A Review on Endophytic Actinomycetes and their Applications

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ABSTRACT

Actinomycetes were also known as actinobacteria and there are Gram-positive, Spore forming, filamentous actinobacteria which usually contain about 57-75% of G+C content in their DNA. Actinomycetes are considered as a unique group of microorganism which locus between the true bacteria and the true fungi. Streptomyces genera occupy 50% of actinobacteria, in which 75% of commercially useful antibiotics produced by these genera. Pectinase, amylase, lipase, protease, cellulose and xylanase are industrially significant enzymes that are produced by the actinomycetes. About 22,500 bioactive compounds were produced by microbes, among this 17%, 38% and 45% were isolated from unicellular bacteria, fungi and actinomycetes respectively. The main aim of this review is to determine the various applications of endophytic actinomycetes.

Keywords: Endophytic actinomycetes; Streptomyces; Antimicrobial activity; Enzymes; Bioactive compounds

INTRODUCTION

Actinomycetes were also known as actinobacteria, derived from the Greek word where akis refers to "lightning" and mykes refers to the fungus which was originally classified as an interposed group between fungi and bacteria [1]. Waheeda K et al. reported that endophytic microorganisms are those which do not cause any immediate and negative effect while colonizing living internal tissue [2]. Actinomycetes are considered as a unique group of microorganism which locus between the true bacteria and the true fungi. Nearly all plant species serves as a source for endophytes and accepted as a promising source of novel medicinal compounds [3]. Using microorganism 23,000 of active secondary metabolites was produced, in that 10,000 were isolated from actinomycetes. Of these, Streptomyces species produces 7,600 bioactive compounds. Depending upon the source of isolation the biological function of actinomycetes varies [4]. As like plants, endophytic microorganism has the capacity to produce secondary metabolites thereby it serves as an optimistic source for the novel compound [5]. Although previous studies reported that several bio-pharmacological compounds were isolated from endophytes with activities such as antiviral, anti-inflammatory, antimicrobial and antitumor activities but there is less information about their antioxidant activities [3]. Axenic plant is a low-stress tolerance which is considered to be a partial absence of endophytic microbes [6]. When the endophyte affects the plant it grows faster in compare to the unaffected plants [7]. If the leaves are pretreated with heteroauxin and zircon solution it results in adequate isolation of actinomycete [5]. Based on the method of isolation, actinomycetes diversity varies [2]. The organization and diversity of endophytic actinomycetes are very much important to know about their ecological roles and also it helps in the screening of beneficial strains. Using terminal restriction fragment length polymorphism (T-RFLP) density of the endophytic actinobacteria in wheat roots can be identified [8]. Varieties of natural products are produced and medicinal property can be improved by the plants which have ethno-botanical history that serves as the reservoir of endophytic actinomycetes [9].
Both endophytic and rhizospheric are plant-associated bacteria which as valuable effects on the host plants. When compared to rhizospheric bacteria, endophytes are more reliable and specific habitat as it resides within tissues of the plant [2]. Pyrosequencing method is used to reveal a wide range of bacteria that live in and around the roots of the plant such as actinobacteria, bacterioide, verrucomicrobia and proteobacteria [10]. Endophytic actinomycetes isolated from the leaves and roots of Zea mays L. are mostly consisted of Streptomyces and other genera like Micromonospora, Streptosporangium, Streptoverticillium, and Nocardia [11].

Nature of Actinomycetes
Gram-positive, Spore forming, filamentous actinobacteria are usually grown in soils that contain about 57-75% of G+C content in their DNA [12,13]. Actinobacteria are mostly chemoorganotrophic that grows at neutral PH and also at alkalophilic and acidophilic conditions. Where some of the actinobacteria are phototrophic, autotrophic and heterotrophic. During active growth there is an incomplete mycelia development in actinobacteria whereas well-matured actinobacteria will have mycelium of two types; one is rhizoids in the substrate and another aerial mycelium on the outside substrate [1]. Geosmin is a volatile compound that produced during secondary metabolism in which it is linked to the “wet earth” characteristic odour of actinobacteria [1]. Several studies were reported that highest population of endophytic actinomycetes were spotted to be in roots and vegetative stage of the rice plant [14]. Most of the actinomycetes act as saprophytes for nutrient production by degrading the organic materials which are known to present in the environment like soil, pond and lake sediments [6]. Population and diversity of endophytic actinomycetes are mainly influenced by edaphic factors, physiological status and cultivars of plants [8].

Antimicrobial Activity
Totally 93 marine actinomycetes were extracted from the 15 marine samples taken from the different sites in the Bay of Bengal. When these actinomycetes were subjected to antibacterial activity, among these actinomycetes 27 isolates showed activity against Staphylococcus aureus (24 mm) and Bacillus subtilis (22 mm) [15]. Paenonia lactiflora, Radix platycodi, Achyranthes bidentata and Artemisia argyi are the medicinal plants having 65 strains of endophytic actinobacteria, but 12 of them were able to repress Staphylococcus aureus [16]. 70 endophytic actinobacteria were isolated from the tomato plant and tested against selected microorganism for their antimicrobial activity, from these 62 isolates exhibited antimicrobial activity. Out of these 62 isolates, 58th and 37th isolates were active against bacteria and fungi respectively. The high spectrum of antimicrobial activity was found to be in Streptomyces sp., which as yellow aerial mycelia [17].

Disease that are developed by the plant pathogenic bacteria can be suppressed by actinomycetes and it also acts against some of the soil borne pathogens like Plectosporium tabacinum, Gaumannomyces graminis var. tritici, Fusarium oxysporum, Aphanidermatum sp., Colletotrichum orbiculare, Phytophthora, Rhizoctonia solani and Verticillium dahlia that affects plants. Intensity of the BLB disease and Xoo infection can be reduced by using Streptomyces sp., and AB131-1, AB131-2, and LBR02 isolates of Streptomyces sp., correspondingly [18]. Y30 and E36 strains are able to produce siderophores, and ACC deaminase activity and also exhibited antagonism activity to Ralstonia solanacearum, which is similar to Steptomyces Virginiæ [19]. Azadirachta indica, Murraya koenigii, Rauwolfia serpentine, Emblica officinalis, Terminalia arjuna, and Terminalia chebula are the selected medicinal plants, able to produce 76 endophytic actinomycetes from their leaves, stems and roots parts. Of these 76 isolates, 21 endophytic actinomycetes has the antimicrobial activity against the tested pathogen. Microbispora rosea (EAAG89) isolates showed maximum activity against Pseudomonas syringae (23 mm) and Staphylococcus aureus (24 mm) and a broad spectrum of antimicrobial activity was found in Streptomyces sp., not even one isolates has antimicrobial activity against Aspergillus niger, Fusarium oxysporum, Escherichia coli and Pseudomonas aeruginosa [9]. 34 actino bacterial were isolated from the medicinal herbs like Plumbago zeylanica L. Adhatoda vasica Nees, Catharanthus roseus L. G. Don., Asparagus racemosus Willd., Abrus precatorius L. and Aloe vera L. Burm. f. Antimicrobial activity were showed by thirteen out of 34 actino bacterial isolates. Leaf of Asparagus racemosus Willd. (Asp-1), Plumbago zeylanica L. (Ck-L1) and Asp-R3 isolates from the root of Asparagus racemosus Willd., from these three isolates were extracted which has activity against Gram-positive bacteria. An antibacterial activity against gram positive and gram negative bacteria was found in 10 isolates such as Av-R1, Av-R2, Av-R4, Av-R5, Av-R8 (from root of Aloe vera L. Burm. f.), Asp-R1, Asp-R2 (root of Asparagus racemosus Willd.), Ck-L2 (leaf of Aloe vera L. Burm. f.), Gj-R3 (the root of Abrus precatorius L.), and Ck-S3 (stem of Plumbago zeylanica L.) [20].

Totally 9 isolates were isolated from the soil sample of Thirthahalli, all of these isolates as activity against selected bacteria but specifically, SRDP-07 was selected because of their prominent inhibitory activity. The ethyl acetate extract of SRDP-07 was found to have activity against Escherichia coli, Shigella flexneri, Klebsiella pneumoniae, Bacillus cereus, Vibrion cholerae and Staphylococcus aureus [21]. Out of 96 actinomycetes, 20 actinomycetes exhibited antifungal activity against Candida albicans (16mm) and Saccharomyces cerevisiae (14 mm), which were
detected from the 15 marine samples of Bay of Bengal [15]. 131 strains of endophytic actinomycetes were isolated from surface-sterilized roots of banana, among the isolates 99 were Streptomyces, 2 were Streptosporangium, 28 were Streptoverticillium and 2 were not determined. Healthy banana plantlets are made resistance against Fusarium wilt by using the strains of Streptomyces grise rubiginosus [22]. Numerous biological functions like plant growth promoters and anti microbes were reported in actinomycetes isolated from phylosphere, soil and rhizosphere [14].

Endophytic actinobacteria are examined to be a substitute to combat multidrug-resistant human pathogens as they serve as a latent source of novel antimicrobial compounds [23]. Hema SN et al. reported that endophytic actinomycetes are isolated from several host plants like wheat, maize, banana, and tomato provide more significant antimicrobial activity pathogens [24]. Actinomycetes have a wide range of antimicrobial compound which acts as an antagonist on nitrogen-fixing mutualistic bacterial growth and performance. Isolates from roots explore higher antagonistic activity against phytopathogen than the stems and leaves [7]. Until now mostly isolated endophytic Streptomyces are excellent anti fungal producers but less report on their lethality to bacteria endophytes are interdependent to their host [25]. Sitti I et al. reported that through induced systematic resistance, the actinomycetes involves in disease repression. Plant diseases can be reduced by actinomycetes using distinct mechanisms [26]. Endophytic actinomycetes from the medicinal plants which have in vitro plant growth promoting rhizobacter (PGPR) traits has to be identified and characterized in order to understand actinomycetes behaviour located in the plant endosporo so that the potential strains can be used to enhance the growth of agricultural plants that are damaged by fungal phytopathogens [27]. 400 actinomycetes were extracted from the soil sample taken from the Western Ghats, out of these isolates 161 has antimicrobial activity against several bacteria and fungi [28].

Ensieh S et al. reported that from eight soil sediment samples 34 actinobacterial (act) isolates were isolated, from these act-1, 3, 6 and 8 exhibited antibacterial activity against Methicillin-resistant Staphylococcus aureus (MRSA), among them act-1 and act 3 are the excellent anti-MRSA metabolite producer. Researchers focus on many screening and identifying procedure in order to isolate the novel antibiotics from actinomycetes which have antimicrobial activity that is opposed to Gram positive and Gram negative bacteria which creates a major problem in the nosocomial environment [29]. BPSAC70 (Streptomyces sp.) is an endophytic strain that is isolated from the tissues of roots shows higher inhibition rate of about 98.3% against Fusarium culmorum than the other fungal pathogens tested [30]. Strong antifungal activity showed by Streptomyces aureofaciens K20 against plant fungal pathogen and also it involves in production of mycolytic enzymes [31]. Nocardiosis sp., GRG1 (KT235640), an endophytic actinomycetes isolated from brown algae exhibited antibacterial activity against urinary tract infections pathogens [32]. 50 endophytic actinomycetes were isolated from the surface sterilized root, stem and leaf tissue of [33], in which all of the isolates showed activity against microbes [33]. Actinobacteria 10CM9 strain is a Streptomyces, that was isolated from the sediment of Erçek lake produced an antimicrobial agent chloramphenicol. Using micro dilution method an antimicrobial activity was carried out for 10CM9 against Staphylococcus aureus, Escherichia coli 0157: H7 and Enterococcus faecium with MIC value of 1 μg/ml, 0.5 μg/ml and 0.5 μg/ml respectively [12]. Micrococcus yunnanensis strain rsk5 obtained from the medicinal plant Catharanthus roseus, which broad spectrum of antibacterial activity against Staphylococcus aureus and other pathogens like Proteus vulgaris, Bacillus megaterium, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis and Bacillus subtilis [23].

**Larvicidal Activity**
Streptomyces sp., was said to have Larvicidal activity, isolated from the medicinal plants like Balotta undulate, Artemisia herba-alba, Echinops spinosus and Mentha longifoli [16]. Insecticidal activity was found in SRDP-07 isolate (Ethyl acetate extract) at 2 mg/ml conc., where the mortality of larva was 100% in their II instar larvae stage [21]. Avermectins, macrotetrolide, and tetranection are the bioactive compounds from actinomycetes in which they can be used as mosquito larvicides and also it involves in controlling the vector mosquitoes in order to anticipate the mosquito-borne diseases [13]. From the soil sample of Western Ghats, 27 active actinomycetes were isolated, in which 12 has effective larvicidal activity. Of these 27, 5 isolates (S12-4, S12-17, S12-8, FMS-20 and DSP-1) as larvicidal activity against Culex quinquefasciatus and low larvicidal activity was found against Aedes aegypti 100% larval mortality was founded in SA12-4 against Anopheles stephensi [28].

**Antimalarial Activity**
Munumbics type A, B, C and D are the antibiotics produced by the Streptomyces sp., isolated from the stem of host plant Kennedia nigriscans. Of these four antibiotics, Munumbics type D has remarkably effective against malaria-causing parasite Plasmodium falciparum [16]. In Artemisia annua plant, artemisinin an antimalarial compound production can be enhanced with the help of Pseudomoncardia sp., Strain YIM 63111 which is a significant endophytic actinobacterium [34].
Antidiabetic Activity
Endophytic actinobacteria (65) was isolated from medicinal plants *Tinospora crispa*, *Caesalpinia sappans* and *Curcuma aeruginosa* out of which 12 isolates exhibited antidiabetic property. When compared to the plant *Tinospora crispa*, isolated endophytic actinomycete BWA65 showed more antidiabetic activity [16].

Enzyme Production
*Streptomyces* sp., plays a vital role in the production of various enzymes among the different genera of actinomycetes. Aquatic actinomycetes differ in their molecular, biochemical and physiological characteristics and metabolic pathways from the terrestrially isolated actinomycetes. In comparison to terrestrial actinomycetes, saline actinomycetes generate a wide variety of biologically active enzymes [4]. In industries, enzyme plays an important role because it increases the rate of reaction many times than the actual chemical reaction. Hydrolytic enzymes like xylanase, pectinase, cellulose and it also as the ability to produce chitinase, are harvested using actinobacter *Micromonospora* L5, extracted from the *Casuarina equisetifolia* nitrogen-fixing nodules. As *Micromonospora* L5 as the ability to produce these enzymes, it plays important role in various fields like biofuel production and solves the issues relating to organic domestic wastes developing [35]. Enzyme production was characterized for the isolated 23 endophytic actinomycetes immigrate in tomato plant and these actinomycetes were checked to analyze the Substrate hydrolysis and enzymatic index (EI) for the enzymes like esterase, caseinase, catalase, gelatinase and catalase, amyrase, pectinase, cellulose and lipase. Thus this study confirms that hydrolysis of lipases as highest EI whereas cellulose hydrolysis showed the lowest value [36]. Alkali-thermotolerant strain *Streptomyces gulbargensis* DAS 131 is a newly isolated strain that is used for the production of Extracellular amylase as it shows highest amylase production. Thus, this enzyme as a wide range of application in various industries like textile, distilling, food and brewing [4]. Cellulase production is mostly carried out by *Streptomyces* sp., such as *Streptomyces rutgersensis*, *Streptomyces drozdowiczii*, *Streptomyces lividans* and *Streptomyces longispororuber* and are used in textiles, biorefineries, wine, brewing and animal feedstocks industries and also it is used pretreatment of wastes produced from industries [4]. Studies have been reported that Actinomadura sp and *Thermophilic actinomadura* sp., which are isolated from compost in Thailand and poultry compost respectively, showed xylanase and extracellular thermostable xylanase production [1,37]. Keratinases that are produced by actinomycetes are considered to be a most important than the other industrially produced enzyme in which this can be used as an alternative for converting unused chicken feather to useful value added products and keratin waste recycling process. L-asparaginase is mostly produced by *Streptomyces* sp., like *Streptomyces albidoflavus*, *Streptomyces karnatakensis* and *Streptomyces griseus* that are extracted from soil sample [4]. Pectinase, amylase, lipase, protease, cellulose and xylanase are industrially significant enzymes that are produced by the actinomycetes [38]. The enzyme which degrades the imperative compounds are produced by the mycelium of actinobacterial that uses this structure for attachment and penetration thereby obtaining nutritional complements [1]. By hydrolysing the b-1, 4-glycosidic linkages of cellulose, it can be degraded by cellulase that is produced by various organisms such as fungi, yeast, actinomycetes and aerobic bacteria. Several studies reported that *Streptomyces* sp., isolated from *Corinaga* mangrove forest has the ability to produce cellulose. Behera BC et al. reported that actinomycetes produce nine cellulose degrading and 58 cellulases from mangrove soil [10].

Degradation Activity
Quorum quenching enzyme (QQE) activity was found in 127 (36.9%) and 68 (51.5%) out of 344 rhizospheric and 132 endophytic isolates respectively. QQ is an enzyme, that helps in degradation of N-acyl-L-homoserine lactone. Through 16S rRNA gene sequence similarity these isolates were founded as *Streptomyces*. Among these isolates actinomycetes, LPC029 showed higher degradation activity of about around 151.30 ± 3.1 nmole/h/ml [39]. Recent studies suggested that endophytic actinobacteria can be used in bioremediation as like endophytes which promote pesticides cleaning through bioemulsifiers using degradation pathway with help of produced metabolites [1]. Fungal mycelia are degraded by the strain K20 by surrounding them and further, it results in disruption of mycelia by making a hole in it [31].

Bioactive Compound Production
Unexpressed bioactive metabolites expression can be enhanced by directing the actinomycetes to chemical and molecular elicitation [40]. Endophytic actinomycetes in affiliation with medicinal plants may contribute in metabolic pathways and attain genetic information in order to yield bioactive compounds [23]. By initiating increased plant responses against disease or directly acting on fungal cell wall thus actinomycetes said to be a favorable biocontrol agent [41]. For Soilborne pathogens, prevalent soil actinomycetes have been used as a biocontrol agent [8]. The use of endophytic *Streptomyces* strains as biological control agents holds a hopeful place
for the agricultural and environmental industries [42]. Several bioactive substances are produced by actinomycetes such as plant growth factors, vitamins, enzymes, alkaloids and enzyme inhibitors which as higher commercial value [26]. Actinoplanes, ActinosynnemaKitasatospora, Actinomadura, Actinosynnema, Dactylosporangium, Gordona, Intrasporangium, Micromonospora, Microbisporea, Nocardia, Rhodococcus, Streptomyces, Streptosporangium, Streptothalloteichus and Thermomonospora are some of the important genera of actinomycetes among them Actinoplanes and Streptomyces has the capacity to produce broad spectrum antibiotics [38]. Genus Streptomyces produces metabolites like nucleoside antibiotics, benzoquinones and aminoglycosides that are useful in agriculture [30]. Mahayarundin et al. reported that microbes exhibit about 22,500 bioactive compounds in which 17%, 38% and 45% were derived from unicellular bacteria, fungi, and actinomycetes respectively [14]. Antherquinone is the newly identified bioactive compound from soil actinomycetes that has dominant anti-inflammatory, antitumor, antiviral and antimicrobial activities. The previous study reported that bioactive compounds that are produced by actinomycetes, said to have anti-tumorogenic and wound healing properties [38]. From root, stem and leaf parts of sugarcane, 102 endophytic actinomycetes were detached, it was characterized and determined for the production of bioactive compounds like siderophore, biosolubilizing activities of insoluble phosphate, leonardite and indole-3-acetic acid (IAA). Out of 102 extracts, 93, 52, 1 and 46 isolate showed production of bioactive compounds respectively [43]. Actinobacteria produced bioactive compounds are more susceptible to the fungi like Bipolaris sorokiniana 98022, Rhizoctonia sp., and Rhizoctonia sp., and also susceptible to phytopathogenic bacterium like Clavibacter michiganensis subsp. michiganensis (V) and Clavibacter michiganensis subsp. michiganensis (L) [17].

Disease Prevention
Efficient antagonistic activity was found specifically in leaf (Ck-L2) and stem (Ck-S3) portion of Plumbago zeylanica L. against the human pathogen [20]. Up to 53.33% of damping off disease that is generated by Rhizoctonia solani can be inhibited by Streptomyces sp., in tomato seeds [18]. In rice plant, Xanthomonas oryzae pv. oryzae (Xoo) causes leaf blight diseases that can be inhibited with the help of endophytic actinomycetes and these actinomycetes has the ability to produce chitinase, HCN, siderophores, and phosphate can also be solubilised [14]. Masafumi Shimizu et al. first reported that to control foliar disease, biocontrol strain from endophytic actinomycete can be used [8]. Root rot, wilt disease and foliar can be treated with the Mycostop and Actinovate that are produced by Streptomyces [30].

Medicinal Uses
The novel therapeutic agent can be identified for the estimation of antibiotic producing a potential of unknown actinomycetes from the underexplored ecological community so that it can be used for the treatment of disease and also acts against drug-resistant microorganisms [44]. Streptomyces genera occupy 50% of actinobacteria, in which 75% of commercially useful antibiotics produced by this genus [12]. Due to the production of wide range of antibiotics like mumumbicins A to D, alnumycin, coronamycins and novel antitumor agents such as lupinacidins A and B, antherquinones by actinomycetes and so it serves as a most important and attractive in the research field [23].

Plant Growth Promoter
Frankia is the first identified endophytic actinomycetes which fix nitrogen to form actinorrhizae. Root zone is the primary site of entry for endophyte to reach the plant tissue and flowers, cotyledons stems are the aerial portion can also be used as entry site [7]. In addition to plant growth promotion endophytic actinobacteria are more beneficial to host plant by involving in biocontrol of phytopathogens and also helps their host to tolerate biotic and abiotic stresses [28]. Except for frankia, all other endophytic actinomycetes fix nitrogen to benefits plant without forming any nodules once they reach the interior part of the root [45]. Streptomyces sp., plays a vital role in nutrient cycling and also it has a remarkable impact on plant health. 31extracellular hydrolytic enzymes and antimicrobial compounds produced by Streptomyces makes them act as an antagonist against phytopathogens [27]. Seed germination and seedling growth of Phaseolus vulgaris L can be enhanced with the help of indole acetic acid that is produced by Centella asiatica isolated Streptomyces [46]. Plant growth-promoting organisms, biocontrol tools of plant diseases and source of agro-active compounds are the potential activities of actinomycetes thereby it protects plants against diseases [31]. Several studies have reported that plant pathogens, nematode and insects can be controlled by endophytic microorganism and under adverse condition, it accelerates seedling emergence and promote plant organization [5].
CONCLUSION

Endophytic actinomycetes serve as a promising source for natural products and also it helps in promoting the plant growth in field condition, so it is highly considered as an important area for future research. They also have the ability to produce several enzymes like lipase, protease, cellulose, amylase, pectinase and xylanase. Thus it plays a vital role in several fields like pharmaceuticals, agriculture, bio refineries, food, textile, pulp and paper to get rid of various challengeable problems. Antibiotic-producing actinomycetes are mostly derived from the medicinal plants. This review mainly focuses on the importance of endophytic actinomycetes as well as their application in several fields so it is considered as the innovative biotechnological tools.

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