Review
ISSN 2413-0516

Diversity and Colonization of Endophytic Actinomycetes in some Medicinal Plants: Review

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(Submitted: 19 December 2021 – Revised version received: 04 January 2022 – Accepted: 25 January 2022 – Published online: 26 June 2022)

Abstract
The diversity of culturable endophytic actinomycetes related to plants and especially medicinal plants are isolated and characterized. The increased colonization and distribution of endophytic actinomycetes in plants have been reported in many studies. Endophytic actinomycetes belong to different genera are distributed in plants species such as *Streptomyces*, which is the dominant genus, *Micromonospora, Microbispora, Nocardiopsis, Rhodococcus* and *Nocardia*. Moreover, there are many novel strains had been described belong to these genera. Endophytic actinomycetes seem to contribute with plant development and displaying beneficial traits that can be exploited in plant maintenance, substances supplements, protection and defense. Colonization of endophytic actinomycetes in plant tissues are largely influenced by plant species and the environmental factors surrounding the host plants such as soil pH, water content, rainfall, soil salinity, and temperatures. Some endophytic actinomycetes may occur in low numbers and sometimes in localized positions within the plant. Thus, this review summarizes an aspect of the diversity and colonization of endophytic actinomycetes, including their mechanism of action, importance, and isolation and identification methods.
Keywords: Endophytic, Actinobacteria, plants, *Streptomyces*, colonization

Introduction

Actinomycetes are high Guanine and Cytosine ratio (>50%) of their DNA content, Gram-positive and filamentous bacteria that form non-septate branching mycelia similar to those of fungi.[1] They had prokaryotic nucleus and their colonies are powdery consistency and stick firmly to agar surface, producing hyphae with conidia or sporangia.[2] Actinomycetes are distributed in various natural habitats, including soil, extreme environments, plants, lichens and fresh and marine water.[3] The endophytic microorganisms colonize the interior of all plant parts, viz: root, stem, or seeds, regardless of their place of origin, without causing any harmful effects on host plant.[4] Their ability to access and thrive in the host tissues make them unique by many specific interactions within the host plants.[5] There are increasing reports for the existence of new endophytic actinomycetes within various tissues of crops and medicinal plants.

In the early stages of endophytic colonization, endophytic actinomycetes are firstly observed in subsequently in the root cortex, and root hairs.[6,7] From their point of entry, endophytic actinomycetes may systemically colonize plants from roots to shoots, shoots to flowers or fruits, and/or from flowers to fruits and seeds, and they may also cause localized colonization inside/outside plant organs.[8] The diversity aspects of endophytic actinomycetes have addressed by few research groups includes thorough collections and sampling of plant specimens (herbs, shrubs, trees, and woody climbers) and subjecting the specimens to surface sterilization and isolations of endophytes.[9]

In last twenty years, many studies have focused on endophytic actinomycetes by investigating their existence into the medicinal plants' organs. Also, they have developed many methods to sterilize the plants organs surfaces and modified many culture media. These studies help understanding the colonizing and diversity of endophytic actinomycetes in medicinal plants.[10,11] Therefore, according to these studies and reviews as well, this review highlights the endophytic actinomycetes colonizing and their diversity in different medicinal plants organs from multiple habitats in many countries.

Colonization of Endophytic Actinomycetes in Plants

The word endophyte is derived from the Greek words endon (within) and phyton (plant), which means "in plant". This term is used as broad as its literal definition and spectrum of potential hosts and inhabitants, e.g., endophytic actinomycetes.[10] Many studies indicated that almost all the plants are colonized by endophytes microorganisms.[12] The diversity of endophytic microorganisms in plants has been reported from different types such as archaea, eubacteria, and fungi. Among bacteria, endophytic bacteria were isolated from different phylum mainly: Actinobacteria, Firmicutes, proteobacteria and Bacteroidetes. Distribution of endophytic actinomycetes varied in all bacterial phyla. However, Proteobacteria were most dominant phyla followed by Actinobacteria.[4]

Gohain et al.[13] (2015) investigated six selective medicinal plants, *Emblica officinalis, Terminalia chebula, T. arjuna, Murraya koenigii, Rauwolfia serpentina* and *Azadirachta indica* for isolation of Endophytic actinomycetes which gained specific niches in plants by colonizing stems, roots, petioles, leaves, fruit, buds and seeds. The ability to enter and thrive in the host plant tissues, make endophytic actinomycetes unique, and showing multidimensional interactions.[11] Furthermore, endophytic actinomycetes can play myriad crucial roles in hosted plants. Some of these roles have been discovered and linked to many enhancements of growth and defense mechanisms towards pathogens infections while others still unknown till now.

Colonizing Mechanisms in Plant Organs

The colonization of endophytic actinomycetes in different plants is multivariate depending on many factors. Thus, understanding these factors approach a better explanation of the relationships between endophytic actinomycetes and hosted plants. Multiple plant host activities are influenced by the presence of endophytic actinomycetes, which can promote plant host growth, evoke defense responses against pathogens attacks.[5] Flourishing colonization by endophytic actinomycetes is affected by different factors including the plant tissue type, the plant genotype, the microbial taxon and strain type, and biotic and abiotic environmental conditions.[14]

The communities of endophytic actinomycetes have been categorized into three groups, which are "obligate" or "facultative" and "passive". Obligate actinomycetes endophytes are depend basically on plants metabolism for survival, and whose transmission amongst plants take place by the action of various vectors or by vertical transmission.[15] Facultative actinomycetes endophytes spend definite stages of their life cycle in host plant independently. Indirectly, they are associated with hosted plants through soil environment and atmosphere.[16] Actinomycetes endophytes which are lacking the capability to colonize and infect, can enter plant via wounds and cracks on the plant. They are documented as a passive mode of endophytic colonization.[17] In hosted plants, emerging lateral roots build naturally forming a 'highway' for actinomycetes endophytes to enter by breaking through the endodermis, cortex, epidermis casparian strip (band around endodermis) and pericycle. From these cells, endophytes can further enter the phloem and xylem vessels that transport photosynthates, nutrients and water.[8]

Actinomycetes endophytes are beneficial guests to their plant hosts. They have albitites to produce chemical diversity of secondary metabolites. Much of the natural products by actinomycetes endophytes have likely evolved due to their interactions with other microorganisms in highly diverse environments.[18,19] For instance, root exudates such as proteins, amino acids, organic acids, abundance of carbohydrates and inorganic nutrients may be involved in recruiting actinomycetes endophytes from the rhizosphere.[14,20] Endophytic actinomycetes have to compete with plant cells for iron, and therefore, siderophore production is increasing availability of minerals in addition to iron chelation which is highly important for endophytic growth.[21]

Defense Mechanisms in Plant Tissues

Plants have a variety of defense mechanisms against pathogens. The response of the host plant drastically differs to the colonization of endophytic actinomycetes and pathogens. On the other hand, endophytic actinomycetes enhance the disease resistance through the mechanism of induced systematic resistance and systemic acquired resistance.[22] Pathogenic microorganisms, which include bacteria, fungi or viruses, are associated molecular patterns. These are essential structures that are conserved and necessary for pathogens survival, but plants have developed multiple families of receptor proteins to recognize them and encourage the plant immune system.[23] Pattern-recognition receptors have enhanced to recognize common pathogens compounds, such as bacterial flagellin or fungal chitin or microbial/pathogen-associated molecular patterns. Pattern recognition is translated into a first line of defense which keeps the most potential invaders under control.[24] Endophytic actinomycetes have been equipped with necessary traits that enable them to invade, colonize and translocate in the plant's interior.[25] Plants may subsequently influence the biocontrol expression of endophytic actinomycetes against the pathogen.[26]

Colonizing Organs in Plant Tissues

Diversity of endophytic actinomycetes from plant species are documented from various regions around the world. As a matter of fact, they used morphological and microscopic identification methods in addition to methods based on sequencing of highly conserved macromolecules, notably 16S rRNA genes, which has provided valuable data for constructing phylogenies above the genus level.[27] Thus, studying the diversity of endophytic actinomycetes by isolating them from inner plant organs, give a supportive hypothesis about their roles in plants. They are able to colonize both intracellularly and extracellularly the interior of plants after inoculation the soil with these microorganisms.[25,26,28] Matsumoto and Takahashi[29] (2017) revealed that specific strains belonging to various genera could be isolated from plant roots but not from soil. Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant.[4] The maximum endophytic actinomycetes have been gained from roots followed by stems and least isolates were in leaves and they have been detected in plant reproductive organs, such as flowers, fruits and seeds, but in small numbers (Table 1). Specifically, in a leaf, endophytic actinomycetes can colonize in xylem vessels, palisade mesophyll cells, upper epidermis cells as well as spaces between spongy cells of mesophyll layer.[30] Alternatively, these endophytic actinomycetes can get into phyllosphere epiphytes through natural openings (e.g., stomata, hydathodes), wounds and cracks generated by wind, insect and pathogen attacks.[31]

Endophytic Actinomycetes Genera Distribution

Even thought, there are many reports demonstrated the distribution of endophytic actinomycetes in several organs in plants mainly in roots, stems or leaves. The isolation of endophytic actinomycetes from herbaceous and woody plants is a matter to speculate, as no consensus exists between the research groups.[9]

The total number of endophytic cultured, uncultured or rare isolates in addition to their genera and families were recorded. Chen et al.[32] (2011) reported 8 endophytic actinomycetes from root of *Elaeagnus angustifolia* while 47 isolates were detected in roots of four different plants, *Phyllanthus niruri, Withania somnifera, Catharanthus roseus* and *Hemidesmus indicus*.[33] Goudjal et al.[34] (2013) identified 27 isolates from roots of *Cleome arabica, Solanum nigrum, Astragallus armatus, Aristida pungens* and *Panicum turgidum* while Rahayu et al.[35] (2019) isolated 7 endophytic actinomycetes from roots of *Zingiber officinale*.

Endophytes Actinomycetes Importance

Actinomycetes are widespread in nature and represent the largest taxonomic group within the domain Bacteria. They are

Table 1. **Number of endophytic actinomycetes isolates from different plant parts**

Country	Plant species	Organ	No. of isolates	References
India	*Brassica juncea*	Root Stem Leaf	13 4 3	Chaudhry et al., 2020[36]
Vietnam	*Cinnamomum cassia* Presl	Root Stem Leaf	29 67 15	Vu et al., 2020[37]
Thailand	*Andrographis paniculata* *Asystasia gangetica* *Berleria lupulina* *Clinacanthus nutans* *Justicia subcoriacea* *Ruellia squarrosa*	Root Stem Leaf	49 1 2	Phongsopitanuna et al., 2020[38]
China	*Thymus roseus* Schipcz	Root Stem Leaf	54 35 37	Musa et al., 2020[39]
India	*Ageratum houstonianum* *Scoparia dulcis* (L.) *Phyllanthus niruri* (L.) *Discorea bulbifera* (L.)	Root Stem Leaf Flower	7 5 2 2	Momin et al., 2019[40]
China	*Camellia sinensis*	Root Stem Leaf	18 9 19	Shan et al., 2018[41]
Indonesia	*Ficus deltoidea*	Root Stem Leaf Fruit	6 7 24 2	Janatiningrum et al., 2018[42]
China	*Dracaena cochinchinensis* Lour	Root Stem Leaf	117 113 74	Salam et al., 2017[43]
India	*Catharanthus roseus*	Root Leaf	6 5	Ranjan and Jadeja, 2017[44]
Brazil	*Vochysia divergens*	Stem Leaf	5 5	Gos et al., 2017[45]
India	*Rauvolfia serpentine* *Gymnema sylvestre* *Stevia crenata* *Bacopa monnieri* *Andrographis paniculata* *Withania somnifera*	Root Stem Leaf	27 17 24	Singh and Gaur, 2016[46]
India	*Syzygium cumini*	Root Stem Leaf	28 21 1	Saini et al., 2016[47]
India	*Azadirachta indica* A. Juss.	Root Stem Leaf	15 13 7	Kaur, 2016[48]
India	*Schima wallichii*	Root Stem Leaf Fruit	9 6 4 3	Passari et al., 2016[49]
Thailand	*Centella asiatica* (L.)	Root Stem Leaf Fruit	9 19 4 4	Phuakjaiphaeo and Kunasakdakul, 2015[50]
India	*Combretum latifolium* Blume	Root Stem Leaf	64 31 22	Rao et al., 2015[51]
India	*Emblica officinalis* Gaertn	Root Stem Leaf	18 9 9	Gangwar et al., 2015[52]

(*Continued*)

Table 1. **Number of endophytic actinomycetes isolates from different plant parts**

Country	Plant species	Organ	No. of isolates	References
India	*Aloe vera* *Mentha arvensis* *Ocimum sanctum*	Root Stem Leaf	28 7 5	Gangwar et al., 2014[53]
Algeria	*Triticum durum*	Root Leaf	16 7	Sadrati et al., 2013[54]
Australia	*Callitris preissii*	Root Stem Leaf	189 3 2	Kaewkla and Franco, 2013[55]
	Eucalyptus camaldulensis	Root Stem Leaf	9 8 2	
	Eucalyptus microcarpa	Root Stem Leaf	169 21 1	
	Pittosporum phylliraeoides	Root Stem Leaf	91 28 33	

abundant in soil and into plant tissues and have been extensively explored for their therapeutic applications.[56] Targeting a compound for a particular biological activity involves the screening of a number of strains against wide targets, the resulting positive is designated as the "lead".[9] Symbiotic actinomycetes residing as endophytes within the plant tissues have generated immense interest as potential source of novel compounds, which may find applications in medicine, agriculture, and environment such as antibacterial, antifungal, antiviral and anticancer.[56] The antimicrobial compounds produced by endophytic bacteria represent a promising alternative protection to plants against phytopathogens.[57] The genera *Streptomyces* and *Micromonospora* are the potential producers of antibiotics. Newer antibiotics are being discovered from actinomycetes and the endophytes are the better choices for antibiotics. Actinomycetes are the potent producers of enzyme inhibitors. Enzyme inhibitors are specific biochemical tools that are potential in the treatment of diseases.[9]

Cell-wall degrading enzymes are important for plants to break plant cell walls and translocate compounds to the apoplast. Genes encoding cell-wall degrading enzymes widely exist in the genomes of endophytic bacteria. For example, genes encoding plant polymer-degrading cellulases, xylanases, cellobiohydrolases, endoglucanase, cellulose-binding proteins, pectinase and chitinase, are found in endophytic actinomycetes.[58,59] Enzymes produced from actinomycetes play an important role in food, fermentation, textile and paper industries.[60]

The use of actinomycetes endophytes in agriculture has immense potential to reduce the environmental impacts caused by chemical fertilizers, especially N fertilizers.[61] Common characteristics of actinomycetes endophytes include the ability to synthesize plant hormones such as indole-3-acetic acid, solubilize phosphate, secrete siderophores, and confer plant tolerance to biotic and abiotic stresses.[62–64] Some actinomycetes endophytes carry genes necessary for biological nitrogen fixation (BNF), potentially enabling them to convert dinitrogen gas (N_2) into usable forms of nitrogen such as ammonium and nitrate within the host plant.[65]

Endophytic *Streptomyces* as a Dominant Genus

Streptomyces accounts for the major dominant genus in many studies, which is the most commonly isolated endophytic actinomycetes. They were targeted for the first time for endophytic actinomycetes isolates. *Streptomyces* isolates were about 66%, which is closely to a study for Kaur[48] (2016) who reported that 65.7% of all endophytic isolates belong to genus *Streptomyces*. Also, Salam et al.[43] (2017) found that 86.8% of all isolates from roots, stems and leaves of *Dracaena cochinchinensis* were identified as *Streptomyces* species. From Tea plants (*Camellia sinensis*), Shan et al.[41] (2018) detected that 51.1% of the isolates were identified as *Streptomyces*. Priya[33] (2012) studied four medicinal plants, *Phyllanthus niruri, Withania somnifera, Catharanthus roseus* and *Hemidesmus indicus* and genus *Streptomyces* was the dominant Endophytic actinomycetes with 56.8% while it was recorded by 37.2% in 15 plant samples, obtained from Taklamakan Desert.[66]

Rare and Alternative Genera of Endophytic Actinomycete

Even though there are many studies support the hypothesis that the genus *Streptomyces* is the most abundant genera in endophytic actinomycetes, there are a wide range of other genera were recorded that are difficult to be isolated or rare.[67] The criteria of isolation are important to find novel endophytes.[68] Furthermore, some studies showed that the diversity of uncultured endophytic actinomycetes is comparable to those of the cultured endophytic actinomycetes.[56]

Gohain et al.[13] (2015) isolated 76 endophytic actinomycetes belong to 16 genera which were *Verrucosispora, Isoptericola, Kytococcus, Streptomyces, Micromonospora, Saccharopolyspora, Kocuria, Micrococcus, Brevibacterium, Amycolatopsis, Timonella, Leifsonia, Microbacterium, Mycobacterium* and *Nocardia*. Another study by Gos et al.[45] (2017) isolated 10 isolates belong to 8 genera from stems and leaves of *Vochysia divergens*, which were *Microbispora, Actinomadura, Microbacterium,* *Aeromicrobium, Micrococcus, Streptomyces, Sphaerisporangium* and *Williamsia*. Even though, Salam et al.[43] (2017) reported 2 major genera of endophytic actinomycetes *Streptomyces* and *Nocardiopsis* in addition to ten rare endophytic actinomycetes, *Arthrobacter, Brevibacterium, Kocuria, Microbacterium, Pseudonocardia, Rhodococcus, Nocardioides, Nocardia, Nocardiopsis,* and *Tsukamurella*. Moreover, 13 different genera of endophytic actinomycetes were detected by Shan et al.[41] (2018). They included the two rare endophytic actinomycetes, *Piscicoccus* and *Mobilicoccus*. Although Janatiningrum et al.[42] (2018) observed only 1 genera was uncultured endophytic actinomycete in addition to 6 genera, *Streptomyces, Verrucosispora, Rhodococcus, Kineospora, Intrasporangium* and *Actinomadura*, that were identified from roots, stems, leaves and fruits from Ficus deltoidea. Musa et al.[39] (2020) obtained 126 endophytic actinomycete isolates belonging to 24 genera from *Thymus roseus*. The genera *Agromyces, Alloactinosynnema, Labedella, Microbacterium, Mycobacterium, Williamsia, Blastococcus, Dietzia, Micromonospora, Pseudarthrobacter* and *Solirubrobacter* belong to rare genera. Similarly, out of 23 genera were obtained by Wang et al.[66] (2021), 6 genera were identified as rare endophytic actinomycetes, *Labedella, Rathayibacter, Leucobacter, Frigoribacterium, Aeromicrobium* and *Kineococcus*.

Qin et al.[69] (2009) reported that medicinal plants of tropical rain forests were the richest source of novel endophytic actinomycetes. Evidently, endophytic actinomycetes which investigated from medicinal plants and tropical rain forests have a considerable attention of the scientific research community (Table 2). Additionally, roots are a major organ that had been studied to its novel strains.

Isolation of Endophytes Actinomycetes

Understanding the colonization of endophytic actinomycetes can be gained by studying their characteristics, and explain their interactions and applications into plant tissues. The most commonly used isolation procedures start with surface sterilization of the plant organs. Ordinary and specific modified cultures and

Table 2. **Novel endophytic actinomycete species isolated from plants**

Plant species	Isolation region	Plant organ	Family	Novel strain	References
Typha angustifolia L.	Yunnan Province, Southwest China	Root	*Streptomycetaceae*	*Streptomyces typhae sp.* nov. p1417T	Peng et al., 2021[70]
Peganum harmala L.	Xinjiang Uygur Autonomous Region of China	Root	*Pseudonocardiaceae*	*Actinokineospora pegani* sp. Nov. TRM 65233T	Lei et al., 2020[71]
Anabasis elatior (C.A.Mey.) Schischk.	Xinjiang, North-west China	Root	*Pseudonocardiaceae*	*Amycolatopsis anabasis* sp. Nov. EGI 650086T	Wang et al., 2020[72]
Excoecaria agallocha Linn	Guangxi Zhuang Autonomous Region, PR China	Stem	*Microbacteriaceae*	*Microbacterium excoecariae* sp. Nov. CBS5P-1T	Chen et al., 2020[73]
Mentha haplocalyx Briq.	Guizhou, PR China	Stem	*Nakamurellaceae*	*Nakamurella flava* sp. Nov. N5BH11T	Yan et al., 2020[74]
Phragmites australis	Taklamakan Desert in Xinjiang Uygur Autonomous Region, China	Leaf	*Nocardioidaceae*	*Aeromicrobium endophyticum* sp. Nov. 9W16Y-2T	Li et al., 2019[75]
Podochilus microphyllus Lindl.	Trat Province, Thailand.	Root	*Pseudonocardiaceae*	*Actinomycetospora endophytica* sp. Nov. A-T 8314T	Sakdapetsiri et al., 2018[76]
Triticum aestivum L.	Langfang, Hebei Province, China	Root	*Glycomycetaceae*	*Glycomyces rhizosphaerae* sp. Nov. NEAU-C8	Li et al., 2018[77]
Anabasis aphylla L.	Xinjiang, Northwest PR China	Root	*Glycomycetaceae*	*Glycomyces anabasis* sp. Nov. EGI 6500139T	Zhang et al., 2018[78]
Glycine max L.	Harbin, Heilongjiang Province, China	Root	*Streptosporangiaceae*	*Nonomuraea glycinis* sp. Nov. NEAU-BB2C19T	Li et al., 2017[79]
Capparis spinosa	Urumqi city, Xinjiang, north-west China	Fruit	*Streptomycetaceae*	*Streptomyces capparidis* sp. Nov. EGI 6500195T	Wang et al., 2017[80]
Grosourdya appendiculata (Blume) Rchb.f.	Nakhorn Ratchasima Province, Thailand.	Root	*Micromonosporaceae*	*Verrucosispora endophytica* sp. Nov. A-T 7972T	Ngaemthao et al., 2017[81]
Glycine max (L.) Merr.	Harbin, Heilongjiang Province, China	Root	*Micromonosporaceae*	*Plantactinospora soyae* sp. Nov. NEAU-gxj3T	Guo et al., 2016[82]

(*Continued*)

Table 2. **Novel endophytic actinomycete species isolated from plants**

Plant species	Isolation region	Plant organ	Family	Novel strain	References
Bruguiera gymnorhiza	Zhanjiang Mangrove Forest National Nature Reserve in Guangdong, China	Stem	*Intrasporangiaceae*	*Phycicoccus endophyticus* sp. Nov. IP6SC6T	Liu et al., 2016[83]
Bruguiera sexangular	Dongzhaigang National Nature Reserve in Hainan, China	Stem	*Propionibacteriaceae*	*Microlunatus endophyticus* sp. Nov. S3Af-1T	Tuo et al., 2016[84]
Prosopis laegivata	San Luis Potosi, Mexico	Root	*Micrococcaceae*	*Kocuria arsenatis* sp. nov. CM1E1T	Román-Ponce et al., 2016[85]
Anabasis elatior (C.A.Mey.) *Schischk.*	Urumqi, Xinjiang province, North-west China	Root	*Microbacteriaceae*	*Frigoribacterium endophyticum* sp. Nov. EGI 6500707T	Wang et al., 2015b[86]
Veratrum nigrum L.	Wuchang, Heilongjiang-province, Northern China	Root	*Micromonosporaceae*	*Plantactinospora veratri* sp. Nov. NEAU-FHS4T	Xing et al., 2015[87]
Salsola affinis C. A. Mey	Urumqi, Xinjiang province, North-west China	Root	*Micrococcaceae*	*Arthrobacter endophyticus* sp. Nov. EGI 6500322T	Wang et al., 2015a[88]
Aloe barbadensis	Pune, Maharashtra, India	Leaf	*Micrococcaceae*	*Micrococcus aloeverae* sp. Nov. AE-6T	Prakash et al., 2014[89]
Artemisia argyi	Yesanpo located in Laishui county, Hebei province, China	Root	*Glycomycetaceae*	*Glycomyces artemisiae* sp. Nov. IXS4T	Zhang et al., 2014[90]
Artemisia annua L.	Yunnan province, south-west China	Root	*Streptomycetaceae*	*Streptomyces endophyticus* sp. Nov. YIM 65594T	Li et al., 2013[91]

isolation procedures are critical steps in working with endophytic actinomycetes. Surface Sterilization of the used part must be carried out. The sterilizing agent should kill any microbe on the plant surface without affecting the host tissue and the endophytic microorganisms. Qin et al.[69] (2009) described the methods of cleaning and sterilization of plant organs. Initially, plant organs samples were air dried, washed to remove the surface soils and adherent epiphytes completely. After that, the samples were subjected to a five-step surface sterilization procedure with 5% Sodium hypochlorite for 4–10 min., 2.5% Sodium thiosulfate for 10 min., 75% ethanol for 5 minutes, sterile water and finally in 10% Sodium Bicarbonate for 10 min. After being thoroughly dried under sterile conditions, the surface- sterilized tissues were subjected to continuous drying at 100°C for 15 min. It is very important that sterility is guaranteed for all tools and steps of this procedure. Optimination of the procedures for each plant tissue, since sensitivity varies with species, age and surface properties must be considered.

The choice of the isolation culture media is crucial as it directly affects the number and type of endophytic actinomycetes that can be isolated from the plant organs. International Streptomyces protocol media were also used for isolation as well as some other commonly used media include Starch Casein Agar, Starch Casein Nitrate Agar, Tryptic Soya-Yeast Extract Agar and Glycerol Aspargine Agar.[13,33,38,40,45]

Major Identification Methods

Culture characteristics should be determined on International Streptomyces protocol (ISP) media.[80] The colors of aerial and substrate mycelia in addition to color of the soluble pigments are important for actinomycete identification. Morphological characteristics are observed by light and scanning electron microscopes and growth at different temperatures (5–60°C) and different pH values are recorded.[81] Some physiological and biochemical characteristics like utilization of different carbon and nitrogen sources by the actinomycetes are important for identification.[82] Cell chemistry was determined by detecting diagnostic sugars in whole-cell hydrolysates, amino acids in the cell wall and cell total content of fatty acids and phospholipids. The G+C content of the DNA, and 16S rRNA gene were performed to confirm the identification of the actinomycetes.[78]

Conclusion and Future Perspective

Actinomycetes endophytes are gained importance due to their ability to colonization mechanisms and distribution in all plant organs. Many studies had investigated endophytic actinomycetes which associated with different plant species and their organs such as roots, stems, leaves, fruits and flowers. They are considered as useful microorganisms due to their roles as a defense barrier against some pathogenic microorganisms. Many medicinal plant hosted many novel taxa, thus, there is a need to specific focusing on isolation of rare endophytic actinomycetes to determine their potent useful and benefit effects on plant growth and production as well as their applications in medical and industrial fields. Finally, it is clear that endophytic actinomycetes play a key role in maintaining plant health by contributing to all biotic and a biotic stress tolerance.

Conflict of Interest

The authors declare that there is no conflict of interest involved in this study.

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