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RESEARCH ARTICLE

ISOLATION OF IAA PRODUCING BACTERIA FROM SOIL AND OPTIMISATION OF CULTURE CONDITIONS FOR MAXIMUM IAA PRODUCTION.

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Abstract

Production of growth hormones has been the major criteria of bacteria in plant growth promotion. Laboratory study conducted to isolate the bacteria having Indole Acetic Acid (IAA) producing activity from soils under different agriculture practices and a virgin soil. The bacterial strains were observed for their IAA production, the major growth promoting trait helpful for the sustainable agriculture. IAA production from different bacterial strains was estimated for 1, 4, 6, 8 days of bacterial growth using salkowski reagent. All six isolates were observed for the production of IAA, among them I4 and I5 isolates were comparatively recorded with high competence. The culture conditions were optimised for two strains to determine the effect of additional carbon, nitrogen sources and pH of the culture media on IAA production. At optimised conditions 35.4µg/ml and 39.7 µg/ml of IAA concentrations were observed in culture media with I4, I5 isolates respectively.

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Introduction:-

Plant root system is a vital zone which could enhance the growth of soil microbes, the term rhizosphere first coined by Lorenz Hiltner (Anton Hartmann et al 2007). The soil bacteria were divided into three classes based on their interactions with plants; they include plant beneficial bacteria, pathogenic bacteria, plant neutral bacteria (bais et al., 2006). The bacteria rapidly colonize plant roots of different crops and promote plant growth and yield were termed as plant growth promoting rhizobacteria shortly PGPR (Joseph et al., 1980). The mechanism of plant growth promotion by the PGPR may be direct or indirect. PGPR could directly promote plant growth by producing various phytohormones or enhancing phosphate availability etc. (Munees et al 2014). Production of auxins especially IAA was considered as major growth promoting trait for plants (Dawwam et al 2013, Geetha et al 2014, Mahwish et al 2015, Farzana et al 2009, Javid et al 2013).

Indole 3- acetic acid been characterised as the most predominant, physiologically active, naturally occurring auxin, produced in larger quantities than any other related compounds (Hariharan et al.,2014). Various microorganisms present in soil capable of producing IAA include various bacterial and fungal species. The rhizospheric bacteria utilise the root exudates to produce IAA as part of their secondary metabolism (Hariharan et al., 2014). Mostly microorganisms produce IAA through tryptophan dependent pathway (Shulamit et al.,1994, Jiahui et al 2015), tryptophan independent pathway also hypothesised by researchers (Ricardo et al.,2015).

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Diverse genera of bacteria produce IAA in their rhizospheric colonising strategy include *Pseudomonas* (Sadafetal 2009, Monita et al., 2014, Farah et al.,2005, Farah et al 2008), *Bacillus* (Sadaf et al.,2009, ElSorra et al.,2007, Farah 2008), *Actinomycetes* (Hariharan et al., 2014), *Azotobacter* (Farah et al.,2005, Farah et al 2008), *Acinetobacter*, *Ralstonia*, *Stenotrophomonas* (lata et al.,2006)*Sphingomonas*, *Microbacterium*, *Mycobacterium*, *Rhizobium*, *Acampepapillosa*, *Sphingomonas*, *Rhodococcus*, *Cellulomonas*, *Micrococcus luteus* (Tsavkelova et al., 2005). IAA producing bacteria from different soils was known to promote growth in different plants (Hariharan et al., 2014, Monita et al.,2014, Farah et al., 2005,Ashrafuzzaman et al., 2009). Different stages of plant growth could be effected by the IAA production from rhizospheric bacteria includes seed germination (Hariharan et al., 2014), root elongation (Hariharan et al., Sadaf et al.,2009, Farah 2005, Loper1986), growth of root hairs (Sadaf et al., 2009), shoot length, shoot length, weight (Hariharan et al.,2014). The production of IAA in culture media varies with culture conditions like pH, incubation period, tryptophan concentration, type of carbon and nitrogen sources etc. (Mohite 2013). In the present study efficient IAA producing bacteria were isolated from different agriculture and virgin soils and the IAA production was optimized by additional carbon, nitrogen sources and pH of the growth media.

Materials and methods:-

Sample collection and bacterial isolation:-

Four soil samples collected for this study viz., virgin soil sample from forest regions of araku hilly region, agriculture field under Podu cultivation from Paderu region, agriculture fields under organic farming from Krushivignan Kendra and the agriculture field under chemical fertilizer and pesticide cultivation practices from anandhapuram. The soil samples are serially diluted and 100 μ l standard inoculum plated on to LB media. The media composition is as follows, 1 litre of media contains Casein enzymic hydrolysate 10.0gms, yeast extract 5.0gms. Sodium chloride 10.0gms, Distilled water 1000ml and pH adjusted to 7.5 \pm 0.2. Plates are incubated at 28 $^{\circ}$ C for 2 days and growth was monitored regularly. The clearly visible and morphologically different dominant colonies were selected from each plate and pure cultured on nutrient agar media.

Estimation of IAA production:-

All pure isolates were inoculated into 250ml conical flasks containing 50ml LB broth media. The liquid cultures are incubated on shaker incubator at 180rpm for 2days at 28 $^{\circ}$ \pm 1 $^{\circ}$ c. Cultures were centrifuged at 12000rpm for 10 min and 500 μ l of supernatant from liquid cultures are taken into 1.5ml tube, 1ml of Salkowski reagent was added. Salkowski reagent was prepared by dissolving 2% of 0.5M FeCl₃ in 35% perchloric acid. Reaction tubes were incubated for 30 min in dark at room temperature. The development of pink colour indicated the IAA production. Optical absorbance was measured at 530 nm.

Standard curve:-

Auxin production was determined by using standard graph of concentrations ranged from 1.25 μ g/ml to 100 μ g. Concentration of IAA in μ g/ml was taken on X-axis and the optical density on Y-axis for making standard curve. And by extrapolating the obtained OD value onto standard graph, the quantity of IAA produced by the bacterial isolates was obtained.

Optimisation of iaa production:-

Effect of incubation time on IAA production:-

The bacteria inoculated into liquid broth of LB medium and incubated on shaker at 120 rpm for eight days at 28 $^{\circ}$ C. IAA production was estimated at regular time intervals.

Effect of additional carbon and nitrogen sources IAA production:-

LB media was prepared and amended with two additional carbon sources Mannitol and sucrose in 1% concentration. The efficiency of additional carbon source in influencing the IAA production was observed. And the additional yeast extract at three different concentrations i.e., 0.1, 0.5 and 1% in LB media prepared. The effect of additional nitrogen source on IAA production at varying concentrations was observed.

Effect of pH on IAA production:-

LB media was prepared and adjusted to 3, 5, 7 and 9 pH conditions and bacterial strains were inoculated. The effect of different pH conditions on IAA production were observed at regular time intervals.

Results and discussion:-

Plant growth promoting bacteria has ability to colonise in rhizospheric region of plant system exert stimulation of plant growth either directly or indirectly. In our study we have isolated 6 bacterial strains from four soil samples and tested for their ability to produce IAA. All six isolates namely I1, I2, I3, I4, I5, I6 were able to produce moderate to high amount of IAA under laboratory conditions (Table. 1). During the time of nine days of incubation period, the IAA production by all isolates was ranged from 0 to 33µg/ml. And 6th day was the optimal day for the IAA production in all cultures (Fig. 1). Among them I4 and I6 have shown highest IAA production of 27.1µg/ml and 33.5µg/ml and were considered as potential IAA producers than the remaining cultures and continued for further optimisation studies. Production of phytohormones phosphate solubilisation nitrogen fixation and etc. were considered as direct effects of growth promotion (Rishi et al., 2015). Among other auxins IAA had been the native and well-studied for their plant growth promoting activities. Many rhizospheric bacteria have reported to produce IAA in in-vitro condition from low to high efficiency. The IAA production was generally effected by the culture conditions, growth stage and substrate availability for bacteria (Farah et al., 2005, Ashrafuzzaman et al., 2009, Mohite 2013). The concentration of tryptophan effected the IAA production of bacteria under culture conditions was well studied (Ashrafuzzaman et al., 2009). The external supply of tryptophan is not essential for the bacterial IAA production even though it has stimulatory effect on amount of IAA production (Farah et al., 2005).

Effect of incubation time on IAA production:-

IAA concentration produced in LB media at pH 7 and 28±2°C observed for four times in period of 8 days includes 1st 3th 5th 7th 9th days respectively. All six strains have shown their highest activity at 6th day of incubation period ranged from 7µg/ml to 33µg/ml. Our findings were in agreement with other researchers that 6th day incubation of bacterial cultures has shown maximum IAA production. (Dipanwita et al., 2015, Nita et al., 2011, Apine et al., 2011), but not agreed with others (Dhara 2009, Hariharan et al., 2014) that 72 hr of incubation on LB medium was optimum for IAA production. And after 6th day incubation there was decline in IAA production. According to Bhosale et al., 2016, cyanobacterial strains could able to produce IAA over three weeks of incubation time. The µg/ml of IAA production on sixth day incubation period were 7.4, 7.8, 17.06, 33.5, 27.11, 22.09 by the isolates I1, I2, I3, I4, I5, I6 respectively.

Effect of additional carbon and yeast extract concentration on IAA production:-

Two additional carbon sources mannitol and sucrose at 1% in culture medium upon IAA production of two bacterial strains I4 and I6 was observed. Mannitol and sucrose have shown significant effect on IAA production. I4 strain have produced highest activity of 34.5µg/ml with additional 1% sucrose in culture medium observed similar with previous reports (Umang et al., 2013, Apine et al., 2011) (Fig. 2). But mannitol addition have results in higher IAA production than the sucrose was observed in few reports (Jeyanthi et al., 2013). The IAA concentration reached 39.7µg/ml for I5 strain in 6th day incubation when additional 1% sucrose present in the culture medium (Fig. 3). Yeast extract at varying concentrations of 0.1%, 0.5% and 1% on culture conditions was observed for its effect in IAA production. Effect of change in nitrogen source effected the IAA production was previously reported (Umang et al., 2013, Apine et al., 2011). The concentration of the nitrogen source present in the media also shows significant effect on IAA production. Apine et al., 2010 standardized the optimum concentration of 0.8% meat extract in the culture medium for highest IAA production. The positive effect of yeast extract added to the culture medium for optimal IAA production have observed in statistical analysis of optimisation of IAA production with changes in media composition (Sasirekha et al., 2012). Here in our study we optimised the concentration of yeast extract on IAA production. I4 strain with 0.5% yeast extract has shown highest IAA production of 35.4µg/ml than the control and 1% yeast extract concentration (Fig. 4). I5 strain has shown highest IAA production of 39.9µg/ml at 1% yeast extract concentration (Fig. 5).

Effect of pH on IAA production:-

Neutral pH that is 7-8 was general optimal condition for IAA production by different bacterial strains. I4 strain was actively producing IAA only at pH7 (Fig. 6) and there was drastic decrease in IAA production when pH was higher or lower than that. The results were in agreement with other reports (Hariharan et al., 2014, Umang et al., 2013, Apine et al., 2011). I5 strain has effective IAA production at pH 5 then the pH 7 (Fig. 7). This result was not agreed with other researchers (Hariharan et al., 2011). Optimal pH for maximum IAA production was reported to be lower than neutral by some previous reports similar to our present result (Nita et al., 2011).

Conclusion:-

All six isolated bacterial strains were potent IAA producers. But two strains namely I4 and I5 were considered best among all other strains, and considered for further analysis. Media optimizations were carried for the effective incubation period for highest IAA production, effect of additional carbon source and effect of nitrogen source concentration and the optimal pH for maximum IAA production. It was concluded that IAA production was maximum at 6th day of incubation with 1% additional sucrose, 0.5% yeast extract and pH 7 in I4 isolate. And I5 isolate have shown its effective IAA producing activity at 6th day incubation period with 1% additional sucrose, 1% yeast extract concentration and pH 5.

Table.1:- Concentrations of IAA for six different isolates in regular intervals.

	IAA production (µg/ml)			
	1st day	4th day	6th day	8th day
I1	0.3	1.657754	7.491979	11.93048
I2	0.6	2.085561	7.812834	12.62567
I3	0	0	17.06417	20.37968
I4	3	18.60963	33.53476	21.23529
I5	3.2	14.38503	27.11765	19.73797
I6	2.9	15.93583	22.09091	18.93583

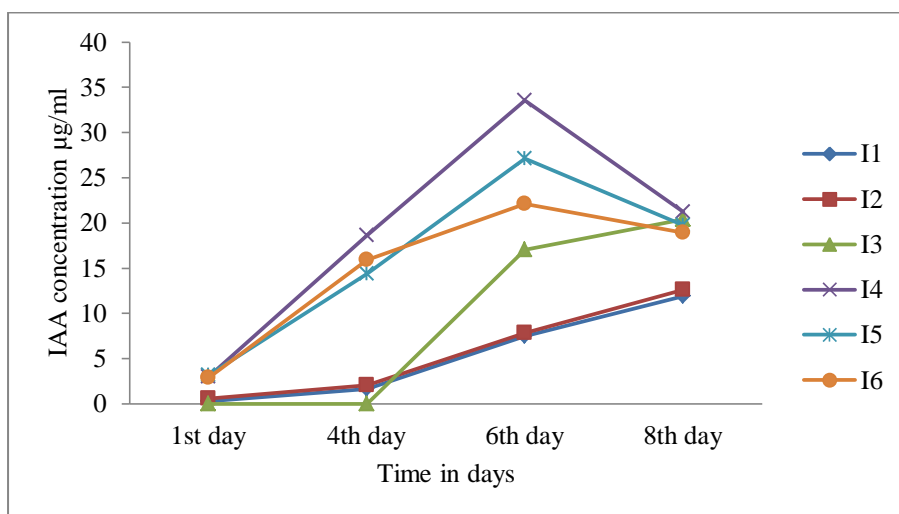


Figure1:- Graph showing concentrations of IAA for six different isolates in regular intervals.

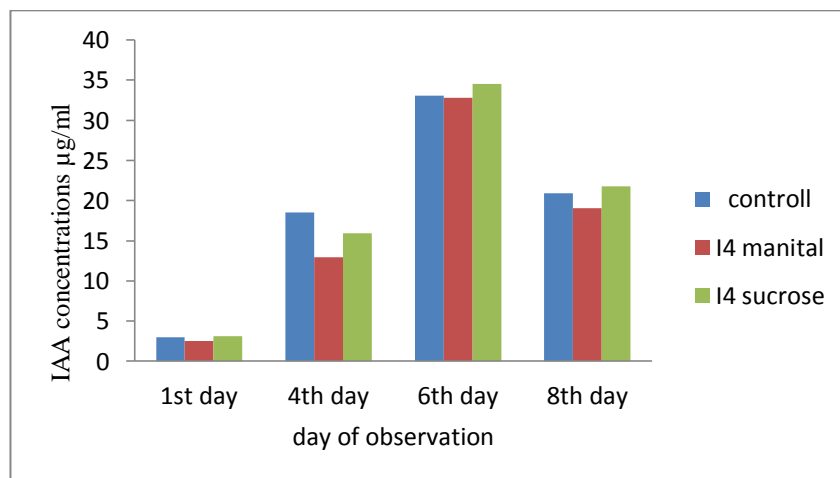


Figure2:- Effect of additional carbon source on IAA production of I4 isolate

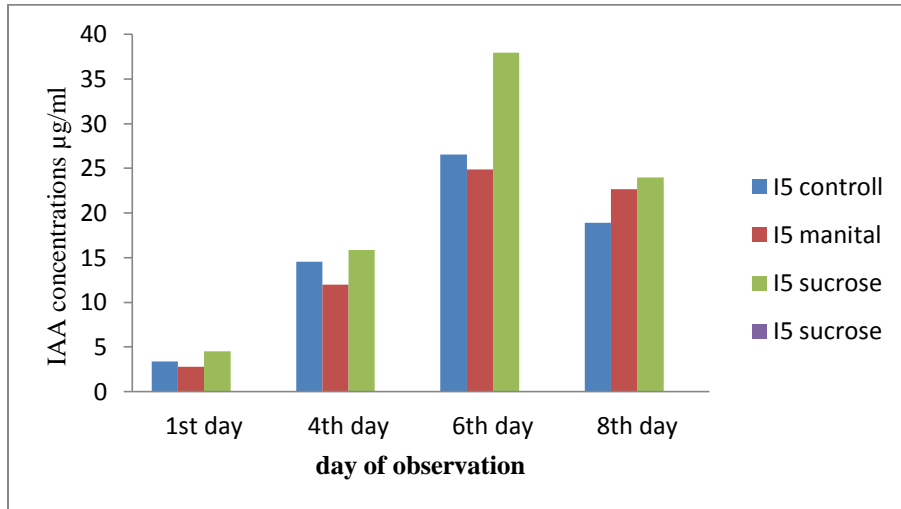


Figure 3:-Effect of additional carbon source on IAA production of I5 isolate

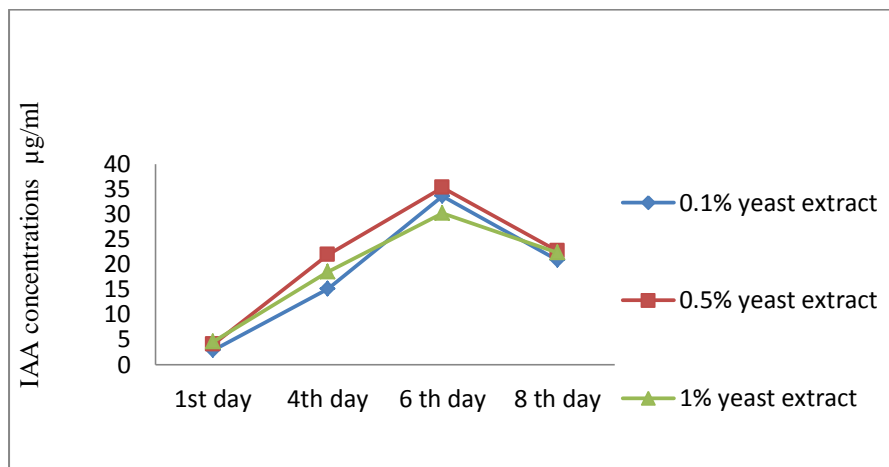


Figure 4:- Effect of yeast extract concentration on IAA production of I4 isolate

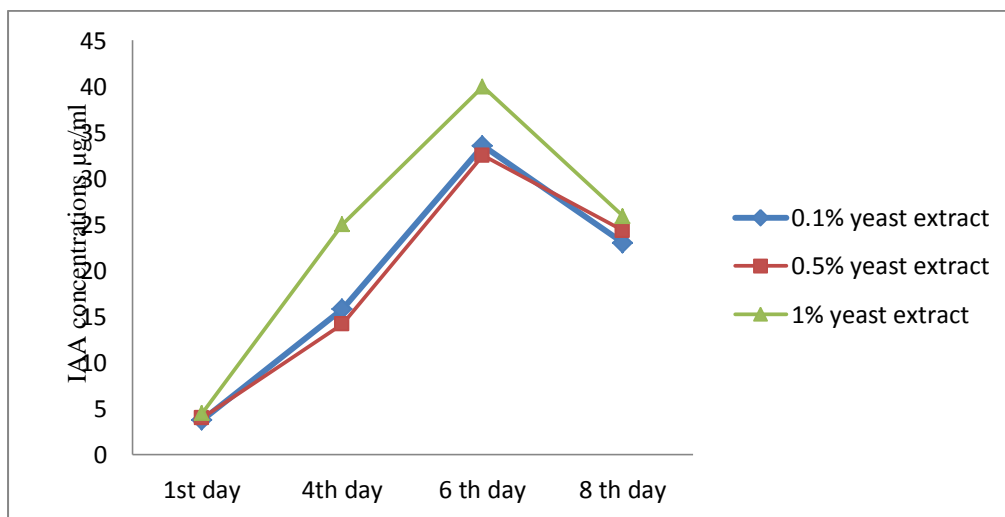


Figure 5:- Effect of yeast extract concentration on IAA production of I5 isolate

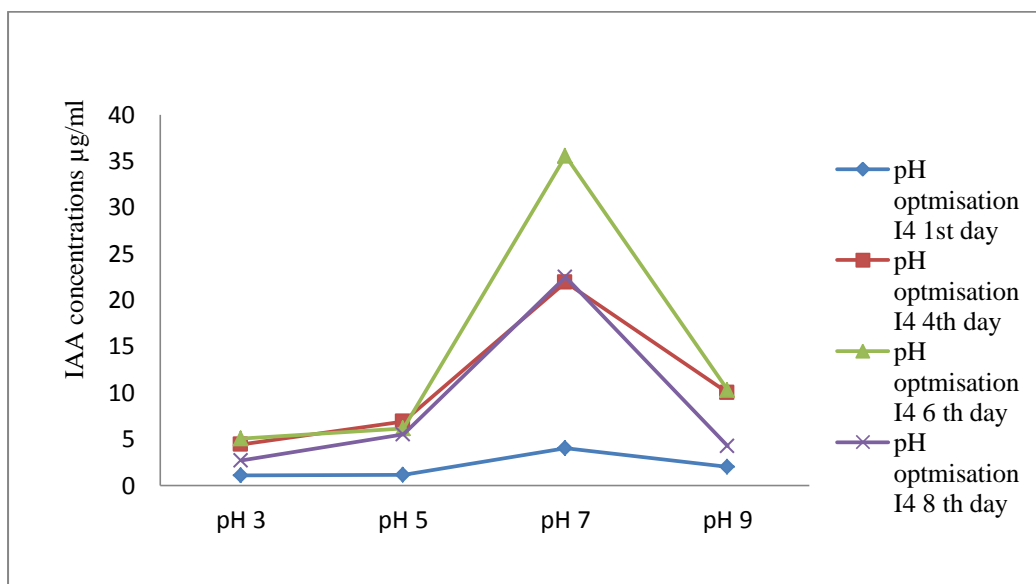


Figure6:- Effect of pH on IAA production of I4 isolate

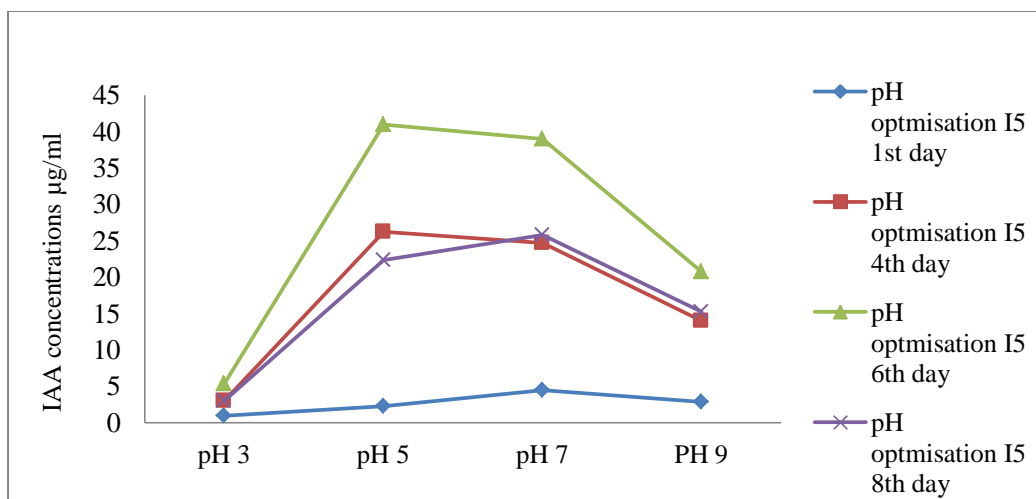


Figure 7:- Effect of pH on IAA production of I5 isolate

References:-

1. Anton, H., Michael, R., and Michael, S. (2008): Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil*. Vol312:7–14.
2. Apine, O. A. and Jadhav, J. P. (2011): Optimization of medium for indole-3-acetic acid production using *Pantoea agglomerans* strain PVM. *Journal of Applied Microbiology*. Vol 110: 1235–1244.
3. Ashrafuzzaman, M., Farid, A. H., Razi, I. M., Anamul, H. M., Zahurul, I. M., Shahidullah, S. M. and Sariah, M. (2009): Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology* Vol 8 (7): 1247-1252.
4. Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S. and Vivanco, J. M. (2006): The role of root exudates in rhizosphere interactions with plants and other organisms. *Ann. Rev. Plant Biol.* Vol57:233-266.
5. Bhosale, A., Puranik, P. and Pawar, S. (2016): Screening and optimization of indole 3 acetic acid producing non-heterocystous cyanobacteria isolated from saline soil. *Sch. Acad. J. Biosci.* Vol4(9):738-744.
6. Dawwam, G. E., Elbeltagy, A., Emara, H. M. Abbas, I. H. and Hassan, M. M. (2013): Beneficial effect of plant growth promoting bacterianisolated from the roots of potato plant. *Annals of Agricultural Science*. Vol 58(2):195–201.

7. Dhara, P. S., Hemangi, G. C., Vijay, M. K., Dilip, D.D. and Balu, A. C. (2009): Isolation and characterization of Indole acetic acid producing *Klebsiella pneumonia* strain from rhizosphere of wheat (*Triticumaestivum*) and their effect on plant growth. *Indian journal of experimental biology*. Vol 47: 993-1000.
8. Dipanwita, D., Chandan, S. and Goutam, P. (2015). Screening and Identification of Best Three Phosphate Solubilizing and IAA Producing PGPR Inhabiting the Rhizosphere of *Sesbaniabispinosa*. *International Journal of Innovative Research in Science, Engineering and Technology*. vol4,issue6.
9. ElSorra, E. I., Domingo, J. I., Manuel, T. and Rainer, B. (2007): Tryptophan-Dependent Production of Indole-3-Acetic Acid (IAA) Affects Level of Plant Growth Promotion by *Bacillus amyloliquefaciens* FZB42. *The American Phytopathological Society. MPMI* Vol. 20(6): 619–626.
10. Farah, A., Iqbal, A. and Mohd, S. K. (2005): Indole Acetic Acid Production by the Indigenous Isolates of *Azotobacter* and Fluorescent *Pseudomonas* in the Presence and Absence of Tryptophan. *Turk J Biol* Vol 29: 29-34.
11. Farah, A., Iqbal, A. and Mohd, S. K. (2008): Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research*. Vol 163:173—181.
12. Farzana, Y., Radziah, O., Kamaruzaman, S. and Mohd. S. S. (2009): Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. *African Journal of Microbiology Research* Vol. 3(11):815-82.
13. Geetha, K., Venkatesham, E., Hindumathi, A. and Bhadrachari, B. (2014): Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vigna Radita* (L.) R. Wilczek. *Int.J.Curr.Microbiol.App.Sci*. 3(6): 799-809.
14. Hariharan, H., Vellasamy, S. and Natesan, B., (2014): Optimization for production of Indole acetic acid (IAA) by plant growth promoting *Streptomyces* sp VSMGT1014 isolated from rice rhizosphere. *Int.J.Curr.Microbiol.App.Sci*. Vol 3(8):158-171.
15. Javid, A. P., Azra, N. K., Zaffar A. R., Rehana, H. and Raies, A. Q. (2013): Screening of beneficial properties of rhizobacteria isolated from Saffron (*Crocus sativus* L.) rhizosphere. *African Journal of Microbiology Research*. Vol. 7(23):2905-2910.
16. Jeyanthi, V. and Ganesh (2013): Production, Optimization and Characterization of Phytohormone Indole Acetic Acid by *Pseudomonas fluorescens*. *International Journal of Pharmaceutical & Biological Archives*. 4(2): 514 – 520.
17. Jiahui, S., Shuqing, L., Nan, Z., Xiaoshuang, C., Xuan, Z., Guishan, Z., Qirong, S and Ruifu, Z. (2015): Analysis and cloning of the synthetic pathway of the phytohormone indole-3-acetic acid in the plant-beneficial *Bacillus amyloliquefaciens* SQR9. *Microb Cell Fact*. 14:130.
18. Joseph, W. K., John, L., Martin, T. and Milton, N.S. (1980): Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*. Vol 286: 886-88.
19. Lata, H., Li, X. C., Silva, B., Moraes, R. M. and Halda, A. L. (2006): Identification of IAA-producing endophytic bacteria from micropropagated Echinacea plants using 16S rRNA sequencing. *Plant Cell, Tissue and Organ Culture*. Vol 85: 353–359
20. Loper, J. E. and Schroth, M. N. (1986): Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet, *Physiology and biochemistry*. Vol 76(4): 387-389.
21. Mahwish, Z., Kaleem, A. M., Sohail, H. and Nasir, R. (2015): Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zeamays* L.). *Front. Microbiol*. Vol 6: 207.
22. Mohite, B. (2013): 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*. Vol 13(3): 638-649.
23. Monita, V. P. and Rajesh, K. P., (2014): Indole-3-acetic acid (iaa) production by endophytic bacteria isolated from saline desert. *The little runoff kutch*. *CIBTech Journal of Microbiology*. Vol 3 (2):17-28.
24. Munees, A. A. B. and Mulugeta, K. (2014): Mechanisms and applications of plant growth promoting rhizobacteria. *Current perspective*. *Journal of King Saud University – Science*. Vol 26:1–20.
25. Nita, B. P., Milind, G., Sangita, S. A., Aparna, B. G., Balasaheb, P. K. (2011): Optimization of Indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. *International journal of environmental sciences*. Vol 2:1
26. Ricardo, C., Tiago, E. F., and Irene, S. S. (2015): The Plant Growth-Promoting Bacteria *Azospirillum amazonense*: Genomic Versatility and Phytohormone Pathway. *BioMed Research International*. Article ID 898592
27. Rishi, K., Garima, P., Nitesh, J. and Pavan, K. A. (2015): Plant Growth Promoting Rhizobacteria: Mechanism and Current Prospective. *Journal of Fertilizers & Pesticides* Vol 6, issue 2.

28. Sadaf, S., Nuzhat, A and Nasreen S. K. (2009): Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. *African Journal of Agricultural Research*. Vol 4 (11):1312-1316.
29. Sasirekha, B., Shivakumar, S. and Sullia, B. (2012): Statistical optimization for improved indole-3-acetic acid (iaa) production by *Pseudomonas aeruginosa* and demonstration of enhanced plant growth promotion. *Journal of Soil Science and Plant Nutrition*. Vol 12:4
30. Shulamit, M., Hadas, S., Ephraim, E., Amnon, L. and Isaac, B. (1994): Biosynthesis of indole-3-acetic acid via the indole-3-acetamide pathway in *Streptomyces* SPP. *Microbiology*. Vol 140:1045-1050.
31. Tsavkelova, E. A., Cherdynseva, T. A., and Netrusov, A. I. (2005): Auxin Production by Bacteria Associated with Orchid Roots. *Microbiology*. Vol 74(1): 46–53.
32. Umang, B., Kamlesh, P. and Ujjval, B. T. (2013): Optimization of Indole Acetic Acid Production by *Pseudomonas putida* UB1 and its Effect as Plant Growth-Promoting Rhizobacteria on Mustard (*Brassica nigra*). *Agric Res* 2(3):215–221.