

Endophytic *Streptomyces*-How they help plants

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ABSTRACT

Streptomyces sp. is genus under Actinomycetes, and is considered largest in the phylum, actinobacteria. There has been considerable research on Actinomycetes and Streptomyces owing to their ability to produce potent antibiotic metabolites of high clinical, biological and economic value. The Streptomyces sp. can be both soil dwelling or endophytic in nature and both have attributes to undertake plant growth promotion owing to their ability to produce bioactive compounds as secondary metabolite. The endophytic Streptomyces in particular assist in plant growth promotion and bio control through direct or indirect mediation. The mode may involve secretion of enzymes or compounds to directly affect the pathogen or colonization of host tissues thus evoking the mechanism of SAR and ISR supportive of the primary and secondary defense mechanism of any plant tissue system. The occurrence of endophytic Streptomyces among all host particularly in medicinal plants, their isolation and study may be highly beneficial as medicinal plants themselves have attributes that assist in biocontrol and plant growth promotion.

1. Introduction

Streptomyces sp. belonging to Streptomyces, is a gram positive organism and is a large genus. There are both soil dwelling as well as endophytic *Streptomyces* present in nature. There is substantial evidence of the therapeutic usage of the antibiotics produced from the members of the genera and phylum actinobacteria in general. Moreover, there have been findings wherein the bioactive compounds and secondary metabolites produced by the members of *Streptomyces* sp. have shown ability like IAA, siderophore production, phosphate solubilisation, mediation of secretion of PR proteins in host, and hydrolytic enzymes etc. This may be instrumental in terms of usage of this novel group of microorganism as agents of bio control and growth promotion in crops and other plants of importance.

However evidence of extensive research regarding *Streptomyces* is negligible in the eastern and North Eastern part of India, in comparison to study and related inventory than the rest of the world. The article aims to provide insight into the basics as per relevance in terms of firsthand knowledge and opportunities for work to be carried out in the outlined locations. As North Bengal and whole of North East India which is located in the folds of the Himalayas, the youngest of the Physical features of the world; it has varied ecological and climatic condition and corresponding establishment of Flora. Hence looking into the economic and novel properties of Streptomyces and other genus within Actinomycetes extensive study can be conducted as these locations may have untapped resources in actinomycetes, which can evidently inhabit the most hostile to affable environmental conditions. Relevance of soil *Streptomyces* is always there but focus has to be there for endophytic *Streptomyces* also.

1.1. Understanding *Streptomyces*:

Streptomyces are a group of Actinobacteria. The genus *Streptomyces* belong to the family Streptomycetaceae under the single order Streptomycetales under the class and phylum

Actinobacteria (Sausa et al., 2016). These are Gram positive antibiotic producing bacteria having some characteristic which make them somewhat close to the fungi.

They are mostly soil inhabiting, saprophytic, have filamentous structure and produce spores (Vurukonda et al., 2018). These characters make them similar to fungi, than with other bacterial group. In the soil they remain in close proximity to the root rhizosphere or may exhibit endophytic nature i.e. they colonize the plant tissues of root, stem or leaf. (Hasegawa et al., 2006). Actinomycetes, particularly the Streptomyces group, are considered to be part of plant growth promoting microorganisms or PGPM. But their efficiency as PGPM has not yet been fully analyzed. Whereas many work have confirmed the beneficial aspect of *Bacillus* and *Pseudomonas* as plant growth promoting rhizobacteria (PGPR) (Sivasakthi et al., 2014) or *Trichoderma* as plant growth promoting fungi (PGPF) (Hermosa et al., 2012), same emphasis was not given to Actinomycetes. But like the PGPR or the PGPF, the soil *Streptomyces* and endophytic *Streptomyces* show activities which prove their efficiency as being beneficial to plants. Recent studies prove their importance as plant growth promoters (Vurukonda et al., 2018). Actinomycetes produce secondary metabolites which are bioactive compounds. Among them almost 70% of the compounds come from the *Streptomyces* (Sausa et al., 2016). Among them antibiotics are mostly important. Almost all the antibiotics that we use are obtained from the Streptomyces and other actinomycetes. But apart from the antibiotics, enzymes, plant hormones, chemical compound with therapeutic activity are also produced by actinomycetes in form of secondary metabolites. (Viaene et al., 2016) Endophytic actinomycetes are the key producers of antimicrobial compounds used for clinical or agricultural process such as munumbicin, kakadumycin and koronamycin etc. Moreover Multi-cyclic indolosequiterpenes having antibacterial properties was obtained from endophytic *Streptomyces* sp. HKI0595. Another compound spoxazomicins exists in endophytic *Streptosporangium oxazolanicum* strain K07-0450r (Hardoim et al., 2015).

2. Role of Streptomycetes in plant growth:

Plant growth promoting microorganisms like *Bacillus*, *Pseudomonas*, *Aspergillus*, *Trichoderma*, *Azotobacter*, and *Burkholderia*, have shown the ability to solubilise phosphate and at the same time evidence is also there, supportive of production of chemicals associated with plant growth promotion like IAA secretion, siderophore production etc (Khan et al., 2014). In the soil system, phosphate, an important nutrient for plant physiology is mostly present in insoluble form, which renders its direct intake by the plants. The resultant affect is evident symptoms of phosphate deficiency in the plant growth pattern. However, the same if available in soluble form can make better plant growth and vigor. Reports supportive of phosphate solubilisation by both endophytic *Streptomyces* and rhizospheric *Streptomyces* isolated from root of wheat is there (Jog et al. 2014).

Fe (iron) which is an essential ion and an important component of various phytochemicals viz cytochrome, ferredoxin, nitrogenous and others exists in a highly insoluble form, which cannot be properly utilized by the plant system on its own. Hence the underground plant system and the soil dwelling bacteria meticulously produce low molecular weight compounds called siderophores, these in turn mediate in extraction of iron from the soil stratum to the plant system (Khan et al., 2014). *Streptomyces* group too have been reported, as doing the same as the other bacteria but to produce hydroxamate type siderophore for iron uptake and usage (Tokala et al., 2002). Similarly Verma et al., (2011) reported that endophytic *Streptomyces* isolated from *Azadirachta indica* (Neem), a known medicinal plant, showed similar activity. Even in case of, leguminous plants which are already in association with the symbiotic bacteria *Rhizobium*, mediatory role played by Streptomycetes has beneficial effect. Evidence has been provided towards enhanced nodule growth as well as bacteroid differentiation in relation to enhanced plant growth (Tokala et al., 2002). Also, reports of many researchers are available of an important phytohormone namely Indole acetic acid associated with plant health and growth being produced by soil actinomycetes. For instance Shrivastava et al. (2008) reported Soil Streptomycetes *Kitasatospora*, as an IAA producer. Likewise, synthesis of IAA by *Streptomyces* spp was also reported by Manulis et al. (1994). Reports by Lin et al., (2013) of their work with isolate of an endophytic strain *Streptomyces* Sp.En-1 from a medicinal plant *Taxus chinensis* affirmed activity indicative of IAA production, with a positive effect in many aspects on an alternative host namely *Arabidopsis thaliana*. Recent reports (Shan et al. 2018) are available supportive of IAA production and also ACC deaminase activity among endophytic spp., after their isolation from different plant tissue systems of root, stem and leaf respectively of *Camellia sinensis* (Tea) plants.

3. Streptomycetes as endophytes:

According to Hallmann et al., (1997) endophytic bacteria are those which can be isolated from surface sterilized plant tissues or extracted from inside the plant and they won't harm the plant visibly. Endophytes are not saprophytes in terms of them associating with the host living tissue and providing some kind of benefit (Haggag, 2010). Among the endophytic actinomycetes *Frankia* was the first to be reported by

Brunchorst in the year 1886. As a nitrogen fixing actinobacterium *Frankia* forms actinorrhizae mainly with plants like *Alnus*, *Kasuarina* which are non leguminous.

Along with *Frankia* there have been instances of isolation of many other endophytic actinomycetes, that too from a range of various host plants. Evidently and collectively the occurrence of Streptomycetes is most common form of association observed with different plants as the host. (Coombs and Franco, 2003). The other actinomycetes that occur to be present in different host are *Micromonospora*, *Microbispora*, *Nocardia*, *Actinomyces*, *Arthrobacter* etc (Hasegawa et al., 2006). Studies have revealed members of actinomycetes mostly *Streptomyces* sp. are present in various crop plants like wheat, maize, rice etc which act as host. And secondly there are many reported isolation of the endophytic actinomycetes from various medicinal plants, which we know have therapeutic characteristic and usage. Medicinal plants like Neem (Verma et al., 2011), Zinger (Alshaibani et al., 2017), *Catharanthus* (Kafer and Khan 2011), *Centella* (Dochhil et al., 2013), *Taxus* (Lin et al., 2013) have been reported to host endophytic *Streptomyces* spp. As the endophytic organisms are present in the root, stem, as well as the leaf and other parts of the plant, colonizing the vascular tissue, the apoplast region and at times within the intracellular spaces of the host tissue system, it is speculated that the active principle, obtained from medicinal plants may be actually due to already existing endophytic association. (Haggag, 2010).

4. Mode of Action:

- a. **Direct mode:** Many plant pathogenic fungi and other microorganisms enter the host plant during disease formation. These microorganisms, using different modes, invade the host plant epidermis and go deep within the plant tissue system. Thereby producing hyphae in due course and subsequent release of toxic constituents within the tissue of the host. According to Hallmann et al., (1997), the endophytes occupy the ecological niche similar to that of phytopathogenic microbes or fungi and competes with the phytopathogens for host and produce antimicrobial compounds which will nullify the toxicity of pathogen exudates and at the same time will produce compounds which will enhance plant growth and vigor. Streptomycetes produce lytic enzymes, which assist to control fungal growth, inside the host plant by destroying the fungal hyphae. Also, they produce many other enzymes like Chitinase, Glucanase, amylase which may in one way or the other destroy the cell wall of pathogenic fungi thereby killing them. Among other members the endophytic streptomycetes particularly have shown these characteristics. Taechowisan et al (2003) reported endophytic *Streptomyces aureofaciens* CMUAc 130 to have shown chitinolytic activity against phytopathogens *Fusarium oxysporum*. Hastuti et al (2012) also confirmed production of chitinase by endophytic *Streptomyces*. These reports were supported by isolation of endophytic *Streptomyces* sp. P4 by Tang and Niamsup (2012) from sweet pea root which produce chitinase and showed chitinolytic activity against *Fusarium oxysporum* f.sp. *lycopersici*,

a phytopathogen of tomato. Minotto *et al.*(2016) have reported endophytic actinomycetes found in the roots of tomato, to produce both enzymes chitinase and glucanase and as such were antagonistic against *Bipolaris sorokiniana*, causative agent of root rot in wheat. Another endophytic *Streptomyces* sp. CEN26 isolates from the root of *Centella asiatica* produced 2, 5-(bis(hydroxymethyl)furan monoacetate(BHMF-OAc). This compound showed inhibition of the infection caused by *Alternaria brassicicola* in cabbage (Phuakjaiphaeo *et al.*,2016).

- b. **Indirect mode:** The enhancement of plant defense mechanisms by PGPM takes place by Induced Systemic Resistance (ISR). Function wise ISR is similar to SAR or Systemic Acquired Resistance. Whereas in SAR the plant defense mechanism, is fully activated in response to a weak primary infection, and upon a secondary infection, (though much stronger), it results in hypersensitive activity followed by necrosis. Furthermore, during the course of SAR in plants, the systematic immunization is caused by the inducing agents, which may be pathogens, biocontrol agents, composts, plant activating compounds etc. , this, later on is instrumental in the early recognition of the pathogen through different types of signaling system. The main characterization of SAR within a host or plant tissue system is accumulation of Salicylic Acid and different PR proteins (Kothari *et al.*2004). As stated both ISR and SAR are functionally same in inhibiting growth or ultimate death of the pathogen, but unlike in SAR, the inducing microbe does not cause any visible symptom. Further, the signaling systems in ISR is most of the time, independent of Salicylic acid and involves Jasmonic Acid and ethylene. Comparatively there is increased level of association of plant defense enzymes like peroxidase, phenylalanine ammonia lyase, polyphenol oxidase and phytoalexins during ISR mediated responses (Compant *et al.*,2005). Mainly plant growth promoting rhizobacteria (PGPR) or PGPF which colonize the root rhizospheric soil of the target plant induce ISR. But reports of *Streptomyces* inducing ISR are also there. Bower *et al.*(1996) reported that pathogenicity of *Streptomyces scabris* can be controlled by suppressive *Streptomyces* strains. Viaene *et al.*(2016) reported that powdery mildew in Oak (*Quercus* sp.) is suppressed by ISR inducing *Streptomyces* Ach 505. Conn *et al.*(2008) reported that endophytic *Streptomyces* isolated from Wheat, activate Jasmonic acid/ ethylene defense pathway in *Arabidopsis* and protect the plants from fungal diseases.

5. Endophytic Streptomyces and their effect in crop plants:

There are many reports of endophytic Streptomyces in crop plants which helps in improvement of overall plant health by endophytic association. There are many reports of presence of endophytic actinomycetes in the crop plants. Coombs and Franco (2003) isolated large number of endophytic streptomyces and other actinobacteria like *Micromonospora*, *Nocardiodes*, *Microbispora* from surface sterilized wheat roots.

88% of the isolates were *Streptomyces* as revealed by 16S rDNA sequencing. Hastuti *et al.*,(2012) reported that rice plants inoculated with endophytic *Streptomyces* sp. showed increased grain yield and higher dry weight. The isolates also inhibited the rice blight pathogen *Xanthomonas oryzae* pv *oryzae* Meguro *et al.*(2006) reported endophytic *Streptomyces* sp. MBR-52 obtained from rhododendron, has accelerated root formation in cucumber as well as in tissue cultured rhododendron seedlings suggesting that *Streptomyces* MBR-52 might release some rooting promoting hormones. Igarasi *et al.*, (2002) isolated Pteridic acid A and Pteridic acid B from endophytic *Streptomyces hygrosopicus* TP-A045 obtained from *Pteridium aquilinum*. Pteridic acids showed plant growth promoting activity like auxin and accelerated formation of adventitious roots in the hypocotyls of kidney beans. Not only plant growth promotion, endophytic *Streptomyces* also showed biocontrol properties. Taechowisan *et al.*, (2005) isolated *Streptomyces aureofaciens* CMUAc130 from root tissue of ginger. The major active ingredients in *Streptomyces aureofaciens* CMU Ac130 were 5,7-dimethoxy-4-p-methoxyphenylcoumarin and 5,7-dimethoxy-4-methoxyphenylcoumarin. Bioassays studies among other experimental work is supportive of evidence of the compounds as having antifungal activities against *Colletotrichum musae* and *Fusarium oxysporum*, causative agents for anthracnose of banana and wilt of wheat. Cao *et al.*,(2004) isolated 56 endophytic *Streptomyces* from tomato roots. 50% of the isolates were antagonistic to phytopathogenic fungi *Rhizoctonia solani*. One isolate *Streptomyces* strain S30 enhanced seed germination activity of tomato by 90% and growth enhancement in the tomato seedlings was also observed. Araujo *et al.*,(1999) isolated 6 *Streptomyces* isolates from Maize root and leaf tissues. The isolates showed antibacterial and anti fungal activity. *Streptomyces* sp.TQR12-4 isolated from a native orange variety of Vietnam, *Citrus nobilis*, produced thermostable antifungal compound which inhibited different pathogens including *Fusarium oxysporum*, *F. Udum*, *Colletotrichum* sp.,(Hong-Thao *et al.*, 2016). Dochhil *et al* (2013) isolated *Streptomyces* sp.CA10 and *Streptomyces* sp. CA26 from *Centella asiatica* . These two endophytes showed the capacity of IAA production and enhanced seed germination of French bean (*Phaseolus vulgaris*).

6. Endophytic Streptomyces in medicinal plants:

Not only the crop plants, Endophytic *Streptomyces* spp. were also isolated from a number of medicinal plants. The *Streptomyces* isolates have a number of activities. Some of them have antimicrobial, antitumor, antifungal and other activity. Sometimes they are isolated from well known medicinal plants like *Catharanthus*, *Zingiber*, *taxus* or *Centella* etc. Taechowisan *et al.*(2016) isolated *Streptomyces* sp VE2 from *Vernonia cineria* and found antibacterial effect of its secondary metabolites upon pathogenic bacteria *Bacillus cereus* , *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *B Subtilis*. Secondary compound Acetogenin having antioxidant activity (squamacin and rollidecin B) was obtained from the strain which was inhibitory to tested bacteria and highest activity was against *B cereus* and *S typhi*. In Indonesia plants like *Alpinia galanga* , *kaempferiae galanga* , *K rotunda*, *Zingiber cassumunar* are traditionally used as antiobesity agents. 35 endophytic isolates obtained from these

plants were all *Streptomyces* and the crude culture filtrate showed pancreatic lipase inhibitory effect against swine lipase (Fitri et al., 2017). Antimicrobial activity of a bacterium isolated from *Catharanthus roseus*, that has a very high level of sequence similarity with *Streptomyces cavourensis* sub sp. *cavourensis* ABI84264.1. by 16 S rDNA sequencing, showed both antibacterial and antifungal activity (Kafer and Khan 2011). Bioactive secondary metabolic diketopiperazine (DKPs), obtained from endophytic *Streptomyces* SUK25 which was isolated from *Zingiber spectabile* showed antimicrobial toxicity against Hepa RG cells. (Alshaibani et al.,2017). Momin and Tripathi (2018) isolated endophytic actinomycetes from 6 medicinal plants. Highest number of isolates were obtained from root tissue of *Mikania micrantha* followed by *costus speciosus*. Eight of the isolates were *Streptomyces* and they showed antifungal activity against *Fusarium oxysporum* and *F. proliferatum*. The isolates also had IAA producing capacity. Wardicki et al.,(2015) reported the endophytic streptomycetes from medicinal plant *Arnica Montana* showed remarkable cytotoxic activity as well as antifungal activity against *Candida parapsilosis* and *Fusarium verticillioides* and both the plant and *Streptomyces* benefitted from each other. Endophytic *Streptomyces* sp. AC 35, closely related to *Streptomyces odorifer* obtained from rhizosphere of *Taxus baccata* produced

bioactive isoflavone aglycone-daidzein, glycitein and genistein and antimycin(Ondrejickova et al., 2016).

7. Conclusion

Though studies have been conducted for *Streptomyces* sp due to their unique ability to produce antibiotic of therapeutic value their importance in agriculture and role in plant physiology, the rhizosphere, rhizosphere and overall ecosystem augmentation cannot be undermined. Extensive study of virgin areas for isolation and study maybe required, mostly in the Eastern Himalayas which has a rich flora, fauna and is home to many Biodiversity hot spots of India. Both soil dwelling and endophytic *Streptomyces* sp are to be studied but focus can be provided to importance of endophytic species as, occurrence as well as ethno botanical usage of medicinal plants among tribals of North Bengal and North East India is till date relatively on the higher side.

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