

Review Article

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Isolation, Characterization and Antimicrobial Potential of Endophytic Actinomycetes

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ABSTRACT

Endophytes are organisms that are intimately associated with various parts of plants. Endophytic actinomycetes represent actinomycetes of various genera that are associated with various parts of the plants. The beneficial interactions of endophytic actinomycetes with plants are being considered as an important area of research. These endophytic actinomycetes are an attractive source of novel bioactive compounds having pharmaceutical and agricultural importance. Endophytic actinomycetes are associated with different parts such as roots, stem and leaves. Their isolation requires the selection of plant parts and their surface sterilization so as to remove adhering dirt and organisms. A range of surface sterilants such as ethanol, sodium hypochlorite, sodium thiosulfate and sodium carbonate are used for this purpose. Media such as potato dextrose agar, yeast extract-malt extract agar, actinomycetes isolation agar and starch casein agar are used for isolation of actinomycetes. Identification of isolated endophytic actinomycetes is usually based on cultural, microscopic, staining, biochemical and genetic studies. The antimicrobial potential of endophytic actinomycetes is tested by primary as well as secondary screening procedures. Methods such as cross streak and dual inoculation method are used in primary screening. Disk diffusion and well diffusion techniques are commonly employed for secondary screening. Crude extracts and purified compounds from endophytic actinomycetes are shown to exhibit a range of bioactivities such as antimicrobial, antiviral, antioxidant and anticancer activity. Among these, antimicrobial potential of endophytic actinomycetes appears to be an important one. Several researchers have worked on isolation and antimicrobial activity of endophytic actinomycetes isolated from different parts of various plants. It is found that endophytic actinomycetes exhibit inhibitory activity against Gram positive bacteria, Gram negative bacteria, yeasts and filamentous fungi. The isolates and their metabolites are shown to exert detrimental effect against drug resistant pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA) and penicillin resistant *S. aureus*. Many isolates are effective against phytopathogenic fungi. These endophytic actinomycetes appear promising resources of bioactive agents and hence, they can be exploited for protection of crops and production of therapeutic agents active against disease causing agents.

Keywords

Plants,
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Introduction

Throughout history, a continuous battle exists between human beings and pathogenic microbes. Infectious diseases caused by bacteria, fungi, viruses and protozoa have been the major cause of morbidity and mortality. Infectious diseases remain the leading cause of death worldwide. Approximately 25% of annual deaths worldwide are believed to be due to these infectious diseases. Antibiotics form an important group of compounds possessing the property of inhibiting and killing other microorganisms. Antibiotics have been produced by bacteria, fungi and actinomycetes. The antibiotic era began with the accidental discovery of Penicillin (from *Penicillium notatum*) by Alexander Fleming. Later, Selman Waksman discovered Streptomycin from *Streptomyces griseus*. The discovery of antibiotics is considered as a turning point in medicine and their use saved countless lives. However, the use of antibiotics has been accompanied by the rapid development of antibiotic resistance in microbes.

Methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus* spp., multidrug-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, multidrug-resistant TB are among the drug-resistant pathogens of today. Moreover, the ability of pathogens to acquire and transmit resistance has made the situation even worse and the therapy more complicated. These drug resistant pathogens impose a substantial burden to human population. Therefore, search for new antimicrobials is a continuous process to keep pace with continually evolving pathogens (Castillo *et al.*, 2002; Tenover, 2006; Dzidic *et al.*, 2008; Davies and Davies, 2010, Joseph *et al.*, 2012; Kekuda *et al.*, 2013; Kekuda *et al.*, 2015).

The microbial metabolites form important sources of life saving environments such as bacterial and fungal infections, Cancer, transplant rejection and high cholesterol. There are over 23,000 known microbial secondary metabolites. A major portion of these metabolites are produced by actinobacteria and fungi while bacteria account for remaining portion. Among microbes producing bioactive secondary metabolites, actinomycetes produced highest number of bioactive metabolites (Saadoun and Al-Momani, 2000; Berdy, 2005; Zhao *et al.*, 2006; Zin *et al.*, 2007; Kekuda, 2014). Many microbes such as bacteria, actinomycetes and fungi have been found to be associated with the internal tissues of plant and are known as endophytes (within plants). The term endophyte was coined by De Bary (1866), which involves the existence of microorganisms inside the plant tissues without having negative effects on host plant. Endophytes occupy cortical tissues of roots and confer defense against invading pathogens while the cortex confers protection to endophytes from the harsh environment of the rhizosphere. In general endophytic bacteria occur at lower population densities than rhizospheric bacteria. Almost all the plants have been found to be associated with one or more endophytes. These endophytes have been isolated from both monocots and dicots, ranging from woody tree species to herbaceous crop plants. Endophytes that colonize inner tissues of plants usually obtain nutrition as well as protection from host plants and in return, they provide enhanced fitness to the host by producing bioactive metabolites which protect the plant. These microbes are shown to produce metabolites such as growth promoters, insect and pest repellents, antimicrobials and protectors in stress conditions. Endophytes have the potential to produce unique secondary metabolites, which can be

exploited in pharmaceutical, agricultural and other industries. Thus, there is a growing interest of researchers in bioprospecting of endophytic microbial communities inhabiting the plants from various ecosystems (Petrini *et al.*, 1992; Rosenblueth and Martínez-Romero, 2006; Zhang *et al.*, 2006; Ryan *et al.*, 2008; Kafur and Khan, 2011; Selim *et al.*, 2012; Taechowisan *et al.*, 2013; Golinska *et al.*, 2015; Passari *et al.*, 2015; Roy and Banerjee, 2015). In this review, methods available for isolation and characterization and evaluation of antimicrobial activity of endophytic actinomycetes strains have been discussed and also literature survey on antimicrobial activity of endophytic actinomycetes recovered from various plants is presented.

Actinomycetes

Actinomycetes (order Actinomycetales) represents high G+C containing, filamentous, Gram positive bacteria that are widespread in nature and distributed in a variety of ecological habitats such as soil, fresh water, marine environment, plants (as endophytes) and others. They are involved in the decomposition of organic matter in soil, including lignin and other recalcitrant polymers, and can degrade agricultural and urban wastes. The actinomycetes and their bioactive metabolites have shown to possess antimicrobial, cytotoxic, plant growth promoting, antiviral, antioxidant, insecticidal, antiprotozoal, anthelmintic, enzyme inhibitory, plant growth promoting and herbicidal agents. They are known to play key role in bioremediation of dyes, petroleum products and pesticides and biosorption of heavy metals. The bioactive metabolites from actinomycetes are also active against antibiotic resistant bacteria and plant pathogenic fungi. Among various genera, the genus *Streptomyces* seems to be

abundant and the members of this genus have produced a wide range of metabolites having several bioactivities (Cao *et al.*, 2004; Zin *et al.*, 2007; Bunyoo *et al.*, 2009; Gonzalez-Franco *et al.*, 2009; Kekuda *et al.*, 2010; Qin *et al.*, 2011; Kumar and Krishnan, 2012; Kekuda *et al.*, 2013; Taechowisan *et al.*, 2013).

Endophytic Actinomycetes and their Potential Roles

Actinomycetes of various genera are found associated with plants. The beneficial interactions of endophytic actinomycetes with plants are being considered as an important area of research. These endophytic actinobacteria are attractive source of novel bioactive compounds and therefore, many research groups are involved in the study of their bioactivities and industrial applications. It is experimentally proven that the endophytic actinomycetes display a wide array of bioactivities. Crude solvent extracts and purified compounds from endophytic actinomycetes are shown to exhibit bioactivities such as antimicrobial, antiviral, antioxidant, antitumor, larvicidal, antimalarial, antidiabetic and plant growth promotory activities (Table 1). The studies have also shown that the endophytic actinomycetes exhibit inhibitory activity against drug resistant pathogens (Castillo *et al.*, 2002; Qin *et al.*, 2011; Joseph *et al.*, 2012; Huang *et al.*, 2012; Golinska *et al.*, 2015; Savi *et al.*, 2015; Passari *et al.*, 2015; Singh and Dubey, 2015).

Location of Endophytic Actinomycetes

Endophytic actinomycetes are shown to associate with their host at a very early stage of the plant development (Minamiyama *et al.*, 2003; Hasegawa *et al.*, 2006). Endophytic actinomycetes have been

isolated from various parts of the plants. The woody plants conferred far greater diversity of actinomycetes in comparison to herbaceous plants. In most cases, the maximum endophytic actinobacteria have been recovered from roots followed by stems and leaves. The high rate of occurrence of actinomycetes in roots is 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 the symbiotic association with them by entering the plant tissues. The greater diversity of endophytes is probable to occur in the tropical and temperate regions. The genus *Streptomyces* seems to be relatively abundant genus (Strobel and Daisy, 2003; Qin *et al.*, 2009; Nimnoi *et al.*, 2010; Huang *et al.*, 2012; Gangwar *et al.*, 2014; Golinska *et al.*, 2015; Rao *et al.*, 2015; Passari *et al.*, 2015).

Isolation of Endophytic Actinomycetes

Searching specific endophytes capable of producing antimicrobials is not necessarily a random process. The first objective is to select one or more plants that may harbor endophyte. This selection process is usually done on the basis of the environment and the age of a plant. Besides, the ethnomedicinal uses of the plants also form a basis for selection of plants (Castillo *et al.*, 2002). Several methods are employed for isolation of endophytic actinobacteria from different plants. The recovery of diverse actinobacteria depends mainly on the methods of isolation. The most commonly employed method for their detection and enumeration involves isolation from surface-sterilized plant tissue. Various factors such as host plant species, age and type of tissue, geographical and habitat distribution, sampling season, surface sterilants, selective media and culture conditions influence isolation of endophytic actinobacteria

(Castillo *et al.*, 2002; Takahashi and Omura, 2003; Kafur and Khan, 2011; Golinska *et al.*, 2015).

Surface Sterilization of Plant Parts

The key as well as obligatory step that is to be followed after selection of plants and their parts such as leaves, stem and roots for isolation of endophytic actinomycetes is to eliminate the microorganisms that are present on the surface. This is accomplished by surface sterilization of plant parts. These explants are washed in running tap water to remove adhered microbes, soil debris or dust particles, followed by surface sterilization using one or more different surface sterilants. The most frequently used surface sterilants are ethanol and a strong oxidant or general disinfectant like household bleach (NaOCl) with 2–5 % (w/v) available chlorine (for 2-4 min). Besides, combination of 5% sodium chlorate (NaClO₃), 2.5 % sodium thiosulfate (Na₂S₂O₃), 75% ethanol and 10% sodium bicarbonate (NaHCO₃) were also used as sterilizing agents. Ethanol concentrations of 70% and 95% have also been used. Hydrogen peroxide has been used for surface sterilization. Surfactants (wetting agents) such as Tween 20, Tween 80 and Triton X-100 can also be added so as to enhance the effectiveness of surface sterilization. Addition of sodium thiosulfate is often recommended as thiosulfate can suppress the detrimental effect of residual NaOCl on surface of explant, which may kill endophytes or does not allow endophytes to form colonies. Soaking of plant tissues in 10% NaHCO₃ solution inhibit the endophytic fungi, which could overgrow and mask the actinobacteria. The strength of sterilizing chemicals depends on permeability of the sample. Otherwise, the internal tissues will be sterilized. Finally, the explants are rinsed with sterile distilled water, divided into small pieces and

inoculated on appropriate agar medium. Alternatively, the surface sterilized plant tissues can be macerated and thoroughly homogenized with phosphate buffer or other suitable liquid medium. This suspension is serially diluted and plated on respective media. The efficacy of the surface sterilization, resulting from lack of microbial growth, can be authenticated by inoculating the last washing water into the same media plates (Cao *et al.*, 2004; Zin *et al.*, 2007; Ghadin *et al.*, 2008; Qin *et al.*, 2009; Qin *et al.*, 2011; Passari *et al.*, 2015; Golinska *et al.*, 2015).

Media used for Isolation of Endophytic Actinomycetes

Several growth media are used by various researchers for the isolation of actinomycetes from plant samples. Media such as Starch casein agar, Starch casein nitrate agar, Humic acid-Vitamin agar, Yeast extract-Malt extract agar, Actinomycetes isolation agar, Potato dextrose agar, Glycerol asparagine salts agar and Tap water-Yeast extract agar are frequently used for isolation of endophytic actinomycetes. The growth media are usually amended with antibiotics such as Nystatin, Nalidixic acid and Cycloheximide which inhibit the growth of fungi and other bacteria (Ghadin *et al.*, 2008; Qin *et al.*, 2011; Golinska *et al.*, 2015; Passari *et al.*, 2015).

It is shown that medium containing low nutrients (for e.g. tap water yeast extract agar) seems to be effective for isolation of endophytic actinomycetes, due to the fact that medium with high nutrient concentration allow the growth of fast growing bacteria which may overgrow slow growing actinomycetes (Qin *et al.*, 2011). Table 2 depicts growth media used by various researchers for isolation of

endophytic actinomycetes from different plants.

Identification of Endophytic Actinomycetes

At present, the identification of actinomycetes is based on polyphasic approach comprising morphological, physiological and molecular studies. Based on these features, each taxon should be described and differentiated from related taxa. Distinguishing phenotypic differences are required for the description of a new species. Characteristics of colonies, the presence of aerial mycelia and substrate mycelia, spore mass color, distinctive reverse colony color and production of diffusible pigment, and sporophore and spore chain morphology are carried out. Electron microscopy is very useful in study in the morphology of hyphae and spores. Staining characteristics and the presence of various enzymes are detected by biochemical tests. Requirement of pH, temperature and utilization of various carbohydrates and amino acids are also screened. Determination of cell wall types and characteristic sugars are also noted. The sequencing of highly conserved macromolecules, notably 16S rRNA genes, has provided valuable data for constructing phylogenies at and above the genus level.

The DNA:DNA relatedness, molecular fingerprinting and phenotypic techniques are methods of choice for delineating taxa at and below the rank of species (Shirling and Gottlieb, 1966; Wayne *et al.* 1987; Rossello-Mora and Amann 2001; Ezra *et al.*, 2004; Zin *et al.*, 2007; Qin *et al.*, 2009; Goodfellow *et al.* 2012; Golinska *et al.*, 2015; Passari *et al.*, 2015). Table 3 shows various parameters used by researchers for identification of isolated actinomycetes.

Methods used to Evaluate Antimicrobial activity of Endophytic Actinomycetes

Several protocols have been used to evaluate antimicrobial potential of endophytic actinomycetes. Methods such as cross streak technique and dual inoculation are used for preliminarily screening for antimicrobial potential of actinomycetes (Gayathri and Muralikrishnan, 2013; Kandpal *et al.*, 2013; Phuakjaiphaeo and Kunasakdakul, 2015; Passari *et al.*, 2015; Rao *et al.*, 2015).

Methods such as disk diffusion and agar well diffusion are performed to investigate antimicrobial potential of crude solvent extracts and purified compounds (Castillo *et al.*, 2002; El-Shatoury *et al.*, 2006; Li *et al.*, 2008; Khafur and Khan, 2011; Singh and Padmavathy, 2015; Saini *et al.*, 2016). Some authors have employed micro-broth dilution technique (Castillo *et al.*, 2002; Mai *et al.*, 2013). Bioautographic method has been used to evaluate inhibitory efficacy of separated components (Savi *et al.*, 2015).

Antimicrobial activity of endophytic actinomycetes

Several species of endophytic actinomycetes exhibit a range of bioactivities which can be exploited commercially. Among bioactivities shown by these endophytes, antimicrobial efficacy seems to be the best one. Crude extracts and purified compounds from endophytic strains of actinomycetes have shown to inhibit a wide range of pathogenic microorganisms of plants and animals including drug resistant pathogens. In a study, 43.4% of isolates of endophytic actinomycetes recovered from leaves and roots of maize showed antimicrobial activity against one or more tested bacteria and yeast (de Araújo *et al.*, 2000). 55% of endophytic actinomycetes recovered from leaves of *Paeonia lactiflora* and *Trifolium repens* inhibited the growth of *Rhizoctonia solani*, a

significant pathogen of plants (Gu *et al.*, 2006).

The ethyl acetate extract of *Streptomyces* strain SUK 06 recovered from *Thottea grandiflora* showed promising antimicrobial activity against several bacterial strains including MRSA and phytopathogenic fungi (Ghadin *et al.* 2008). Endophytic actinomycetes isolated from pharmaceutical plants in Yunnan province, China exhibited inhibitory activity against Gram positive and Gram negative bacteria (Li *et al.*, 2008). A strain designated as *Streptomyces* sp. Tc052 isolated from roots of *Alpinia galanga* was shown to possess strong antimicrobial activity with MIC value of 64-128µg/ml (Taechowisan *et al.*, 2008). An endophytic strain designated as CEN26, isolated from *Centella asiatica*, displayed high inhibitory activity against *Alternaria brassicicola* and resulted in morphological disorders of the pathogen, swollen hyphae and frequent septa of the treated pathogen mycelia (Phuakjaiphaeo and Kunasakdakul, 2015). Further, Table 4 reveals the antimicrobial activity of endophytic actinomycetes recovered from different plants by various researchers.

Endophytic actinomycetes against drug resistant pathogens

It has been shown that endophytic actinomycetes and their metabolites have the capability to inhibit drug resistant pathogenic microorganisms. Munumbicins A, B, C and D were isolated from *Streptomyces* NRRL 3052 (an endophyte in the stem of medicinal plant *Kennedia nigricans*) demonstrated inhibitory activity against multidrug-resistant *Mycobacterium tuberculosis* (Castillo *et al.*, 2002). The ethyl acetate extract of *Streptomyces* strain SUK 06 recovered from *Thottea grandiflora* showed promising antibacterial activity against MRSA (Ghadin *et al.* 2008). In a

study, 12 out of 65 strains of endophytic actinomycetes recovered from *Achyranthes bidentata*, *Paeonia lactiflora*, *Radix*

platycodi and *Artemisiae argyi* have shown inhibitory activity against penicillin-resistant *staphylococcus aureus* (Zhang *et al.*, 2012).

Table.1 Some bioactivities of endophytic actinomycetes

Endophyte	Bioactivity	Reference
<i>Streptomyces</i> NRRL 30562	Antimicrobial, antimalarial	Castillo <i>et al.</i> (2002)
<i>Streptomyces</i> sp. Tc052	Antioxidant	Taechowisan <i>et al.</i> (2009)
<i>Streptomyces aureofaciens</i> CMUAc130	Antifungal	Taechowisan <i>et al.</i> (2005)
<i>S. aureofaciens</i> CMUAc130	Anti-inflammatory	Taechowisan <i>et al.</i> (2007)
<i>S. aureofaciens</i> CMUAc130	Antitumor	Taechowisan <i>et al.</i> (2007)
Endophytic streptomycetes	Antitumor, antimicrobial	Li <i>et al.</i> (2008)
<i>Streptomyces</i> sp. 34 and <i>Leifsonia xyli</i> 24	Plant growth promotion	Passari <i>et al.</i> (2015)
<i>Streptomyces</i> sp.	Plant growth promotion and disease control	Sreeja and Gopal (2013)
<i>Streptomyces albovinaceus</i> and <i>S. badius</i>	Insecticidal	Tanvir <i>et al.</i> (2014)
Strain BWA65	Alpha-glucosidase inhibitor	Pujiyanto <i>et al.</i> (2012)
<i>Streptomyces</i> sp.	Antioxidant	Zhou <i>et al.</i> (2014)
<i>Micromonospora lupine</i> sp. nov.	Antitumor	Igarashi <i>et al.</i> (2007)

Table.2 Media used by various researchers to isolate endophytic actinomycetes

Medium used	Reference
Inhibitory Mold Agar-2 (IMA-2)	Phuakjaiphaeo and Kunasakdakul (2015)
Yeast extract agar and Yeast extract- Malt extract agar	Piza <i>et al.</i> (2015)
Potato dextrose agar	Mai <i>et al.</i> (2013), Savi <i>et al.</i> (2015)
Starch casein nitrate agar, Actinomycetes isolation agar, Tap water yeast extract agar, Yeast malt extract agar and Glycerol asparagine agar	Passari <i>et al.</i> (2015), Roy and Banerjee (2015)
Starch casein agar, Actinomycetes isolation agar, Tap water Yeast extract agar, Tryptone soya agar, Potato dextrose agar, CYC agar, Arginine glycerol salts agar	El-Shatoury <i>et al.</i> (2006)
Tryptic soy agar	Gangwar <i>et al.</i> (2014)
S medium	Cao <i>et al.</i> (2004)
ATCC 172 agar	Huang <i>et al.</i> (2012)
Glucose-asparagine agar	Huang <i>et al.</i> (2012)
Water agar	Ezra <i>et al.</i> (2004)
Humic acid-Vitamin agar	Taechowisan <i>et al.</i> (2008), Huang <i>et al.</i> (2012), Sunaryanto and Mahsunah (2013)
Organic medium 2 Gause	Machavariani <i>et al.</i> (2014)
Soya bean agar	Shenpagam <i>et al.</i> (2012)
Starch casein agar	Zin <i>et al.</i> (2007), Ghadin <i>et al.</i> (2008), Bunyoo <i>et al.</i> (2011), Kafur and Khan (2011), Kandpal <i>et al.</i> (2012), Vijayan <i>et al.</i> (2014), Saini <i>et al.</i> (2016)

Table.3 Methods used for characterization of endophytic actinomycetes

Host plant	Strain(s)	Characterization	Reference
<i>Monstera sp.</i>	<i>Streptomyces sp.</i> (MSU-2110)	Microscopic and 16S rDNA gene sequencing	Ezra <i>et al.</i> (2004)
<i>Paeonia lactiflora</i> and <i>Trifalium repens</i>	<i>Pseudonocardia</i> and streptomycetes	Morphological and 16S rRNA gene sequencing	Gu <i>et al.</i> (2006)
<i>Thottea grandiflora</i> , <i>Polyalthia spp.</i> And <i>Mapania sp.</i>	Streptomycetes	16S rRNA gene sequencing	Zin <i>et al.</i> (2007)
<i>Thottea grandiflora</i>	<i>Streptomyces</i> SUK 06	The morphological and 16S rRNA sequence analysis	Ghadin <i>et al.</i> (2008)
<i>Alpinia galanga</i>	<i>Streptomyces sp.</i>	Morphology and amino acid composition of whole cell extract	Taechowisan <i>et al.</i> (2008)
<i>Acacia auriculiformis</i>	<i>Streptomyces</i> , <i>Actinoallomurus</i> , <i>Amycolatopsis</i> , <i>Kribbella</i> and <i>Microbispora</i>	16S rRNA sequencing	Bunyoo <i>et al.</i> (2011)
<i>Artemisia annua</i>	<i>Pseudonocardia antimicrobica sp. nov.</i>	Cell-wall peptidoglycan, fatty acid, G+C content, 16S rRNA gene sequence, DNA–DNA relatedness,	Zhao <i>et al.</i> (2012)
<i>Curcuma domestica</i>	<i>Streptomyces</i> strain KY01	Morphological and 16S rRNA gene sequencing	Sunaryanto and Mahsunah (2013)
<i>Zingiber officinale</i>	<i>Streptomyces sp.</i> GMT-8	Morphology, chemotaxonomy and 16S rDNA sequencing	Taechowisan <i>et al.</i> (2013)
<i>Ocimum sanctum</i>	<i>Nocardiopsis</i> species	Morphological and 16S rRNA gene sequencing	Singh and Padmavathy (2015)
<i>Macleaya cordata</i>	<i>Streptomyces mobaraensis</i>	Morphological, biochemical and molecular studies	Wang <i>et al.</i> (2014)
<i>Lycopersicon esculentum</i>	<i>Streptomyces</i>	Morphological and cultural characteristics	Sreeja and Gopal (2013)
<i>Vochysia divergens</i>	<i>Microbispora sp.</i> , <i>Streptomyces sampsonii</i> , <i>Micromonospora sp.</i>	16S rRNA sequencing	Savi <i>et al.</i> (2015)
<i>Cinnamomum sp.</i>	<i>Streptomyces rochei</i>	Morphological, physiological, biochemical and 16S rRNA gene analysis	Roy and Banerjee (2015)

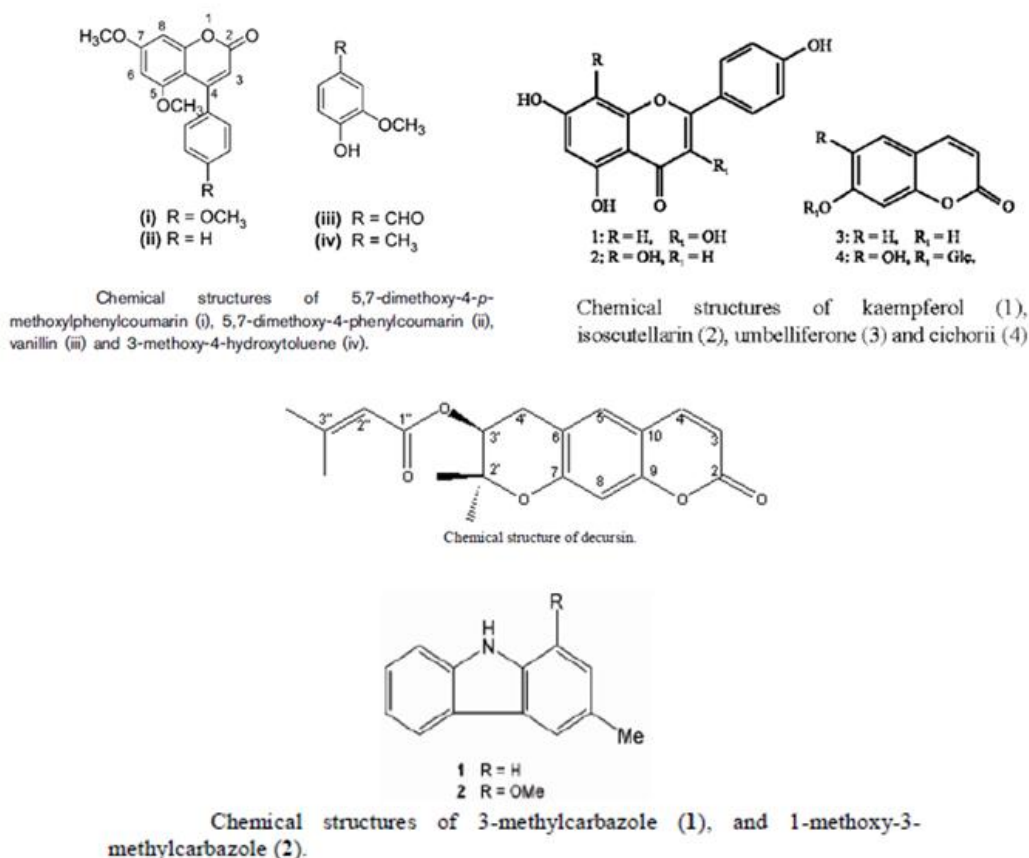
Table.4 Antimicrobial activity of endophytic actinomycetes

Host plant	Strain(s)	Activity against	Reference
<i>Thottea grandiflora</i> , <i>Polyalthia</i> spp. and <i>Mapania</i> sp.	Streptomycetes	Phytopathogenic fungi	Zin <i>et al.</i> (2007)
<i>Eucalyptus globus</i>	<i>Streptomyces</i>	Gram negative bacteria	Muthiah <i>et al.</i> (2009)
<i>Azadirachta indica</i>	<i>Streptomyces</i> and others	Bacteria and fungi	Verma <i>et al.</i> (2009)
<i>Acacia auriculiformis</i>	<i>Streptomyces</i> and others	Bacteria and fungi	Bunyao <i>et al.</i> (2011)
<i>Aloe vera</i> , <i>Mentha arvensis</i> and <i>Ocimum sanctum</i>	<i>Saccharopolyspora</i> 0-9	Phytopathogenic fungi	Gangwar <i>et al.</i> (2011)
<i>Spermacoce verticillata</i>	<i>Streptomyces</i> sp., <i>Microbispora</i> sp. and <i>Nocardia</i> sp.	Gram positive bacteria	Conti <i>et al.</i> (2012)
<i>Zingiber officinale</i> and <i>Alpinia galanga</i>	<i>Streptomyces aureofaciens</i> CMUAc130	Fungi	Taechowisan and Lumyong (2003)
<i>Lycopersicon Esculentum</i>	<i>Streptomyces</i>	<i>Ralstonia solanacearum</i>	Sreeja and Gopal (2013)
<i>Zingiber officinale</i>	<i>Streptomyces</i> sp. GMT-8	Gram positive and Gram negative bacteria	Taechowisan <i>et al.</i> (2013)
Sugarcane and banana	<i>Streptomyces</i> sp. BAR1 and others	Bacteria and fungi	Gayathri and Murulikrishnan (2013)
<i>Macleaya cordata</i>	Strain BL7	<i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i>	Wang <i>et al.</i> (2014)
<i>Glycine max</i>	Strain JDA 1 and JDA 12	Phytopathogenic fungi	Dalal and Kulkarni (2014)
Neem, eucalyptus and coffee seeds	Strain NEK5, EE9 and CE1	Phytopathogenic fungi	Vijayan <i>et al.</i> (2014)
<i>Combretum latifolium</i>	<i>Streptomyces</i> and others	Bacteria and fungi	Rao <i>et al.</i> (2015)
<i>Vochysia divergens</i>	Strain LGMB259	Bacteria, <i>Candida albicans</i> and MRSA	Savi <i>et al.</i> (2015)
<i>Centella asiatica</i>	Strain CEN26	<i>Alternaria brassicicola</i>	Phuakjaiphaeo and Kunasakdakul (2015)
<i>Ocimum sanctum</i>	<i>Nocardioopsis</i> No.5	Bacteria	Singh and Padmavathy (2015)
<i>Cinnamomum</i> sp.	<i>Streptomyces rochei</i>	Bacteria	Roy and Banerjee (2015)
<i>Syzygium cumini</i>	Isolate J-10	<i>Staphylococcus</i> and others	Saini <i>et al.</i> (2016)
<i>Miconia albicans</i> (Sw.) Triana	<i>Amycolatopsis orientalis</i>	Bacteria and <i>C.albicans</i>	Pizza <i>et al.</i> (2015)

Table.5 Inhibitory activity of purified compounds from endophytic actinomycetes

Compound	Endophytic strain	Activity against	Reference
24-demethyl-bafilomycin C ₁	<i>Streptomyces</i> sp. CS	Antimicrobial	Lu and Shen (2003)
Cedarmycins A and B	<i>Streptomyces</i> sp. TP-A0456	Antifungal	Igarashi (2004)
Celastramycins A and B	<i>Streptomyces</i> sp. MaB-QuH-8	Antimicrobial	Pullen <i>et al.</i> (2002)
Saadamycin	<i>Streptomyces</i> sp. Hedaya-48	Antifungal	El-Gendy and El-Bondkly (2010)
24-demethyl-bafilomycin A ₂	<i>Streptomyces</i> sp. CS	Antimicrobial	Lu and Shen (2004)
Alnumycin	<i>Streptomyces</i> sp.	Gram positive bacteria	Birber <i>et al.</i> (1998)
Dinactin and nonactin	<i>Streptomyces</i> sp. Is9131	Antimicrobial	Zhao <i>et al.</i> (2005)
Antimycin A ₁₈	<i>Streptomyces albidoflavus</i>	Antifungal	Yan <i>et al.</i> (2010)
Demethylnovobiocins	<i>Streptomyces</i> sp. TP-A0556	Antimicrobial	Igarashi (2004)
6-Prenylindole	<i>Streptomyces</i> sp. TP-A0595	Antifungal	Igarashi (2004)
Munumbicins A, B, C and D	<i>Streptomyces</i> NRRL 3052 (from <i>Kennedia nigricans</i>)	Gram positive bacteria and multidrug-resistant <i>Mycobacterium tuberculosis</i>	Castillo <i>et al.</i> (2002)
Kakadumycin A	<i>Streptomyces</i> sp. NRRL 30566 (from <i>Grevillea pteridifolia</i>)	Gram-positive bacteria	Castillo <i>et al.</i> (2003)
Coronamycins	<i>Streptomyces</i> sp.(MSU-2110)	Yeasts and molds	Ezra <i>et al.</i> (2004)
5,7-dimethoxy-4-pmethoxyphenylcoumarin (i), 5,7-dimethoxy-4-phenylcoumarin (ii), Vanillin (iii) and 3-methoxy-4-hydroxytoluene (iv)	<i>Streptomyces aureofaciens</i> CMUAc130 (from <i>Zingiber officinale</i>)	<i>Colletotrichum musae</i> and <i>Fusarium oxysporum</i>	Taechowisan <i>et al.</i> (2005)
Actinomycin D	<i>Streptomyces</i> sp. Tc022	<i>Colletotrichum musae</i> and <i>Candida albicans</i>	Taechowisan <i>et al.</i> (2006)
Kaempferol, Isoscutellarin, Umbelliferone, Cichoriin	<i>Streptomyces</i> sp. Tc052 (from <i>Alpinia galanga</i>)	Gram positive and Gram negative bacteria, <i>Candida albicans</i> and <i>Colletotrichum musae</i>	Taechowisan <i>et al.</i> (2008)
Decursin	<i>Streptomyces</i> sp. GMT-8 (from <i>Zingiber officinale</i>)	Gram positive and Gram negative bacteria	Taechowisan <i>et al.</i> (2013)
3-acetonylidene-7-prenylindolin-2-one (new prenylated indole derivative)	<i>Streptomyces</i> sp. neu-D50	Phytopathogenic fungi	Zhang <i>et al.</i> (2014)

Fig.1 Chemical structures of some compounds from endophytic actinomycetes



An active fraction, partially purified by TLC and HPLC, from a potent endophytic strain recovered from *Euphorbia hirta* exhibited inhibitory activity against MRSA strain (Syed *et al.*, 2015). An endophytic actinomycetes strain designated LGMB259 from *Vochysia divergens* was shown to be potent in inhibiting MRSA (Savi *et al.*, 2015).

Antimicrobial activity of purified compounds from endophytic actinomycetes

Several compounds have been isolated and characterized from endophytic actinomycetes. These compounds are generally isolated and characterized by chromatographic and spectral techniques (Castillo *et al.*, 2002; Ezra *et al.*, 2004;

Taechowisan *et al.*, 2013). Table 5 shows antimicrobial activity of purified compounds isolated from endophytic actinomycetes. Figure 1 shows the chemical structures of some compounds from endophytic actinomycetes having antimicrobial activity.

In conclusion, it is estimated that <1% of all bacteria are currently known and it indicates that millions of microbial species remain to be discovered. The natural condition of plants seems to be in a close interaction with endophytes. The plant endosphere is considered as a complex micro-ecosystem in which different niches can be colonized by different types of microorganisms representing rich sources of novel bioactive metabolites. Endophytes appear to play an important role in ecological systems by shaping plant communities and mediating

ecological interactions. Endophytes in particular actinomycetes seem promising to increase crop yields, remove contaminants, inhibit pathogens, and produce fixed nitrogen or novel substances. Endophytes are a rich and reliable source of genetic diversity and biological novelty as they produce wide array of compounds having useful activities. More research on the formulation, development of novel technologies and methodologies is needed for employing them in the agricultural, medical and pharmaceutical fields. An extensive research on characterization and identification of the diverse population of endophytic actinobacteria associated with plants also provide greater insight into the plant-endophyte interaction. There is a pressing need for search of new therapeutic drugs, particularly anti-infective compounds due to the rapid increase of resistance in major known pathogens. Therefore, screening and isolation of promising strains of microbes, particularly endophytic actinomycetes, with antimicrobial properties has increased the interest of researchers in both basic and applied fields.

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