Isolation, Screening and Molecular Characterization of *Streptomyces* sp. for the production of IAA

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ABSTRACT:

Indole acetic acid is one of the most significant physiologically active auxins. Indole Acetic Acid (IAA) is a typical product of L-tryptophan metabolism and is produced by several microorganisms, particularly Plant Growth- Promoting Rhizobacteria. The bacterial enzyme tryptophanase can cause the oxidation of Tryptophan, an essential amino acid. The present work deals with isolation and screening of Streptomyces sp. for IAA production. A total of 18 Streptomyces spp. were isolated from the rhizosphere soil of maize. Colorimetric estimation was carried out for the estimation of IAA at 530nm using Salkowsky reagent. The equations obtained from the standard curve were used to determine the IAA concentration produced by the isolates. The isolate SDSRO-2 used to have the highest IAA production (Indole-3-Acetic Acid). The culture filtrate of spore size 10^6 mg/ml was used for the In vitro seed treatment. The growth parameters such as percentage of seed germination, root length, shoot length, and vigour index were measured with the control. The isolate SDSRO-2 showed good results compared to control treated with distilled water.The conclusion is that the selected strain of Streptomycessp. SDSRO-2 can be used to promote the growth of the crop plants.

KEYWORDS: *Streptomyces* sp. Indole-3- acetic acid, Molecular characteristics, *In vitro* seedling tests.

INTRODUCTION:

Actinomycetes are Gram-positive, aerobic, spore-forming microorganisms that form aerial and substrate mycelium. They are classified as belonging to the Actinomycetales order. They are the most common creatures, forming thread-like soil filaments and emitting the distinct, "earthy" odor of soil[1]. Actinomycetes were long been recognized as a significant source of biological compounds of agricultural use. Employing microbial consortia in the form of bio fertilizers to reduce the usage of chemical fertilizers is now a hot topic for research in the fields of agriculture, microbiology, and biotechnology[2].

Phytohormones are those of carbonic composites which are able to control physiological processes of plants in low densities[3]. Plants respond to many environmental factors such as light, gravity, water, inorganic nutrients, and temperature. Different hormones affect different plant processes[4]. These microorganisms support is essential for growth and development through a wide range of mechanisms (direct and indirect), including the recycling of nutritional elements, atmospheric nitrogen fixation, mineral solubilization (phosphorus), and phytohormone production (e.g. auxins, cytokinins, and gibberellins). These microorganisms also assist the plant in trying to cope with a variety of biotic and abiotic stresses through a variety of mechanisms, such as 1 aminocyclopropane carboxyinsecticides, and associated agrochemicals, without affecting the plant yield[5]. 1 Aminocyclopropane carboxylate (ACC) deaminase activity can cause the deposition of harmful ions in certain plants and restricts water intake. It also releases enzymes and produces siderophores. Plant growth-promoting (PGP) strains can be a useful strategy for increasing crop output and reducing the negative effects of salt stress[6]

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Owing to its significance as a phytohormone, auxin controls practically all aspects of plant growth and development[7]. Based on the coleoptiles curvature bioassay, the name "auxin," which comes from the Greek word "auxein," which means "to grow or to expand," was developed to characterize compounds having signaling action. The true auxin was ultimately revealed to be heteroauxin, which was chemically determined to be indole-3-acetic acid (IAA).

The potential of the bacterial isolates from the rhizosphere region of various crops to synthesize indole acetic acid as a secondary metabolite is induced by the availability of substrates in this region[8]. Actinomycetes have primarily been exploited in the pharmaceutical industry since the 1940s, but relatively few have been created as industrial plant products for use in agriculture[9]. *Streptomycetes* were long been thought of as purely free-living soil organisms, but more subsequently, the significance of their complex interactions with plants and other species has been recognized[10].

Materials and methods:

Isolation and Screening of *Streptomyces* spp. for IAA production

A soil sample from the rhizosphere was obtained from a field of maize. 1% CaCO3 was incorporated into one g of soil to grow actinomycetes till 10^{-6}, the soil was serially diluted on a medium of starch, casein, and nitrate. Plates were inoculated with 1.0 ml of a soil sample suspension individually and incubated for 14 days at 28° C. Actinomycetes colonies were carefully isolated from the plates after 14 days to avoid bacterial or fungal contamination. For 14 days, the isolated plates were maintained at 28 °C. Purified actinomycetes colonies were incubated at 28 °C for seven days. Actinomycete pure cultures were maintained in suspensions of 20% glycerol at -20° C[11].

Screening of *Streptomyces* sp. for IAA production

Colorimetric estimation of *Streptomyces* spp. for IAA production

The Patten and Glick (2002)[12] approach was modified in order to calculate the IAA content. In 100 ml flasks with 25 ml ISP-2 media supplemented with 0.2% L-Tryptophan at 28º C for 5 days on a rotary shaker, 100 micro liter spore suspension (10^6 CFU/ ml) was produced. Centrifugation was employed to separate the cells for 15 minutes at 10,000 rpm. Finally, the supernatant was treated with 2 ml of Salkowski reagent. In a UV-Vis Spectrophotometer, the pink auxin complex's absorbance was measured at 530 nm. The standard IAA curve was produced using dilutions of a standard IAA solution and the uninoculated medium with the reagent as a control[13].

IAA Standard curve

The IAA concentration obtained by isolates was calculated using equations derived from the standard curve. The isolates were inoculated with 45 ml of ISP-2 medium that was 0.2% L-tryptophan enriched, and were then incubated in a Shaker for five days to determine the IAA concentration. For the production of IAA, 1 ml of filtered supernatant and 1 ml of salkowski reagent was added and left for 30 minutes in dark conditions for the development of pink color. The absorbance is read at a wavelength of 530 nm using a spectrophotometer. IAA concentration in the media was calculated using the IAA standard curve[14].

Fermentation

The isolate which showed highest IAA production was grown in 250ml Erlenmeyer flask containing 100ml of ISP-2 broth medium. The flask was inoculated with *Streptomyces* sp. SDSRO-2 and incubated at 28±2° C for 7days. After incubation, the broth was filtered through Whatmann filter paper and the cell free suspension was used for seed treatment for the PGP activity[15].

Inoculum preparation for *In vitro* seed germination assay

Streptomyces isolate SDSRO-2 was grown on the ISP2 agar at 30°C for one week. The spore suspension was adjusted to 10^6 spores/mL.

***In-vitro* activity of *Streptomyces* sp. SDSRO-2 for IAA production**

Four varieties of seeds were used, Chilli, Wheat, Maize and Sorghum seeds were surface sterilized by using the method of Khalid *et al.* (2004). Sterilized seeds were soaked for 1h in the suspension and dried under laminar flow hood overnight. For control, seeds were dipped in distilled water only. Germination assay was performed by using *Streptomyces* isolate SDSRO-2 treated, Chilli (*Capsicum annuum*), wheat (*Triticum aestivum*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) seeds were surface sterilized according to the protocol described by Indananda *et al*. (2010). Four healthy seed varieties were cleaned soaked in distilled water for an hour, surface sterilized with 70% ethanol for 5 minutes, treated with 2% H2O2 for 5 minutes,

and then given five washes with sterile distilled water. The seeds were placed on moist sterile tissue paper and stored at 28°C. The seeds were supplied with 2 ml distilled water for 2–3 days to promote seed germination under dark conditions. After one week, effect of *Streptomyces* isolate SDSRO-2 on root length, shoot length and number of roots were observed.

16S rRNA Analysis

Using a spin column kit (made by HiMedia, India, or comparable manufacturers), chromosomal DNA was extracted. 16S rRNA gene of bacteria (1500 bp)[18]. Exonuclease 1 and Shrimp Alkaline Phosphatase (Exo-SAP) were used to purify 18 after it had been amplified using polymerase chain reaction in a thermal cycler[19]. The ABI 3500xL genetic analyzer used the Sanger technique to sequence purified amplicons. The closest culture sequence from the National Centre for Biotechnology Information (NCBI) database, along with sequencing files (.ab1) that had been edited with CHROMASLITE (version 1.5), were examined using the Basic Local Alignment Search Tool (BLAST), which identifies regions of local similarity between sequences. Utilising sequence databases[20], the programme compares nucleotide or protein sequences to determine the statistical significance of matches. The BLAST algorithms are used to infer functional and, in addition to identifying members of gene families. Start by looking for type strain sequences that may be closely related using the BLASTN tool (ii). Pair-wise alignment will show how similar the query sequence and the sequences found in step I are to one another[21]. The top five to ten hits found in the mentioned database are therefore included with each isolate's report. It is thus advised to do further multiple sequence alignment and phylogenetic analysis to accurately determine species and their evolutionary relationships[22,23].

Results:

Isolation of *Streptomyces* spp.

Rhizosphere soil sample was collected from the maize agriculture field. Collected soil was serially diluted till $10^{6.}$ The plates were incubated at 27±2° C for 14 days. After incubation Actinomycetes colonies were observed (Figure 1).

Figure 1: Actinomycetes colonies on SCN plates

Eighteen isolates were subjected for IAA production and were designated as SDSRO-1 to SDSRO-18. All the isolates showed varied morphological characteristics. The colony morphology appeared granular, powdery, rugose and radiating.

Colorimetric estimation of IAA by *Streptomyces* spp.

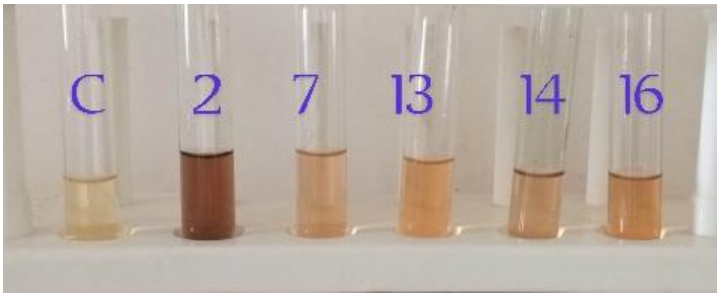

Figure 2: Colorimetric estimation of IAA by *Streptomyces* spp.

All the 18 isolates were tested for IAA production. IAA production for each isolate of *Streptomyces* was quantified using a colorimetric estimation. Before IAA measurement, all *Streptomyces* isolates were grown in 150 ml ISP2 liquid medium supplemented with 0.2% L-Tryptophan and incubated in an agitated incubator at 120 rpm at room temperature (27±2 °C) for 5 days. Salkowski reagent is combined with approximately 1 ml of culture supernatant, and the mixture was then incubated for 30 minutes in the dark. IAA production was observed by changing color from pink to red (Fig. 2). IAA concentrations of all the isolates were calculated based on the standard curve (Fig. 3). Each sample was tested in triplicates.

Table 1: *In vitro* quantitative estimation of IAA by *Streptomyces* spp.

No.	Isolate code	IAA concentration* (µg/ml)
1	SDSRO-1	2.08
2	SDSRO-2	9.15
3	SDSRO-3	5.63
4	SDSRO-4	2.7
5	SDSRO-5	3.3
6	SDSRO-6	1.8
7	SDSRO-7	6.32
8	SDSRO-8	1.05
9	SDSRO-90	5.05
10	SDSRO-10	2.32
11	SDSRO-11	0.56
12	SDSRO-12	2.12

13	SDSRO-13	4.56
14	SDSRO-14	5.32
15	SDSRO-15	2
16	SDSRO-16	4.95
17	SDSRO-17	1
18	SDSRO-18	1.34

Note: * The data were calculated from duplo measurement.

The concentration of IAA produced by the 18 *Streptomyces* isolates in a 5 days old ISP2 medium containing 0.2 ml of 0.2% of L-Tryptophan.

Among 18 isolates which were able to produce IAA. The isolate SDSRO-2 is found to be very high producer (9.15µg/ml) than the other rest whereas the least producer of IAA was SDSRO-11 (0.56µg/ml) (Table 1).

IAA standard curve

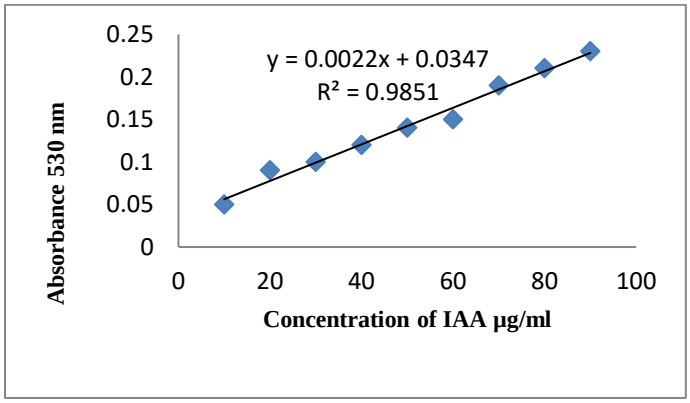

Figure 3: IAA Standard curve

Quantitative estimation of IAA by *Streptomyces* isolates

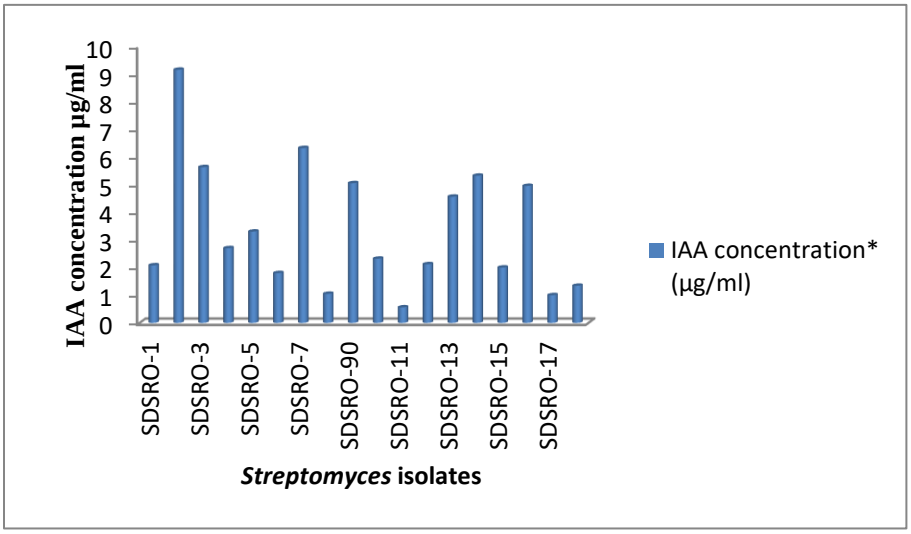

Figure 4: Quantitative tests for IAA production by *Streptomyces* isolate

All the *Streptomyces* isolates were positive for IAA production. The isolate SDSRO-2 was highest producer of IAA and isolate SDSRO-11 was very least producer of IAA (Figure 4).

Effect of culture filtrate treatment on seeds germination and vigour index

Table 2: Effect of culture filtrate treatment on seeds germination and vigour index

Seeds		Root length (cm)	Shoot length(cm)	% of seed germination	Vigour index
Chilli	**Control**	0.47±0.035	0.85±0.070	57.75±3.181	124.2±0.0136
	Treatment	1.80±0.141	2.20±0.282	85±7.021	212.5±0.0132
Wheat	**Control**	1.75±0.070	2.25±0.777	50±2.071	126±0.0321
	Treatment	5.65±0.353	4.55±0.494	90±4.074	474.12±0.013
Maize	**Control**	1.6±0.565	4.75±0.353	65±2.021	159.25±0.0213
	Treatment	7.9±2.262	8.25±1.060	90±3.01	405±0.0221
Sorghum	**Control**	1.8±0.989	7.9±0.848	55±3.535	212.62±0.0142
	Treatment	4.0±1.838	11.35±1.343	92.5±3.535	434.75±0.0121

The significant effect of plant growth promotion of chilli seedlings by the culture filtrate of the isolate SDSRO-2 showed substantial increase in root length (1.80±0.141cm) and shoot length (2.20±0.282cm). The seedling vigour index was found to be (212.5±0.0132) (Table 2).

The effect of plant growth promotion activity of wheat seedlings by the culture filtrate of the isolate SDSRO-2 showed increase in root length (5.65±0.353cm) and shoot length (4.55±0.494cm). The seedling vigour index was found to be (474.12±0.013) (Table 2).

The effect of plant growth promotion activity of maize seedlings by the culture filtrate of the isolate SDSRO-2 showed increase in root length (7.9±2.262cm) and shoot length (8.25±1.060cm). The index of seedling vigour found to be (405±0.0221) (Table 2).

The effect of plant growth promotion activity of sorghum seedlings by the culture filtrate of the isolate SDSRO-2 showed increase in root length (4.0±1.838cm) and shoot length (11.35±1.343cm). The seedling vigour index was found to be (434.75±0.0121) (Table 2).

***In vitro* seedling test of *Streptomyces* sp. SDSRO-2**

In vitro seedling test was carried out on Chilli, Wheat, and Maize and Sorghum seeds. The seeds were treated with seven days old cell-free culture suspension of the potent isolate S-2 and control was treated with distilled water for 7 days. The seeds treated with SDSRO-2 were showed highest plant growth promoting parameters compared to control (Fig.6)**.**

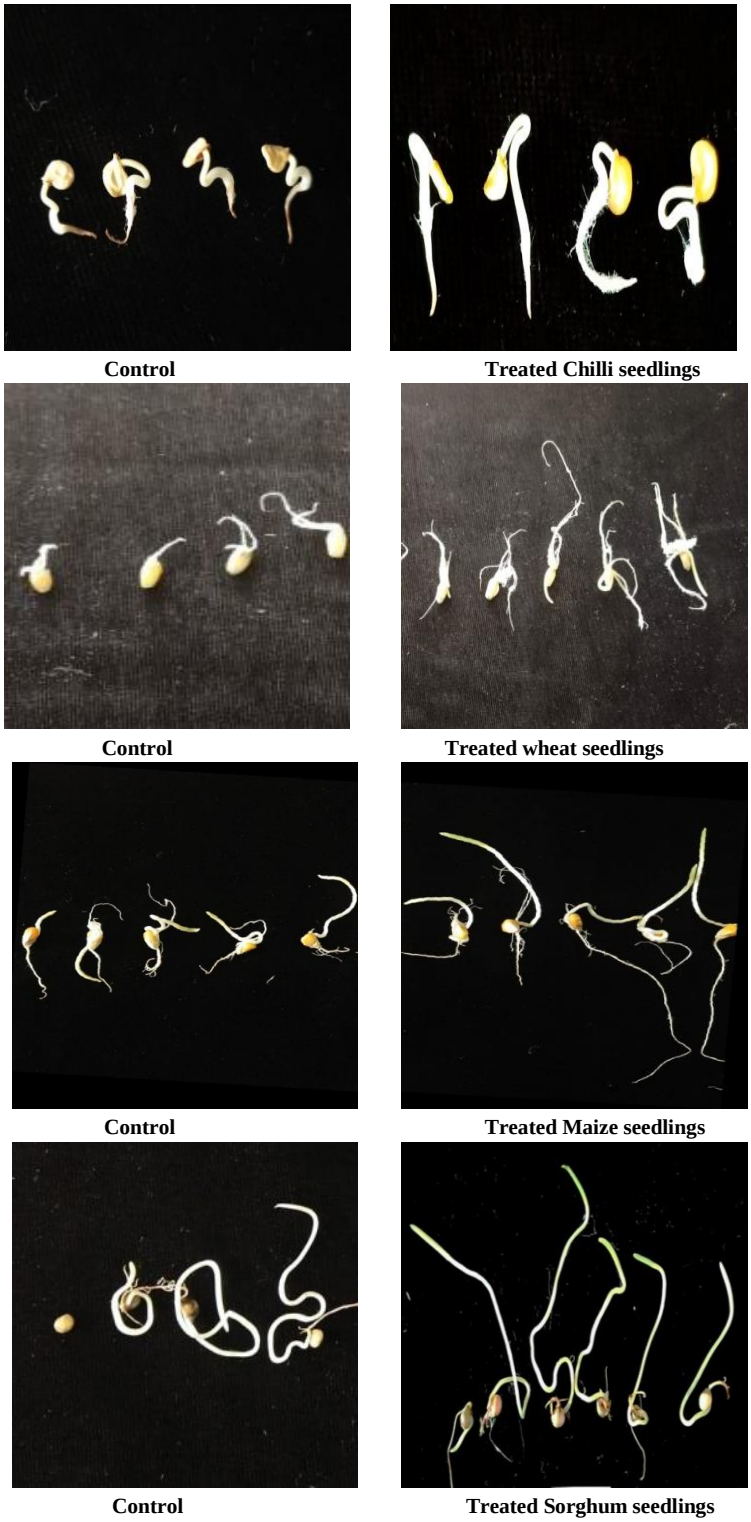

Figure 6: *In vitro* seedling tests on Chilli, Wheat, Maize and Sorghum seeds

16S rDNA gene sequence analysis of SDSRO-2

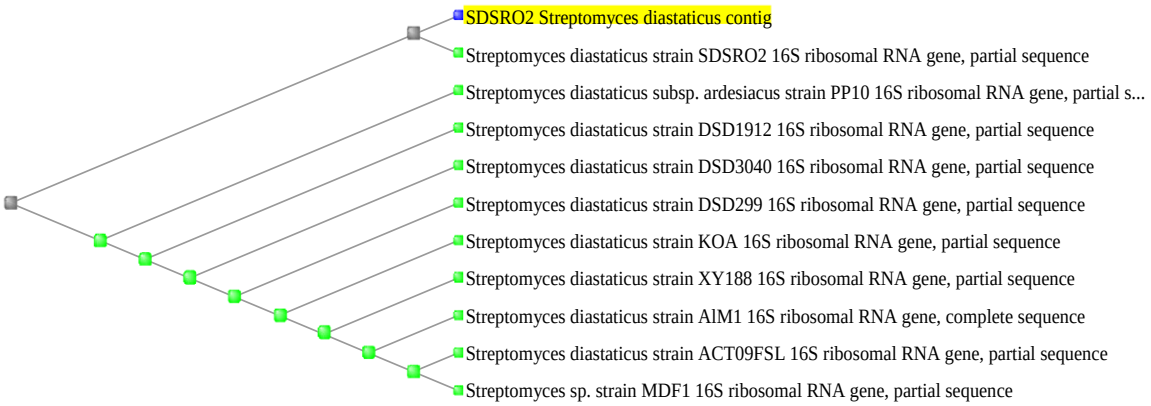

Figure 7:Phylogenetic tree showing the position of *Streptomyces* sp. The 16S rDNA gene sequence analysis was used to identify SDSRO-2. The neighbor-joining technique was used to build the tree. The strain of *Streptomyces diastaticus* served as the out group.

Streptomyces sp. SDSRO-2 was identified as *S.diastaticus* according to its 16S rRNA gene sequence (Fig.7).

Discussion:

The most dominant phylum in rhizosphere that has an important economic impact due to its secondary metabolites production is actinobacteria[24]. More than 30% of total rhizospheric soil micro biota belongs to actinobacteria. Genus *Streptomyces* represents more than 95% of all rhizospheric actinobacteria[25,26].

In the present study, we isolated 18 *Streptomyces* spp. All the 18 isolates were screened for IAA production. Among the 18 isolates SDSRO-2 was the highest producer of IAA (9.15µg/ml) (Table 1, Fig. 4). The potent isolate SDSRO-2 was then subjected for *In vitro* seedling test for the observations of different growth parameters of the four varieties seeds. Chilli (*Capsicum annuum*), wheat (*Triticum aestivum*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) seeds were used for the treatmentscompared with control. The growth parameters of all four varieties of seedlings after inoculation with SDSRO-2 were significantly higher than control. Similar results were obtained by Sheela Chandra *et al*. (2018) in green house conditions.

Auxins are synthesized by various regions of plants to control various developmental processes. Indole-3-acetic acid, a phytohormone that is crucial for plant growth, can help its host survive stressful situations including drought and pathogen infections[28, 29]. Similarly, it promotes lateral root development and height in plants, as well as seedling growth and cell differentiation[30]. IAA synthesis by several rhizospheric bacteria has already been described, as the various IAA biosynthetic pathways utilized by them[31].

Idris *et al*. (2007) also showed that the secretion of IAA can be enhanced by the addition of tryptophan in medium. The increased amount of IAA produced by these bacteria in the medium

used was due to the presence of L-tryptophan. When compared to seedlings treated with sterile distilled water as a control, SDSRO-2 inoculating Chilli, Wheat, Maize, and Sorghum seedlings had greater levels of activity that promoted plant development. The IAA results of this investigation are consistent with earlier findings of bacterial production of IAA.

According to studies, the endophytic *Streptomyces* species (*S.astrovirens, S.olivaceoviridis, S.rimosus, S.rochei, and S.viridis*) that generate IAA promoted root elongation, growth, and seed germination in plants[33,34]. According to Matsukawa *et al*. (2007) and Chinenyenwa *et al*. (2020), IAA exhibited higher cell differentiation, sporulation, and hyphal extension in *Streptomyces atroolivaceus*. When compared to control, the *streptomyces* isolate SDSRO-2 directly increased the vigour index and seed germination of sorghum, wheat, and chilli seeds.

Conclusion:

This research emphasized the importance of Actinomycetes, which are naturally capable of producing beneficial substances like IAA for plant development in agricultural fields. Specifically, it helped us comprehend the significance of the *Streptomyces* species role as PGP (Plant Growth Promoting) activity, which is often present in soil rhizosphere. To enhance crop yield, it may be beneficial to improve the nation's existing agricultural practices. It has been demonstrated to be an effective method for more sustainable agriculture that is also inexpensive and environmentally beneficial.

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References:

1. *Bhatti AA, Haq S, Bhat RA. Actinomycetes benefaction role in soil and plant health. Microbioogy Pathology. 2017;111: 458-467.*
2. *Sumaira Anwar, Basharat Ali , Imran Sajid. Screening of rhizospheric Actinomycetes for Various In-vitro and In-vivo Plant Growth Promoting (PGP) traits and for agroactive compounds. Front. Microbiol. 2016; 7:1334.*
3. *Waheeda Parvin, Radziah Othman, Hawa Jaafar, Mahbubur Rahman and Wong Mui Yun. Chromatographic detection of phytohormones from the bacterial strain UPMP3 of Pseudomonas aeruginosa and UPMB3 of Burkholderia cepacia and their role in oil palm seedling growth. International Journal of Biotechnology Research. 2015; 3(5), pp. 073-080.*
4. *Manju Sahu, Moumita Sinha, Isukapatla Arjun Rao, Smriti Sahu, Bharati Ahirwar. Thin Layer Chromatography Analysis of Different Plant Growth Hormones. Research Journal of Pharmacy and Technology 2017; 10(12): 4273-4281. doi: 10.5958/0974-360X.2017.00783.1*
5. *Ahmad F., Ahmad A. I., Khan M. S. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. J. Microbiol. Res. 2008; 163:173-181.*
6. *Rihab D jebaili , Marika Pellegrini , Massimiliano Rossi , Cinzia Forni , Maria Smati , Maddalena Del Gallo and Mahmoud Kitouni. Characterization of Plant Growth-Promoting*

Traits and Inoculation Effects on Triticum durum of Actinomycetes Isolates under Salt Stress Conditions. Soil System. 2021; 5(26): 1-20.

7. *Enders TA, Strader LC. 2015. Auxin activity: Past, present, and future. American Journal of Botany. 2015; 102:180-196.*
8. *B. Mohite. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of Soil Science and Plant Nutrition. 2013; 13(3): 638-649.*
9. *Minuto A., Spadaro D., Garibaldi A., Gullino M. L. Control of soilborne pathogens of tomato using a commercial formulation of Streptomyces griseoviridis and solarization. J. Crop Protection. 2006. 25:468-475.*
10. *Seipke R. F., Barke J., Brearley C. A single Streptomycetes Symbiont Makes Multiple Antifungals to Support the Fungus Farming Ant Acromyrex octospinosus. PLOS ONE. 2011; 6(8):22028.*
11. *Hamada AbdElgawad , Walid Abuelsoud , Mahmoud M. Y. Madany , Samy Selim ,Gaurav Zinta , Ahmed S. M. Mousa , Wael N. Hozzein. Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomolecules. 2020; 10(12):1675.*
12. *Pattern CL, Glick BR. Role of Pseudomanas putida indole acetic acid in development of the host plant root system. Applied Environmental Microbiology. 2002; 68: 3795–801.*
13. *Vaghasiat, H.L., Patel, G.M., Chudasama, R.S., Bhott, K.R.. Screening of IAA from rhizospher microflora of field crops. Bioscience Discovery. 2011; 02: 94-100.*
14. *Gusmiaty, M Restu, A and R Y Payangan. Production of IAA (Indole Acetic Acid) of the rhizosphere fungus in the suren community forest stand. IOP Conf. Ser. Earth Environmental Science. 2019; 343(1):012-058.*
15. *Ei Mon Myo, Beibei Ge , Jinjin Ma , Hailan Cui , Binghua Liu , Liming Shi , Mingguo Jiang , Kecheng Zhang. Indole-3-acetic acid production by Streptomyces fradiae NKZ-259 and its formulation to enhance plant growth. BMC Microbiology. 2019; 19(1):155.*
16. *A. Khalid, M. Arshad and Z.A. Zahir. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology 2004; 96: 473–480.*
17. *Indananda C, Matsumoto A, Inahashi Y, Takahashi Y, Duangmal K, Thamchaipenet A. Actinophytocola oryzae gen. nov., sp. nov., isolated from root of Thai glutinous rice plants, a new member of the family Pseudonocardiaceae. International Journal of Systematic and Evolutionary Microbiology. 2010; 60: 1141–1146.*
18. *Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical Microbiology.2004;17(4):840-62*
19. *Darby AC, Chandler SM,Welburn, SC, Douglas AE. Aphid-symbiotic bacteria cultured in insect cell lines. Applied Environmental Microbiology. 2005; 71(8):4833-4839.*
20. *Gertz EM, BLAST Scoring Parameters, 16 March 2005. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of Molecular Biology. 1990; 215(3):403-410. PubMed PMID: 2231712.*
21. *States, D.J., Gish, W., Altschul, S.F. Improved sensitivity of nucleic acid database searches using application-specific scoring matrices. Methods. 1991; 3:66-70.*
22. *Karlin, S. and Altschul, S.F. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proceedings of the National Academy of Sciences. 1990; 87:2264-2268.*
23. *Myers, E. W. & Miller, W. (1988). Optimal alignments in linear space. Computer Applications in the Biosciences. 1988; 4:11-17.*

24. *Yadav, A.N.; Verma, P.; Kumar, S.; Kumar, V.; Kumar, M.; Sugitha, T.C.K.; Singh, B. P.; Saxena, A.K. and Dhaliwal, H.S. Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In: New and future developments in microbial biotechnology and bioengineering. Elsevier. 2018.pp. 13–41.*
25. *Ventura, M.; Canchaya, C.; Tauch, A.; Chandra, G.; Fitzgerald, G.F.; Chater, K.F. and van Sinderen, D. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiology and Molecular Biology. Rev. 2007; 71(3):495–548.*
26. *Hazem H. Elsayed. Screening of rhizospheric actinobacteria isolated from different cultivated plants in Egypt. African Journal of biological Science.2022; 18(2): 33-41.*
27. *Sheela Chandra, Kazim Askari, Madhumita Kumari. Optimization of indole acetic acid production by isolated bacteria from Stevia rebaudiana rhizosphere and its effects on plant growth. Journal of Genetic Engineering and Biotechnology. 2018; 16:581-586.*
28. *Bardi L, Malusà E. Drought and nutritional stresses in plant: alle-viating role of rhizospheric microorganisms. In: Haryana Nikhil, PunjShreya, editors.Abiotic stress: new research. Hauppauge, NY, USA:Nova Science. 2012; p. 1–57*
29. *Sathya A, Vijayabharathi R, Gopalakrishnan S. Plant growth-pro-moting actinobacteria: a new strategy for enhancing sustainable pro-duction and protection of grain legumes. 3 Biotechnology. 2017; 7:102–112.*
30. *Vurukonda, S., Giovanardi, D and Stefani, E. Plant growth promoting and biocontrol activity of Streptomyces spp. as endophytes. International Journal of Molecular Sciences.2018; 19:952.*
31. *Vijayabharathi, R., Gopalakrishnan, S., Sathya, A.,Vasanth Kumar, M., Srinivas, V and Mamta, S. Streptomyces sp. as plant growth-promoters and host-plant resistance inducers against Botrytis cinerea in chickpea. Biocontrol Science and Technology. 2018; 28(12): 1140-1163.*
32. *ElSorra E. Idris, Domingo J. Iglesias, Manuel Talon, and Rainer Borriss. Tryptophan-Dependent Production of Indole-3-Acetic Acid (IAA) Affects Level of Plant Growth Promotion by Bacillus amyloliquefaciens FZB42. Molecular Plant-Microbe Interactions. 2007; 20(6):619–626*
33. *El-Tarabily KA. Promotion of tomato (Lycopersicon esculentum Mill.) plant growth by rhizosphere competent L-aminocyclopropane- L-carboxylic acid deaminase–producing Streptomycete actinomycetes. Plant Soil. 2008; 308:161–174.*
34. *Khamna, S.; Yokota, A.; Peberdy, J.F. and Lumyong, S. Indole-3-acetic acid production by Streptomyces sp. isolated from some Thai medicinal plant rhizosphere soils. EurAsian Journal of BioSciences. 2010; 4(1):23–32.*
35. *E. Matsukawa, Y. Nakagawa, Y. Iimura, M. Hayakawa, Stimulatory effect of indole-3- acetic acid on aerial mycelium formation and antibiotic production in Streptomyces spp, Journal of Actinomycetologica. 2007; 21:32-39.*
36. *Chinenyenwa Fortune Chukwuneme, Olubukola Oluranti Babalola, Funso Raphael Kutu & Omena Bernard Ojuederie. Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize, Journal of Plant Interactions. 2020; 15(1):93-105.*