Int.J.Curr.Microbiol.App.Sci (2016) *5*(3): 159-180

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 3(2016) pp. 159-180**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.503.021

Plant Growth Promotion (PGP): on *Cicer arietinum* L. Under Elaborated Treatment Conditions

Debasis Mitra[1,2] and Arup Kumar Mondal[2]*

[1]Department of Biotechnology, Graphic Era University, 566/6 Bell Road, Clement Town, Dehradun, Uttarakhand 248002, India
[2]Department of Biotechnology, Vidyasagar University, Oriental Institute of Science and Technology, Rangamati, West Bengal 721102 India
**Corresponding author*

Keywords

PGP, *Bacillus sp*., Elaborate treatments, Seedling, Vigour.

Article Info

Accepted:
15 February 2016
Available Online:
10, March 2016

A B S T R A C T

Generally microorganisms isolated from rhizospheric zone of plants have the congruency of high level phosphate solubilization (PS) and Plant Growth Promotion (PGP) for increase in crop production. In order to identify the swathe of rhizobacteria from rhizospheric zone of *Lycopersicon esculentum*, we used PYD and Bacillus isolation medium (BSM). Selected bacterial strain (Ari^{D6A}) showed good potential in PGP and PS. It was screened for PGP factors likes PS, Production of IAA, HCN and cell wall degrading enzyme. We also did genus level identification base on morphological and biochemical property of the isolated strain. In this work, we designed different types of regimen treatments. In this unique work we showed that Ari^{D6A} having a positive influence on *Cicer arietinum L*. in every treatment conditions with best performance. The isolate showed that seed germination of control (70.09-71.33%), treated (71.70-88.03%) and also shoot-root length as well as enhanced vigour index of C-SEO1: 1551.424, C-SEO2: 1626.008, SES1: (2310.68-3192.38), SES2: (2151.00-3202.00), SOS1: (2816.96-2968.35), SOS2: (2226.30-3057.37). Additionally, Ari^{D6A} solubilized phosphate at a concentration of 193.75 µg/ ml in PVK broth after 4 days of culturing and quantitative amount of IAA production was noted 46.30ppm. Ari^{D6A} could produce high level of HCN and SA good amount qualitatively. The biochemical test and characteristics suggest that Ari^{D6A} may belong to genus *Bacillus*. Overall study, lead us to suggesting the potentiality of isolate in PGP. This work can help in developing newer dimensions in PGP treatment and sustainable agriculture.

Introduction

Different plant growth promoting rhizospheric microorganisms, including associative and symbiotic bacterial such as *Pseudomonas sp., Azospirillum sp., Azotobacter sp., Rhizobium sp., Klebsiella sp., Enterobacter sp., Alcaligenes sp., Arthrobacter sp., Burkholderia sp., Bacillus sp.,* and *Serratia sp.* groups have been used for their beneficial effects on plant health and development of natural crop production

(Höflich *et al.*, 1994; Kloepper *et al.*, 1992). These microorganisms are generally known as plant growth promoting rhizosphere rhizobacteria (PGPR). Plant growth promoting rhizobacteria accounts for about 2-5% of total the rhizobacteria involved in plant growth promotion (Antoun *et al.*, 2001; Bakker *et al.*, 1987). In PGP, directly & indirectly treatments are the main treatment condition. Those two types conditions are already describe by (Goldstein, 1995). The direct promotion of the plant growth by PGP entails either providing the plant with a compound that is synthesized by the bacterium such as phytohormones or facilitating the uptake of certain nutrients from the environment and soil (Abdul *et al.*, 1973). Rhizospheric bacterial population mainly promote plant health, (Cattelan *et al.*,1999) they stimulate plant growth directly by produce or change the concentration of plant growth regulators (Kloepper *et al.*,1980; Ramette *et al.*, 2003) like gibberellic acid, IAA and cytokinins etc. The ability to produce ACC deaminase to reduce the level of ethylene in the roots of the developing plants, thereby increasing the root length and growth (Glick, 1995; Vessey, 2003), asymbiotic N_2 fixation (Chen *et al.*, 2006), antagonism against phytopathogenic microorganisms by producing siderophores, b-1-3-glucanase, chitinases, antibiotics, fluorescent pigment, cyanide (Summanen, 1993), solubilization of mineral phosphates and other nutrients (Johri *et al.*, 1999; Sharma *et al.* 2003). Main part in plant growth, auxins are key hormones that control plant growth and development of plant health (Davies, 1995; Lukkani *et al.*, 2014; Asghar *et al.*, 2004). PGP activity are known to produce anti-biotic, anti-fungal metabolites, enzyme, phenolic compound, signaling compound, and other determinants of defense in response to pathogen attack (Patt *et al.*, 1967; Elkoca *et al.*, 2008; Maurilio *et al.*, 2013). It is reported that, huge amount of chemical fertilizers used for the production of crops results in increased soil salinity and that microbes Play vital role by mediating nutrient transformation from the soil to plants (Patten *et al.*, 2002; Barreto *et al.*, 2011). But, in our earlier study, Several *Pseudomonas sp.* and *Bacillus sp.* have been reported among of the most efficient phosphate solubilizing bacteria and as it important bio inoculants due to their multiple bio fertilizing activities of better improvement soil nutrient status with secretion of plant growth regulators and suppression of soil borne pathogens (Verma, 2010; Vessey, 2003). Phosphorous release from insoluble phosphate reported for several soil microorganisms has been attributed mainly to the production of organic acids and their chelating capacity (Fiske *et al.*, 1925; Goldstein, 1995). In the present study, we intent to show the effect of different bacterial treatments on Cicer arietinum under different plant growth parameters.

Materials and Methods

A rhizobacteria isolated from rhizospheric zone (soil sample collection Place: Ghatal, Paschim Medinipur, West Bengal 721212 India; latitude: $22^0$39´45N, longitude: $87^0$44´2E; altitude: 16 feet; soil type: alluvial soil) of *Lycopersicon esculentum* (25days old plant), was characterized on the basis of morphological and biochemical determination describe by Harley & Presscott; (Dubey, 2007). The test soil sample (0.50gm/ 5ml dH_2O) is added in 5mL distilled water and vigorous shaking at 200rpm. The fresh shaking upperpart water sample was serially diluted (10^{-6}) and dilution sample was plated onto selected PYD medium (bacterial peptone: 2.00gm, yeast extract: 2.00gm, dextrose: 5.00gm, agar: 15.00gm, distilled water: 1litter, pH:

6.8±0.2 at 25^0C). After 48hours on 28±0.2°C, in this culture plate's 180-200 bacterial colony appeared. For our designed works, total ten bacteria strain are selected and re-plated in culture media for morphological and biochemical test.

Then, we selected one best multi-activity *Bacillus sp.* strain (Ari^{D6A}) for our PGP works. For the confirming of *Bacillus sp.* bacterium, Ari^{D6A} was streaked onto selected BSM medium (bacterial peptone: 1.00gm, mannitol: 10.00gm, NaCl: 2.00gm, $MgSO_4$: 0.10gm, disodium phosphate: 2.5gm, mono potassium phosphate: 0.25gm, sodium pyruvate: 10.00gm, bromo mt-blue: 0.12gm, agar: 15gm, dH_2O: 1000ml, pH: 7.2) (Shyam, 2007).

Then preservation for future, added 20% glycerol in pure culture and store at -20^0C. Fresh sample were raised from glycerol stock and incubated at 28^0C with shaking at 200 rpm to provide aeration for the bacteria. The cells (3×10^8 cfu/ ml) were harvested by centrifuged (REMI, India) at 10,000 rpm for 20 min. and washed twice with phosphate buffer (pH: 7), then dispensed in 5 ml PB (McClung *et al.*, 1947).

Determination of carbohydrate utilization, assays were performed by nine carbohydrates with phenol red as indicator. For quick confirming, the ability to use some Carbohydrate utilization was identify by Triple Sugar Iron Agar (TSIA) slant, the production of H_2S and gas were also observed and describe by Dubey, 2007. The utilization of citrate as only sole carbon source and ammonium ions as the sole nitrogen source was measured using Simmons Citrate Agar slants (Anija, 2003). The Voges-Proskauer test shows that if the bacterium has butanediol fermentation and can split glucose to acetoin via pyruvate (Dubey, 2007). In this time, KOH (potassium hydroxide) is added, acetoin will be converted to diacetyl, which reacts with alpha-naphtol and forms a pink complex. Incubate at 28ºC for 48 hours, add 0.2 ml of 40% KOH and then 0.6 ml of alpha-naphtol solution. Suspend one colony from the pure culture, which is to be investigated, in VP-MR medium (Anija, 2003). The purpose is to see if the microbe has catalase, with protective, chemical hydrogen peroxide. Growth from 48hours culturing of the microbe is smeared on a microscope slide. A drop of 3% hydrogen peroxide is added. Then we will noted perfect test results. This biochemical test describe by Dubey, 2007. For the re-test of Hydrogen sulfide (H_2S), isolated bacteria produced (H_2S) which can be easily & scientifically detected on Kligler's agar by formation a black precipitate at the stabbing side describe by Kligler, 1917. It can be examined by using the pH indicator phenol red in the medium (Dubey, 2007; Anija, 2003). An inoculum from a pure culture is transferred aseptically to a sterile tube of phenylalanine agar to streak the slant. After 5 days incubation, five drops of 10% ferric chloride and five drops of 0.1N HCl are added. Then all tubes are gently shaken. A positive result is indicated if a green color develops within 7 minutes.

Apply the bacteria in the form of a streak onto the egg yolk agar (Pancreatic Digest of Casein: 15.0gm, Vitamin K_1: 10.0gm, Sodium Chloride: 5.0gm, Papaic Digest of Soybean Meal: 5.0gmYeast Extract: 5.0gm, L-Cystine: 0.4gm, Hemin: 5.0mg, Egg Yolk Emulsion: 100.0ml, Agar: 20.0gm, adjust Final pH: 7) (Meyer *et al.*, 1992). A positive lecithinase test is noted by the appearance of a white, opaque and diffuse zone that extends into the medium surrounding the bacterial colonies. A negative lecithinase test is indicated by the absence of a white, opaque zone extending from the edge of the colony, describe by Nautiyal *et al.*, 2001.

Immediately following inoculation, place the medium, in an inverted position (agar side up), in an arobic atmosphere and incubate at 37^0C for 48 hours. Observe plates for the appearance of lecithinase test after 48 hours of incubation. But, Cultures should not be discarded as negative until after 7 days of incubation (Koneman, 2006; Trivedi *et al.,* 2008). Starch Hydrolysis is primarily used to distinguish positive hydrolyzed. For the test of Starch Hydrolysis, Prepared SHM medium (bacterial peptone: 1.00gm, sodium chloride: 5.00gm, starch: 5.00gm, beef extract: 3.00gm, agar: 15gm, dH_2O: 1lit.) and then added 100µl bacterial culture onto the culture plate describe by Dubey, 2007. After that plates are incubate at 28^0C for 24 hours, then results are noted. The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and CO_2 gas. Prepared UTM (bacterial peptone: 1.00gm; dextrose: 1.00gm NaCl: 5.00gm; potassium phosphate: 2.00gm; urea: 20.00gm; phenol red: 0.012gm; dH_2O:1lit.), then added 200µl bacterial culture. After 24 hours (red to pink) result is appeared. Oxidase test performed by highly modified detection ket (HiMedia, India).

Analyzed for its production of IAA, take 48 hours old isolated bacterial pure culture then adding 1 ml of Kovac's reagent, after 5 mints result is appeared (Sawar *et al.,* 1995; Maurhofer *et al.,* 1994). IAA production by isolated was assayed calorimetrically using $Fecl_3$ acid reagent in the presence of tryptophan describe by Dubey, 2007.

Culture growth conditions: 250ml of nutrient broth containing 0.1% DL-tryptophan was inoculated with 300µl of 48 hours old bacterial cultures and incubated in refrigerated incubator Shaker at 30±0.2°C and 200 rpm for 48 hours in dark. The bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4°C. Estimation of indole-3-acetic acid (IAA) in the supernatants was done using colorimetric assay (Loper *et al.,* 1987; Prathibha *et al.,* 2013).

Estimation: One milliliter of supernatant was mixed with 4 ml Salkowski reagent (Sigma-Aldrich, India) and absorbance of the resultant pink color was read after 30 mint, 520nm in UV/visible Spectrophotometer, India. Appearance of pink color in test tubes indicated IAA production described by Gordon and Weber (Gordon et al., 1951). The IAA production was calculated from the regression equation of standard curve and the result was expressed as ppm over control (Bakker *et al.,* 1987; Bakker *et al.,* 2003).

Protease activity (PA) or casein degradation was determined from clear zone test in S_MA (skim milk powder: 100.00gm, peptone: 5.00gm, agar: 15.00gm, pH: 7.2) (Cattelan *et al.,* 1999). Colonies were screened for cellulose activity by plotting in CMC agar (MERCK, India) describe by Dubey, 2007.

Phosphate solubilization ability of the isolated was evaluated in PVK medium incorporated with tri-calcium phosphate (TCP) $[Ca_3(PO_4)_5]$ as insoluble phosphate. Quantitative phosphate solubilizing was estimated by *Fiske and Subarow* method. The flasks containing 25 ml medium was inoculated with bacterial culture in triplicates and incubated at 30±0.2°C at 250 rpm for 4 days in Incubator Shaker. Simultaneously, the uninoculated control was also kept under similar conditions. The cultures were harvested by centrifugation at 8,000 rpm for 15 min. The phosphorus in supernatant was estimated by vanado-molybdate-yellow color method (Fiske *et al.,* 1925). To a 0.5 ml aliquot of the supernatant, 2.5 ml Barton's reagent was added and volume was made to 50 ml with

distilled water. The absorbance of the resultant color was read after 15 min at 430 nm in UV/Visible Spectrophotometer. The total soluble phosphate was calculated from the regression equation of standard curve. The values of soluble phosphate liberated were expressed as µg /ml^{-1} over control (Jackson, 1973).

Detection of ammonia production (AP) was done by adding 1.5ml Nessler's reagent to a 48 hours culture in 4% peptone broth and recording the presence of the yellowish brown colour (Murray, 2011). Isolated strain was grow overnight in 10% trypone soy agar supplemented with glycine (4.4 gly /L) (Sigma, India) (Bakker *et al.,* 2003).

A Whatman No. 1 filter paper soaked in 2% sodium carbonate and 0.4% picric acid solution was placed to the underside of the Petri dish lids (Macfaddin *et al.,* 1980). The plates were sealed with parafilm® and incubated at 28°C for 3 days and the production of HCN was determined by the change in colour of filter paper from yellow to red-brown (Cook, 1993).

Plant growth promotion by the selective isolate apply on *Cicer arietinum L*. as following seed, and soil treatment. Seeds were obtained from fresh seeds shop (*IP address: 66.249.79.135*). The seeds were surface sterilized in 70% ethanol for 30 second, 2% sodium hypo chloride for 5 min. and followed 5 times washing in sterile double distilled water. In our designed work, different type's treatments are shown with freshly in Table 1.

The experiment soil sample was autoclave at 15 lb/ $inch^2$ pressure for 15 mints. 100gm per 50µl to 1000µl of bacterial inoculants, containing (3×10^8 cfu/ml), are separately centrifuged (cooling centrifuged REMI C-24 BL, India) at different speed and time (noted on Table 2).

After centrifuged supernatants are discarded. Bacterial palate were washed three time with CMC solution (1mg CMC/ 100ml dH_2O) describe by Ramette *et al.,* 2003. Then the bacterial pallet solution is added separately with the sample-A &sample-NA kept in the culture tube (Abdul *et al.* 1973).

Seedling vigor index was calculated as per the recommendations of ISTA (1976). Seeds were placed in between two wet paper towels specially made for germination test, 24 seeds each were placed equidistantly and the papers were rolled and kept for incubation at 25 ± 2^0C (Patt *et al.,* 1976; Ramamoorthy *et al.,* 2002).

After seven days of incubation, seeds were evaluated for germination percentage. The length and shoot length was measured in centimeter scale and vigour index was calculated by adding mean root length and shoot length and multiplied by percentage of germination (Abdul *et al.,* 1973).

Observations were recorded for the seed germination. Shoot length, root length and germination percentage were calculated.

Vigour Index (VI) = (Mean root length + Mean shoot length) X (Percentage of germination).

Finally, to determine the effect of high temperature and pH, the extent of growth of the isolate was assed and determined measured by 660 nm in UV/Visible Spectrophotometer (O.D. value) of spent culture of the aforementioned condition. Drug resistance and sensitivity of the Ari^{D6A} to various antibiotic was assayed by disc diffusion method (The concentration of tested antibiotic and results are given) on BSM plates (Bauer *et al.,* 1966).

Results and Discussion

In generally, Soil sample from rhizospheric zone of *Lycopersicon esculentum* was used to isolate thermophilic, Plant growth promoting and nitrogen-fixing with highly enzyme production bacteria (Kloepper *et al.*, 1992). In our study, the soil samples (10gm) are collected from rhizospheric zone of *Lycopersicon esculentum*. Then, these samples were plated on Nutrient agar medium (NAM) (Bacterial Peptone: 5.00gm, Beef extract: 3.00gm, NaCl: 5.00gm, Agar: 15.0gm, pH: 6.8).

After 48 hours incubation, we observed that on the plate number of colony formation 3×10^8 cfu/ml of bacteria in NAM of 10^6 fold serial dilution (Dubey, 2007). Then, we selected ten number of colony, re-culture in PYD medium and finally Ari^{D6A} bacterial culture was selected for ours work. All morphological and biochemical test was done, results are shown in Table 3.

Ari^{D6A} obtained in the present study was gram positive, rods and was subjected to PGP properties determining test i.e. phosphate solubilization, IAA production, Cell wall degrading enzyme production, ammonia production, HCN and SA production test. Phosphate solubilization, Cell wall degrading enzyme production was indicated by halos zone around the colonies. Reports are noted on Table 4.

Take 48hours growing tested bacterial culture then added 1mL of Kovac's reagent, after 2 minutes get the positive results. For confirm test, indol diffused test strip white paper turn to colour change (Agrawal *et al.*, 2013) i.e. so that indol is diffused and this test results is positive (Figure 17). IAA production by isolated was assayed by calorimetrically method (graphical figure not shown).

Phosphate solubilizing ability of the isolate was checked on Pikovskaya (PVK) medium incorporated with TCP [$Ca_3(PO_4)_2$] (Ngoma *et al.*, 2013). Formation of transparent halo zone around the developing colonies indicated phosphate solubilizing ability of the isolate. Maximum TCP (Tricalcium phosphate) solubilization in liquid medium was observed and quantitative estimation reported that isolate can soluble 193.75±00.13µg/ml in natural condition. Plate Result are shown in Figure 14.

Bacterial isolate was tested for the production of ammonia in peptone broth (4%). Freshly grown cultures were inoculated in 5 ml peptone water in each tube and incubated for 48hrs. at 38±2°C. Nessler's reagent (2 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Lata, 2003).

Isolates was screened for the production of hydrogen cyanide. Briefly, as following reference, that test at last stage plates were sealed with parafilm and incubated at 28±2°C for 3 days (Lorck, 1948; Lukkani et al., 2014). After that time, development of yellow to red colour indicated HCN production (Niranjan *et al.*, 2005). Isolate was able to produce protease in skimmed milk agar medium describe by Anija, 2003. Diameters of halo zone of bacterial colonies on S_MA medium were measured after 48 hours and note as positive result in protease activity. Cellulose activity was determined by CMC agar and after 48 hours Ari^{D6A} bacterial strain show a clear zone around the colony i.e. strain cellulose activity is positive (Figure 15).

Ari^{D6A} bacterial strain are definitely helpful microbes in plant growth promotion (PGP). In our study, we make a different types of treatment show in flow chart of Table 1.

Table.1 Treatment status on PGP

SET I		SET II	
Test tube name/ Number	**Treatment status**	**Test tube name/ Number**	**Treatment status**
$_{A}C^{1}$	**C-SEO1**	$_{NA}C^{1}$	**C-SEO2**
$_{A}C^{2}$		$_{NA}C^{2}$	
$_{A}C^{3}$		$_{NA}C^{3}$	
$_{A}C^{4}$		$_{NA}C^{4}$	
$_{A}SE^{5}$	**SES1**	$_{NA}SE^{5}$	**SES2**
$_{A}SE^{6}$		$_{NA}SE^{6}$	
$_{A}SE^{7}$		$_{NA}SE_{7}$	
$_{A}SE^{8}$		$_{NA}SE^{8}$	
$_{A}SO^{9}$	**SOS1**	$_{NA}SO^{9}$	**SOS2**
$_{A}SO^{10}$		$_{NA}SO^{10}$	
$_{A}SO^{11}$		$_{NA}SO^{11}$	
$_{A}SO_{12}$		$_{NA}SO^{12}$	

Legends: A: sterilized soil sample; NA: non-sterilized soil sample; C: control; SE: seeds: SO: soil; C-SEO1: Control of set I; SES1: Seed treatment of S-I; SOS1: soil treatment of S-I; C-SEO2: control of set II; SOS2: Soil treatment of S-II; SES2: seed treatment of S-II.

Table.2 Condition of Treatments

Tube Name	Soil Treatment		Seeds Treatment	
	Centrifuged (rpm-time)	**Inoculants: (Soil(gm.)/ Ari^{D6A} (µl/mL)**	**Centrifuged (rpm-time)**	**Inoculating: [Seeds+ Ari^{D6A} (µl/mL)+ CMC]**
$_{A}SO^{9}$&$_{NA}SO^{9}$	8000rpm-10mints	100gm/50µl	—	
$_{A}SO^{10}$ &$_{NA}SO^{10}$	8000rpm-10mints	100gm/100µl	—	
$_{A}SO^{11}$ &$_{NA}SO^{11}$	8000rpm-10mints	100gm/500µl	—	
$_{A}SO^{12}$ &$_{NA}SO^{12}$	8000rpm-10mints	100gm/1000µl	—	
$_{A}SE^{5}$&$_{NA}SE^{5}$	—		8000rpm-5mints	5minutes
$_{A}SE^{6}$&$_{NA}SE^{6}$	—		8000rpm-10mints	10minutes
$_{A}SE^{7}$&$_{NA}SE^{7}$	—		8000rpm-20mints	20minutes
$_{A}SE^{8}$&$_{NA}SE^{8}$	—		8000rpm-30mints	30minutes

Table.3 Biochemical & Morphological Test

Morphological Tests		**Biochemical Tests**	
Test Name	**Result**	**Test Name**	**Result**
Gram nature	+ve	MR/VP	−/+
Shape	Rods	Lecithinase test	+
Growth condition	Aerobic	Citrate Utilization	+
Growth medium	NAM-BSM-PYD	Starch Hydrolysis	+
Endospore formation	−	Phenylalanine Deaminase	±
Growth temperature	4-60^{0}C	Catalase	+
pH growth	4-9	Oxidase	+
Motility	−	Urease	±
ColonyColour	Whitish	H_2S Production	+

Legend: +ve: positive, -ve: negative.

Table.4 Plant Growth Promotion Ability

Test	**IAA**			**PS**	**PA**	**CMC**	**HCN**	**SA**	**AP**
Results	46.30ppm	**CT**	**NT**	+^{c}	+^{b}	+^{b}	+^{b}	+^{a}	+^{b}
		+^{b}	+^{c}						

Legend: +ve: positive, -ve: negative, a: low activity, b: medium activity, c: high activity, NT: normal test, CT: confirm test

Table.5 Antibiotic Test Study

Antibiotic	Short name	Used (μg)	Result
Ampicillin	AMP	10	S
Cephalothin	CEP	30	R
Novobiocin	NV	05	S
Erythromycin	E	15	S
Teicoplanin	TEI	10	S
Penicillin	P	10	S
Azithromycin	AZM	15	S
Linezolid	LZ	30	S
Methicillin	MET	05	R
Clindamycin	CD	02	R
Amoxyclav	AMC	30	R
Tetracycline	TE	30	S
Vancomycin	VA	30	S
Amikacin	AK	30	R
Cotrimoxazole	COT	25	S
Chloramphenicol	C	30	S
Ofloxacin	OF	05	S
Oxacillin	OX	01	R
Gentamicin	GEN	10	S
Clarithromycin	CLR	15	S

Legend: S: Sensitive, R: Resistance

Table.6 Carbohydrate Utilization

Carbohydrate Utilization								
Glucose	Lactose	Maltose	Fructose	Dextrose	Mannose	Glycerol	Sucrose	Galactose
+/AG	±	+/A	A	±	AG	+	±	+/A

Legend: +ve: positive, -ve: negative, ±: variation, A: Acid, G: gas, AG: acid+gas

Table.7 Different Types of PGP Treatment Full Results Index

CONTROL RESULTS								
SL. No.	TUBE NAME	SHOOT HEIGHT [cm.]	SHOOT WEIGHT [gm.]	ROOT HEIGHT [cm.]	ROOT WEIGHT [gm.]	DRY ROOT WEIGHT [gm.]	DRY SHOOT WEIGHT [gm.]	NUMBER OF LEAVES
1.	$_{A}C^{1}$	11.50±0.03	0.790	12.00±0.04	0.620	0.080	0.685	44
2.	$_{A}C^{2}$	12.00±0.05	0.700	08.00±0.50	0.330	0.050	0.480	48
3.	$_{A}C^{3}$	11.50±0.03	0.760	09.00±0.70	0.580	0.100	0.530	48
4.	$_{A}C^{4}$	13.00±0.05	0.590	10.00±0.06	0.410	0.050	0.435	39
5.	$_{NA}C^{1}$	12.50±0.04	0.680	13.00±0.03	0.970	0.325	0.453	37
6.	$_{NA}C^{2}$	11.80±0.08	0.585	12.00±0.05	0.690	0.100	0.370	42
7.	$_{NA}C^{3}$	13.00±0.03	0.890	07.00±0.90	0.460	0.060	0.565	38
8.	$_{NA}C^{4}$	12.50±0.05	0.760	11.00±0.06	0.425	0.055	0.440	41
TREATMENT RESULTS								
9.	$_{A}SE^{5}$	12.50+2.00+2.50	0.855	13.50±0.01	1.090	0.140	0.560	60
10.	$_{A}SE^{6}$	17.00+9.00	0.880	13.00±0.05	0.740	0.105	0.685	61
11.	$_{A}SE^{7}$	16.00±0.01	0.955	11.00±0.09	0.820	0.145	0.636	56
12.	$_{A}SE^{8}$	15.00±0.05	1.100	13.00±0.05	0.980	0.125	0.695	70
13.	$_{NA}SE^{5}$	15.00±0.05	0.995	25.00±0.00	0.815	0.125	0.690	59
14.	$_{NA}SE^{6}$	13.00±0.08	1.160	17.00±0.08	0.650	0.105	0.830	65
15.	$_{NA}SE^{7}$	13.00+3.30	0.805	15.00±0.03	0.895	0.105	0.576	60
16.	$_{NA}SE^{8}$	13.50±0.09	0.790	14.00±0.05	0.585	0.070	0.525	55
17.	$_{A}SO^{9}$	14.00±0.05	0.730	18.00±0.05	0.785	0.100	0.495	51
18.	$_{A}SO^{10}$	16.00+7.00+3.00	1.080	12.50±0.08	0.750	0.105	0.720	55
19.	$_{A}SO^{11}$	13.00±0.09	0.800	14.00±0.06	0.755	0.145	0.540	56
20.	$_{A}S0^{12}$	14.50±0.05	0.765	16.00±0.07	0.830	0.125	0.570	48
21.	$_{NA}SO^{9}$	13.00+2.00	1.005	16.00±0.01	0.810	0.140	0.670	65
22.	$_{NA}SO^{10}$	16.50±0.05	1.050	14.00±0.04	0.745	0.105	0.700	59
23.	$_{NA}SO^{11}$	14.00+6.00+4.00	1.405	13.50±0.09	0.985	0.100	0.986	58
24.	$_{NA}SO^{12}$	14.00±0.00	1.005	22.00±0.01	0.650	0.080	0.655	51

Legend: sl. No. 1 to 4: control of set I; sl. No. 5 to 8: control of set II

Table.8 Vigour Index of Well-Developed *Cicer Arietinum L.*

Name (control & tube name)	Control		SES1			SES2		SOS1		SOS2		
	C_S^1	C_S^2	$_{A}SE^5$	$_{A}SE^6$	$_{A}SE^7$	$_{NA}SE^5$	$_{NA}SE^6$	$_{A}SO^9$	$_{A}SO^{10}$	$_{NA}SO^9$	$_{NA}SO^{10}$	$_{NA}SO^{11}$
% of seed germination	71.33	70.09	75.76	84.01	84.12	80.05	71.70	88.03	77.10	81.08	74.21	81.53
VI	1551.427	1626.088	2310.680	3192.380	2439.480	3202.000	2151.000	2816.960	2968.350	2513.480	2226.300	3057.375

Legend: C_S^1:Control of Set-I; C_S^2:Control of Set-II

Figure.1 After 12 Days Shoot Height

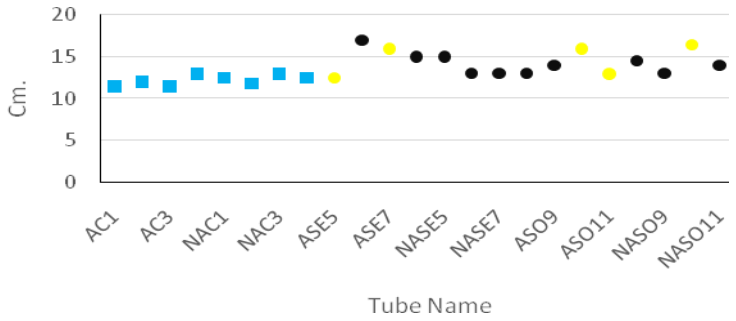


Figure.2 Root Height of Applied Plant

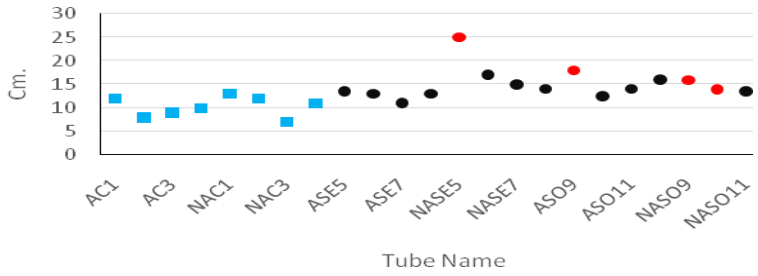


Figure.3 Shoot Weight of Treatment Plant

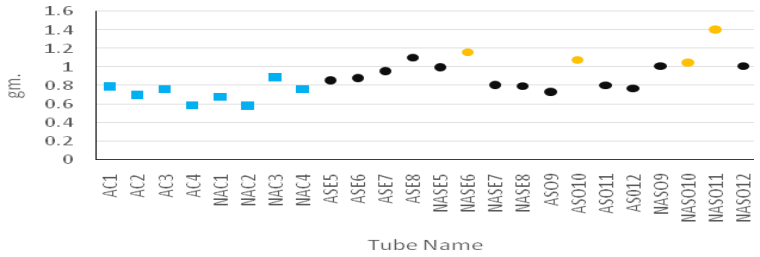


Figure.4 Root Weight of *Cicer arietinum L.*

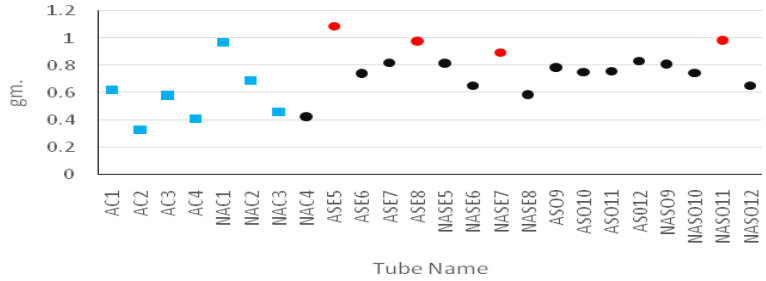


Figure.5 Treatment Time

Figure.6 Seed Germination

Figure.7 PGPR Result after 7 Days

Figure.8 PGPR Data after 12 Days

Figure.9 Set of C-SEO1 & C-SEO2

Figure.10 SES1 & SES2 Treated PGPR Results

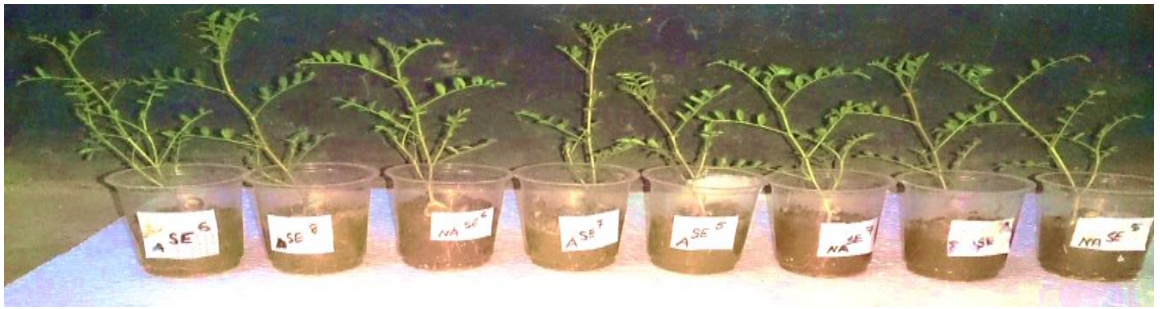


Figure.11 SOS1 & SOS2 Treated Results Performed by AriD6A Strain

Figure.12 Control & Treated Root and Shoot Plant (AC1 to ASE8)

Figure.13 NASE5 to NASO12 Treatment plant

Figure.14 PsSon Spread Culture Plate

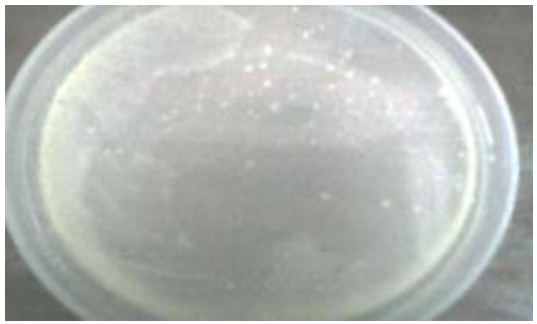


Figure.15 Cell Wall Degrading Enzyme Production

Figure.16 Starch Hydrolysis

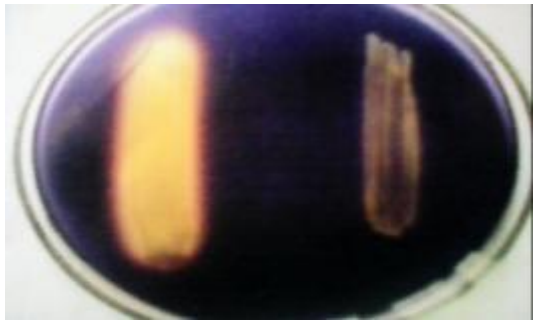


Figure.17 IAA Production Test

Figure.18 Optimum Temperature Growth

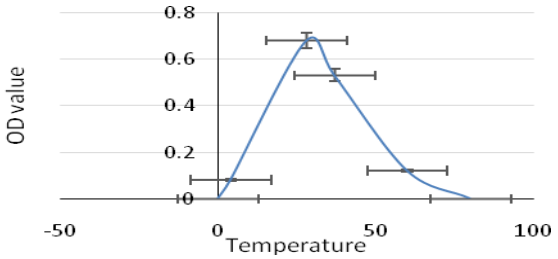


Figure.19 Bacterial Growth Under Ph

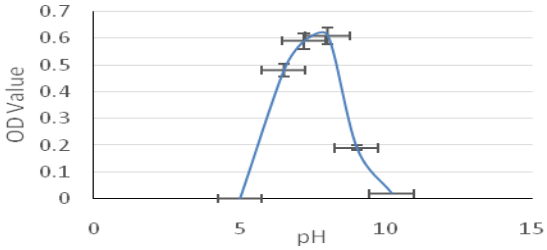
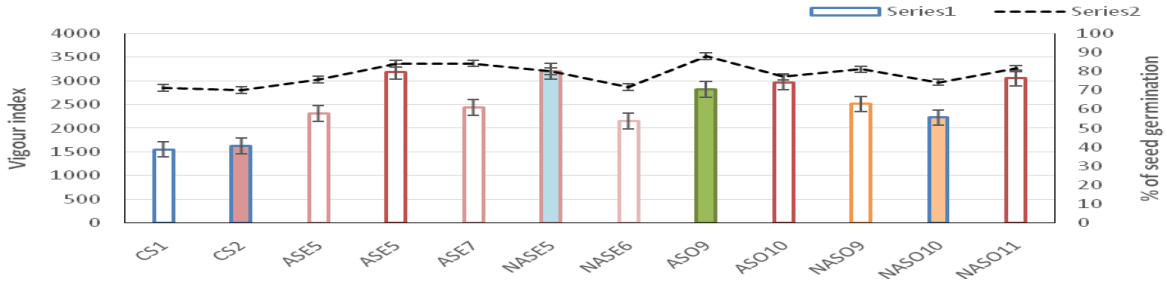


Figure.20 Elaborated Working Vi and Seed Germination Results



(C_S^1: Control of Set-I; C_S^2: Control of Set-II; series1: vigour index; series2: seed germination

Then as following chart treatment status making centrifuged, seed treatment and soil treatment. Before treatment *Cicer arietinum* L. fresh seeds sample are collected from near Midnapore railway station seed store.

Fifty seed are dissolved in double distilled water for overnight, then test for seed germination. After 36 hours, all seeds are germinated. As following Table 1, all treatments were complete with successfully. After 7 days and 12 days, all plants are shown healthy with compare then control set. This unique work suggested that this plants treated shoot height of $_{A}SE^{6}$, $_{A}SE^{7}$, $_{A}SO^{10}$, $_{NA}SO^{10}$; root height of $_{NA}SE^{5}$, $_{A}SO^{9}$, $_{NA}SO^{9}$, $_{NA}SO^{10}$; shoot weight of $_{NA}SE^{6}$, $_{A}SO^{10}$, $_{NA}SO^{11}$, $_{NA}SO^{10}$& root weight of $_{A}SE^{5}$, $_{A}SE^{8}$, $_{NA}SO^{11}$, $_{NA}SE^{7}$ better results comparison with the control.

In PGPR soil treatment: 8000rpm for 10mints (inoculate: 100gm/50µl, 100gm/100µl and 100gm/500µl) is best principle for give a well health plant. In PGPR seed treatment: centrifuged 8000rpm for 5mints (treatment time: 5minutes), 8000rpm for 10mints (treatment time: 10minutes) and 8000rpm for 20mints (treatment time: 20minutes) could give a best bio inoculants.

All plant promoting results and after 48 hours (in 8 hours sun lights) all resulting plants data are noted and that will be performed by Ari^{D6A} whichare shown in Table 7., and graphical results in Figure (1-4) & Figure 7 to Figure 13. Treatment time and seed germination (after 36 hours) picture in Figure 5 to Figure 6. All over the study, we suggested well health plants vigour index data show in Table 8. & Figure 20. It is screened and suggested that which treatment is best for PGPR.

In the present study, antibiotic test was determined by disc diffusion method on BSM plates. Tested antibiotic kit serial number is IC002-Icosa G-1 (HiMedia, India). Firstly, prepared BSM media plate, then 150µl pure test bacterial culture spread onto the plate. Carefully kit is placed on the plate. After 48 hours, all results are noted& shown in Table 5. Carbohydrate metabolism denotes the various biochemical processes responsible for the formation, breakdown and interconversion of carbohydrates by organisms (Dubey, 2007). Carbohydrate utilization in order to determine the physiological active of the selected bacterial strain, a series of carbohydrates are used in utilization test. Results are shown in Table 6.

To determine if the organism is capable of breaking down starch into maltose through the activity of the extracellular α-amylase enzyme, incubate starch containing media plates with isolate at 37^{0}C for 48 hours & after that results are shown in Figure16. Bacteria have adapted to a wide range of temperatures. In this study, isolated bacterial strain were to test in Temperature (0-80^{0}C) and pH (5-10). After 48 hours (28^{0}C), results show that bacterial growth level in temperature 28^{0}C-37^{0}C and pH (6.5-8) is naturally high (Figure18 and Figure 19).

In conclusion, the present study focused on the characterization of bacterial isolate and use it as an ecofriendly bio fertilizer in plant growth promotion under in vitro treatment condition. Plant growth promoting rhizobacteria (PGPRs) are a groups of microorganism which can influence plant growth parameters of host plants and can be used by a treatment condition as Biofertilizers. This work demonstrates that appropriate conditions treatment, could give high level of natural crop production i.e. which treatment conditions are favorable for a plant health and this treatment can

maintain all effects & higher uptake nutrient by plants. This study suggest that isolate PGP, PS, production of IAA, enzyme production and antibiotic production that may benefit the plant for its application and development. Therefore, best treatment results indicate that isolated rhizobacteria may be used as a promising microbial bio inoculant for plant growth and productivity. Isolated bacteria need to 16sRNA sequencing & higher level analysis for field apply as a bio fertilizer.

Acknowledgement

We are very happy to take this opportunity to express our gratitude to Board of Management of Oriental Institute of Science and Technology, West Bengal, India &Dr. Paratpar Sarkar, Professor, Dept. of Biotechnology, Graphic Era University, Dehradun. We are also grateful to Dr. Arijit Mukhopadhyay, OIST, India for supervised& providing us research facilities. First author thanks, Soma Sasmal (BCA, MCA) Dept. of Computer Science & Engineering, Kalyani University India for her helping in graphical tools-technique. The acknowledgement cannot end without mentioning the constant support rendered by our family members.

References

Abdul Baki, A.A., Anderson, J.D. 1973. Vigor determination in soybean by multiple criteria. *Crop Sci.*, 13: 630–633.

Agrawal, D.P.K., Agrawal, S. 2013. Characterization of Bacillus sp. strains isolated from rhizosphere of tomato plants (*Lycopersicon esculentum*) for their use as potential plant growth promoting rhizobacteria *Int. J. Curr. Microbiol. App. Sci.*, 2(10): 406–417.

Shyam, S., Yadav, W., Chen. 2007. Chickpea Breeding and Management – Page IV. ISBN-13: 978 184593 214 5.

Anija, K.R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. ISBN: 81-224-1494-X. (https://books.google.co.in/books?isbn=812241494X)

Antoun, H., Kloepper, J.W. 2001. Plant-Growth-Promoting Rhizobacteria and Medicinal Plants. New York. 1477–1480. (https://books.google.co.in/books?isbn=3319134019)

Asghar, H.N., Zahir, Z.A., Arshad, M. 2004. Screening rhizobacteria for improving the growth, yield, and oil content of canola (Brassica napus L.) *Australian J. Agri. Res.*, 55: 187-194.

Bakker, A.W., Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas sp-mediated plant growth stimulation. *Soil Biol. Biochem.*, 19: 451–457.

Bakker, P.A.H.M., Ran, L.X., Pieterse, C.M.J., Van Loon, L.C. 2003. Understanding the involvement of induced systemic resistance in rhizobacteria-mediated biocontrol of plant diseases. *Can. J. Plant Pathol.*, 25: 5–9.

Barreto, V., Seldin, L., Araujo, F.F. 2011. Plant Growth and Health Promoting Bacteria. Eds. Maheshwari DK. 18: 21- 44. doi:10.1007/978-3-642-13612-2.

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45(4): 493–496.

Cattelan, A.J., Hartel, P.G., Fuhrmann,

J.J. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sci. Soc. Am. J.*, 63: 1670–1680.

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai WA, Young, C.C. 2006. Phosphate solubilizing bacteria from sub-tropical soil and their tri calcium phosphate solubilizing abilities. *App. soil ecol.*, 34: 33–41.

Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Ann. Rev. Phytopathol.*, 31: 53–80.

Davies, P.J. 1995. The plant hormone concept: concentration, sensitivity, and transport, pp. 13–18. In P. J. Davies (ed.), Plant hormones: physiology, biochemistry, and molecular biology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Dubey, R.C., Maheshwari, D.K. 2007. *Prac. Microbiol*., ISBN: 81-219-2153-8; 2007. (http://www.gettextbooks.com/isbn/9788121921534/)

Elkoca, E., Kantar, F., Sahin, F. 2008. Influence of nitrogen fixing and phosphate solubilizing bacteria on nodulation, plant growth and yield of chickpea. *J. Plant Nutr.*, 33: 157–171.

Fiske, C.H., Subbarow, Y. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.*, 66: 375–400.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Canadian J. Microbiol.*, 41(2): 109–117, 10.1139/m95–015.

Goldstein, A.H. 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. *Biol. Agri. Horticulture*, 12: 185–193.

Gordon, S.A., Weber, R.P. 1951. Colorimetric estimation of indole acetic acid. *Pl. Physiol.*, 26: 192–195.

Höflich, G., Wiehe, W., Ku¨hn, G. 1994. Plant growth stimulation with symbiotic and associative rhizosphere microorganisms. *Experientia*, 50: 897–905.

Jackson, M.L. 1973. Estimation of phosphorus content. Soil chemical analysis. Printer Hall, New Delhi India.

Johri, J.K., Surange, S., Nautiyal, C.S. 1999. Occurrence of salt, pH and temperature tolerant phosphate solubilizing bacteria in alkaline soils. *Curr. Microbiol.*, 39: 89–93.

Kligler, I.J. 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. *Am. J. Public Health*, 7: 1042–1044.

Kloepper, J.W., Beauchamp, C.J. 1992. A review of issues related to measuring of plant roots by bacteria. *Canadian J. Microbiol.*, 38: 1219–1232.

Kloepper, J.W., Leong, J., Teintze, M., Scroth, M.N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286: 885–886.

Koneman, E.W. 2006. Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. (Ref: Handbook of Microbiological Media, Fourth Edition). J.B. Lippincott Company, Philadelphia, PA.

Lata, Saxena, A.K. 2003. Characterization of plant growth promoting rhizobacteria. In: Training manual on Biofertilizer Technology (eds.) A K Saxena. IARI Delhi. pp. 24–25.

Loper, J.E., Schroth, M.N. 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of

sugar beet. *Phytopathol.*, 76: 386–389.

Lorck, H. 1948. Production of hydrocyanic acid by bacteria. *Plant Physiol.*, 1: 142 –146.

Lukkani, N.J., Reddy, E.C.S. 2014. Evaluation of plant growth promoting attributes and biocontrol Potential of native fluorescent *pseudomonas sp*.against *aspergillusNiger*causing collar rot of ground. *Int. J. Pl. Ani. Environ. Sci.*, 4(4): 256–262.

Macfaddin, J.F. 1980. Biochemical Tests for Identification of Medical Bacteria. Williams and Wilkins, Baltimore. pp 173–183.

Maurhofer, M., Hase, C., Mevwly, P., Metraus, J.P., Defaga, G. 1994. Influence of the gacA gene and of pryverdine production. *Phytopathol.*, 84: 139–146.

Maurilio, L.F., Miguel, A.O.N. 2013. The use of LAB isolated from intestinal treated on nile tilapia, as growth promoter in fish fed low protein diets. *Lat. Am. J. aquat. Res.*, 41(3): 490–497. doi: 103856/vol41-issue3-fulltext-12.

McClung, S.L., Toabe, R. 1947. The Egg Yolk Plate Reaction for the Presumptive Diagnosis of *Clostridium sporogenes* and Certain Species of the Gangrene and Botulism Groups. *J. Bacteriol.*, 53(2): 139–147.

Meyer, J.M., Azelvandre, P., Georges, C. 1992. Iron metabolism in Pseudomonas: salicylic acid, a siderophore of Pseudomonas fluorescens CHA0. *BioFactors*, 4: 23–27.

Murray, P.R. 2011. Manual of Clinical Microbiology, 10th ed. American Society for Microbiology, Washington, D.C. ISBN: 9781555814632, doi: 10.1128/9781555816728.

Nautiyal, C.S., Mehta, S. 2001. An Efficient Method for Qualitative Screening of Phosphate Solubilizing Bacteria. *Microbiol*., 43: 51–56.

Ngoma, L., Esau, B., Babalola, O.O. 2013. Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. 12(26): 4105–4114. doi: 10.5897/AJB2013.12345.

Niranjan, R., Shethy, S.H., Reddy, S.M. 2005. Plant growth promoting rhizobacteria. Z.A. Siddiqui (ed.), PGPR: Biocontrol and Biofertilization. pp. 197–216. Springer, Dordrecht, The Netherlands.

Patt, T.E., Cole, G.C., Hanson, R.S. 1976. Methylobacterium, a new genus of facultatively methylotrophic bacteria. *Int. J. Syst. Bacteriol*., 26: 226–229.

Patten, C.L., Glick, B.R. 2002. Regulation of indoleacetic acid production in Pseudomonas putida GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. *Can. J. Microbiol*., 48: 635–642.

Patten, C.L., Glick, B.R. 2002. Role of Pseudomonas putida indole acetic acid in development of the host plant root system. *Appl. Environ. Microbiol.*, 68: 3795– 3801.

Prathibha, K.S., Siddalingeshwara, K.G. 2013. Effect of plant growth promoting Bacillus subtilis and Pseudomonas fluorescence as Rhizobacteria on seed quality of sorghum. *Int. J. Curr. Microbiol. App. Sci.*, 2(3): 11–18. (http://www.ijcmas.com/Archives-4.php).

Ramamoorthy, V., Raguchander, T., Samiyappan, R. 2002. Induction of defense related proteins in tomato roots treated with p. Fluorescenspfl, fusariumoxysporum Sp. and f. Lycopersiciv. *Plant and soil*, 239: 55–68.

Ramette, A., Frapolli, M., Défago, G., Moënne-Loccoz, Y. 2003. Phylogeny of HCN synthase-encoding hcnBC genes in biocontrol fluorescent pseudomonads and its relationship with host plant species and HCN synthesis ability. *Mol. Pl. Microbe Int.*, 16(6): 525–35. (http://www.ncbi.nlm.nih.gov/pubmed/12795378).

Sawar, M., Kremer, R.J. 1995. Determination of bacterially derived auxins using a microplate method. *Letters in App. Microbiol.*, 20: 282–285.

Sharma, A., Johri, B.N. 2003. Growth promoting influence of siderophore–producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron depriving conditions. *Microbiol. Res.*, 158: 243– 248.

Summanen, P., Baron, E.J., Citron, D.M., Strong, C.A., WexleH, M.H., Finegold, M.S. 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Co., Belmont, CA.

Trivedi, P., Sa, T. 2008. Pseudomonas Corrugata (NRRL-B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. *Curr. Microbiol.*, 56(2): 140–144.

Verma, J.P., Yadav, J., Tiwari, K.N., Lavakush, S.V. 2010. Impact of plant growth promoting rhizobacteria on crop production. *Int. J. Agric. Res.*, 5: 954–983.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant soil*, 255: 571–586.

How to cite this article:

Debasis Mitra and Arup Kumar Mondal. 2016. Plant Growth Promotion (PGP): on *Cicer arietinum* L. Under Elaborated Treatment Conditions. *Int.J.Curr.Microbiol.App.Sci.* 5(3): 159-180. doi: http://dx.doi.org/10.20546/ijcmas.2016.503.021