European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 15-01-2020; Accepted: 16-02-2020; Published: 19-02-2020
www.biosciencejournals.com
Volume 8; Issue 1; 2020; Page No. 30-36

Isolation and characterization of *Streptomyces s*pp showing antagonistic activity against fungal pathogens effecting soybean crop

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Abstract
A total 93 cultures of *Actinomycetes* were screened for their antagonistic activity against major fungal pathogens of soybean crop. Viz., *Colletotrichum lindemuthianum*, *Fusarium oxysporum,* and *Rhizoctonia solani*. Dual culture assay was performed**.** Glucose Casaminoacid yeast extract agar (GCY) plates were prepared. A 5mm fungal discs were placed on the one edge (1cm from the corner) with *Actinomycetes* streaked on the other edge of the plate (1cm from the corner) followed by incubation at 28 ± 2°C for four days. Control plate growth has covered the whole plate. The inhibition of fungal mycelium was measured as the distance of inhibition in cm. All the plates were maintained in triplicates and mean values were taken. Three isolates namely NAVS13, KNSS3, CHSS3 inhibited *C. lindemuthianum, F. oxysporum,* and *R. solani*. The isolates were further evaluated for Cellulase, Lipase, Protease, HCN, phosphate solubilization, production of growth hormone IAA, production of Siderophore, Chitinase, β-1, 3-Glucanase, and physiological properties. The isolates were subjected to molecular characterization and identified as *Streptomyces rochei, Streptomyces cavourensis* and *Streptomyces alfalfa.*

Keywords: soybean, actinomycetes, fungal pathogens, PGP traits, antagonistic activity

Introduction

Soybean (*Glycine max* (L.) is originated from East Asia. It is cultivated as one of the important pulse crops in India with a production of 15.68Metric tons in an area of 3.89 lakh hectares. However, the production can be still enhanced by limiting the problems due to diseases caused to Soybean especially from *Colletotrichum lindemuthianum, Fusarium oxysporum,* and *Rhizoctonia solani*. The fungal diseases cause more than 50 % loss to Soybean cultivation leading to huge economic losses (Agrio 2000) [1]. In order to combat the diseases, the farmers are highly dependent on various harmful chemicals which are not recommended when the environment and human health is concerned.

The environment-friendly options include the use of plant growth promoter's produced by microbes and vermicompost (Rupela *et al.,* 2005) [9]. It is evident from the past research that certain bacterial species such as *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp, and *Streptomyces* spp., acted as bio-control agents against the pathogenic fungi. All these strains were isolated from the fields of cultivation and from the vermicompost (Gopalakrishnan *et al.,* 2011a) [2]. The soil contains a wide range of bacteria that are useful to the plants by enhancing the production of plant growth promoting substances, acts as antagonists against pathogenic fungi (Alekhya *et al.,* 2017) [2]. Vermicomposting is the process of biological degradation and conversion of agricultural and household wastes to use manure to plants. Vermicomposting improves the recycling of waste with environmentally safe and pollution free approach. It has the capacity to inhibit the growth of plant pathogens, to mobilize the nutrients, etc. (Postma *et al.,* 2003; Gopala krishnan and Rupela 2011a, c.) [18, 9] Hence, in the present study *Actinomycetes* sps were collected from both soil and vermicompost to check the presence of useful plant growth promoting substances that will enhance the disease resistance capacity of plants and nutrient uptake capability.

Materials & Methods

The soil samples were collected from Soybean (Glycine max (L.) Merr.) cultivated fields in both Kharif and Rabi seasons. The samples were collected up to 30cm depth of the rhizosphere soil and stored in sterile plastic bags, kept at room temperature. Samples were collected from different villages of Guntur district, Andhra Pradesh, India and are given codes as NAVS, KNSS, CHSS

Isolation of *Actinomycetes*

Ten grams of rhizosphere soil and vermicompost samples were added separately in to 90ml saline solution (0.85% of NaCl). The flasks were placed on orbital shaker with 100rpm for 1 hour at room temperature. At the end of the shaking, the samples were serially diluted up to10^{-4} to 10^{-6} respectively. Taken 0.1 ml of suspension spread on the *Actinomycetes* Isolation Agar (AIA) plate using spread plate technique. The plates were incubated at 28 ± 2°C for four days. The colonies were observed for morphology and physical properties, Grams staining (Sreevidya *et al.,* 2016) [23]. The pure cultures were subcultured as three replicates and maintained on Starch Casein Agar slants at 4°C for further studies.

In-Vitro Antifungal Activity by Dual Culture Assay (DCA)

A total 93 cultures of *Actinomycetes* were screened for their antagonistic activity against major fungal pathogens of Soybean crop. Viz., *Colletotrichum lindemuthianum*, *Fusarium oxysporum,* and *Rhizoctonia* solani acquired from MTCC (Microbial Type Culture Collection and Gene Bank, Chandigarh, India). Dual culture assay was performed as per the protocol of Anjaiah *et al.,* 1998 [4]**.** Glucose

Casaminoacid yeast extract agar (GCY) plates were prepared. A 5mm fungal discs were placed on the one edge (1cm from the corner) with *Actinomycetes* streaked on the other edge of the plate (1cm from the corner) followed by incubation at 28 ± 2°C for four days. Control plate growth has covered the whole plate. The inhibition of fungal mycelium was measured as the distance of inhibition in cm. All the plates were maintained in triplicates and mean values were taken.

Plant growth promoting and enzymatic activity

Cellulase production

Production of cellulase was estimated as per the protocol of Hendricks *et al.,* 1995 [12]. Cellulose Congo red agar plates were prepared and 6mm wells were made with borer. *Actinomycetes* (20 to 50µL) cultures were added and incubated at 28 ± 2°C for five days. Formation of the halo zone around the *Actinomycetes* colonies indicates the production of cellulase. Production was recorded on a 0-5 scale as follows: 0 = no halo zone; 1 = halo zone of 1–10 mm; 2 = halo zone of 11–20 mm; 3 = halo zone of 21–30 mm; 4 = halo zone of 31–40 mm; and 5 = 41–50 mm.

Lipase production

Production of lipase was estimated as per the protocol of Bhattacharya *et al.,* 2009 [6]; Gopalakrishnan *et al.,* 2011 a [2]. Tween 80 agar media was prepared and 6mm wells were made with borer. *Actinomycetes* (20 to 50µL) cultures were added and incubated at 28 ± 2°C for five days. Formation of halo zone around the cultures was identified as positive for Lipase production. Observations were record as 0-5 scale as follows: 0 = no halo zone; 1 = halo zone of the 1–10 mm; 2 = halo zone of 11–20 mm; 3 = halo zone of 21–30 mm; 4 = halo zone of 31–40 mm; and 5 = 41–50 mm.

Protease production

Production of protease was measured as per the protocol of Bhattacharya *et al.,* 2009 [6]: Gopalakrishan *et al.,* 2011 a. Casein agar media were prepared and 6mm wells were made with borer. *Actinomycetes* (20 to 50µL) cultures were added and incubated at 28 ± 2°C for five days. At the end of the incubation, the plates were observed for the halo zone around the colonies. Production was recorded on a 0-5 scale as follows: 0 = no halo zone; 1 = halo zone of 1–10 mm; 2 = halo zone of 11–20 mm; 3 = halo zone of 21–30 mm; 4 = halo zone of 31–40 mm; and 5 = 41–50 mm.

β- 1, 3-glucanase assay

The protocol for the β-glucanase assay was carried out according to the protocol of Singh *et al.,* 1999. The *Actinomycetes* were inoculated into Tryptic soy broth supplemented with 2% colloidal chitin. The three-day-old cultures were centrifuged at 10,000×g for 12mins and supernatant were collected. To 1mL of the supernatant, 0.1mL of Laminarin (2%wt /Vol) was added and incubated at 40°C for 1hour. Further, the reaction was stopped by adding 3mL of Di Nitro salicylic acid (DNS) reagent and kept them on boiling water bath for 10minutes. The color change from yellow to red indicated the presence of glucose produced by the activity of β-glucanase. Concentration of Enzyme activity was measured by taking absorbance at 530 nm.

Chitinase assay

Production of chitinase was estimated as per the protocol of Gopalakrishnan *et al.,* 2011 [2]; Hsu and Lockwood 1975. Minimal media containing chitin was prepared and 6mm well was made with the borer. *Actinomycetes* culture (20-30µL) was inoculated and incubated at 28 ± 2°C for five days. The chitinase produced by *Actinomycetes* breaks down the chitin present in the medium and this was measured and recorded as scale 0 to 5 where 0= No change; 1 = halo zone of 1–5 mm; 2 = halo zone of 6–10 mm; 3 = halo zone of 11–15 mm; 4 = halo zone of 16–20 mm; and 5 = 21–25 mm.

Indole acetic acid (IAA) production

Actinomycetes isolates were inoculated on Starch casein broth amended with L-tryptophan and incubated at 28±2 ºC with 180 rpm for five days. The culture was centrifuged at 10000×g for 15minutes, the supernatant was collected. 2mL of salkowsky's reagent was added into 1mL of supernatant and incubated for 30 min in a dark place. The color change to pink color after incubation indicates the presences of IAA. The amount of IAA produced by the culture was estimated by measuring absorbance at 530 nm. A standard curve was plotted to quantify the IAA (µg ml^{-1}) Patten and Glick1996; Gopalakrishnan *et al.,* 2011a [2].

Hydrocyanic acid (HCN) production

Presence of HCN was estimated qualitatively by the method of Lorck 1948. For this purpose, Bennet's agar with glycine (4.4 g l^{-1}) was prepared, poured into plates and *Actinomycetes* was streaked. Sterile Whatman filter paper no.1 (8 cm diameter) was soaked in 1% Picric acid and sprayed with 1mL of 10% Sodium carbonate and stuck underneath the Petri dish lids. The plates were sealed with Parafilm and incubated at 28±2 ºC for five days. The appearance of reddish brown color on the filter paper indicates the positive for HCN production. The intensity of color was measured and noted as scale 0 to 3 which indicates 0 = no color change; 1= positive (or) light reddish brown; 2 = medium reddish brown and 3 = dark reddish brown.

Siderophore production

The production of Siderophore was carried out by using the protocol of Macgnan *et al.,*2008; Schwyn and Neilands 1987 [21]. Kings'B broth was prepared and inoculated with *Actinomycetes* and incubated at 150 rpm in room temperature for four to five days. After incubation, the culture was centrifuged at 5000×g for12minutes and supernatant was collected. To 1mL of the supernatant, an equal amount of Chrome Azurole Sulphate (CAS) solution was added and incubated in the dark for 30minutes. The color change from blue to pink indicated the presence of siderophore. The amount of siderophore produced by the culture was estimated by measuring absorbance at 630 nm. The Siderophore production was estimated using the formula

$$\% \; of \; Siderophore \; units = A_r - A_s / A_r \times 100$$

Where, $A_r =$ Reference of OD value (un inoculated media + CAS solution)

$A_s =$ reference standard value(culture supernate + CAS solution)

Phosphate solubilization
Phosphate solubilization was estimated as per the protocol of Gyaneshwar *et al.,* 1998 [11]; Alok Ranjan *et al.,* 2013. Rock phosphate buffer media was prepared and 6 mm wells were made with borer. *Actinomycetes* (20 to 50µL) cultures were added and incubated at 28 ± 2°C for five to seven days. At the end of the incubation, the plates were observed for the halo zone around the colonies, production was recorded on a 0-5 scale as follows: 0 = no halo zone; 1 = halo zone of 1–10 mm; 2 = halo zone of 11–20 mm; 3 = halo zone of 21–30 mm; 4 = halo zone of 31–40 mm; and 5 = 41–50 mm.

Molecular identification of selected *Actinomycetes*
The pure cultures of *Actinomycetes* were grown in starch casein broth medium and genomic DNA was isolated according to the protocol of Bazzicalupo and Fani 1995 [5]. The isolated DNA was tested qualitatively and quantitatively and then sent to Barcode biosciences, Bangalore for 16s rRNA sequencing. The sequence obtained from Biosciences was compared with similar sequences of Gen bank by using the BLAST program Altschul *et al.,*1990 [3]. The obtained results were aligned using the Clustal W software Thomposon *et al.,*1997. The phylogenetic tree was constructed by the neighbor-joining method using Mega X software. (Saitou and Nei 1987; Tamura *et al.,* 2007) [20, 24].

Experimental Method
1. DNA was isolated from the culture provided by the scientist. Its quality was evaluated on 1.0 %agarose gel, a single band of high-molecular weight DNA has been observed.
2. Fragment of 16S rRNA gene was amplified by 16SrRNA-F and 16SrRNA-R primers. A single discretePCR amplicon band of 1300 bp was observed when resolved on agarose gel.
3. The PCR amplicon was purified to remove contaminants.
4. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 16SrRNA-Fand 16SrRNA-R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.
5. Consensus sequence of 16S rRNA gene was generated from forward and reverse sequence data using aligner software.
6. The 16S rRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 6.

Results
Selection of *Actinomycetes* with antagonistic activity against fungal pathogens
A total of 93 isolates were isolated from different soil samples in the rhizosphere of Soybean. Among these 93 isolates, only three isolates were significantly inhibiting fungal pathogens. In dual culture assay, the three isolates significantly inhibited the *Fusarium oxysporum* (FOC), in the range of 1.7- 2.7cm, exhibited the antagonistic activity against *Colletotrichum lindemuthianum* in the range 0.9-1.8cm, inhibited *Rhizoctonia solani* in the range of 1.2-2.5cm when compared to control.

Plant growth promoting and enzymatic activity
In *in-vitro* studies, selected isolates showed effect on plant growth promoting attributes such as cellulase production (except DVS-11), lipase and protease production in all isolates, β-1,3-glucanase production up to 25.9-32.3, chitinase production, IAA production range is between them 18.2 to 81 µg/ml. HCN, siderophore, phosphate solubilization is found in all the isolates.

Molecular Identification
16S rDNA sequences of three promising antagonistic *Actinomycetes* were analyzed. When running the sequences in NCBI BLAST all the three isolates showed similarity to *Streptomyces* sp., with 98% similarity. Then compared with the sequences in NCBI and EzTaxon using BLAST program, aligned sequences with Clustal W software and constructed the phylogenetic tree (Dendrogram) by the neighbor-joining method. Sample which was labelled as NAVS-13 was found to be *Streptomyces rochei*, sample which was labeled as KNSS-3 was found to be *Streptomyces cavourensis*, sample which was labeled as CHSS-3 was found to be *Streptomyces alfalfae* showed high similarity with Top based on nucleotide homology and phylogenetic analysis.

Table 1: Antagonisticactivityagainst three fungal pathogens

Isolates	*Colletotrichum lindemuthianum*	*Fusarium oxysporum*	*Rhizoctonia Solani*
NAVS-13	1.6	1.9	1.5
KNSS-3	1.9	1.6	1.6
CHSS-3	1.8	1.7	1.7
Control	0	0	0
Mean	1.32	1.3	1.20
SE (Standard Error)	0.51	0.50	0.46
SD (Standard Deviation)	0.89	0.87	0.80
CV (coefficient of variation)	0.79	0.76	0.64

In vitro selected actinomycetes are showing antagonistic activity against major fungal pathogens in soybean crop. Values are the mean of 3 experiments (n = 3), each with 3 replicates per treatment. Means were subjected to factorial ANOVAs separate means.

Table 2: Enzymatic assay

Isolates	Cellulase (mm)	Lipase (mm)	Protease (mm)	HCN	IAA µg ml-1	Siderophore units	Phosphate (mm)	Chitinase (mm)	β- 1,3 glucanase units@
NAVS-13	18 mm	16 mm	18 mm	2	18.3	52.6	16 mm	17 mm	28.9
KNSS-3	16 mm	12 mm	19 mm	0	64.3	39.8	20 mm	16 mm	38.6

CHSS-3	14 mm	16 mm	18 mm	1	29.6	42.9	18 mm	18 mm	42.8
Control	0	0	0	0	0	0	0	0	0
Mean	12	11	13.75	0.75	28.05	33.82	13.5	12.75	27.57
SE (Standard Error)	4.71	4.37	5.299	1.85	15.62	13.39	5.28	4.93	11.13
SD (Standard Deviation)	66.67	57.33	84.25	2.75	27.06	23.19	83.66	72.91	371.82
CV (coefficient of variation)	68.04	68.84	66.75	127.65	96.51	68.59	67.76	66.97	69.93

In vitro PGP traits of selected *Actinomycete* isolates were observed. HCN, hydrocyanic acid; IAA, indole acetic acid; siderophore, cellulase, lipase and protease production as: 0-no halozone; 1-halozone of<1mm; 2- halozone of 1–3mm; 3-halozone of 4–6mm and 4-halozone of 7mm and above; (ii) HCN production as: 0-no colour change; 1-light reddish brown; 2-medium reddish brown and 3-dark reddish brown; (iv) -1,3-glucanase (U)– one unit is an amount of enzyme that liberated 1 mol of glucose hour^{-1} at defined conditions. Value son secondary (right) axis indicates IAA production. Values are the mean of 3 experiments (*n*=3), each with 3 replicates per treatment. Means were subjected to factorial ANOVAs separate means.

Table 3: salinity concentration (Nacl%)

Isolates N	**0%**	**2%**	**4%**	**6% N**	**8%**	**10%**	**12%**
NAVS-13	2	3	2	2	3	2	1
KNSS-3	3	2	2	3	2	1	1
CHSS-3	2	2	1	3	3	1	2
Control	0	0	0	0	0	0	0
Mean	1.75	1.75	1.25	2.00	2.00	1.00	1.00
SE (Standard Error)	0.72	0.72	0.55	0.81	0.81	0.47	0.47
SD (Standard Deviation)	1.58	1.58	0.83	2.00	2.00	0.66	0.66
CV (coefficient of variation)	71.9	71.9	66.33	70.71	70.71	81.65	81.65

The selected *Actinomycetes* were tested in different salts concentrations. Values are the mean of 3experiments (*n*=3), each with 3 replicates per treatment. Means were subjected to factorial ANOVAs separate means.

Table 4: Effect of Temperature and pH

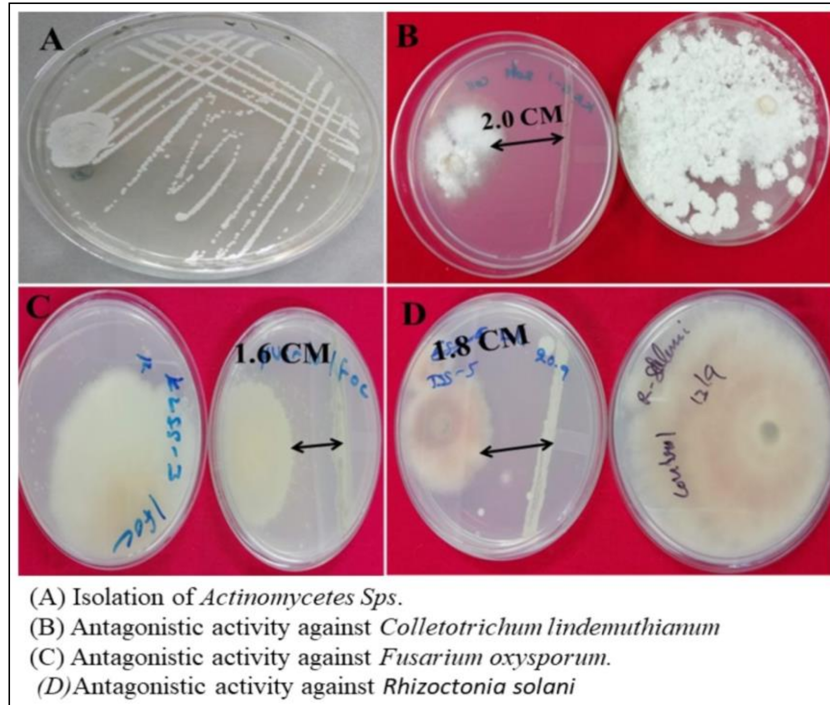
Isolates	**20oC**	**30oC**	**40oC**	**50oC**	**pH-5**	**pH-7**	**pH-9**	**pH-11**	**pH-13**
NAVS-13	2	2	2	00	00	2	2	2	00
KNSS-3	3	3	3	00	00	3	3	3	00
CHSS-3	3	2	1	00	00	3	2	2	00
Control	00	00	00	00	00	00	00	00	00
Mean	2.0	2	1.5	00	00	2	1.75	1.75	00
SE (Standard Error)	0.81	0.72	0.74	00	00	0.816	0.72	0.72	00
SD (Standard Deviation)	2.0	2	1.67	00	00	2	1.58	1.58	00
CV (coefficient of variation)	70.71	71.90	86.07	00	00	70.71	71.9	71.9	00

The selected *Actinomycetes* are tested at different temperature and pH conditions. Values are the mean of 3 experiments (*n*=3), each with 3 replicates per treatment. Means were subjected to factorial ANOVAs separate means.

Table 5: Effect of carbon sources

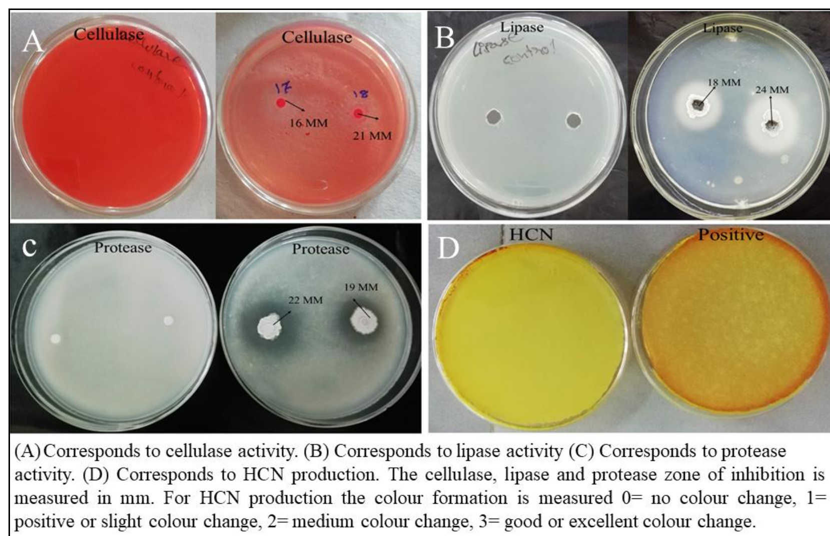
Isolates	**Glucose**	**Sucrose**	**Fructose**	**Starch**
NAVS-13	2	2	2	00
KNSS-3	3	3	3	00
CHSS-3	3	2	1	00
Control	00	00	00	00
Mean	2.0	2	1.5	00
SE (Standard Error)	0.81	0.72	0.74	00
SD Standard Deviation)	2.0	2	1.67	00
CV (coefficient of variation)	70.71	71.90	86.07	00

The selected actinomycetes Effect of carbon sources. Values are the mean of 3 experiments (n = 3), each with 3 replicates per treatment. Means were subjected to factorial ANOVAs separate means Antagonistic activity against three fungal pathogens



(A) Isolation of *Actinomycetes Sps*.
(B) Antagonistic activity against *Colletotrichum lindemuthianum*
(C) Antagonistic activity against *Fusarium oxysporum*.
*(D)*Antagonistic activity against *Rhizoctonia solani*

Fig 1



(A) Corresponds to cellulase activity. (B) Corresponds to lipase activity (C) Corresponds to protease activity. (D) Corresponds to HCN production. The cellulase, lipase and protease zone of inhibition is measured in mm. For HCN production the colour formation is measured 0= no colour change, 1= positive or slight colour change, 2= medium colour change, 3= good or excellent colour change.

Fig 2

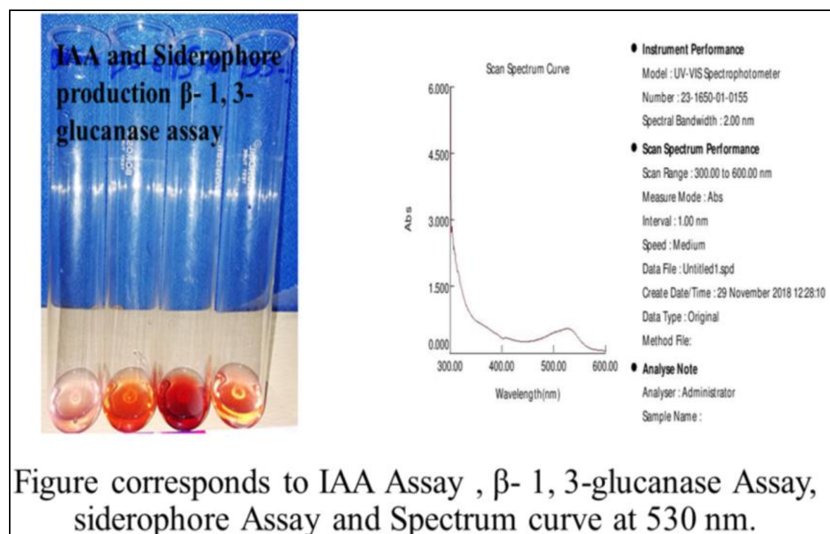
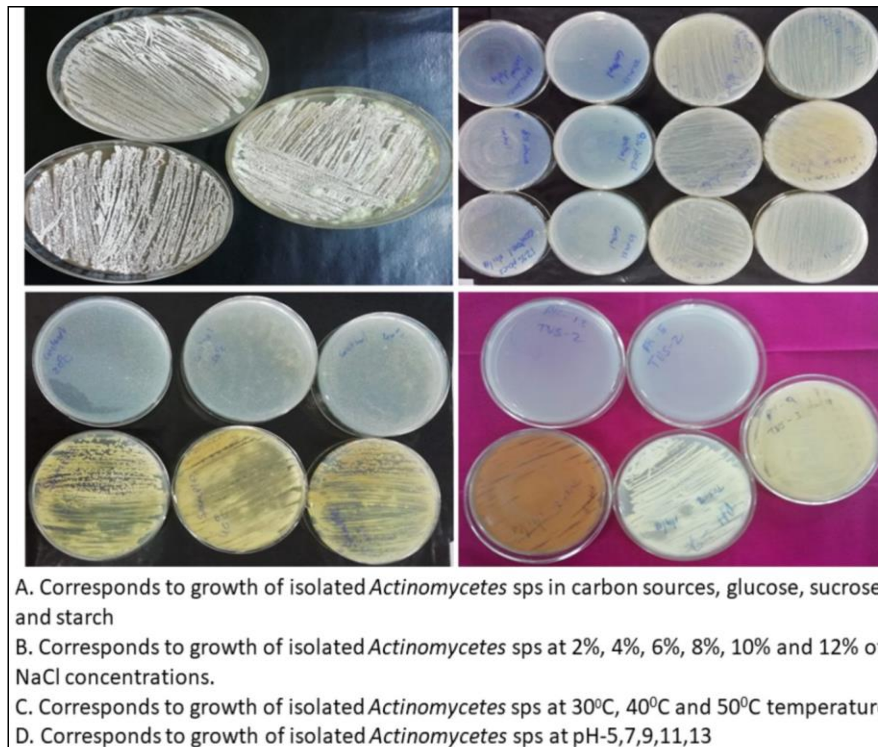


Figure corresponds to IAA Assay , β- 1, 3-glucanase Assay, siderophore Assay and Spectrum curve at 530 nm.

Fig 3



A. Corresponds to growth of isolated *Actinomycetes* sps in carbon sources, glucose, sucrose and starch
B. Corresponds to growth of isolated *Actinomycetes* sps at 2%, 4%, 6%, 8%, 10% and 12% of NaCl concentrations.
C. Corresponds to growth of isolated *Actinomycetes* sps at 30^0C, 40^0C and 50^0C temperature.
D. Corresponds to growth of isolated *Actinomycetes* sps at pH-5,7,9,11,13

Fig 4

Conclusion

Out of the 93 *Actinomycetes* isolates obtained from different soil samples collected in the fields of Soybean, and vermicompost only 29 isolates were found to have antagonistic activity. When the 29 *Actinomycetes* isolates were further evaluated for antagonistic activity against three fungal pathogens namely *C.lindemuthianum, F.oxysporum and R.solani* using dual culture assay method, three isolates namely NAVS13, KNSS3, CHSS3 inhibited *C.lindemuthianum, F.oxysporum,* and *R.solani*. From these results it can be concluded that NAVS13, KNSS3 and CHSS3 have good antagonistic activity and inhibit the three major fungal pathogens. The isolates were further evaluated for Cellulase, Lipase, Protease, HCN, phosphate solubilization, production of growth hormone IAA (Correa *et al.,* 2005), production of Siderophore, Chitinase, β-1, 3-Glucanase and physiological properties. The isolates were identified by molecular characterization as *Streptomyces rochei, Streptomyces cavourensis*and *Streptomyces alfalfa.*

Reference

1. Agrios GN. Significance of plant diseases. Plant Pathology. Academic Press, London, 2000, 25-37.
2. Alekhya G, Gopalakrishnan S. Biological control and plant growth-promotion traits of Streptomyces species under greenhouse and field conditions in chickpea. Agricultural Research. 2017; 6(4):410-420.
3. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of molecular biology. 1990; 215(3):403-410.
4. Anjaiah V, Koedam N, Nowak-Thompson B, Loper JE, Höfte M, Tambong JT, *et al*. Involvement of phenazines and anthranilate in the antagonism of Pseudomonas aeruginosa PNA1 and Tn 5 derivatives toward Fusarium spp. and Pythium spp. Molecular Plant-Microbe Interactions. 1998; 11(9):847-854.
5. Bazzicalupo M, Fani R. The use of RAPD for generating specific DNA probes for microorganisms. In. Clap JP (ed.) Methods in molecular biology, species diagnostic protocols: PCR and other nucleic acid methods, 1995.
6. Bhattacharya A, Chanda S, Barik S. Lipase and protease producing microbes from the environment of sugar beet field. Indian Journal of Agricultural Biochemistry. 2009; 22(1):26-30.
7. Donate-Correa J, León-Barrios M, Pérez-Galdona R. Screening for plant growth-promoting rhizobacteria in Chamaecytisusproliferus (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant and Soil. 2005; 266(1-2):261-272.
8. Gopalakrishnan S, Kiran BK, Humayun P, Vidya MS, Deepthi K, Jacob S, *et al*. Biocontrol of charcoal-rot of sorghum by actinomycetes isolated from herbal vermicompost. African Journal of Biotechnology. 2011; 10(79):18142-18152.
9. Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Rupela O, *et al*. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of Fusarium wilt of chickpea. Crop Protection. 2011; 30(8):1070-1078.
10. Gopalakrishnan S, Srinivas V, Alekhya G, Prakash B, Kudapa H, Rathore A, Varshney RK. The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum Streptomyces sp. in chickpea. Springer Plus. 2015; 4(1):31.
11. Gyaneshwar P, Kumar GN, Parekh LJ. Effect of buffering on the phosphate-solubilizing ability of microorganisms. World Journal of Microbiology and Biotechnology. 1998; 14(5):669-673.
12. Hendricks CW, Doyle JD, Hugley B. A new solid medium for enumerating cellulose-utilizing bacteria in

soil. Appl. Environ. Microbiol. 1995; 61(5):2016-2019.
13. Hsu SC, Lockwood JL. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl. Environ. Microbiol. 1975; 29(3):422-426.
14. Lorck H. Production of hydrocyanic acid by bacteria. Physiologia Plantarum. 1948; 1(2):142-146.
15. Macagnan D, Romeiro RDS, Pomella AW, deSouza JT. Production of lytic enzymes and siderophores, and inhibition of germination of basidiospores of Moniliophthora (ex Crinipellis) perniciosa by phylloplane actinomycetes. Biological Control. 2008; 47(3):309-314.
16. Pal KK, Tilak KVBR, Saxcna AK, Dey R, Singh CS. Suppression of maize root diseases caused by Macrophominaphaseolina, Fusarium moniliforme and Fusarium graminearum by plant growth promoting rhizobacteria. Microbiological research. 2001; 156(3):209-223.
17. Patten CL, Glick BR. Bacterial biosynthesis of indole-3-acetic acid. Canadian journal of microbiology. 1996; 42(3):207-220.
18. Postma J, Montanari M, van den Boogert PH. Microbial enrichment to enhance the disease suppressive activity of compost. European journal of soil biology. 2003; 39(3):157-163.
19. Rupela OP, Gupta AP, Kapoor KK. Is high yield possible with biological approaches. Management of organic wastes for crop production. CCS Haryana Agricultural University, Hisar, Haryana, 2005, 193-203.
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution. 1987; 4(4):406-425.
21. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical biochemistry. 1987; 160(1):47-56.
22. Singh PP, Shin YC, Park CS, Chung YR. Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology. 1999; 89(1):92-99.
23. Sreevidya M, Gopalakrishnan S, Kudapa H, Varshney RK. Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. brazilian journal of microbiology. 2016; 47(1):85-95.
24. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular biology and evolution. 2007; 24(8):1596-1599.
25. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research. 1997; 25(24):4876-4882.