

PRODUCTION OF INDOLE ACETIC ACID BY
Azotobacter, Rhizobium **AND PHOSPHATE**
SOLUBILISING BACTERIA

A MINOR RESEARCH PROJECT

SUBMITTED TO

UNIVERSITY GRANT COMMISSION,
NEW DELHI

IN

MICRO BIOLOGY

BY

DR. Y.C. ATTAR

M.Sc., Ph.D.

DEPARTMENT OF MICROBIOLOGY
RAJARAM COLLEGE,
KOLHAPUR, (MAHARASHTRA)

DECEMBER - 2015

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefits plants by producing growth promotion. A large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have reported as PGPR to enhance plant growth (Saharan B. S. et al., 2011). PGPR influence plant growth by two ways: indirectly and directly. Indirectly through production of substances inhibitory to phytopathogens and directly through phosphate solubilisation, nitrogen fixation, production of phytohormones, siderophores, etc (Lenin G. et al., 2012).

Indole Acetic Acid (IAA) is a member of phytohormone group and is generally considered as the most important native auxin (Ashrafuzzaman M. et al., 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic response, cellular response such as cell expansion, division and differentiation and gene regulation (Ryu R. et al., 2008). Phosphate solubilizing bacteria are known to solubilize insoluble phosphate and make it available to plants (Rodriguez H. et al., 1999). They are also capable of producing phytohormones like IAA. Most bacteria use tryptophan as a precursor for IAA production (Saharan B.S. et al., 2011). This study was mainly aimed to assess the potential of isolates in producing IAA by replacing tryptophan with natural precursors such as soybean flour, watermelon seed powder and pumpkin seed powder. The research was also aimed to study other activities of most potent isolates such as gibberellic acid production, Siderophore production, salt tolerance ability, effect on plant growth and insecticide tolerance.

OBJECTIVES

- Screening of IAA producing *Azotobacter*, *Rhizobium* and phosphate solubilizing bacteria using tryptophan
- Screening of isolates for IAA production using natural sources of tryptophan
- Purification and Identification of potent isolates
- Study of effective isolates for various characteristics
- Study of effect of potent isolates on growth of seedlings

RESULTS

A total of 30 aerobic nitrogen fixing and 10 phosphate solubilizing bacterial cultures were isolated after enrichment. Out of 30 aerobic nitrogen fixing bacteria, 18 isolates belong to Genus *Azotobacter*, in which 16 isolates produced IAA. The remaining 12 isolates belong to Genus *Rhizobium*, in which 9 isolates produced IAA. 7 out of 10 phosphate solubilizing bacterial cultures had the ability to synthesize IAA which was tested using Salkowski's reagent.

Effective IAA producing isolates such as 5 *Azotobacter*, 5 *Rhizobium* and 7 phosphate solubilizing spp. were screened for utilization of natural crude precursors like soybean flour, pumpkin seeds, water melon seeds, sprouted soybean, molasses, cooked soybean as a source of tryptophan for IAA production. It is observed that for synthesis of IAA, *Azotobacter* species utilized soya flour; however *Rhizobium* and PSB isolates utilized watermelon seeds. Also by using efficient isolates, efficiency of IAA production was optimized with respect to precursor concentration, time required for production, rate of aeration and agitation. For *Azotobacter* species optimum concentration of soybean flour is 4% and optimum incubation time is 8 days for maximum IAA production. For *Rhizobium* and PSB optimum concentration of watermelon seeds is 6% and time required for maximum IAA production is 6 days. Agitation rate of 200 rpm enhanced the IAA production than that observed under static conditions.

Amongst all the isolates studied for IAA and GAA production phosphate solubilizing bacterial spp named as *Enterobacter cloacae* subsp. *cloacae* strain YCA was found to give high yield hence it was further exploited for its ability to synthesize Siderophore, HCN, ammonia, organic acid and pesticide degradation ability.

DISCUSSION

It is concluded that potent isolates *Azotobacter chroococcum*, *Rhizobium meliloti* and *Enterobacter cloacae* subsp. *cloacae* strain YCA when inoculated with soybean flour and watermelon seed powder respectively showed elevation in plant growth over uninoculated control. So we can exploit these organisms as biofertilizers for fertile and sterile soil. *Enterobacter cloacae* subsp. *cloacae* strain YCA was found to produce secondary metabolites such as amylase, cellulase and inulinase which are helpful for utilization of reserve food material.

The results of research work were presented in poster form entitled as

“Production of Indole Acetic Acid by using Azotobacter spp. from Natural Precursors”

in **Nation Conference on Challenges and Opportunities in Life Sciences** organized by Department of Biochemistry, Microbiology & Biotechnology, Shivaji University, Kolhapur in Feb. 2013 as well as in **International Conference on Emerging Horizons in Biochemical Sciences and Nanomaterials (EHBCSN-2013)** organized by Department of Chemistry and Microbiology, Shri Shivaji Mahavidyalaya, Barshi entitled as

“Screening of Phosphate Solubilizing Bacteria for Indole-3-Acetic Acid Production using Natural source of Tryptophan”

and in another **National Conference on Recent Trends and future Prospects in Multidisciplinary Approaches in Microbiology RTFPM-2014** organized by Rajaram College, Kolhapur entitled as

“Degradation of Pesticides Phorate by PCR Bacteria”

where my poster won 2nd prize. This research work was also published in **International Journal of Pure and Applied Biosciences 3 (1): 71-80 (2015)**, under the title

“Study of Phosphate solubilizing Enterobacter cloacae sub sp. cloacae strain YCA for production of plant growth promoting substances”



International Journal
of Pure & Applied
Bioscience

ISSN: 2320 – 7051

International Journal of Pure & Applied Bioscience

HOME ABOUT US INSTRUCTIONS TO AUTHORS ARCHIVES CONTACT US DOWNLOAD



Welcome to IJPAB

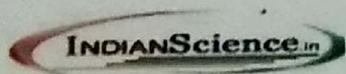
International Journal of Pure & Applied Bioscience (IJPAB) is a peer reviewed, bimonthly, open access online official international journal. It publishes original reviews, research articles and short communications from the field of Biotechnology, Microbiology, Enzymology, Biochemistry, Bioinformatics, Biophysics, Cell biology, Cellular and Developmental Biology, Ecology, Entomology, Ethnobotany, Ethno pharmacology, Ethno veterinary, Evolutionary Biology, Genetics and Genomics, Horticulture, Molecular biology, Morphology, Nutrition, Pathogen Resistance, Pest Management and Plant Pathology all other related areas.

IJPAB's mission is to provide a platform established with the aim of motivating to the scientific persons as authors, researchers, professionals, teachers and their students, assisting them improve their knowledge.

International Journal of Pure & Applied Bioscience (IJPAB) has an editorial policy of publishing original, high scientific quality and interesting reviews, research articles and short communications. International Journal of Pure & Applied Bioscience (IJPAB) to publish research articles in all areas of human study without financial restriction to readers using the open access model of publication.

Authors can submit valuable research work/ knowledge as manuscript for forthcoming issue. Get decision on your manuscript within week from the date of submission. For submission, send your manuscript at: editor@ijpab.com or submit@ijpab.com or submitijpab@gmail.com

Indexed and Abstracted in:



Recent News

IJPAB is indexed in International Scientific Indexing (ISI) with Impact Factor Value of 1.106 (2013-14)

IJPAB Now indexed in Thomson Reuters

Volume 3 Issue 1 Released

Volume 3 Issue 2 call for paper

IJPAB Now Indexed In Google Scholar

IJPAB Now Indexed in GIF

Impact factor: 2.636 (SJIF)



THOMSON REUTERS

Current Issue **NEW**

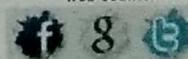
Volume-3, Issue-1, Jan.-Feb.- 2015

Archive Issue

[Click Here >>](#)

Home | About us | Instructions to Authors | Archives | Contact Us | Download

web counter



Study of phosphate solubilising *Enterobacter cloacae* sub sp. *cloacae* strain YCA for production of plant growth promoting substances

Yasmin Chand Attar^{1*}, Savita Dnyandeo Mali², Prajakta Prakash Kamble³

^{1,2,3} Department of Microbiology, Rajaram College, Kolhapur

*Corresponding Author E-mail: yasminattar33@gmail.com

ABSTRACT

Phosphates solubilising bacterial spp. isolated from saline soil near Kolhapur city were tested for IAA production using pure tryptophan. All isolates were then screened for production of IAA using natural sources of tryptophan such as soybean flour, pumpkin seed powder and watermelon seed powder. IAA production was tested qualitatively and quantitatively. Most effective phosphate solubilising bacterial spp. was identified as *Enterobacter cloacae* subsp. *cloacae* YCA on the basis of its morphological, biochemical characteristics and 16s rRNA gene sequencing. The isolate was found to give high yield of IAA (0.89 mg/ml) from watermelon seed powder and it was also found to produce other plant growth promoting substances such as siderophore, ammonia, organic acids and hydrogen cyanide. The isolate was moderate halophile as it tolerates NaCl and KCl up to 6%. The amount of soluble phosphorus was also found to increase over initial level in the pot where isolate was inoculated. Results of pot trials using saline and fertile soil indicate the potential use of our isolate in enhancing plant growth.

Keywords: Indole-3-acetic acid, Nutrient broth, Kartznelson and Bose medium, Siderophore, Saline soil.

Abbreviations: Indole-3-acetic acid (IAA), Nutrient broth (NB), Kartznelson and Bose (KB) medium.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. A large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have reported as PGPR to enhance plant growth¹. PGPR influence plant growth by two ways; indirectly and directly. Indirectly through production of substances inhibitory to phytophagogens and directly through phosphate solubilisation, nitrogen fixation, production of phytohormones, siderophores etc².

IAA (indole-3-acetic acid) is the member of the group of phytohormones and is generally considered as the most important native auxin³. It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, and differentiation, and gene regulation⁴. Phosphate solubilising bacteria are known to solubilise insoluble phosphate and make it available to plants⁵. They are also capable of producing phytohormones like IAA. Most bacteria use tryptophan as a precursor for IAA production¹. This study was aimed to assess the potential of 10 phosphate solubilising bacterial isolates in producing IAA by replacing tryptophan with natural precursors such as soybean flour, watermelon seed powder and pumpkin seed powder.

MATERIAL AND METHOD

Chemicals

Orthophosphoric acid, ethyl acetate, picric acid, sodium carbonate and other chemicals were obtained from Himedia (India). All chemicals used were of highest purity and of analytical grade.

Collection of sample

Soil samples were collected from different areas nearby Kolhapur city. The collected samples were stored at controlled conditions until use.

Enrichment and isolation of phosphate solubilising bacteria

Soil samples were inoculated in sterile KB broth and incubated at 28°C for 5 days. Repeatedly three transfers were given. Bacterial cultures from final enriched broth showing clear zone on KB agar medium indicates their ability to solubilize phosphate. These isolates were purified and coded. All cultures were maintained on respective slant media and stored at 4°C till further use.

Screening of isolates for IAA production from tryptophan

Isolates were screened for IAA production from tryptophan by inoculating in NB containing 0.1% tryptophan. Production of IAA in culture filtrate was detected colorimetrically at 540nm using orthophosphoric acid and Salkowski's reagent⁶.

Screening of isolates for IAA production using natural sources of tryptophan

Positive cultures were then screened for their ability to produce IAA in presence of natural precursors such as soybean flour, watermelon seed powder, and pumpkin seed powder instead of tryptophan.

2.6 Optimization of natural precursor concentration for IAA production

Effective isolate was inoculated in sterile NB containing various concentrations of natural precursors like 2%, 4%, 6% and 8% and incubated at 28°C for 6 days and IAA produced was estimated using Salkowski's reagent.

Characterization and identification of potential isolate

Phenotypic characters such as morphology (Gram staining and motility) and biochemical characteristics (sugar fermentations, Voges-Proskauer, citrate utilisation, arginine hydrolysis, indole production, methyl red and lysine decarboxylase tests) of potential isolate were studied by using standard procedures as per Bergey's manual.

The 16s rRNA gene of the efficient isolate was sequenced. The nucleotide sequence was determined by automated sequencer. The nucleotide sequence was submitted to NCBI. It was aligned and analyzed by using BLAST program. The homologous sequence was used for phylogenetic analysis. The phylogenetic tree was constructed with Clustalx software⁷.

Production and extraction of IAA

The potent isolate was inoculated in nutrient broth containing 6% watermelon seed powder. After 10 days, the fermented broth was centrifuged at 10,000 rpm for 30 min. Supernatant was acidified to pH 2.5 to 3 with 1 N HCl and IAA was extracted by adding equal volume of ethyl acetate. Ethyl acetate fraction of IAA was then evaporated to dryness and solubilised with minimum volume of methanol⁸. Presence of IAA was confirmed by paper chromatography and TLC⁹. Quantification of IAA was carried out from standard graph obtained by using pure IAA.

Study of salt tolerance ability of the isolate

Salt tolerance ability of the isolate was studied by inoculating culture in KB agar medium containing different concentrations of various salts such as NaCl (1 to 7%), KCl (1 to 7%), Na₂CO₃ (0.1 to 1%) and NaHCO₃ (0.1 to 1%).

Ammonia production

Bacterial isolate was tested for the production of ammonia in peptone water. Freshly grown culture was inoculated in 10 ml peptone water and incubated for 72 hrs at 28 ± 2°C. Production of ammonia was detected by addition of Nessler's reagent¹⁰. Development of brown to yellow colour indicates production of ammonia by isolate.

Hydrogen cyanide (HCN) production

Qualitative determination of HCN production by the efficient isolate was done by streaking the culture on nutrient agar medium supplemented with 4.4 gm glycine/lit. Whatman filter paper no. 1 soaked in sodium carbonate in 0.5% picric acid solution was placed on the top of the plate and plate was sealed with paraffin and incubated at 28°C for 72hrs¹¹. If Whatman filter paper becomes orange coloured it indicates production of HCN.

Siderophore production

The isolate was inoculated in NB and incubated at 28°C for 24 hrs. 5% inoculum was inoculated in low iron modified broth medium and incubated at 28°C for 4 days with constant shaking at 200 rpm on rotary shaker. After incubation broth was centrifuged and supernatant was analysed for presence of siderophore by CAS test¹². 1 ml filtrate was added with 1-5 ml 2% FeCl₃ solution. Development of red colour of filtrate indicates presence of siderophore.

Organic acid production

The major mechanism for solubilisation of insoluble inorganic phosphates by micro-organisms is through production of organic acids. Hence, the organic acid production profile of the PSB was examined, by paper chromatographic technique¹³. One ml of 24 h. old culture of isolate was inoculated to 50 ml Pikovskaya's broth¹⁴ and incubated at 28°C for 10 days. The broth was centrifuged at 10,000 rpm for 10 min. The supernatant so obtained was concentrated to nearly 1/10th of the original volume in a water bath maintained at 60°C. The concentrated material was then used for determination of organic acids by paper chromatography in comparison with standard organic acid¹⁵. Standards of organic acids were prepared at 20 mg/ml stock. About 10 µl of standards and 15µl of culture supernatant was spotted on Whatman No. 1 chromatographic paper and dried with a hair dryer. A descending chromatography was run using a solvent mixture of n-butanol, acetic acid and water in 12:3:5 ratios in a chromatographic chamber pre saturated with solvent for 6 h. The chromatogram was run for 16 h and air dried for 3 days. The air dried paper was sprayed with 0.04% bromocresol green. The paper was dried at room temperature. The Rf value of yellow spot of organic acid developed on a blue background was measured and compared with the Rf values of the standard organic acids for identification.

Study of effect of isolate on plant growth

Plate test

Sterile NA plate containing precursor was inoculated with culture and surface sterilized healthy jowar seeds were kept on the surface of agar. Control plate without culture was also seeded with surface sterilized jowar seeds and plates were incubated.

Pot test¹⁰

Pots were prepared for both saline and fertile soils separately as follows

Set I using fertile soil

- Pot A : Only Fertile soil
- Pot B : Fertile soil + culture
- Pot C : Fertile soil + culture + precursor (watermelon seed powder)

Set II using saline soil

- Pot D : Only saline soil
- Pot E : Saline soil + culture
- Pot F : Saline soil + culture + precursor (watermelon seed powder)

Seeds were surface sterilized and then sown in above pots. The pots were kept in sunlight and observed for seed germination and growth of seedlings. Seedling growth was measured daily.

RESULT AND DISCUSSION

The present study is done in order to isolate phosphate solubilising bacteria capable of producing large amount of indole acetic acid. Totally, 10 phosphate solubilising bacteria were isolated on KB agar.

After purification the isolates were coded as A, B, C, D, E, F, G, H, I and J. All isolates were studied for IAA production by Salkowski's method (Table no. 1). Out of 10 isolates, isolate J was selected as it produced maximum IAA (0.03mg/ml) within 6 days of incubation. Isolates F,E,I,G,C and A also produced IAA but in remarkably less quantity, while isolates B,D,H were unable to produce IAA even after 15 days of incubation.

Seven isolates showing positive results were further tested for quantitative estimation of IAA in presence of natural sources of tryptophan. All isolates utilised watermelon seed powder and pumpkin seed powder for IAA production; however in presence of watermelon seed powder isolate J produced IAA rapidly and in more quantity (0.05mg/ml) than other isolates as depicted in table no. 2. However, soyabean flour was utilized by none of the isolates.

This potential isolate namely J, was identified by studying morphological and biochemical characteristics as per Bergey's manual. It was found to be Gram negative motile rod positive for glucose, mannitol, lactose, sucrose, citrate utilization, Voges-Proskauer and arginine hydrolysis test and negative for indole, methyl red and lysine decarboxylase test. These biochemical tests demonstrated only the genus *Enterobacter*. Confirmation of this isolate was done by 16s rRNA gene sequencing. The nucleotide sequence was submitted to NCBI. It was aligned and analyzed and named as *Enterobacter cloacae subsp. cloacae YCA*. (Genbank accession no. BankIt 1742549 *Enterobacter* KM 186607). Its phylogenetic position in relation to other species of same genus is illustrated in Fig.1.

The isolate when studied for IAA production using different concentrations of watermelon seed and pumpkin seed powder showed high yield of IAA (0.05mg/ml) at 6% watermelon seed powder. When concentration was increased above 6%, IAA production was found to decrease (Table no. 3). This decrease may be due to feedback inhibition¹⁶.

Studies on optimization of incubation time showed a higher rate of IAA production on the 10th day (0.89mg/ml) as shown in table no.4, in presence of watermelon seed powder at 6% concentration. IAA produced was extracted and identified by paper chromatography and thin layer chromatography by comparing with R_f value of standard IAA (Fig 2).

The isolate was moderate halophile as it tolerates NaCl and KCl up to 6%, NaHCO₃ up to 1% and Na₂CO₃ up to 0.5% as per Table no. 5.

It also produced ammonia, HCN, siderophore and organic acid. The produced ammonia helps in inducing plant growth as it is the most common assimilatory form of nitrogen. The production of defensive secondary metabolites like HCN and siderophore will suppress plant diseases thus indicating *Enterobacter cloacae subsp. cloacae YCA* as a bio-control agent. Since siderophore is secreted under iron deficient conditions, this ability of the isolate will help in providing iron to plants in utilizable form¹⁷. Organic acid secreted was detected as acetic acid which reduces the pH and contributes in phosphate mineralization. Thus enhances phosphate availability to plants¹⁸.

The results of pot trials of set I showed that, shoot length of jowar seedlings in pot C was higher (30cm) in presence of isolate as compared to pot A (18cm) and pot B (20cm) on 29th day (Fig. 5) (Table no. 6). Jowar seeds were not found to germinate in saline soil hence pot trials in saline soil were carried out using chavali seeds. For SET II, the isolate was also found to promote growth of seedlings in pot F (5.6cm), as compared to pot E (3.6cm) and pot D (dry) as shown in (Fig. 6) (Table no. 7). Soluble phosphorus content was found to increase in both pots of fertile as well as saline soils where isolate was inoculated over uninoculated control. (Table no. 8). Pot trials indicate that our isolate is beneficial for growth of plants in fertile as well as saline soil when applied with watermelon seed powder.

Table No.1: Screening and isolation of IAA producing PSB

PSB Isolates	Medium	Incubation time	IAA mg/ml
J	NB	6 days	0.03
F	with 0.1% tryptophan	6 days	0.01
E,I,G,C,A		6 days	0.005
B,D,H		15 days	-

Table No. 2: Screening of isolates for IAA production using natural sources of tryptophan

Natural Precursors	PSB isolates	Incubation time	IAA in mg/ml
Soybean flour	J,F,E,I,G,C,A	15 days	-
Watermelon seed powder	J	6 days	0.05
	F,E,I,G,C,A	10 days	0.03
Pumpkin seed powder	J	6 days	0.01
	F,E,I,G,C,A	10 days	0.005

Table No. 3: Optimization of precursor concentration for IAA production using *Enterobacter cloacae* sub sp. *cloacae* strain YCA

Natural Precursor	IAA in mg/ml			
	Concentration of precursor			
	2%	4%	6%	8%
Watermelon seed powder	0.01	0.03	0.05	0.04
Pumpkin seed powder	0.01	0.02	0.03	0.01

Table No. 4: Study of incubation time for IAA production from *Enterobacter cloacae* sub sp. *cloacae* strain YCA in presence of watermelon seed powder

Medium	Incubation time (days)	Optical density (at 540nm)	Concentration of IAA (mg/ml)
NB with 6% Watermelon seed powder	6	0.11	0.05
	7	0.13	0.11
	8	0.20	0.29
	9	0.37	0.72
	10	0.43	0.89
	11	0.39	0.79
	12	0.25	0.42

Table No. 5: Salt tolerance ability of *Enterobacter cloacae* sub sp. *cloacae* strain YCA

Name of salt	Concentration of salt in %						
	1	2	3	4	5	6	7
NaCl	+	+	+	+	+	+	-
KCl	+	+	+	+	+	+	-
	0.1	0.3	0.5	0.7	0.8	0.9	1
Na ₂ CO ₃	+	+	+	-	-	-	-
NaHCO ₃	+	+	+	+	+	+	+

+ Growth
- No growth

Table No. 6: Effect of *Enterobacter cloacae* sub sp. *cloacae* YCA on the growth of jowar plants in fertile soil

Time (days)	Fertile soil only	Fertile soil + isolate	Fertile soil + isolate + precursor
	Height of seedlings in cm		
3 rd day	1.0	1.0	1.0
5 th day	1.0	1.5	2.9
7 th day	5.0	3.0	3.0
9 th day	4.6	5.0	6.0
11 th day	6.5	8.0	8.0
13 th day	7.0	11.0	12.0
15 th day	9.0	15.0	16.0
17 th day	10.0	16.0	18.0
19 th day	11.0	16.5	19.0
21 th day	11.3	17.0	20.0
23 th day	13.0	17.5	23.0
25 th day	15.0	18.0	26.0
27 th day	17.0	19.0	29.0
29 th day	18.0	20.0	30.0

Table No. 7 Effect of *Enterobacter cloacae* sub sp. *cloacae* YCA on the growth of Chavali plants in saline soil

Time (days)	saline soil only	Saline soil + Isolate	Saline soil + Isolate + Precursor
	Height of seedlings		
6 th day	1.0 mm	1.0 mm	2.0 mm
8 th day	3.0 mm	6.0 mm	1.2 cm
10 th day	5.0 mm	9.0 mm	2.0 cm
12 th day	9.0 mm	1.2 cm	3.2 cm
14 th day	1.1 mm	1.3 cm	3.3 cm
16 th day	1.2 mm	2.0 cm	4.0 cm
18 th day	Dry	2.4 cm	4.4 cm
20 th day	Dry	2.7 cm	4.9 cm
22 nd day	Dry	3.1 cm	5.2 cm
24 nd day	Dry	3.6 cm	5.6 cm

Table No. 8 Effect of *Enterobacter cloacae* sub sp. *cloacae* YCA on the soluble phosphorus content of soil

	Fertile soil	Saline soil
Initial phosphorus	2 kg/acre	3 kg/acre
After 30 days		
Soil + Isolate	3 kg/acre	6 kg/acre
Soil + Isolate + Precursor	5 kg/acre	7 kg/acre

Fig.1: A tree showing phylogenetic affinity of the potent isolate [i.e. *Enterobacter cloacae* subsp. *cloacae* YCA] to other members of Genus: *Enterobacter*

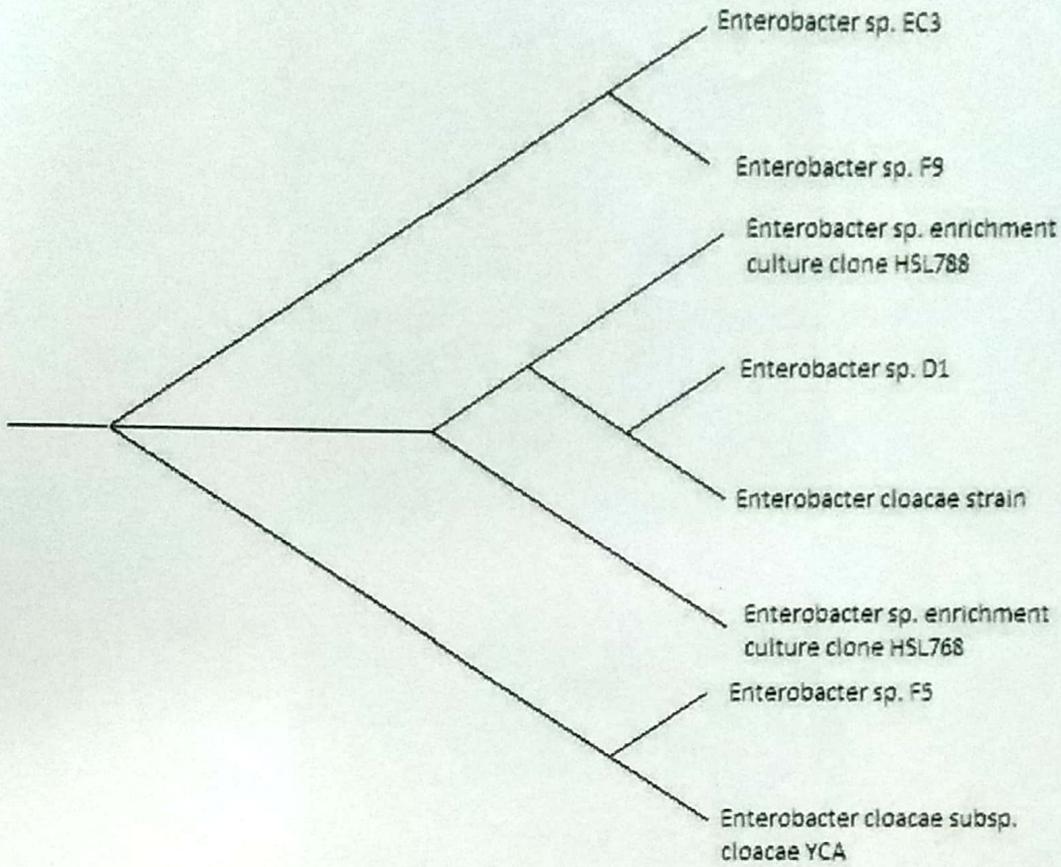


Fig.2: Silica gel plate showing pink spot of pure IAA and extracted IAA

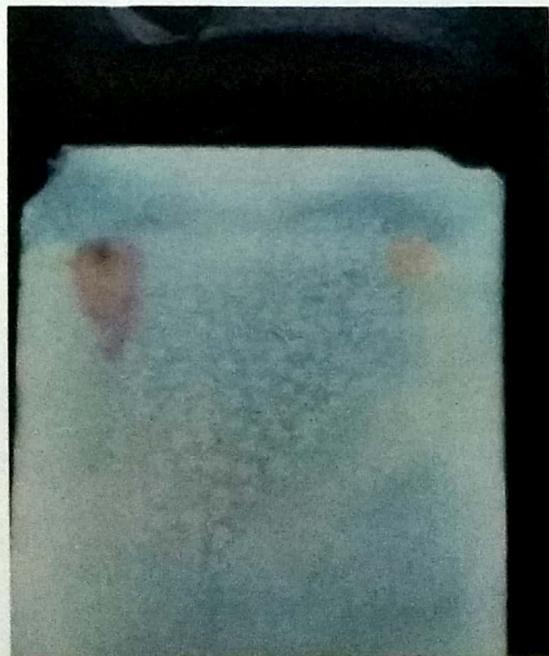


Fig.3: Plate test in presence of *Enterobacter cloacae* sub sp. *cloacae* strain YCA



Fig. 4: Plate test in absence of *Enterobacter cloacae* sub sp. *cloacae* strain YCA



Fig.5: Pot trials of fertile soils in presence of *Enterobacter cloacae* sub sp. *cloacae* strain YCA and watermelon seed powder

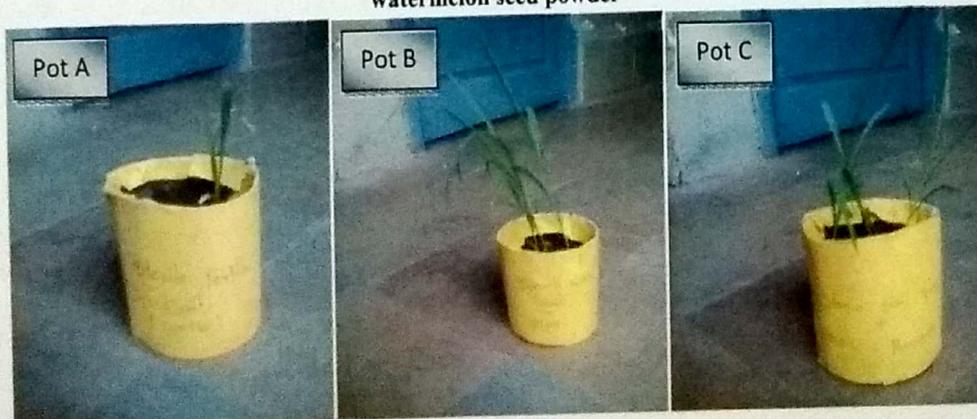
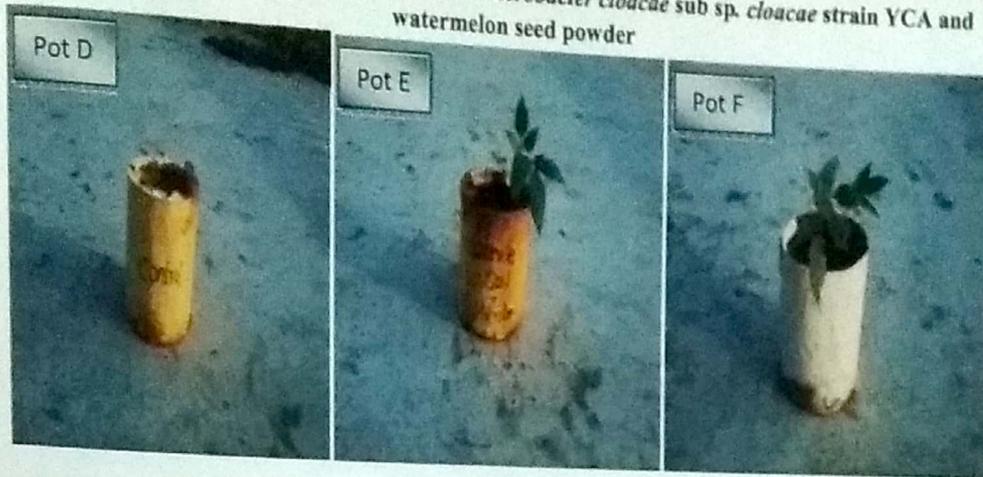


Fig.6. Pot trials of saline soil in presence of *Enterobacter cloacae* sub sp. *cloacae* strain YCA and watermelon seed powder



CONCLUSION

Amongst various natural precursors, watermelon seed powder in 6% concentration was found to be more effective for IAA production from *Enterobacter cloacae subsp. cloacae strain YCA* and yield obtained was 0.89 mg/ml after 10 days of incubation. This isolate was found to produce HCN, organic acid, ammonia and siderophore. It resulted in significant increase in shoot length in both saline and fertile soil in presence of precursor. The isolate was also found to be moderate halophile. The present study suggests that phosphate solubilization and IAA production in presence of easily available and cheap precursor i.e. watermelon seed powder is a beneficial aspect of *Enterobacter cloacae subsp. cloacae strain YCA* in enhancing crop yield. Thus using it as a biofertilizer along with watermelon seeds for fertile and saline soil will be an ecofriendly, economically feasible and promising alternative in the hands of farmers.

Acknowledgment

This project was supported by University Grant Commission. The authors wish to thank University Grant Commission for financial support.

REFERENCES

1. Saharan, B. S. and Nehra, V., Plant Growth Promoting Rhizobacteria: A Critical Review. *Life Sciences and Medicine Research.*, **21**: 1-30 (2011)
2. Lenin, G. and Jayanthi, M., Indole acetic acid, gibberellic acid and siderophore production by PGPR isolates from rhizospheric soils *Catharanthus roseus*. *Int. j. pharm. biol. sci. arch.*, **3**: 933-938 (2012)
3. Ashrafuzzaman, M. Hossen, F. A. Ismail, M. R. Hoque, M. A. Islam, M. Z. Shahidullah, S. M. and Meon, S., Efficiency of plant growth promoting Rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.*, **8**: 1247-1252 (2009)
4. Ryu, R. and Patten, C. L., Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by 4 TyrR in *Enterobacter cloacae* UW5. *Am Soc Microbiol.*, **190**: 1-35 (2008)
5. Rodríguez, H. and Fraga, R., Phosphate solubilizing bacteria and their role in Plant Growth Promotion. *Biotechnol Adv.*, **17**: 319-339 (1999)
6. Gordon, S.A. and Weber, R.P., Colorimetric estimation of indole acetic acid. *Plant Physiol.*, **26**: 192-195 (1951)
7. Jeanmougin, F. J. D. Thompson, J. D. and Gouy, M., Multiple sequence alignment with Clustal X. *Trends Biochem Sci.*, **23**: 403-405 (1998)
8. Loper, J. E. and Schroch, M. N., Influence of bacterial source of IAA of root elongation of sugar beet. *Phytopathology.*, **76**: 386-389 (1986)

9. Datta, C. and Basu, P. S., Production of indole acetic acid in root nodules and culture by a *Rhizobium* species from root nodules of the fodder legume *Melilotus alba* DESR. *Acta Biotechnol.*, **18**: 53-62 (1998)
10. Yadav, J. Verma, J. P. and Tiwari, K. N., Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum L.*) under in Vitro conditions. *Biological Forum — An International Journal.*, **2**: 15-18 (2010)
11. Samuel, S. and Muthukkaruppan, S. M., Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil. *Current Botany.*, **2**: 22-25 (2011)
12. Schwyn, B. and Neilands, J.B., Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, **160**: 47-56 (1987)
13. Vikram, A. Hamzehzarghani, H. Alagawadi, A. R. Krishnaraj, P. U. and Chandrashekar, B. S., Production of Plant Growth Promoting Substances by Phosphate Solubilizing Bacteria Isolated from Vertisols. *Journal of Plant Sciences.*, **2**: 326-333 (2007)
14. Mehta, S. and Nautiyal, S.C., An efficient method for qualitative screening of phosphate solubilizing bacteria. *Curr. Microbiol.*, **43**: 51-56 (2001)
15. Gaur, A. C., Phosphate Solubilizing Microorganisms as Biofertilizers. 1st Ed., Omega Scientific Publishers, New Delhi, India, ISBN: 81-85399-09-3 (1990)
16. Ghosh, P.K. Saha, P. Mayilraj, S. and Maiti, T.K., Role of IAA metabolizing enzymes on production of IAA in root, nodule of *Cajanus cajan* and its PGP *Rhizobium* sp. *Biocatal Agric Biotechnol.*, **2**: 234-239 (2013)
17. Ali, S. S. and Vidhale, N. N., Evaluation of siderophore produced by different clinical isolate *Pseudomonas aeruginosa*. *Int J Microbiol Res.*, 131-135 (2011)
18. Inui - Kishi, R. N. Kishi, L. T. Picchi, S. C. Barbosa, J. C. Olivério Lemos, M. T. Marcondes, J. and Lemos, E. G., Phosphorus solubilizing and IAA production activities in plant growth promoting rhizobacteria from Brazilian soils under sugarcane cultivation. *ARNP J Eng Appl Sci.*, **7**: 1446-1454 (2012)