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A brief review on biocontrol potential and PGPR traits of *Streptomyces* sp. for the management of plant diseases

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Abstract
Streptomyces is the largest genus of Actinobacteria and the type genus of the family *Streptomycetaceae*. *Streptomyces* are soil saprophytes and are responsible for earthy odour of freshly ploughed soil due to production of geosmin. Now a days farmers became more and more dependent on agrochemicals but increasing negative effects. So alternative approaches like use of biocontrol agents for management of plant disease is very important. In that *Streptomyces* is also contributing for plant growth promotion activity and plant disease suppression. *Streptomyces* also produces some of the secondary metabolites such as indole acetic acids and chitinase. *Streptomyces* acts as both plant growth promoting rhizobacteria and also plant disease suppressor by various mechanisms like increase in the supply of nutrients such as phosphorus, sulphur, iron, copper, production of IAA, cytokinin and siderophor production; plant growth promoting through multi-dimensional manner by producing plant hormones; controlling fungi and bacterial disease through antibiosis and competition.

Keywords: *Streptomyces*, PGPR, enzymes, biocontrol

Introduction
The Streptomycetes are gram positive bacteria with a filamentous form that present in a wide variety of soil including composts, water and plants. The most characteristic of Streptomycetes is the ability to produce secondary metabolites such as antibiotics. They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g. neomycin and chloramphenicol. Another characteristic of Streptomycetes is making of an extensive branching substrate and aerial mycelium. Carbon and nitrogen sources, oxygen, pH, temperature, ions and some precursors can affect production of antibiotics. This review also addresses the different methods to study the antimicrobial activity of *Streptomyces sp.* Because of increasing microbial resistance to general antibiotics and inability to control infectious disease has given an impetus for continuous search of novel antibiotics all the word.

They form thread-like filaments in the soil which give them an advantage in colonizing the rhizosphere effectively. As a rhizobacteria, they Biotechnology influence plant growth, antagonize plant pathogens and makes nutrients available for the plants (Maheshwari and Shimizu, 2011) [14]. Actinomycetes are important, not only as degraders of organic matter in the natural environment, but also as producers of antibiotics and other useful compounds of commercial interest (Saugar *et al*. 2002; Bentley *et al*. 2002 and Basilio *et al*. 2003) [17, 6, 5].

Many actinomycetes are important source of enzymes, such as Chitinase (*Streptomyces viridificans*), cellulases (*Thermomonospora* spp.), peptidases, proteases (*Nocardia* spp.), Xylanases (*Microbispora* spp.), ligninases (*Nocardia autotrophica*), amylases (*Thermomonospora curvata*), sugar isomerases (*Actinoplanes missouriensis*), pectinase, hemicellulase and keratinase. The production of these hydrolytic enzymes makes it possible for actinomycetes to break down organic matter in their natural environment.

First time the genus *Streptomyces* was introduced by Waksman and Henrici in 1943 (Williams *et al*. 1983) [20]. Genus *Streptomyces* belongs to the Streptomycetaceae family (Arai, 1997) [4]. In general Streptomycetaceae family can be distinguished by physiological and morphological characteristics, chemical composition of cell walls, type of peptidoglycan, phospholipids, fatty acids chains, percentage of GC content, 16 SrRNA analysis and DNA-DNA hybridization (Korn-Wendisch and Kutzner, 1992) [12]. Streptomycetaceae family are in Actinobacteria phylum and Actinomycetales order within the classis Actinobacteria and the genus *Streptomyces* is the sole member of this family (Anderson and Wellington 2001) [3]. In terms of number and variety of identified species, *Streptomyces* represents one of the largest taxonomic items of recognized *Actinomycetes.*

Streptomycetes are aerobes, chemoorganotrophic bacteria and they need organic carbon source, inorganic nitrogen sources, and mineral salts and don't need vitamins and growth factors. *Streptomyces* requirements has been investigated by kutzner. Most of *Streptomyces sp*. are mesophile and grow in temperatures 10-37 but three species *Streptomyces thermonitrificans*, *S. thermovulgaris* and *S. thermoflavus* are thermophile and grow in temperature 45-55. Streptomycetes grow in pH 6.5-8.0. Streptomycetes are not only more resistant to drought and form arthrospore but also require less moisture than other bacterial and are very sensitive to water logged conditions. Some of reports described that drained soils (sandy loam, calcareous) have more *Streptomyces* than heavy clay soils.

Streptomyces is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae. Over 500 species of *Streptomyces* bacteria have been described. *Streptomyces* species are chemoorganotrophic, filamentous gram-positive bacteria but not acid-alcohol fast, not fungi and occur in the same habitats as fungi and are superficially similar. They have genomes with high GC content 69-78 percent. The filaments and spores are very small usually 1 μm or less in diameter. The spores are formed by the fragmentation of the filaments and are borne in straight, wavy, or helical chains. The colonies are slow-growing and often have a soil-like odour because of production of a volatile metabolite, geosmin. Firstly colonies are relatively smooth surfaced but later they develop a weft of aerial mycelium that may appear floccose, granular, powdery, or velvety. They produce a wide variety of pigments responsible for the colour of the vegetative and aerlial mycelia. *Streptomyces* species are nonmotile, catalase positive, reduce nitrates to nitrites and degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and Ltyrosine. The cell wall peptidoglycan contains major amounts of L-diaminopimelic acid (L-DAP). They have no mycolic acids,contain major amounts of saturated, iso- and anteiso-fatty acids, possess either hexa- or octahydrogenated menaquinones with nine isoprene units as the major isoprenolog, and have complex polar lipid patterns that typically contain diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidyl inositol mannosides.

The work done in Abroad and India pertaining to biocontrol potential and PGPR traits of *Streptomyces* Sp. for the management of plant diseases in recent years are is critically reviewed and presented here under.

Adhilakshmi *et al*. (2013) [1] collected different isolates from Tamil Nadu among them 5 were prominant further these were evaluated for their antagonists activity. MDU has most effective in inhabiting the fungal growth with the inhibition zone of 49 mm, whereas CBE and PDK 48 and 46 mm. The application of *Streptomyces* in both greenhouse and field condition. Among them, CBE and PDK were highly effective in controlling stem rot and increasing pod yield under greenhouse condition. Among different treatments, soil application and seed treatment showed less disease incidence and higher pod yield in both greenhouse condition and field condition.

Simi *et al*. (2016) [18]. Prepared the culture filtrate of 10 isolates among them RCE-10 and RP1A at 25 and 50 per cent concentration completely inhibited mycelia growth of *Sclerotium rolfsii*. RCE 22 and RP9A 16 did not inhibit the fungal growth at any concentration. Green house evaluation was done for RP1A isolate, they have made different treatments like talc formulation and soil application. Among all treatments seed treatment with talk based formulation gives significantly reduces disease severity and yield followed by talc formulation. Crude extract also showed significantly reduced disease incidence than soil application over the pathogen inoculation. Among five isolates RP1A-12 showed 100 per cent inhibition of mycelium followed by RP1A-15.

Kamal and Sharma, (2014) [11] checked the antagonistic activity of *Streptomyces* strain CPP-53 against different pathogens by dual culture assay was checked. It was found that the strain CPP-53 showed antagonistic activity against *Fusarium oxysporum*, *Colletotrichum tranctum*, *Helminthosporium oryzae* and *Colletotrichum capsici* pathogen. They also tested in greenhouse condition in which treatment with *Fusarium* showed death of plants and also with more disease but in case of CPP-53 inoculated strain reduced disease incidence, but the strain CPP-53 treated increase growth of plant root growth and dry mass of plant.

Of the eight isolates studied by Alekhya and Gopalakrishnan (2014) [2] on chickpea diseases, all showed inhibition on *R. bataticola* strain RB-6, *R. bataticola* strain RB-24 (except BCA-657), *R. bataticola* strain RB-115 (except CAI-70), *B. cinerea* (except BCA-657) and FOC (except CAI-98), whereas on sorghum diseases, all showed inhibition on *M. phaseolina* (except CAI-70), *F. proliferatum* strain FM-242 (except BCA-657 and BCA-687) and *F. andiyazi* strain FM-943 (only for CAI-671, CAI-679, CAI-67 and CAI-70).

Alekhya and Gopalakrishnan (2014) [2] used the cell-free extracts of isolates for evaluation for their secondary metabolite production capabilities, among which BCA-690 (except *S. rolfsii*) and CAI-70 (except *R. bataticola* strain RB-115) exhibited antagonistic activity against eight pathogens. Of the remaining six isolates, BCA- 657 (except FOC and *S. rolfsii*) and BCA-679 (except *R. bataticola* strain RB-115 and *S. rolfsii*) showed antagonistic activity against seven pathogens whereas the remaining four isolates showed antagonistic activity against only four pathogens. The biochemical and PGP traits such as chitinase, cellulase, lipase, protease, HCN, siderophore and IAA were estimated for the promising isolates. All the isolates produced chitinase (except BCA-657, CAI-67 and CAI-70), cellulase, lipase (except CAI-70), protease (except CAI-67, CAI-70 and CAI-98), HCN, siderophore (except CAI-67) and IAA. Of the eight isolates, CAI-98 was found to produce the highest chitinase whereas BCA-690 produced the highest amount of cellulase, lipase (also BCA-671 and BCA- 679), protease, HCN, siderophore and IAA (52.9 μg mL-1).

Sangdee *et al*. (2016) [16] selected seven actinomycete like colonies to test against the plant pathogenic fungi *C. gloeosporioides* isolates Cg1 and Cg2 and *B. maydis* isolates Bm1 and Bm2. The results showed that all the selected isolates inhibited the growth of the tested fungi in the range 70.4-100.0 per cent. The selected actinomycete like colonies isolate SRF1 showed the highest percentage of mycelial growth reduction in the tested fungi, which was significantly greater than with the other isolates. The inhibition zone produced by the isolate SRF1 varied from 28-30 mm. The isolate SRF1 was chosen for further evaluation of the antagonistic activity against *C. capsici*, *Pyricularia* sp., *Fusarium* sp., *Curvularia* sp. and *S. roftsii*. The results showed that isolate SRF1 inhibited the mycelial growth of *C. capsici*, *Pyricularia* sp., *Fusarium* sp. and *Curvularia* sp. in the range of 65.5-91.6 per cent, while there was no effect on the mycelial growth of *S. roftsii*.

Manhas and Kaur (2016) [15] in their present study demonstrated inhibition of mycelia growth of *A. brassicicola*

grown in broth supplemented with culture supernatant of DH16. Incomparison to control, the mycelia dry weight of pathogen in PDB was significantly lowered in the presence of culture supernatant and this suppression of mycelia growth was found to be depended on the concentration of bioactive metabolites present in the culture supernatant. More than 50 per cent inhibition was achieved at concentration of 5 per cent and complete inhibition of mycelia growth occurred at 20 per cent anti-fungal metabolites. Furthermore, the change in pH of the spent medium varied between 6.5 and 7.15, which suggested that the resulted mycelia inhibition was not due to pH change.

Manhas and Kaur (2016) [15] also conducted the *In vitro* biocontrol potential of cells and culture supernatant of streptomycete against *A. brassicicola* was studied as seed treatment in radish. Seed germination, number of healthy seedlings and seedling vigour were found to differ significantly in treated and non-treated seeds. In the seeds treated with pathogen alone, the percentage of seed germination and healthy seedlings, fresh and dry weights, and seedling vigor were significantly lower as compared to the uninoculated control seeds. On the other hand, treatment of pathogen infested seeds with culture supernatant at the highest concentration of 20 percent significantly improved seed germination and seedling vigor to 80 per cent and 1538, respectively and were comparable to control. The percentage of healthy seedlings (90 percent) and their fresh and dry weights were also significantly higher in treated seeds. Similarly, treatment of pathogen infected seeds with antagonist also significantly improved all the parameters as compared to pathogen infested seeds. Additionally, seeds treated with streptomycete/culture supernatant only were found to be healthier than the uninoculated control seeds. Strain DH16 showed significant stimulatory effect and an increase of 21-35 percent over control in various growth parameters of seedlings was observed.

In the investigation of Gopalakrishnan *et al*. (2015) [8] strains were evaluated for their PGP traits in chickpea under field conditions. The plots treated with *Streptomyces* strains showed significantly enhanced agronomic performance of all the traits measured at 30 and 60 DAS. As seen at 30 DAS in both 2012-13 and 2013-14 seasons, the *Streptomyces* treated plots showed significantly enhanced agronomic traits including the nodule numbers (35-83 percent), nodule weight (27-64 percent), root weight (9-14) and shoot weight (32-50 per cent) over the un-inoculated control. Similarly at 60 DAS, the *Streptomyces* strains significantly enhanced the pod number (29-95 percent), pod weight (30-135 percent), leaf area (32-34 percent), leaf weight (25-63 percent) and stem weight (23-68 percent) and at crop maturity, stover yield (25-75 percent), grain yield (11-26 percent), total dry matter (19-32), pod weight (6-51 percent), seed number (20-52) and seed weight (3-47 percent) in both 2012-13 and 2013-14 seasons over the un-inoculated control plots.

In the top 15 cm rhizosphere soils, at crop maturity, the *Streptomyces* strains treated plots significantly enhanced the microbial biomass carbon (35-55), dehydrogenase activity (27-56 percent), total N (411), available P (51-106 percent) and organic carbon (9-16 percent) in both 2012-13 and 2013-14 seasons over the un-inoculated control plots reported by Gopalakrishnan *et al*. (2015) [8]. Of the six *Streptomyces* strains studied in the present investigation, CAI-85, CAI-93 and KAI-180 (in descending order) were found superior to CAI-155, CAI-140 and CAI-13 in terms of their effects on root and shoot development, nodule formation and crop productivity.

Srividya *et al.* (2012) [19] in her study demonstrates the efficiency of actinomycetes isolates from Solanaceae rhizosphere to produce lytic enzymes *viz*., Chitinase, *β*-1, 3 and *β*-1, 4 glucanase, lipase and protease in addition to their possible role in the destruction of broad spectrum soil borne fungal phytopathogens-*Alternaria alternata* OTA36; *Alternaria brassicola* OCA1; *Alternaria brassicaceae* OCA3; *Colletotrichum gloeosporioides* OGC1; *Rhizoctonia solani* and *Phytophthora capsici*. The PGPR traits of the organism were determined in terms of IAA, siderophore, PO4 solubilization etc. and germination index of chilli. The antifungal potential of extracellular mycolytic enzymes produced by soil-borne Actinomycete could be exploited for its future use as a biofungicide

Srividya *et al.* (2012) [19] used the isolate 9p for seed coating with/without the *Colletotrichum* spores and was tested for the growth promotion, bio control efficiency and germination properties of the isolate. Treatment of the chilli seeds with isolate 9p showed 100per cent germination index similar to untreated. The treatment of the seed with co-inoculation of the pathogen with 9p showed 75 percent reduction in seed mortality by the treatment as compared to the seed treated with pathogen alone. This treatment also showed 87.5 percent germination index suggesting both the bio control and PGPR aspect of the bacteria. Inoculation of 9p with seeds showed the presence of more lateral roots as compared to uninoculated control seeds. There are reports of antagonism of chilli phytopathogen *Pythium debaryanum* by soil fungi from chilli rhizosphere.

Lyu *et al*. (2017) [13] used the crude extract of *Streptomyces sp.* 3-10 to test the inhibition of spore germination of *B. cinerea*. For *B. cinerea*, the inhibitory efficacy depended greatly on concentration of the crude extract. In the treatments of the crude extract at 1 and 5μg/ml, the conidia germinated by 93-99 per cent at 6-12 h post-incubation (hpi), not significantly different (P>0.05) from the germination rate of 99 per cent in the control treatment. The average values of germ-tube length reached 146.6 and 106.5μm for the two concentrations of the crude extract, respectively. In the treatments of the crude extract at 10, 50, and 100μg/ml, the percentages of germinated conidia at 12 hpi were reduced by 12, 90, and 99 per cent, respectively, and the average germ tube length was reduced by 51, 89, and 92 percent, respectively, compared to the control treatment.

At 3-7 days post inoculation (20 ºC) of crude extract, reveromycin A from *Streptomyces sp.* 3-10, the strawberries in the control treatment were severely diseased. The percentages of diseased strawberries reached 100, 88.9, 100, and 94.4 percent for the control inoculations with *B. cinerea*, *M. hiemails*, *R. stolonifer*, and *S. sclerotiorum*, respectively. The disease severity index values reached 78.5, 62.5, 88.1, and 82.6 in these treatments, respectively. In contrast, most strawberries in the treatments of the crude extract and reveromycin A from strain 3-10 at 10, 50, and 100μg/ml, as well as in the treatments of the fungicides at 10, 50, and 100μg a.i./ml appeared healthy. The percentages of diseased strawberries were lower than 40per cent and the disease severity index values were lower than 35 in these treatments. Reveromycin A at 50 and 100μg/ml completely suppressed strawberry fruit rot caused by all the four fungi. Statistical analysis indicated that for each pathogen, the treatments of the CE, reveromycin A and fungicide differed significantly (P<0.05) from the control treatment both in disease incidence

and in disease severity index. Under the same concentration, the crude extract and reveromycin A from strain 3-10 did not significantly (P>0.05) differ from the corresponding fungicide (Lyu *et al*. 2017) [13].

Gopalkrishnan *et al.* (2013) [7] selected five strains of Streptomyces (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90), reported earlier as potential for biocontrol traits against Fusarium wilt in chickpea (Gopalakrishnan *et al*. 2011) [9], were further studied in sorghum and rice. These Streptomyces were characterized for enzymatic activities, physiological traits and further evaluated in greenhouse and field for their plant growth promotion (PGP) of sorghum and rice.

Out of the five isolates CAI-127 shows the more chitinase and lipase activity then followed by CAI-121. KAI- 90 shows less chitinase and lipase activity.CAI-24, KAI-32 and KAI-90 shows both chitinase and lipase activity. All the isolate shows β-1-3-glucanase that is ranges from 0.0-2.5 units.

After evaluation of different isolates for enzyme activity again tested for field evaluation for rice and sorghum crop. In the rice field, the actinomycetes significantly enhanced tiller numbers, panicle numbers, filled grain numbers and weight, stover yield, grain yield, total dry matter, root length, volume and dry weight over the uninoculated control. Again these isolates were evaluated for sorghum also under the laboratory condition. Here also it increases the plant height, leaf area, stem weight, root length, root volume and also root dry weight when compare with control. The mechanism by which the actinomycetes enhanced morphological and yield traits of rice could be attributed not only to their enzymatic activities such as siderophore and IAA (direct stimulation of PGP) and/or chitinase, cellulase, lipase, protease, hydrocyanic acid and -1, 3-glucanase production capabilities (indirect stimulation of PGP) but also to their ability to survive under harsh environments

Hata *et al*. (2015) [10] selected active antagonistic actinomycetes isolates for the antagonistic and plant growth-promoting activities. All isolates showed zone of inhibition that indicated positive results were measured by subtracting the actinomycete colony diameter from the combined positive zone diameter and the actinomycete colony diameter against *Xanthomonas oryzae* pv. *oryzicola*. The highest zone of inhibition (17.67mm) were observed in case of SS8 isolate and the followed by TKSC3 (14.00mm). And lowest inhibition were observed in case of TKSB1 (2.33 mm) and TKSB2 (2.00 mm). Again these isolates were selected for the hydrolytic enzyme activity like amylase, protease, lipase and cellulose. Most of all isolates shows the all enzyme activity. The amylase activity values ranges from 3.33-15.00 in all isolates. Protease activity values ranges from 0.67-18.33 and lipase and cellulose activity values ranges from 4.83-14.83 and 1.67-10.50 in mm. sometime, some of the isolates not determine enzyme activity. Results from the preliminary screening showed that actinomycetes, especially Streptomycetes, could offer a promising source for both biocontrol and plant growth-promotion agents agains *Xanthomonas oryzae* pv. *oryzicola* pathogen in rice.

Mrunalini (2015) selected three actinobacterial isolates commonly present in both Neem and Tulsi leaves. These three isolates were designated as 5, 7 and 9. All isolates has shown significant PGPR activity. Especially Isolate no.7 showed positive results for all PGPR studies whereas, Isolate no. 9 showed negative response for Phosphate solubilization, ACC and siderophore production. Among three (5, 7 and 9) isolstes, all the isolates showed branched hyphae with spores, positive for methyl red oxidase, nitrate reductase and catalase reactions. Isolates 7 and 9 showed the alkality and acidity reactions to TSI test. Isolate 7, though it was assigned to the genus *Streptomyces*, the strain did not match with any of the species and therefore it may be a novel species which may be designated as mrinalini 7strain. Molecular characterization by 16S rRNA ribotyping and phylogeny analysis was carried out in order to identify *Streptomyces sp.* mrinalini 7 up to species level. Mrinalini 7 strain showed the best PGPR activity, its ability of plant growth promotion has been studied by inoculating into Tomato plant and its activity has been compared with the uninoculated control plant. The PGPR isolates significantly affected the production of tomato seedlings. Results revealed that the root length and shoot length increased in PGPR treated plants over uninoculated control. A significant increase in both fresh and dry root, shoot and fruit biomass of tomato seedling were observed in response to PGPR isolates over control.

Conclusion

Now a days farmers became more and more dependent on agrochemicals but this increased use created lot of adverse effects. So, alternative approaches *viz*., use of biocontrol agents for management of plant disease is very important. Among biocontrol agents *Streptomyces* is contributing for plant growth promotion activity and plant disease suppression by producing secondary metabolites *viz*., indole acetic acid, chitinase etc. Further, research and understanding of mechanisms of PGPR mediated phytostimulation would pave the way more for identification of competent rhizobacterial strains which may work under diverse agroecological conditions.

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