Effect of Jeevamrutha on Seed Germination of *Ocimum basilicum* L. under Different Cadmium Concentrations

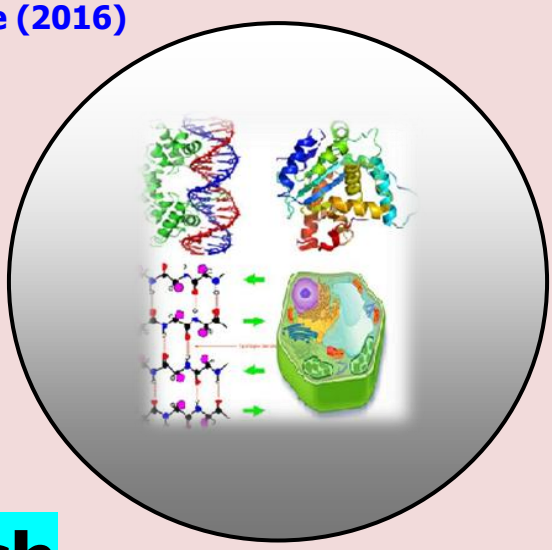
By

Ankit Samuel Singh and Eugenia P. Lal

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 35 (2) 2018 Pages No. 386-392



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 35, No. 2: 386-392, 2018
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 35/02/6001/2018

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

S. S. Sakure
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER

Received: 21/05/2018 **Revised: 21/06/2018** **Accepted: 22/06/2018**

Screening and Optimization of IAA Producing Actinomycetes Isolated from Rhizosphere of different Plants

S. S. Sakure and *S.B. Kshemkalyani

Department of microbiology, S.B.B. alias Appasaheb Jedhe College, Pune, India
***Department of microbiology, Ahemadnagar College, Ahemadnagar, Pune, India.**

ABSTRACT

A total of 32 actinomycetes isolates were isolated from rhizosphere soil of different plants and screened for their potential to produce IAA. Five isolates showing maximum IAA production and were selected for optimization study. The highest IAA producing isolate (33µg/ml of IAA) was A3 recovered from rhizospheric soil sample of Aloevera near Pune.IAA production by actinomycetes A3, D, BF5, Turmeric and H5 was optimized by studying environmental parameters. There was maximum IAA production when the isolates were cultivated in tryptone yeast extract broth supplemented by tryptophan 2 mg/ ml, pH 8 at 30º C for 7 days. TLC analysis confirmed the IAA production in cell filtrate of A3.Pot study revealed significant increase in root and shoot length and root and shoot dry weight using A3. These results suggest that IAA producing A3 could be a promising candidate for utilization in growth improvement of plant for sustainable agricultural development .Morphological, cultural and 16s rDNA partial gene sequencing of A3 similarity with Streptomyces spp.

Key words: Indole -3- acetic acid, TLC, Optimization, Tryptophan and Rhizosphere.

Abbreviations-
IAA- Indole-3-acetic acid
TLC-Thin layer chromatography

INTRODUCTION

Actinomycetes are Gram positive bacteria, with a high guanine (G) plus cytosine (C) ratio in their DNA (>55mol %), which are phylogenetically related from the evidence of 16S ribosomal cataloguing and DNA: rRNA pairing studies (Goodfellow and Williams, 1983). The actinomycetes are a group of bacteria which possess many important and interesting features. They are of considerable value as producers of antibiotics and of other therapeutically useful compounds. They exhibit a range of life cycles which are unique among the prokaryotes and appear to play a major role in the cycling of organic matter in the soil ecosystem (Veiga*et al*., 1983). Therefore, actinomycetes hold a prominent position due to their diversity and proven ability to produce new compounds, because the discovery of novel antibiotic and non-antibiotic lead molecules through microbial secondary metabolite screening is becoming increasingly important. Active actinomycetes may be found in medicinal plant root rhizosphere soils and may have the ability to produce new inhibitory compounds against

phytopathogens. Plant root exudates stimulate rhizosphere growth of *Streptomycetes* that are strongly antagonistic to fungal pathogens.*Streptomyces sp.* Strain5406 has been used in China to protect cotton crops against soil-borne pathogens.

The auxins are group of Indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth. Indole – 3-acetic acid (IAA) is the main member of the auxins family that controls many important physiological processes including cell enlargement and division, tissue differentiation; it stimulates spore germination and mycelia elongation in the Streptomyces. Several Streptomyces species, such as *S. olivaceoviridis,S. rimosus, S. rochei*and *Streptomyces* spp. from the tomato rhizosphere, have the ability to produce IAA and improve plant growth by increased seed germination, root elongation and root dry weight. Microbial isolates recovered from the rhizosphere of different crops appear to have greater potential to synthesize and release IAA as secondary metabolites. Production of IAA by microorganisms varies greatly among different species and strains and depends on the availability of substrate(s) .Many bacteria isolated from the rhizosphere have the capacity to synthesize IAA in vitro in the presence or absence of physiological precursors such as tryptophan. Different bacterial pathways to synthesize IAA have been identified and high degree of similarity between IAA biosynthesis pathways in plants and bacteria was observed

Tryptophan is believed to be the primary precursor for the formation of IAA in plants and microorganisms. This study aims to screen and optimize IAA producing actinomycetes and to study the effect of highest IAA producer on growth of plant *in vitro*.

MATERIALS AND METHOD

Sample collection and transport (Crawford D. L, et al., 1993.)

Soil samples were collected from rhizosphere of medicinal plants such as Turmeric, Aloevera, Hibiscus and soil near brick factory plants rhizosphere from Pune region. All the samples were collected in sterilized autoclave bags, transferred to laboratory and maintained at 4^{o}C.

Sample pretreatment and isolation:

Heat treatment was given to all the soil samples by keeping them in hot air oven at 50^{o}C for 1hr. The soil suspension was serially diluted and 0.1ml aliquots of each dilution was spread on CSA (casein starch agar) and AIA (actinomycetes isolation agar) medium and incubated at 28^{o}C for 5-6 days. After incubation plates were observed for growth of actinomycetes (Sakthi velayudham et al, 2012).

***In vitro* screening for IAA production**

The Actinomycetes isolates, grown on tryptone yeast extract agar and incubated at 30°C for 5 days were transferred to 5ml tryptone yeast extract broth containing 2mg/ml L-tryptophan (Bano & Musarrat). These cultures were incubated at 30°C with shaking at 125 rpm for 7 days. Then harvested by centrifugation for 15 min. one ml of supernatant was mixed with 2mL of Salkowasky's reagent. The appearance of a pink color indicated IAA production. Optical density was read at 535 nm. The level of IAA produced was estimated against the IAA standard.

Extraction and purification of IAA

Isolates were cultivated in tryptone yeast extract broth & it was centrifuged for 15 min. The supernatant was collected & mixed with ethyl acetate (1:2).After vigorous shaking it was allowed to stand for 10 min.IAA was extracted within solvent layer .The procedure was repeated 3 to 4 times.

Thin layer chromatography

Propanol: water (8:2) was used as solvent system. The extracted sample & standard IAA (10 mg/100 ml) were spotted on TLC plate. Chromatogram was developed with Salkowasky's reagent.

Optimization of IAA production

The best IAA producer isolates of IAA were chosen for further studies to optimize the environmental conditions for IAA production. Isolates were grown on various media supplemented with 2mg/ml L – tryptophan. Media used for study of best medium for production of IAA were ,Tryptone soya yeast extract, Nutrient media, ISP-2 ,ISP-4 ISP-1 .The effect of L – tryptophan concentration was studied by cultivating the strains in tryptone yeast extract broth supplemented with different concentrations of L – tryptophan (2, 4, 6,8,10 mg/ml).The effect of pH and temperature on IAA production was studied by cultivating the strain in nutrient broth containing 2mg/ml L-tryptophan at different temperatures (20-45°C) and pH levels ranging from 2 – 10 for 7 days.

Pot assay

Seeds were surface disinfected by dipping in 95 % ethanol and 0.2 % (w/v) mercuric chloride solutions as described by Russell et.al . The Treated seeds were rinsed thoroughly with sterilized water. Sterilized seeds were immersed for 20 minutes in culture rich broth grown in CSA broth for 72 hrs, and then the seeds were removed and allowed to dry. Uninoculated seeds were used as control. Coated seeds were sown in pot containing sterile soil. Water was added in equal quantity in the pots as per daily requirement and observed for growth of maize plants.(D. A. Gamit et al.,IJR in Pure and Applied Microbiology).

Molecular identification of potential isolates:

16s rDNA partial gene sequencing was done by isolating and purifying the genomic DNA of potential isolates. This work was carried out in association with Genombio technologies, Pune. The nucleotide sequence obtained were compared using the BLASTN programme on the page of National centre for biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and BLAST analysis was realized.

RESULT AND DISCUSSION

All isolated strains were filamentous, Gram positive, non motile and aerobic in nature, having Catalase and Oxidase activities. Slide culture observation indicated its similarity with genus *Streptomyces*. All tested actinobacterial cultures utilized every tested rhizospheric sugar indicating higher probability of rhizosphere survival.

A total of 33 isolates were screened for IAA production. Out of this 18 isolates have the ability to utilize tryptophan and produce the IAA. The IAA producing isolates were categorized into three groups according to the amount of IAA produced. Seven isolates produced low concentration (<17µg/ml), six isolates produced moderate concentration (17-25µg/ml), and five isolates produced high concentrations (>26µg/ml) of IAA. These five isolates, A3, Tu, D, H5, and BF4 were used for optimization studies. The highest producer isolate (33 µg/ml of IAA) was A3 recovered from rhizospheric soil sample of Alovera plant.

Out of five medium tested, highest amount of IAA production was recorded in tryptone yeast extract broth and nutrient media (42 µg/ml). Studies on Days of incubation time indicate that the production of IAA increased with time till 11-12 days then decreased as shown in graph for selected isolates.

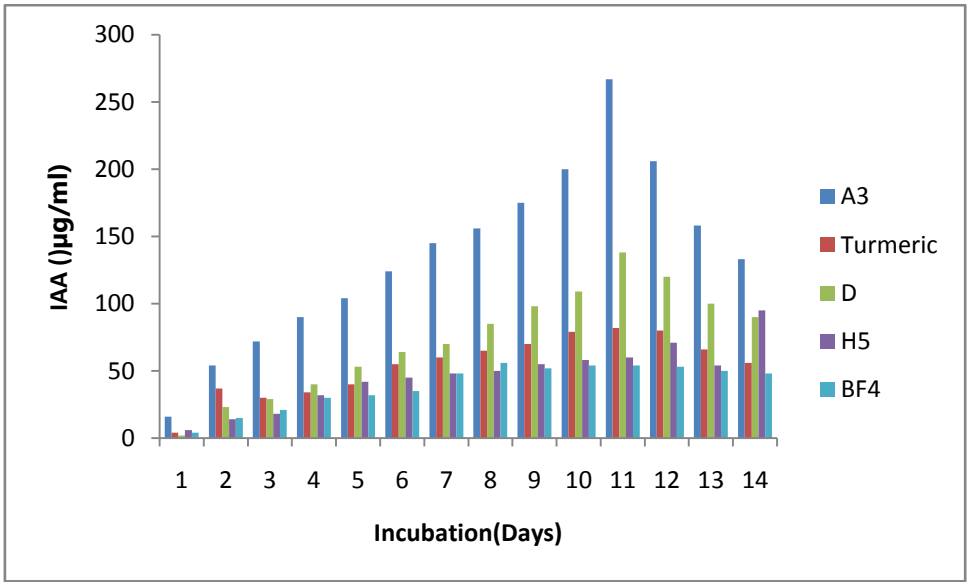


Figure 1. Effect of incubation time on IAA production.

Various concenentrations of tryptophan (2, 4, 6, 8,10mg/ml) were used to check the effect of
These concentrations on IAA production. Maximum IAA production was found at 10mg/ml concentration of tryptophan. There is gradual increase in IAA production except for H5 isolate

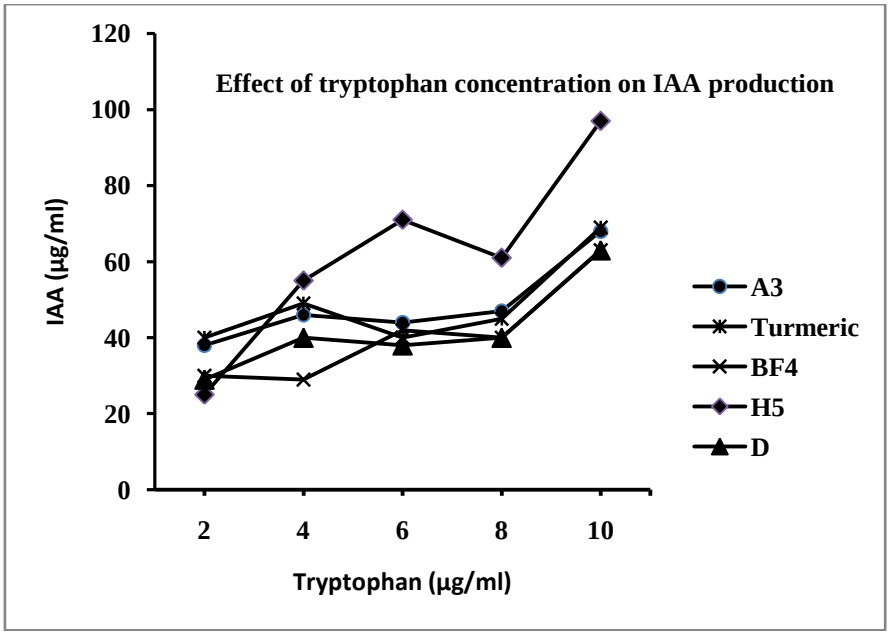


Figure 2 Production of IAA in different tryptophan concentrations by five different isolates of actinomycetes.

The effect of different values of pH (2, 4, 6, 8, and 10) of the culture medium on the biomass and IAA production was investigated. IAA formation production was increased gradually with the increase of pH values reaching maximum at pH 8 which is followed by decreasing at pH values more than pH 8. These actinomycete isolates also shows maximum growth at pH -8

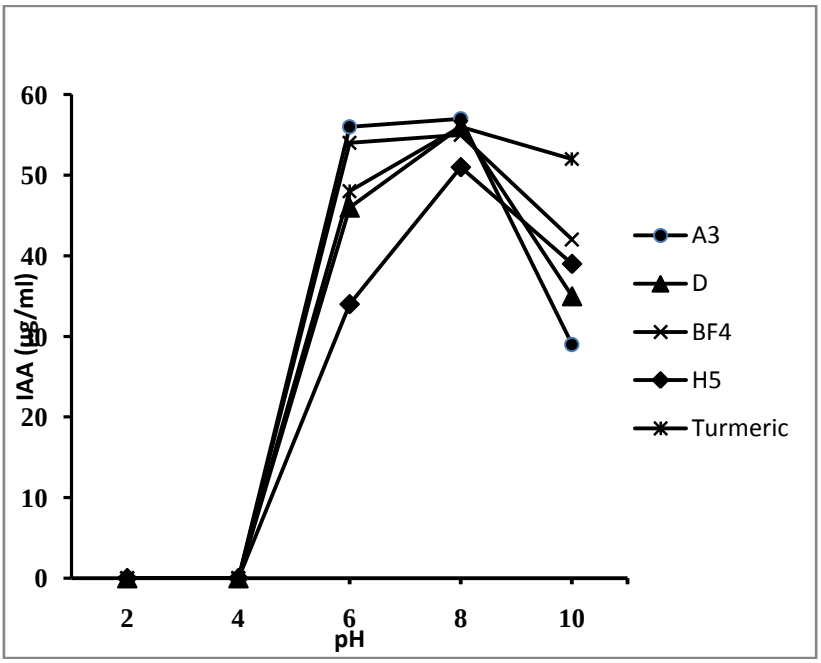


Figure 3. Production of IAA at different pH values by five different isolates of actinomycetes.

The effect of different temperatures (20, 25, 30, 35, and 40) on IAA formation was investigated. The optimum temperature for IAA formation was at 30º.

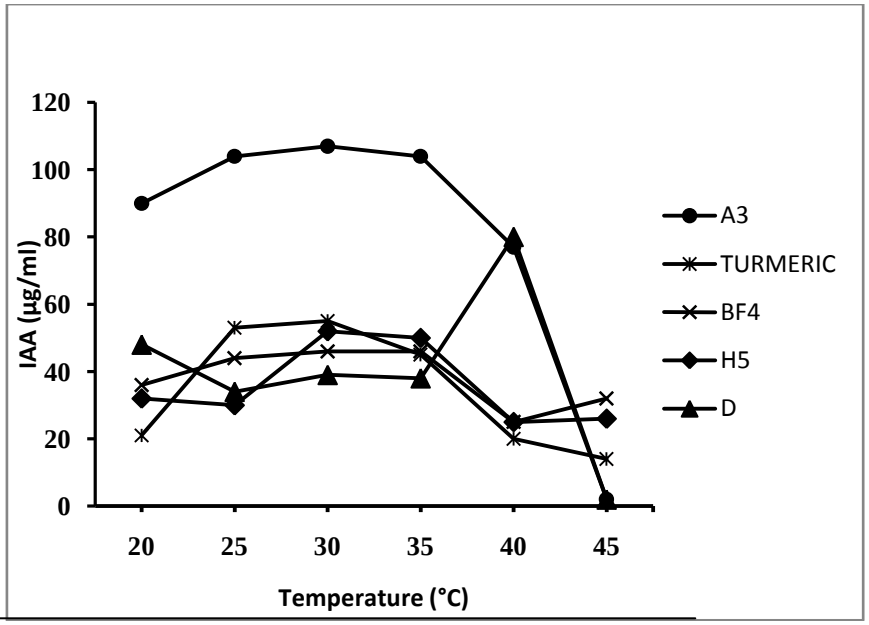


Figure 4. Production of IAA at different temperature values by five different isolates of actinomycetes.

Plant hormones are chemical messengers that affect plant ability to respond to its environment. IAA is the member of the group of phytohormone and is generally considered the most impotent native auxins. It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, differentiation and gene regulation. Diverse actinomycetes species possess the ability to produce the auxin phytohormone IAA, these actinomycetes produce auxins in the presence of a suitable precursor such as L-tryptophan.

The IAA was extracted by ethyl acetate and extract were eluted on the TLC sheet against standard IAA substance and the Rf was recorded as 0.91.Morphological , cultural and biochemical characteristics confirmed that isolates A3 as *Streptomyces* spp.

The actinomycetes isolates which were collected from rhizosphere soil sample in this investigation were tested for their ability to produce IAA. Several reports have shown that

Different actinomycetes species from many crop rhizosphere soils have this ability.

A total of five isolates were able to produce high concentrations of IAA ranged between 17 to 26 µg/ml. The most active isolate obtained in this study is collected from the rhizosphere of *Aloevera*. The culture filtrate of the highest active isolates A3 was use to extract IAA for characterization by TLC. Chromatograms of culture extracts and standard IAA, sprayed with Salkowasky's reagent, showed almost the same R_f values. The TLC findings are in agreement with other reports. IAA production was affected by the initial pH value of the broth medium. IAA production increased gradually with the rise of pH values until 8.Acidic or high alkaline pH is unsuitable for IAA production because Streptomyces grow poorly in these conditions.

The molecular identification by 16SrDNA partial sequencing and morphological characteristics confirmed that isolates A3 confirmed as *Streptomyces* spp. The IAA production by the tested isolates was maximum at 30º C. Our results are consistent with the previous finding of Aldesuquy et al. who recorded temperatures in the range between25 to 30º C were suitable for growth and IAA production by Streptomyces sp. The maximum IAA were achieved at 10 mg/l tryptophan concentration. It has been reported that IAA production by plant growth promoting rhizobacteria can vary amount different sp. And it is also influenced by culture condition, growth stage and substrate availability.IAA can increase colonization of plant surfaces by the epiphytic an endophytic bacteria that enhances plant growth and yield.

Pot study using isolate A3 showed significant increase in root length and root dry weight of maize.

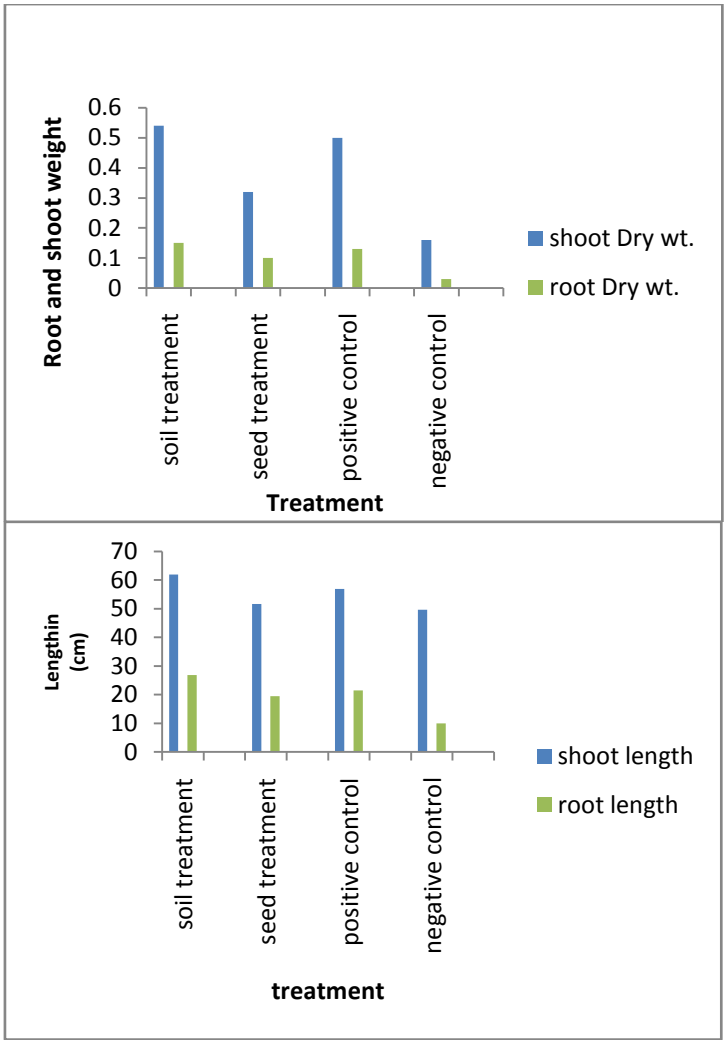


Figure 5 Effect of treatment on growth parameters of maize.

From this study, it is clear that rhizosphere soil can provide a rich source of IAA producing bacteria and has the ability to produce a significant amount of IAA in a tryptophan supplemented medium. It is concluded that presence of such growth promoting rhizoflora accountable for the beneficial effects on crop growth and yield. The significance of the study could be stated as the potential of these IAA producing isolates and optimization study for IAA production will flourish the growth & ultimately IAA production in the field and prevent environmental pollution by avoiding excessive application of industrially produced fertilizers to cultivated fields. Streptomyces A3 can be very effective and potential microbial inoculant and can be used as plant growth promoting rhizobacteria for enriching the soil fertility and enhancing the crop yield.

ACKNOWLEDGEMENTS

The authors are grateful to DR.Shashank Pole, Principal S.B.B. alias Appasaheb Jedhe College, Pune for providing basic research facilities. The authors are thankful to Madhuri Bhandwalkar, Komal Mohite and Sucheta Ghurphale for their cooperation.

REFERENCES

Brenner, D.J., Krieg, N. and Staley, J.R. (2005). Bergey's Manual of systematic bacteriology, vol 2. Springer, New York.

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, S. T. Williams (1994). Bergey's Manual of Determinative Bacteriology ninth edn, Baltimore, Philadelphia, Hong Kong, London, Munich, Sydney, Tokyo: Williams and Wilkins.

Mohamed Hemida Abd- Alla, EI- Sayed A.EI- Sayed, Abdel- Hamied M. Rasmey. Indole-3-acetic acid (IAA) production by Streptomyces atrovirens isolated from rhizospheric soil in Egypt

Hariharan Harikrishnan, Vellasamy Shanmugaiah and Natesan Balasubramanian. Optimization for production of Indole acetic acid (IAA) by plant growth promoting Streptomyces sp VSMGT1014 isolated from rice rhizosphere.

Bharucha, U. D., Patel, K. C. and Trivedi, U. B. (2013). *In vitro* screening of isolates for its plant growth promoting activities from the rhizosphere of Alfalfa (*Medicago Sativa*) J. Microbiol. Biotech. Res., 3 (5): 79-88.

Mohite, B. Isolation and characterization of . Indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth.

Jog, R., G. Nareshkumar and S. Rajkumar (2010). Plant growth promoting potential and sol enzyme production of the most abundant Streptomyces spp. from wheat rhizosphere. Ph. D. thesis, Manisha S. Bendale, NMU, Jalgaon.

Nandini Jodhawat, Sanju Purohit, Ruchika Sharma and Swarnjeet Kaur (2012). Antagonistic potentiality of secondary metabolite producing actinomycetes isolated from soil of western Rajasthan Int. J. Pharm. Sci. Rev. Res., 14(2), 53-56.

Nita, B. Patil, Milind Gajbhiye, Sangita S. Ahiwale, Aparna B. Gunjal, Balasaheb P. Kapadnis (2011). Optimization of Indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. International Journalof Environmental Sciences. 2(1): 307-314

Sasikumar Arunachalam Palaniyandi, Seung Hwan Yang, Karthiyaini Bamodharan and Joo-Won Suh (2013). Genetic and functional characterization of culturable plant-beneficial actinobacteria associated with yam rhizosphere. Journal of basic microbiology, 1-11.

Sakthi Velayudham, Kasi Murugan (2012). Diversity and Antibacterial Screening of Actinomycetes from Javadi Hill Forest Soil, Termilnadu, India. Journal of Microbiology Research. 2(2): 41-46.

Shirling, E.B. and Gottlieb, D. (1966). Methods for characterization of Streptomyces species, International journal of systematic bacteriology. 16(3): 313-340.

Thangapandian, V., Ponmuragan, P. and Ponmuragan, K. (2007). Actinomycetes diversity in the rhizosphere soil of different medicinal plants in Kolly Hills Termilnadu, India, for secondary metabolite production. Asian Journal of Plant Science 6, 66-70.

Vaishali, A. Pawar, Pooja R. Pawar, Ashok M. Bhosale and Sourabh V. Chavan (2014). Effect of *Rhizobium* on Seed Germination and Growth of Plants, Journal of Academia and Industrial Research (JAIR), Volume 3. ISSN: 2278-5213.

Corresponding author: Dr. S. S. Sakure, Department of microbiology, S.B.B. alias Appasaheb Jedhe College, Pune, India
Email: sakuresunita@gmail.com Mobile no.9325510030

Indexed, Abstracted and Cited in Indexed Copernicus International and 20 other databases of National and International repute