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EFFECT OF SOIL *STREPTOMYCES* ON SEED GERMINATION

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ABSTRACT

Microorganisms are abundant in soil possessing ability to produce economically valuable substances like enzymes, antibiotics, hormones, etc. Actinomycetes especially *Streptomyces* predominate organic soil. In the present study *Streptomyces gibosonni, Streptomyces caerulius, Streptomyces viridochromogenes, Streptomyces gresioluteus* and *Streptomyces clavifer* were isolated from different soil samples. Their effect on growth of crop plants and herbs was analysed. *S.viridochromogenes* and *S.clavifer* found to be inhibiting seed germination of crop plants while *S.gibosonni* and *S.grieseoleutus* induced seed germination.

KEYWORDS

Streptomyces, seed germination, Phosphinothricin, *Actinomycetes.*

INTRODUCTION

The out burst of population has given rise to series of problems, mainly the need for surplus food. The agricultural yield has to increase through usage of chemical fertilizer, pesticides and herbicides which may lead to some undesirable consequences such as soil pollution, water pollution, biomagnification and excessive persistence.

There is considerable interest in application of organic products like biological fertilizers, pesticides and herbicides in fields. This is an eco-friendly approach and helps in promotion of plant growth and yield, by producing phyto-hormones, degradation of complex molecules such as cellulose, lignin, xylene, etc [1]. Natural products such as secondary metabolites of certain microbes have been investigated as biological pesticides and herbicides which are used as alternative for agrochemicals.

Soil microorganisms peculiarly have the capacity to produce compounds that are potentially promoting plant growth and yield. In addition certain organisms inhibit plant and microbial growth by the production of phthoxazolins, phosphinothricin, gougerotin, etc [2,3]. *Streptomyces* produce many extra-cellular active compounds such as indole acetic acid, phosphate solubilizing substances, chitinase and intracellular siderophores which induce germination of seeds and their growth [4,5]. In this regards *Streptomyces* is isolated

from soil and their plant growth promoting activity and herbicidal activity are investigated in this work.

MATERIALS AND METHODS

I. *Site and Sample collection*

The soil samples were collected from 4 different places of Mettur hills at a depth of 4 – 7 cm using a soil borer. A total of 15 samples were obtained for isolation and dried under room temperature for 15 – 30 days before isolation.

II. *Isolation*

The soil samples were decimally diluted and pour plated on Starch Casein Nitrate medium incorporated with streptomycin 40µl/ml and griseofulvin 50 µl/ml [6]. The plates were incubated at 28˚C for 7-8 days to obtain actinomycetes growth.

III. *Identification*

Identification of the strains were carried out based on their morphological, physiological and biochemical characters to the genus level following the direction mentioned in the Manual of International Co-operative Project for description and deposition of *Streptomyces* culture [7] and Bergey's Manual of Systematic Bacteriology [8].

For morphological characterization the isolates were inoculated in SCN medium as single streak, covered with slide cover and incubated at 28˚C. On the 7th, 14th and 21st day the slide covers were removed and observed under microscope directly. Also the growth was mixed with saline and smeared on a clean glass slide, heat fixed, then stained with Gram staining reagents, then finally observed under oil immersion objectives.

IV. *Biochemical tests*

The physiological and biochemical characters of each isolate were identified by performing the following tests:

TEST	MEDIUM	TIME OF INCUBATION	REAGENT USED
Starch hydrolysis (amylase activity)	Starch agar	7 days	Plate flooded with 1% iodine solution
Casein hydrolysis (protease activity)	Skimmed milk agar	7days	–
Indole production	Peptone broth	7 days	Kovacs reagent
Acetoin production	MR-VP broth	7 days	40% KOH 5% α – naphthol
Catalase production	Starch casein broth	7 days	Hydrogen peroxide

V. *Chemotaxonomic analysis of whole cell sugars*[9,10]

One ml of 1N H_2SO_4 was add to 50mg freeze dried actinomycete in a vial heated at 100˚C for 2 hrs. The mixture was centrifuged at 300rpm for 10 minutes. The pH of the supernatant was adjusted to 5 with saturated barium hydroxide, followed by centrifugation at 6000rpm for 10 minutes. The supernatant was filter through Millipore filter; 5ml of the standard 1 % sugar solution (galactose, glucose, arabinose and ribose) were spotted separates onto a TLC silica gel plate. The plate was developed in the solvent acetonitrite. Water (92.5: 7.5 for 20min and dried for 2 hrs. than it was sprayed with aniline phthalate and heated at 100˚C for 4 minutes on a hot plate.

VI. Analysis of cell wall amino acids[11]

One ml of 6N HCL was added to 50mg freeze dried actinomycetes in a vial with a screw cap and heated overnight at 100˚C. The mixture was completely dried at 45 in vaccum. Two ml of distilled water was added to the vial and then dried off. This step was repeated several times to remove HCL. The final dried material was dissolved in 0.2ml of water. Five ml of the sample and 1ml of standard diamino pimelic acid solution were separately spotted on to a TLC silica gel plate. The plate was developed in the solvent (methanol: distilled water 6N HCL, pyridine = 80:26:4:10) for 3 hrs, air dried in a chemical hood for 2hrs and sprayed with 0.1% ninhydrin, followed by heating at 120˚C for 10minutes on a hot plate.

VII. Growth pattern studies

The isolates of *Streptomyces sp.* were incubated at 4˚C, 25˚C, 37˚C and 45˚C.Colony morphology was observed after 5-7 days. Likewise the growth was recorded in different salt (NaCl) concentrations ranging from 2 to 13%.

VIII. Screening for plant growth promoting and herbicidal activity Crude extract Primary inoculum was prepared and inoculated in SCN broth, incubated at 28˚C for 7 days in shaker. Culture filtrates were used for bioassay.

IX. Screening for plant growth promoting activity

It was performed by placing a sterile filter paper moistened with 2.5ml crude extract in a Petri dish. Seeds of black gram, maize, radish and *Bromus lyticus* (grass) were scattered on the filter paper and incubated at 28˚C in dark for 4 days and observed for growth.

X. Screening for herbicidal activity

9cm diameter of semi filter paper was soaked in 2.5ml of individual culture filtrate and placed in a Petri dish. Then seeds of the above mentioned plants were placed on its surface. The set up was incubated at 28˚C for 4 days in dark and observed for growth.

XI. Detection for presence of phosphinothricin tripeptide (PTT) [12]

Bioassay for the PTT production were carried out with the sensitive strain of *B.subtilis ATCC 6633*. In this a 6mm paper disk soaked with the culture filtrate and applied to a confluent lawn of *B.subtilis ATCC 6633*. The plates were incubated at 37˚C for 24 hrs and the results were observed.

XII. Qualitative determination of Di-amine putricine producing Actinomycetes

The isolates were plated on Moller's Decarboxylase agar medium with arginine (2gm/l)and phenol red (0.02gm/l). The plates were incubated at 28±2˚C in dark for 4 days. Growth of the decarboxylating isolates were detected by presence of red halo around and beneath the actinomycete colonies.

XIII. Purification of PTT

The sample containing phosphinothricin was passed through a column of amberlite IR120 (H+). The adsorbed material was eluted with 2N NaOH and subjected to TLC using BuOH – AcOH – H2O (3:1:2) as developing solvent. The spots were visualized with ninhydrin corresponding to phosphinothricin (at 480nm). Purified compound used to find out herbicidal activity as mentioned earlier.

RESULTS

The soil samples were screened to isolate actinomycetes with plant growth regulating activities, either promoting or inhibiting their growth. Seven potential isolates with gross morphological differences of *Streptomyces* were isolated. The isolates were

coded as SAV-01 to 07. Later the strains were identified based on their morphological, biochemical and chemotaxonomic characters mentioned in Bergey's Manual of Systematic Bacteriology for the genus *Streptomyces.*

All isolates were Gram positive, filamentous and sensitive to lysozyme. All of them used glucose as their carbon source but failed to utilize sorbitol as their carbon source. *S.gibosonni* and *S.viridochromogenes* used wide range of carbohydrates as their carbon source such as glucose, arabinose, mannitol and surcrose with the latter utilizing also fructose. Except SAV-02 no isolate produced brown diffusible pigment.(Table 1& 2). All the isolates grew well at 28˚C - 37˚C except SAV-03 and *S.caeruleus.* All starins were tolerant to NaCl concentration of 2-5% in the medium, *S.gibosonni* and SAV-02 tolerated upto 7% .SAV-02 and *S. caeruleus* were able to hydrolyze casein but not starch (Table 3,4& 5). The chemotaxonomic nature of the Streptomycetes isolate was studied by Thin Layer Chromatographic method. The results showed that all the isolates possess L - di-aminopimelic acid and not sugar residues.

Table 1
Morphological characters of Streptomyces sp.

Isolates	Microscopic observation		Macroscopic observation	
	Grams reaction	**Morphology**	**Colony morphology**	**Pigmentation (back view)**
S.gibosoni	+	Filamentous	Dry, powdery, Gray white	Yellow
Streptomyces spp.	+	Large, filamentous	Brownish yellow	Brown
Streptomyces spp.	+	Filamentous	Dry white	Absent
S.caeruleus	+	Filamentous	Dry, dark gray	Absent
Sviridochromogenes	+	Long rods	Dry, powdery, gray white	Absent
S. grieseoleutus	+	Large, filamentous	Grayish white	Absent
S.clavifer	+	Long rods	Yellowish white	Absent

Table 2
Biochemical characters for identification of Streptomyces sp

Isolates	Biochemical tests							
	In	MR	VP	Cat	Nit	Lyz	Sta hyd	Cae hyd
S.gibosoni	-	-	+	+	-	S	+	+
Streptomyces sp	-	-	-	+	+	S	-	+
Streptomyces sp	-	-	-	-	-	S	+	+
S.caeruleus	-	-	-	-	-	S	-	+
S.viridochromogenes	-	-	-	+	+	S	+	+
S. grieseoleutus	-	-	-	+	-	S	+	+
S.clavifer	-	-	-	-	+	S	+	+

In – Indole **MR – Methyl red**
Vp – Voges Prouskauer **Nit - Nitrate**
Cat – Catalase **Lyz – Lyzozyme sensitivity**
Sta hyd – Starch hydrolysis **Cae hyd – Casein hydrolysis**

Table 3
Effect of different temperatures on growth of the Streptomyces sp.

	Growth at different temperatures (˚C)			
Isolates	**4**	**28**	**37**	**45**
S.gibosoni	-	++	+	-
Streptomycetes spp	-	++	++	-
Streptomycetes spp	-	++	-	-
S.caeruleus	-	++	-	-
S.viridochromogenes	-	++	+	-
S. grieseoleutus	-	++	+	-
S.clavifer	-	++	++	-

- - no growth
+ - poor growth
++ - good growth

Table 4
Effects of different NaCl concentrarions on growth of the Streptomyces sp.

	Growth at different % of NaCl					
Isolates	**2**	**5**	**7**	**9**	**11**	**13**
S.gibosoni	++	++	+	-	-	-
Streptomycetes spp	++	++	+	-	-	-
Streptomycetes spp	++	++	-	-	-	-
S.caeruleus	++	++	+	-	-	-
S.viridochromogenes	++	++	-	-	-	-
S. grieseoleutus	++	-	-	-	-	-
S.clavifer	++	++	-	-	-	-

- - no growth

+ - poor growth
++ - good growth

Table 5
Carbohydrate utilization test for the Streptomyces sp isolated

Isolates	Glucose	Arabinose	Fructose	Mannitol	Sorbitol	Sucrose
S.gibosoni	+	+	-	+	-	+
Streptomyces spp	+	-	-	+	-	-
Streptomyces spp	+	-	-	-	-	-
S.caeruleus	+	-	+	-	-	-
S.viridochromogenes	+	+	+	+	-	+
S. grieseoleutus	+	-	+	+	-	-
S.clavifer	+	-	+	-	-	-

- - no growth
+ - poor growth
++ - good growth

The isolate SAV-01 was identified as *Streptomyces gibosonni,* SAV-04 as *S.caeruleus,* SAV-05 as *S.viridochromogenes* and SAV-06 and SAV-07 were identified as *S.grieseoleutus* and *S.clavifer* respectively. The isolates SAV-02 and SAV-03 didnot match with any of the previously described species and were preserved for further genetic studies.

The culture filtrates of these strains were used to study their effects on seed germination of black gram, maize, radish and *Bromus lyticus*. Isolate SAV 01 and SAV 06 induce germination of radish, maize, and gram. The range of germination after 7 days was observed. The length of shoot and root growth was found to be 1.3, 4.1., 0.67, 2.65., and 0.18, 4.1cm respectively. Similarly SAV06 treated seeds germinate to give 0.7, 3.9., 1.07, 3.5., 0.55, 3.15cm length of shoot and root growth. Very less growth was obtained in ease of seed treated with culture filtrate S*. caeruleus* (Table 6).

Table 6

The effects of culture filtrate of Streptomyces sp. on seed germination

Name of the seed and number	Radish (5n)						Black gram (5n)						Maize (5n)						Bromus lyticus
	Shoot growth(cm)			Root growth (cm)			Shoot growth (cm)			Root growth(cm)			Shoot growth(cm)			Root growth(cm)			Shoot& root growth
Name of the culture filtrate	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	
Streptomyces gibasonii	1.3	0.14	0.07	4.1	0.27	0.13	0.67	0.09	0.04	2.65	0.26	0.13	1.12	0.12	0.06	4.07	0.22	0.11	A
Streptomyces sp	0.6	0.05	0.02	2.6	0.18	0.09	0.7	0.08	0.04	2.22	0.05	0.02	0.5	0.11	0.05	2.77	0.15	2.17	P
Streptomyces. sp	0.4	0.05	0.02	1.9	0.17	0.08	0.6	2 .05	0.02	1.8	0.14	0.07	0.65	0.05	0.02	2.5	0.11	0.05	P
S. caeruleus	0.8	0.08	0.04	1.7	0.1	0.05	0.47	0.05	0.02	1.55	0.12	0.66	0.5	0.08	0.04	0.62	0.12	0.06	P
S. viridochromagens	07	0.09	0.04	2.72	0.15	0.12	0.62	0.05	0.02	2.5	0.11	0.05	1.02	0.12	0.06	2.65	0.26	0.13	A
S. griseleutus	0.6	0.09	0.04	2.9	0.09	0.04	1.07	0.09	0.04	3.55	0.05	0.02	0.53	0.05	0.02	3.15	0.19	0.09	A
S. clarifer	0.7	0.05	0.02	2.5	0.05	0.02	0.57	0.09	0.04	2.12	0.18	0.09	0.55	0.1	0.05	2.75	0.14	0.12	A
Control	0.8	0.08	0.02	3.9	0.12	0.06	0.62	0.12	0.06	2.1	0.14	0.07	0.87	0.09	0.04	2.52	0.25	0.12	P

***5n – No of seeds,* M – Mean value,* SD- Standard Deviation,* SE- standard Error**
*** P- Present,* A- Absent**

The isolate *S.viridochromogenes* and *S.clavifer* inhibited the growth of radish, maize, black gram and also of the herb *Bromus lyticus.* The isolates SAV-02, SAV-03 and SAV-4 did not enhance or inhibit seed germination of any crop or herb.

Those organisms inhibiting seed germination were tested against the lawn culture of *B.subtilis* with a filter paper disc impregnated in the appropriate culture filtrate. Here zone of inhibition was obtained for those isolates that inhibit seed germination.

DISCUSSION

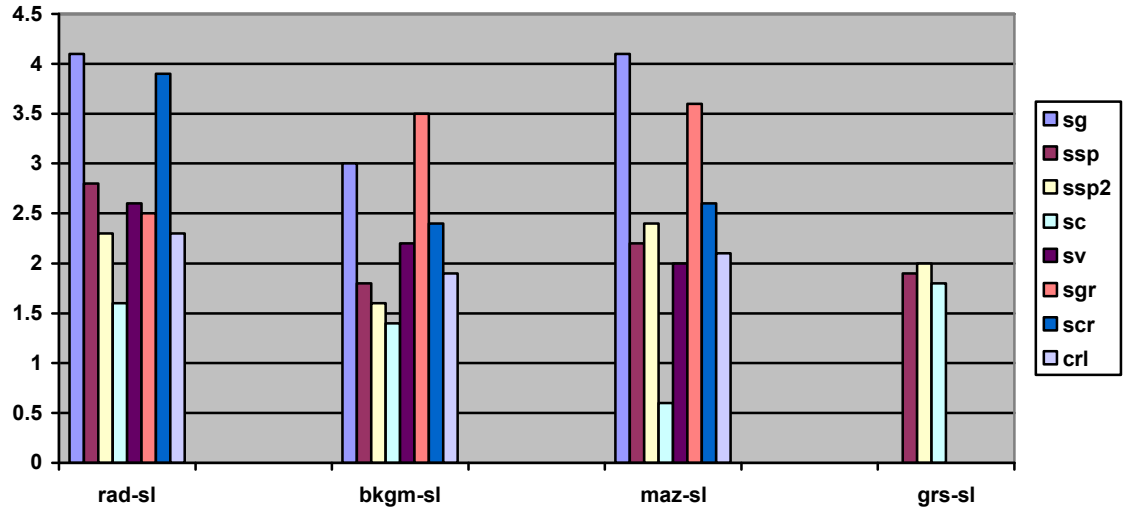
Those organisms inducing seed germination which is *S. gibosonni* and *S.grieseoluteus* when plated in Moller's medium showed dark red halo after 4 days of incubation at 28˚C. The compound responsible for red halo and growth is Putriscine. *S. gibosonni* and *S.grieseoluteus* produced putriscine which induced germination of the seeds. Polyamines such as putriscine, spermine and spermidine are ubiquitous polycationic compounds produced by most organisms and are found to be multi-functional and interact with biomolecules like DNA or proteins[13]. It is a polyamine involved in the control of cell cycle, cell division, morphogenesis, in phytochrome and plant hormone mediated processes as well as in plant responses to various stress factors [14].

Biosynthesis of cephamycin, an antibiotic is stimulated by putrescine in *Nocardia lactumdurans* [15]. It was found that incorporation of polyamines in the primary media enhanced the germination and early seedling growth in sunflower [16]. The putrescine activity improved seed germination, seed growth criteria (shoot and root length, dry and wet stress), hydrolytic enzyme activity.

The strains *S.viridochromogenes* and *S.clavifer* showed inhibitory effects. The compound responsible for seed germination inhibition and sensitive to *B.subtilis* is phosphinothricin. It is a tripeptide which is composed of two L-alanine residues and an analouge of glutamic acid [17,18]. Phosalacine, a new herbicidal antibiotic containing phosphinothricin from the culture filtrate of *Kitasatesporia phosalacinea KA338* was obtained by certain workers[19]. Reports show that an endophytic Streptomyces sp. 35 showed inhibitory activity against germination of wheat, mung bean and *Pasphleum notatum* [20]. Also it has been shown previously that *S.hygroscopicus* produce phosphinothricin which inhibit plant growth by accumulation of ammonium ions due to inhibition of glutamate synthatase enzyme activity [21], while the intact molecule has little or no inhibitory effect [22] .The heterologous expression of phosphinothricin tripeptide biosynthetic gene cluster from *S.viridochromogenes* DSM40376 were investigated [23]. This result correlated to our finding.

CHART 1

Effect of Streptomyces culture filtrates on various crop seed germination (growth of shoot in cm)

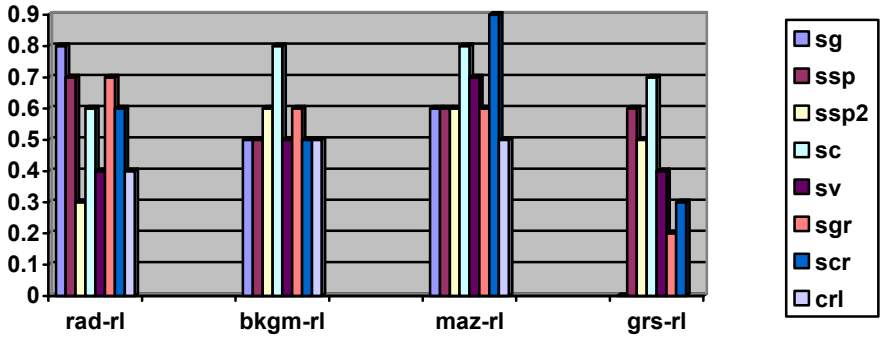


sg – *Streptomyces gibosoni*
ssp – *Streptomyces sp*
ssp2 – *Streptomyces sp*
sc – *Streptomyces caeruleus*
sv – *Streptomyces viridochromogenes*
sgr – *Streptomyces grieseoluteus*
scr – *Streptomyces clavifer*

rad-sl – radish shoot length
bkgm-sl – blackgram shoot length
maz-sl – maize shoot length
grs-sl – grass shoot length

CHART 2

Effect of Streptomyces culture filtrates on various crop seed germination (growth of root in cm)



sg – *Streptomyces gibosoni*
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sv – *Streptomyces viridochromogenes*
sgr – *Streptomyces grieseoluteus*
scr – *Streptomyces clavifer*

rad-rl – radish root length
bkgm-rl – black gram root length
maz-rl – maize root length
grs-rl – grass root length

CONCLUSION

From this work it is finally concluded that *S.gibosonni* and *S.grieseoletus* induce seed germination by the production of polyamines while S.*viridochrogenes* and *S.clavifer* inhibited the germination of seeds by the production of phosphinothricin. This study will lead and support to produce biopesticides and biocompounds for improving growth and yield of economically valuable plants.

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