Optimization for the production of Indole acetic acid by *Streptomyces* sp. SDSRO-2 isolated from Rhizosphere soil of maize

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Abstract:

The primary auxin family member generated by plants is indole-3-acetic acid, which is crucial for a variety of plant developmental stages including leaf creation, embryo development, root initiation and development, abscission, phototropism, geotropism, and fruit development. The current study focuses on the isolation, characterization, and optimization of IAA synthesis from such bacteria found in the rhizosphere soil of maize. The optimum conditions for IAA generation were tested at various pH levels, temperatures, and culture medium including a variety of carbon and nitrogen sources. One of the 18 identified isolates was evaluated as an effective PGPR based on several characteristics that promote plant development. The *Streptomyces* isolate SDSRO-2 produced IAA more effectively at pH 8 (3.16 g/ml) and 32°C (5.5 g/ml). The optimal carbon source for IAA synthesis (0.37 g/ml) was found to be dextrose (1%). From the current study, SDSRO-2 appeared as noble alternatives for IAA generation further which also resulted in root and shoot length in agricultural plants including Chilli, Wheat, Maize and Sorghum, therefore could potentially be employed as bio-inoculants for plant growth promotion.

Keywords: 1. Optimization, 2.Indole-3-acetic acid, 3.*Streptomyces* sp., 4.Rhizosphere soil, 5.Thin Layer Chromatography.

Introduction:

The term "PGP" or plant growth-promoting refers to the beneficial effects that certain microorganisms can have on the growth and health of plants. Actinomycetes are known to exhibit plant growth-promoting (PGP) activity by producing a range of bioactive compounds that can enhance plant growth and development. Some examples of PGP activity of actinomycetes are, Production of phytohormones: Actinomycetes have been reported to produce various phytohormones such as auxins, cytokinins, gibberellins, and abscisic acid, which are known to promote plant growth and development [1].

Actinomycetes can solubilize minerals such as phosphate, iron, and potassium, making them more available to plants [2]. This can enhance plant growth and development by increasing nutrient uptake. Some actinomycetes such as Frankia, can fix atmospheric nitrogen and convert it into a form that can be used by plants [3]. This can enhance plant growth by increasing the availability of nitrogen, which is a crucial nutrient for plant growth. Actinomycetes are known to produce a range of bioactive compounds such as antibiotics and enzymes that can suppress the growth of plant pathogens and promote plant health [4]. This can indirectly promote plant growth and development by reducing the impact of plant diseases. In the presence of l-tryptophan, a variety of microorganisms, including soil bacteria, epiphytic and endophytic bacteria, and some cyanobacteria, were discovered to produce indole-3-acetic acid (IAA) [5]. Microorganisms from rhizospheres of various plants synthesize and release auxin as secondary metabolites because of rich substrates exuded from the roots

in rhizosphere compared with nonrhizospheric soils [6]. The release of l-tryptophan in root exudates may result in its conversion into IAA by rhizosphere microbes [7]. Several of these groups are implicated in the plant pathogenesis, while others stimulate plant growth [5]. The *Streptomyces griseoviridis* K61 and *Streptomyces lydicus* WYEC108 were used commercially for IAA production under the trade name Mycostop [8].
In the present research work optimization parameters were carried out for the IAA production by the potent *Streptomyces* isolate SDSRO-2. Incubation period, pH, temperature, L-tryptophan concentration, and carbon sources were employed as optimization factors.

Materials and Methods

Isolation of *Streptomyces* sp.

Isolation of the *Streptomyces* strain capable of IAA production carried out from the agricultural soil by serial dilution plate technique. The SCN medium was used for the isolation and inoculated with SDSRO-2 followed by incubation at 35° C for 5-6 days. Morphologically distinct colonies of *Streptomyces* spp. were selected for screening of IAA-producing ability. The most efficient *Streptomyces* sp. SDSRO-2 isolate was selected and used for further studies [9].

Screening of *Streptomyces* isolates for IAA production

Colorimetric method was used to measure IAA production by each Actinomycete isolates. When evaluating IAA, a loopful of inoculum was inoculated on 20 mL of ISP2 liquid medium supplemented with 0.2 mL of 0.2% L-tryptophan and cultured for 10 days at room temperature (27°C) in an agitated incubator at 120 rpm. Each culture's supernatant was mixed with 2 mL of Salkowski reagent in an amount of around 1 mL. The combined liquids were then incubated for around 30 minutes in the dark. Based on the standard curve, the IAA concentration was determined. Each sample performed a duplo test [10].

IAA production and optimization parameters for the production of IAA

IAA production and optimization parameters for IAA production, the culture medium was inoculated with *Streptomyces* isolate SDSRO-2. For the analysis, many factors including incubation duration, temperature, pH, L-tryptophan concentration, and carbon source were used. ISP-2 was used as the main IAA production medium. One of the most crucial physicochemical factors in the formation of IAA is pH. The impact of pH 5–9 on the production of IAA by various isolates was investigated [11]. The development of bacteria is impacted by varying temperatures and IAA production depends on the proper growth of microorganisms, temperature is also a crucial factor in IAA synthesis. Thus, tests on IAA production were conducted at 4, 25, 32, and 50°C [12]. At a concentration of 1.0%, Dextrose, starch, glycerol, and maltose, four distinct sugars, were examined [13].

Effect of selected isolates in crop plants for plant growth activities

Seeds of four crop plants like. Chilli, Maize, Wheat and Sorghum seeds were purchased from market. Healthy seeds were sterilized by washing them with tap water to remove all the dirt and dust followed by washing twice with distilled water to remove any tap water. Seeds were then washed with 70% ethanol 3 times followed by rinsing them with distilled water 5 times. The selected PGPRs were grown in SCN for 5days at 37° C in an incubator shaker at 120 rpm. The surface sterilized seeds were inoculated in broth for 5–6 days. 10 seeds for each crop plant were then washed in autoclaved distilled water to remove broth and placed at an equal distance in the sterilized petriplates with blotting paper using sterile forceps. Uninoculated seeds were treated with distilled water and worked as control. After seven days, the plates were taken, and the plants were carefully uprooted. To evaluate the growth characteristics, the lengths of the shoots and roots were measured [14].

Results and Discussion:

IAA (indole-3-acetic acid) is a plant growth hormone produced by many microorganisms, including *Streptomyces*. The optimization parameters for IAA production by *Streptomyces* can vary depending on the strain and the culture conditions. Here are some commonly used parameters for IAA production by *Streptomyces*:

Streptomyces isolates were screened for IAA production. Out of 18 isolates, SDSRO-2 was selected for the efficient producer of IAA (data not shown here) hence isolate SDSRO-2 was used for optimization for IAA production.

Effect of incubation time on IAA production by SDSRO-02

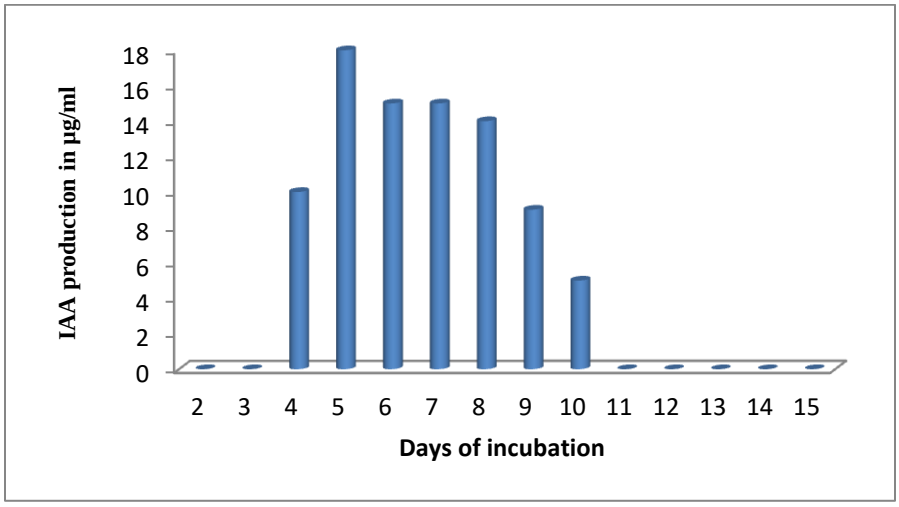

Figure 1: Effect of incubation time on IAA production by SDSRO-02

The isolate SDSRO-02 was observed for growth on ISP-2 broth. Thin layer of mycelia was observed on the fourth day of incubation. The broth was subjected for IAA production from fourth day to fourteenth day of incubation. No IAA production was observed on first, second and third day. On fifth day maximum IAA production was observed (18µg/ml). Gradual decrease of IAA production was observed from day ninth (18µg/ml). IAA production was zero on the eleventh day of incubation (Fig 1).

Effect of pH on IAA production of SDSRO-02

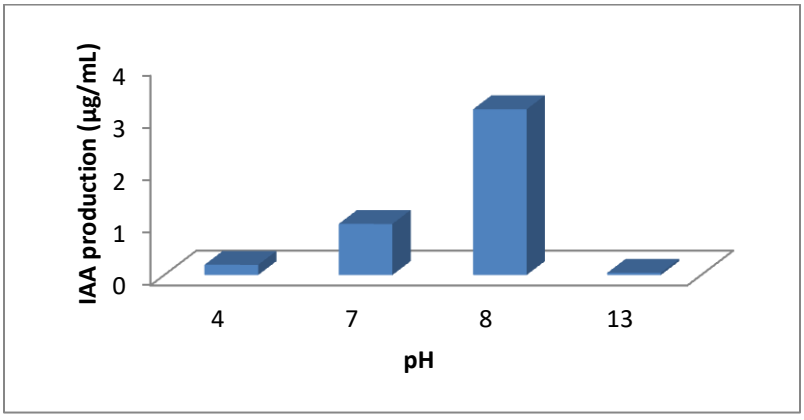

Figure 2: Effect of pH on IAA production of SDSRO-02

Impact of pH on IAA production of SDSRO-02 was studied at pH 4, pH 7, pH 8 and pH 13. IAA synthesis was not observed at pH 4. Average IAA production was observed at pH 7, in addition to the development of pink colour (0.97 g/ml). At a pH of 8, red pigment (3.16 g/ml) showed good IAA production. IAA synthesis was not seen at pH 13. (Fig. 2). [15] also got similar results. Lower pH limits the growth of plants, as concentration of metal ions could reach toxic levels in the soil at low pH, number of physiological and metabolic activities taking place in the rhizosphere can be affected by soil pH and metal cations present in vicinity. The optimal pH for IAA production by *Streptomyces* can vary depending on the strain. For example, the optimal pH for IAA production by *Streptomyces* sp. S37 was reported to be 7.0 [16],while *Streptomyces* sp. B7-2 showed the highest IAA production at pH 6.0 [17] .

Effect of temperature on IAA production of SDSRO-02

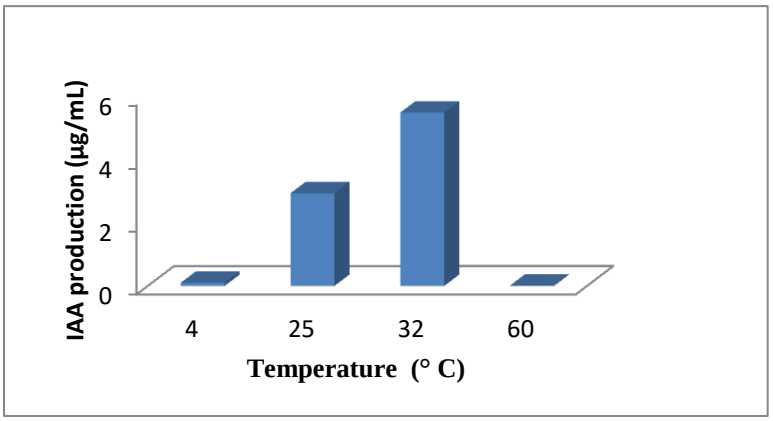

Figure 3: Effect of temperature on IAA production of SDSRO-02

Effect of temperature on IAA production of SDSRO-02 was studied at 4°C, 25°C, 32°C and 60ºC. At 4°C, no IAA production was observed. At 25°C, average production of IAA was observed with light pink color. IAA concentration was found to be 2.94µg/ml. At 32°C good IAA production was observed by changing color from pink to red. IAA concentration was found to be 5.50µg/ml. At 60°C, no IAA production was observed. So for IAA production 32°C was the best temperature. Similar results were obtained by *Streptomyces* sp. S37 showed the highest IAA production at 30°C [16] while *Streptomyces parvulus* JK-1 showed the highest IAA production at 28°C [18].

The optimal temperature for IAA production by *Streptomyces* can also vary depending on the strain. Incubation time: The incubation time for IAA production by *Streptomyces* can vary depending on the strain and culture conditions. *Streptomyces* sp. G8 showed the highest IAA production at 72 hours of incubation [19] while *Streptomyces enissocaesilis* showed the highest IAA production at 96 hours of incubation [20].

Effect of L-Tryptophan concentration on IAA production

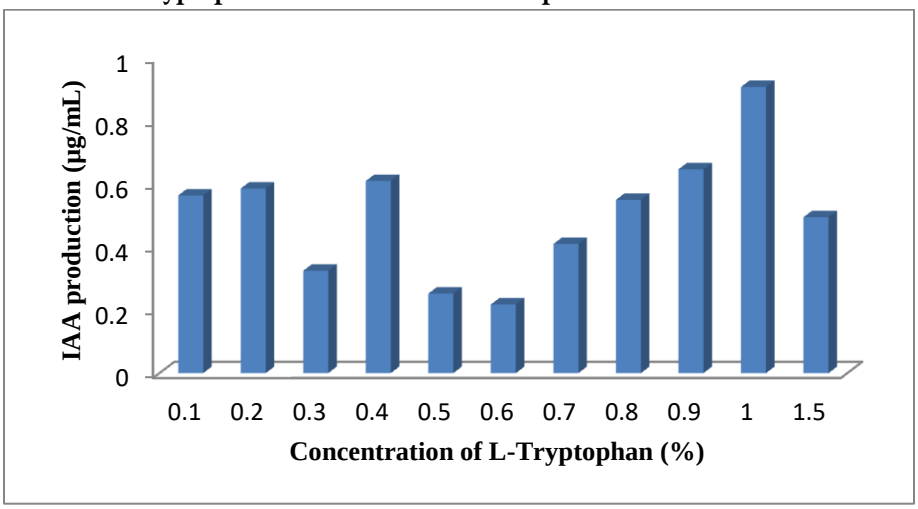

Figure 4: Effect of L-Tryptophan concentration on IAA production

L-tryptophan is considered as a precursor for IAA production. In the present investigation, we have used different concentrations of L-tryptophan between 0.1% - 1.5%. The spectrophometric analysis showed gradual increase in the IAA production with the increase in L-Tryptophan concentration. 1% of L-tryptophan concentration in the medium showed maximum IAA production (0.9085µg/ml) (Fig. 4).

Effect of Carbon sources on IAA production

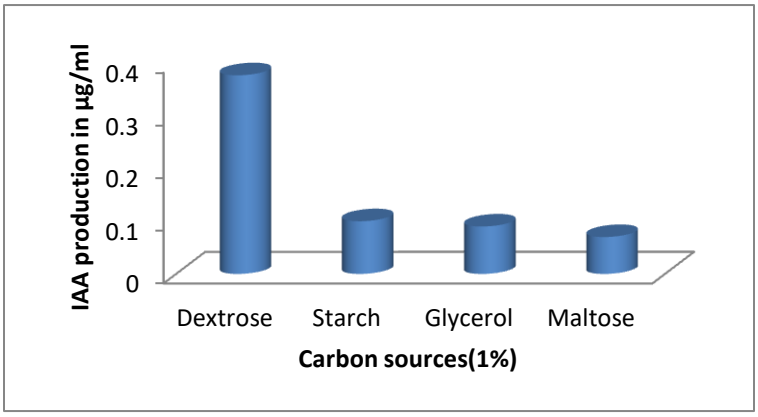

Figure 5: Effect of Carbon sources on IAA production

Effect of carbon sources on IAA production was studied using Dextrose, Starch, Glycerol and Maltose. In Dextrose- Good production of IAA (0.37µg/ml) was observed with production of red color. Whereas in case of starch, low IAA production was observed (0.1µg/ml). Glycerol - Low IAA production was observed (0.07µg/ml). Maltose - Very low IAA production was observed (0.09µg) (Fig. 5).

Glucose, sucrose, maltose, fructose, and lactose are commonly used carbon sources for IAA production by *Streptomyces.* Glucose is the most commonly used carbon source for IAA production by *Streptomyces* species such as *Streptomyces* sp. G8 and *Streptomyces* sp. S37 [16]

Scanning Electron Microscopic image of SDSRO-2

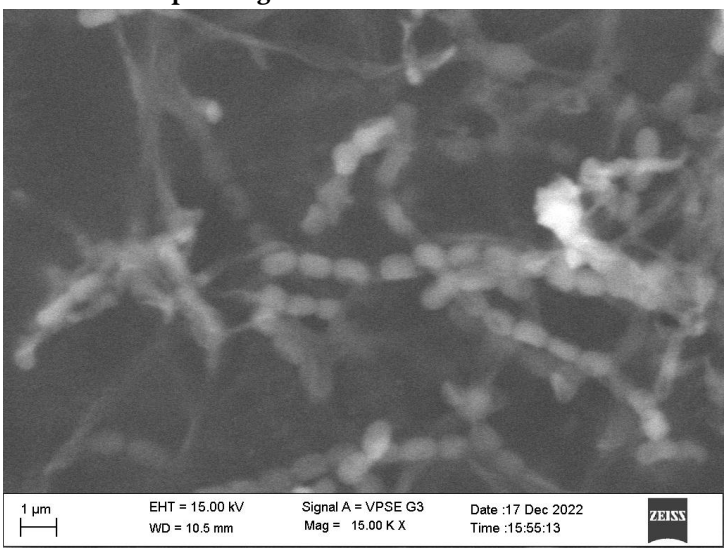

Figure 6: Scanning electron microscopic view of SDSRO-2

The potential *Streptomyces* isolate SDSRO-2 was identified by scanning electron microscopic observation (Fig. 6).

Thin Layer Chromatography

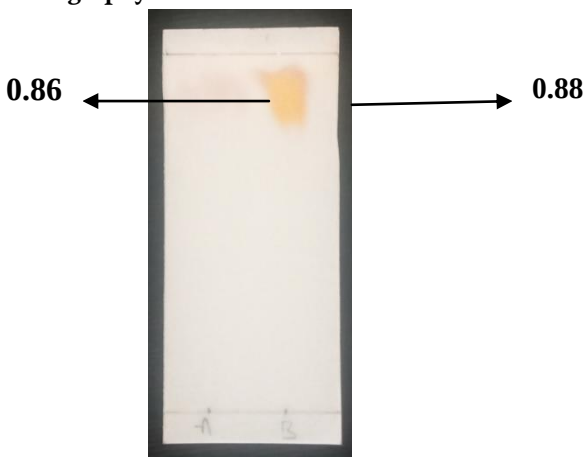

Figure7: Thin layer chromatography of Standard IAA (B) and IAA crude extract (A)

The strain *Streptomyces* sp. SDSRO-2 efficacy to produce IAA was confirmed by TLC analysis. As shown in Figure 7, TLC plate was treated with Salkowsky reagent. The ethyl acetate solvent extract from culture filtrate showed pink color spot on the TLC plate at the Rf value of Standard IAA (0.88) and Crude extract (0.86).

***In vitro* seedling tests of SDSRO-02 Chilli, Maize, Wheat and Sorghum**

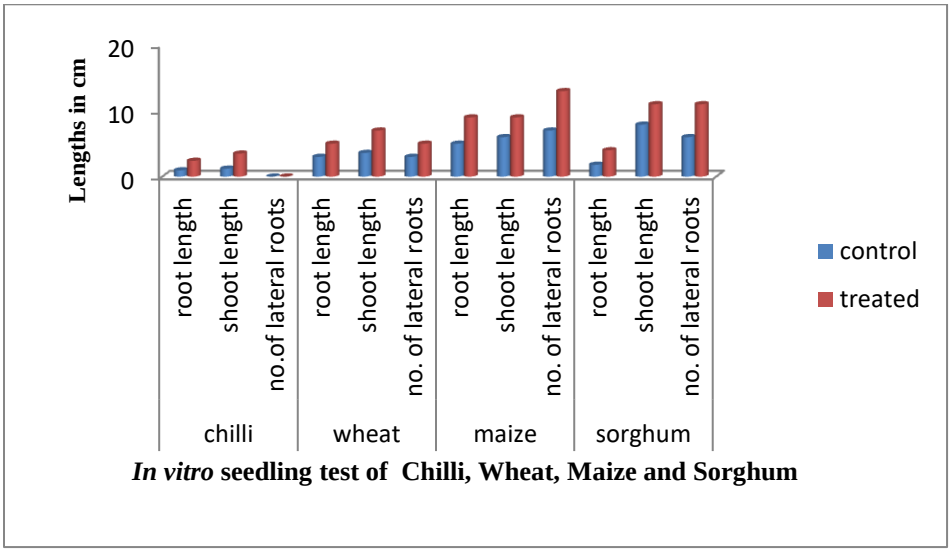

Figure 8 : *In vitro* seedling tests of SDSRO-02 on Chilli, Wheat, Maize and Sorghum seeds

Four varieties of seeds were treated with the *Streptomyces* isolate SDSRO-2 in order to evaluate the effects of the growth parameters of SDSRO-2. Calculations were performed of the lengths of the lateral roots, shoots, and roots themselves. In maize, the root lengths were longer (9 cm). Moreover, sorghum had longer shoots (11 cm), whereas maize had more lateral roots [13], as seen in Fig. 8 correspondingly [21] and [10] both came to similar conclusions. The ability of rhizobacterial isolates to produce IAA was thought to be a useful screening method for helpful microorganisms because of their significant impact on growth promotion [22].

Conclusion:

The *Streptomyces* isolates known to promote plant development are strongly associated to the propensity to synthesize IAA. The results of the current investigation demonstrated that SDSRO-2 produced the most IAA at pH 8 (3.16 g/ml) and 32 °C (5.5 g/ml). The optimal carbon source for IAA synthesis (0.37 g/ml) was discovered to be dextrose (1%). The maximum amount of IAA (0.9g/ml) could be produced at 1% L-Tryptophan concentration. The highest IAA production (18 g/ml) occurred on the sixth day of incubation. SDSRO-2 emerged from the current investigation as a competent substitute for the manufacturing of IAA.

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Conflicts of Interest: The authors declare no conflict of interest.

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