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Studies on IAA Producing *Pseudomonas* and *Serratia spp.* Isolated from Agricultural and Garden Soil of Akola Region

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A B S T R A C T

Indole-3-acetic acid (IAA) is the main member of the auxin family that controls many important physiological processes including cell enlargement and division. In the present study *Serratia spp.* and *Pseudomonas spp.* were isolated from the soil samples of the Akola region. 10 soil samples from agriculture and garden of various locations were collected. A total of 25 isolates were obtained which included 10 *Serratia spp.* and 15 *Pseudomonas spp.* The isolates where then subjected for their ability to produce indole acetic acid by standard procedure using L-tryptophan. Results showed that the isolate P6 (*Pseudomonas* spp) and S7 (*Serratia spp*) have ability to produce IAA in significant amount. Further studies on optimization suggest that – IAA production was maximum at 40°C for S7 and 37° C for P6 at 72 hrs of incubation at pH 9 and 7 respectively. The Mannitol was found to be best carbon source for IAA production. The 1% and 2% tryptophan was found to be optimum for maximum IAA production for P6 and S7 respectively.

Introduction

In order to meet the challenges of providing food to the ever increasing population, there is an urgent need to boost crop yield. As the rate of population increased, there is also an excessive increase in the usage of chemical fertilizers and pesticides for various purposes. Although achieving the satisfactory results by the application of chemical fertilizers and pesticides, the disadvantages of chemical fertilizers and pesticides are now threatening the agricultural processes, such as pollution of large water resources, destruction of microorganisms, acidity of the soil and reduction in soil fertility (Ahmad *et al.*, 2008). Thus, in the recent years, scientists have diverted their attention towards exploring the potential of beneficial microbes and their use in plant growth promotion for sustainable agriculture.

Plant hormones regulate or influence a range of cellular and physiological process, such as cell division, cell enlargement, bud dormancy, flowering, fruit ripening, seed dormancy, seed germination and leaf abscission. Indole-3-acetic acid (IAA) is the main member of the

auxin family that controls many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity (Taele *et al.*, 2006). Various microorganisms including bacteria, fungi, and algae are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment. Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus* and *radyrhizobium japon-icum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 1996).

The species of genus *Pseudomonas* are widely distributed in nature and act as plant growth-promoting rhizobacteria by nitrogen fixation, mineral solubilization, as well as transformation of nutrients, production of phytohormones and siderophores, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Lugtenberg and Kamilova, 2009). Although IAA production has been reported in *Pseudomonas species*, there is not much information on statistical optimization of nutritive conditions for its production (Karnwal, 2009).

The IAA produced by bacteria colonizing the rhizosphere of the plants is proposed to act in conjunction with endogenous IAA in plant to stimulate cell proliferation, elongation, and enhancement of host's uptake of minerals and nutrients from the soil. IAA also serves as a regulating agent for microbial cell differentiation (Suzuki and Oyaizu, 2003; Leveau and Lindow, 2005). Tryptophan is believed to be the primary precursor for the formation of IAA in plants and microorganism (Monteiro *et al.*, 1988). Different bacterial pathways to synthesize IAA have been identified and a high degree of similarity between IAA biosynthesis pathways in plants and bacteria was observed (Spaepen *et al.*, 2007).

Indole -3-acetic acid (IAA) is the common natural auxin that shows all auxin doing actions and extensively affects plant's physiology. Thus the present study aimed to isolate the *Pseudomonas* spp and *Serratia* spp from soil samples of Akola region and check the effect of various parameters on its production.

Materials and Methods

Collection of soil samples

The soil samples were collected from different areas of Akola region. The samples were collected in sterile plastic bag and brought to the laboratory for further work.

Isolation and identification of *Pseudomonas* and *Serratia* spp.

The soil samples were inoculated on *Pseudomonas* isolation agar and Nutrient agar the colonies showing similarity with the two were selected and purified and maintained on nutrient agar slant. Further the identification was done by cultural morphological and morphological and biochemical characteristics.

Determination of IAA production by isolates

The isolates were inoculated separately in the nutrient broth supplemented separately in the nutrient broth supplemented with L-tryptophan and incubated for 96 hrs. After 24 hours interval 10ml amount of media removed and centrifuged at 5000 rpm for 15 minutes. The 1 ml of supernatant was mixed with 2ml of Salkowski's reagent (50 ml of 35% perchloric acid and 1 ml of 0.5 M $FeCl_3$

solution) and 2 drops of orthophosphoric acid. The mixture was incubated in dark for 1 hr. Red colour development was recorded with spectrophotometer at 530 nm.

Preparation of standard curve for IAA estimation

Standard curve was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.00 ml of standard IAA solution in test tubes.

The volume was made to 2 ml with distilled water and then 4 ml of Salkowaski reagent was added and tubes were incubated for 25 minutes at room temperature and optical density was measured at 530 nm. Standard curve was prepared by plotting absorbance at 530 nm against concentration of IAA solution.

Effect of temperature on IAA production

Effect of temperature on IAA production was studied by inoculating the culture in the production media and incubating at different temperatures as room temp, 37^{o}C, 40^{O}C.The IAA production was calculated as per previously described.

Effect of pH on IAA production

The production media was adjusted for pH 3, 5, 7 and 9 by addition of 1N HCl and 1N NaOH. The media were then inoculated with cultures, incubated and IAA produced was determined spectrophotometrically at 530 nm as previously described.

Effect of carbon sources on IAA production

The production media was supplemented with 1%of sugar like glucose, sucrose and mannitol were then inoculated after autoclaving with cultures incubated and IAA was estimated at 530 nm by spectrophotometer as described.

Effect of L- tryptophan concentration on IAA production

Production media was supplemented with different concentration of L- tryptophan as 0.05%, 1%, 2% and 3%. It was inoculated with cultures after autoclaving and after incubation IAA was determined at 530 nm by spectrophotometer.

Results and Discussion

In the present study a total of 25 bacterial isolates were obtained from the soil samples. Primarily on the basis of colour on the media colonies were selected and coded as S for *Serratia* and P for *Pseudomonas*. The isolates were then identified by cultural, morphological and biochemical characteristics by standard conventional methods.

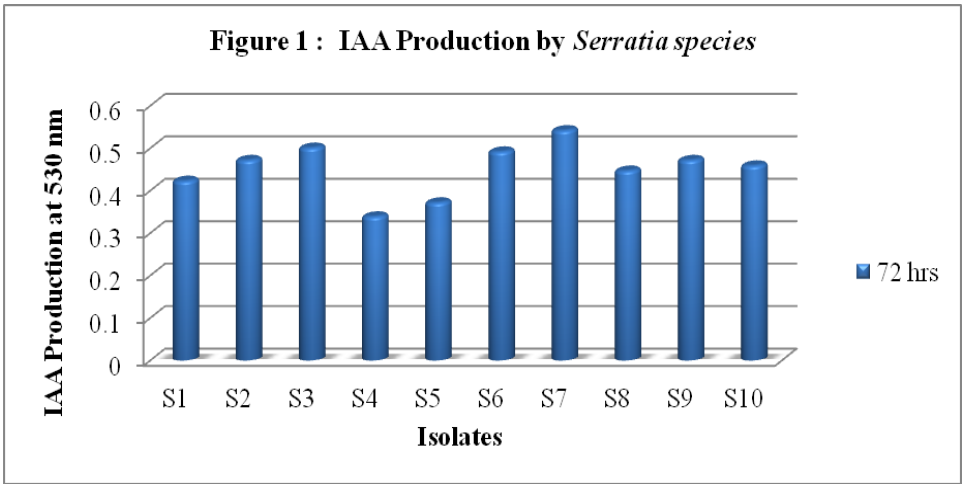
In the isolates 10 *Serratia spp* and 15 *Pseudomonas spp* found IAA producers by using Salkowski reagent. The intensity of pink colour development at 530 nm after addition of reagent was noted for each isolate. The results were recorded for IAA production after each 24 hrs. It was found that IAA production was maximum at 72 hrs of incubation time (Fig. 1 and 2). The two isolates S_7 and P_6 each from *Serratia spp* and *Pseudomonas spp* were selected for further study as both the isolates showed maximum IAA production amongst all isolates. This is in agreement with other studies who also supported the production of IAA by *Pseudomonas* and *Serratia spp*. (Karnwal, 2009; Malik and Sindhu, 2011; Kamble and Galerao, 2015; Bharucha *et al*., 2013; Reetha *et al*., 2014; Serepa *et al*., 2015; Lwin *et al*., 2012).

The quantitative estimation of IAA needs the standard graph for which the known concentrations of standard IAA procured from Himedia were prepared and estimated with spectrophotometer at 530 nm (Fig. 3).

In the study effect of various parameters like temperature, pH, carbon sources and tryptophan concentration was studied on IAA production of S_7 and P_6.

The effect of temperature studied shows that IAA production was maximum for isolates P_6 (*Pseudomonas spp*.) at 37°C which was 59 µg/ml after 72 hrs of incubation. At room temperature P_6 showed 55µg/ml IAA production while at 40°C it was 52 µg/ml. In a similar way IAA production at various temperature for isolate S_7 (*Serratia spp*) showed that IAA was produced maximally at 40°C temperature which was 92ug/ml. At room temperature it was 75 µg/ml and at 37°C it was 68µg/ml (Fig. 4).

According to Sudha *et al*., (2017) also 37°C temperature was optimum for IAA production for *Rhizobium* and *Bacillus* spp. Bharucha *et al*., (2013) studied IAA production at 30° C from *Pseudomonas putida*. Sachdev *et al*., (2009) reported. Maximum IAA production at 37°C after 72 hrs of incubation. Kamble and Galerao (2015) reported very few cultures of *Pseudomonas spp* producing IAA at 45°C.



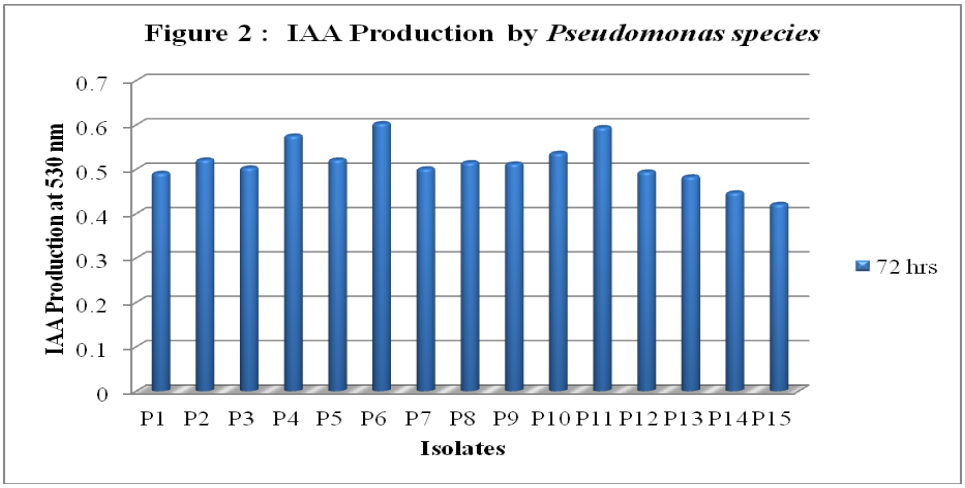


Fig. 3 : Standard graph for IAA estimation

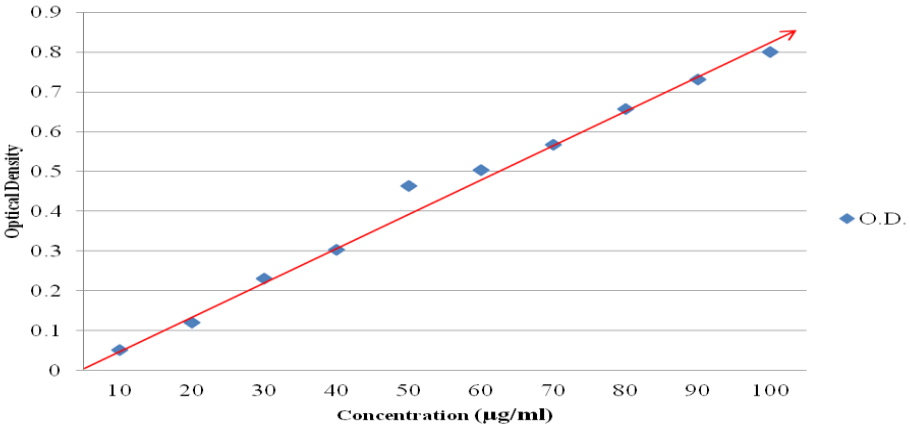


Fig. 4 : Effect of Temperature on IAA production

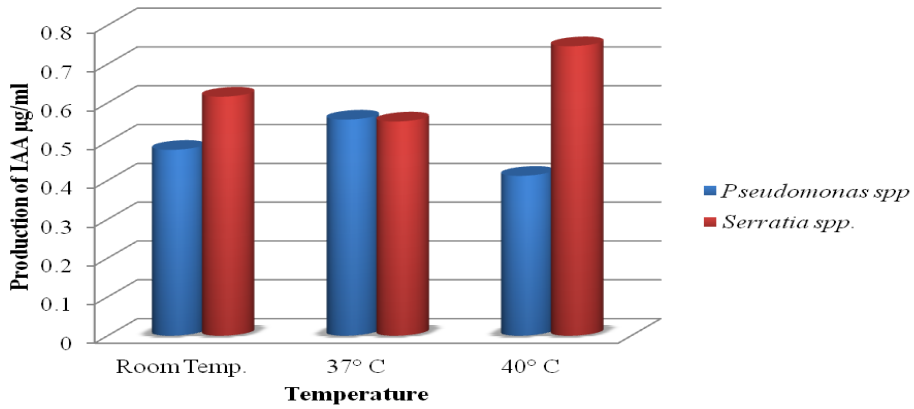
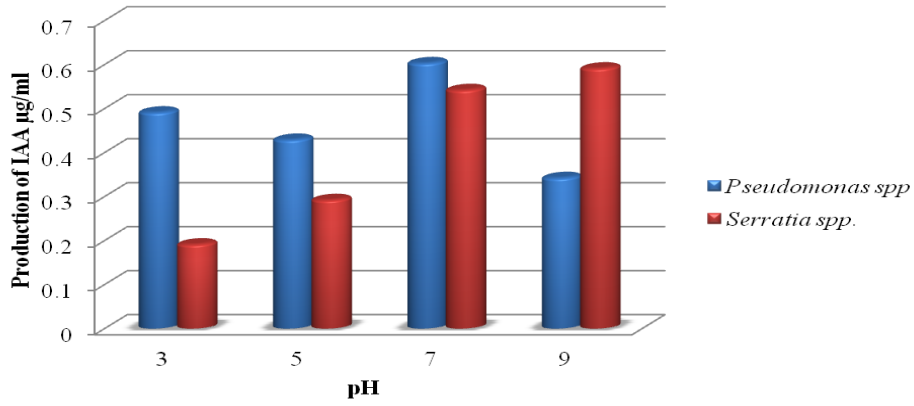
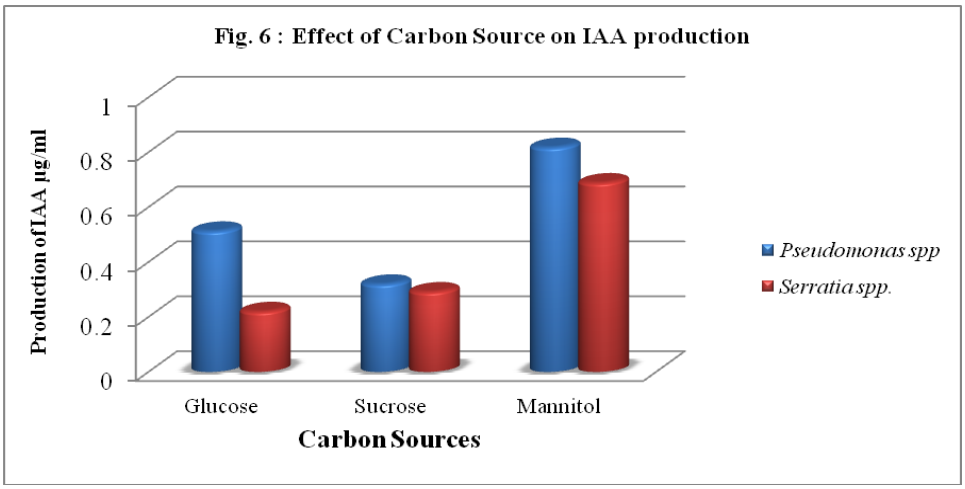
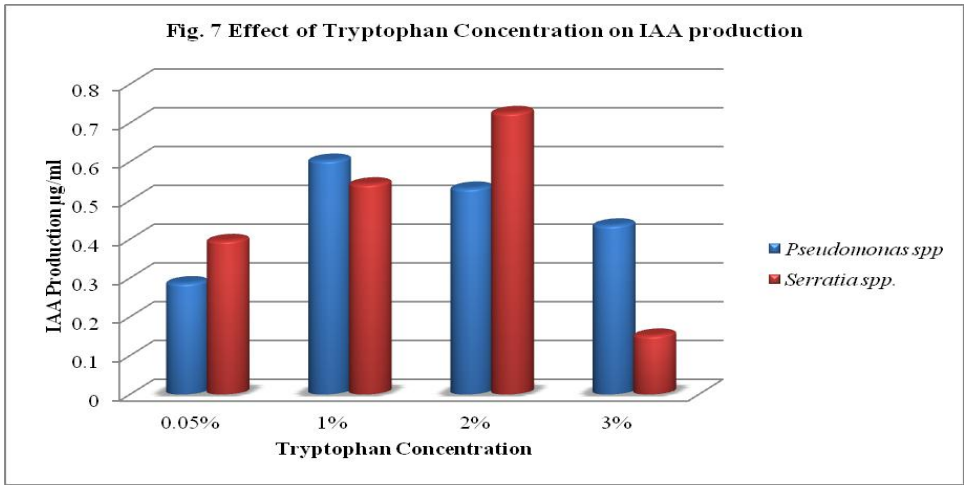
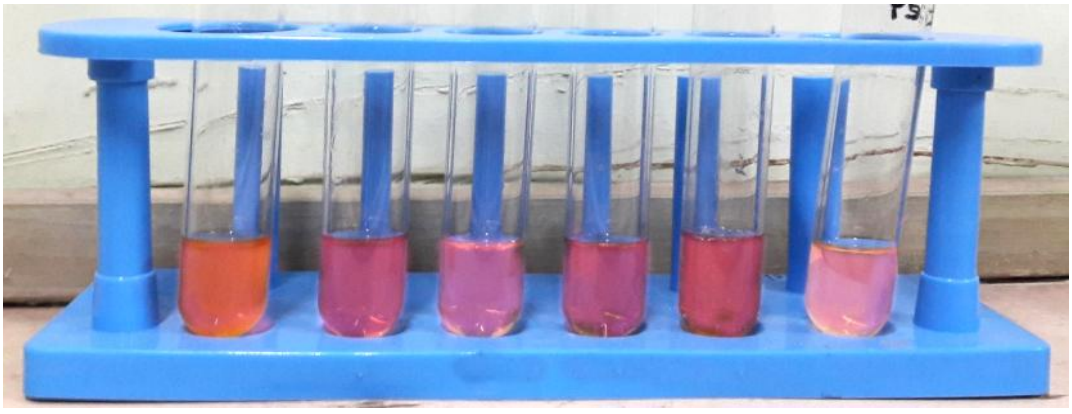


Fig. 5 : Effect of pH on IAA production









IAA Produced by Isolates

In the study pH 3, 5, 7 & 9 were adjusted with the production medium and its effect on IAA production was studied (Fig. 5). It was found that P6 showed maximum IAA production 74 µg/ml at pH 7 while S7 showed maximum IAA production 42 µg/ml at pH 9, after 72 hrs of incubation our results are in correlation with the Barucha *et al*., (2013) who also reported maximum IAA production at pH 7.5 for *Pseudomonas spp*. While it is similar to the reports of Kamble and Galerao (2015), who also reported maximum IAA production at pH 7. Mohite (2013) reported pH 9 was optimum for the IAA production for one of the strain.

Effect of three different carbon sources like glucose, sucrose and mannitol at 1% concentration was also studied. Mannitol was found to be the best carbon source which gave maximum production of IAA than glucose and sucrose (Fig. 6). This is in agreement with the Sridevi *et al*., (2008) who also revealed that mannitol and glutamic acid were best promotor for IAA production. Shilts *et al*., (2005) and Mohite (2013) also reported mannitol as best carbon source. Bharucha *et al*., (2013) reported source is the best carbon source for IAA production which is concordance with the present study.

L-tryptophan is considered as a precursor of IAA production because its addition to medium increase IAA production (Ahmad *et al*., 2005). To check this effect, different concentrations of L-tryptophan between 0.05% - 3% were added to the medium for IAA production (Fig. 7). It was found that with increase in tryptophan concentration IAA production was also increased. For isolate P_6 1% tryptophan concentration showed highest production of 74 µg/ml for IAA than other concentrations. While for isolate S_7 the optimum concentration of tryptophan was recorded 2% at which 89 µg/ml of IAA was found to be produced. Mohite (2013) also reported 0.1%, 1.5% and 0.05% showed maximum IAA production while Bharucha *et al*., (2013) reported 0.2 as optimum concentration of tryptophan for IAA production. Khalid *et al*., (2004) showed variable amount of auxins produced by the rhizobacteria *in vitro* and amendment of the culture media with L-tryptophan stimulates IAA biosynthesis

In conclusion, the isolate P6 (*Pseudomonas* spp) & S7 (*Serratia spp*) showed potential of IAA production. Thus the isolates P6 and S7 would be beneficial in agricultural biotechnology in increasing crop production. Studies on optimization suggest that – IAA production was maximum at 40°C for S7 and 37° C for P6 at 72 hrs of incuation at pH 9 and 7 respectively. The Mannitol was found to be best carbon source for IAA production.The 1% and 2% tryptophan was found to be optimum for maximum IAA production for P6 and S7 respectively.

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