Chapter 5

Plant Growth Promotion by Endophytic Actinobacteria Associated with Medicinal Plants

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Abstract

There is a lot of prospect for production of novel bioactive metabolites for application in medicine, pharmaceutical, agricultural and other industry from endophytic bacteria associated with medicinal plants. Actinobacteria are spore forming that can form a stable and persistent population in various ecosystems. Actinobacteria especially Streptomyces are prolific producers of several agriculturally important secondary metabolites that can be use as plant growth promoting and biocontrol agents. Endophytic actinobacteria associated with medicinal plants can directly promote the growth of plants through production of indole acetic acid, siderophore, solubilization of inorganic phosphate and fixing of free nitrogen. They can promote the plant under stress conditions by production of aminocyclopropane-1-carboxylic acid deaminase. They can also act as an agent for improving phytoremediation of toxic metals and organic pollutants. They may indirectly promote the plant growth by production of antifungal antibiotics and cell wall degrading enzymes. It is expected that endophytic actinobacteria associated with medicinal plants may produce bioactive metabolites that differ significantly from the soil dwelling actinobacteria. They may also participate in the host metabolic pathway and gain some genetic information and produce secondary metabolites similar to the host plants. Intensive research on characterization and identification of the untapped bioresource from endophytic bacteria, especially actinobacteria, is of outmost important for application in agriculture as the use of synthetic chemical pose serious risk to human health and environment. The use of plant growth promoting endophytic actinobacteria can emerge as novel sustainable and alternative tools.
Keywords: Endophytic actinobacteria; medicinal plants; *Streptomyces*; plant growth promoting activities

1. Introduction

Ethno-medicinal plants are the backbone of traditional medicine that has been used by mankind to treat a number of diseases since time immemorial. Numerous studies on the bioactivity of medicinal plants are still underway, since they constitute a rich source for production of novel secondary metabolites, for application in pharmaceutical, agricultural and other industries. In the past, research on medicinal plants focussed primarily on their ingredients; however, recently the focus has shifted to include the structure and function of several medicinal plant microbiomes. Endophytic bacteria associated with the medicinal plants may directly or indirectly involve in the production of bioactive phytochemicals [1]. Surprisingly, not only the plants themselves were able to produce compounds with phytotherapeutic properties, but their associated microbes, in particular endophytes, could as well [1,2].

Endophytes are microorganisms that reside within the interior tissues of plants without exhibiting negative effects on the host plant or the environment. However, some seemed to be latent pathogens and, conditionally, either induce or participate in host plant infection [3]. Almost all the plants have been found to be associated with one or more endophytes. They are able to associate with the host at a very early stage of plant development [4]. Endophytic bacteria have been isolated from various parts of the plants. However, majority of them are isolated from roots tissues followed by stem and leaf. The woody plants conferred far greater diversity in comparison to herbaceous plants. Plants that grow at tropical region harbour greater diversity than that grow at temperate region [5,6,7].

It is widely believed that the potential of secondary metabolites with biological activities from endophytic bacteria is just as great as that achieved from soil bacteria [8]. As a consequence of long term association of endophytes with the host plant, bacteria may participate in metabolic pathways and/or may gain some genetic information from the host plant, and produce biologically active compound(s) similar to the host plant [7,9,10,11]. Endophytes associated with medicinal plants have great potential to produce unique secondary metabolites, which can be exploited for application in pharmaceutical, agricultural and other industries.

2. Actinobacteria

Actinobacteria are aerobic spore forming gram-positive bacteria containing high guanine-cytosine (57-75%) in their genome, and belong to the order Actinomycetales that grow as branching filaments consisting of vegetative mycelia and aerial hyphae. They are ubiquitous and form a stable and persistant population in various ecosystems and play an important ecological role in soil nutrient cycling [12,13,14]. They are well known for the production of wide range of secondary metabolites, for use not just in pharmaceutical industries but agriculture as
well. The most extensively studied actinobacteria belong to genera *Streptomyces*. Actinobacteria are prolific producers of several agriculturally important secondary metabolites and several members have been considered as plant growth promoting (PGP) and biocontrol agents [15, 16,17].

Actinobacteria can stimulate plant growth directly or indirectly. The main mechanisms by which they directly contribute to the plant growth are production of phytohormones such as indole-3-acetic acid (IAA), cytokinins and gibberellins; enhancing plant nutrition by solubilization of minerals such as phosphorus (P) and iron, production of siderophores and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase [17,18]. They indirectly benefit the plant by biocontrol of deleterious pathogens through the production of antibiotics and volatile compounds (VOCs), synthesis of fungal cell wall degrading extracellular enzymes, induction of systemic resistance and competition for nutrients and niches [17,18,19].

3. Actinobacteria from Medicinal Plants

Actinobacteria are able to associate with the host plant as endophyte at the early stage of plant development [4]. Outer plant tissues have greater diversity compared to other plant tissues [8,20,21,22]. Majority of actinobacteria are isolated from root tissues followed by stem and leaf [23-30]. The high rate of occurrence of actinobacteria in roots may be due to the fact that the actinomycetes are natural dwellers of soil and hence easily come in contact with the roots of plants, and may form symbiotic association with the host by entering the plant tissues [5-7].

Among endophytic actinobacteria recovered from medicinal plants, *Streptomyces* accounts for the most abundant genus [5-7,26,28,30-34] followed by *Micromonospora, Actinopolyspora, Nocardia, Saccharopolyspora, Streptosporangium, Promicromonospora* and *Rhodococcus* [25,26,30,31]. Some rare genera, like *Dietzia, Microtetraspora, Actinocorallia, Verrucocispora, Isoptericola* and *Kytococcus* [35,36] were also reported from medicinal plants.

Actinobacteria isolated from medicinal plants are producers of growth promoting metabolites, insect and pest repellents, antimicrobials against plant pathogens and protectors in stress conditions [7,10,11,16,28,37]. They also exhibited antimicrobial activity against multi drug resistant pathogens [38], antiviral [39], larvicidal [40], antimalarial [41,42] and other important activities such as antitumor [43], antidiabetic [44] and antioxidant [30].

4. Direct Plant Growth Promotion

Endophytic bacteria especially actinobacteria may directly contribute to growth of plants through PGP activities such as P solubilization, IAA, ACC deaminase and siderophore
production, and nitrogen (N) fixation [27,29,44-46] (Table 1). Out of 81 endophytic actinobacteria isolates from medicinal plant *Rhynchotoechum ellipticum*, 36 strains were positive for IAA production in the range of 7.4 to 52.3 µg/ml. Majority of IAA producer belong to genus *Streptomyces* and some to *Actinomycetes, Microbacterium, Micromonospora, Leifsonia, Brevibacterium, Pseudonocardia, Promicromonospora, Kocuria and Amycolatopsis* [30]. Similarly, Gangwar et al. [47] also found actinobacteria, mostly *Streptomyces* sp, capable of producing IAA in the range of 9.0–38.8 µg/ml. Khamna et al. [48] reported that *Streptomyces* spp. isolated from medicinal plants produced IAA in the range of 11–144 µg/ml. Liu et al. [49] reported that 88 % endophytic actinobacteria isolated from medicinal plants *Ferula songorica* could fix free N while 19 % solubilize P. Dochhil et al. [50] demonstrated that IAA producing endophytic *Streptomyces* sp. CA10 and CA26 isolated from a folk ethno-medicinal plant *Centella asiatica* enhanced seed germination and seedling growth of French bean.

Endophytic bacteria having multiple PGP activities could successfully colonized the internal tissues and promote the growth of plants under greenhouse and field conditions. *Streptomyces* sp. En-1 endophytic to medicinal plant *Taxus chinensis* synthesize IAA via indole-3-acetamide (IAM) pathway. The strain could successfully colonize the intercellular tissue and promote the growth of *Arabidopsis* [51]. Endophytic bacteria strains *Sphingomonas, Pantoea, Bacillus* and *Enterobacter* isolated from the roots of elephant grass could solubilize inorganic P, fix N, and produce IAA and ammonia. Similarly, those strains were able to successfully colonized the roots of *Hybrid Pennisetum* and significantly promote the growth under salt stress conditions [52]. Endophytic bacterial strains *Paenibacillus* and *Bacillus* sp. (isolated from medicinal plant *Lonicera japonica*) possessing positive results for P solubilization, IAA, Siderophore, ACC deaminase productions enhance the growth and chlorophyll content of wheat plants under pot conditions [53]. Similarly, treatment of chilli and tomato with endophyte *Streptomyces* sp. having multiple PGP activities significantly enhance the growth under greenhouse conditions [36].

ACC deaminase producing endophytic bacteria can promote the growth of host plants by degrading the ACC, precursor of stress hormone ethylene, before its oxidation by plant ACC oxidase thus blocking stress ethylene production. As a result, bacteria can protect the host when plant is exposed to either biotic or abiotic stress conditions. ACC deaminase producing endophytic actinobacteria can effectively protect the host plants growth inhibition by flooding, high salt, drought, presence of pathogens, high levels of toxic metals and organic pollutants and low temperature [54,55].

Plant growth promotion by ACC deaminase producing endophytic bacteria was demonstrated by Sun et al. [56]. ACC deaminase producing wild type *Burkholderia phytofirmans* could promote the elongation of the roots of canola seedlings. Whereas, ACC deaminase (acds) gene deleted mutant strain failed to promote the growth of root growth. Tomato plant
treated with the ACC deaminase producing endophytic bacteria \textit{(Pseudomonas fluorescens} and \textit{Pseudomonas Migulae}) were more healthier than those plants treated with the mutant strains deficient in \textit{acds} gene when grown under salt stress conditions (165 mM and 185 mM) [57]. The strains also delayed the flowers senescence in \textit{Dianthus caryophyllus}, whereas senescence were quicker when treated with \textit{acds} gene deleted strain [58]. Endophyte \textit{Bacillus} sp. from medicinal plant \textit{Phyllanthus amarus} positive for IAA, ACC deaminase and siderophore production enhance seed germination, vigor index and growth of \textit{Phyllanthus amarus} under salt stress conditions (160 mM) [59].

Endophytes have a great potential for use as an agent for improving phytoremediation and biomass production of non-food crops [60]. Endophytic strain \textit{Pseudomonas} sp. A3R3 could successfully colonize the interior tissue of \textit{Allysum serpillifolium} and \textit{Brassica juncea} and increased the plant biomass and Ni accumulation in both plant when grown in Ni contaminated soils [61].

\textbf{Table 1.} PGP activities of endophytic actinobacteria from medicinal plants

<table>
<thead>
<tr>
<th>Endophytic actinobacteria</th>
<th>Host</th>
<th>PGP activities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Streptomyces} sp. En-1</td>
<td>\textit{Taxus chinensis}</td>
<td>IAA production</td>
<td>51</td>
</tr>
<tr>
<td>\textit{Streptomyces albosphorus}, \textit{Streptomyces cinereus}, \textit{Micromonospora} sp. O6, \textit{Saccharopolyspora} sp. O9</td>
<td>\textit{Aloe vera, Mentha arvensis, Ocimum sanctum}</td>
<td>P solubilization, IAA production</td>
<td>47</td>
</tr>
<tr>
<td>\textit{Streptomyces roseosporus}</td>
<td>\textit{Mentha arvensis, Ocimum sanctum}</td>
<td>P solubilization, IAA production</td>
<td>47</td>
</tr>
<tr>
<td>\textit{Streptomyces aureus}, \textit{Streptomyces griseorubroviolaceous}, \textit{Streptomyces globisporus},</td>
<td>\textit{Mentha arvensis, Ocimum sanctum}</td>
<td>IAA production</td>
<td>47</td>
</tr>
<tr>
<td>\textit{Streptomyces viridis}</td>
<td>\textit{Aloe vera, Mentha arvensis, Ocimum sanctum}</td>
<td>IAA production</td>
<td>47</td>
</tr>
<tr>
<td>\textit{Streptomyces olivaceus}, \textit{Streptomyces} sp. BPSAC101, \textit{Streptomyces} sp. BPSAC121, \textit{Streptomyces thermocarboxydu}</td>
<td>\textit{Rhynchotoechum ellipticum}</td>
<td>P solubilization IAA and ammonia production</td>
<td>30</td>
</tr>
<tr>
<td>\textit{Brevibacterium} sp. S10S2, \textit{Janibacter} sp. R4S4, \textit{Microbacterium} sp. S4S17</td>
<td>\textit{Ferula sinkiangensis}</td>
<td>IAA and siderophore production, N fixation</td>
<td>29</td>
</tr>
<tr>
<td>\textit{Kocuria} sp. R7S1</td>
<td>\textit{Ferula sinkiangensis}</td>
<td>IAA production, N fixation</td>
<td>29</td>
</tr>
</tbody>
</table>

\textbf{5. Indirect plant growth promotion}

Endophytic bacteria, especially actinobacteria, can indirectly promote the growth of plant by production of antifungal antibiotics, cell wall degrading enzymes and VOCs. Actinobacteria are prolific producers of several agriculturally important secondary metabolites for use as biocontrol agents. Of about 23,000 bioactive secondary metabolites discovered in end-
Endophytic actinobacteria isolated from medicinal plant *Ferula sinkiangensis* inhibit the growth of fungal pathogen *Alternaria alternate* [29]. Of 81 endophytic actinobacteria isolated from medicinal plant *Rhychnotoechum ellipticum*, 72 inhibit the growth of *Fusarium proliferatum*, *F. oxysporum f. sp. ciceri* and *F. oxysporum*. Majority of the strain showing antifungal activities belong to *Streptomyces* spp. viz; *Streptomyces olivaceus*, *Streptomyces* sp. *BPSAC101*, *Streptomyces* sp. *BPSAC121* and *Streptomyces thermocarboxydus*. *Streptomyces olivaceus* and *Streptomyces* sp. *BPSA 121* produce antifungal antibiotics ketoconazol, fluconazole and miconazole (*Table 2*). Seventeen strains showed positive results for presence of antibiotics biosynthetic gene cluster PKSI, PKSII and NRPS [30]. Antibiotic 6-prenylindole produced by endophyte *Streptomyces* sp. exhibit significant antifungal activity against plant pathogens, viz; *A. brassicicola* and *F. oxysporum*. Antifungal compound fistupyrone from *Streptomyces* sp. inhibit the infection of *A. brassicicola* in spring onion [64]. Li et al. [65] reported that antibiotic staurosporine extracted from endophytic *Streptomyces* strain CNS-42 showed a potent effect against *F. oxysporum f. sp. cucumerinum*. The *in-vivo* biocontrol assays showed a significant reduction in disease severity and increases in biomass and growth of cucumber plant. Endophytic *Streptomyces* sp. showed antifungal activity against *Alternaria* sp., *Colletotrichum truncatum*, *Geotrichum candidum*, *F. oxysporum* and *F. udum* [46,63]. Four peptide antifungal compounds Munumbicin A-D obtained from *Streptomyces* sp NRRL 3052, endophytic actinobacteria from medicinal plant snakevine, inhibit important agricultural fungal pathogens such as *P. ultimum*, *R. solani*, *Phytophthora cinnamomi* and *Sclerotinia sclerotiorum* (*Table 3*). The peptide compounds contained common amino acids such as threonine, aspartic acid (or asparagine), glutamic acid (or glutamine), valine and proline, in varying ratios [40].

Production of fungal cell wall degrading enzymes by endophytic bacteria especially actinobacteria, and their biocontrol activities against important plant fungal pathogens have been well documented in a number of literatures [27,30,50]. Endophytic bacteria isolated from ethnomedicinal plants exhibited antifungal activity against *F. oxysporum* through production of chitinase, pectinase and cellulase [44]. Similarly, cellulase and pectinase producing endophytic strains *Paenibacillus* and *Bacillus* sp. isolated from medicinal plant *Lonicera japonica* inhibit the growth of *F. oxysporum* [53]. Cell wall degrading enzymes and HCN producing endophytic actinobacteria such as *Streptomyces* sp. DBT204, *Streptomyces* sp. DBT 207, *Nocardiopsis* sp., and *Streptomyces thermocarboxydus* inhibit the growth of *R. solani*, *F. oxysporum*, *F. proliferatum*, *F. graminearum* and *Colletotrichum capsici* [36]. Chitinase producing endophytic *Streptomyces* sp. isolated from maize plant showed antifungal activity against *Fusarium* sp., *Pythium aphanidermatum*, *R. solani* and *Sclerotinia sclerotiorum*. The strain reduced the...
damping-off incidence caused by *P. aphanidermatum* in cucumber under greenhouse conditions [66]. Similarly, 3 endophytic actinomycetes isolated from cucumber roots identified as *Actinoplanes campanulatus*, *Micromonospora chalcea* and *Streptomyces spiralis* significantly promoted plant growth, yield and reduced seedling damping-off, and root and crown rots of mature cucumber caused by *P. aphanidermatum* under greenhouse conditions [67,68]. The three isolates causes plasmolysis, hyphal lysis and reduced the conidial germination of fungal pathogens by production of cell wall degrading enzymes such as chitinase, glucanase and cellulase. The strains could successfully colonize the internal tissues of roots, stems and leaves under field conditions [67,69,70].

**Table 2**: Antifungal metabolites production by endophytic actinobacteria from medicinal plants

<table>
<thead>
<tr>
<th>Endophytic actinobacteria</th>
<th>Host</th>
<th>Antifungal metabolite</th>
<th>Target pathogen(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces</em> sp. NRRL 3052</td>
<td><em>Kennedia nigriscans</em></td>
<td>Munumbicins A, B, C and D</td>
<td><em>Pythium ultimum, Rhizoctonia solani, Phytophthora cinnamomi, Sclerotinia sclerotiorum</em></td>
<td>40</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. MSU-2110</td>
<td><em>Monstera sp.</em></td>
<td>Coronamycin</td>
<td><em>Pythium ultimum, Fusarium solani, Rhizoctonia solani</em></td>
<td>71</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. NRRL 30562</td>
<td><em>Kennedia nigriscans</em></td>
<td>Munumbicins E-4 and E-5</td>
<td><em>Pythium ultimum, Rhizoctonia solani</em></td>
<td>41</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. Hedaya 48</td>
<td><em>Aplysina fistularis</em></td>
<td>Saadamycin/5,7-Dimethoxy-4-pmethoxylphenyl coumarin</td>
<td><em>Fusarium oxysporum</em></td>
<td>72</td>
</tr>
<tr>
<td><em>Streptomyces aurantiacus</em></td>
<td><em>Impariens chinensis</em></td>
<td>-</td>
<td><em>Fusarium oxysporum, Curvularia lunata, Botrytis cinerea</em></td>
<td>73</td>
</tr>
<tr>
<td><em>Streptomyces chryseus</em></td>
<td><em>Potentilla discolor</em></td>
<td>-</td>
<td><em>Botrytis cinerea</em></td>
<td>73</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. SAUK6020</td>
<td><em>Achyranthes aspera</em></td>
<td>-</td>
<td><em>Fusarium graminearum</em></td>
<td>73</td>
</tr>
<tr>
<td><em>Streptomyces albogriseolus</em></td>
<td><em>Cynanchum auriculatum</em></td>
<td>-</td>
<td><em>Fusarium graminearum, Curvularia lunata, Botrytis cinerea</em></td>
<td>73</td>
</tr>
<tr>
<td><em>Streptomyces ochraceiscle- roticus</em></td>
<td><em>Salvia militiorrhiza</em></td>
<td>-</td>
<td><em>Curvularia lunata Botrytis cinerea</em></td>
<td>73</td>
</tr>
<tr>
<td><em>Micromonospora peucetia</em></td>
<td><em>Ainsliaea henryi</em></td>
<td>-</td>
<td><em>Curvularia lunata Botrytis cinerea</em></td>
<td>73</td>
</tr>
<tr>
<td><em>Micromonospora</em> sp. SAUK6030</td>
<td><em>Stellera chamaejasme</em></td>
<td>-</td>
<td><em>Curvularia lunata Botrytis cinerea</em></td>
<td>73</td>
</tr>
</tbody>
</table>
Table 3: Antifungal activity of the Munumbicin A-D from *Streptomyces* NRRL 3052 against indicated pathogens (MIC µg/ml) [Adapted from Catillo et al. [40]]

<table>
<thead>
<tr>
<th>Fungal pathogens</th>
<th>Munumbicin A</th>
<th>Munumbicin B</th>
<th>Munumbicin C</th>
<th>Munumbicin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pythium ultimum</td>
<td>2.0</td>
<td>0.2</td>
<td>4.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>-</td>
<td>8.0</td>
<td>1.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Phytophthora cinnamomi</td>
<td>-</td>
<td>6.2</td>
<td>1.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>8.0</td>
<td>0.2</td>
<td>8.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

6. Conclusions and future perspectives

Intensive research on characterization and identification of the diverse population of endophytic actinobacteria associated with medicinal plants is of utmost importance, in order to explore the enormous untapped bioresource for bioactive metabolites, for application in modern medicine, agricultural, pharmaceutical and other industries. It is expected that endophytes may produce novel secondary metabolites that differ significantly from soil-dwelling actinobacteria or other bacteria. As the use of synthetic pesticide and fertilizers pose serious...
threat to human health and environment, the use of plant growth promoting endophytic actinobacteria can emerge as alternative tools for sustainable, organic and environmental friendly agricultural crop production.

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8. References


