Among actinorhizal symbioses, three of the eight host families (Betulaceae, Casuarinaceae, and Myricaceae) are nodulated by *Frankia* via intracellular infection pathway. In five of the families (Elaeagnaceae, Rhamnaceae, Rosaceae, Datiscaceae, and Coriariaceae), early nodule initiation occurs via intercellular colonization (Wall and Berry 2008). There are some studies in actinorhizal plants with *Pseudomonas cepacia* and *Bacillus* spp. that are known as PGPR or helper bacteria stimulating the nodulation and growth of *Alnus* spp. (Knowlton and Dawson 1983; Probanza et al. 1996, 1997). In recent times, the actinomycetes were gaining importance as inhabitants of the rhizosphere and as endophytes of many plants of interest and other non-*Streptomyces* genera, such as *Micromonospora* as endophyte of root nodules, and in actinorhizal and leguminous plants (Ghodhbane-Gtari et al. 2010; Trujillo et al. 2006, 2007).

10.4.2.1 Synergistic Effect of Actinomycetes on *Ochetophila trinervis–Frankia* Symbiosis

Ochetophila trinervis (Gillies ex Hook. & Arn.) Poepp. ex Miers, for many years known as *Discaria trinervis* (Kellerman et al. 2005) (family Rhamnaceae), is a native actinorhizal plant from South America (Tortosa 1983). In northwest Patagonia, it grows along watercourses. *O. trinervis* plants are nodulated by the nitrogen-fixing actinomycete *Frankia*, and this interaction is an example of an actinorhizal symbiosis (Chaia 1998; Wall 2000), with intercellular root invasion and an infection pathway (Valverde and Wall 1999) that implies no root hair deformation process. Although this symbiosis is well studied, little is known about the interaction with other actinomycetes of the rhizosphere. For this reason, we studied the effect of rhizosphere actinomycete co-inoculation on this symbiosis. In these studies, the helper effect could be demonstrated in experimental assays under controlled conditions, using *O. trinervis* plants growing in tube, pot, and pouch systems, and inoculated with *Frankia* and co-inoculated with saprophytic actinomycete strains (Solans 2008). It could be observed that saprophytic strains *Streptomyces* MM40, *Actinoplanes* ME3, and *Micromonospora* MM18 act as helper bacteria on both actinorhizal (Solans 2007) and rhizobial N_2-fixing symbioses (see next section). These strains clearly produce phytohormones (Solans et al. 2011) and have enzymatic activity for cellulose, hemicellulose, pectin, and lignocellulose (Solans and Vobis 2003), but the real responsible metabolites are still unknown.

10.4.3 Rhizobial Symbioses

Plant co-inoculation with rhizobia and other PGPR received considerable attention for legume growth promotion (Zhang et al. 1996; Bai et al. 2002a, b; Cassán et al. 2009). Results from many studies concerning the effect of co-inoculation on legume growth are summarized by Morel et al. (2012). Several genera of bacteria have been identified as "helpers" of the rhizobia–legume symbiotic process (Beattie 2006), including actinomycete genera (Solans and Vobis 2013). It can be said that there is great potential of PGPR to improve legume–rhizobia symbiosis under different conditions, although, more studies are needed on this interaction. Research has proposed different mechanisms involved in the PGPR–rhizobia–legume interaction, and these were described by Morel et al. (2012) and Mehboob et al. (2013). Based on our previous results on the nitrogen-fixing actinorhizal symbiosis by *O. trinervis*, we investigated the effect of these helper actinomycetes on co-inoculation of different legume–rhizobia symbioses: forage and grain legumes. Graham and Vance (2003) summarized the importance and uses of legumes in different natural and agricultural environments, such as the nutritional importance of forage legumes (alfalfa, clover, birdsfoot trefoil, vetch) and grains (Morel et al. 2012). Forage legumes are important sources of protein, fiber, and energy, rich in calcium and phosphorus, while the grain

legumes have a high protein and oil content in seeds, and are rich in essential amino acids (Graham and Vance 2003).

10.4.3.1 Synergist Effect of Actinomycetes on *Medicago sativa–Sinorhizobium meliloti* Symbiosis

Among the legumes, alfalfa (*Medicago sativa*) is the largest input of forage species in Argentina, and the world, due to the spread of cultivation, the quality and palatability of forage provided, and the ability to grow in a wide range of climatic and soil conditions. Saprophytic actinomycetes with promoting nodulation effect on *O. trinervis–Frankia* symbiosis were studied in the legume system, *Medicago sativa–S. meliloti* (Solans et al. 2009). In these assays, the plants co-inoculated with actinomycetes and rhizobium showed an increase of nodulation and plant growth compared with plants with single inoculations, under low level of N fertilization (0.07 mM), while high levels of N (7 mM) that inhibit nodulation by rhizobia, surprisingly the stimulation of nodulation, was observed in co-inoculated plants. These results showed that the interaction of actinomycetes could interfere with the autoregulation of nodulation in alfalfa mediated by high N. Beneficial effect of actinomycetes on growth and nodulation occurs only in alfalfa–*Rhizobium* symbiosis, since no effect was observed when inoculated alone. These results show the potential use of actinomycetes in plants of agronomic interest. Some endophytic actinomycetes have the potential to enhance the lucerne symbiosis compared to plants treated with *Rhizobium* alone, similar to what we observed with rhizospheric actinomycetes in our study (unpublished).

10.4.3.2 Synergistic Effect of Actinomycetes on *Lotus tenuis–Mesorhizobium loti* Symbiosis

Besides alfalfa, as forage species, *Lotus* is used for basic studies and as an alternative model to traditional limited cultivation of fodder in Argentina. In our country, studies about *Lotus* are related to the Salado River basin in the province of Buenos Aires and are mainly referred to *Lotus tenuis* Waldst et Kit (Estrella et al. 2009; Escaray et al. 2012). This species of legume is widely accepted and cultivated by livestock producers in the Flooding Pampa region (Cahuepé 2004; Coria et al. 2005) due to its nutritional value, high productivity, natural resistance, and adaptation to soils in this area, which are characterized by waterlogging and alkalinity (Vignolio and Fernandez 2006; Paz et al. 2012). The genus *Lotus* has peculiar characteristics as a legume, such as a high tannin content that prevents bloat. It responds to small applications of fertilizer, so it is considered an alternative forage production, cheaper than alfalfa, becoming more competitive production of meat or milk in some systems. Part of the success of *L. tenuis* as naturalized species in the Flooding Pampa region is related to their ability to form symbiotic associations with nitrogen-fixing bacteria *M. loti* (Sannazzaro et al. 2011) and also to its interaction with PGPR such as *Azospirillum* sp. Previous results obtained in the IIB-INTECH (Instituto de Investigaciones Biotecnológicas, Instituto Tecnológico de Chascomús) indicate the existence of PGPR strains as *Azospirillum* spp. associated to rhizosphere of *L. tenuis* (Cassán et al. 2003). Associating with PGPR and their metabolic products could result in better implementation and establishment of *Lotus* and more efficient use of soil mineral sources. However, there is very few or none information in relation to saprophytic bacteria different to *Azospirillum*, such as actinomycetes in the rhizosphere of *L. tenuis* plants. Therefore, we studied the influence of actinomycetes on *L. tenuis* (Solans et al. 2015). *L. tenuis* plants co-inoculated with *M. loti* and saprophytic actinomycete strains (MM40, ME3, MM18) showed a promoting effect on nodulation and biomass. Also, under high N level, the co-inoculated plants presented root nodules, even under these levels of fertilization (inhibitory for nodulation by rhizobia), similar to alfalfa under the same conditions and with the same actinomycete strains (Solans et al. 2009). The effect of actinomycete co-inoculation on

germination and early development was evaluated in *Lotus*, using the same strains, previously studied (Table 10.1). In general, the greatest co-inoculation effect was observed in the triple co-inoculation with the three actinomycetes together, showing a synergistic effect on the early development by *M. loti*. In general, a positive effect of co-inoculation with actinomycete strains could be observed in *L. tenuis* plants growing in pouches system (Table 10.2). This is a "helper" effect of actinomycetes on *Rhizobium* in *L. tenuis–M. loti* symbiosis.

Also, the co-inoculated plants developed flowers, whereas the single-inoculated plants with *Mesorhizobium* did not. A remarkable effect was the flower production in some plants (Fig. 10.1), mainly in those co-inoculated in triple or double combination with actinomycetes (RSAM and RSA). Only RSAM treatment presented 100 % of flowering, and the rest presented values between 17 % (RS, RM) and 33 % (R, RSA). This effect on flowering in co-inoculated *Lotus* plants was not observed in previous studies in alfalfa or *Ochetophila* plants. However, in preliminary assays with other strain of rhizobia (*M. loti* MAFF 303099) and with the same actinomycetes, the co-inoculated plants developed flowers in RS, RA, and RSAM treatments (Fig. 10.1).

The synergistic effect on nodulation and plant growth of N-fixing symbioses by the actinomycete co-inoculation showed here is expressed on nodulation level upon a preexisting symbiotic plant–microbe interaction, as we already found in our other studies with *O. trinervis*, alfalfa, and *Lotus* plants. Also, the phenomenon of stimulation of nodulation by actinomycetes appeared to be independent of the infection pathway and of the nodule developmental program.

Table 10.1 Effect of actinomycete co-inoculation on early development of *L. tenuis* growing in pots after 15 days

Treatments	Shoot length (cm)	Root length (cm)	Nodule number ($plant^{-1}$)	Shoot dry weight (mg)	Root dry weight (mg)	% Nodulation
R	2.5 (0.6)a	4.1 (1.1)a	1.8 (1.3)a	3.3 (0.6)a	2.9 (1.2)a	40
RS	2.9 (0.8)a	6.3 (1.2)a	4.6 (2.9)a	4.4 (1.6)a	3.5 (1.1)a	60
RA	7.2 (0.3)b	9.4 (0.6)b	9.4 (0.7)b	9.9 (0.6)bd	6.2 (0.4)b	100
RM	4.9 (1.6)ab	5.8 (1.4)a	2.6 (1.1)a	7.3 (2.4)cd	4.6 (0.9)b	60
RSAM	8.0 (0.4)b	12.0 (0.8)b	10.4 (0.7)b	11.4 (0.8)b	4.7 (0.3)b	100

R *Mesorhizobium loti* NZP2213 as rhizobia, *RS* rhizobia + *Streptomyces* MM140, *RA* rhizobia + *Actinoplanes* ME3, *RM* rhizobia + *Micromonospora* MM18, *RSAM* rhizobia + *Streptomyces* + *Actinoplanes Micromonospora*. Values represent mean (SE), $n = 7$. Different letters in the same column denote significant differences ($p \leq 0.05$)

Table 10.2 Effects of co-inoculation with rhizoactinomycetes on growth and nodulation of *Lotus* plants growing in pouches after 11 weeks

Treatments	Shoot length (cm)	Root length (cm)	Nodule number (p/plant)	Shoot dry weight (mg)	Root dry weight (mg)	Nodule dry weight (mg)
R	16.2(1.7)a	14.2(1.1)a	11.6(1.7)a	72.6(13.3)a	31.0(4.1)a	6.7 (1.1)a
RS	26.9(1.3)b	17.5 (1.1)abc	22.8 (3.0)b	200 (19.9)b	74.6(7.9)b	15.1(1.4)b
RA	25.9(0.8)b	18.5 (0.5)c	29.2 (2.3)b	243 (14.6)b	77.4(6.7)b	18.5(0.9)b
RM	21.7(1.8)ab	16.5 (1.2)ab	14.7 (1.8)ab	137.5(14.8)ab	58.7(6.4)ab	12.2(1.4)ab
RSAM	22.9(2.2)b	14.8 (1.3)bc	23.4 (4.3)b	245.5(21.8)b	89.6(7.2)b	19.4(1.6)b
RSA	26.7(1.7)b	16.4 (0.8)bc	25.6 (3.1)b	239.7(23.7)b	73.3(7.3)b	16.4(1.6)b

*R Mesorhizo*bium *loti* NZP2213 as rhizobia, *RS* rhizobia + *Streptomyces* sp., *RA* rhizobia + *Actinoplanes* sp., *RM* rhizobia + *Micromonospora* sp., *RSAM* rhizobia + *Streptomyces* + *Actinoplanes* + *Micromonospora* spp., *RSA* rhizobia + *Streptomyces* + *Actinoplanes* spp. Values represent means (SE), $n = 7$. Different letters in the same column denote significant differences ($p \leq 0.05$)

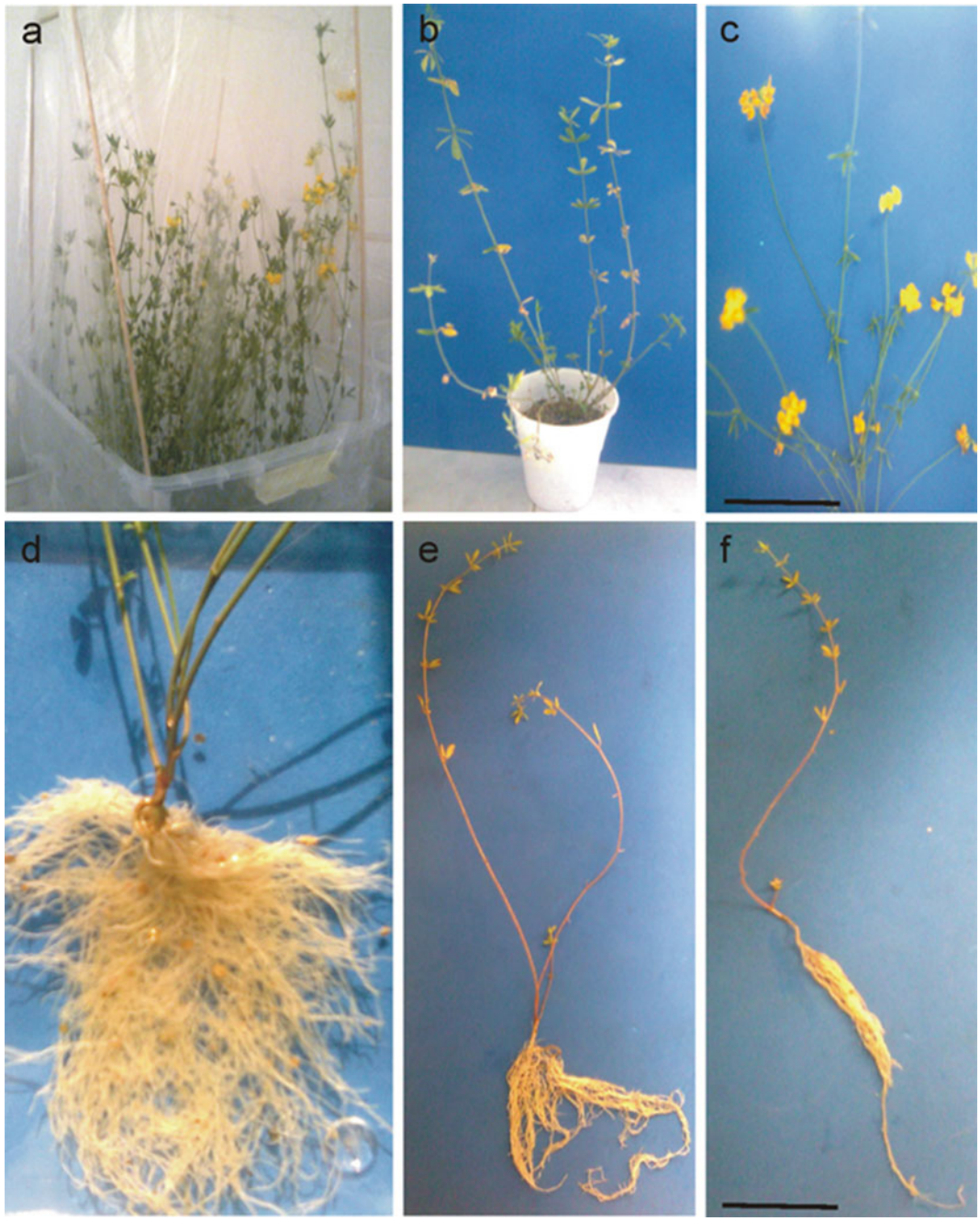

Fig. 10.1 Development of flowers in *L. tenuis* plants inoculated with actinomycetes grown in pots during 11 weeks post-inoculation. (**a**) Plants grown in culture chamber. (**b**) Plant grown in pot. (**c**) Flower development in plant co-inoculated with *M. loti* + *Streptomyces* + *Actinoplanes* + *Micromonospora* (*RSAM*). (**d**) Roots with nodules in co-inoculated plant. (**e**) Control plant without inoculum. (**f**) Plant inoculated with actinomycetes alone. Scale: (**a–c**) 10 cm; (**d–f**) 3.5 cm

10.4.3.3 Synergistic Effect of Actinomycetes on Soybean–*Bradyrhizobium japonicum* Symbiosis

The soybean (*Glycine max*) is an annual summer legume native of Southeastern Asia, which is used as human food and livestock feed as well as for several industrial purposes (Liu 1999; Ali 2010). This legume is one of the main crops cultivated for oil extraction, fish food, and very important as biodiesel oil (Morel et al. 2012). The utilization of actinomycetes as potential soybean co-inoculants was evaluated by Gregor et al. (2003). The combination of *Streptomyces kanamyceticus* and *B. japonicum* increased nodulation and shoot nitrogen composition of soybean by up to 55 % and 41 %, respectively (Gregor et al. 2003). These results show that no relationship exists between nodule number and nodule occupancy or shoot N content, but that co-inoculation with *S. kanamyceticus* may improve the occupancy of an applied strain. The problem of successful soybean inoculation by an applied strain of *B. japonicum* still exists. It is clear from this study that the co-inoculation of soybeans with an appropriate actinomycete strain may be a novel approach in overcoming competition by the native *Bradyrhizobium* and the establishment of an applied strain. In case of soybean, inoculation of selected endophytic actinomycetes (*Streptomyces* sp.), isolated from sweet pea, showed 83 % antagonistic ability against fungal plant diseases when compared to un-inoculated control and improved nitrogen uptake when grown under controlled conditions (Thapanapongworakul 2003). Further, the endophytic *Streptomyces* sp. was also found compatible with *Bradyrhizobium* (Thapanapongworakul 2003).

The utilization of actinomycetes as potential soybean co-inoculants was also evaluated by Soe et al. (2012). They reported the highest shoot N accumulation, nitrogen fixation, and seed weight of soybean because of dual inoculation of *Streptomyces* strain P4 and *B. japonicum*. *Streptomyces* P4 was one of the effective actinomycetes which could be used in combination with selective root nodule bacterial strains for improved production of leguminous crops. Co-inoculation of *Streptomyces* sp. T4 with *B. japonicum* USDA110 significantly increased shoot nitrogen accumulation and seed weight of soybean in field trials (Soe et al. 2012). Soe and Yamakawa (2013) examined whether low-density co-inoculation of Myanmar *Bradyrhizobium yuanmingense* strain MAS34 and *Streptomyces griseoflavus* P4 would enhance nodulation, N_2 fixation, and seed yield in two soybean varieties. From these studies, it was obviously shown that the symbiotic interaction of actinomycetes with selected indigenous bradyrhizobial strains significantly improved nodule dry weight and N fixation than the single inoculation in all tested soybean varieties. This synergistic efficacy of tested strains was found in all soybean varieties. This was supported by the reports of Akarapisan et al. (2008), Soe (2009), and Soe et al. (2012), who found that dual inoculation of bradyrhizobial strains and endophytic actinomycetes (*Streptomyces* sp. P4) may increase the nodulation and N_2 fixation in different soybean varieties. They propose that this combination is expected to be useful biofertilizers for soybean production.

Following this line of evidences, a huge diversity of rhizospheric actinomycetes was isolated out of soybean root rhizosphere and nodule surfaces from different agricultural productive soils in Argentina (Solans et al. unpublished results). The diversity of this collection of actinomycetes was similar in terms of a different genus as the one obtained from *O. trinervis* rhizosphere (Solans and Vobis 2003) and from the soil rhizosphere of *Lotus* (Solans et al. unpublished). It is worth noting that selected strains from the collection in the case of soybean-enhanced nodulation and plant productivity in field assays using *Glycine max–Bradyrhizobium japonicum*, and we have seen stipulations of 10 % in field performance (Josza and Wall, personal communication).

10.4.3.4 Synergistic Effect of Actinomycetes on Pea–*Rhizobium leguminosarum* Symbiosis

Tokala et al. (2002) reported on a novel plant–microbe interaction between *Streptomyces lydicus* strain WYEC 108 and peas (*Pisum sativum* L.) and that this plant–microbe rhizosphere interaction involving a root-colonizing actinomycete and the pea plant is very important to the health and growth of this nodulating legume. Colonization leads to an increase in the average size of the nodules that forms and improves the vigor of the bacteroids within the nodules by enhancing nodular assimilation of iron and possibly other soil nutrients. They found that *S. lydicus* had significant effects on the field pea symbiosis by increasing the number of nodules and height and weight of the shoot. They concluded that this soil isolate was probably involved in the mechanisms of colonization and nodulation.

10.4.3.5 Actinomycetes as Helper Bacteria in Nodulation

Series of studies by our research group brought a comparison for the effect of actinomycete co-inoculation with rhizobia on the nodulation kinetics of various N_2-fixing symbioses and depicted in Fig. 10.2, and the system explaining these effects using actinorhizal with *O. trinervis*, rhizobial with *M. sativa*, and *L. tenuis* was shown in Fig. 10.2a–c, respectively. The analysis of the kinetics of nodulation in the cases of co-inoculations suggests that the effect of the actinomycetes operates at the beginning of the infection and nodulation process of plant roots, sustaining the initial nodulation rate for a longer time than when only N_2-fixing bacteria is inoculated. A positive effect regarding stimulation of nodulation and plant growth was found ($p \leq 0.05$). The stimulus in the kinetics of nodulation was similar to that found in the actinorhizal *O. trinervis* plants, the rhizobial *M. sativa* plants, and in *L. tenuis* plants.

Our results showing similar results by co-inoculation of the same rhizospheric actinomycetes isolated from *O. trinervis*,

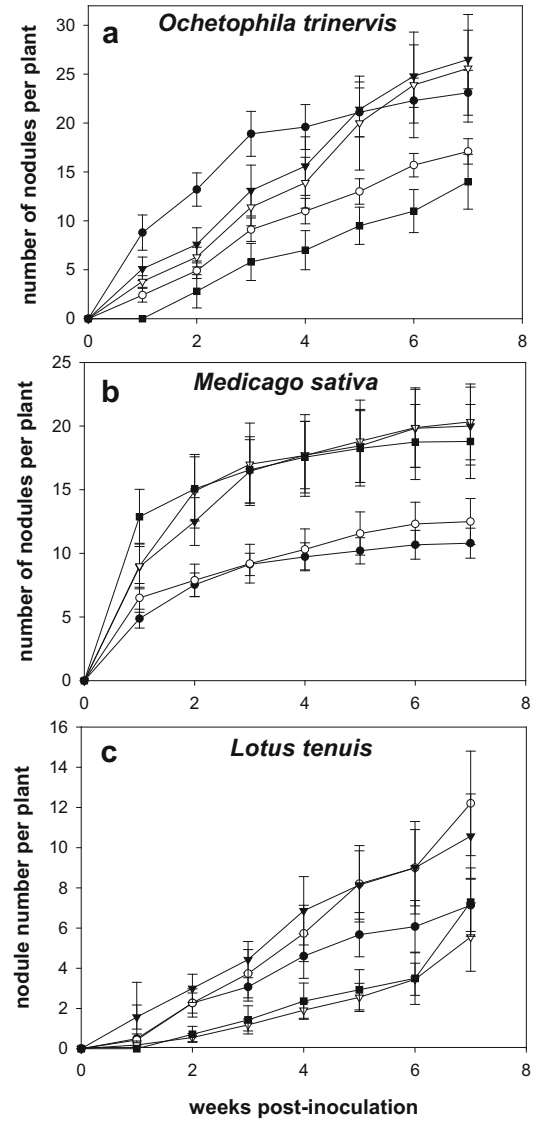

Fig. 10.2 Nodulation kinetics of N_2-fixing plants with actinomycete co-inoculations, growing in pouches system during 7 weeks post-inoculation. (**a**) In *O. trinervis* plants: *F Frankia* (●), *FS Frankia* + *Streptomyces* (○), *FA Frankia* + *Actinoplanes* (▼), *FM Frankia* + *Micromonospora* (▽) and *FSA Frankia* + *Streptomyces* + *Actinoplanes* (■); (**b**) In *M. sativa* plants: *R Rhizobium* as *S. meliloti* (●); *RS S. meliloti* + *Streptomyces* (○), *RA S. meliloti* + *Actinoplanes* (▼), *RM S. meliloti* + *Micromonospora* (▽) and *RSA S. meliloti* + *Streptomyces* + *Actinoplanes* (■); (**c**) In *L. tenuis* plants: *R Rhizobium* as *Mesorhizobium loti* NZP2213 (●), *RS Rhizobium* + *Streptomyces* (○), *RA Rhizobium* + *Actinoplanes* (▼), *RM Rhizobium* + *Micromonospora* (▽) and *RSAM Rhizobium* + *Streptomyces* + *Actinoplanes* + *Micromonospora* (■). Values represent mean ± SE, $n = 7$ (Modified from Solans and Vobis 2013)

Streptomyces MM40, *Actinoplanes* ME3, or *Micromonospora* MM18 (Solans and Vobis 2003) when used as co-inoculants on different rhizobia–legume symbioses as alfalfa, *Lotus*, and soybean suggest that the helper effect is nonplant species or symbiosis specific but somehow generalist. The finding that co-inoculation of these actinomycetes modifies the nodulation kinetics (Fig. 10.2) suggests that the helper effect is a general effect on the plant–microsymbiont interaction. Hence, it can be concluded that there are helper actinomycetes which act in nitrogen-fixing symbiosis, stimulating nodulation nonspecifically, in actinorhizal or legume symbiosis, independently of the infection pathway (intercellular or intracellular) and of the nodule development program (indeterminate or determinate).

10.5 Conclusions and Future Perspectives

The need to maximize the capacity of *Rhizobium*–legume symbiosis is not only due to certain environmental biotic and abiotic stress factors which adversely affect this system but also because of economic and environmental concerns relating to the use of chemical fertilizers in agriculture. Although the application of efficient and effective rhizobial inocula to legumes is a well-recognized cost-effective and eco-friendly approach, it does not guarantee for consistent performance. Hence, application of competent and beneficial rhizosphere bacteria as "helper" bacteria or co-inoculant comes out as a mean capable of improving the performance of rhizobia and legumes for ultimate increase in the amount of nitrogen to be fixed by this system.

Many evidences have been accumulated showing that co-inoculation with beneficial microorganisms, having different mechanisms of plant growth promotion, has additive or synergistic effect on plant growth and crop yield (Morel et al. 2012). Diverse mechanisms are implicates in the co-inoculation benefits, and some of them have been discussed in Barea et al. (2005). Probably, the most reported mechanism that explains the improved rhizobia–legume association by other PGPR is the production of plant hormones (phytohormones), such as gibberellic acid (GA_3) or auxin-type phytohormones (mainly indole-3-acetic acid; IAA) (Beattie 2006). However, the main mechanism involved in improved rhizobia–legume association is still under investigation (Dobbelaere and Okon 2007). It might be possible that multiple mechanisms, rather than only one, are acting. This is known as the "additive hypothesis" (Bashan et al. 2004; Bashan and de-Bashan 2010). Morel et al. (2012) showed that on average, an increase of 4–5 % in crop yield has an important impact in agricultural production. The data obtained in different growth systems (gnotobiotic laboratory conditions, hydroponics, greenhouse, and field) show that co-inoculation produces a major increase in legume yield compared with single inoculation overwhelming the agronomic expectations. Inoculation and co-inoculation experiments must be done in field to provide a realistic assessment of the performance of a living formulation in practical farming conditions.

From above discussion, it is clear that actinomycetes as helper bacteria have promising potential for use as co-inoculant with rhizobia to improve *Rhizobium*–legume symbiosis in a way that could harness the benefit of sustainable increased production of legumes under diverse conditions. This is mainly based in eco-friendly microorganisms that control pest and improve plant growth. In such scenario, the use of biofertilizers, rhizobia, or consortium of plant-beneficial microbes (rhizobia and symbiotic enhancers) in formulations provides a potential solution. The data showed in this chapter support that the design of new formulations with cooperative microbes might contribute to the growth improvement of legumes. The co-inoculation has a positive effect in growth stimulation of legume crops; however, we believe it is necessary to continue studying this subject, especially with actinomycete strains, up to now little used as inoculants in bacterial consortia, even though they are very attractive as potential inoculants in agriculture, as they produce very hardy spores that can survive for prolonged periods in soil and in storage containers.

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Role of ACC Deaminase in Stress Control of Leguminous Plants

11

Francisco X. Nascimento, Márcio J. Rossi, and Bernard R. Glick

Abstract

Leguminous plants produce grains that are used to feed both animals and humans, and they also play a role in the maintenance of soil fertility. However, under stressful conditions, like other plants, leguminous plants produce high levels of ethylene, a phytohormone known to inhibit both plant development and nodulation. Bacteria that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which converts ACC (the precursor of ethylene in all higher plants) to ammonia and α-ketobutyrate, can decrease inhibitory plant ethylene levels. Bacterial ACC deaminase plays an important role in plant growth promotion in a variety of stress conditions. This chapter discusses the role of ACC deaminase-containing plant growth-promoting bacteria, including rhizobia and actinomycetes, to improve the growth of legumes in environmentally stressful conditions.

Keywords

ACC deaminase • Ethylene • Rhizobia • Leguminous plants • *Actinobacteria*

11.1 Introduction

Environmentally friendly agricultural and remediation practices are necessary to increase food production, without the use of potentially harmful chemicals, and to recover degraded and polluted areas. In this sense, leguminous plants produce highly nutritious grains for human and animal consumption; show an increased phytoremediation potential (Ansari et al. 2015); participate in biological nitrogen fixation, which

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has a beneficial impact on soil nitrogen content; and consequently, reduce the need for pollutant fertilizers (Zahran 1999). As a consequence of environmental conditions, many soils inhibit leguminous plant development; these include high salinity, heavy metal and organic contaminant pollution, low or high water drainage, temperature extremes, and nutrient imbalance. In addition, numerous biotic agents can limit the growth of leguminous plants.

11.2 Plant Stress and Ethylene Production

Phytohormones play a key role in plant's response to stress (Schmelz et al. 2003; Khan et al. 2012). Ethylene (Bleecker and Kende 2000; Lin et al. 2009) regulates many plant developmental processes such as germination, root and shoot elongation, abscission, senescence, flowering, and fruit ripening (Abeles et al. 1992) and the responses to environmental stress (Abeles et al. 1992; Van Loon and Glick 2004). One of the most impacting effects of ethylene on plant growth occurs as a result of stress conditions. In this instance, the stressed plant first produces a small peak of ethylene that activates the transcription of various plant defensive genes (Glick et al. 2007). Subsequently, the stressed plant synthesizes a high level of ethylene (termed stress ethylene) that ultimately can lead to plant premature senescence and death (Hyodo 1991). In fact, some of the effects of stress cannot solely be attributed to the stress itself but are also due to autocatalytic ethylene synthesis (Van Loon 1984).

In plants, ethylene biosynthesis occurs via methionine-dependent pathway where methionine is converted to *S*-adenosyl methionine (SAM) by the enzyme SAM synthase and SAM is converted to ACC by the action of the enzyme ACC synthase. Finally, ACC is converted to ethylene by the enzyme ACC oxidase. The limiting step in plant ethylene biosynthesis is generally considered to be the conversion of SAM to ACC, indicating the key role of ACC in plant ethylene production (Yang and Hoffman 1984); however, since expression of ACC oxidase is also induced, this step provides another point at which this pathway may be regulated (Barry et al. 1996).

11.3 Ethylene Affects the Nodulation Process of Leguminous Plants

Ethylene inhibits both leguminous plant growth under stressful conditions and the nodulation process (Guinel and Geil 2002; Gage 2004; Ferguson et al. 2010). Studies employing exogenous ethylene or ethylene biosynthesis/perception inhibitors have demonstrated the inhibitory role of ethylene in the nodulation process in plants such as *Phaseolus vulgaris*, *Pisum sativum*, *Medicago sativa*, *Trifolium repens*, and *Melilotus alba* (Grobbelaar et al. 1971; Goodlass and Smith 1979; Peters and Crist-Estes 1989; Lee and La Rue 1992; Tamimi and Timko 2003). Experiments with mutant/transgenic plants have also contributed to the understanding of the role of ethylene in regulation of the nodulation process. Penmetsa and Cook (1997) showed that *Medicago truncatula sickle* mutants, insensitive to ethylene, formed an increased number of nodules when compared to its wild-type form. Later, Penmetsa et al. (2008) identified *sickle* mutants to be defective in a gene homologous to the *Arabidopsis* EIN2 (ethylene insensitive 2) gene. Transgenic *Lotus japonicus* plants expressing mutant ethylene receptors showed increased nodule formation (Nukui et al. 2004; Lohar et al. 2009). Silencing of two *L. japonicus* EIN2 homologous genes also resulted in an augmented nodule number (Miyata et al. 2013).

Ethylene can inhibit numerous steps of the nodulation process such as infection thread formation, nodule morphology, and nodule positioning (Ferguson and Mathesius 2014). Oldroyd et al. (2001) suggested that ethylene inhibits the calcium spiking responsible for the perception of bacterial Nod factors in *M. truncatula*. Lee and La Rue (1992) showed that exogenous ethylene did not lead to a

decreased number of infections, but rather nearly all of the infections were blocked when the infection thread was in the basal epidermal cell or in the outer cortical cells. Heidstra et al. (1997) postulated that a gradient of ethylene restricts nodules radially to positions opposite to the xylem poles. Curiously, radial restriction of nodule positioning is not observed in ethylene-insensitive mutants (Penmetsa and Cook 1997; Lohar et al. 2009; Chan et al. 2013), further confirming the hypothesis.

11.4 Bacterial Modulation of Ethylene Levels

Some microorganisms have mechanisms to regulate plant ACC and, consequently, ethylene levels. For instance, many *Bradyrhizobium* strains produce rhizobitoxine, an enol-ether amino acid that acts as an inhibitor of the enzyme ACC synthase (Sugawara et al. 2006). The production of the ACC deaminase enzyme is another mechanism that microorganisms use to regulate plant ACC and ethylene levels (Glick 1995). In this sense, ACC deaminase has been shown to be key to bacterial plant growth-promoting abilities (Glick 2014; Nascimento et al. 2014). This enzyme converts the ethylene precursor ACC to ammonia and α-ketobutyrate (Honma and Shimomura 1978), but unlike rhizobitoxine, ACC deaminase is found in a wide range of microorganisms, from bacteria to fungi (Nascimento et al. 2014).

11.4.1 ACC Deaminase

ACC deaminase belongs to the tryptophan synthase beta superfamily (fold type II) of pyridoxal phosphate-binding proteins. It is a multimeric enzyme with a subunit molecular mass of approximately 35–42 kDa per subunit (Glick et al. 2007). While able to cleave a few other substrates (Nascimento et al. 2014), the enzyme mainly cleaves ACC and is inhibited by some L-amino acids such as L-serine (Glick 2014). The enzymes from *Pseudomonas putida* GR12-2, *Pseudomonas* sp. ACP, *Pseudomonas* sp. UW4, *Methylobacterium radiotolerans* JCM 2831, *Methylobacterium nodulans* ORS 2060, and *Amycolatopsis methanolica* 239 have been characterized (Honma and Shimomura 1978; Hontzeas et al. 2004; Fedorov et al. 2013; Ekimova et al. 2015). These studies showed that ACC deaminase exhibits K_m values ranging from 0.8 to 3.4 mM, showing a low affinity for ACC, pH optima ranging from 8.0 to 8.5, and temperature optima ranging from 37 to 60 °C. Despite the fact that ACC deaminase utilizes plant ACC as a substrate, the enzyme is not secreted but rather it is located in the cytoplasm of the bacterium (Jacobson et al. 1994). Recently, Nascimento et al. (2014) reported that ACC deaminase genes (*acdS*) are widespread in *Actinobacteria* and *Proteobacteria* (α, β, γ) genomes. The authors also suggest that *acdS* genes are mostly vertically inherited; however, horizontal transfer events can also occur between more distantly related strains.

11.4.2 ACC Deaminase in Plant-Microbe Interactions

A detailed model for the role of ACC deaminase-producing bacteria in plant growth has been proposed (Glick et al. 1998; Glick 2014). Bacteria producing ACC deaminase can take up and metabolize ACC that is exuded by the plant (Penrose and Glick 2001), thus, leading to a reduction of the plant ACC pool available for conversion to ethylene. Glick et al. (1998) and Glick (2014) also postulated that IAA (indole acetic acid, auxin) plays an important role in the plant growth-promoting effects of ACC deaminase-producing bacteria. IAA synthesized and secreted by the bacterium is taken up by the plant and can stimulate plant cell proliferation and/or elongation (Patten and Glick 2002; Duca et al. 2014). In addition, IAA can stimulate ACC synthase transcription (Kim et al. 1992; Kende 1993; Kende and Zeevaart 1997). Hence, bacteria that produce both IAA and ACC deaminase possess a significant advantage over bacteria that only produce IAA, since they can decrease ACC

(ethylene) levels resulting from increased IAA action (Glick 2014). ACC deaminase is central to the functional interactions of various plant-associated bacteria, including rhizobacteria and endophytes. Rhizobacteria such as *P. putida* GR12-2, *Pseudomonas* sp. UW4, *Variovorax paradoxus* 5C2, and *Agrobacterium tumefaciens* D3 no longer promote root elongation after its *acdS* gene is deleted or disrupted (Glick et al. 1994; Li et al. 2000; Belimov et al. 2009; Hao et al. 2011). Similarly, the endophytes *Burkholderia phytofirmans* PsJN, *Burkholderia unamae* MTl-641, *Pseudomonas fluorescens* YsS6, and *Pseudomonas migulae* 8R6 do not promote plant growth when their *acdS* genes are deleted (Onofre-Lemus et al. 2009; Sun et al. 2009; Ali et al. 2012). Moreover, the symbiotic efficiency of *Rhizobium leguminosarum* bv. *viciae* and *Mesorhizobium sp*. MAFF303099 is decreased upon *acdS* gene deletion (Ma et al. 2003; Uchiumi et al. 2004).

11.4.3 ACC Deaminase in Rhizobia

Rhizobia play a crucial role in leguminous plant growth promotion by their ability to infect the root tissues of their compatible host legumes and induce the formation of nitrogen-fixing nodules (Zahran 1999). ACC deaminase genes (*acdS*) are prevalent in many rhizobial species, including α- and β-rhizobia (Nascimento et al. 2014). In α-rhizobia *acdS* genes are found in *Azorhizobium*, *Bradyrhizobium*, *Methylobacterium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (*Ensifer*). In β-rhizobia *acdS* genes are found in *Burkholderia* and *Cupriavidus* representatives. Studies by Ma et al. (2003) showed that a gene encoding an LRP-like protein (termed *acdR*) controls *R. leguminosarum acdS* transcription. *R. leguminosarum acdR* gene deletion resulted in a loss of ACC deaminase activity (Ma et al. 2003). Analysis of completely sequenced bacterial genomes showed that *acdR* is found in most *Proteobacteria* strains possessing an *acdS* gene (including *Azorhizobium*, *Bradyrhizobium*, *Methylobacterium*, *Rhizobium*, *Sinorhizobium*, *Burkholderia*, and *Cupriavidus*) suggesting that *acdR* is a common regulator of *acdS* gene transcription (Nascimento et al. 2014). Most *Mesorhizobium* strains do not possess *acdR* genes. In *Mesorhizobium* spp., *acdS* genes are only expressed in symbiotic conditions under the transcriptional control of the *nifA* promoter and NifA protein (Uchiumi et al. 2004; Nukui et al. 2006; Nascimento et al. 2012a). Analysis of the upstream regions of the *acdS* gene in many *Mesorhizobium* spp. indicates the presence of a putative NifA binding site, suggesting that NifA regulation of *acdS* expression may be common within the *Mesorhizobium* genus (Nascimento et al. 2012a). Studies using rhizobial *acdS* deletion mutants as well as rhizobial strains expressing exogenous *acdS* genes have demonstrated the important role of ACC deaminase in the nodulation process. Ma and coworkers (2003) reported that *R. leguminosarum* bv. *viciae* 128C53K *acdS* gene deletion reduced its nodulation abilities in *P. sativum* cv. Sparkle by 25 %. Upon transcriptomic analyses, Uchiumi et al. (2004) found that *Mesorhizobium* sp. MAFF303099 *acdS* gene was upregulated under symbiotic conditions.

Intrigued by this fact, Uchiumi and colleagues created a *Mesorhizobium* sp. MAFF303099 *acdS* insertion mutant and observed that it had decreased symbiotic abilities. The *acdS* mutant strain formed fewer nodules in *L. japonicus* than its wild-type counterpart and also showed decreased nodule occupancy abilities. The expression of an exogenous ACC deaminase gene in *Sinorhizobium meliloti* resulted in an increased ability to nodulate *M. sativa* plants (Ma et al. 2004). In this instance, *S. meliloti* Rm1021 expressing the *acdS* and *acdR* gene from *R. leguminosarum* bv. *viciae* 128C53K was able to induce 35–40 % more nodules when compared to its wild-type form. Similarly, Conforte et al. (2010) showed that an engineered strain of *Mesorhizobium* sp. MAFF303099 expressing ACC deaminase under free-living conditions displayed increased nodulation efficiency and competitiveness. Nascimento et al. (2011) also demonstrated that *Mesorhizobium ciceri* LMS-1 expressing an exogenous ACC deaminase was able to form an increased number of nodules in *Cicer arietinum*

plants. By expressing an exogenous ACC deaminase, the transformed strain enhanced its nodulation profile by 127 % compared to the wild-type strain and consequently increased chickpea biomass by 125 %. Using a similar strategy, Brígido et al. (2013) demonstrated that by expressing an exogenous *acdS* gene, a salt-sensitive *Mesorhizobium* strain was able to induce nodules in chickpea plants to the same extent as a salt-tolerant strain, further emphasizing the role of ACC deaminase in the nodulation abilities of these strains. Recently, Kong et al. (2015) observed that *S. meliloti* CCNWSX0020 expressing an exogenous *acdS* gene had increased nodulation abilities in *Medicago lupulina* plants. Although *S. meliloti* CCNWSX0020 possesses a functional *acdS* gene in its symbiotic plasmid and shows some level of ACC deaminase activity, the expression of an exogenous ACC deaminase nevertheless increases its nodulation abilities. Hence, selecting for or improving ACC deaminase activity might be important for developing rhizobial inoculum with increased nodulation abilities. Most rhizobial strains have a very low level of ACC deaminase activity compared to free-living (rhizospheric or endophytic) bacteria that possess this enzyme (Glick et al. 2007). This has been interpreted as indicating that there are at least three types of ACC deaminase-producing bacteria. In free-living bacteria that interact relatively non-specifically with plants, the high level of ACC deaminase activity protects plants from a wide range of environmental stresses. By contrast, rhizobia interact in a highly specific manner with their plant hosts, so that only a low level of ACC deaminase is required to locally lower root ethylene levels and facilitate nodulation. The third type of ACC deaminase-producing bacteria includes mesorhizobia where ACC deaminase functions only inside root nodules, presumably delaying nodule senescence.

11.4.4 ACC Deaminase in *Actinobacteria*

ACC deaminase genes are found in a range of *Actinobacteria* such as *Streptomyces*, *Amycolatopsis*, *Mycobacterium*, *Rhodococcus*, and others (Nascimento et al. 2014). In addition, ACC deaminase activity has been demonstrated for some members of *Actinobacteria*. Belimov et al. (2001) and later Hontzeas et al. (2005) detected a high level of ACC deaminase activity in *Rhodococcus* sp. Fp2 and *Rhodococcus* sp. 4N-4 both isolated from polluted soils. Inoculation with *Rhodococcus* sp. Fp2 increased the root dry weight of *P. sativum* cv. Sparkle (Belimov et al. 2001) while *Rhodococcus* sp. 4N-4 increased *Brassica juncea* plant growth (Belimov et al. 2005). Similarly, Dell'Amico et al. (2008) indicated that *Mycobacterium* sp. ACC14 possessed ACC deaminase activity and was able to promote *Brassica napus* growth. El-Tarabily (2008) and Palaniyandi et al. (2013) showed that some *Streptomyces* species from tomato and yam rhizospheres could produce ACC deaminase. Moreover, El-Tarabily (2008) showed that *Streptomyces filipinensis* 15 and *S. atrovirens* 26 could reduce tomato endogenous ACC levels in both roots and shoots, resulting in increased plant growth. Several *Nocardia*, *Rhodococcus*, and *Microbacterium* strains possessing ACC deaminase activity were obtained from the rhizosphere of pea, lentil, and chickpea from western Canada (Hynes et al. 2008). Furthermore, *Nocardia brasiliensis* 2–12 and *Microbacterium esteraromaticum* 6–9 were able to promote canola root elongation. Dastager et al. (2010) indicated that the cowpea plant growth-promoting bacterium *Micrococcus* sp. NII-0909 produced ACC deaminase under free-living conditions. In a survey done near the Yellow Sea in Korea, Siddikee et al. (2010) found that several halotolerant actinobacteria strains, isolated from both the soil of barren fields and the rhizosphere of six naturally growing halophytic plants, produced ACC deaminase and increased canola plant growth. The bacteria producing ACC deaminase were identified as *Brevibacterium epidermidis*, *Brevibacterium iodinum*, *Micrococcus yunnanensis*, *Arthrobacter nicotianae*, and *Corynebacterium variabile*. *Citricoccus zhacaiensis* B-4, an osmotolerant actinobacterium isolated from the banana rhizosphere, also produces ACC deaminase and improved the percent germination, seedling vigor, and germination rate of onion

seeds (cv. Arka Kalyan) at osmotic potentials up to −0.8 MPa (Selvakumar et al. 2015).

Not only rhizospheric but also endophytic *Actinobacteria* are able to produce ACC deaminase. In this sense, *Curtobacterium*, *Okibacterium*, and *Rhodococcus* strains associated with flowering plants of *Thlaspi goesingense* were also found to possess ACC deaminase activity (Idris et al. 2004). While many studies show that functional ACC deaminase genes are present in *Actinobacteria*, not much is known about *acdS* gene regulation in these strains. In a recent study, Nascimento et al. (2014) suggested that *Actinobacteria* possess a different mechanism regulating ACC deaminase transcription. *Actinobacteria* do not possess the common *acdR* (LRP-like) gene found in many rhizobia and other *Proteobacteria* but, in turn, contain a GNTR (gluconate operon transcriptional repressor)-like gene (termed *acdAR*) in the vicinity of *acdS*. This different transcriptional regulation may account for the increased ACC deaminase activity presented by *Actinobacteria* (Nascimento et al. 2014).

11.5 ACC Deaminase Facilitates Plant Growth Under Stress Conditions

11.5.1 Nonleguminous Plants

11.5.1.1 Flooding

Tomato plants grown from seeds bacterized with *Pseudomonas* spp. expressing ACC deaminase showed a substantial tolerance to flooding stress which resulted in overall plant growth, increased leaf chlorophyll content, and substantially decreased ethylene production in leaf petiolar tissue (Grichko and Glick 2001). In addition, *Pseudomonas* strains expressing an exogenous ACC deaminase under free-living conditions were able to increase tomato resistance to waterlogging while the non-transformed strains did not (Grichko and Glick 2001). Farwell et al. (2007) showed that *B. napus* was more tolerant to flooding stress in the field when the plants were inoculated with *Pseudomonas* sp. UW4 producing ACC deaminase. Similarly, transgenic *B. napus* plants expressing *Pseudomonas* sp. UW4 *acdS* gene also showed increased resistance to waterlogging under field conditions (Farwell et al. 2007). Barnawal et al. (2012) reported that bacteria with ACC deaminase activity protected *Ocimum sanctum* plants from waterlogging-induced stress ethylene production, reduced chlorophyll concentration, higher lipid peroxidation, and reduced foliar nutrient uptake. In these experiments, the bacterium *Achromobacter xylosoxidans* Fp2 induced growth and herb yield by 46.5 % and reduced plant ACC concentrations by 53 % compared to waterlogged plants without bacterial inoculation.

11.5.1.2 Drought

The ACC deaminase-producing *Achromobacter piechaudii* ARV8 significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress by reducing stress ethylene levels (Mayak et al. 2004a). Similarly, the auxin- and ACC deaminase-producing *Bacillus licheniformis* K11 reduced drought stress symptoms in pepper (Lim and Kim 2013).

11.5.1.3 Salt Stress

Mayak et al. (2004b) first reported the ability of the ACC deaminase-producing bacterium *A. piechaudii* ARV8 to promote tomato plant growth under high-salinity conditions. Subsequently, Gamalero et al. (2010) were the first to prove the role of ACC deaminase activity in bacterial plant growth promotion under high-salinity conditions by showing that a *Pseudomonas* sp. UW4 *acdS* deletion mutant was unable to promote cucumber plant growth and resistance to high salinity compared to its wild-type counterpart. Similar results were obtained by Ali et al. (2014) demonstrating that the endophytic strains *P. fluorescens* YsS6 and *P. migulae* 8R6 *acdS* deletion mutants displayed decreased plant growth promotion abilities and therefore were unable to promote tomato resistance to high-salinity conditions. On the other hand, the wild-type ACC deaminase-producing *P. fluorescens* YsS6 and *P. migulae* 8R6

increased tomato plants' fresh and dry biomass, chlorophyll content, and flower numbers in the presence of very high salt concentrations (i.e., up to 185 mM). Inoculation of actinomycetes producing ACC deaminase resulted in increased canola salinity tolerance (Siddikee et al. 2010), thus suggesting that these bacterial species also have the ability to enhance plant growth under saline stress. Based on inoculation assays using ACC deaminase-producing bacteria, many other studies demonstrate the important role of ACC deaminase in facilitating the growth of various plants in the presence of inhibitory salt levels (Zahir et al. 2009; Jalili et al. 2009; Palaniyandi et al. 2013; Chang et al. 2014; Singh et al. 2015) even under field conditions (Nadeem et al. 2009).

11.5.1.4 Temperature Stress

Recently, Subramanian et al. (2015) demonstrated that the expression of an exogenous ACC deaminase gene in the psychrotolerant bacterial strains *Flavobacterium* sp. OR306 and *Pseudomonas frederiksbergensis* OS211 resulted in an increased plant growth-promoting potential. They found that stress ethylene, ACC accumulation, and ACC oxidase activity were significantly reduced in tomato plants subjected to chilling stress when they were first inoculated with the ACC deaminase-producing transformants.

11.5.1.5 Heavy Metals and Organic Contaminants

Canola seeds inoculated with *Kluyvera ascorbata* SUD165, displaying ACC deaminase activity, presented an increased resistance to the toxic effects of nickel. In addition, the presence of *K. ascorbata* SUD165 had no influence on the amount of nickel accumulated per gram of dry weight in either roots or shoots of canola plants suggesting that the bacterial plant growth-promoting effect was not attributable to a reduction of nickel uptake by seedlings but rather by the bacterium's ability to lower stress ethylene levels induced by nickel (Burd et al. 1998). Subsequently, Farwell et al. (2006) demonstrated that the inoculation of canola plants in the field with ACC deaminase-producing bacteria led to increased canola plant growth. In the presence of arsenate, canola plants inoculated with ACC deaminase-producing *Enterobacter cloacae* CAL2 grew to a greater extent than uninoculated canola plants (Nie et al. 2002). Moreover, transgenic canola plants expressing bacterial ACC deaminase showed increased tolerance to arsenate toxicity (Nie et al. 2002). Belimov et al. (2005) and Dell'Amico et al. (2008) demonstrated that ACC deaminase-producing bacteria, including some *Actinobacteria* strains, improved the growth of metal-accumulating *B. juncea* and *B. napus* in the presence of toxic cadmium concentrations. The bacterial endophytes *P. fluorescens* G10 and *Microbacterium* sp. G16 increased biomass production and Pb uptake in *B. napus* plants (Sheng et al. 2008). Similar results were obtained by Zhang et al. (2011), supporting the notion that ACC deaminase-producing bacteria can protect *B. napus* from lead stress. A study performed by Truyens and coworkers (2012) showed that ACC deaminase-producing bacteria were predominant in *Arabidopsis thaliana* seedlings exposed to cadmium stress for several generations, while being mostly absent in *A. thaliana* growing without added cadmium. The authors suggest that certain endophytic bacteria are selected for transfer to the next generation and that their presence may be important for subsequent germination and early seedling development. These results reinforce the role of ACC deaminase in plant protection against heavy metal stress conditions. Bacteria with ACC deaminase activity can also be used as inoculants to protect plants from organic contaminants. For example, Reed et al. (2005) have shown that the bacterium *Pseudomonas asplenii* AC, genetically transformed to express a bacterial *acdS* gene, increased *Phragmites australis* seed germination and plant growth in the presence of creosote. Sheng et al. (2009) demonstrated that the ACC deaminase-producing actinomycete *Microbacterium* sp. F10a increased both wheat growth and phenanthrene and pyrene removal from soil in a low-temperature environment.

11.5.1.6 Biotic Stresses

When *P. fluorescens* strain CHA0, a root-colonizing bacterium with biological control activity, was transformed to produce ACC deaminase, it showed an increased ability to promote canola root elongation and protect cucumber against *Pythium* damping-off and potato tubers against *Erwinia* soft rot (Wang et al. 2000). By reducing stress ethylene levels through ACC deaminase production, *Methylobacterium sp.* CBMB20 increased tomato plant resistance to *Pseudomonas syringae* pv. tomato, *Ralstonia solanacearum*, and *Xanthomonas campestris* pv. vesicatoria (Indiragandhi et al. 2008; Yim et al. 2013, 2014). Toklikishvili et al. (2010) showed that various rhizosphere bacteria producing ACC deaminase could increase tomato plant resistance to pathogenic strains of *A. tumefaciens* and *Agrobacterium vitis*. The bacterial strains *Pseudomonas* sp. UW4, *B. phytofirmans* PsJN, and *Azospirillum brasilense* Cd1843 carrying a plasmid-encoded *acdS* gene were able to reduce the mass of *Agrobacterium*-induced tumors. Recently, Nascimento et al. (2013) showed that *Pseudomonas* sp. UW4 enhanced *Pinus pinaster* resistance to the pinewood nematode, *Bursaphelenchus xylophilus*, responsible for pine wilt disease. In this case, an *acdS* deletion mutant of *Pseudomonas* sp. UW4 was unable to protect pine seedlings against the pinewood nematode, while the wild-type ACC deaminase-producing strain reduced nematode infectivity and overall wilting disease symptoms. These results were obtained despite the fact that *Pseudomonas* sp. UW4 did not present nematicidal activities, suggesting that reducing deleterious ethylene is key to increase pine resistance to the nematode.

11.5.2 Leguminous Plants

11.5.2.1 Drought

Inoculation with *Pseudomonas* strains producing ACC deaminase significantly decreased the drought stress-imposed effects on the growth and yield of *P. sativum*. After drought stress imposition, pea plants showed decreased shoot growth and reduced grain yield; however, in the presence of ACC deaminase-producing bacteria, these effects were diminished. Furthermore, inoculation of stressed plants resulted in better grain yield (40–62 % higher) than the uninoculated non-stressed control (Arshad et al. 2008). Similar results were obtained in a field study by Zahir et al. (2008) who demonstrated that at the lowest soil moisture level (25 % field capacity), *P. fluorescens* ACC-5 producing ACC deaminase increased *P. sativum* fresh weight, dry weight, root length, shoot length, number of leaves per plant, and water-use efficiency when compared with the uninoculated controls. Belimov et al. (2009) showed that *Variovorax paradoxus* 5C-2 improved growth, yield, and water-use efficiency of drought-stressed peas. Conversely, an ACC deaminase minus mutant of *Variovorax paradoxus* 5C-2 was unable to protect pea plants from drought stress-induced symptoms. Also, *V. paradoxus* 5C-2 increased the nodulation profile of symbiotic nitrogen-fixing bacteria under both control and drought stress conditions, probably by reducing deleterious ethylene levels that regulate the nodulation process. The ACC deaminase-containing *Bacillus subtilis* LDR2 greatly protected *Trigonella* plants from severe drought stress by reducing deleterious ethylene levels (Barnawal et al. 2013). The *Bacillus* strain also acted synergistically with beneficial microbes like *S. meliloti* and the mycorrhizal fungi *Rhizophagus irregularis*, improving their colonization rate, which further increased *Trigonella* plant growth.

11.5.2.2 Salt

Pseudomonas fluorescens TDK1, possessing ACC deaminase activity, enhanced *Arachis hypogea* growth and saline resistance (Saravanakumar and Samiyappan 2007). In addition, the authors indicated that *P. fluorescens* TDK1-treated plants presented higher plant height, number of pods per plant, pod-filling percent, and seed weight, in two consecutive field trials. Ahmad et al. (2011) observed that salinity stress significantly reduced *Vigna*

radiata plant growth, but co-inoculation using both *Pseudomonas* strains (containing ACC deaminase) and rhizobia enhanced plant growth, hence, reducing the inhibitory effect of salinity. Later, Ahmad et al. (2013) demonstrated that co-inoculation of *Rhizobium* with ACC deaminase-producing *Pseudomonas* strains greatly diminished the adverse effects of salinity on *V. radiata* growing under field conditions. Brígido et al. (2013) demonstrated that ACC deaminase plays a significant role in *Mesorhizobium* nodulation and plant growth promotion under salinity stress. Chickpea *Mesorhizobium* isolates expressing an exogenous ACC deaminase gene showed increased nodulation abilities and protected chickpea plants from salinity stress-induced symptoms. Interestingly, by utilizing an exogenous ACC deaminase that was derived from a free-living bacterium, Brígido et al. (2013) found that a salt-sensitive *Mesorhizobium* strain was able to induce nodules to the same extent as a salt-tolerant strain, both under conditions of high salinity. *Actinobacteria*, as well as *Proteobacteria*, that produce ACC deaminase can promote the growth of leguminous plants under high-salinity conditions. Barnawal et al. (2014) showed that *Arthrobacter protophormiae* SA3 producing ACC deaminase promoted *P. sativum* growth under salt stress by reducing the endogenous ACC levels and hence the stress ethylene levels. In addition, they observed that *A. protophormiae* SA3 improved the colonization of plants by beneficial microbes like *R. leguminosarum* and *Glomus mosseae*. This tripartite synergistic interaction induced a high level of protection of pea plants against salt stress.

11.5.2.3 Metal Contamination

Recently, Kong et al. (2015) showed that *Medicago lupulina* plants nodulated by *S. meliloti* expressing an exogenous ACC deaminase gene presented a greater dry weight, a decreased ethylene level in roots, and a higher total copper uptake but a lower level of copper translocation to aerial parts, compared with plants nodulated with the wild-type strain under copper stress conditions. The authors also showed that under severe copper stress, inoculation with the ACC deaminase-overproducing *S. meliloti* led to a higher expression of plant antioxidant enzymes in the roots, suggesting that either ethylene or ACC may play a role in modulating some plant responses to copper stress.

11.5.2.4 Biotic Stresses

Using a *M. ciceri* strain expressing an exogenous ACC deaminase gene, Nascimento et al. (2012b) demonstrated that this ACC deaminase played an important role in the plant growth-promoting and biological control abilities of *Mesorhizobium*. By producing ACC deaminase under free-living conditions, *M. ciceri* LMS-1 increased its nodulation abilities by ~150 % and the biomass of two different chickpea cultivars (in unsterilized soil) by 45 %. The transformed *Mesorhizobium* LMS-1 also decreased the impact of *Fusarium* root rot and manganese toxicity on the chickpea plants, while the wild-type strain was unable to exert any protective effect against either *Fusarium* or excess manganese.

11.6 Concluding Remarks

Regulating ACC and ethylene levels is key to promoting leguminous plant nodulation and growth under stressful conditions. Hence, the use of bacteria that produce the enzyme ACC deaminase presents a valuable tool to increase leguminous plant growth and resistance. In this sense, rhizobial strains with ACC deaminase activity, and consequently increased nodulation activity, can enhance the nitrogen fixation potential of leguminous plants. Moreover, ACC deaminase-producing actinomycetes have increased resistance to environmental stresses, as well as biological control activity. Ultimately, developing inoculants based on ACC deaminase-producing bacterial consortia (rhizobia and/or actinomycetes) is extremely beneficial for optimizing the productivity of leguminous plants.

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Induction of Systemic Resistance in Crop Plants Against Plant Pathogens by Plant Growth-Promoting Actinomycetes

12

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Abstract

Plants possess the ability to acquire and express an enhanced defense mechanism against pathogen attack after being treated with biocontrol agents or chemical stimulant. The role of induced systemic resistance by biocontrol agents against pathogen colonization has been revealed in several crop plants. Actinomycetes are one of the most promising sources of biocontrol agents at present gaining increased attention in the field of biological control. The secondary metabolites produced by actinomycetes play a vital role in plant growth promotion as well as suppression of pathogen growth and development in host plant. In this chapter, traits involved in plant growth-promoting actinomycetes (PGPA)-mediated induced systemic resistance (ISR) will be discussed.

Keywords

Induced systemic resistance • Actinomycetes • Endophytes • Biological control • Plant pathogens

12.1 Introduction

Plant disease management is done mainly by means of chemicals. Various chemical pesticides have been reported to be effective against a wide range of pathogens but not considered as long-term solution because of concerns about health and environmental hazards, expensiveness, residue persistence, pest resurgence, and elimination of natural enemies. Therefore, the need for alternative method of control of plant diseases has become vital. The development of biological control for plant diseases is accepted as a durable and eco-friendly alternative for agrochemicals. Beneficial microorganisms either bacteria or fungi that survive in the plant rhizosphere region exhibit direct and/or indirect mechanism as plant growth promoters and biocontrol agents. Direct mechanisms include making the availability of phosphorus for plant uptake, nitrogen fixation, production of siderophores and plant growth hormones such as auxins, cytokinins,

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and gibberellins, and lowering plant ethylene levels using 1-aminocyclopropane-1-carboxylate (ACC) deaminase that accumulated during biotic and abiotic stresses (Glick 1995; Mayak et al. 2004). Indirect mechanisms include the production of antibiotics, viz., 2,4-diacetylphloroglucinol (DAPG), phenazine, pyoluteorin, and pyrrolnitrin against pathogens, reduction of iron availability to phytopathogens in the rhizosphere, synthesis of cell wall-lysing enzymes, competition with harmful microbes for space in the rhizosphere region, and induction of systemic resistance (Ramamoorthy et al. 2001). Among several other modes of actions, induced resistance is one of the promising mechanisms by which the beneficial microbes including endophytes restrict the pathogen growth and development inside the plant system. The elicitation of systemic resistance by these microorganisms or biocontrol agents is denoted as induced systemic resistance (ISR), whereas the pathogen-induced resistance is denoted as systemic acquired resistance (SAR) (Van Loon et al. 1998). Induced systemic resistance by several nonpathogenic, plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) has been extensively reviewed by many researchers (Ramamoorthy et al. 2001; Choudhary et al. 2007; Bakker et al. 2007; Choudhary and Johri 2009; Pieterse et al. 2014). However, the Gram-positive and filamentous actinomycetes (bacto-fungoid in nature) are proved to be more effective in terms of plant growth promotion as well as biocontrol activity against several plant pathogens than PGPR or PGPF (Krechel et al. 2002; Weller et al. 2002; Coombs and Franco 2003; Coombs et al. 2004; Cao et al. 2005; Conn et al. 2008; Lehr et al. 2008; Gopalakrishnan et al. 2011; Misk and Franco 2011). Plant growth-promoting actinomycetes (PGPA) particularly *Streptomyces* are known to be a potent producer of various secondary metabolites including antibiotics and cell wall-degrading enzymes against many fungal and bacterial pathogens (Ezra et al. 2004; Lu et al. 2008; Goodfellow and Fiedler 2010; Nachtigall et al. 2011). In addition, *Streptomyces* have the ability to compete and metabolize carbon and nitrogen sources available in the rhizosphere region (Schlatter et al. 2009).

El-Tarabily et al. (2000) characterized the chitinolytic activity of *Streptomyces viridodiasticus* and *Micromonospora carbonacea* against *Sclerotinia minor* and reported that these isolates secreted high level of chitinase in vitro and significantly reduced the incidence of basal drop disease of lettuce under greenhouse conditions. Chitinase isolated from *Streptomyces* sp. M-20 showed antagonistic activity against *Botrytis cinerea* (Kim et al. 2003). Hoster et al. (2005) reported the antagonistic activity of *Streptomyces* chitinase against *Aspergillus nidulans*, *B. cinerea*, *Fusarium culmorum*, and *Sclerotinia sclerotiorum*. Chitinases produced by *Stenotrophomonas maltophilia* inhibited the mycelial growth of *Fusarium*, *Rhizoctonia*, and *Alternaria* (Jankiewicz et al. 2012). El-Tarabily (2003) reported that an endophytic strain, *Actinoplanes missouriensis*, was found to be effective in reducing the root rot incidence in lupin caused by *Plectosporium tabacinum*. Lee et al. (2008) studied the bioefficacy of *Microbispora rosea* subsp. *rosea* and *Streptomyces olivochromogenes* against *Plasmodiophora brassicae* and reported reduction of club root disease incidence in Chinese cabbage. Shimizu et al. (2009) reported that *Streptomyces* sp. -MBCu-56 was found to be effective against cucumber anthracnose pathogen, *Colletotrichum orbiculare*. Shinde et al. (2014) reported antagonistic activity of *Actinopoymorpha* spp. IABT-A7 on sheath blight disease (*Rhizoctonia solani*) and plant growth promotion in rice. Recently, Srivastava et al. (2015) reported the bioefficacy of *Streptomyces rochei* SM3 against *S. sclerotiorum* in chickpea. Exploitation of PGPA as inducers of ISR may pave way for increasing the plant's innate immunity against wide range of pathogens. This chapter elucidates the mechanisms of ISR mediated by PGPA in crop plants against plant pathogens.

12.2 Induced Systemic Resistance

It is a well-known fact for more than ten decades that plants can defend themselves through induced resistance against plant pathogens (Chester 1933). Besides, resistance can also be induced through inoculation of biocontrol agents against pathogen colonization (Vallad and Goodman 2004; da Rocha

and Hammerschmidt 2005). But for that, plants must possess all the necessary genes in order to express a range of defense activities against pathogen attack. Similarly, the inducer or biocontrol agents also should have the ability to induce defense compounds in crop plants. Based on the nature of inducing agents and signaling pathways involved, this induced resistance is categorized into two types, viz., SAR and ISR (van Loon et al. 1998). SAR is defined as the expression of hypersensitive response or localized necrotic lesion on host plant upon infection caused by a virulent pathogen in order to arrest further growth of the pathogen, whereas ISR is the enhanced level of defensive responses elicited by PGPR in response to pathogen attack (van Loon et al. 1998). However, the biocontrol agents should produce elicitors or inducers; conversely, plant also should have a corresponding receptor and a signaling pathway in order to activate the ISR.

Compounds like DAPG (Iavicoli et al. 2003), chitin (Zhang et al. 2002), ergosterol (Kauss and Jeblick 1996), glucans (Mithöfer et al. 1996), lipopolysaccharides (LPS) (Erbs and Newman 2003; Silipo et al. 2005), proteins and peptides (Harman et al. 2004), salicylic acid (Van Loon et al. 1998), sphingolipids (Umemura et al. 2004), and volatile organic compounds (Ryu et al. 2004) produced by biocontrol agents can act as an elicitor during the event of ISR. Cohen et al. (2005) reported that the production of nitric oxide by *Streptomyces* also induced plant defense against pathogen attack. However, literature on actinomycetes-mediated ISR is sparse. Pathogenesis-related (PR) protein genes and salicylic acid (SA) signaling pathway play key roles in SAR (Hammerschmidt 1999), whereas in the case of ISR, jasmonic acid (JA) and ethylene (ET) signaling play major roles. The study of Mahmoudi et al. (2011) reported that the signaling compounds that are responsible for the expression of pathogenicity gene in *Pectobacterium carotovorum* have been degraded by *Streptomyces*.

12.3 Role of Signaling Pathways in ISR

The application of genomics, transcriptomics, proteomics, and metabolomics has provided thorough evidence into how host plants protect themselves from the invasion of pathogens. By using these techniques, various researchers (De Meyer et al. 1999; Van Wees et al. 1999; Ahn et al. 2007; Verhagen et al. 2004; Tjamos et al. 2005) have demonstrated the changes in the expression of defense genes when challenged with PGPR against plant pathogens as well as the involvement of SA, JA, and ET signaling pathways in controlling the systemic resistance in *Arabidopsis*.

Plants possess the ability to acquire and express an improved level of resistance to pathogen attack after being treated with strains of PGPR. The role of signaling pathways in PGPR that intervened ISR was first established in *Arabidopsis* using *Pseudomonas fluorescens* strain WCS417r (Pieterse et al. 1996), and this strain was found to be effective against various plant pathogens including *Pseudomonas syringae* pv. *tomato* DC3000, *Xanthomonas campestris* pv. *armoraciae*, *Fusarium oxysporum* f. sp. *raphani*, and *Peronospora parasitica* (van Peer et al. 1991; Leeman et al. 1995; Duijff et al. 1998). Although the pathogen-induced SAR is governed by SA, JA and ET play a vital role in the regulation of ISR against pathogen attack (Thomma et al. 2001). For instance, the possible role of JA and ET signaling pathway in PGPR-mediated ISR was tested using JA (*jar1-1*) and ethylene (*etr1-1* and *ein2*) response mutant strains of *Arabidopsis*. However, these mutants failed to express ISR activity in *Arabidopsis* inoculated with WCS417r against *P. syringae* pv. *tomato* (Pieterse et al. 1998). This indicates that both JA and ET are essential for activating the ISR signaling pathway in crop plants. In addition to this, NPR1 (non-expresser of PR genes), a regulator, is also required not only for the SA-dependent SAR but also for the JA- and ET-dependent ISR activated by several PGPR and PGPF (Dong 2004; Ahn et al. 2007; Hossain et al. 2008; Stein et al. 2008; Vlot et al. 2009; Weller et al. 2012).

12.4 Antagonistic Potential of Actinomycetes

Actinomycetes carries various modes of actions for suppressing the plant pathogens in nature

Table 12.1 Examples for the antagonistic potential of actinomycetes

Actinomycetes	Pathogens	References
Streptomyces spp.	*Phoma medicaginis* var. *medicaginis*	Samac et al. (2003)
Streptomyces hygroscopicus	*Bipolaris sorokiniana* and *Sclerotinia homeocarpa*	Hodges et al. (1993)
Streptomyces strain 93	*Pythium* and *Phytophthora*	Jones and Samac (1996)
Streptomyces spp.	*Streptomyces scabies*	Liu et al. (1996)
Streptomyces spp.	*Phytophthora medicaginis* and *Phytophthora sojae*	Xiao et al. (2002)
Streptomyces spp.	*Phytophthora erythroseptica*,	Zin et al. (2007)
	Pythium ultimum, *S. sclerotiorum*, *Mycosphaerella fijiensis*, and *R. Solani*	
S. rochei	*Fusarium oxysporum* f. sp. *lycopersici*	Kanini et al. (2013a)
Streptomyces aureofaciens	*Colletotrichum musae*, *F. oxysporum*	Taechowisan et al. (2005)
Streptomyces ACTA1557 and ACTA1383	*R. solani*	Kanini et al. (2013b)
A. missouriensis	*Plectosporium tabacinum*	El-Tarabily (2003)
M. rosea subsp. *rosea* and *S. olivochromogenes*	*Plasmodiophora brassicae*	Lee et al. (2008)
Streptomyces sp.	*Colletotrichum orbiculare*	Shimizu et al. (2009)
Actinopoymorpha spp.	*R. solani*	Shinde et al. (2014)
S. rochei	*S. sclerotiorum*	Srivastava et al. (2015)
Streptomyces, *Microbispora* and *Nocardioides* spp.	*Gaeumannomyces graminis var. tritici* and *R. solani*	Coombs et al. (2004)
Streptomyces sp. strain S96	*F. oxysporum*	Cao et al. (2005)
Streptomyces albovinaceus, *Streptomyces griseus*, and *Streptomyces virginiae*	*Moniliophthora perniciosa*	Macagnan et al. (2008)
Streptomyces toxytricini vh6 and *Streptomyces flavotricini* vh8	*R. solani*	Patil et al. (2011)

including antibiosis, competition for nutrients and space, production of lytic enzymes, production of nitrous oxide, quorum quenching, and induction of systemic resistance (Mahadevan and Crawford 1997; Cohen and Mazzola 2006; Quecine et al. 2008; Mahmoudi et al. 2011; Verma et al. 2011). Plant physiological and biochemical characters are changed when they are treated with PGPR or PGPF. These changes make the plants less suitable to subsequent attack by pathogens, and plants are benefitted from induced responses by reducing subsequent pathogen load. Induced resistance is frequently viewed as an alternative tactic to constitutive resistance. Hence, understanding of the relationship between constitutive and induced resistance will be an important thing in defining how best to use induced resistance in management of plant diseases. Finally, to use the antibiotic production, growth promotion activity, and induced responses of PGPA as an effective pathogen management tool, one should evaluate these effects on plant performance and yield under field conditions. Examples for the antagonistic potential of actinomycetes against various plant pathogens are given in Table 12.1.

12.5 Mechanisms of ISR Mediated by PGPA

Plants possess a number of insoluble defensive structural barriers against pathogen colonization that are inducible in nature including the formation of cell wall appositions, deposition of callose and hydroxyproline-rich glycoproteins (HRGP), and accumulation of phenolic compounds such as

lignin and suberin and minerals such as silicon and calcium (Humphreys and Chapple 2002; Collins et al. 2003; Vorwerk et al. 2004; Zhu et al. 2004; Ton et al. 2005). In addition to the occurrence of hypersensitive response, strengthening of structural barriers, and production of phytoalexins (low-molecular weight antimicrobial compounds synthesized upon pathogen infection), induction of various defense genes encoding peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT), superoxide dismutase (SOD), chitinase, β-1,3-glucanase, lipoxygenase, proteinase inhibitors, and phenylalanine ammonia lyase (PAL) (van Loon 1997; Chen et al. 2000; Pozo et al. 2005) plays a vital role in ISR. Mechanisms of ISR mediated by strains of *P. fluorescens* have been shown in several crop plants, including rice (Nandakumar et al. 2001; Saravanakumar et al. 2007), tomato (Ramamoorthy et al. 2002), mango (Vivekananthan et al. 2004) and groundnut (Senthilraja et al. 2013).

El-Tarabily et al. (2009) conducted an experiment to test the bioefficacy of three endophytic actinomycetes, *Actinoplanes campanulatus*, *Micromonospora chalcea*, and *Streptomyces spiralis*, against *Pythium aphanidermatum* in cucumber and found that all the isolates produced high levels of cell wall-degrading enzymes including β-1,3, β-1,4, and β-1,6 glucanases and thereby significantly reduced the incidence of damping off in cucumber. Patil et al. (2011) quantified the activity of PAL and total phenolics in tomato plants inoculated with *S. toxytricini* vh6 and *S. flavotricini* vh8 against *R. solani* and found that actinomycete-treated plants expressed enhanced level of PAL and phenolic compounds including gallic, ferulic, cinnamic, gentisic, chlorogenic, and salicylic acid when compared to control. Cheng et al. (2014) found that *Streptomyces felleus* YJ1 has strong antagonistic activity and ability to synthesize enhanced level of defense enzymes such as SOD, POD, PPO, and PAL against *S. sclerotiorum* in oilseed rape under greenhouse conditions.

Lehr et al. (2008) studied bioefficacy of *Streptomyces* GB 4-2 isolated from forest soil against *Heterobasidion abietinum*, root rot pathogen of Norway spruce seedlings. They observed that *Streptomyces*-inoculated plant roots showed thickened cell wall with increased xylem and lignin formation. In another study, Hasegawa et al. (2004) reported that mountain laurel inoculated with *Streptomyces padanus* strain showed increased callose deposition in cell wall. An endophytic actinomycete *Streptomyces galbus* R-5, isolated from rhododendron, induced disease resistance and callose appositions in the cell walls of tissue-cultured rhododendron seedlings (Suzuki et al. 2004). These results indicate that the observed cell wall appositions were the mechanisms behind the enhanced disease resistance in plants that are pre-inoculated with *Streptomyces*. Root inoculation with *Streptomyces* GB 4-2 also induces systemic resistance against foliar pathogen *B. cinerea* in Norway spruce needles, besides inducing the local defenses (Lehr et al. 2008). Shimizu et al. (2005) quantified the upregulation of PDF1.2 gene in *Arabidopsis* pre-inoculated with *S. galbus* MBR-5 against *Colletotrichum higginsianum*. Baz et al. (2012) observed induction of cytosolic Ca^{2+} and biphasic oxidative burst by *Streptomyces* sp. OE7 as a defense response in BY2 tobacco cell suspensions against the challenge of *P. carotovorum* and *Pectobacterium atrosepticum* and also observed the delayed induction of scopoletin production and programmed cell death. In addition, OE7 triggered the synthesis of PAL and increased accumulation of EREBP1 and AOX genes that are governed by the JA/ET pathway. However, there was no change in the accumulation of PR1b and NIMIN2a that are governed by SA pathway.

Conn et al. (2008) inoculated *Arabidopsis* (ecotype Columbia-0 and mutants *npr1-1*, *jar1-1*, *etr1-3*, and *NahG*) seeds with *Actinobacteria* endophytes (EN27, *Streptomyces* sp. AY148075; EN28, *Streptomyces* sp. AY148076; EN43, *Micromonospora* sp. AY291589; EN46, *Nocardioides albus* AY148081; and EN2, *Microbispora* sp. AY148073) in order to determine the role of SAR and JA/ET pathways in induction of defense genes against *Erwinia carotovora* and *F. oxysporum*. Seeds inoculated with EN27 and EN28 expressed 19-fold induction of the *PR-1* transcript and 23-fold induction

of *PDF1.2*, respectively, compared with control plants. Seeds inoculated with EN46 induced the activation of *PR-1* and *PR-5*, whereas strain EN43 showed no activity on *PR-1* and *Hel* expression; however, it suppressed the *PR-5* and *PDF1.2* expression when compared to controls. When the *Arabidopsis* (Columbia-0) inoculated with all these endophytes against *E. carotovora* subsp. *carotovora*, strains EN43 and EN46 showed highest induction of *PDF1.2* which is mediated by JA/Et pathways. However, only the EN43 demonstrated the induction of *Hel*, *PR-1*, and *PR-5* transcripts when compared to other endophyte-treated plants. Similarly, the endophyte-treated Columbia-0 plants were challenged with *F. oxysporum* to induce defense genes. Plants treated with EN28 induced the expression of *Hel*, *PR-1*, and *PR-5* to some extent compared with controls, whereas EN27-treated plants induced the expression of *PDF1.2*.

Similarly, the mutant plants were inoculated with endophytes against *E. carotovora* subsp. *carotovora*. There was no significant induction of resistance genes in *NahG* plants inoculated with strain EN27 when compared to Columbia-0 plants. Though the strain EN27 induced resistance in JA and ET mutant plants, the level of expression of *PDF1.2* and *Hel* was lower when compared to *jar1-1* and *etr1-3* mutants. *F. oxysporum*-infected *NahG* and *npr1-1* expressed comparatively less defense activity in both the SAR and JA/ET pathways. Similar results were observed in mutants inoculated with EN27 against *F. oxysporum*. On the other hand, *F. oxysporum*-infected *jar1-1* and *etr1-3* plants expressed considerable amount of gene expression when compared with SAR mutants. It was noticed that *jar1-1* and *etr1-3* expressed appreciable amount of PR-1 gene, and the activity was further improved in plants inoculated with EN27. However, the level of induction of *PDF1.2* and *Hel* was significantly reduced in EN27-treated plants. This study revealed that EN27 induced resistance to *E. carotovora* by a NPR1-independent pathway and to *F. oxysporum* by a NPR1-dependent pathway. This indicates that the expression of defense genes in response to streptomycetes treatment shares both ISR and SAR pathways (Conn et al. 2008). These results show that *Streptomyces* could induce the plants to synthesize an array of defense molecules locally and systemically in order to suppress the pathogen growth.

12.6 Conclusion

Activation of ISR by biocontrol agents in order to enhance the defensive capacity of crop pants is proved to be more effective against a wide range of pathogens. The role of endophytic actinomycetes in the field of biological control has gained increased interest, as they are also capable to elicit an array of defense compounds in the host plant to restrict the pathogen colonization. Hence, it is suggested that integration of PGPA-mediated ISR in crop improvement will eventually help the scientists in developing a durable resistant variety against a wide range of plant pathogens. However, it needs comprehensive understanding of the interactions between actinomycetes and plant pathogens in relation to SA-, JA- and ET-dependent pathways in host plants. Certainly, the availability of high-throughput molecular techniques would help to design an effective and ecologically safe biocontrol strategy by involving actinomycetes in different cropping systems.

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Actinomycetes as Mitigators of Climate Change and Abiotic Stress

13

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Abstract

Agricultural productivity is affected worldwide due to anthropogenic and climate change-induced abiotic stresses, posing a threat to food security. Use of microorganisms for abiotic stress management in agriculture is emerging as economically viable and environmental-friendly option. Actinomycetes, the Gram-positive bacteria with filamentous structure that are common associates of plants (as rhizosphere inhabitants and as plant endophytes), are receiving attention for their potential application in stressed ecosystems. Many actinomycetes exhibit plant growth-promoting (PGP) properties including indole acetic acid (IAA) production, phosphate solubilization, siderophore production, biocontrol of phytopathogens, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. Besides, they can grow under diverse stress conditions such as moisture stress, high temperature, salinity, alkalinity, and wide pH range. Recently, many reports have documented the role of actinomycetes in alleviating salinity and drought stress in crop plants. However, there is a need to further strengthen the research to explore their potential to improve plant productivity under diverse environmental stress conditions by conducting extensive pot and field trials and to understand the underlying mechanisms.

Keywords

Microorganisms • Actinomycetes • *Streptomyces* • Drought • Salinity • Plant growth promotion

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13.1 Introduction

With the change in global climate pattern (global warming and changes in precipitation patterns) and anthropogenic activities, incidences of environmental stresses such as extreme temperatures, drought, flooding, salinity, metal stress, nutrient stress are on increasing trend causing and bound to impact agricultural productivity worldwide. These factors are likely to cause serious impacts on crop yields and impose severe pressure on soil and water resources. According to an estimate by the Food and Agricultural Organization (FAO), abiotic stress factors may result in 30 % land loss in the next 25 years and up to 50 % loss by the year 2050, if corrective measures are not taken (Munns 2002). Abiotic stress factors can cause reduction in average yields for major crops by more than 50 % (Mahajan and Tuteja 2006). Climate change models have predicted the negative effects of warmer temperatures and frequent droughts on net agricultural productivity during the twenty-first century (Clair and Lynch 2010). Rainfed agriculture is considered more vulnerable to climate change due to uncertainty of rainfall, increasing frequency of droughts, midseason droughts, decrease in number of rainy days, extreme and untimely rainfall, and natural calamities such as hail storms (Srinivasarao et al. 2015).

There is urgent need to develop strategies to combat climate change effects and provide easy solutions to the farmers for sustainable agricultural production. Researchers all over the world are attempting to develop measures to combat environmental stresses in agricultural crops. Genetic improvement of crop plants through plant breeding and genetic engineering has been achieved for enhanced tolerance to abiotic stresses. However, these strategies are long drawn and cost intensive. Further, it is not practically feasible to genetically improve all the crop plants for different types of stresses. Therefore, alternate strategies that are quick, cheap, and environmental friendly are needed to address the issue. One of the economically viable and environmental-friendly solutions to this problem is the use of plant-associated beneficial microorganisms to combat the harmful effects of abiotic stresses on plant growth and productivity (Venkateswarlu and Grover 2009). These organisms include inhabitants of rhizosphere, rhizoplane, phyllosphere, endophytes, and symbionts that operate through a variety of mechanisms, such as triggering stress response that alleviates stress tolerance and induction of novel genes in plants. Besides bacteria and fungi, viruses are also reported to induce abiotic stress tolerance in host plant (Grover et al. 2011). Plant beneficial microorganisms form important components of integrated nutrient management and organic agricultural practices. Organic inputs also promote soil microbial activities and play important role in the sustainable management of soils for enhancing agronomic productivity and sequestering carbon (Srinivasarao et al. 2009, 2013, 2014).

13.2 Actinomycetes: The Hidden Treasure

Actinomycetes (also known as *Actinobacteria*) are a heterogeneous and widely distributed group of Gram-positive bacteria (order *Actinomycetales*) with a high guanine (G) plus cytosine (C) ratio in their DNA (>55 mol %). They are moldlike, rod-shaped, filamentous bacteria with a branching tendency and form important group of rhizosphere and endophytic microbial community (Gayathri and Muralikrishnan 2013). Actinomycetes are known to produce chemically diverse metabolites and play important role in nutrient cycling. In addition, they produce hydrolytic enzymes which not only degrade diverse substrates but can also inhibit the growth of phytopathogens. Actinomycetes can degrade recalcitrant polymers occurring naturally in plant litter and soil, including lignocelluloses, chitin, and pectin, thus helping in C-cycling (Strap 2011). Historically, the most commonly described actinomycetes genera are *Streptomyces* and *Micromonospora*. The genus *Streptomyces* has been extensively explored for bioactive

natural products. Approximately two-thirds of natural antibiotics have been isolated from actinomycetes, and about 75 % of them are produced by members of the genus *Streptomyces*. Recently, this group of bacteria has received lot of attention, owing to its soil dominance and strong antimicrobial potential and plant growth promotion (Franco-Correa et al. 2010; Jog et al. 2014).

13.3 Actinomycetes as Plant Growth Promoters

Actinomycetes are effective rhizosphere colonizers and can endure unfavorable environmental conditions by forming spores (Alexander 1977). Biocontrol potential of actinomycetes, especially *Streptomyces*, has been tested against a wide range of plant pathogens (Shimizu et al. 2009; Shimizu 2011; Wang et al. 2013). Despite the well-documented history of *Streptomyces* in biocontrol, *Streptomyces* species have been poorly investigated specifically for their potential as plant growth-promoting microorganisms (PGPM) (Doumbou et al. 2001) as compared to other plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* and *Bacillus*. However, PGP potential of actinomycetes has been widely reported in the last two decades (Table 13.1). Many studies have reported the presence of PGP traits such as phosphate solubilization, indole acetic acid (IAA), and siderophore production in *Actinobacteria* (Jog et al. 2012; Cruz et al. 2014). Application of streptomycete culture filtrates resulted in significant increase in growth parameters (shoot fresh mass, dry mass, length, and diameter) and yield components (spikelet number, spike length, and fresh and dry mass of the developing grain) of wheat plants (Aldesuquy et al. (1998). Igarashi et al. (2002) purified pteridic acids A and B from the culture broth of endophytic *Streptomyces hygroscopicus*. These metabolites were found to be as effective as IAA in promoting the formation of adventitious roots in hypocotyls of kidney beans. Meguro et al. (2006) reported an endophytic strain of *Streptomyces* sp. MBR-52 that accelerated emergence and elongation of adventitious roots in tissue-cultured seedlings of rhododendron. Similarly, El-Tarabily (2008) reported PGP of tomato (*Lycopersicon esculentun* Mill.) by rhizosphere-competent, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing *S. filipinensis* and *S. atrovirens*. El-Tarabily et al. (2009) further reported that IAA and indole-3-pyruvic acid producing strains of three endophytic actinomycetes *A. campanulatus*, *M. chalcea*, and *S. spiralis* could enhance growth of cucumber plants significantly.

Table 13.1 Plant growth promotion by actinomycetes

Actinomycetes	Crop	Observed effects	References
Streptomyces hygroscopicus	Kidney beans	Formation of adventitious roots in hypocotyls	Igarashi et al. (2002)
Streptomyces sp. MBR-52	Rhododendron	Accelerated emergence and elongation of adventitious roots in tissue-cultured seedlings	Meguro et al. (2006)
S. filipinensis and *S. atrovirens*	Tomato	Plant growth promotion	El-Tarabily (2008)
Actinoplanes campanulatus, *Micromonospora chalcea*, and *Streptomyces spiralis*	Cucumber	Plant growth promotion	El-Tarabily et al. (2009)
Streptomyces sp.	Sorghum	Enhanced agronomic traits of sorghum	Gopalakrishnan et al. (2013)
	Rice	Enhanced stover yield, grain yield, total dry matter, and root biomass	
Streptomyces padanus AOK30	Mountain laurel	Enhanced protection against *Pestalotiopsis sydowiana*	Meguro et al. (2012)

Gopalakrishnan et al. (2013) evaluated five strains of *Streptomyces* for growth promotion of sorghum and rice. These *Streptomyces* strains demonstrated multiple mechanisms of actions including antibiosis, production of PGP hormone IAA and lytic enzymes (lipase, β-1-3-glucanase, and chitinase), tolerance to NaCl (up to 6 %), wide pH range (5 and 13), and temperatures (20 and 40 °C). In the greenhouse conditions, all the strains significantly enhanced all the agronomic traits of sorghum, and under field conditions, the application of *Streptomyces* significantly enhanced stover yield, grain yield, total dry matter, and root biomass of rice. Application of streptomycetes also improved soil health parameters in terms of microbial biomass carbon and nitrogen, dehydrogenase activity, total N, available P, and organic carbon (Gopalakrishnan et al. 2013). In another study, Cruz et al. (2014) evaluated the effectiveness of actinomycetes on the growth and yield of upland rice. Five actinomycetes strains produced both IAA and ACC deaminases and were able to increase root dry weight of upland rice under growth room condition. Actinomycetes inoculation in combination with full doze of fertilizers significantly increased P uptake (80–136 %) and grain yield (up to 62 %).

An endophytic actinomycete, *Streptomyces padanus* AOK30, could protect mountain laurel against infection by *Pestalotiopsis sydowiana*, a causal agent of pestalotia disease. Meguro et al. (2012) attempted to identify the genes differentially expressed in seedlings of mountain laurel after application of *S. padanus* AOK30 through semiquantitative real-time polymerase chain reaction (RT-PCR) analysis. This demonstrated the increased expression of defense-related genes as well as distinct classes of glutathione S-transferase, although endochitinases were exclusively suppressed. These results clearly indicated that the *S. padanus*-colonizing seedlings primed plant defense responses toward pathogen infection. All these studies demonstrate the potential of actinomycetes as PGP agents for diverse crops.

13.4 Actinomycetes as Mitigators of Climate Change and Abiotic Stresses

Microbe-induced systemic tolerance against abiotic stress/stresses in plants has gained considerable attention recently. The term induced systemic tolerance (IST) has been proposed for PGPR-induced physical and chemical changes that result in enhanced tolerance to abiotic stress (Yang et al. 2009). Systemic tolerance occurs when plants develop enhanced defensive capacity in response to an appropriate signal perception from pathogens or abiotically challenging environments. Therefore, identification, characterization, and utilization of such microbes for possible alleviation of stresses in plants can be a potential strategy to combat abiotic stresses highly relevant under climate change scenario (Grover et al. 2011, 2015; Srivastava et al. 2015). Previous reports have demonstrated that PGPR can increase plant's tolerance to abiotic stresses such as drought, high temperature, salinity, flooding, and freezing. Bacteria belonging to different genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, and *Enterobacter* have been reported to promote growth of host plant under abiotic stress conditions. These microorganisms have opened up opportunities to alleviate stresses and enhance agricultural productivity (Mayak et al. 2004; Ali et al. 2009; Sandhya et al. 2009; Grover et al. 2011, 2014; Choudhary 2012). Actinomycetes are closely associated with plants as rhizosphere inhabitants and/or as endophytic symbionts but, however, only recently have attracted attention of the researcher for their role in abiotic stress alleviation in host plants. Abiotic stress tolerance of actinomycetes to abiotic stresses like drought, high temperature, salinity, and wide pH range has been documented in recent reports (Yandigeri et al. 2012; Sakure et al. 2015). Figure 13.1 is the conceptual presentation of PGP and abiotic stress-alleviating traits of actinomycetes.

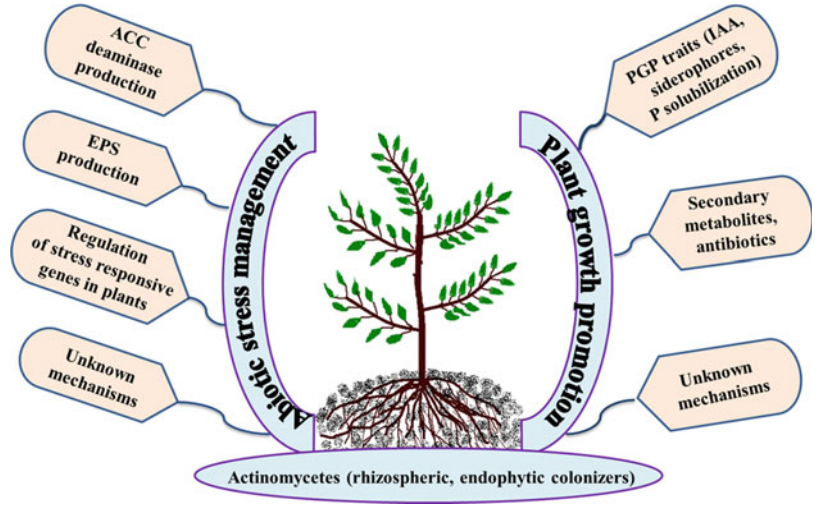

Fig. 13.1 Plant growth-promoting and abiotic stress-alleviating traits of actinomycetes

13.4.1 Actinomycetes for Alleviation of Salinity Stress in Plants

Salinity is considered as one of the major abiotic factors limiting crop yields in arid and semiarid regions. In agriculture, salinity is defined as the presence of higher level of salt than essential plant limit in soil (Yadav et al. 2011). It chemically denotes dissolved mineral salt such as cations and anions of Na^+, Ca^+, Mg^+, K^+, Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, and NO_3^-. According to the USDA report, electrical conductivity of 4 dSm^{-1} or above is classified as saline soil (Seidahmed et al. 2013). Soil salinity causes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, and Zn) deficiency, and oxidative stress in plants and thus limits water uptake from soil. Salinity effects in plants are the results of complex interactions among morphological, physiological, and biochemical processes affecting almost all aspects of plant development including seed germination, vegetative growth, and reproductive development. Excessive intracellular accumulation of sodium in cells under saline conditions can rapidly cause osmotic stress leading to molecular damage, growth arrest, and even death of the plant. Under salinity stress conditions, photosynthesis is also affected due to reduction in leaf area, chlorophyll content, stomatal conductance, and photosystem II efficiency (Netondo et al. 2004). Salinity also adversely affects enzymatic activities and reproductive development. All these factors cause adverse effects on plant growth and development at physiological, biochemical, and molecular levels (M'sehli et al. 2011; Srivastava and Kumar 2015).

Many PGPR including *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Serratia*, *Rhizobium*, *Aeromonas*, *Achromobacter*, and *Azospirillum* have been reported to alleviate salinity stress effects in different crops including vegetables, cereals, pulses, and cotton (Srivastava and Kumar 2015). *Streptomyces* strains can tolerate high concentrations of NaCl, and tolerance up to 13 % NaCl has been reported in previous studies (Tresner et al. 1968). Tolerance to high salinity in *Streptomyces* has been associated with intracellular accumulation of free neutral amino acids and under conditions of extreme salt stress, with selective internal concentration of potassium (Killham and Firestone 1984). Many studies have reported role of actinomycetes in alleviating salinity stress in plants (Table 13.2).

Gupta et al. (2010) reported the requirement of NaCl for better solubilization of tricalcium phosphate among the *Streptomyces* isolates. However, high salt concentration (more than 0.2 %) decreased phosphate solubilization. Aly et al. (2003) evaluated the effect of NaCl and salt-tolerant *Streptomyces niveus* on some physiological traits of the salt-sensitive maize cultivar

Table 13.2 Actinomycetes-mediated abiotic stress management in plants

Actinomycetes	Crop	Stress	Observed effects	References
Streptomyces niveus	Maize	Salinity	Increased salt tolerance of plant	Aly et al. (2003)
Streptomyces sp.	Wheat	Salinity	Increased germination, plant growth, and development	Aly et al. (2012)
Streptomyces sp.	Wheat	Salinity	Improved germination rate, percentage and uniformity, shoot length, and dry weigh	Sadeghi et al. (2012)
Streptomyces sp. PGPA39	Tomato	Salinity	Increase in plant biomass and chlorophyll content and a reduction in leaf proline content	Palaniyandi et al. (2014)
Streptomyces rochei SM3	Chickpea	Salinity	Suppressed chickpea mortality due to *Sc. sclerotiorum* infection, increased biomass accumulation, concentrations of phenolics, and antioxidant activities	Srivastava et al. (2015)
Streptomyces pada AOK-30	Mountain laurel	Drought	Increase resistance to drought. Structural modification of cell wall, accelerated callose accumulation, and cell wall lignifications of sieve cells	Hasegawa et al. (2004, 2005)
Streptomyces coelicolor DE07, *Streptomyces olivaceus* DE10, and *Streptomyces geysiriensis* DE27	Wheat	Drought	Improved seedling vigor, plant growth, and yield	Yandigeri et al. (2012)
Citricoccus zhacaiensis B–4	Onion	Drought	Improved germination and seedling vigor	Selvakumar et al. (2015)

Giza 122 under greenhouse conditions. Irrigating plants with saline water (20, 40 and 60 mM) increased Na concentration and decreased N, P, K, and Mg concentrations in shoots and roots. Increasing salinity decreased chlorophyll (Chl) concentrations of leaves but not carotenoids. Marked increase was noticed in total-soluble sugars, total free amino acids, and proline concentrations of both shoots and roots, whereas the total-soluble proteins, DNA and RNA concentrations, were reduced. Shoot growth and IAA biosynthesis were inhibited by increasing salinity. Applying S. *niveus* to the experiment also influenced most test characters by increasing the salt tolerance of the plant specifically during co-inoculation with *Azotobacter chroococcum*. However, the number of these microorganisms reduced under saline conditions which lead to the conclusion that applying co-inoculation slightly improved the salt tolerance of the test cultivar. In another study, Aly et al. (2012) observed that soaking wheat seeds in *Streptomyces* sp. increased wheat germination significantly. Moreover, soil inoculations with *Streptomyces* sp. alone or *Streptomyces* sp. + *Azotobacter vinelandii* increased the growth and development of wheat in normal and saline conditions. Inoculation significantly increased root depth, shoot length, and shoot and root dry weights. The amounts of P, N, Mg, K, and proteins present in wheat shoots, grown in normal and saline soil, also increased by soil inoculation. Increasing NaCl concentration increased proline content, but soil inoculation decreased the adverse effects of NaCl and decreased proline concentration compared to control at the same salinity level. Under saline conditions (100 and 200 mM NaCl), application of *Streptomyces* sp. SF5 improved seed germination (85 and 68.3 %, respectively) in wheat (*Triticum durum* L.) over un-inoculated control (83.3 and 53.3 %) (Ameur and Ghoul 2014). Inoculation also improved root and stem length under saline conditions.

Sadeghi et al. (2012) tested a *Streptomyces* isolate exhibiting biocontrol properties for its PGP traits under saline conditions. Exposure to elevated osmotic strengths up to 300 mM NaCl increased bacterial dry weight and cfu/ml significantly. The isolate could produce IAA and siderophore (increased under salt stress conditions) and could solubilize tricalcium phosphate (decreased under salt stress conditions). Soil treatment with *Streptomyces* increased the growth performances of wheat under normal and saline conditions. Significant increases in the germination rate, shoot length, and shoot dry weight were observed along with N, P, Fe, and Mn concentrations in wheat grown in normal and saline soil. Another actinomycete strain, *Streptomyces* sp. PGPA39 exhibiting IAA production, P solubilization, and ACC deaminase activity, and salt tolerance to 1 mol l^{-1} NaCl could promote the growth of *Arabidopsis* seedlings under in vitro conditions as evident from a significant increase in plant biomass and number of lateral roots (Palaniyandi et al. 2014). Salinity stress-alleviating activity of PGPA39 was evaluated using "Micro Tom" tomato plants with 180 mmol l^{-1} NaCl stress under gnotobiotic condition. A significant increase in plant biomass and chlorophyll content and a reduction in leaf proline content were observed in PGPA39-inoculated tomato plants under salt stress compared with control and salt-stressed non-inoculated plants (Palaniyandi et al. 2014).

Srivastava et al. (2015) attempted to study the mechanism underlying actinomycetes-mediated stress tolerance in chickpea. They used *Streptomyces rochei* strain SM3 for treating chickpea seeds and challenged the pre-inoculated seedlings with *Sclerotinia sclerotiorum* and NaCl. Treatment with SM3 suppressed the plant mortality due to *S. sclerotiorum* infection (48 %) and increased biomass accumulation (20 %) in the salt-stressed condition over untreated control. Physiological responses in chickpea under the challenging conditions showed that phenylalanine ammonia lyase activities increased in SM3-treated plants. Further, accumulation of higher concentrations of phenolics that led to enhanced lignifications in SM3-treated plants compared to non-SM3-treated plants challenged with the same stresses. SM3-treated plants showed catalase activities and proline accumulation under both the stresses compared to non-treated plants. Investigation at genetic level further showed that the strain SM3 triggered the ethylene-responsive ERF transcription factor (CaTF2) under the challenged conditions. This study concluded that actinomycetes *S. rochei* SM3 triggered the ET-mediated defense pathway in chickpea and activated the phenylpropanoid pathway for alleviating the stresses caused by *S. sclerotiorum* and salt in chickpea.

13.4.2 Actinomycetes for Alleviation of Drought Stress in Plants

Drought is the major environmental stress that influences plant growth at cellular and molecular levels limiting the plant growth, crop quality, and productivity in the arid and semiarid regions. Drought is almost an inevitable phenomenon in all years in the arid regions (mean annual rainfall, <500 mm), whereas, in semiarid regions (mean annual rainfall, 500–750 mm), droughts occur in 40–60 % of the years due to deficit seasonal rainfall or inadequate soil moisture availability between two successive rainfall events. Even in the dry subhumid regions (annual rainfall, 750–1200 mm), contingent drought situations occur due to break in monsoon conditions (Srinivasarao et al. 2015). Plants growing under these conditions undergo water limitation and nutrient deficiencies. Yield declines in wheat and paddy due to increasing moisture stress; reduction in number of rainy days and increased air temperature have been reported in many parts of South Asia (Challinor and Wheeler 2008). Rhizospheric microorganisms adapted to adverse conditions may compensate for such detrimental conditions. Microorganisms mitigate water loss by synthesizing extracellular polysaccharides (EPS) to create a barrier between themselves and the dry environment, by increasing the intracellular concentration of compatible solutes that permit cellular machinery to function under stress conditions, and by the upregulation of

genes associated with protein stabilization (heat-shock and chaperone proteins), with countering oxidative threats and with regulatory response to desiccation (LeBlanc et al. 2008).

Inoculation with native beneficial micro-organisms may increase drought tolerance of plants growing in arid or semiarid areas (Yandigeri et al. 2012). Role of actinomycetes in mitigating the effects of drought stress has been reported scantily (Table 13.2). For example, endophytic *Streptomyces pada* AOK-30 exhibited the potential to increase resistance of mountain laurel (*Kalmia latifolia* L.) to drought. The improved tolerance was associated with structural modification of cell wall, a higher osmotic pressure of plant cells owing to accelerated callose accumulation, and cell wall lignifications of sieve cells induced by AOK-30 treatment (Hasegawa et al. 2004, 2005). Yandigeri et al. (2012) isolated drought-tolerant endophytic *Actinobacteria*, *Streptomyces coelicolor* DE07, *S. olivaceus* DE10, and *S. Geysiriensis* DE27 from arid and drought-affected regions. These isolates exhibited PGP traits such as IAA production and intrinsic water stress tolerance from −0.05 to −0.73 MPa. Significant enhancement of wheat seedling vigor was recorded by the inoculation of these endophytic *Actinobacteria*. Seed treatment with culture and cell-free extract of the endophytes could significantly increase the wheat yield. However, use of cultures yielded better results than cell-free extract. Further, co-inoculation of two endophytes (*S. olivaceus* DE10 and *S. geysiriensis* DE27) recorded highest yield. Selvakumar et al. (2015) isolated an osmotolerant *Actinobacterium Citricoccus zhacaiensis* B–4 from banana rhizosphere. This isolate expressed PGP traits, viz., IAA, GA3 production, phosphate, zinc solubilization, ACC deaminase activity, and ammonia production under polyethylene glycol-induced osmotic stress and non-stress conditions. In vitro osmotic conditions and biopriming with the *Actinobacterium* improved the germination and seedling vigor of onion seeds at osmotic potentials up to −0.8 MPa, suggesting it as a viable option for the promotion of onion seed germination under drought-stressed environments.

13.5 Conclusion

Anthropogenic activity and climatic variability-induced abiotic stressors are limiting agricultural productivity worldwide. Microbial deployment in agriculture could be a low-cost and environmental-friendly strategy to combat abiotic stresses in crop plants. Actinomycetes, known to exhibit plant beneficial traits, are found in close association with plants and can survive under abiotic stress conditions. All these traits make them suitable candidates to be deployed in agriculture as stress mitigators. Potential of actinomycetes in combating salinity and drought stress in plants has been demonstrated. However, their application needs to be tested under other/multiple abiotic stresses like high and low temperature, flooding, carbon dioxide (CO_2), etc. Further, there is need to study the underlying mechanisms. Till date, the mechanisms behind bacterial-induced abiotic stress tolerance are not fully understood. Actinomycetes being classified as Gram-positive bacteria may exhibit the similar mechanisms, although their morphological difference (filamentous structure) from bacteria might display some altered/additional mechanisms for inducing abiotic stress tolerance in plants. Understanding these mechanisms will contribute to the long-term goal of exploiting plant–microbe interactions in stressed ecosystems to boost crop productivity.

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Perspectives of Plant Growth-Promoting Actinomycetes in Heavy Metal Phytoremediation

14

Z. Zarin Taj and M. Rajkumar

Abstract

Phytoremediation is an emerging technology that uses plants and their associated microbes to clean up pollutants from the soil, water, and air. In recent years, heavy metal phytoremediation assisted by plant beneficial actinomycetes has been highly used for cleaning up metal-polluted soils since these bacteria play an essential role in plant growth, metal/nutrient acquisition, metal detoxification, and alleviation of biotic/abiotic stress in plants. Direct plant growth promotion by actinomycetes is based on hormonal stimulation and improved nutrient acquisition by plants. Similarly, diverse mechanisms, viz., soil acidification and production of metal mobilizing/immobilizing substances by actinomycetes, are involved in heavy metal uptake by plants, which is often directly connected with the efficiency of phytoremediation process. Based on these beneficial plant-actinomycetes interactions, it is possible to develop microbial inoculants as environmentally friendly bio-tool for use in heavy metal phytoremediation. In this study, we highlight the diversity and plant growth beneficial features of actinomycetes and discuss their potential role on plant growth and phytoremediation process in metal-polluted soils.

Keywords

Plant-associated microorganisms • Actinomycetes • Heavy metals • Rhizosphere • Siderophores • 1-Aminocyclopropane-1-carboxylic acid

14.1 Introduction

The development of numerous technologies and industrialization ends up with the result of release of heavy metals as pollutants into the environment (Doble and Kumar 2005; Rajkumar et al. 2009). Particularly, the contamination of

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soil with heavy metals is a major worldwide problem in the current decade (Kamran et al. 2014). The heavy metal accumulation in soil adversely affects both the ecosystem and human health. Although some metals are essential for life, they are highly toxic to microorganisms (Fig. 14.1), plants, animals, and humans at higher concentrations. They affect various physiological and biochemical process by displacing other metal ions, blocking essential functional groups, disintegrating cell organelles (Vangronsveld and Clijsters 1994), acting as genotoxic substance, and disrupting the physiological process such as photosynthesis, respiration, protein synthesis, and carbohydrate metabolism.

Application of various physical, chemical, and biological strategies for decontaminating the polluted sites is a challenging task because heavy metals cannot be degraded and thus persist in the environment indefinitely. In order to clean up the contaminated sites, heavy metals should be concentrated and extracted from the contaminated sites by conventional methods for proper disposal or reuse. Although various strategies (such as land filling, excavation, fixation, solidification, and leaching) have been applied to remediate the contaminated sites, most of these methods are either extremely costly or simply involve the isolation of the contaminated sites or adversely affect the soil biological activity and fertility (Pulford and Watson 2003; Wu et al. 2010). Currently, the biological-based technique has been extensively used as an alternative method to remove pollutants from air, soil, and water or to render pollutants harmless (Chowdhury et al. 2015). "Phytoremediation" is one of the key processes of bioremediation that involves the use of plants and their associated microbes to relief, transfer,

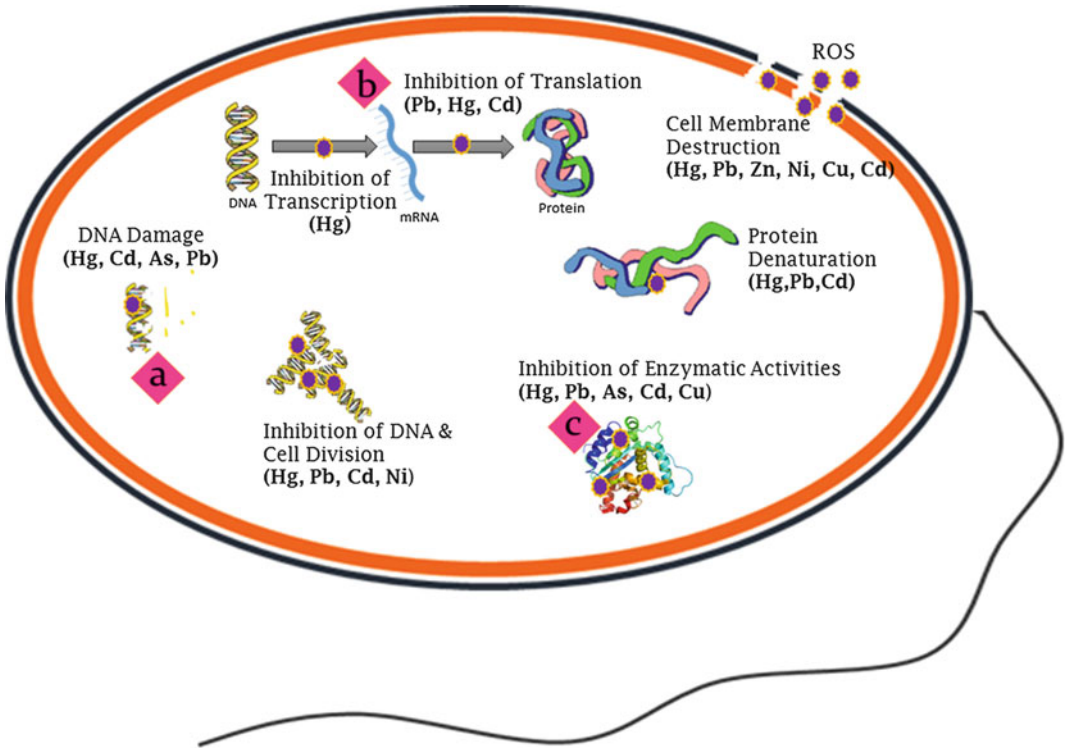

Fig. 14.1 The major molecular mechanism involved in heavy metal toxicity. (*a*) Production of reactive oxygen species by auto-oxidation and Fenton reaction causes DNA damage, cell membrane disruption (e.g., Fe and Cu) (Valls and de Lorenzo 2002), (*b*) blocking of essential mechanisms by damaging biomolecules (e.g., Cd and Hg), (*c*) displacement of essential metal ions (Fe) in biomolecules by heavy metals (Cu and Cd)

stabilize, or degrade the pollutants from soil, sediments, surface waters, and groundwater (Elekes 2014; Paz-Ferreiro et al. 2014; Laghlimi et al. 2015). The concept of phytoremediation was first proposed by Chaney (1983), which paved the way for the development of process of removing environmental contaminants using plants. The success of phytoremediation is dependent on the potential of the plants to tolerate the metal stress and produce high amount of biomass within a relatively short period. In recent years, plant-associated beneficial microbes have been used to enhance heavy metal phytoremediation process (Rajkumar et al. 2012). The plant-associated microbes accelerate phytoremediation process in metal-polluted soils by promoting plant growth and play a significant role in altering heavy metal accumulation in plants through producing various metabolites (e.g., siderophores, organic acids, and plant growth regulators) and various reactions in the rhizosphere (e.g., acidification, chelation, precipitation, and oxidation-reduction reactions). In turn, plant roots release nutrients through exudation which support the growth, survival, and colonization potential of microflora, involved in phytoremediation process.

Actinomycetes are gram positive, aerobic, sporulating, and filamentous bacteria which are ubiquitous in soils. Actinomycetes gain their importance among the researchers due to the production of enormous secondary metabolites and enzymes including antibiotics, degrading enzymes, enzyme inhibitors, immunosuppressants, phytotoxins, phytohormones, pesticides, and insecticides (Erikson 1949; Bèrdy 1995; Park et al. 2002; Hamaki et al. 2005; Imada 2005; Doumbou et al. 2011). They also directly promote plant growth by producing phytohormones (auxin, cytokinins, and gibberellins) and siderophore, solubilizing phosphate, fixing atmospheric nitrogen, and suppressing stress-ethylene production in plant through 1-amino cyclopropane-1-carboxylate (ACC) deaminase activity (Misk and Franco 2011; Sadeghi et al. 2012; Harikrishnan et al. 2014a) (Table 14.1). Moreover, the actinomycetes possess many properties that make them good candidates for application in bioremediation of soils contaminated with inorganic and/or organic pollutants. They produce extracellular enzymes that degrade a wide range of complex organic compounds. They play an important role in the recycling of organic carbon and are able to degrade complex polymers by production of extracellular degrading enzymes and peroxidases (Goodfellow and Williams 1983; Ball et al. 1989; Pasti et al.1990; Mason et al. 2001). Therefore, the utilization of metal-resistant actinomycetes, which are associated with plants, could be of particular importance as they can provide/solubilize nutrients such as Fe and P to plants, which could reduce the toxic effects of heavy metals. In addition, the metabolites produced by actinomycetes (e.g., siderophores and organic acids) bind Fe and other heavy metal ions and thus enhance their bioavailability in the rhizosphere of plants (Braud et al. 2009; Rajkumar et al. 2010). The resulting increase in plant growth and heavy metal accumulation by plants enhance the efficiency of phytoremediation in metal-contaminated soil.

This paper details recent advances in understanding plant and actinomycetes interaction and describes how their beneficial partnerships can be exploited as a strategy to accelerate plant growth and phytoremediation potential in heavy metal-polluted soils.

14.2 Actinomycetes and Heavy Metal Interaction

Microbial mechanisms conferring both plant growth promotion and heavy metal resistance have significant environmental importance because of their potential use in phytoremediation. In order to survive in metal-polluted environment, actinomycetes have evolved a number of mechanisms, by which they tolerate high concentrations of heavy metals (Pavel et al. 2013). Actinomycetes have been shown to alter heavy metal toxicity/bioavailability through various metal-independent mechanisms including (a) reduction of cellular sensitivity, (b) siderophore-heavy metal complexation, (c) intracellular metal sequestration,

Table 14.1 List of plant growth-promoting actinomycetes

Bacterial strains	PGP traits	References
Streptomyces sp.	Siderophore production, IAA, and GA3 production	Goudjal et al. (2015)
Streptomyces sp.	Gibberellic acid, IAA, abscisic acid, kinetin, and benzyladenine	Rashad et al. (2015)
Streptomyces aurantiogriseus	IAA production, antagonistic against *Rhizoctonia solani* in rice sheath blight	Harikrishnan et al. (2014a, b)
Streptomyces sp., *Micromonospora* sp., *Nocardia* sp., *Actinomadura* sp., *Microbispora* sp., and *Actinoplanes* sp.	Antagonistic against soil-borne pathogens of soybean	Dalal and Kulkarni (2014)
Streptomyces sp.	Production of IAA and siderophore	Rafik et al. (2014)
Actinomycetes	Production of HCN, IAA, siderophore, and phosphate solubilization	Damle and Kulkarni (2014)
Streptomyces sp.	ß-1,3-Glucanase, IAA, and HCN production	Gopalakrishnan et al. (2013, 2014)
Streptomyces sp.	Siderophore production	Lee et al. (2012)
S. rochei, *S. carpinensis*, *S. thermolilacinus*	Production of siderophore, IAA, and phosphate solubilization	Jog et al. (2012)
Rhodococcus sp.	IAA production	Costa and Melo (2012)
Rhodococcus erythropolis	Enhancing plant growth under Cr^{6+} toxicity	Patel et al. (2012)
Frankia sp., *Actinoplanes* sp., *Micromonospora* sp., and *Streptomyces* sp.	Production of IAA, gibberellin, and zeatin	Solans et al. (2011)
Streptomyces and non-identified *non-Streptomyces* strains	Control egg hatching of the nematode *Meloidogyne incognita*	Ruanpanum et al. (2010)
Actinomadura glauciflava, *Nonomuraea rubra*, and *Nocardia alba*	Protease activity, ammonia, IAA, and siderophore production	Nimnoi et al. (2010)
Streptomyces sp.	Siderophore production, phosphate solubilization, and N_2 fixation	Franco-Correa et al. (2010)
Leifsonia soli	Plant growth promotion by ACC deaminase production	Madhaiyan et al. (2010)
Microbacterium azadirachtae	IAA production, P solubilization, ACC deaminase activity, and sulfur oxidation	Madhaiyan et al. (2010)
Streptomyces sp.	Production of zeatin, gibberellic acid, and IAA and antagonism against *Pseudomonas savastonii*	Ghodhbane-Gtari et al. (2010)
Actinoplanes campanulatus, *Micromonospora chalcea*, and *Streptomyces spirali*	Reduction of root crown rots induced by *Pythium aphanidermatum* in cucumber	El-Tarabily et al. (2010)
Actinomadura sp.	Production of antifungal compounds, IAA, and siderophores	Khamna et al. (2009)
Micromonospora aurantiaca	Strong antagonistic activity against *Pythium ultimum* and *Fusarium oxysporum* and IAA and P solubilization activity	Hamdali et al. (2008a, b)
Streptomyces kasugaensis	Antagonistic activity against *Pyricularia oryzae*	Schluenzen et al. (2006)
Micromonospora carbonacea	Cell wall degradation of *Sclerotina minor*	El-Tarabily et al. (2000)
Streptomyces cacaoi	Antagonism against fungi	Copping and Duke (2007)
S. olivaceoviridis and *S. rochei*	Auxin, gibberellin and cytokinin production	Aldesuquy et al. (1998)
Micromonospora endolithica	P solubilization activity	El-Tarabily et al. (1997)

and (d) exclusion through permeability barriers. Several actinomycetes can adopt to resist the toxicity of heavy metals by altering the sensitivity of cellular components. Particularly, the mutations and DNA repair mechanisms may contribute to the protection toward plasmid and genomic DNA. Similarly, the metal-resistant components such as metallothioneins produced by actinomycetes can effectively bind heavy metals (Stillman 1995; Garbisu and Alkorta 2003) by which they can mobilize or immobilize and thus reduce their toxicity to tolerate heavy metal. For instance, glutathione offers resistance to the cell by suppressing the free radical formation from Cu(II) and Fe(II) and also to Ag(I), Cd(II), and Hg(II) (Rouch et al. 1995; Bruins et al. 2000). Similarly, the production of siderophores by actinomycetes can also play an important role in complexing toxic metals and in decreasing their toxicity. Siderophores are the iron-chelating secondary metabolites produced by various microorganisms under iron-limiting conditions. Actinomycetes are abundant producer of siderophores which plays a key role in the remediation of heavy metals. Many siderophores (e.g., desferrioxamine B, desferrioxamine E, rhodotorulic acid) are relatively stable biomolecules, protected from environmental peptidases and lytic enzymes by modifying structural composition (Sessitsch et al. 2013). In general, the siderophores produced by rhizosphere microbes form complexes with Fe(III) at the soil interface, desorb Fe from soil matrix, and thus increase Fe solubility and bioavailability in the soil solution. The siderophores also possess affinity to other trace element ion (Hider and Kong 2010) by which the bacteria reduce the harmful effects of metal and help in phytoremediation process. Dimkpa et al. (2009a, b, c) reported that the bacterial culture filtrates containing three hydroxamate siderophores secreted by *Streptomyces tendae* F4 significantly promoted plant growth and enhanced the uptake of Cd and Fe by cowpea relative to the control. Similarly, a recent study by Ji et al. (2012) observed that the production of siderophore desferrioxamine B (DFOB) accounted for the increased uptake of Fe and Pu by bacteria and reported that Pu^{4+}-DFOB and Fe^{3+}-DFOB complexes inhibit uptake of the other ions and compete for shared binding sites or uptake proteins. These results suggest that Pu-siderophore complexes can generally be recognized by Fe-siderophore uptake systems of microbes. Similarly, siderophores also played an important role in biocontrol of plant pathogens and in enhancement of plant growth promotion (Shanmugaiah et al. 2015).

The mechanism of metal tolerance exhibited by the actinomycetes is also due to the ability of its cell wall to bind with metal ions and accumulate in intracellular at higher concentrations (Lin et al. 2011; Singh et al. 2014; El Baz et al. 2015). For instance, a recent study by Lin et al. (2011) demonstrated the intracellular accumulation of Zn^{2+} and Cd^{2+} in a novel species, *Streptomyces zinciresistens*, under in vitro conditions and reported the interaction of heavy metals with amino, carboxyl, hydroxyl, and carbonyl groups accounted for the observed metal biosorption. In addition, certain actinomycetes reduce mobility of heavy metals through oxidation or reduction reactions. Such transformation especially plays a key role in the reduction of the toxicity of certain elements such as Cr and Hg in soils. For example, a *Streptomyces* sp. isolated from riverine sediments was shown to reduce the mobile and toxic CrO_4^{2-} to non toxic Cr^{3+} (Amoroso et al. 2000). In a similar study, Ravel et al. (1998) demonstrated the Hg reducing potential of *Streptomyces* sp. isolated from the Baltimore Inner Harbor, at a site heavily contaminated with metal. They reported that this bacterium significantly reduced Hg(II) to elemental and volatile Hg and thereby reduce their toxicity to tolerate Hg.

Actinomycetes can also reduce the heavy metal bioavailability through producing extracellular polymeric substance (EPS). The EPSs are high-molecular-weight polymers which are composed of sugar residues. Lead, cadmium, and uranium are the most common heavy metals which bind to the EPS which results in the restriction of heavy metal entry in the cell. Albarracin et al. (2008) investigated biosorption potential of a copper-resistant *Actinobacterium*,

Amycolatopsis sp. ABO, and found that these isolates were able to accumulate 25 mg/g of Cu. Intracellularly copper was distributed in cytosolic fraction (86 %), cell wall (11 %), and ribosome/membrane fraction (3 %).

The cells exposed to excess concentration of heavy metal has to manage with the production of toxic reactive oxygen species including superoxide anions in the Fenton reaction (Stohs and Bagchi 1995). These molecules are detoxified via superoxide dismutases (SODs) which dismutate the superoxide to O_2 and H_2O_2 (Fridovich 1995). Subsequently, the hydrogen peroxide is detoxified in a catalase-mediated reaction. Schmidt et al. (2005) isolated a strain *Streptomyces acidiscabies* which showed tolerance to various metals (Ni, Cu, Cd, Cr, Mn, Zn, and Fe) conferred by Ni-containing SODs. The gene *sodN* code for the Ni-containing SODs is not only activated by Ni but also Cu, Fe, and Zn. Summers (1985) has reported that Hg-resistant *Streptomyces* sp. was able to detoxify the Hg through converting Hg^{2+} to volatile Hg^0 by mercuric reductase enzyme.

The largest mechanism of metal-resistant system in microbes is active transport or efflux system. Some efflux systems involve ATPases, and others are chemiosmotic ion/proton pumps. These mechanisms actively pump back toxic ions that have entered the cell out of the cell via active transport (ATPase pump) or diffusion (chemiosmotic ion/proton pump). As, Cr, and Cd are the three metals most commonly associated with efflux resistance. It has been shown that a particular family of *Actinobacteria* including *Streptomyces* and *Mycobacterium* sp. use efflux-like mechanism for metal removal and antibiotic tolerance. An example is the ABC transport system for antibiotics, which can also be used as efflux pump for many metals (Borges-Walmsley et al. 2003). Albarracin et al. (2005) explained that the Cu resistance mechanisms of actinomycetes could be similar to that encountered in other bacteria such as the PcoABCDRS system of *Escherichia coli* or its homologue CopABCDRS of *Pseudomonas* sp. and *Xanthomonas campestris* (Nies 1999). Thus, the conservation of Cu pumps along evolution may indicate that uptake, reduction, or efflux of copper in actinomycetes could be also due to P-type ATPases. The schematic representation of metal-resistant mechanisms of actinomycetes in metal-polluted soil is presented in Fig. 14.2. Taken together, these reports clearly indicate the potential of actinomycetes to tolerate/reduce heavy metal toxicity and suggest that suitability of these microbes for heavy metal bioremediation.

14.3 Heavy Metal Phytoremediation

The emerging technology of bioremediation which paved the potential way for removal of heavy metals is phytoremediation. The term phytoremediation denotes the broaden area of remediation of polluted environment using plants which includes:

1. *Phytoextraction*: Cultivation of metal hyperaccumulating plants to remove the metals by concentrating them in harvestable parts of the plant
2. *Rhizofiltration*: Adsorption/precipitation of metals onto roots or absorption by roots of aquatic metal-tolerant plants
3. *Phytostabilization*: Immobilization of metals in the soils by adsorption onto roots or precipitation in the rhizosphere
4. *Phytovolatilization*: Conversion of pollutants to volatile form and their subsequent release to the atmosphere
5. *Phytohydraulics*: Absorption of large amount of water by fast-growing plants and prevent expansion of contaminants into adjacent uncontaminated areas
6. *Rhizodegradation*: Decomposition of organic pollutants by rhizosphere microorganisms
7. *Phytoresaturation*: Revegetation of barren area by fast-growing plants that cover soils and thus prevent the spreading of pollutants into environment (Masarovičová and Kráľová 2012)

Although a large number of plants are tolerating/accumulating high concentrations of heavy metals, the adverse environmental conditions

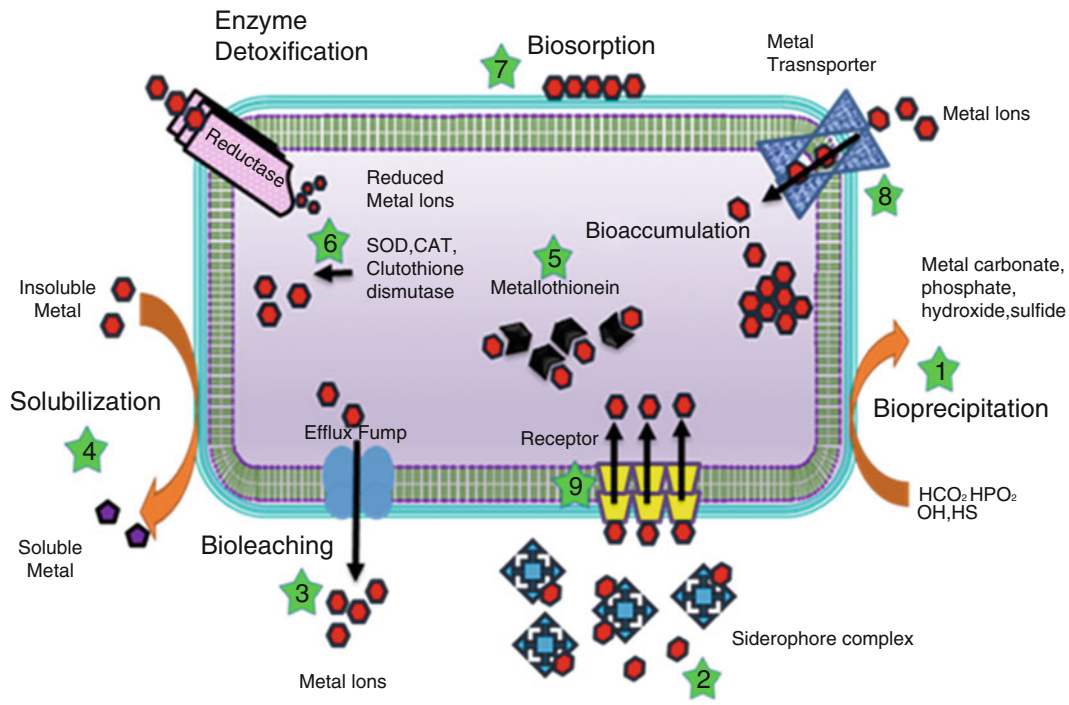

Fig. 14.2 Microbial interactions with heavy metals in polluted soils. (*1*) Precipitation/crystallization of metals occurs due to the production of secondary metabolites; (*2*) secretion of siderophore decreases metal bioavailability by complexation reaction; (*3*) plasmid-DNA-encoded efflux transporters (e.g., ATPase pumps or chemiosmotic ion/proton pumps) expel the accumulated metals outside the cell; (*4*) organic acids secreted by bacteria solubilize the insoluble metal minerals; (*5*) synthesis of metallothioneins and cysteine-rich proteins binds to the metals with greater affinities; (*6*) detoxification of metal by production of enzymes such as reductase and superoxide dismutase; (*7*) metals bind to the cell wall components of anionic functional groups and extracellular polymeric substance secreted by the bacterium; (*8*) metals enter into the cell by metal transporters either through ATP hydrolysis or chemiosmotic gradient across the cytoplasmic membrane

particularly poor soil quality, higher concentrations of metals, multi-metal-contaminated soils, etc., generally impair the plant metabolism and thus reduce growth, survival, and overall phytoremediation potential in polluted soils. To overcome this limitation, the plant-associated bacteria have been extensively used as inoculants that confer plant metal tolerance, improve plant growth and health, mobilize/immobilize heavy metals, and are able to maintain a stable relationship with plants in metal-polluted soils. The following sections summarize the effects of plant-associated actinomycetes on plant growth in metal-polluted soils (Fig. 14.3).

14.4 Plant Growth-Promoting (PGP) Potential of Actinomycetes

Actinomycetes are recognized as a potential group of rhizobacteria which influence the plant growth, yield, and nutrient uptake by an array of mechanisms including the production of auxins, ACC deaminase, nitrogen fixation, siderophore production, and phosphate solubilization. Actinomycetes directly regulate plant physiology by mimicking synthesis of plant hormones, whereas other microorganisms increase mineral and nitrogen availability in the soil as a way to

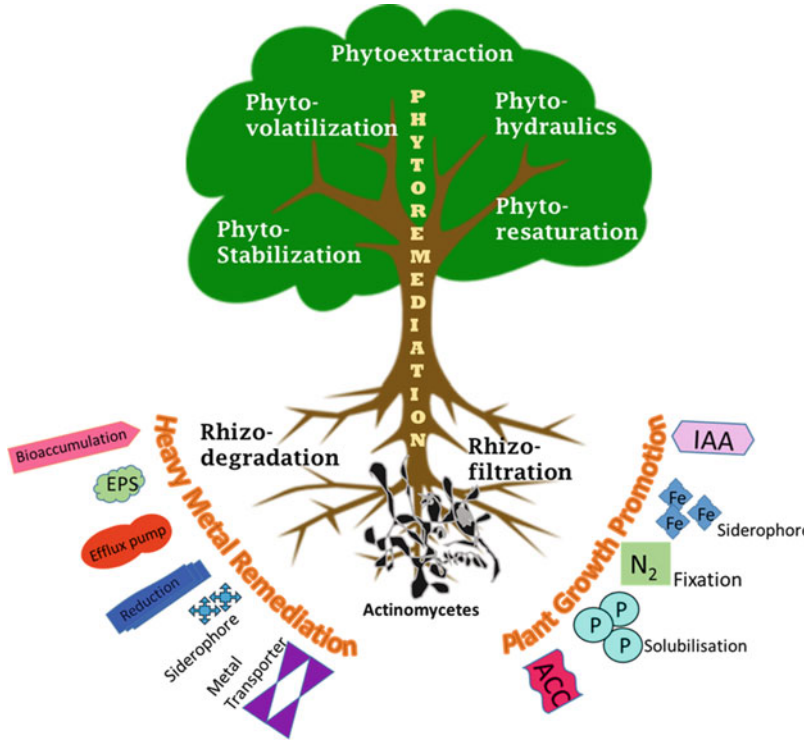

Fig. 14.3 Various types of microbial assisted heavy metal phytoremediation

augment growth. The isolates could exhibit more than two or three PGP traits, which may promote plant growth directly, indirectly, or synergistically (Yasmin et al. 2007).

14.4.1 Indole-Acetic Acid (IAA)

Auxins are classified as the main phytohormone which regulate growth, ontogeny, morphogenesis, and adaptive and repair processes in plants (Shatheesh Kumar 2011). It was shown that auxins play an important role in root formation, elongation, promotion of ethylene production, and fruit ripening (Table 14.2). Among the numerous auxins that can be produced by plants and microorganisms, IAA, have received increasing attention as potential compounds to improve the plant growth and development. Experiments with *Citrus reticulata* revealed that the inoculation with the *Nocardiopsis* of actinomycetes increased the shoot height, shoot fresh weight, and root fresh weight from 20.2 % to 49.1 %, 14.9 % to 53.6 %, and 1.6 % to 102 %, respectively (Shutsrirung et al. 2013). This effect was attributed to the increased level of IAA (222.8 μg/mL) produced by the strain that was able to promote the plant growth. Harikrishnan et al. (2014a) assessed the ability of IAA producing *Streptomyces aurantiogriseus* to promote the growth of rice (*Oryza sativa*) plants and reported that *S. aurantiogriseus*, which produces high levels of IAA, increased the root and shoot length from 3.3 to 9 cm and 3.63 to 10.2 cm, respectively. Likewise, Cruz et al. (2015) also observed that the inoculation with IAA producing actinomycetes increased the growth and yield of rice under greenhouse conditions. The inoculation with *Streptomyces* sp. that had been isolated from wheat field has also been studied in detail (Sadeghi et al. 2012). These bacteria significantly reduced the toxicity of salt stress in wheat plants and promoted the plant growth and nutrient (N, P, Fe, and Mn) uptake under in vivo conditions. Here, it was suggested that IAA production together with other plant growth-promoting mechanisms, such as phosphate solubilization and siderophore

Table 14.2 Phytohormones produced or modulated by *Actinobacteria* (Modified from Hamedi et al. 2015)

Phytohormone	Functions in plant[a]	*Actinobacteria*	References
Indole-3-acetic acid	Stimulates seed and tuber germination, initiates lateral and adventitious root formation, and affects biosynthesis of metabolites	*Micromonospora*, *Streptomyces*, and *Frankia*	Solans et al. (2011), Hirsch and Valdes (2010), Goudjal et al. (2015), and Harikrishnan et al. (2014a)
Brassinolide	Increases content of chlorophyll, stimulates protein synthesis, activates certain enzymes, and regulates cellular differentiation	*Streptomyces*	Merzaeva and Shirokikh (2010)
Salicylic acid	Induces SAR, prolongs life of flowers, inhibits ethylene biosynthesis, and facilitates pollination of certain plants	*Streptomyces*	Lin et al. (2011)
Cytokinins	Key role in plant morphology, leaf senescence, and source-sink relationships, key regulators of the plant growth defense	*Micromonospora*, *Streptomyces* and *Actinoplanes*	Scherlacha and Hertweck (2009), Mohandas et al. (2013)
Jasmonic acid	Induces ISR against necrotrophs, activates phylloptopsis, tuber formation, fruit ripening, and pigment formation	*Streptomyces*	Merzaeva and Shirokikh (2010)
Gibberellins	Stimulate stem elongation by stimulating cell division and elongation. Stimulate bolting/ flowering	*Micromonospora*, *Frankia*, *Actinoplanes*, and *Streptomyces*	Solans et al. (2011), Mohandas et al. (2013), Rashad et al. (2015)
Serotonin (5-hydroxytryptamine)	Structural analog of auxins and plant metabolize serotonin to IAA	*Streptomyces*	Tsavkelova et al. (2005)
Abscisic acid	Phylloptopsis, closure of stomata and aging	*Streptomyces*	Bach and Rohmer (2012), Rashad et al. (2015)

[a]Liu et al. (2009)

production, accounted for the observed increase in growth of the test plants. Several of the plant-associated actinomycetes have also been reported to protect the plants from various soil-borne pathogens (Verma et al. 2011; Harikrishanan et al. 2014a, b). For instance, Verma et al. (2011) reported that the inoculation of spore suspension of *Streptomyces* strain AzR-051 significantly promoted plant growth and antagonized the growth of *Alternaria alternata*, causal agent of early blight disease in tomato plant.

14.4.2 Siderophore Production

Among the various plant growth-promoting traits, the production of siderophores by bacteria is of special significance because of its metal-chelating properties which play pivotal roles in increasing the Fe concentration in the rhizosphere soils and its uptake by plants. Valencia-Cantero et al. (2007) demonstrated the potential of siderophore-producing actinobacterial strain *Arthrobacter maltophilia* to protect *Phaseolus vulgaris* (common bean) form alkaline stress and reported this effect may be due to increased level of siderophores produced by the *A. maltophilia* that were able to increase Fe availability in the rhizosphere of the plants. Rungin et al. (2012) reported that the inoculation of an endophytic *Streptomyces* sp. GMKU 3100 to rice and mung bean plants significantly increased root and shoot biomass and length of test plants compared with non-inoculated and siderophore-deficient mutant treatments. This study indicates that siderophores of *Streptomyces* sp. GMKU played a major role in making

sequestered iron available to the plant. Since the siderophores in rhizosphere soil may form complexes with other heavy metal ions and minimize the toxic effects of free metal ions, the heavy metal-siderophore complex is considered as less toxic than the free form of heavy metals. Dimkpa et al. (2008) have pointed out metal-chelating properties of siderophores, accounted for reduced heavy metal toxicity and increased auxin production in plants. They attributed the alleviation of metal toxicity to siderophore and metal complexation, thus protecting auxin from the toxic effects of free form of toxic metals.

14.4.3 ACC Deaminase Activity

Another important way in which the actinomycetes might influence the host plant growth is the utilization of ethylene precursor ACC as the sole source of nitrogen into α-ketobutyrate and ammonia. Actinomycetes containing ACC deaminase metabolize ACC, thereby lowering stress-ethylene level and enhancing plant growth (Glick 2005). *Kibdelosporangium phytohabitans* sp. KLBMP 1111^T, a novel endophytic actinomycete isolated from root of the oilseed plant *Jatropha curcas*, has the ability to utilize ACC as a sole source of nitrogen via ACC deaminase enzyme. It also has the ability to produce siderophore and IAA (Xing et al. 2012). Halotolerant non-*Streptomycete Actinobacteria* such as *Micrococcus yunnanensis*, *Corynebacterium variabile*, and *Arthrobacter nicotianae* isolated from saline coastal region of Yellow river were reported to exhibit ACC deaminase activity and were able to significantly promote the growth of canola plants under salt stress condition (Siddikee et al. 2010). Similarly, El-Tarabily (2008) demonstrated that *Streptomyces filipinensis* no. 15 was able to reduce the level of ACC in roots and shoots promotes the growth of the tomato plants. They attributed this effect to the ability of actinomycetes to lower endogenous ACC level and low stress-ethylene accumulation.

14.4.4 Nitrogen Fixation

Nitrogen fixation is a process by which atmospheric nitrogen (N_2) is converted into ammonia (NH_3) (Wagner 2011), which can be assimilated by plants for the synthesis of nitrogenous biomolecules. A few species of *Arthrobacter*, *Agromyces*, *Corynebacterium*, *Mycobacterium*, *Micromonospora*, *Propionibacteria*, and *Streptomyces* have been shown to possess N_2 fixation trait. Similarly free-living or symbiotic *Frankia* can also enhance plant growth and development in different soils and climate regions through nitrogen fixation. Particularly, the actinorhizal nitrogen fixation (symbiotic association between *Frankia* and dicotyledonous plants) plays a major role in establishing the plantations at adverse sites (Diagne et al. 2013). Similarly, some species of *Thermomonosporaceae* and *Micromonosporaceae* family also demonstrated to fix atmospheric nitrogen (Valdés et al. 2005). Similarly, *Streptomyces thermoautotrophicus* has been reported to utilize N_2 as a sole nitrogen source when growing chemolithoautotrophically with CO or H_2 and CO_2 under aerobic conditions at 65 °C (Gadkari et al. 1992).

14.4.5 Phosphate Solubilization

Phosphorus is the second most important nutrient for plants, after nitrogen. It exists in soil as mineral salts or incorporated into organic compounds. Phosphate deficiency is one of the limiting factors in crop production. Microbes are able to solubilize insoluble phosphates in metallic complexes or in hydroxyapatite and release free phosphates (Rodríguez and Fraga 1999). Recent studies investigating the role of actinomycetes in plant growth promotion have demonstrated that the bacterial colonization often results in increased P solubilization and its uptake by plants. For instance, the increased plant growth and P uptake have been reported on the inoculation of *Streptomyces griseus* (Hamdali et al. 2008a, b), *Streptomyces*

mhcr0816 and mhce0811 (Jog et al. 2012), *Microbacterium* sp. F10a (Sheng et al. 2009) in wheat plant, *Streptomyces*, and *Thermobifida* in *Trifolium repens* (Franco-Correa et al. 2010).

Although previous studies suggest that the inoculation of plants with beneficial actinomycetes could be a suitable approach for plant growth promotion, several authors have pointed out that single plant growth-promoting trait was not solely responsible for the plant growth. A large number of studies confirm the existence of cumulative effects of microbes such as the production of IAA, ACC deaminase activity, nitrogen fixation, siderophore production, and phosphate solubilization. For instance, Selvakumar et al. (2015) recently reported the potential of osmotolerant *Actinobacterium Citricoccus zhacaiensis* B–4 on the growth of onion plants under PEG-induced drought stress and reported that *Actinobacterium* improved the seedling vigor and germination rate of onion seeds (cv. Arka Kalyan) at osmotic potentials up to −0.8 MPa. They attributed this effect to the ability of the bacterium to exhibit various plant growth-promoting traits including the production of IAA and GA3, solubilization of phosphate and zinc, and ACC deaminase activity. Similarly, Mrinalini and Padmavathy (2014) also demonstrated that endophytic *Streptomyces* sp. Mrinalini7, isolated from neem plant, was able to promote the growth of tomato seedling through several plant growth-promoting traits such as IAA, ACC deaminase, phosphate solubilizing, siderophore, and ammonia production. These examples illustrate mechanisms, by which actinomycetes improve the plant growth and reflect the suitability of these microbes for improving heavy metal phytoremediation process.

14.5 Actinomycetes in Heavy Metal-Polluted Soils

Heavy metal contamination not only affects the plant growth and development but also influences the growth, survival, and activity of plant-associated microbes in polluted sites. However, numerous studies have demonstrated that actinomycetes isolated from metal-polluted soils exhibit multiple-metal tolerance as they have adopted to such environment and play an important role in metal detoxification process in the rhizosphere soil that determines the plant quality and yield (Ahemad and Kibret 2014). For instance, Gremion et al. (2003) characterized the metabolically active bacteria in heavy metal-contaminated rhizosphere soil of *Thlaspi caerulescens* using 16S ribosomal DNA and reverse-transcribed 16S rRNA clone libraries and reported that the dominant part of the metabolically active group of bacteria was *Actinobacteria* in both bulk and rhizosphere soil. Likewise, numerous studies have demonstrated the *Actinobacteria* as a consistently dominant group together with α *Proteobacteria* in metal-contaminated soils (Lazzaro et al. 2008; Karelova et al. 2011; Tipayno et al. 2012), which suggest a potential adaptation of the actinomycetes population to the heavy metal stress condition. The strains *Streptomyces* sp. A160 and S164 and *Streptomyces fradiae* A161 isolated from the soil of the Bay of Bengal showed the resistance to Cu up to 480 mg/L. Further, these strains also exhibited antibacterial and antifungal activity against wide range of pathogenic microbes. Moreover, the filamentous nature of the actinomycetes makes them as a potential heavy metal accumulator (Panday et al. 2004). Recently, Daboor et al. (2014) isolated heavy metal-resistant *Streptomyces chromofuscus* K101 from Nile River and assessed its heavy metal absorption potential. They found that *S. chromofuscus* was able to absorb high concentrations of metals with the order of $Zn^{2+}>Pb^{2+}>Fe^{2+}$ in single or mixture metal reaction. Similarly, Hamedi et al. (2015) assessed cadmium accumulation potential of *Promicromonospora* sp. UTMC 2243 and found that the isolate was able to remove 96.5 % of Cr from aqueous solution. Vinod et al. (2014) reported Cr, Cu, Pb, and Zn accumulation potential of metal-resistant *Streptomyces roseisederoticus* (V5), *Streptomyces flavochromogenes* (V6), *Streptomyces vastus* (V7), and *Streptomyces praguaeneses* (V8) isolated

from the rhizosphere soil of *Casuarina equisetifolia*. It was found that the *S. roseisederoticus* (V5) exhibited highest biosorption capacity for Cr, whereas *S. flavochromogenes* (V6) exhibited highest biosorption for Pb.

Several authors have pointed out that actinomycetes and their interactions with heavy metals (e.g., heavy metal biosorption/bioaccumulation, oxidation/reduction, and metal mobilization/immobilization) greatly influence the biomass production and quantity of metal accumulation in plants growing on metal-contaminated field soils. The following sections describe how the metal-resistant actinomycetes influence the plant growth and heavy metal uptake by plants in polluted soils (Table 14.3).

14.6 Role of Actinomycetes in Heavy Metal Phytoremediation

The functioning of plant and microbial interaction can be influenced by properties of rhizosphere soil. Actinomycetes play significant roles in plant growth under adverse environmental conditions by solubilizing plant nutrients, maintenance of soil structure, mobilization/immobilization of toxic chemicals, and controlling of plant pathogens (Giller et al. 1998; Elsgaard et al. 2001; Filip 2002; Jing et al. 2007). Besides, actinomycetes and their host plants can form specific associations in which the plant provides nutrients through root exudation that induces the growth, survival, and colonization potential of rhizosphere microbes. The metal-tolerant actinomycetes, such as *Streptomyces*, *Amycolatopsis*, and *Rhodococcus* (Trivedi et al. 2007; El Baz et al. 2015; Sunil et al. 2015), have been found to have potential to improve the plant growth and heavy metal mobilization or immobilization in metal-polluted soils. The abundant presence of actinomycetes in the metal-contaminated rhizosphere soil and its ability to withstand extreme environment make it suitable as a potential microbe which assisted the plants in remediation of heavy metal (Reinicke et al. 2013). Specifically, the metal-resistant actinomycetes have been reported to possess several traits that can alter heavy metal uptake by plants through acidification or by producing metal mobilizing/immobilizing substances. Experiments with *Sorghum bicolor* (sorghum) revealed that the inoculation of heavy metal-resistant *Streptomyces mirabilis* P16B-1 significantly increased the new tip growth and biomass of the sorghum plants as compared to the controls (Schutze et al. 2013). Similarly, Trivedi et al. (2007) demonstrated the potential of a psychrotrophic actinomycete *Rhodococcus erythropolis* to protect *Pisum sativum* (pea) from the toxicity of Cr in high concentrations and reported that this effect may be due to the reduction of Cr^{6+} to Cr^{3+} and various PGP traits such as the production of IAA, ACC deaminase activity, phosphate solubilization, and siderophore production. Khan et al. (2015) reported the greater potential of the Cr-resistant bacterium, *Microbacterium arborescens* HU33 associated with *Prosopis juliflora*, to protect ryegrass (*Lolium multiflorum*) from the toxicity of high concentrations of heavy metals such as Cr, Cd, Cu, Zn, and Pb grown on the tannery effluent contaminant soil. They attributed this effect to the ability of the bacterium to produce of IAA, siderophore, ACC deaminase, and solubilize P. Further, they reported that the inoculation of bacteria enhanced the heavy metal uptake of ryegrass plants. Javaid and Sultan (2012) reported that *Streptomyces* sp. isolated from the farmlands were shown to reduce toxic form of chromium [Cr(VI)] to less toxic form of Cr (III). This study suggests that by inoculating the plants with Cr-reducing actinomycetes, it should be possible to improve plant growth and Cr (VI) bioremediation.

An experiment with *Arthrobacter creatinolyticus* isolated from the rhizosphere of *Spartina densiflora* also revealed that the inoculation of microbial consortia along with *A. creatinolyticus* significantly increased the seed germination and plant growth under Cu and NaCl stress. In this case, enhanced plant growth could be correlated with various PGP traits such as N_2 fixation and phosphate solubilization (Andrades-Moreno et al. 2014). Likewise,

Table 14.3 Examples of actinomycetes involved in phytoremediation of heavy metals

Actinomycetes	Source of strain	Plants	Metals	Role of actinomycetes in phytoremediation	PGP traits of actinomycetes	References
Microbacterium arborescens HU33	*Prosopis juliflora*	Rye grass	Cr, Cd, Cu, Zn, and Pb	Increased accumulation in root and shoot	ACC deaminase, P solubilization, IAA, and siderophore	Khan et al. (2015)
Streptomyces sp. HM1	Rhizospheric soil	*Zea mays*	Cd	Increased metal tolerance	Increases chlorophyll content, PGP traits	El Sayed et al. (2015)
Streptomyces mirabilis P16B-1	Soil from uranium mining area	*Sorghum bicolor*	U, Cu, Ni, Cd, Co, and Zn	Decreased metal bioavailability	Promote plant growth	Schutze et al. (2013)
Arthrobacter creatinolyticus	Rhizospheric soil	*Spartina densiflora*	Cu	Resistance toward Cu	N_2 fixation and P solubilization	Andrades-Moreno et al. (2014)
Streptomyces sp.	Farm lands	NA	Cr	Converted toxic [Cr(VI)] to less toxic Cr (III)	IAA	Javaid and Sultan (2012)
Arthrobacter sp, *Micrococcus* sp., and *Microbacterium* sp.	Copper mine wasteland	*Brassica napus*	Cu, Zn, Pb, Cd, and Ni	Increased metal uptake and reduced metal stress	ACC deaminase and P solubilization	He et al. (2010)
Cellulosimicrobium cellulans	Rhizospheric soil	Green chilli	Cr	Reduced Cr uptake	IAA and P solubilization	Chatterjee et al. (2009)
Rhodococcus erythropolis	Psychrotrophic metal-contaminated soil	*Pisum sativum*	Cr	Decreased uptake of metals by plants	Promote plant growth – ACC, IAA, siderophore	Trivedi et al. (2007)
Microbacterium arabinogalactanolyticum	Rhizospheric soil	*Alyssum murale*	Ni	Increased Ni accumulation	N_2 fixation	Abou-Shanab et al. (2006)
Arthrobacter spp. UMCV	Rhizosphere soil	Common bean	Fe	Converted Fe^{3+} to soluble Fe	Siderophore	Valencia-Cantero et al. (2007)
Frankia sp.	Rhizospheric soil	*Alnus glutinosa*	Ni	Decreased metal availability	Increased nodulation	Wheeler et al. (2001)
Arthrobacter mysorens	Rhizospheric soil	Barley	Cd and Pb	Decreased toxicity	Increased uptake of nutrients	Belimov and Dietz (2000)

Wheeler et al. 2001 also observed that the inoculation of *Frankia* sp. significantly increased yield of their host *Alnus glutinosa* in the presence of Ni. Although previous studies have demonstrated a significant role of actinomycetes in facilitating the heavy metal uptake by plants, the molecular mechanisms involved in microbe-mediated heavy metal uptake by plants remain unknown. Moreover, there are some opposing viewpoints that the inoculation of actinomycetes reduced heavy metal accumulation in plants. For instance, Chatterjee et al. (2009) reported that the inoculation of Cr-reducing actinomycetes *Cellulosimicrobium cellulans* increased the plant growth and reduced Cr uptake in chilli plants. These contrasting effects may be due to microbial metal mobilization/immobilization potential, rhizosphere soil properties, the differences in the ability of plants to uptake heavy metals, metal toxicity, and its bioavailability.

14.7 Conclusions

The seriousness of heavy metal pollution in the environment dragged the attention of researchers toward sorting out of solutions for the removal of contaminants and a safer life. Though many conventional technologies have been employed, phytoremediation gains much importance because of its safe and eco-friendly method for remediation of these toxic heavy metals. Actinomycetes associated with the plant proved as a potential candidate in assisting phytoremediation. The metal-resistant beneficial actinomycetes not only improve the plant growth in metal-polluted soils but also protect their host plant from metal toxicity and alter heavy metal accumulation in plant tissues. The beneficial effects caused by actinomycetes indicate that inoculation with these microbes might have potential to improve phytoremediation efficiency in metal-contaminated soils. However, almost all the previous research on actinomycete-assisted phytoremediation were carried out in lab or greenhouse conditions; hence, further work including the interactions among actinomycetes, heavy metals, and plant is essential to apply this strategy in metal-polluted field level. Similarly, since the molecular background of mechanisms involved by actinomycetes in plant growth promotion and heavy metal uptake by plants is not yet been fully explored, more research has to be explored in order to make an actinomycete-assisted phytoremediation more effective.

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Role of Actinomycete-Mediated Nanosystem in Agriculture

15

K.S. Subramanian, Iniyakumar Muniraj, and Sivakumar Uthandi

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.

Keywords
Actinobacteria • Plant growth promotion • Soil fertility • Biotic and abiotic stresses • Biosynthesis of nanoparticles • Legumes

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 mechansisms 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

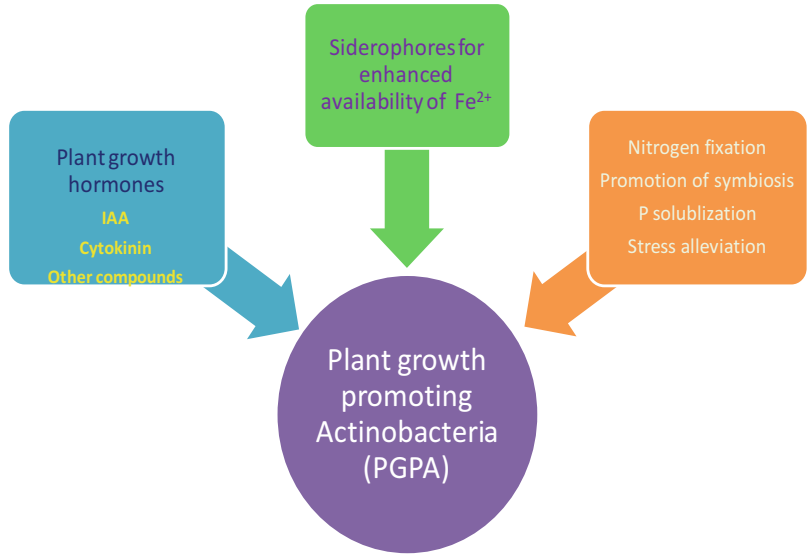

Fig. 15.1 Plant growth-promoting properties of actinobacteria

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 $\mu g.ml^{-1}$) was secreted by *Streptomyces* sp. isolated from the rhizosphere region of medicinal plants (Khamna et al. 2009). Maximal IAA secretion of 143 $\mu g.ml^{-1}$ 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 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.

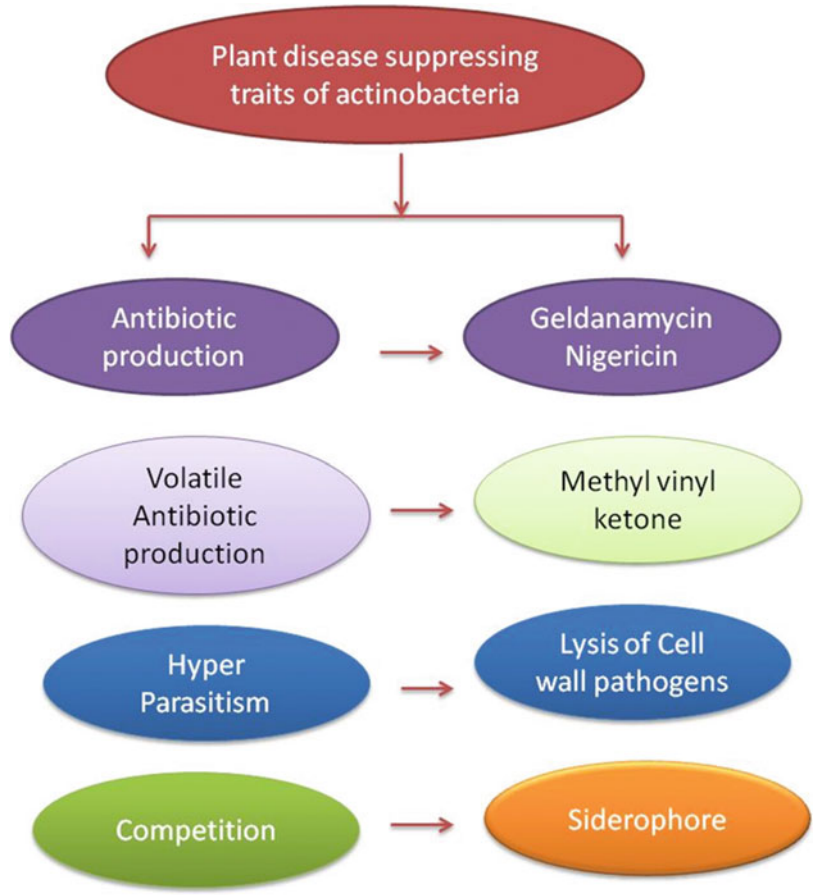

Fig. 15.2 Mechanisms of plant disease suppression adopted by actinobacteria

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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Use of Genomic Approaches in Understanding the Role of Actinomycetes as PGP in Grain Legumes

16

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Abstract

The advancement in molecular technologies has given a breakthrough to explore the untapped and novel microbial isolates for characterization in every aspect as we can consider microbes as an important primary natural store house for key secondary metabolites and enzymes. Actinomycetes are the most fruitful source of microorganisms for all types of bioactive secondary metabolites, including agroactive-antibiotic molecules that are best recognized and most valuable for their role in agriculture and industries. In agriculture, actinomycetes are used as biocontrol agents against some pests and pathogenic organisms as well as plant growth-promoting (PGP) agents for crops. Use of different molecular methods, e.g., metagenomics, metatranscriptomics, genetic fingerprinting, proteogenomics, and metaproteomics, are more significant for classifying and discovering the immense diversity in microbial population and for understanding their interactions with other abiotic and biotic environmental elements. The opportunity of accessing inexpensive sequencing techniques has led to the assemblies of copious genomic data for actinomycetes, such as *Streptomyces* and related species, with the goal of discovering novel bioactive metabolic and their utility as PGP; however, the use of actinomycetes in agriculture using genomic approaches is in its initial stages.

Keywords

Actinomycetes • Plant growth-promotion • Grain legumes • Whole genome sequence • Molecular technologies

16.1 Introduction

The analysis of microbial communities with the recent advances in culture-independent molecular techniques, including sequencing

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technologies and genomics information, has begun a new era of microbial ecology. Multiple techniques in molecular approaches based on direct analysis of lipids, proteins, and nucleic acids from environmental samples have uncovered structural and functional information about microbial communities. Molecular techniques, such as genetic fingerprinting and whole genome sequencing (WGS), are important tools for discovering, characterizing the diversified microbial population, and understanding their chemistry with other abiotic and biotic factors in environs.

Molecular and advanced technologies have a massive role in investigating the knowledge by exploring the actinomycetes across the microbial world. Advancement of WGS has made a scientific breakthrough, which unchains the understanding of latent biochemical and molecular topographies of uncultured microbe present in composite environs. The tactic of WGS of microbes bare the chemistry of the cryptic clusters of biosynthetic-related genes that are sometimes present but hidden, because those are not well recognized for producing any bioactive secondary metabolites (Fraser et al. 2002). In recent days, a total number of six genera of actinomycetes viz. *Corynebacterium*, *Mycobacterium*, *Arthrobacter*, *Frankia*, *Rhodococcus*, and *Streptomyces* have enough information about complete genome sequences to extemporize the core analysis of potential secondary metabolite and gene diversity (James and William 2013). Utilizing inexpensive sequencing techniques has led to the gathering of enormous genome sequencing data for *Streptomyces* and related species (Liu et al. 2013) with the goal of discovering novel bioactive metabolites. This chapter summarizes recent progress in the potential applications of actinomycetes using genomic approaches in agriculture. How they can be combined for a comprehensive evaluation of actinomycetes has been illustrated with example studies.

16.2 Role of Molecular Approaches for Identification of Actinomycetes

For identification and characterization of any biological organism, nucleic acid-based molecular approach is considered the most powerful approach and provides significant information about the organisms and the relationship with others (Kumar et al. 2014a). In past decades, classification and identification of organisms by approach of molecular systematics was based on nucleic acid hybridization studies. Gradual introduction of nucleic acid-based sequencing techniques in molecular systematics has been proved to be authentic (O'Donnell et al. 1993).

Following the polymerase chain reaction (PCR) technology, for better separation of the PCR products in polyacrylamide matrix, urea-formamide denaturing gradient gel electrophoresis (DGGE) (Myers et al. 1985) and temperature gradient gel electrophoresis (TGGE) (Riesner et al. 1989) were well adapted in the laboratory for studying microbial ecology. Later, for getting a sequence-based DNA fingerprint of microbial populations, temperature gradient gels were found to be quite promising (Muyzer 1999). Heuer et al. (1997) used DGGE and TGGE to study the genetic diversity of actinomycetes in different soils and to monitor shifts in their abundances in the potato rhizosphere. They used actinomycetes group-specific primers for the direct amplification of 16S rDNA and an indirect nested PCR approach using a forward actinomycetes group-specific primer and a reverse bacterial primer, followed by PCR with two bacterial primers. Use of DGGE or TGGE of the products obtained with the nested PCR made it possible to estimate the abundance of the actinomycetes populations relative to the abundance of the other bacteria present in the soil.

Sequence of 16S rDNA in systematic as well as phylogenetic studies of actinomycetes and

other bacteria is another most commonly used approach. Primarily, the study of identification and evolution of actinomycetes based on 16S rDNA has been initiated by amplifying the 16S rRNA gene implying PCR strategy and followed by direct sequencing of amplified DNA fragments (Shiva 2001). Generally, thereafter, the obtained sequences are further explored in global database using bioinformatics tools to find out the identity and genetic information of query sequence if available, followed by analysis of phylogenetic correlation which reveals the identification of the actinomycetes up to the genus level and an overview on evolutionary aspect.

In a study by Intra et al. (2011), 16S rRNA gene sequences were used to identify the diversified actinomycetes groups exists in a collection of environmental samples. Detailed comparison of 16S rRNA gene of 30 actinomycetes isolates determined that majority of the isolates (87 %) within the environmental samples belong to the genus *Streptomyces* spp., whereas one each belongs to *Saccharopolyspora* and *Nocardiopsis* and two to *Nocardia*. However, 16S rRNA sequencing does not always give clear resolution to distinguish between closely related genera (Girard et al. 2013). To order the actinomycetes, they presented a novel method based on the conserved sequence of genes *Ssg*A and *Ssg*B proteins. The wide conserve feature between members of the same genus in amino acid (aa) sequence of the *SsgB*, e.g., only one aa variation was found in between all the *Ssg*B orthologues of identified in *Streptomyces*, whereas it has low sequence identity as 40–50 % even between genera of closely related morphologically complex actinomycetes, provides concrete data for better resolution in classification systems. Recently, real-time PCR (RT-PCR) technology is being used for the specific detection and quantification of selected PGP genes of actinomycetes. Quantitative real-time PCR of selected PGP genes of actinomycetes revealed the selective up-regulation of indole acetic acid (IAA)-related and siderophore-related genes by *Streptomyces* sp., CAI-68 and of β-1,3-glucanase genes (Gopalakrishnan et al. 2015).

16.3 Utility of Advanced Genomic Approaches in Actinomycetes

The genomic technologies are promising tools to explore the untapped and novel microbial isolates for characterization in all aspects as we can consider its significance as natural store house of excellent enzymes and bioactive secondary metabolites. Fluorescence-activated cell sorting (FACS) technology is a quick method for separating the cells in a suspension on the basis of fluorescence and cell size. This technology can be performed for isolation of actinomycetes cell from a complex microbial population. Gel MicroDrop technology is an efficient enzyme-fluorescence technology, basically used for detection of positive clones by capturing of emitted fluorescence from catalytic broke down of biotinylated substrate by specific top articular enzymes present in the positive clone (Short et al. 2003).

Recently, high throughput screening (HTS) technique is considered to be rapid and economical for classifying of any microbial population by enzymatic characterization, but it is extended very little for actinomycetes. HTS consists of drop-based microfluid platform and gives an array data of insoluble substrates specific for the desired enzymes (Chang et al. 2013). Compared with all HTS-based methods, currently the proteomics approach is well accepted in the way of discovering new microbial flora and fauna. Wang et al. (2012a) first reported on *Streptomyces* sp. products α-glucosidase inhibitor miglitol using HTS method. They considered 12 actinomycete strains as to be producers of α-glucosidase inhibitors in which strain PW409 showed effective inhibitory and was used for fermentation and separation of bioactive compound using HPLC. In mass spectrometry, two compounds, miglitol and 1-deoxynojirimycin, were identified. The method can be utilized for discovering new α-glucosidase inhibitors or identifying from other inhibitory strains.

However, the common PCR methods used in microbial detection, the amplified DNA fragment, is always not prominent in visibility for

the important microbial species, which are relatively less abundant. Nowadays, two promising and pioneering approaches, preamplification inverse-PCR (PAIPCR) and substrate-induced gene expression screening (SIGEX), are used extensively to overcome this problem in characterization of actinomycetes from metagenomic DNA shuffling in a particular microflora (Kennedy et al. 2010). MALDI-LTQ-Orbitrap is one of the proteomics-based techniques for identifying desired proteins from suspensions and complex matrices. This technique is worked based on the principal of chromatography separations in both media liquid and gas with the coordination of MALDI and ion trap system (Akeroyd et al. 2013). Electrospray ionization mass spectrometry (ESI-MS) is another promising ionization technique that can measure proteins as little as femtomole quantities (Smith et al. 2013). Few in silico techniques, e.g., 3DQSAR, CoMSIA, and CoMFA, are likely to be promising to characterize potent enzymes from database and have the ability to predict superior enzymes and its in silico reaction with substrate and development of microenvironment during reaction (Murumkar et al. 2009).

16.4 Application of Actinomycetes in Agricultural Crops: Genomic Approach

16.4.1 Actinomycetes as Source of Bioactive Compounds

Actinomycetes specifically *Streptomyces* are the most fruitful source for all types of bioactive secondary metabolites. Approximately 60 % of the new insecticides and herbicides reported in the past 5 years originate from *Streptomyces* (Tanaka and Omura 1993; Roshan et al. 2013; Kumar et al. 2014b). It also is estimated that as many as three-quarters of *Streptomycetes* spp. are able to produce antibiotics (Alexander 1977). Actinomycetes produce a variety of secondary metabolites and have a wide range of uses, including antimicrobial, antifungal, herbicidal, antineoplastic, and plant growth-promoting agents. Gopalakrishnan et al. (2011) reported the potential of selected actinomycetes isolates as biological control of *Fusarium* wilt and dry root rot diseases in grain legumes. They reported five most promising antagonistic isolates of *Streptomyces*'s species (CAI-24, CAI-121, CAI-127, KAI-32, and KAI-90) and characterized for the production of siderophore, hydrocyanic acid (HCN), protease, cellulase, IAA, etc. These actinomycetes are likely to be the potential organisms for discovery of novel secondary metabolites for various biocontrol applications.

16.4.2 Actinomycetes as a Source of Nitrogen Fixation

Generally, population of actinomycetes is largely higher in rhizosphere in comparison of non-rhizosphere soils (Miller et al. 1989, 1990). Root colonization of *Streptomyces griseoviridis* in SEM studies showed a higher density in the rhizosphere of lettuce than in non-rhizosphere soil (Kortemaa et al. 1994). A similar result was found when interaction of *Streptomyces lydicus* WYEC 108 and nodules of pea was studied in SEM. *S. lydicus* was found to be colonized at nodulation sites, and then the vegetative hyphae moved onto root hairs and from the external surface of the root cells to the inside of the root cells, intermittently (Tokala et al. 2002). The PCR-DGGE analysis of DNA from colonized nodules showed the presence of a *Streptomyces* band in addition to other bands corresponding to the plant and *Rhizobium*.

Rhizobia with legumes are considered under PGPR genera and play a bigger role in nitrogen fixation. Nitrogen is the most essential nutrient for plant productivity and growth, and it is a vital element for all forms of life. Although 78 % of the atmospheric volume contains dinitrogen they remain unavailable to the plants. Plant growth-promoting actinomycete *Frankia* has the ability to fix atmospheric nitrogen to ammonia

(available form for plants) and provide it to plants by symbiotic association with non-leguminous trees and shrubs (Zahran 2001; Ahemad and Kibret 2014). The nitrogenase (*nif*) genes responsible for nitrogen fixation are found in systems: free living or symbiotic (Reed et al. 2011). The *nif* genes include structural genes responsible for electron donation, iron-molybdenum cofactor biosynthesis, and involved in activation of the Fe-protein, and regulatory genes responsible for the synthesis and function of the enzyme.

16.4.3 Molecular Basis of Nitrogen Fixation

Agrobacterium rhizogenes and *Agrobacterium tumefaciens* are available for studying down-regulation of plant genes by RNAi in some actinorhizal plants (Svistoonoff et al. 2003; Gherbi et al. 2008a, b). In transcriptome analyses, it is found that the common symbiosis (SYM) pathway shared by rhizobium-legume and arbuscular mycorrhizal for nodulation symbiosis is present in *Frankia* (Gherbi et al. 2008a, b; Markmann et al. 2008; Hocher et al. 2011). For the induction of calcium oscillations in this pathway, a receptor, potassium channels, and nuclear pore proteins are required. A putative calcium/calmodulin-dependent protein kinase (CCaMK) also is present and might thus recognize calcium "actinorhizal signatures" (Singh and Parniske 2012). The similar genes linked to a NOD-specific pathway used by legumes for the nodulation process also is present in *Frankia*. This overlapping of legume and actinorhizal root nodule symbiosis RNS supports the hypothesis of a common genetic ancestor with a genetic predisposition for nodulation in the nitrogen-fixing clade (Soltis et al. 1995).

Because traditional approaches are not yet available for studying *Frankia* genetics, most work has proceeded through the cloning of genes via heterologous hybridization to genes from other organisms, most notably those involved in nitrogen metabolism. These genes include the cloning and sequencing of *nif*H (Normand and Bousquet 1989; Normand et al. 1988), *nif* D (Twigg et al. 1990; Normand et al. 1992), part of *nif*K (Twigg et al. 1990), *nif*B, *nif*X, *nifW*, and *nif*Z, open reading frames that correspond to the *Azotobacter vinelandii* orf 3 and *Azorhizobium caulinodans* orf l (Arigoni et al. 1991). At least 20 *nif* genes are involved in N_2 fixation in the well-characterized *Klebsiella pneumoniae*, and many of these genes have homologs in other diazotrophs (Dean and Jacobson 1992).

The nitrogenase and associated proteins are highly conserved in prokaryotes. Nitrogenase of *Frankia* also is O_2 labile, requires Mg ATP and reducing power, and produces NH_4^- and H_2 gas in an ATP-dependent fashion (Benson et al. 1979). There is no alternative N_2 fixing systems akin to the vanadium or iron-based nitrogenases reported from *Frankia*. Because *Frankia* grow and respire slowly, the delivery of substrates to nitrogenase and the maintenance of a low O_2 level in the proximity of nitrogenase are important problems encountered by *Frankia* strains. The structural genes for the Fe protein and the Mo-Fe protein of nitrogenase are encoded by the *nif*H and the *nif*D and *nif*K genes, respectively. Hybridization results have indicated that *nif*HDK in some *Frankia* strains are clustered on the chromosome (Mullin and An 1990), five genes about 4 kbp downstream from *nif*HDK have been sequenced, four of which belong to a single operon consisting of at least orf 3, orf l, *nif*W, and *nif*Z; *nif*B is located immediately downstream from *nif*Z and may be transcribed as part of another operon. The *nif*B, *nif*W, and *nif*Z are all involved in FeMo-cofactor biosynthesis (Dean and Jacobson 1992). Nucleotide and amino acid sequence analyses of *nif*H, *nif*D, and other *nif* genes confirm the similarity of *Frankia* nitrogenase with the classical Mo-Fe protein based systems (Normand and Bousquet 1989; Normand et al. 1988; Simonet et al. 1986). The most common genes present in bacteria for symbiosis and N_2 fixation is as follows (Table 16.1).

Table 16.1 Most common genes present in bacteria and actinomycetes involved in symbiosis and nitrogen fixation

Genes	Function of gene product
Nodulation genes	
*nod*A	Acyltransferase
*nod*B	Chitooligosaccharide deacetylase
*nod*C	N-acetylglucosaminyltransferase
*nod*D	Transcriptional regulator of common nod genes
*nod*IJ	Nod factors transport
*nod*PQ	Synthesis of Nod factors substituent
*nod*X	Synthesis of Nod factors substituent
nof EF	Synthesis of Nod factors substituent
Other nod genes	Several functions in synthesis of Nod factors
nol genes	Several functions in synthesis of Nod factors substituent and secretion
NOE genes	Synthesis of Nod factors substituent
Nitrogen fixation genes	
nif HDK	Nitrogenase
*nif*A	Transcriptional regulator
nif BEN	Biosynthesis of the Fe-Mo cofactor
nif B, *nif* W, and *nif* Z	Fe-Mo cofactor biosynthesis
*fix*ABCX	Electron transport chain to nitrogenase
*fix*NOPQ	Cytochrome oxidase
*fix*LJ	Transcriptional regulators
*fix*K	Transcriptional regulator
*fix*GHIS	Copper uptake and metabolism
*fdx*N	Ferredoxin
Other genes	
exo	Exopolysaccharide production
hup	Hydrogen uptake
gln	Glutamine synthase
dct	Dicarboxylate transport
nfe	Nodulation efficiency and competitiveness
ndv	β-1,2 glucans synthesis
lps	Lipopolysaccharide production

Source: Laranjoa et al. (2014)

16.5 Characterization of Actinomycetes Through Whole Genome Sequencing

Advancement of WGS has unchained the understanding of whole biochemical and molecular potentiality prevailing even in those microbes incompetent in laboratory culture from a composite environment. The WGS of microbes revealed the chemistry of the cryptic clusters of biosynthetic-related genes that are sometimes present but hidden, because those are not well recognized for any secondary metabolites synthesis (Fraser et al. 2002). Currently, six genera of actinomycetes, namely *Frankia*, *Arthrobacter*, *Corynebacterium*, *Mycobacterium, Rhodococcus* and *Streptomyces*, have enough information on whole genome sequences to extemporize the basic analysis of potential secondary metabolite and gene diversity (James and William 2013).

16.5.1 Gene Cluster Diversity Within Actinomycete Groups

The genomes of actinomycetes revealed that they have gene clusters for a high number of natural products, although a lot of these are very complex to tie to products in the laboratory. The evaluations of these gene clusters are more difficult, because the existed domains of the most common biosynthetic machinery, non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs), are repeated and highly similar. It is predictable that for being diverse lifestyles that habitats use secondary metabolites differently by different genera of actinomycetes.

In many genera, it was found that the siderophores are the most conserved secondary metabolite clusters, whether they are NRPS-independent or NRPS products. The study of Doroghazi and Metcalf (2013) showed that genomes 41 of 102 common actinomycetes contain at minimum one gene cluster for siderophore biosynthesis, which is NRPS-independent (aerobactin-like), whereas 31 genomes of 34 actinomycetes in the group of *Nocardia*, *Mycobacterium*, and *Corynebacterium* do not have this class of siderophores, but except *Corynebacterium kroppenstedtii* all contain the gene cluster for mycolic acid. In general, the more pathogenic genus *Mycobacterium* and *Corynebacterium* contain gene clusters for higher proportions of conserved secondary metabolite, whereas in *Streptomyces* and *Rhodococcus*, the

essentially saprophytic genera are less conserved. This may be due to pathogens that are inhabited in the increased homogeneity of environments compared with free-living bacteria. The pattern of host-association in *Frankia* is different where no overlap secondary metabolic capabilities are present. It is assumed that over the evolutionary period, the location of the gene clusters of natural product will change through horizontal gene transfer and it would make change in the genomes and phylogenetic trees because of genome rearrangements (Fischbach et al. 2008; Osbourn 2010).

Based on genomic data only, *Streptomyces* spp. are the most important actinobacterial groups for secondary metabolites (Table 16.2). *Streptomyces* consists of large numbers of biosynthetic gene clusters related to secondary metabolite with a large variety of classes. The common classes of PKS and NRPS are present in the majority of the *Streptomyces*, followed by terpenoids, aerobactin-like non-NRPS siderophores, and lanthipeptides. Most of the genomes contain the genes for butyrolactone biosynthesis. All *Streptomyces* spp. contain the genes responsible for the biosynthesis of the aerobactin-like siderophore desferrioxamine. Except for *S. griseus*, all *Streptomyces* contain gene cluster for the spore pigment type II PKS, whereas *S. griseus* contains type III PKS for a different spore pigment (Ohnishi et al. 2008). Only half of the strains, including *S. griseus* and *S. coelicolor* A3 (2), contain the gene cluster lanthipeptide *Sap*B that is required for aerial mycelia formation on rich media (Kodani et al. 2004). This genus has very low amount of overlap gene clusters of PKS and

Table 16.2 Recent genome publications for *Streptomyces* species

Species and strain	Motivation for sequencing	References
S. albulus CCRC 11814	Produces ε-poly-L-lysine antibiotic	Dodd et al. (2013)
S. albus J1074	Widely used host for heterologous expression of bioactive natural products; Small genome	Zaburannyi et al. (2014)
S. albulus PD-1	Produces ε-poly-L-lysine and poly-L-diaminopropionic acid antibiotics	Xu et al. (2014b)
S. bottropensis ATCC 25435	Produces bottromycin antibiotics	Zhang et al. (2013)
S. collinus Tu 365	Produces elfamycin-family antibiotic kirromycin	Rückert et al. (2013)
S. exfoliatus DSMZ 41693	Degrades poly3-hydroxyalkanoate	Martínez et al. (2014)
S. fulvissimus DSM 40593	Produces ionophore antibiotic valinomycin	Myronovskyi et al. (2013)
S. gancidicus BKS 13–15	Not known	Kumar et al. (2013)
S. mobaraensis DSM 40847	Industrial producer of transglutaminase	Yang et al. (2013)
S. niveus NCIMB 11891	Produces novobiocin, an aminocoumarin antibiotic	Flinspach et al. (2014)
S. rapamycinicus NRRL 5491	Produces immunosuppressant drug rapamycin	Baranasic et al. (2013)
S. rimosus ATCC 10970	Oxytetracycline	Pethick et al. (2013)
S. roseochromogenes subsp. *oscitans* DS 12.976	Produces clorobiocin, an aminocoumarin antibiotic	Rückert et al. (2014)
Streptomyces sp. Mg1	Causes lysis and degradation of *Bacillus subtilis* cells and colonies. Sequenced using the PacBio platform	Hoefler et al. (2013)
Streptomyces sp. PRh5	An endophyte isolated from wild rice root	Yang et al. (2014)
S. violaceusniger SPC6	Tolerant to multiple stresses. Small genome	Chen et al. (2013)
S. viridochromogenes Tu57	Produces oligosaccharide antibiotic avilamycin	Grüning et al. (2013)
S. viridosporus T7A	Produces oligosaccharide antibiotic avilamycin	Davis et al. (2013)

Source: Harrison and Studholme (2014)

NRPS. Although a large amount of polyketides and non-ribosomal peptides has been discovered already from *Streptomyces*, there are only a few reports for terpenoids in streptomycetes. However, a number of terpene synthases has been discovered in genomes of *Streptomyces* sp., suggesting that a large diverge group of terpenoids has remained to be discovered in members of this genus.

16.6 Genomics and Genetic Information of *Streptomyces*

With the goal of discovering novel bioactive compounds, the huge genomic data of *Streptomyces* and other related species has led to cheap genome sequencing techniques (Liu et al. 2013). However, productive "genome mining" is possible only when the gene clusters clone and express in any heterologous host or to force expression by genetic modification (Gomez-Escribano and Bibb 2014). Therefore, unavoidably there will be a lag between the initial state of genome characterization by sequencing and harder to depict the novel useful products by biochemical investigations. *Streptomyces* PRh5, an endophyte of wild rice, produces nigericin, an antibiotic active antagonized to mycobacteria is recently discovered (Yang et al. 2014). Genetic information of *Streptomyces* species and few other actinomycetes genera are as follows:

Streptomyces sp. strain CT34: total assembly size of the genome of *Streptomyces* sp. strain CT34 is 8,066,430 bp, with coverage of 99.85 %. The genome contains an average GC content of ~71.39 % with 7781 protein coding genes of an average length of 875 bp. The predicted total gene length is about 6,809,991 bp, which makes up 84.42 % of the genome. The analysis of genome data revealed that it comprises 30 gene clusters for secondary metabolites biosynthesis, including four for terpene biosynthesis, three of each for siderophores, PKSs (2T2-PKSs, 1T3-PKS), NRPSs, bacteriocins, and butyrolactones; two for lantipeptides, one of each for mixed lantipeptide/PKS (T1-PKS), mixed PKS (T4-PKS)/PKS (T1-PKS), mixed oligosaccharide/terpene, mixed non-ribosomal peptide synthetase (NRPS)/polyketide synthase (T1-PKS), and ectoine; and four unspecified clusters. A putative gene cluster of 12,108 bp comprises ten ORFs encoding proteins for catalytic activity and auxiliary functions and one ORF for the biosynthesis of prepeptide related to new linaridin RiPP is found to be present in the genome (Zhai et al. 2015).

Streptomyces sp. strain TOR3209: The draft genome sequence of *Streptomyces* sp. TOR3209 is 8,066,796-bp long with an average GC content of 72.59 %. It comprises 4 rRNA genes, 73 tRNA genes, and 7564 protein encoding genes with an average length of 937 bp. The sequence annotation revealed that among all of the genes, 187 genes are associated with transport, biosynthesis, and catabolism of secondary metabolites and 322 genes of unknown function. It is found that among the protein-encoding genes, several genes are involved with the regulation mechanism of rhizosphere microecology, because they take a part in the production of flavonol, flavone, hormones, terpenoid, quinone, antibiotics, and other active substances. In addition, 29 degradation pathways of toxic substances are present in TOR3209 genome. These distinguished features are not present in other microorganisms. The degradation pathways for toxic substances that are difficult to degrade include naphthalene, carbazole, fluorene, anthracene, xylene toluene, trinitrotoluene, atrazine, and ethylbenzene. These pathways may help to resolve toxic substances around crop roots. The genomic information of *Streptomyces* sp. strain TOR3209 has great significance to the research field oriented with the regulation mechanism of rhizosphere microecology (Hu et al. 2012).

Streptomyces sp. strain AW19M42: The total size of the genome is 8,008,851 bp and has a GC content of 70.57 %, similar to that of other sequenced *Streptomyces* isolates. A total of 7727 coding DNA sequences are predicted. In addition, 62 tRNAs and 8 copies of the rRNA operons are identified in the genome of *Streptomyces* sp. strain AW19M42 (Bjerga et al. 2014).

Streptomyces albus strain J1074: The total genome size of *S. albus* strain J1074 is 6,841,649 bp. It is one of the smallest

Streptomyces genomes along with *Streptomyces cattleya* (Zaburannyi et al. 2014). However, the strain contains a mega plasmid *p*SCAT of 1,809,491 bp. Analysis of chromosomal genes revealed that *S. albus* contains highest known GC content ~73.3 % within the Streptomycetes. It is found that *S. albus* have a tendency to reduce the number of orthologous groups of genes. Unlike those of other Streptomycetes genomes, it has the single chromosome includes 66 tRNA genes (41 species) and 7 rRNA operons (16S-23S-5S). The presence of seven rRNA operons may help the strain for its exceptionally fast growth rate and versatility (Klappenbach et al. 2000).

Streptomyces acidiscabies strain 84–104: The size of the draft genome sequence of *S. acidiscabies* is approximately 11,005,945 bp in length (Huguet-Tapia and Loria 2012). The genome encodes 10,070 putative proteins. Reciprocal BLAST analysis with other *Streptomyces* genomes is noted that *S. acidiscabies* 84–104 contains 75 tRNA genes and shares 3006 orthologs with *S. scabies*, *S. coelicolor*, *S. griseous*, *S. avermitilis*, and *S. bingchenggensis*. *S. acidiscabies* and *S. scabies* shares 357 orthologs, including many of which are in asyntenic (Huguet-Tapia et al. 2011).

Streptomyces albus strain NBRC 13014 T: The total size of the assembly of *S. albus* NBRC 13014T genome is 7,594,701 bp, with a GC content of 72.7 %. The genome contains at least one type-II PKS, two NRPS, two hybrid PKS/NRPS, and four type-I PKS gene clusters (Komaki et al. 2015). The type-II PKS gene cluster is required for synthesize of xantholipin-like compounds, because its CLF and KS showed 78 % and 89 % aa sequence identities to XanE and XanF, respectively (Zhang et al. 2012).

Streptomyces auratus strain AGR0001: The genome of *S. auratus* strain AGR0001 contains a linear chromosome of 7,885,420 bp, with average GC content of 71.45 %. The chromosome of *S. auratus* strain AGR0001 comprises 66 tRNA genes, 8 rRNA operons, and 7102 protein-coding genes that encode at least 3935 proteins with assigned putative functions. At least 33 putative gene clusters were identified for the biosynthesis of PKS, NRPS, or terpene in the genome of *S. auratus* strain AGR0001 (Han et al. 2012).

S coelicolor strain A3(2): *S. coelicolor* strain A3(2), a producer of most natural antibiotics, is a representative of the group of soil-dwelling, filamentous bacteria. The linear chromosome of this organism is approximately 8,667,507-bp long, containing the largest number of genes. The genome contains a total number of 7825 predicted protein genes, including more than 20 clusters that are identified as responsible for coding of predicted known secondary metabolites (Bentley et al. 2002).

Streptomyces globisporus strain C-1027: The analysis of draft sequence of whole genome of *S. globisporus* C-1027 revealed that the chromosome is 7,693,617-bp long with GC content of 71.63 %. The chromosome contains 56 tRNA genes, 5 rRNA operons, and at least 7231 putative protein CDSs account for 88.22 % of the genome. A number of clusters related to biosynthesis of varied secondary metabolites, including putative PKS genes, NRPS genes, NRPS-PKS hybrid genes, terpene cyclase genes, and lantibiotic biosynthesis, are found to be present in the genome of *S. globisporus* strain C-1027. The complete genome sequence of *S. globisporus* C-1027 will aid with understanding the biosynthesis-regulatory mechanisms of C-1027 and identifying new natural bioactive compounds by uncovering hidden metabolic pathways (Wang et al. 2012b).

S. griseus strain IFO 13350: The complete genome sequence of *S. griseus* is of 8,545,929 bp in length with no plasmids. The analysis of *S. griseus* chromosome showed that it contains at least 7138 ORFs; of them a total of 4464 ORFs are associated with known or putative functions, and the remaining 2674 ORFs are hypothetical proteins. The chromosome contains 66 tRNA genes (42 species) and 6 rRNA operons (16S-23S-5S). The average GC content of the chromosome is 72.2 %, but the ~300-kb regions at both ends (including the 133-kb TIR sequence) contained lower GC content. The replication origin *oriC* is found to be located at positions of 4,324,631–4,325,203 bp. Nineteen DnaA box-like sequences are predicted to be present

in the middle of the chromosome (52 kb away from the center toward the right end) (Ohnishi et al. 2008).

Streptomyces zinciresistens strain K42: The initial genome sequence data of *S. zinciresistens* strain K42 showed that it comprises 8,228,741 bp with high GC content of 72.46 %. It contained a single plasmid of 30,979 bp. The 5S, 16S, and probably multiple copies of 23SrRNAs, 7307 protein-coding sequences (CDSs), and 69 tRNA genes are annotated. The genome has 2019 proteins with orthologs in *S. coelicolor*, *S. avermitilis*, *S. griseus*, and *S. scabiei*. It has 2520 hypothetical proteins, which may give the high degree genome specificity of strain K42. A total of 61 diverse secondary metabolic genes, of them 31 genes predicted to be involved in biosynthesis of antibiotics could be identified in the genome of K42 (Lin et al. 2011).

Kocuria rhizophila strain DC2201: *K. rhizophila*, a divergent bacterial group of soil actinomycete belongs to the suborder *Micrococcineae*. Until now, a limited amount of genomic information has been available for *K. rhizophila*. Annotation of the whole genome sequence of *K. rhizophila* DC2201 (NBRC 103217) revealed that it contains a single circular chromosome of 2,697,540 bp with high GC content of ~71.16 %. It has 2357 predicted protein-coding genes; most of those (87.7 %) are orthologous to actinobacterial proteins with fairly good conservation of synteny with related actinobacterial genomes. In contrast, the genome seems to encode very few numbers of proteins required for lateral gene transfer, transcriptional regulation, and secondary metabolism (one each of NRPS and type III PKS), indicating the small genome size. The presence of a large number of genes related with membrane transport, especially drug efflux pumps and amino acid transporters, and of possible metabolic pathways for the transformation of phenolic compounds generated after degradation of plant materials, may contribute to the tolerance in various organic compounds and to organism's utilization of root exudates (Takarada et al. 2008).

Amycolatopsis orientalis HCCB10007: The complete genome of *A. orientalis* HCCB10007 contains an 8,948,591-bp circular chromosome and a 33,499-bp dissociated plasmid. In total, 8121 protein-coding sequences are predicted to be present in the genome. In addition, 26 gene clusters related to secondary metabolism, including the 64-kb vancomycin cluster encoded a halogenase, a methyltransferase, and two glycosyltransferases (Xu et al. 2014a).

Rhodococcus imtechensis RKJ300: The genome of *R. imtechensis* RKJ300 is 8,231,486 bp with GC content of 67.22 %. The genome comprises of 8059 predicted coding regions (CDSs), 49 tRNAs, and 5 rRNA genes (Vikram et al. 2012).

16.7 Conclusion

Researchers have great interest in the selection of actinomycetes, because they are importance in sustainable agriculture and are able to antagonize most deleterious phytopathogens. A large number of bioactive compounds have been isolated from different actinomycetes, mostly from *Streptomyces* spp. Different molecular approaches and bioinformatics tools are dynamic for discovering and characterizing the vast actinomycetes diversity. Furthermore, WGS can unzip the chemistry of the cryptic clusters of biosynthetic-related genes that are sometimes present but crypted, because those are not well acknowledged for synthesizing any antimicrobial compounds. In near future, those bioactive products synthesized-related genes may arise as the key of antagonism of major phytopathogens as well as PGP in crops.

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Exploration of Plant Growth-Promoting Actinomycetes for Biofortification of Mineral Nutrients

17

A. Sathya, R. Vijayabharathi, and S. Gopalakrishnan

Abstract

Mineral malnutrition, especially Fe and Zn, affects more than two million people around the world and increases vulnerability to illness and infections. These malnourished people live in developing countries and rely upon staple foods routinely with inability to either afford for dietary diversification or pharmaceutical supplementation or industrial fortification of minerals. Biofortification is a strategy that can tackle hidden hunger merely through staple foods that people eat every day. This strategy can be achieved through agronomic practices and conventional breeding and genetic engineering approaches, and each has their own pros and cons. The sustainability of such grain fortification with higher seed mineral concentration is soil health dependent, especially on the availability of mineral in the rhizosphere. Microorganisms, the invisible engineers in improving the soil health by solubilizing trace elements and by driving various biogeochemical cycles of soil, have the ability to serve as a key solution for this complex issue. In specific, plant growth-promoting (PGP) microbes reside in root-soil interface and employ the use of siderophores, organic acids, and exopolysaccharides for increasing the mineral availability and subsequent mobilization to the plants. Increasing the seed mineral density with the use of such PGP microbes, especially actinomycetes, is in its infancy. Hence, this chapter is aimed to bring a view on the role of microbes, especially actinomycetes, with metal-mobilizing and PGP traits for biofortification as this strategy may

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act as a complementary sustainable tool for the existing biofortification strategies.

Keywords
Biofortification • Minerals • Iron • Zinc • Grain legumes • Soil fertility • Plant growth-promoting microbes • Actinobacteria

17.1 Introduction

Peace and welfare of the human society depends fundamentally on a sufficient, balanced, and secure supply of food. But in the present scenario, undernourishment is one of the serious problems faced by poor people living in developing countries. Recent reports of FAO states that chronic undernourishment is estimated about 805 million people around the world during 2012–2014, of which about 791 million are in developing countries. Though an overall reduction of 203 million undernourished people has occurred from the last two decades, still one in eight people in these regions, or 13.5 % of the overall population, remain chronically underfed (FAO et al. 2014). The resulting food insecurity is closely linked with nutritional insecurity/malnutrition. During the discussion of world hunger, protein energy malnutrition (PEM), also called classical hunger, is highly referred because most of the hungry and undernourished people live on a mono-carbohydrate diet such as maize or rice. Incidences of PEM have been the cause for the death of 35 % of the children below 5 years of age (FAO et al. 2012). Though meat-based diet is an option to overcome PEM, its continuous supply to the developing countries is unrealistic because of high cost, high energy requirement, land and water resources for the maintenance of animal-based food systems, and also religious constraints (Pimentel and Pimentel 2003).

From the past two decades, the definition of malnutrition also covers "hidden hunger," a form of hunger also called micronutrient deficiency, caused by chronic lack of vitamins and minerals (WHO 2004). The consequences of hidden hunger will not be visible immediately, and it continues to affect the entire population though the food supply is adequate in preventing classical hunger (Kennedy et al. 2003). According to the Global Hunger Index 2014, there are two billion people suffering from hidden hunger (von Grebmer et al. 2014). Besides individual health, development, and productivity, it has subsequent socioeconomic consequences affecting overall economic growth and national income (Arcand 2001). Hence, FAO recommended to introduce nutritional-related indicators additionally in one of the dimensions of food security called "utilization" which is denoted from 2013 onward in "The State of Food Insecurity in the World" (FAO et al. 2013). The current indicators of the utilization dimension include:

1. Percentage of children under 5 years of age affected by wasting
2. Percentage of children under 5 years of age who are stunted
3. Percentage of children under 5 years of age who are underweight
4. Percentage of adults who are underweight
5. Prevalence of anemia among pregnant women
6. Prevalence of anemia among children under 5 years of age
7. Prevalence of vitamin A deficiency in the population
8. Prevalence of iodine deficiency in the population (FAO et al. 2014)

Among the micronutrient deficiencies, mineral malnutrition has higher prevalence than vitamin deficiency as it holds various facets such as (1) high impact for iron (Fe), zinc (Zn), and iodine (I) (WHO 2002), (2) less impact for calcium (Ca) and selenium (Se) (WHO 2004), and impact at subpopulations or at regional levels for

magnesium (Mg) and copper (Cu) (White and Broadley 2009). Among them, Fe deficiency (FeD) and Zn deficiency (ZnD) are the prevalent mineral deficiencies and ranked 9th and 11th, respectively, among the 20 leading health risks. FeD leads to anemia, impaired physical activity, impaired mental development, and maternal mortality with stillbirths and child deaths, while ZnD has been documented mainly on infants and children with growth disorders, delayed sexual development, increased susceptibility to infection, and immune suppression (Stein 2009). So, a food that supplements for both PEM and hidden hunger is highly important for the current situation.

With this ground information, this book chapter will bring the role of agriculture in the history of hidden hunger especially mineral malnutrition, currently available interventions and their pros and cons, and how a microbe-mediated process, especially actinomycetes, can help in overcoming the root causes of hidden hunger.

17.2 Agriculture: A Hidden Cause for Hidden Hunger

The first green revolution begun during the 1960s is the science-based transformation of Third World agriculture which increased the cereal production more than twice and offered solution to the threats of mass starvation in the 1960s and of continuing food shortages during the 1960s and 1970s. This includes the development and use of high-yielding varieties of cereal grains, expansion of irrigation infrastructure, and distribution of hybridized seeds, synthetic fertilizers especially NPK, and pesticides. The continuous use of high-yielding cultivars that have higher response to fertilizers made the soils deficient in their native nutrients especially micronutrients because NPK fertilizers do not supply any of the necessary micronutrients like organic manures. This revolution is also responsible for biodiversity loss due to the loss of many wild and locally adapted cultivars. On the other end, there was a decrease in pulse production and other secondary staples as the developed technology is mostly targeted on cereals resulting in relative price increases for non-cereal crop products (Welch 2002a, b; Graham et al. 2007). For instance, in the Philippines, intensive rice monoculture systems led to the loss of wild leafy vegetables and fish that the resource-poor people had previously harvested from rice paddies (Pingali and Roger 2012). In case of India, the increased pulse prices have been associated with a consequent decline in its consumption across all income groups. This supply-mediated price effects limited the access and hence insufficient minimum daily requirements of micronutrients (Kataki 2002). However, these hidden causes were not prioritized by agricultural researchers and also nutritionists during the revolutionary period.

Though the history of iron deficiency has started before the 1930s (Haden 1938), a steady increase in the extent of iron deficiency anemia in humans was noticed during the 1980s, especially among the resource-poor populations who benefited from the greater cereal productivity of the green revolution (Graham 2008). In case of ZnD, it was initially reported during the 1960s by Prasad et al. (1963) and later in the 1980s (Prasad 1991). Efforts of this research group were largely ignored, and the impact of ZnD was recognized only during the 1990s by their further findings (Prasad 2003). This might be due to the lack of quick and simple diagnostics for ZnD in humans than anemia, and it continued to be largely ignored. During this decade, other micronutrient deficiencies affecting large population such as iodine, selenium, and vitamin A were also given importance (Ren et al. 2008).

It is understood from the previous section that, logically, agricultural farming systems are part of the root causes of hidden hunger, as success of the modern agriculture by the continuous use of high-yielding cultivars made the soils deficient in their native nutrients. This is proved by the study of Garvin et al. (2006) by analyzing micronutrient density of 14 different hard red winter wheat (HRWW) genotypes representing different production eras ranging from 1873 (the year of introduction of HRWW) through the modern breeding era starting in the early 1940s until 2000, in Hutchinson and Manhattan, Kansas,

USA. A significant negative regression for seed of Fe, Zn, and Se content on both yield and variety release date was observed. Further evaluation by Fan et al. (2008) confirmed the similar trend in which analysis of mineral concentration from the archived wheat grain and also soil samples over 160 years from Broadbalk wheat experiment was done. This experimental station was established at Rothamsted, England, in 1843 to test the effect of different combinations of inorganic fertilizers and organic manures on wheat yield. The determined micronutrient concentration and the observed trends over time in the context of cultivar, yield, and harvest index revealed that the concentrations of Fe, Zn, Cu, and Mg have remained stable during 1845 to the mid-1960s; later, reductions were observed which coincides with the introduction of semidwarf, high-yielding cultivars. Multiple regression analysis data registered that increasing yield and harvest index were the significant contributors for the downward trend of grain mineral concentration.

These experiments clearly indicate the low mineral availability of soils and observed mainly in developing countries such as Pakistan, China, India, Iran, and Turkey (Cakmak et al. 1999; Alloway 2009). It has been shown that the Indian soils are deficient by 11.2 % in extractable Fe and by 48.1 % in extractable Zn with an expectation of this deficiency to increase up to 63 %. This is due to the difference in total vs. available soil minerals and observed as 4000–273,000 mg/kg vs. 0.36–174 mg/kg for Fe and 7–2960 mg/kg vs. 0.1–24.6 mg/kg for Zn (Gupta 2005; Singh 2009). This was further emphasized by studies in Turkey, where Zn concentration of wheat grains grown on Zn-sufficient soils ranged between 20 and 30 mg/kg, whereas on the Zn-deficient soils, this range decreased to 5–12 mg/kg (Kalayci et al. 1999; Erdal et al. 2002).

17.3 Interventions for Hidden Hunger

The interventions for hidden hunger include many facets, and a detailed view on this was given by Stein (2009) which was here depicted as an overview in Fig. 17.1. The interventions such as dietary diversification or pharmaceutical supplementation or industrial fortification of minerals could not be affordable by millions of poor people residing in developing countries. In addition, such supplementation is coming up with some restrictions in food intake pattern and requirement of additional supplements for active therapy. For instance, iron supplements should not be taken during the medication with antacids or calcium supplements and food such as high-fiber foods, drinks with caffeine, cheese and yogurt, eggs, milk, and spinach, but it has to be taken with either vitamin C supplement or citrus juice to enhance iron absorption into the body. On the other hand, the strategy called biofortification can tackle hidden hunger as it merely targets staple foods that people eat every day. Biofortification is a process by which crops are bred in a way that increases their nutritional value especially minerals and vitamins. The currently available strategies for biofortification are agronomic biofortification, conventional plant breeding, and genetic engineering. The agronomic approach employs the application of mineral fertilizers and/or the improvement of the solubilization and mobilization of mineral elements in the soil (White and Broadley 2009; Graham et al. 2012).

The other two biofortification strategies have the highest impact than agronomic fortification, and crops such as iron beans, iron pearl millet, zinc rice, and zinc wheat have been developed and released across many parts of the world through HarvestPlus, a Global Challenge Program of the Consultative Group on International Agricultural Research (CGIAR) (HarvestPlus 2013). Though genetic biofortification has the highest impact than agronomic fortification, it carries some potential risks such as exposure to cancer and non-specificity of Fe/Zn genes, if the biofortification strategies were aimed at decreasing anti-nutrients and increasing Fe/Zn concentration, respectively (Shahzad et al. 2014). Strengths, weaknesses, opportunities, and threats (SWOT) analysis on these strategies has identified that mineral availability in the soil is the common weakness for conventional breeding

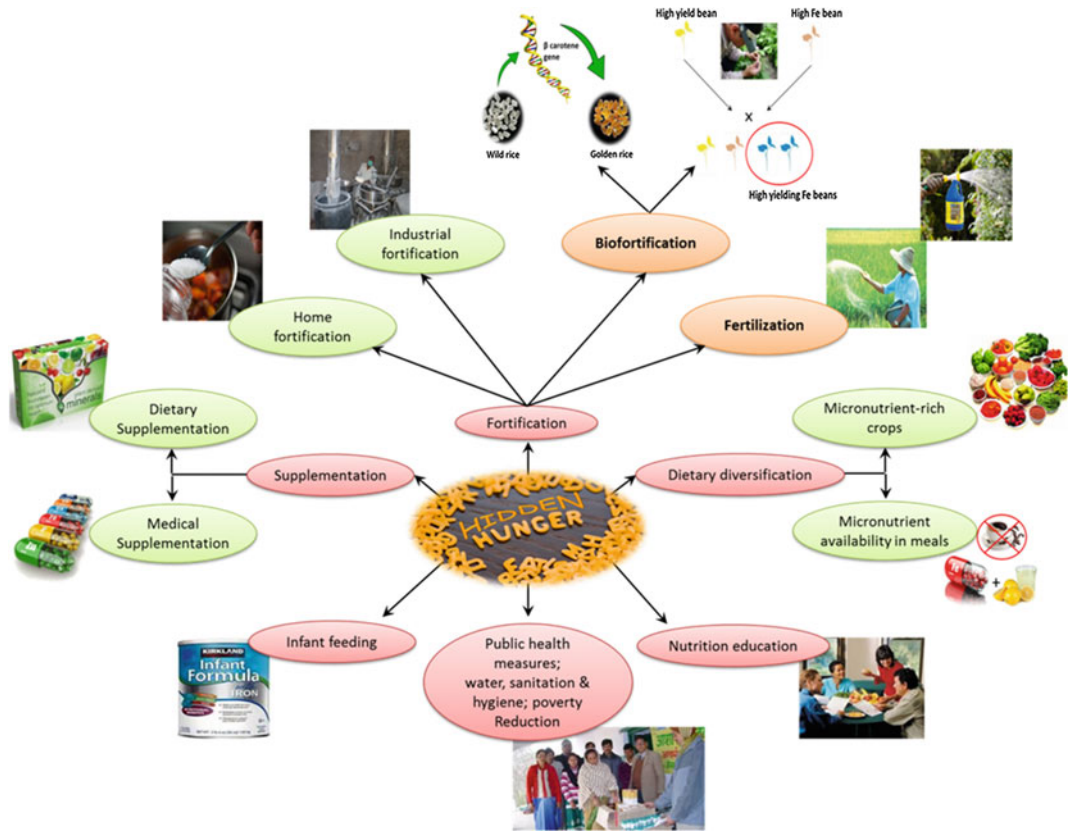

Fig. 17.1 Available interventions for hidden hunger. Interventions of hidden hunger indicated in *pink* and *green shapes* are the major and substrategies, respectively. *Orange shapes* are the strategies targeted through agriculture

and genetic engineering approaches (Carvalho and Vasconcelos 2013). Previous reports have also stated that the key barrier to micronutrient absorption in plants occurs in the root-soil interface (Welch 2001). Hence, it is apparent that enhancing the availability of mineral nutrients is a key process for any kind of biofortification targeting staple crops.

17.4 Microbes: Hidden Players of Soil Fertility

Microbes are the largest population that exists in soil with a high diversity index, and its population (number/g soil) includes bacteria (10^8–10^9), actinomycetes (10^7–10^8), fungi (10^5–10^6), algae (10^4–10^5), and protozoa (10^3–10^4) (Hoorman and Islam 2010). Besides the small voluminous nature, they are the key drivers of biogeochemical cycles involving macroelements (C, N, S, and P) and microelements (Fe, Zn, Mg, Cu, Se, and B) (Bloem et al. 1997). In the case of mineral elements, microorganisms enhance the solubility of trace elements through a variety of mechanisms and engineer the plant rhizosphere and improve the soil health.

17.4.1 Plant Growth-Promoting Microorganisms

Population density of microbes is generally high in rhizospheric soil (10–100-fold) than bulk soil due to the influence of plant roots as they secrete numerous nutrients such as sugars,

organic acids, vitamins, amino acids, fatty acids, nucleotides, phenols, and sterols (Uren 2007). These microbial groups may reside at various proximity of roots, viz., near the roots (rhizosphere), root surface (rhizoplane), and inside the root tissue either as free living (endophytes) or as symbionts in specialized root structures or nodules. Many microorganisms living in any of these proximities have the capacity to promote plant growth either directly by influencing nitrogen fixation, P solubilization, Fe chelation, and phytohormone synthesis or indirectly by suppressing phytopathogens and inducing host plant resistance against biotic and abiotic stresses. These are referred as plant growth-promoting microorganisms and broadly used with the terminology plant growth-promoting rhizobacteria (PGPR) (Glick 1995; Bhattacharyya and Jha 2012). PGPR are reported from a wide range of plants such as cereals (de Souza et al. 2013; Majeed et al. 2015), pulses (Medeot et al. 2010; Wahyudi et al. 2011), vegetables (Abhishek et al. 2013; Agrawal and Agrawal 2013), fruits (Mehta et al. 2013; Thokchom et al. 2014), medicinal plants (Ahmed et al. 2014; Egamberdieva et al. 2015) and tree species (Donate-Correa et al. 2005; Barriuso et al. 2008; Singh et al. 2011) and also environmental conditions of temperate (Trivedi and Pandey 2008), arid (Silini-Chérif et al. 2012), and semiarid regions (Kavamura et al. 2013) and also high altitudes (Zahid et al. 2015). They were also documented in polluted soils containing petroleum, sewage sludge, dye, and heavy metals (Belimov et al. 2001; Liu et al. 2014). This indicates the omnipresence of PGPR on various natural and contaminated soils and climatic conditions.

17.5 Metal-Mobilizing PGPR in Biofortification

Among the microbes, PGPR reside in metalliferous soil with higher metal solubilizing and extracting capacity which can play decisive role in the context of soil mineral density and biofortification. Many of such isolates reported for one or multiple plant growth-promoting (PGP) traits such as production of indole acetic acid (IAA), siderophore, and 1- aminocyclopropane-1-carboxylate (ACC) deaminase; solubilization of Zn, P, and K; and N_2 fixation. Some of the examples are *Enterobacter*, *Pseudomonas*, *Bacillus*, *Rhizobium*, *Bradyrhizobium*, and *Streptomyces*. From the literature data, it is understood that PGP actinomycetes were not explored much in this area than other microbial groups, though they are higher stress-tolerant microbes and are found to produce higher ACC deaminase, a stress-relieving enzyme (Ma et al. 2011; Rajkumar et al. 2012; Nascimento et al. 2014), and demonstrated for plant growth-promoting potentials in many cereals, legumes, and vegetable crops (Gopalakrishnan et al. 2013, 2014; El-Tarabily and Sivasithamparam 2006; El-Tarabily 2008). Some of the representative reports stating the potential of PGP actinomycetes with metal mobilization traits were given in Table 17.1.

Microbes with metal-mobilizing and PGP traits were evaluated mostly on nonedible/hyper-accumulating plants and on toxic metals in the area of phytoremediation. Such works on edible crops were a few. A metal-resistant PGP bacterium, *Bacillus weihenstephanensis* SM3, has been found to promote higher growth rate and Zn, Cu, and Ni uptake in *Helianthus annuus* upon its inoculation (Rajkumar et al. 2008). Similar effects were also identified by *Pseudomonas* sp., on *Ricinus communis* at contaminated sites (Rajkumar and Freitas 2008). A metal-tolerant PGP fungus *Trichoderma virens* PDR-28 has been found to enhance the growth rate of maize and also the absorption of Cd, As, Zn, Cu, and Pb (Giridhar Babu et al. 2014). On pea, a metal-tolerant PGP *Rhizobium* sp. was shown to produce better growth performance and Zn uptake in a metal-amended soil (Wani et al. 2008). Similarly, PGP *Enterobacter* sp. has been observed to increase the growth and metal (Zn, Cr, and Ni) accumulation in *Brassica juncea* (Kumar et al. 2008).

Metal-mobilizing property of microbes is aided by its substances such as siderophores, organic acids, biosurfactants, polymeric

Table 17.1 Metal mobilization potential of PGP actinomycetes

Actinomycetes	Source	Identified PGP/metal mobilization traits	Plant studied	Exhibited effects	References
Nonedible crops					
Microbacterium oxydans AY509223	Rhizosphere of *Alyssum murale* grown in Ni-rich serpentine soil	Ni mobilization	*A. murale*	Increased Ni uptake in the low (36 %), medium (39 %), and high (27 %) Ni soils	Abou-Shanab et al. (2008)
Streptomyces sp., *Agromyces* sp.	Rhizosphere of willows growing on a contaminated site in Arnoldstein, Austria	Siderophore, IAA, Zn, and Cd immobilization (except for *Agromyces* sp.)	*Salix caprea*	Increased plant leaf biomass, decreased Cd and Zn uptake (except for *Agromyces*)	Kuffner et al. (2008)
Edible crops					
Azotobacter chroococcum HKN-5	Agronomic soils in Hong Kong	N fixation, P and K solubilization, metal mobilization	*Brassica juncea*	Increased plant aboveground biomass	Wu et al. (2009)
Rhodococcus sp. Fp2	Cr-contaminated site situated in the Indian Himalayan Region	Metal detoxification mechanism	*Pisum sativum*	Increased plant growth	Trivedi et al. (2007)
Rhodococcus erythropolis MtCC 7905					
Streptomyces acidiscabies E13	Former uranium mine, Wismut, in eastern Thuringia, Germany	IAA and siderophore: desferrioxamine E, desferrioxamine B, and coelichelin	*Vigna unguiculata*	Increased height and biomass	Dimkpa et al. (2008)
Streptomyces tendae F4	Former uranium mine, Wismut in eastern Thuringia, Germany	Siderophore: desferrioxamine B, desferrioxamine E, and coelichelin	*Helianthus annuus*	Enhanced Cd and Fe uptake by plants through facilitating their mobilization	Dimkpa et al. (2009)
Azotobacter spp.	Manganese mine spoil dump near Gurgaon, India	Extracellular polymeric substances or cell wall lipopolysaccharides	*Triticum aestivum*	Immobilized Cd and Cr and decreased their uptake	Joshi and Juwarkar (2009)
Arthrobacter sp. MT16, *Azotobacter vinelandii* GZC24, *Microbacterium* sp. JYC17, and *Microbacterium lactium* YJ7	Cu-tolerant plant species growing on a Cu mine wasteland, Nanjing, China	ACC deaminase, siderophore, IAA, P solubilization	*Brassica napus*	Increased root length promotion	He et al. (2010)
Streptomyces mirabilis P16B-1	Heavy metal-contaminated soil derived from a former uranium mining site in Ronneburg, Germany	Siderophore: ferrioxamines E, B, D, and G	*Sorghum bicolor*	Increased plant biomass	Schütze et al. (2014)

Modified from Ma et al. (2011)

substances, and glycoprotein and the reaction such as metal reduction and oxidization and biosorption. The mechanism behind the metal mobilization process through these substances was reviewed in detail (Ma et al. 2011; Rajkumar et al. 2012; Sessitsch et al. 2013).

17.5.1 PGPR in Biofortification of Cereal and Leguminous Crops

The research frontiers mentioned on biofortification through PGPR are studied to certain extent at international and national level but not extensively. Initial studies of Rana et al. (2012a) on wheat under glasshouse conditions documented that combination of rhizobacterial strains *Bacillus* sp. AW1 and *Providencia* sp. AW5 enhanced 14–34 % of plant biometric parameters along with the increase of 28–60 % in mineral content with the higher counts for Fe. Further studies on wheat field trials revealed that PGP *Providencia* sp., having P, Zn, and Fe solubilization capacity, increased the Fe content by 105 % (Rana et al. 2012b). Recently, they investigated the effect of PGPR (*Brevundimonas diminuta* PR7, *Ochrobactrum anthropi* PR10, and *Providencia* sp. PW5) and cyanobacteria (*Anabaena oscillarioides* CR3), alone and in combination on mineral enrichment and yield in rice-wheat sequence, for a period of 2 years. In rice, combination of *Providencia* sp., *B. diminuta*, and *O. anthropi* recorded higher enhancement of about 13–16 % of Fe, Zn, Cu, and Mn. In the case of wheat, *Providencia* sp. alone registered higher enrichment of Fe and Cu by 45 % (Rana et al. 2015). Co-inoculation of some cyanobacteria *Anabaena* with *Azotobacter* or *Providencia* on 11 maize hybrids showed a positive correlation with Zn concentration in the flag leaf (Prasanna et al. 2015). A PGP strain *Pseudomonas aeruginosa* isolated from roots of *Vigna mungo* has PGP traits and Zn solubilization potential. Under pot trials on wheat, it increased soil enzyme activities and grain Zn content by about 85 % in comparison to the control plants grown in Zn-deficient soil (Sirohi et al. 2015). Similarly, PGP *Pseudomonas putida* B17 and B19 exhibited the translocation efficiency of the Fe from roots to grains and led to the increased grain Fe content by twofolds (Sharma et al. 2013).

As like cereals, in leguminous crops also few studies were carried for biofortification by PGPR, but they have an additional advantage over cereals, because their characteristic pattern of high protein and minerals helps in overcoming both classical and hidden hunger. In the realm of biofortification, a recent study had revealed that arbuscular mycorrhizal (AM) fungal colonization on chickpea roots had enhanced the crop growth, productivity, plant nutrient uptake, and grain fortifications with enhanced protein, Fe, and Zn under a rainfed low-input cropping system (Pellegrino and Bedini 2014). A collection of AM fungal inoculum (*Acaulospora* spp., *Acaulospora cavernata*, *Acaulospora spinosa*, *Claroideoglomus etunicatum*, *Diversispora spurca*, *Funneliformis coronatum*, *Funneliformis geosporum*, *Funneliformis mosseae*, *Glomus* spp., *Rhizophagus clarus*, *Rhizophagus irregularis*, *Scutellospora aurigloba*, *Scutellospora calospora*, and *Septoglomus viscosum*) had shown 8 % and 36 % increase in Fe and Zn, respectively. Verma et al. (2013) had documented the effect of two PGPR isolates, *Mesorhizobium* sp., and *Pseudomonas* sp., on chickpea yield under greenhouse and field conditions of Varanasi, Uttar Pradesh. The efficiency of *Mesorhizobium* sp., in enhancing N_2 fixation and *Pseudomonas* sp., in enhancing P and Fe acquisition has also been registered. Similar results were reported by Rudresh et al. (2005) using a consortium of *Rhizobium* sp., phosphate solubilizing *Bacillus megaterium* subsp. *phospaticum* and *Trichoderma* sp. on chickpea under greenhouse and field conditions of Bangalore, Karnataka. Recent study of Khalid et al. (2015) on chickpea further supports the ability of PGP bacterial strains with siderophore-producing capacity in increasing Fe concentration by 81 and 75 % in grain and shoot over the control treatments under greenhouse conditions. Some of the PGP *Streptomyces* from our microbial collection showed increase in the grain Fe and Zn content by 18 % and 9 %,

respectively, in chickpea (unpublished results). Though the actinomycetes were not reported in the context of biofortification, previously demonstrated effects on their metal mobilization property along with PGP reveal that actinomycetes are able to mobilize/solubilize minerals and metals in a wide range of food crops including cereals, oil seed, and leguminous crops (Table 17.1). It is also noted that actinomycetes employ multiple PGP traits necessary for mineral mobilization such as production of various siderophores and extracellular polymeric substances along with IAA and ACC deaminase. Still, potential actinomycete isolates have to be explored for enhanced mineral solubilization/mobilization rates under field conditions. So it is postulated that use of such potential PGP actinomycetes can improve mineral density of grains in not only staple crops but also in other secondary staple crops. This further protects the soil fertility and biodiversity loss, the major threats raised during the adaptation of hybridized crops, and hence offers sustainable solution for biofortification.

17.6 Conclusions

The information available for microbes in enhancing soil macro- and micronutrients is voluminous. However, the focus of biofortification of grain minerals through PGP microbes, particularly on actinomycetes, is in its infancy, and only a limited number of reports are available. On the other hand, many microbial groups from PGP microbes have been evaluated for the metal-mobilizing property in the context of microbe-mediated phytoremediation in nonfood crops, since they can act quickly and enhance the remediation rates. Though phytoremediation and biofortification can be considered as two sides of one coin and employ the central core of metal mobilization and accumulation to the harvestable or edible parts of plants, metal-mobilizing microbes especially PGP actinomycetes are not evaluated for the latter. Only the microbes from rhizospheric soil were evaluated on wheat and maize in case of cereals and on pea and chickpea in case of legumes. Though appreciable quantities of Fe and Zn have been observed in grains through the use of PGP microbes, most of the studies are done under glasshouse conditions. Further characterization of PGP microbes, especially of actinomycetes, from rhizospheric and metalliferous soil under various field conditions helps in understanding the role of metal-mobilizing PGP bacteria in accumulating grain minerals. The success of this strategy can bring a complementary sustainable tool for the existing biofortification strategies and substantially reduce the chemical fertilizer inputs and reduce protein and mineral malnutrition incidences in developing countries.

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Evaluation of Plant Growth-Promoting Actinomycetes on *Vigna*

18

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Abstract

The legume genus *Vigna* are grown in warm temperate and tropical regions globally but are particularly crucial to human nutrition in large parts of tropical Africa and Asia. It can also serve as forage crops. Among the *Vigna* species, the Asian *Vigna* has received little research initiatives than African *Vigna* such as cowpea and mung bean. From the last decade, the research initiatives are getting increased for both the *Vigna* species in the context of genetic resource analysis and genome mapping. The production status has remained stagnant in many countries due to long list of pest and pathogen attacks and abiotic stresses. Use of plant growth-promoting microbes for improving the productivity of *Vigna* species is still in its infancy, and there were very few field evaluation studies conducted. This chapter brings an overview of several reports which documented the various facets of plant growth-promoting microbes, particularly of actinomycetes, in increasing growth performance and productivity of *Vigna*.

Keywords

Vigna • Plant growth promotion • Actinomycetes • Phosphate solubilizers • *Burkholderia* • *Streptomyces*

18.1 Introduction

Legumes belonging to the family Fabaceae or Leguminosae are the second most important crops next to cereals among the food crops. Legumes are the third largest family among the flowering plants, consisting of approximately 650 genera and 20,000 species (Doyle 1994). They are the important sources of protein for

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vegetarians and comprise twice the amount on average than cereals. It also provides significant amounts of micronutrients, including iron, zinc, calcium, and vitamins. In addition, legumes are excellent sources of nutraceutical constituents such as phenolics, flavonoids, isoflavones, lignans, and tannins. These compounds have antioxidant, antimutagenic, and anticarcinogenic activities. Hence, their consumption is recommended by several health organizations for a broad spectrum of health benefits (Letreme 2002; Duranti 2006). Along with the nutritional benefits, their accessibility and affordability to lower-income populations and resource-poor people around the world made them to be recognized as "poor man's meat" (Swaminathan 1974). A list of some legumes and their origin is shown in Table 18.1 (Nene 2006).

As one of the strategies of "Green Revolution," high inputs of artificial N fertilizers into farmlands (up to 100 million tons per year) were used for higher crop productivity. However, they could not serve for sustainable aspect of food production as they are produced using energy from fossil fuels. On the other end, biological nitrogen fixation (BNF) accounts for about 65 % of N currently used in agriculture. Due to the N-fixing ability of legumes, they are used in crop rotations which have a positive impact on soil fertility and subsequent crop productivity. Legumes meet their own N needs via BNF, and major part of fixed N is harvested as grains, while the soil and the succeeding crops benefitted by N in the form of root and shoot residues (Bhattacharyya and Jha 2012). Though nonsymbiotic systems are also contributing for N fixation, the contribution of legume-rhizobia symbiosis (13–360 kg N ha^{-1}) is far greater than the nonsymbiotic systems (10–160 kg N ha^{-1}) (Bohlool et al. 1992). This leads to the substantial reduction of the N requirement from external sources. The quantity of N fixed by some legumes is shown in Table 18.2. Hence, the production and consumption of more legumes in human diets could aid in the reduction of global warming, eutrophication, acidification, and land degradation besides reducing protein-energy malnutrition and micronutrient deficiencies in developing countries (Davis et al. 2010).

Even though legumes are high in numbers, selected cash crops such as soybean, pea, and cowpea alone have been exploited. Severe genetic erosion of the legume species is occurring currently due to anthropogenic activities and also due to the introduction of genetically modified crops. A total of 2206 legume species have been listed in International Union for Conservation of Nature (IUCN) red list (Walters and Gillet 1998). In addition, the production of

Table 18.1 Geographical origin and domestication of various pulses grown in Indian subcontinent

Legume	Binomial name	Geographical origin and domestication
Chickpea	*Cicer arietinum*	Turkey-Syria
Pigeon pea	*Cajanus cajan*	India
Lentil	*Lens culinaris*	Southwest Asia (Turkey-Cyprus)
Black gram	*Vigna mungo*	Indian subcontinent
Green gram	*Vigna radiata*	Indian subcontinent
Lablab bean	*Lablab purpureus*	Indian subcontinent
Moth bean	*Vigna aconitifolia*	Indian subcontinent
Horse gram	*Macrotyloma uniflorum*	Indian subcontinent
Pea	*Pisum sativum*	Southern Europe
Grass pea	*Lathyrus sativus*	Southern Europe
Cowpea	*Vigna unguiculata*	West Africa
Faba bean	*Vicia faba*	West Asia

Source: Nene (2006)

Table 18.2 Reported quantum of nitrogen fixed by legumes

Legume	Fixed nitrogen quantity (kg N ha^{-1})
Soybean	33–643
Groundnut	126–319
Black gram	125–143
Cowpea	25–100
Pigeon pea	77–92
Green gram	71–74

Source: Peoples and Crasswell (1992)

common legumes has remained unmet with the consumption rate (Ali and Kumar 2000). The crop yield is constrained due to limited adaptability of available cultivars and by a long list of pathogen attacks like powdery mildew, downy mildew, rust, *Ascochyta* blight, *Botrytis* gray mold, white mold, damping-off, anthracnoses, root rot, collar rot, and vascular wilts and pest attacks from chewing and sap-sucking insects followed by attacks from parasitic weeds, viruses, bacteria, and nematodes (Rubiales et al. 2015).

Pesticides and fertilizers of chemical origin can overcome yield losses by pathogen and pest attacks and increase the productivity. However, it also has safety risks, loss of natural enemies, outbreaks of secondary pests, insect resistance, environmental contamination, and biodiversity loss (Lacey and Shapiro-Ilan 2008). The increasing costs, negative effects of pesticides, fertilizers, and consumer preference on pesticide-free food products necessitate the idea of biological options for crop protection and production. Usage of animal manure, crop residues, composts, and microorganisms (*Rhizobium*, *Azotobacter*, *Azospirillum*, blue-green algae, *Pseudomonas*, *Bacillus*, and actinomycetes) can play key roles as it provides natural nutrition, reduces the use of inorganic fertilizers, develops biodiversity, increases soil biological activity, maintains soil physical properties, and improves environmental health (Hue and Silva 2000; Vessey 2003). This book chapter will bring a note on one of the legume genus *Vigna* and the importance of microbial inoculum, in particular actinomycetes, in its exploration.

18.2 *Vigna*

The genus *Vigna* are hot weather herbaceous legumes first evolved in Africa as the major species (Vaillancourt et al. 1993). Recent report of Thulin et al. (2004) further suggests this through molecular studies that *Vigna* may have evolved from *Wajira*, the African genus as it is basal compared to *Vigna* and *Phaseolus*. Detailed description on taxonomy of *Vigna* documents that there are 98 species and six subgenera in which the subgenus *Vigna* has the highest number of species of about 38 (Maxted et al. 2006). Most of the *Vigna* species are nutritionally enriched and are particularly crucial to human and animal nutrition in large parts of tropical Africa and Asia (Vijayakumari et al. 1998; Ullah et al. 2014). However, the domesticated *Vigna* species such as cowpea (*Vigna unguiculata*) and mung bean (*Vigna radiata*) are vital in terms of production. The production stands at about 4.5 million metric tons/10 million ha for the former and is 2.5–3 million metric tons/5 million ha for the latter species (Tomooka et al. 2005). Other species of interest in specific countries are listed in Table 18.3. A complete description on genetic resources of available *Vigna* species was given by Tomooka et al. (2011), and it is understood that *Vigna* has huge biodiversity of wild and cultivated species.

The genus *Vigna* is also peculiar for its resistance against many abiotic stresses. Reports of Iwasaki et al. (2002) and Singh et al. (2015) registered tolerance of *Vigna* species such as *V. umbellata* and *V. unguiculata* for the heavy metals Al and Mn. They are well tolerant for salinity (Sehrawat et al. 2015; Win et al. 2011), and many crops were developed with enhanced salt tolerance using proline biosynthetic pathway genes PSCS and PSCSF129A of *V. aconitifolia* and the list is given in Table 18.4. Besides this, recent report of De-Abreu et al. (2014) brought the involvement of various proteins for salt stress tolerance in *V. unguiculata* through proteomic approaches. The proteome data registered that cowpea cultivars adopt different strategies to

Table 18.3 Representatives of other *Vigna* species of interest and their producing countries

Common name	Binomial name	Producing country
Azuki bean	*V. angularis*	China and Japan
Rice bean	*V. umbellata*	Northern India and Southeast Asia
Moth bean	*V. aconitifolia*	South Asia
Bambara groundnut	*V. subterranea*	Africa

Table 18.4 Genes of *V. aconitifolia* used for developing transgenic plants and its developed traits

Genes[a]	Target plant	Enhanced tolerance and phenotype of transgenic plants
PSCS	Tobacco	Enhanced biomass, flower and seed development
		Proline accumulation and increased enzyme activities
	Wheat	Enhanced proline accumulation
	Carrot	Salt stress tolerance
	Larix leptoeuripaea	Enhanced tolerance for cold and salinity
	Medicago	Enhanced proline accumulation
	Chickpea	Enhanced proline accumulation and salt stress tolerance
	Sugarcane	Enhanced proline accumulation and salt stress tolerance, lesser oxidative damage
	Rice	Enhanced salt stress tolerance
		Enhanced salt and drought stress tolerance
		Enhanced salt stress tolerance up to 200 mM NaCl
PSCSF129A		Enhanced proline accumulation and salt stress tolerance
	Pigeon pea	Enhanced proline accumulation and salt stress tolerance

Source: Kumar et al. (2015)
[a]Genes involved in proline biosynthetic pathway

alleviate salt stress. In salt-tolerant cultivar Pitiúba, proteins involved in photosynthesis and energy metabolism, such as rubisco activase, ribulose-5-phosphate kinase (Ru5PK), glycine decarboxylase, and oxygen-evolving enhancer (OEE) protein 2, were profoundly expressed. On the other hand, in salt-sensitive cultivar TVu, downregulation of OEE protein 1, Mn-stabilizing protein-II, carbonic anhydrase, and Ru5PK was noticed which led to energy reduction and hence decline in plant growth.

The African *Vigna*, cowpea, is a mandate crop of the International Institute of Tropical Agriculture (IITA) and subsequently receiving considerable attention from the international agricultural research community by the initiatives such as Cowpea Genomics Initiative (Chen et al. 2007), Bean/Cowpea Collaborative Research Support Program (http://www.isp.msu.edu/CRSP), Generation Challenge Programme (http://www.generationcp.org), and Network for the Genetic Improvement of Cowpea for Africa – NGICA (http://www.entm.purdue.edu/NGICA/). However, the Asian *Vigna* is called as "slow runners" by Borlaug (1973) as its research and development is not focused by international institutes. However, the importance for the Asian *Vigna* has recently increased with some significant scientific advances in particular to genetic resource analysis and genome mapping (Kaga et al. 2005, 2008; Tomooka et al. 2006). A detailed review by Nair et al. (2013) on one of the Asian *Vigna* mung bean conveys its key role in enhancing the food and nutritional security via breeding and other agronomic practices. Besides the magnitude of research attention, seed yield of cowpea and other Asian and African *Vigna* species remains low in farmer's fields except few countries (Singh 2005; Matsunaga et al. 2008; Saxena 2011) due to various biotic and abiotic stresses (Kumar and Kumar 2015).

18.3 Plant Growth-Promoting Microbes

Microbes with agriculturally favorable traits categorized as plant growth-promoting (PGP) microbes are of great importance in agricultural practice. In case of legumes, the practice of mixing natural rhizospheric soil with seeds is the recommended method of legume inoculation during the nineteenth century. The reason behind this practice is that rhizospheric soil is an enriched source of microorganisms (10–100-folds than the bulk soil) such as bacteria, fungus, algae, and protozoa. Rhizospheric soil is usually rich in nutrients than bulk soil as it accumulates

organic acids, amino acids, fatty acids, phenols, nucleotides, putrescine, sterols, vitamins, sugars, and plant growth regulators/promoters released from the root exudates (Uren 2007).

The rhizobacteria were categorized depending on their proximity to the roots as (i) bacteria living near the roots (rhizosphere), (ii) bacteria colonizing the root surface (rhizoplane), (iii) bacteria residing in root tissue (endophytes), and (iv) bacteria living inside cells in specialized root structures or nodules; the latter group is further divided into two groups – the legume-associated rhizobia and the woody plant-associated *Frankia* sp. Microbes belonging to any of these categories and improving plant growth either through direct (N fixation, phosphate (P) solubilization, iron chelation, and phytohormone production) or indirect (suppression of plant pathogens and induction of host plant resistance against phytopathogens and abiotic stresses) mechanisms are referred as plant growth-promoting rhizobacteria (PGPR). This includes the genera *Bacillus*, *Pseudomonas*, *Erwinia*, *Caulobacter*, *Serratia*, *Arthrobacter*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Rhizobium*, *Streptomyces*, and *Rhodococcus* (Glick 1995).

The microbial flora present in soil and other sources such as vermicompost and farmyard manure (FYM) plays an important role in plant growth promotion. Application of organic manure such as FYM and phosphate solubilizer significantly increased the rhizospheric microbial flora and yield of green gram (Chesti and Tahir 2012). Application of microbial species isolated from vermicompost enhanced the growth of green gram in terms of shoot length, root length, number of leaves, and yield (Gopinath and Prakash 2014; Geetha et al. 2014). *Rhizobium* was found to enhance germination of seed of green gram (Vaishali et al. 2014). Fernandes and Bhalerao (2015) reported that the seed treatment of green gram with *Azotobacter* enhanced the plant morphological and biochemical parameters. As compared to green gram, the combination of *Rhizobium*, phosphobacteria, and *Azospirillum* increased the plant growth, morphology, and biochemical constituents of cowpea (Sivakumar et al. 2013). Besides this, indirect growth-promoting effects were also observed. Aswini and Giri (2014) evaluated *Trichoderma viride*, *Bacillus subtilis*, and *Pseudomonas fluorescence* for the control of seed-borne root diseases in green gram and achieved 86 %, 65 %, and 47 % control, respectively. Similarly, a combination of *T. viride* and *P. fluorescence* was utilized to control green gram root pathogen *Macrophomina phaseolina* in vitro, under glasshouse and field conditions. In this combination, the defense-related enzymes of green gram such as peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase were significantly increased (Thilagavathi et al. 2007). In addition, Siddiqui and Mahmood (1999) reported that the microbes such as *Streptomyces*, *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Clostridium*, *Desulfovibrio*, *Pseudomonas*, and *Serratia* were used for the control of nematodes in soil.

Among the PGP microbes, actinobacteria are one of the key groups because, as per the literature survey, they account for ~60 % of new antibiotics among the microbial compounds of about 60–80,000. In specific, the single genus *Streptomyces is* the major producer of secondary metabolites (39 % of all microbial metabolites) (Berdy 2012). *Streptomyces* spp. were reported as potential biocontrol agents against root fungal pathogens (Bhattacharyya and Jha 2012). Actinomycetes isolated form herbal vermicompost including *Streptomyces tsusimaensis*, *Streptomyces caviscabies*, *Streptomyces setonii*, *Streptomyces africanus*, and a *Streptomyces* sp. were found to reduce disease symptoms up to 20 % on *Fusarium* wilt of chickpea grown in wilt-sick plots (Gopalakrishnan et al. 2011). But the potential of actinomycete group has not been much explored in *Vigna* in specific at field conditions. Use of such microbial inoculum in exploring the productivity of *Vigna* is discussed below.

18.4 Role of Phosphate Solubilizers on *Vigna*

Plant P availability is limited particularly in tropical soils, despite its high soil content (Collavino et al. 2010). Usually, most soil P exists as insoluble metal chelates and requires substantial amounts of chemical phosphate fertilizers which are rapidly converted into insoluble P sources. This leads to regular application of P fertilizers, which are costly and environmentally undesirable (Vassilev et al. 2006). It is noted that unavailability of P has more influences on growth performance of *Vigna* because tropical soil is the optimal soil for growing *Vigna* species. In this context, microbial solubilization of soil-insoluble P into soluble forms is considered by various researchers. Phosphate-solubilizing bacteria (PSBs) belonging to the genera *Bacillus*, *Pseudomonas*, *Xanthomonas*, and *Serratia* enhanced the nodule, root, and shoot parameters of green gram under greenhouse conditions (Vikram and Hamzehzarghani 2008). Microbes with the phosphate-solubilizing potential and additional trait of phytohormone production are other key resources. This was noticed by Muthezhilan et al. (2012) using *Pseudomonas* sp. AMET1148 for increased shoot and root length on *V. radiata* and *V. mungo*. Shahab et al. (2009) also noticed similar effects in *V. radiata* by the inoculation of PSB *Pseudomonas aeruginosa* CMG860 with additional capacity of producing auxin (IAA, 57–288 µg/ml; IBA, 22–34 µg/ml). Nonsymbiotic PGPR belong to *Pseudomonas*, *Escherichia*, *Micrococcus*, and *Staphylococcus* with IAA-producing capacity (1.16–8.22 µg/ml), and other PGP traits such as P solubilization and siderophore or hydrogen cyanide production are evaluated for growth-promoting effects of *V. radiata*. Significant positive correlation was noticed for bacterial IAA production and endogenous IAA content of roots ($r = 0.969$; $P = 0.01$) and leaves ($r = 0.905$; $P = 0.01$) under axenic conditions. Bacterization of *V. radiata* seeds significantly enhanced shoot length (up to 48 %) and shoot biomass (up to 43 %) under axenic conditions. Bacterial strains applied under wire house conditions also improved shoot length, pod number, and grain weight up to 58 %, 65 %, and 17 %, respectively, over the control treatments. It is understood that free-living PGPR have the ability to influence endogenous IAA content and growth of leguminous plants (Ali et al. 2010).

Zaidi and Khan (2006) studied the effect of microbial treatment including PSB (*B. subtilis*), phosphate-solubilizing fungus (*Aspergillus awamori*), and AM fungus (*Glomus fasciculatum*) along with nitrogen-fixing *Bradyrhizobium* sp. (*Vigna*) on *V. radiata* under glasshouse conditions. The triple inoculation of AM fungus, *Bradyrhizobium* sp., and *B. subtilis* significantly increased dry matter yield, chlorophyll content in foliage, and N and P uptake along with the seed yield of 24 % than the control treatment. Nodule occupancy was observed between 77 and 96 % but with a greater variation in the rhizobial populations. In a similar study, two potential PSBs, *Pantoea agglomerans* and *Burkholderia anthina*, with the maximum P solubilization of 720 µg/ml were identified among the 31 PSB isolates. Inoculation of these P solubilizers enhanced shoot and root length, shoot and root dry matter, and P uptake of *V. radiata* under greenhouse conditions (Walpola and Yoon 2013). Benefit of microbial co-inoculation is further supported by Bahadur and Tiwari (2014) who studied the effect of nutrient management in mung bean through sulfur (S) and biofertilizers. Though significant increase of growth performance was noticed on S treatment, it decreased the soil PSB and actinomycete population. Co-inoculation of *Rhizobium* and PSB showed significant growth response along with the significant increase of microbial counts for total bacterial population (41.7×10^6/g soil), *Rhizobium*-like organism population (13.9×10^3/g soil), and *Azotobacter* population (12×10^3/g soil).

18.5 Role of Siderophore Producers on *Vigna*

Iron, an essential micronutrient for plants, is present in soils ranging from 0.2 to 55 % (20,000–550,000 mg/kg) and occurs as either the divalent (ferrous or Fe^{2+}) or trivalent (ferric or Fe^{3+}) forms which is determined by redox potential of the soil and the availability of other minerals (Bodek et al. 1988). Under aerobic environments, iron exists as insoluble hydroxides and oxyhydroxides, which are not accessible to both plants and microbes. Generally, microbes have the ability to synthesis low molecular weight compounds called siderophores which are capable of sequestering Fe^3 and also other metals at high affinity and influence their availability to plants. In addition, the siderophores help for antagonistic activity by depriving the availability of Fe to the pathogens (Rajkumar et al. 2010). Sharma and Johri (2003) and Sharma et al. (2003) observed that inoculation of siderophore-producing *Pseudomonas* sp. GRP3 is documented to reduce chlorosis, the iron deficiency symptom in *V. radiata* under pot conditions, with and without iron-limiting nutritional status. Significant increase of chlorophyll content and catalase and peroxidase, the key protoheme enzymes, was noticed. This indicates net physiologically available iron to the plant. In a study by Sindhu et al. (1999), *Pseudomonas* sp. was isolated from the rhizosphere of *V. radiata* with a wide range of antifungal activities against *Aspergillus* sp., *Curvularia* sp., *Fusarium oxysporum*, and *Rhizoctonia solani* in vitro. Culturing with Fe-deficient succinate medium, Luria-Bertani and King's B medium, suggested that the antifungal activity was supported in two ways, by competing for nutrients especially through siderophore and by producing antifungal metabolites. Co-inoculation of green gram with these antagonistic *Pseudomonas* MRS13 and MRS16 and *Bradyrhizobium* sp. (*Vigna*) S24 registered a significant increase in nodule weight, plant dry weight, and total plant N as compared to single inoculation with *Bradyrhizobium* S24. This suggests that the nodule-promoting effects of *Pseudomonas* sp. lead to an increase in symbiotic N fixation and plant growth. In a similar study, Saxena (2010)documented antifungal activity of *P. fluorescens* BAM-4, *Burkholderia cepacia* BAM-6, and *B. cepacia* BAM-12 isolated from the rhizosphere of *V. radiata* against a range of phytopathogenic fungi. The antagonistic activity might be exerted by siderophores (BAM-4 and BAM-6 strains) and chitinase (all the three strains). Morphological abnormalities of pathogens such as fragmentation, swelling, perforation, and lysis of hyphae were confirmed by scanning electron microscopic images. Bacterization with these isolates provided protection against *Macrophomina phaseolina* and also enhanced seed germination, shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, leaf area, and rhizosphere colonization. On par with the control treatments, yield parameters such as pods, number of seeds, and grain yield per plant are also significantly enhanced. Co-inoculation of *Pseudomonas* along with *Bradyrhizobium* reduced the disease symptoms induced by *Rhizoctonia solani* in green gram under greenhouse conditions. The nodule parameters and vegetative biomass are enhanced in infected plants also (Sahu and Sindhu 2011).

Actinomycetes, one of the key biocontrol agents, use siderophores as one of the disease control mechanisms. Siderophores such as desferrioxamine B were produced by *Streptomyces pilosus* and *Streptomyces coelicolor*, desferrioxamine E by *S. coelicolor* (Jurkevitch et al. 1992), and peucechelin by *Streptomyces peucetius* (Kodani et al. 2015). *Streptomyces griseoviridis* is available in the market as a biocontrol agent with trade name of Mycostop, Subtilex, and System3 (Kumar and Pundhir 2009). Though some microbes are evaluated for disease of *Vigna* species, the siderophore-producing actinomycetes were not studied extensively. Further studies in this context will bring potential biocontrol agents for diseases in *Vigna*.

18.6 Role of PGPR Under Stress Conditions on *Vigna*

The gaseous plant hormone ethylene plays a key role in plant development, from seed germination to fruit ripening. However, its triggered production during stress environments ends in plant's premature death. Many rhizospheric microbes are known to control ethylene through ACC deaminase (ACCd) which cleaves ACC, the immediate precursor of ethylene into ammonia and α-ketobutyrate, and helps in alleviating stress consequences of crops (Penrose and Glick 2003). Strains, such as *Rhizobium leguminosarum* bv. *viciae*, *Rhizobium hedysari*, *Rhizobium japonicum*, *Mesorhizobium loti*, *Bradyrhizobium japonicum*, *Sinorhizobium meliloti*, *Bacillus* sp., and *Pseudomonas* sp., had been known to produce ACC deaminase (Duan et al. 2009; Glick 2014; Hafeez et al. 2008; Uchiumi et al. 2004). Inoculation with these bacteria had shown to promote root elongation, shoot growth, enhanced rhizobial nodulation, and mineral uptake (Glick 2012). Shaharoona et al. (2006) observed the similar traits by inoculation of a rhizobacteria possessing ACC deaminase activity isolated from maize rhizosphere along with the co-inoculation of *Bradyrhizobium* on mung bean under pot conditions. Besides the free-living microbes, Jaemsaeng et al. (2013) documented the similar influences of endophytes with ACC deaminase activity. Sixteen strains among the 67 endophytic actinomycetes showed ability of ACC deaminase production and the expression of *acd*S, the ACC deaminase synthetic gene. Native endophytic *Streptomyces* sp. GMKU336 with ACC deaminase and a mutant without ACC deaminase activity was individually inoculated into mung bean plants grown under stress conditions of salinity and flooding. Mung bean plants inoculated with the wild type could survive under salinity at 100 mM NaCl and flooding stresses and significantly enhanced root/shoot growth and leaf chlorophyll content than un-inoculated and ACC deaminase-deficient mutant treatments. The actinobacterial strains such as *Micrococcus*, *Corynebacterium*, *Arthrobacter*, *Rhodococcus*, and *Streptomyces* spp. with exemplified ACC deaminase activity were found to improve plant growth in other crops also (Palaniyandi et al. 2013).

A nickel (Ni)-resistant *Streptomyces acidiscabies* E13 simultaneously produced three different hydroxamate siderophores, and it was observed that they can bind nickel besides binding with Fe. Culture filtrates containing hydroxamate siderophores significantly increased cowpea growth parameters, irrespective of the iron status of the plants, under Ni stress. The presence of reduced iron was found to be high in siderophore-containing treatments in the presence of Ni. Measurements of Fe and Ni contents of cowpea roots and shoots indicated that the siderophore-mediated plant growth promotion reported here involves the simultaneous inhibition of Ni uptake and solubilization and supply of Fe to plants (Dimkpa et al. 2008).

Ahmad et al. (2012a) conducted a pot trial to evaluate the effect of combined application of *Rhizobium phaseoli* (M6 and M9) and PGPR (*Pseudomonas syringae* Mk1, *P. fluorescens* Mk20, and *P. fluorescens* Biotype G, Mk25) to improve the productivity of mung bean under salt-stressed conditions. Inoculation with either rhizobia or PGPR alone enhanced growth performance and yield components significantly. However, the co-inoculation of rhizobia and PGPR was more effective by increased shoot fresh weight (145 %), root fresh weight (173 %), number of pods/plant (150 %), pod fresh weight (182 %), total dry matter (269 %), relative water content (19 %), water use efficiency (51 %), K concentration in leaves (33 %), Na concentration in leaves (56 %), and nitrogen concentration in grains of mung bean (99 %), compared with the un-inoculated control.

Pesticide accumulation in soils has occurred as result of repeated applications beyond the recommended doses and by their slow degradation rate. It affects plant growth by altering plant root's architecture and transformation of microbial compounds to plants and vice versa. Besides this, growth and activity of free-living or

endophytic nitrogen-fixing bacteria have also been affected (Mathur 1999). Several studies have documented the effects of various pesticides on the reduction of microbial diversity and density on various soil types (El Abyad and Abou-Taleb 1985; Moorma 1988; Martinez-Toledo et al. 1996). But several microbes have the capacity to degrade the pesticides and promote plant growth (Kumar et al. 1996). Ahemad and Khan (2011) evaluated the effect of fungicides (hexaconazole, kitazin, and metalaxyl), insecticides (imidacloprid and thiamethoxam), and herbicides (metribuzin and glyphosate), at the recommended and the higher dose rates on PGP activities of *Bradyrhizobium* sp. MRM6 isolated from nodules of green gram plants under in vitro conditions. The highest toxicity was observed at three times higher recommended doses along with decline of PGP traits. In further studies, they observed that a PGP *P. aeruginosa* PS1 with tebuconazole tolerance increased the growth parameters of the green gram plants, two and three times the recommended field rate of tebuconazole. The increased parameters are root N, shoot N, root P, shoot P, and seed yield (Ahemad and Khan 2012b).

18.7 Conclusion

The knowledge of using of microbial inoculum has started many centuries ago as an agricultural practice, but its application at field level is very low in the current scenario. This is due to variations in the microbial activity under field conditions with the complex interaction of soil nutrients, climatic factors, and stress conditions. In the context of *Vigna*, very limited numbers of reports are available for the use of PGPR and also under field conditions, and the available reports can bring only a bird's-eye view. Despite this, actinobacteria, one of the key groups in PGPR, are not extensively studied in *Vigna*, though it was evaluated in many leguminous crops such as pea, chickpea, and soybean. So research initiatives to explore the potential of PGP actinobacteria have to be considered, and the strains should be evaluated in intensive field trials for developing biofertilizers to improve the productivity of *Vigna*.

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19 Plant Growth-Promoting Actinomycetes: Mass Production, Delivery Systems, and Commercialization

K.R.K. Reddy, G. Jyothi, Ch. Sowjanya, K. Kusumanjali, N. Malathi, and K.R.N. Reddy

Abstract

The present global scenario demands researchers to come up with superior technological alternatives to chemical fertilizers and pesticides to enhance grain yield and to increase the quality and quantity of food grains as indiscriminate use of these synthetic inputs has largely affected soil, groundwater, agricultural commodities, animals, and plants. Possible alternatives could be the use of nontoxic and environmentally friendly microbial-based products/formulations for agriculture, maintaining a safe environment and creating a healthy society. Rhizospheric microbes, particularly actinomycetes, have drawn huge attention due to its ability in plant growth promotion and disease and insect pest control, without having any detrimental effect on the environment. The aim of this chapter is to provide handful information on mass production techniques, delivery systems, and commercialization of actinomycete-based products globally.

Keywords

Plant growth promotion • Actinomycetes • Mass production/multiplication • Delivery systems • Commercialization

19.1 Introduction

Actinomycetes are a widely distributed group of microorganisms in nature, and its distribution has been observed in several plant rhizospheres by many authors (El-Naggar et al. 2006; Khamna et al. 2009; Abd-Alla et al. 2013; Dalal and Kulkarni 2014). Actinomycetes are also known as saprophytic soil inhabitants (Takizawa et al. 1993). Many of the actinomycete strains existing in soil belong to the genus *Streptomyces* (Goodfellow and Simpson 1987; Suzuki et al. 2000), and more than 60% of the sources of antifungal and antibacterial compounds or plant growth-promoting (PGP) substances that have been used for agricultural purposes originated from this genus (Alam et al. 2012).

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Serious efforts worldwide in the search of natural products for crop nutrition and crop protection have progressed significantly and found that the genus *Streptomyces* appears to be a potential candidate to discover new approaches for agricultural use (Behal 2000). Soil actinomycetes particularly *Streptomyces* sp. enhance soil fertility and have antagonistic activity against a wide range of soilborne plant pathogens (Aghighi et al. 2004). Actinomycetes are widely efficient in the control of plant pathogens and play an important role in the decomposition of organic material and production of secondary metabolites for pharmacological and commercial interest (Colombo et al. 2001). Two-thirds of natural antibiotics have been isolated from the genus *Streptomyces* (Newman et al. 2003; Jimenez-Esquilin and Roane 2005).

Many actinomycetes have distinct biological attributes and have the ability to biosynthesize a wide range of antibiotics as secondary metabolites (Lechevalier and Waksman 1962; Franklin et al. 1989) and growth-promoting substances such as IAA and siderophore (El-Tarabily 2008; Khamna et al. 2009). Currently, the agroindustry is more interested in actinomycetes as a source of biologically active compounds, plant growth promoters, and biocontrol tools (Bull et al. 1992; Behal 2000; Basilio et al. 2003; Terkina et al. 2006). Though previous studies showed that the *Streptomyces* sp. has biocontrol and plant growth enhancement ability (Aldesuquy et al. 1998), in many agricultural and horticultural crops, they are poorly investigated specifically for their potential as plant growth-promoting rhizobacteria (PGPR). This is surprising as *Streptomyces*, generally accounting for an abundant percentage of the soil microflora, are particularly effective colonizers of plant root systems and are able to endure unfavorable growth conditions by forming spores (Alexander 1977). *Streptomyces griseoviridis* is a good example for colonization of plant rhizosphere and also acts as an antagonistic microorganism effective in the biocontrol of plant diseases (Tahvonen and Lahdenpera 1988). The active root-colonization ability of *S. griseoviridis* was tested on turnip rape and carrot (Kortemaa et al. 1994). Several properties associated with actinomycetes like colonizing plant surface, antibiosis against plant pathogens, synthesis of extracellular proteins, and degradation of phytotoxins might explain their ability to act as biocontrol tools. Some endophytic actinomycetes are reported to act as plant growth promoters and also disease-suppressing agents (Pillay and Nowak 1997; Sreeja and Gopal 2013; Dalal and Kulkarni 2014). Many *Streptomyces* species are reported to produce substantial amounts of growth-regulating substances, including auxins, gibberellins, and cytokinins (Aldesuquy et al. 1998). However, this chapter provides useful information on how actinomycetes are used as plant growth promoters and biocontrol agents, various fermentation techniques used for mass production of PGP actinomycetes and its secondary metabolites, delivery systems developed, and its commercialization globally.

19.2 Actinomycetes as Plant Growth Promoter

The actinomycetes, mainly *Streptomyces* species, are an important group of soil bacteria because of their ample capacity to produce PGP substances, secondary metabolites such as antibiotics, and enzymes (Inbar et al. 2005; Abd-Alla et al. 2013). Manulis et al. (1994) studied the production of the plant hormone indole-3-acetic acid (IAA) and the pathways of its synthesis by various *Streptomyces* spp. including *Streptomyces violaceus*, *Streptomyces scabies*, *Streptomyces griseus*, *Streptomyces exfoliates*, *Streptomyces coelicolor*, and *Streptomyces lividans*. In our laboratory studies, we found that *Streptomyces atrovirens* (SBTA 23) isolated from groundnut rhizosphere produces various PGP substances and that this strain is very effective in improving plant growth in various crops (Fig. 19.1) (unpublished).

El-Sayed et al. (1987) and El-Shanshoury (1991) reported IAA synthesis in *Streptomyces* sp. El-Tarabily (2008) studied plant growth promotion by various *Streptomyces* sp. and

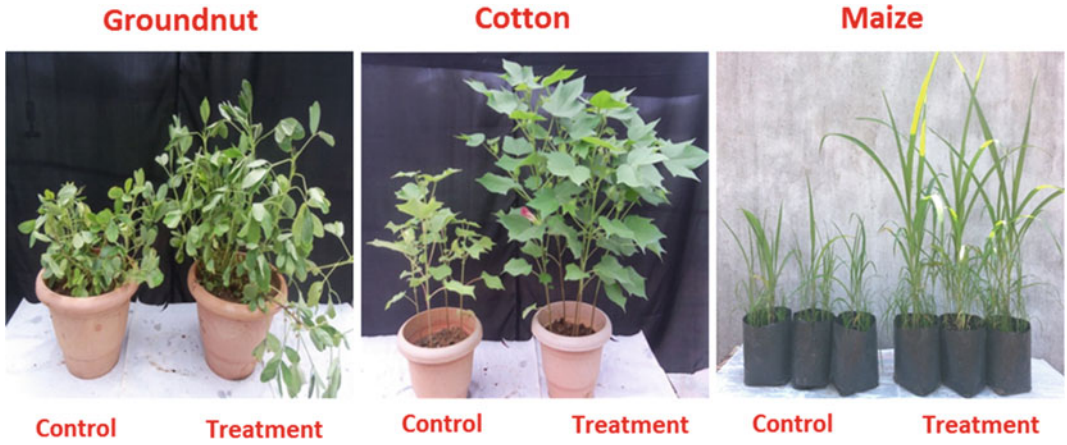

Fig. 19.1 Plant growth promotion by *S. atrovirens* (SBTA 23) in various crops

reported that the plant growth promotion was more pronounced with *Streptomyces filipinensis* than *S. atrovirens* in greenhouse experiment due to the ability of *S. filipinensis* to produce both 1-aminocyclopropane-1-carboxylate (ACC) deaminase and IAA, while *S. atrovirens* produce only ACC deaminase. IAA is a common natural auxin and is a product of L-tryptophan metabolism in microorganisms. Approximately 80 % of rhizospheric bacteria can secrete IAA (Bhavdish et al. 2003). The auxins are also a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination, and seedling growth (El-Tarabily 2008). Khamna et al. (2009) isolated actinomycetes from rhizosphere soil of *Curcuma mangga* and reported *Streptomyces* CMUPA101, *Streptomyces* CMU-SK126, and *Streptomyces* CMU-H009 for their ability to produce antifungal compounds, siderophore, and IAA. Ningthoujam et al. (2009) reported that actinomycetes are prolific producers of various bioactive compounds such as antibiotics, siderophores, chitinases, and phytohormones and have phosphate-solubilizing abilities.

Several bacterial endophytes have been reported as potential biocontrol agents that may improve and promote plant health. The endophytic actinomycetes are reported to produce phytohormones such as IAA or iron-chelating molecules such as siderophores (Shenpagam et al. 2012). Many species of *Streptomyces* including *S. violaceus*, *S. scabies*, *S. griseus*, *S. exfoliates*, *S. coelicolor*, and *S. lividans* were reported to secrete IAA when fed with L-tryptophan (Manulis et al. 1994). Igarashi et al. (2002) isolated *Streptomyces hygroscopicus* from *Pteridium aquilinum* and found that it produces novel pteridic acids A and B as plant growth promoters with auxin-like activity. Gangwar et al. (2012) reported that 17 endophytic actinomycete isolates produced IAA in the range of 18–42 μg/ml. Verma et al. (2011) reported the PGP potentials of three endophytic *Streptomyces* strains recovered from surface-sterilized root tissues of *Azadirachta indica* that prolifically produce IAA and siderophores which play vital roles in plant growth promotion and suppression of *Alternaria alternata*.

Dalal and Kulkarni (2014) isolated 15 endophytic actinomycetes belonging to *Streptomyces* sp., *Micromonospora* sp., *Nocardia* sp., *Actinomadura* sp., *Microbispora* sp., and *Actinoplanes* sp. from soybean and screened for their PGP activity, viz., production of plant growth regulators (auxins, gibberellins, and cytokinins), siderophores, and HCN. They reported that nine endophytic isolates, JDA 3, JDA 4, JDA 5, JDA 6, JDA 7, JDA 9, JDA 10, JDA 12, and JDA 15, were found to exhibit PGP traits. Khamna et al. (2010) reported the isolation of *Streptomyces* sp. with IAA-producing capacity from the rhizosphere

soils of 14 Thai medicinal plants. Sharma et al. (2011) isolated plant growth-promoting endophytic actinomycetes from the disinfected surfaces of plant tissues. Coombs et al. (2004) isolated 38 strains of actinomycetes belonging to *Streptomyces*, *Microbispora*, *Micromonospora*, and *Nocardia* from surface-sterilized root tissues of healthy wheat plants. Okazaki et al. (1995) reported a total of 246 strains of actinomycetes of plant origin belonging to *Streptomyces*, *Microbispora*, *Nocardia*, *Micromonospora*, *Actinomadura*, and several others. Similarly, Takahashi and Omura (2003) successfully isolated 32 strains of *Streptomyces*, 33 strains of *Microbispora*, and 10 strains of other rare actinomycetes from fallen leaves of nine genera of higher plants. Rosenblueth and Martinez-Romero (2006) listed eight genera of plant-associated actinomycetes including *Arthrobacter*, *Curtobacterium*, *Kocuria*, *Nocardia*, and *Streptomyces*. The bio-efficacy of endophytic actinomycetes on plant growth promotion and management of bacterial wilt in tomato was studied by Sreeja and Gopal (2013). This revealed that *Streptomyces* sp. produces some specific compounds that directly influence plant growth.

19.3 Actinomycetes as Biocontrol Agent

Actinomycetes have been known as efficient biocontrol agents that naturally exist in soil and have the ability to control various plant pathogens, among which, *Streptomyces* sp. have been reported as potential antagonistic agent against soilborne fungal plant pathogens. El-Abyad et al. (1993) described the use of three *Streptomyces* sp., *S. pulcher*, *S. canescens*, and *S. citreofluoresce*, in the control of bacterial, *Fusarium*, and *Verticillium* wilts, early blight, and bacterial canker of tomato. Baniasadi et al. (2009) studied antifungal activity of actinomycetes isolated from soil samples of Iran against *Sclerotinia sclerotiorum*, the causal agent of stem rot in sunflower. Maximum activity was observed in crude extract of *Streptomyces* 363 propagated in submerged fermentation. These findings suggested that it can be used as proper candidate for field biocontrol studies. Gopalakrishnan and coworkers reported the biocontrol ability of several *Streptomyces* sp., *S. tsusimaensis*, *S. caviscabies*, *S. setonii*, and *S. africanus*, for inhibitory activity against soilborne pathogens such as *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina* under greenhouse conditions. Antagonistic activity of these PGP actinomycetes on Fusarium wilt-sick fields has also been demonstrated (Gopalakrishnan et al. 2011a, b). Osman et al. (2007) reported the antagonistic and insecticidal activity of *Streptomyces* sp. isolated from different soils and geographical areas in Egypt. *S. atrovirens* (SBTA 23) isolated from groundnut rhizosphere soils exhibited insecticidal activity against *Spodoptera litura* and antifungal activity against various fungal pathogens (Fig. 19.2a, b) (unpublished). Francisco et al. (2013) isolated

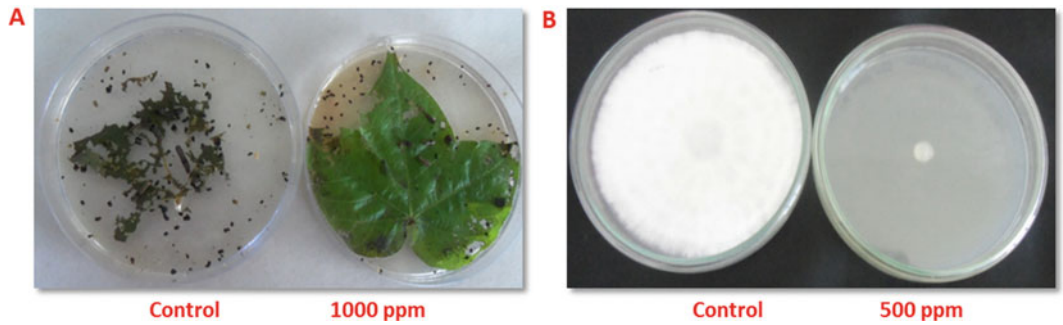

Fig. 19.2 Biocontrol activity of secondary metabolites of *S. atrovirens* (SBTA 23) against *Spodoptera litura* (**a**) and *S. rolfsii* (**b**)

40 strains of endophytic actinomycetes from the healthy maize plants and found two selected isolates 16R3B and 14F1D/2 are effective in the control of 71% and 36% of damping-off incidence, respectively. Similarly, Suseelabhai (2014) isolated actinomycetes from the rhizosphere of black pepper and ginger and tested its antagonistic activity against various phytopathogens such as *Phytophthora capsici*, *Phytophthora palmivora*, *Phytophthora nicotianae*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *Sclerotium rolfsii*, *Pythium myriotylum*, and *Ralstonia solanacearum*.

Srividya et al. (2012) identified a potent actinomycete isolate 9p with broad-spectrum antifungal property against four phytopathogens tested, *Alternaria brassicae*, *C. gloeosporioides*, *Rhizoctonia solani*, and *Phytophthora capsici*. Concurrent production of protease, lipases, siderophore, and IAA coupled with antifungal activity suggests the PGP and broad-spectrum biocontrol potential of this isolate. The strain 9p exhibited mixed antagonism type of mechanisms of biocontrol through the production of mycolytic enzymes. Suwan et al. (2012) isolated 119 isolates of actinomycetes and screened for antifungal activity against anthracnose of long cayenne chili pepper caused by *C. gloeosporioides*. Greenhouse studies revealed the application of NF-NSP1 resulted in significant disease reduction of 66.66 % when compared to uninoculated control. Fifty strains of actinomycetes were isolated from soil samples of Manisa Province, Turkey, and its surroundings and assessed for their antibacterial activity against four phytopathogenic and six pathogenic bacteria. Results indicated that 34 % of all isolates are active against test organisms, *Agrobacterium tumefaciens*, *Erwinia amylovora*, *Pseudomonas viridiflava*, *Clavibacter michiganensis*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Sarcina lutea* (Oskay et al. 2004). Valois et al. (1996) isolated about 200 actinomycete strains and screened for the ability to grow on fragmented *Phytophthora* mycelium and to produce metabolites that inhibit *Phytophthora* growth. Strains producing glucanases were selected that hydrolyze glucans from *Phytophthora* cell walls and cause lysis of *Phytophthora* cells.

19.4 Mass Production Methods of Actinomycetes

Streptomyces sp. are commercially important microbes that produce bioactive compounds such as growth-promoting substances (Khamna et al. 2009; Abd-Alla et al. 2013), antibacterial and antifungal compounds (Anitha and Rabeeth 2009; Srividya et al. 2012), and enzymes (Gombert et al. 1999) (Table 19.1) which are used for plant growth promotion and pest and disease control and also useful in some biological processes mainly biodegradation and waste treatment (Colombo et al. 2001; Claessen et al. 2002). Recent advancements in the field of biotechnology and biochemical engineering and the production of bioactive compounds from microorganisms, cells, and enzymes are considered as better alternatives compared to chemical agents. The biological agents are far better because of their higher selectivity, mild operating conditions, and easy availability of the substrates, mainly from the agriculture wastes.

19.4.1 Solid-State Fermentation (SSF) and Submerged Fermentation (SmF)

Many biotechnology industries rely on SmF, where the microorganisms are grown in liquid media for mass production of live organism as well biologically active secondary metabolites mainly because the processes associated with scale-up are much simplified, compared to those required for scale-up in SSF (Johns 1992; Nigam and Singh 1994). Submerged fermentation also allows greater control of parameters, such as pH, heat, and nutrient conditions (Johns 1992; Nigam and Singh 1994). However, there are some issues associated with secondary metabolite production in submerged fermentation, such as sheer forces, increasing viscosity due to metabolite secretion, and reduction in metabolite stability (Robinson

Table 19.1 Bioactive compounds produced by actinomycetes

Bioactive compound	Organism	Substrate		Productivity		References
		SSF	SmF	SSF	SmF	
IAA	*Streptomyces* sp. ASU1 4	–	Liquid broth	–	22 µg/ml	Abd-Alla et al. (2013)
IAA and siderophores	*Streptomyces* sp.	–	Liquid broth	–	5.47 (IAA) and 143.95 (siderophore) µg/ml	Khamna et al. (2009)
Cephamycin C	*Nocardia lactamdurans*, *Streptomyces clavuligerus* NT4	Soybean flour	Production media	15.75 mg/g	13.65 mg/ml	Kagliwal et al. (2009); Bussari et al. (2008)
Tetracycline	*Streptomyces viridifaciens*, *Streptomyces clavuligerus*	Sweet potato residue agro-waste	Cellulosic substrates	2129 µg/g 300 µg/g	N/A	Yang and Ling (1998)
Pravastatin	*Streptomyces* sp.	Rice bran and rice husk	Nutrient broth	15 mg/l/h	80–85 %	Park et al. (2003); Kostova et al. (2004)
L-Asparaginase	*Streptomyces* sp.	Soybean meal	Yeast extract medium	49.23 U/ml	24.61 U/ml	Basha et al. (2009)
Avermectin B1b	*Streptomyces avermitilis* 41445		SM2 growth medium		17 mg/ml	Siddique et al. (2013)
Neomycin	*Streptomyces fradiae*	Nylon sponge and orange peelings	Glucose, soya peptone, meet extract, NaCl, and ZnSO4	13,903 µg/ml	250 µg/ml	Machado et al. (2013)
Dibutyl phthalate	*S. atrovirens*	–	Glycerol casein broth	–	56.4 µg/ml	Un published

et al. 2001). The use of SSF for the production of secondary metabolites is at present underutilized due to its detrimental effect on formation as secondary metabolites are extremely sensitive to environmental factors; and these factors are more difficult to control in large-scale SSF. But, the use of SSF should be considered by industry, especially when large quantities of secondary metabolites are required in short fermentation periods, with minimal expenditure on media and downstream processing (Robinson et al. 2001).

19.4.2 Submerged Fermentation (SmF) of Actinomycetes

SmF utilizes free flowing liquid substrates and secretes secondary metabolites into fermentation broth. SmF is primarily used in the extraction of secondary metabolites that have to be used in liquid form. The advantage of this technique is that purification of products is easier. Several studies have reported on the utilization of fed-batch cultivation for antibiotic production. The effects of stirring conditions, oxygen transfer (Rosa 2002), and dissolved oxygen concentrations (Yegneswaran et al. 1991) are studied in fed-batch cultivations for the production of clavulanic acid by *S. clavuligerus*. The fed-batch processes may be used to maintain the microorganism in the stationary phase longer as well as to increase the total mass of cells; therefore, production is increased.

Arijit et al. (2013) found improvement of pectinase production through submerged fermentation by *Streptomyces* sp. GHBA10 isolated from mangrove samples. Maximum yield of

amylase (162.4AU) was obtained by *Thermoactinomyces sacchari* in arginine-glycerol-salt (AGS) medium (starch 2.5 % and glucose 3 %) using submerged fermentation with 5 % inoculum, 150 rpm, temperature 60 °C, and pH 7.5 (Ayub et al. 2014). Ribeiro et al. (2012) reported the production of amylolytic enzymes with *Streptomyces* sp. SLBA-08 strain using sisal waste and sugarcane bagasse. Maximum α-amylase activity was observed using sisal waste (2.7 %) and urea (0.8 %) in submerged fermentation after 3 days of cultivation at 30 °C. Siddique et al. (2013) reported the production of avermectin B1b, a component of commercially available abamectin, was obtained as fermentation product of *S. avermitilis* by submerged fermentation by using SM2 growth medium containing soluble cornstarch, yeast extract, KCl, $CaCO_3$, and $MgSO_4$. Maximum production of 17 mg/L was observed with medium pH of 7, 10 % inoculum, and temperature 31 °C for 10 days of fermentation period.

Synthesis of L-glutaminase was identified in two *Streptomyces* sp., *S. avermitilis* GLU1 and *S. labedae* GLU2, using submerged fermentation by altering the physiological parameters such as incubation period, temperature, initial pH, inoculum size, and NaCl concentration. The highest activity of 8.41 and 12.23 U/ml was observed with 4 % Nacl, 7–8 pH, and 30 °C (Abdallah et al. 2012). The secondary metabolite, dibutyl phthalate, having antifungal and insecticidal activity was isolated from *S. atrovirens* SBTA 23 by submerged fermentation. Maximum yield (560 mg/L) was attained in the glycerol casein medium with pH 7.0, inoculum 10 %, and 28 °C for 7 days (unpublished).

19.4.3 Solid-State Fermentation of Actinomycetes

Solid-state fermentation is a fermentation process on a solid substrate, which has low moisture content (lower limit ~12 %) and occurs in a non-septic and natural state (Nigam and Singh 1994). The main advantage of using these substrates is that nutrient-rich waste materials are utilized very slowly and steadily, supporting the controlled release of nutrients. Interest in SSF has been increasing because of its important applications in producing various bioactive compounds. SSF produces a high product concentration but has a relatively low energy requirement (Yang and Yuan 1990). The mycelia morphology associated with the microorganisms predominately used for secondary metabolite production is well suited to growth on a solid support. This can also have a detrimental effect on product formation in liquid media, because highly viscous liquid media are required for successful metabolite production and this can interfere with oxygen transfer. Among several factors that are important for microbial growth and activity, the most critical include substrate, particle size, and moisture level (Lui and Tzeng 1999). The advantage of SSF is to bring cultivated microbes in tight contact with the insoluble substrate and to achieve the highest nutrient concentration from the substrate for fermentation (Bhargav et al. 2008). Substrate for SSF can be divided into three groups, starchy substrates, cellulose or lignocellulose, and those with soluble sugar. Many antibiotics such as penicillin, cephamycin C, neomycin, iturin, cyclosporin A, and cephalosporins are produced by SSF. Cephamycin C is produced by a variety of microorganisms including *Streptomyces cattleya*, *S. clavuligerus*, and *N. lactamdurans*. Wheat raw supplemented with cottonseed de-oiled cake and sunflower cake was used for the production of cephamycin C using SSF (Kota and Sridhar 1999). Wheat raw supplemented with raspberry proved to be optimum for the production of neomycin by SSF. The major difference between SSF and SmF is the free water content in the substrate. Therefore, SSF technology can be exploited as an alternative, allowing better oxygen circulation (Elibol and Mavituna 1997). However, Pandey (1992) reported that bacterial culture can be well managed and manipulated for SSF process. It seems that the high yield in SSF as compared to SmF is due to the growth of the microorganisms similar to their natural habitat, resulting in higher metabolic activities.

19.5 Delivery Systems and Commercialization of Actinomycete-Based Products

The use of actinomycetes and its secondary metabolites presents an attractive way to replace chemical fertilizers, pesticides, and supplements, which results in significant increase in plant growth and pest and disease control in agricultural crops (Sousa et al. 2008). A lot of research work is being carried out nationally and internationally on actinomycete inoculants that promote growth through at least one of the following mechanisms: suppression of plant disease, improved nutrient acquisition, or phytohormone production. But, very few actinomycete-based products are commercialized. Although biocontrol with PGPR is an acceptable green approach, the proportion of registration of biocontrol agents for commercial availability is very slow. Development of formulations with increased shelf life and broad spectrum of action with consistent performance under field conditions could pave the way for commercialization of the technology at a faster rate. Biocontrol agents are easy to deliver, improve plant growth, activate resistance mechanism in the host, and increase biomass production and yield. These antagonists act through antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism, and competition for space and nutrients.

The agroindustry shows a marked interest on actinomycetes as a source of agro-active compounds of PGPR and of biocontrol tools (Behal 2000; Tanaka and Omura 1993). In fact, about 60 % of the new insecticides and herbicides reported in the past 5 years originated from *Streptomyces* sp. (Tanaka and Omura 1993). It is also estimated that as many as three-quarters of all *Streptomyces* species are capable of antibiotic production (Alexander 1977). Actinomycetes produce a variety of antibiotics with diverse chemical structures such as polyketides, β-lactams, and peptides in addition to a variety of other secondary metabolites that have antifungal, antitumor, and immunosuppressive activities (Behal 2000). Kasugamycin is a bactericidal and fungicidal metabolite discovered by Umezawa and coworkers in *Streptomyces kasugaensis* (Umezawa et al. 1965). Polyoxin B and D metabolites were isolated from *Streptomyces cacaoi* var. *asoensis* in 1965 by Isono et al. (1965) as a new class of natural fungicides. Siddique et al. (2013) reported that avermectin B1b, a component of commercially available abamectin, was obtained as fermentation product of *S. avermitilis* which has frequently been used as insecticidal agent. Very few actinomycete-based commercial formulations are available in the market (Table 19.2) mainly for pest and disease control. Unfortunately, no product is commercialized for plant growth promotion though a lot of research is carried out on the production of growth-promoting substances by actinomycetes.

19.6 Conclusion

In this chapter we have tried to provide useful information on mass production technologies, delivery systems, and commercialization of actinomycete-based products. Based on the available literature, we can conclude that the novel secondary metabolite-based products being used in agriculture for pest and disease control are made out of microbes especially from *Streptomyces* sp. Due to their readily degradable nature, highly specific and less-toxic to nontarget organisms that can replace conventional biocides or chemical fertilizers, pesticides which results in significant increase in plant growth and pest and disease control in agricultural crops. The biological agents are always better because of their higher selectivity, nontoxicity, and easy availability from many agriculture wastes itself. Moreover, identification of the mechanisms of action of bioagents may lead to the discovery of novel phenomena of the importance in PGP and biocontrol. Development of PGP actinomycete-based products/formulations with increased shelf

Table 19.2 List of actinomycete-based products commercialized globally

Organism	Brand name	Manufacturer	Delivery system	Pesticide class
S. griseoviridis	Mycostop	Verdera, Finland	Powder	Fungicide
Streptomyces lydicus WYEC 108	Actinovate SP, Actinovate AG, Actinovate STP, Actinovate Lawn and Garden, Actino-Iron	Novozymes BioAg Inc., USA	Powder	Fungicide
S. lydicus WYEC 108	Actino-Iron Lawn and Garden	Novozymes BioAg Inc., USA	Granules	Fungicide
Saccharopolyspora spinosa	Tracer, Entrust	Dow AgroSciences, USA	Liquid	Insecticide
S. hygroscopicus and *Streptomyces viridochromeogenes*	Bialaphos	Toku-E, USA	Powder	Herbicide
S. atrovirens	Incide SP	Sri Biotech Laboratories India Ltd., India	Powder	Insecticide
S. atrovirens	Actin	Sri Biotech Laboratories India Ltd., India	Liquid	Fungicide

life and broad spectrum of action with consistent performance under field conditions could pave the way for commercialization of the technology at a faster rate.

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