The Pharma Innovation Journal 2021; 10(7): 18-27

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(7): 18-27

www.thepharmajournal.com
Received: 10-05-2021
Accepted: 12-06-2021

Anjulata Suman Patre
Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Jyotsna K Peter
Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Corresponding Author:
Anjulata Suman Patre
Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Assessment of physicochemical parameters and characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* L

Anjulata Suman Patre and Jyotsna K Peter

Abstract
The field used in this study was from a site situated of Allahabad School of Agriculture, University campus of SHIATS. Before starting an experiment, composite of soil samples from the surface 0 to 20 cm depth were collected and analyzed for physical and chemical characteristics. The data recorded during course of investigation were analyzed statistically using sample standard deviation one way analysis of variance. *Pseudomonas aeruginosa* was characterized as a gram negative rod shaped bacteria showing positive biochemical test. *Pseudomonas aeruginosa* was assayed for phosphate, Zinc, Potassium solubilization in different media it produce IAA, Auxin, HCN and it showed PQQ independent activity. Phosphate solubilization index was higher in NBRIP (3.44±0.19) as compared to PVK media (1.51±0.02).

Keywords: physicochemical parameters, *Pseudomonas aeuroginosa*, PGP activity

Introduction
Cucumber (*Cucumis sativa* L) is one of the monoecious annual crops in the Cucurbitaceae family that has been cultivated by man for over 3,000 years (Adetula and Denton, 2003; Okonmah, 2011) [1, 41]. It is a creeping vine which bears cylindrical edible fruit when ripe. Cucumbers are improvement because the cucumbers are susceptible to many pathogens for which there are currently no sources of resistance (Staub *et al.,* 1997) [55]. It is a creeping vine which bears cylindrical edible fruit when ripe. Cucumbers belong to the same botanical family as melons (including watermelon and cantaloupe) and squashes (including summer squash, winter squash, zucchini and pumpkin). Cucumber plants naturally thrive in both temperate and tropical environments, and generally require temperatures between 60-90°F/15-33°C. For this reason, they are native to many regions of the world. Cucumbers are usually more than 90% water, due to abundance of vitamins, minerals and organic acids in modern nutrition have especial importance (Daneshvar, 2004; Peyvast, 2009) [14, 45]. Its juice is often recommended as source of silicon to improve the health and complexion of the skin. Cucumber is a very good source of vitamins A, C, K, and B6, potassium, pantothenic acid, magnesium, phosphorus, copper and manganese (Vimala *et al.*, 1999) [62]. The ascorbic acid and caffeic acid contained in cucumber help to reduce skin irritation and swollen (Okonmah, 2011) [41]. The cucumber is originally from Indian subcontinent but is now grown on most continents. There are three main varieties of cucumber: “slicing”, “pickling” and “burpless”. Within these varieties, several different cultivars have emerged. Cucumbers are botanically categorized as berries, which are available in many different sizes, shapes and colors. They range from thick, stubby little fruits (10-12 cm long) to Dutch greenhouse varieties (of upto 50 cm long). The most popular variety is the long smooth salad cucumber which has a smooth, dark-green skin.

Biofertilizers, more commonly known as microbial inoculants, are artificially multiplied cultures of certain soil organisms that can improve soil fertility and crop productivity. Biofertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both, in association with plant roots and without it, solubilise insoluble soil phosphates and produces plant growth substances in the soil. They are in fact being promoted to harvest the naturally available, biological system of nutrient mobilization (Venkatashwarlu, 2008) [61]. Biofertilizers are products containing living cells of different types of microorganisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as

nitrogen fixation and solubilization of rock phosphate (Rokhzadi *et al*., 2008) [50]. Beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases (Ei-Yazeid *et al*., 2007) [19]. The role of soil microorganisms in sustainable development of agriculture has been reviewed (Lee and Pankhurst, 1992; Wani *et al.*, 1995) [35, 63].

Pseudomonas is aerobic non-sporing gram negative bacilli, motile by flagella. They are pathogenic to plants and animals (Champs *et al*., 1993) [11]. *Pseudomonas* are widely distributed in nature and act as plant growth-promoting rhizobacteria by nitrogen fixation, mineral solubilization, as well as transformation of nutrients, production of phytohormones and siderophores, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Lugtenberg and Kamilova, 2009) [36]. IAA production by the potential biocontrol agent *Pseudomonas aeruginosa* (Karnwal, 2009) [31]. *Pseudomonas* sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR (Rodriguez and Fraga, 1999; Misko and Germida, 2002) [49, 38]. *Pseudomonads* produce HCN which control the growth of root-rot pathogens (Thomshow and Weller, 1995) [60].

An era of synthetic chemicals came with the several insecticide and fungicide which successfully manage the infestation caused by insects, fungi and other microflora, but the descriptive use of chemicals and their residual toxicity adversely affects the non target animals including human being besides affecting the seed quality. Hence, the safe and feasible approach is the use of biofertilizer and vermicompost which are safe, ecofriendly, economical and easily available. Cucumber (*Cucumis satival L.*) is the most important vegetable crop in several developing countries. Vermicompost and biofertilizer are the most important input required for Cucumber cultivation. *Pseudomonas aeruginosa* as a bioferlizer have ability to solubilize phosphorus, zinc, potassium and induce some substances like indole acetic acid (IAA), Gibberelic acid and Siderophore that could contribute to the improvement of Cucumber growth.

Materials and Methods

The isolate bacterium (*Pseudomonas aeruginosa)* was procured from the Microbial Culture Collection Bank (MCCB), Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad. The characterization was done by cultural, morphological and biochemical analysis as per Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1984) [27]. *Pseudomonas aeruginosa* MCCB00186 was examined for beneficial traits of PGPR *viz.*, solubilization of insoluble inorganic phosphate, Zinc, Potassium and production of plant growth promoting substances like IAA, HCN.

Results and Discussion

Cultural, morphological, biochemical and physiological characteristics of *Pseudomonas aeruginosa MCCB00186*

Pseudomonas aeruginosa MCCB00186 was procured from the Microbial Culture Collection Bank (MCCB), Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, SHIATS. *Pseudomonas aeruginosa* MCCB00186 was characterized for cultural, morphological and biochemical characterization as per Bergey's Manual of Systematic Bacteriology Holt *et al.* (1984) [27]. The *Pseudomonas aeruginosa* colony was Grey colored on nutrient agar medium with Entire margin, Convex, Translucent, Circular in shape and characterized as Gram negative, rod shaped bacteria and showed negative reaction for MR-VP, Indol production, carbohydrate fermentation (Sucrose, Glucose, Lactose, D-Manitol, Trehalose, D-Galactose, D-Xylose, sorbital, L-Rhamanose, D- Fructose and L-Arbinose) and positive for (oxide, catalase, citrate utilization, nitrate reduction, urease, gelatin liquefaction, motility test, ONPG, PQQ test, starch hydrolysis).The *Pseudomonas aeruginosa* showed confluent growth at low temperature *i.e* 10°C while failed to grow at 50°C and 60°C. Growth of strain was subjected to grow at pH range of 5, 6, 7, 8, 9, 11 and 12. All pH supported the growth of strain. Salt tolerance was examined using NaCl concentration of 1, 2, 3, 4, 5% and it revealed that *Pseudomonas aeruginosa was* able to tolerate all the NaCl concentration.

In a study conducted by Shivakumar *et al.* (2013) [53] *Pseudomonas aeruginosa* is a gram negative, motile rod, oxidase positive, liquefied gelatin and produced diffusible pigments. Which is in agreement with present investigation. In a similar research conducted by Boruah *et al.* (2012) [9] isolated *Pseudomonas aeruginosa* ASO 3 from Geleky, Assam, India. Which was biochemically characterized as. Gram negative, rod shaped that produse *fluorescent* green pigment but showed negative in starch hydrolysis, H2S production, MR-VP. Yadav *et al.* (2010) [65] IAA production was increased on increasing the concentration of tryptophan from 100 to 200 µg ml^{-1} Bacterial strain *P. aeruginosa* BHUPSBO2 showed maximum significant concentration of IAA 21.35 µg ml^{1}. Karnwal (2009) [31] *P. aeruginosa* AK2 gram-negative, citrate-positive, oxidase-positive, catalase-positive, indole-positive, and hydrolyze starch and casein positive produced siderophores and were able to use glucose, mannitol, fructose, arabinose, trehalose, glycerol, xylose and starch as carbon source. These isolates were screened for their ability to produce IAA and other PGPR activity. Many researchers have reported the similar kind of study namely Dias *et al.* (2013) [18], Audipudi *et al.* (2014) [5], Prasad (2014) [47].

PGPR promotes plant growth and yield. They are capable of increasing availability of phosphorus to plant either by mineralization of inorganic phosphate by production of acids. This micro organism secret different types of organic acids, thus lowering the pH in rhizosphere consequently dissociate the bound forms of phosphate like $Ca_3 (Po_4)_2$ calcareous soil.

During phosphate solubilization, pH change due to organic acid production or H^+ ion dissociated from insoluble phosphates. Acidity causes more solubilization of the insoluble phosphate.

The hydroxyl and carboxyl group of organic acid. Chelate the cations (Al, Fe, Ca) bond to phosphate, the latter being converted to soluble forms it leads to decrease in pH.

PSB produces IAA, GA_3 and cytokinin which enhance plant metabolism. PSB also secretes acid phosphatases that mineralizes and mobilizes phosphorus in soil.

Hydrogen cyanide produced by micro organism posses important antifungal features that eventually control root fungi pathogen. Cyanide acts as general metabolism inhibitor the host plants are generally not harmfully affected by enoculation with HCN producing bacteria.

Table 1: Cultural, Morphological, Biochemical and Physiological characteristics of *Pseudomonas aeruginosa* MCCB00186

		Characteristics	*Pseudomonas aeruginosa* MCCB00186
Cultural characteristics		Colony colour	Greyish (Nutrient agar medium)
		Margin	Entire
		Elevation	Convex
		Shape of colony	Irregular large
		Opacity	Translucent
		Pigmentation	Blue-green (Nutrient agar medium)
		Consistency	Mucoid
		Texture	Glossy
Morphological charcteristics		Gram reaction	Gram negative
		Cell shape	Short rod
		Cell arrangement	Mono bacillus
Biochemical characteristics		Oxidase test	+
		Catalase test	+
		MR	-
		VP	-
		Indole test	-
		Citrate utilization test	+
		Starch hydrolysis test	+
		Nitrate reduction test	by pass reaction
		Urease test	+
		Gelatin liquefaction	+
		Motility test	+
		ONPG	+
Carbohydrate fermentaion		D-xyloase	$A^{-}G^{-}$
		D-fructose	$A^{-}G^{-}$
		Glucose	$A^{-}G^{-}$
		Sucrose	$A^{-}G^{-}$
		Manitol	$A^{-}G^{-}$
		Sorbitol	$A^{-}G^{-}$
		Lactose	$A^{-}G^{-}$
		Trehaluose	$A^{-}G^{-}$
		D-galactose	$A^{-}G^{-}$
		L-rhamnose	$A^{-}G^{-}$
		L-arabinose	$A^{-}G^{-}$
Physiological characteristics	Temperature(°C)	0	-
		5	-
		10	+
		20	+
		30	++
		40	+++
		50	-
		60	-
	pH	5	-
		6	+
		7	++
		8	+
		9	-
		10	-
		11	-
		12	-
	NaCl (%) Concentration	1	+
		2	+
		3	+
		4	+
		5	+
		6	+
		7	-
		8	-
		9	-
		10	-

A^{+} = Acid positive, G^{+} = Gas positive, A^{-} = Acid negative
G^{-} = Gas negative, Conc. = Concentrated
+ = light growth, ++ = strong growth

Characterization of *Pseudomonas aeruginosa* MCCB 00186 as a plant growth promoting rhizobacteria

Pseudominas aeruginosa MCCB00186 was screened for plant growth promoting features which were assayed through phosphate solubilization, potassium solubilization, zinc solubilization, IAA and HCN production, PQQ independent activity.The strain showed a positive assay for the afore mentioned PGPR traits therefore, the strain was selected for biofertilizer production and examination (Table 2).

In a research conduct by Shivakumar *et al.* (2013) [53] PGPR characterization of *P. aeruginosa* was examined *P. aeruginosa* FP6 evidenced to produce hydrogen cyanide HCN and IAA. According to Boruah *et al.* (2013) [9] isolate *P. aeruginosa* for tea industry in Assam, India that had positive PGPR characterization of IAA and HCN production. Isfahani and Besharati (2012) [29] used *P. sp.* as PGPR on Cucumber that was able solubilize phosphate. Yadav *et al*. (2010) [65] examined the PGPR characterization of *P. aeruginosa* BHU PSB 02 and *P. putida* BHU PSB04 Both isolate were able to produce IAA, Phosphate Solubilization and ammonia production that enhanced the growth of chickpea. Servanan *et al.* (2003) [52] isolated Zinc solubilizing *Bacillus sp.* and *Pseudomonas spp.* Using Zinc oxide, Zinc sulphide and Zinc carbonate in plate broth assay *P. sp* ZSB S-2 produced clear zone of 3.30 cm with Zn oxide (highest). According to Suresh *et al*. (2010) [57] are in accordance to the present investigation in which 10 *fluorescent Pseudomonas* were found positive for IAA and HCN production. Patten and Glick (1996) [43] reported Bacteria belonging to the genera *Pseudomonas* sp. that produced auxin which helps in stimulating plant growth as well as production of HCNAs per Moreira *et al.* (2011) [39] all the strains were able to solubilize at least one of the low soluble inorganic phosphates in the solid GELP medium except for INPA 03-11B. Karnwal (2009) [31] showed that *Pseudomonas fluorescens* AK1 and *Pseudomoas aeruginosa* AK2 had better PGPR activity than other isolates. Many researchers have reported the similar kind of study namely Ahmed and Shahab (2010) [3]. Dias *et al.* (2013) [18]. Young *et al.* (2005) [66]. PGPR promotes plant growth and yield. They are capable of increasing availability of phosphorus to plant either by mineralization of inorganic phosphate by production of acids. This micro organism secret different types of organic acids, thus lowering the pH in rhizosphere consequently dissociate the bound forms of phosphate like Ca_3 $(Po_4)_2$ calcareous soil.

Phosphate solubilizing micro organism convert insoluble phosphate into soluble forms (*Pseudomonas aeruginosa*) through process of acidification, chelation, enchange reactions and production of gluconic acid, succinic, acetic, glutamic, oxaloacetic, pyruvic, malic, fumaric acid and α- ketoglutaric acid.

During phosphate solubilization, pH change due to organic acid production or H^+ ion dissociated from insoluble phosphates. Acidity causes more solubilization of the insoluble phosphate.

The hydroxyl and carboxyl group of organic acid. Chelate the cations (Al, Fe, Ca) bond to phosphate, the latter being converted to soluble forms it leads to decrease in pH.

PSB produces IAA, GA_3 and cytokinin which enhance plant metabolism. PSB also secretes acid phosphatases that mineralizes and mobilizes phosphorus in soil.

Hydrogen cyanide produced by micro organism posses important antifungal features that eventually control root fungi pathogen. Cyanide acts as general metabolism inhibitor the host plants are generally not harmfully affected by enoculation with HCN producing bacteria.

Table 2: Determination of PGPR characteristics of *Pseudomonas aeruginosa* MCCB00186

Assay	***Pseudomonas aeruginosa* MCCB00186**
Phosphate solubilization	+
Potassium solubilization	+
Zinc solubilizing	+
Indole acetic acid production	+
HCN production	+
PQQ independent activity	+

Qualitative determination of by Phosphate solubilizion index (PSI) and Phosphate solubilizion efficiency (PSE) of *Pseudomonas aeruginosa* MCCB 00186 on two selective media

Phosphate solubilization index (PSI) of *Pseudomonas aeruginosa* on PVK medium recorded at 2d, 4d, 6d, 8d and 10d were (1.31±0.16, 1.40±0.06, 1.49±0.06, 1.51±0.02) respectively while in NBRIP medium the PSI at 2d, 4d, 6d, 8d, and 10d were (2.22±0.19, 2.55±0.19, 2.88±0.19, 3.44±0.19) respectively. Phosphate solubilization efficiency (PSE) of *Pseudomonas aeruginosa* on PVK medium analyzed at 2d, 4d, 6d, 8d and 10d were (31.67±16.07, 39.40±5.63, 49.47±6.37, 51.85±3.20, 62.35±7.73) respectively. While PSE on NBRIP for same days were recorded as 122.2±19.2,155.3±19.1, 188.8±19.4, 211.0±19.1, 244.2±19.4), respectively (Table 3).

In a study conducted by Karpagam and Nagalakshmi (2013) [32] 6 bacteria among 37 isolates showed highest phosphate solubilization index (PSI) ranged from 1.13 - 2.23. Phosphate solubilization by *Pseudomonas sp.* BRS-2 was conducted by Islam *et al.* (2011) [28] that showed the phosphate solubilization index of 2.5. Mardad *et al.* (2013) [37] examined PSB6 was the most efficient phosphate solubilizer on NBRIP plates with a solubilization index of 4.40. Babana *et al.* (2006) [6] reported solubilization index of rock phosphate solubilizer on NBRIP agar plates solubilization index 3.60. Phosphate solubilization by *Pseudomonas aeruginosa* was conducted by Tambekar *et al.* (2009) [59] that showed positive phosphate solubilized. Many researchers have reported the similar kind of study namely Halder *et al.* (1990) [25], Goldstein (1995) [23], Kim *et al.* (1998) [33].

Phosphate solubilizing micro organism convert insoluble phosphate into soluble forms (*Pseudomonas aeruginosa*) through process of acidification, chelation, enchange reactions and production of gluconic acid, succinic, acetic, glutamic, oxaloacetic, pyruvic, malic, fumaric acid and α- ketoglutaric acid.

During phosphate solubilization, pH change due to organic acid production or H^+ ion dissociated from insoluble phosphates. Acidity causes more solubilization of the insoluble phosphate

Phosphorus plays a significant role in physiological and biochemical. Plant activities like photosynthesis transformation of sugar to starch and transporting of genetic traits phosphorus can also causes early ripening in plants decreasing green moisture, improving crop quality and is most sensitive nutrient to soil pH. Phosphate solubilization ability of PSB has direct correlation with pH of the medium.

Table 3: Zone of clearance (mm) of *Pseudomonas aeruginosa* MCCB 00186 on two selected media

Time days	Halo zone measurement (mm)			
	PSI		PSE	
	NBRIP	PVK	NBRIP	PVK
0	0±0 e	0±0c	0±0 e	0±0
2	2.22±0.19 d	1.31±0.16 b	122.2±19.2 d	31.67±16.07 b
4	2.55±0.19 cd	1.40±0.06 ab	155.3±19.1 cd	39.40±5.63 b
6	2.88±0.19 bc	1.49±0.06 ab	188.8±19.4 bc	49.47±6.37 ab
8	3.11±0.19 ab	1.51±0.02 ab	211.0±19.1 ab	51.85±3.20 ab
10	3.44±0.19 a	1.62±08 a	244.2±19.4 a	62.35±7.73 a
$F_{cal.}$	149.52	161.06	72.56	21.52
$F_{tab.}$	2.178	2.178	2.178	2.178
F_{test}	S	S	S	S
CD-(0.05%)	0.729	0.340	73.336	34.105
S. Ed.(±)	0.141	0.063	14.343	6.671

Data are means ± S.D (n=3)
Different letters in each column denote significant differences (P< 0.05) according to a Tukey's HSD test
Each letter shows relative degree of significant each other
a = most significant value
d = is least significant value

Quantification of phosphorus solubilized by *Pseudomonas aeruginosa* MCCB00186 in PVK broth at different time intervals

Solubilized phosphate was estimated using PVK media by *Pseudomonas aeruginosa* at different time intervals which gradually increased at different time intervals which gradually increased at different time intervals of 2d, 4d, 6d, 8d, and 10d. Solubilized phosphate (mg/ml) was 0.333 at 2d, 0.343 at 4d, 0.373 at 6d, 0.463 at 8d, and 0.486 at 10d.

In a study conducted by Bhakthvatchalu *et al.* (2013) [8] characterized multiple plant growth promoting traits of *Pseudomonas sp.* a potential stress tolerant biocontrol agent. Results found were in contrast with present investigation. Phosphate solubilization in broth medium was 270µg/ml while in present investigation it ranged from (31.0 to 46.8 µg/ml). Study conducted by Babana and Antoun *(*2006) [6] is in agreement with present research who recorded (90mg/l) phosphate solubilization by *Pseudomonas sp*. similar result also reported by Santhaguru and Saravanan (2012) [51]. Study conducted by Parani and Saha (2012) [42] is in contrast with present research who recorded 968.5 mg/l phosphate solubilization by *Pseudomonas sp*.

Phosphorus (P) is an essential macronutrient often limiting the plant growth due to its low solubility and fixation in the soil. Improving soil fertility by releasing bound phosphorus by microbial inoculants is an important aspect for increasing crop yield. Phosphorus release from insoluble phosphates reported for several soil microorganisms has been attributed mainly to the production of organic acids and their chelating capacity. Direct periplasmic oxidation of glucose to gluconic acid is considered as the metabolic basis of inorganic phosphate solubilization by many Gram- negative bacteria as a competitive strategy to transform the readily available carbon sources into less readily utilizable products by other microbes.

Table 4: Quantification of phosphorus solubilized by *Pseudomonas aeruginosa* MCCB 00186 in PVK broth at different time intervals

Time intervals (days)	Quantitative estimation of solubilized phosphate by *Pseudomonas aeruginosa* in PVK medium(mg/ml)
2	0.33±0.17 e
4	0.34±0.16 d
6	0.37±0.17 c
8	0.463±0.18 b
10	0.49±0.18 a
$F_{cal.}$	2698.87
$F_{tab.}$	2.228
F_{test}	S
CD-(0.05%)	0.001
S. Ed.(±)	2.419

Data are means ± S.D (n=3)
Different letters in each column denote significant differences (P< 0.05) according to a Tukey's HSD test
Each letter shows relative degree of significant each other
a = most significant value
e = is least significant value

Qualitative determination of Zinc solubilization index (ZSI) and Zinc Solubilization efficiency (ZSE) of *Pseudomonas aeruginosa* MCCB 00186 modified PVK medium

Pseudomonas aeruginosa MCCB 00186 was effectively solubilize the insoluble was Zn compound used Zinc sulfate under the assay conditions.Zinc solubilization was assayed for *Pseudomonas aeruginosa* on modified PVK media at different time intervals of 2d, 4d, 6d, 8d, and 10d. Solubilization index (cm) was recorded in 2d(1.73), 4d(1.37), 6d(2.23), 8d(2.43) and 10d(2.60) which was gradually increased from 2d to 8d but it was same at 10d while solubilization efficiency (%) of Zinc was recorded (100) in 2d, (150) in 4d, (183.33) in 6d, (216.66) in 8d, and (233.33) in 10d (Table 5).

The data was analysed for significance (p<0.005) using one way ANOVA followed by *post hoc* Tukey's HSD comparison. No significant difference was found in intent of

ZSI and ZSE over increasing time duration from 0 to 10 hours. Thus, increasing time interval failed to be a limiting factor for solubilization of Zinc by *P. aeruginosa* MCCB00186

In a study conducted by Servanan *et al. (*2003) [52] isolated Zinc solubilizing *Bacillus sp.* and *Pseudomonas spp.* Using Zinc oxide, Zinc sulphide and Zinc carbonate in plate broth assay *Pseudomonas sp.* ZSB S-2 produced clear zone of 3.30 cm with Zn oxide (highest). Zinc phosphate solubilization by *Pseudomonas sp.* was also conducted by Simine *et al.* (1998) [54] that showed gluconic acid and α – keto gluconic acid produced is broth helped in solubiliz of Zinc salt. Similar study was also performed by Bapiri *et al.* (2012) [7] *Pseudomonas aeruginosa* obtained the highest potential in Zinc carbonate ($ZnCo_3$) containing medium producing a clearing zone of 1.70 cm halo diameter/colony diameter at 2.99 and zone area of 4.10 cm^2. Pawar *et al.* (2015) [44] evaluated zinc solubilization of *Pseudomonas sp.* were showed highest solubilization efficiency and solubilization index (S.E. = 203.33 and S.I. = 3.03). The zinc phosphate solubilization by *Pseudomonas aeruginosa* was investigated by Desai *et al. (*2012) [15] reported that zinc solubilization 2.64cm clering zone. Cozzi *et al.* (1969) [12] reported that the complexing agents produced by microbes stabilized the inorganic ions in the solution and kept them from precipitation or in some cases, prevented from being oxidized or reduced and converted to insoluble forms.

Zinc is an essential micro nutrient required for growth and metabolism of micro organism and plants. Zinc is present in enzmes system as co- factor and metal activator of many enzymes. Bacterial enzymes contain zinc is the actine centre or in structurally important site. Zinc occur in soil as sphalerits, olivine, horn blende, augite and biotile. Rhizobacteria play important role in converting this unavailable zinc to available form. Organic based nutrition is best and more efficient the basic principle behind this approach is decreasing the pH to 5 or below and making zinc soluble as a consequence the available zinc will get increased in the soil system. pH is a vital factor in solubilization. Rhizobacteria can contribute to zinc emmobilization by precipitation and adsorption.

Table 5: Zinc solubilization index (ZSI) and Zinc solubilization efficiency (ZSE) of *Pseudomonas aeruginosa* MCCB 00186on modified PVK medium

Time days	Zone of clearance (mm) in modified PVK media	
	ZSI	**ZSE**
	1.73± 0.15b	135±30.41 c
4	1.37± b	159.00±8.54 c
6	2.23± a	204.66±23.79 b
8	2.43± a	225.32±9.60 ab
10	2.60± a	246.33±11.53 a
$F_{cal.}$	12.27	17.89
$F_{tab.}$	2.228	2.228
F_{test}	S	S
CD-(0.05%)	0.450	34.41
S. Ed.(±)	0.0228	168.73

Data are means ± S.D (n=3)
Different letters in each column denote significant differences (P< 0.05) according to a Tukey's HSD test
Each letter shows relative degree of significant each other
a = most significant value

Qualitative determination of Potassium solubilizion index (KSI) and Potassium solubilizion efficiency (KSE) of *Pseudomonas aeruginosa* MCCB 00186 on Alexandrove medium

Pseudomonas aeruginosa MCCB 00186 was examined for potassium solubilizing index and potassium solubilizing efficiency on Alexandrove medium. PSI as well as PSE was found to gradually increase over times, but thus were no significant variation in PSI and PSE over time (Table 6).

The data was analysed for significance (p<0.005) using One way ANOVA followed by comparison Tukey's HSD (*post hoc*). No significant difference was found in intent of KSI and KSE over increasing time duration from 0 to 10 hours. Thus, increasing time interval failed to be a limiting factor for solubilization of potassium by *Pseudomonas aeruginosa.*

In a study conducted by Prajapati and Modi (2012) [46] isolated and characterized potassium solubilizing bacteria from ceramic industry soil. He found heighest solubilization zone 1.57cm by KSB8 which is in agreement with present investigation. Present investigation very well supported the findings of Archana *et al.* (2008) [4] who found 1.20 cm solubilization index by KSB16. Bacteria have also been shown to accelerate the dissolution of silicates by the production of excess proton and organic ligands and in some cases by the production of hydroxyl anion, extra cellular polysaccharides (EPS) and enzymes. Berthelin and Belgy (1997) [10] found that a variety of extra cellular polysaccharides significantly enhanced the dissolution of plagioclase. The production of organic acids such as acetate, citrate and oxalate by microorganism can increase mineral dissolution rate Hazen *et al.* (1991) [26]. Moreover results also suggest that the weathering ability of the bacteria which involves the production of protons, organic acids, siderophores and organic ligands Grayston *et al.* (1996) [24].

Bacteria can decompose potassium by production of organic acid. Therefore phosphate solubilzing bacteria can also decompose potassium. Potassium is the third comportant plant nutrient and it play important role in activation of several metabolic process incuding protein synthesis, enzymes, it also causes resistance towards diseases and insect. It is applied to soil as natural or synthesis fertilizers but only 2% of this is available to plant. Most common soil components of potassium, 90 to 98% are feldspar and mica. There rhizobacteria decomposes alimino silicat minerals and releases a portion of potassium contained therein. Bacteria can decompose potassium by production of organic acid therefore, PSB can solubilizer or mineralize potassium.

Table 6: Variation in Potassium solubilization index (KSI) and Potassium solubilization efficiency (KSE) by *Pseudomonas aeruginosa* MCCB 00186 Aleksandrov medium

Time days	Zone of clearance (mm) in Aleksandrov media	
	KSI	KSE
0	0±0 b	0±0 b
2	2.30±0.17 a	130.6±17.3 a
4	2.53±0.27 a	153.0±27.9 a
6	2.51±0.22 a	153.0±21.5 a
8	2.65±0.39 a	165.2±38.9 a
10	2.79±0.41 a	180±40.9 a
$F_{cal.}$	42.034	16.60
$F_{tab.}$	2.178	2.178
F_{test}	S	S
CD-(0.05%)	0.506	49.870
S. Ed.(±)	0.228	22.890

Data are means ± S.D (n=3)
Different letters in each column denote significant differences (P< 0.05) according to a Tukey's HSD test
Each letter shows relative degree of significant each other
a = most significant value
b = is least significant value

Table 7: Quantitative estimation of available Potassium by *Pseudomonas aeruginosa* CCB 00186

Time intervals (days)	Quantitative estimation of solubilized potassium by *Pseudomonas aeruginosa* in Alexandrove medium (mg/ml)
2	0.30±0.21 c
4	0.915± 0.21 bc
6	1.225±0.21 b
8	1.535±0.21 ab
10	1.995±0.43 a
$F_{cal.}$	10.806
$F_{tab.}$	2.57
F_{test}	S
CD-(0.05%)	0.709
S. Ed.(±)	0.055

Data are means ± S.D (n=2)
Different letters in each column denote significant differences (P< 0.05,) according to a Tukey's HSD test
Each letter shows relative degree of significant each other
a = most significant value
c = is least significant value

Qualitative estimation of IAA production by *Pseudomonas aeruginosa* MCCB 00186

Pseudomonas aeruginosa was examined for plant growth promoting traits. *Pseudomonas aeruginosa* showed positive IAA production.

In a study conducted by Audipudi *et al.* (2014) [5] the results revealed that all 10 isolates were positive to IAA, Siderophore production, Phosphate solubilisation and Ammonia production. Except CEFR 6, none of the isolates was found negative for HCN production.

Quantitative estimation of IAA production by *Pseudomonas aeruginosa* MCCB 00186

In a study conducted by Santhaguru and Saravanan (2012) [51] studied diversity of plant growth promoting rhizobacteria including *Pseudomonas sp.* from rhizospheric soil of some tropical grasses in southern Tamil Nadu. Results obtained were in accordance with present investigation. IAA production of *Pseudomonas sp.* pf13 was 59.42 μg IAA/mg protein while in present investigation it ranged from 56.55 to 374.9 μg/ml 2d to 10d. Similar result was also found by Dey *et al.* (2004) [17] who recorded 3.6 mg/ml IAA by *Pseudomonas* (PGPR1). Results obtained by Kannaprinam and Ramakumar (2011) [30] is in contrast of present research who recorded (26.5 μg/ml IAA) presence of L-tryptophan. Present investigation very well supported the finding of Bhakthvatchalu *et al.* (2013) [8] who recorded (80 μg/ml IAA) by Pseudomonas aeruginosa in presence of L-tryptophan compared to its abscens (16 μg/ml). Similar results also rported by Ahmad *et al.* (2005) [2].

HCN production by *Pseudomonas aeruginosa* MCCB 00186

Pseudomonas aeruginosa was examined for plant growth promoting traits. *Pseudomonas aeruginosa* showed positive HCN production.

In a study conducted by Ahmed and Shahab (2010) [3] are reliable for current investigation that determined a total of 2212 base which showed 95% homology with pqq BC gene of *Pseudomonas aeruginosa* LESB58 and 97% homology to pqq AB gene of *Pseudomonas aeruginosa* PAO1 and 77% of pqq A-D gene of *Pseudomonas aeruginosa* strain B 16. Study conducted by Suresh *et al.* (2010) [57] are in accordance to the present investigation in which 10 *fluorescent Pseudomonas* were found positive for IAA and HCN. Patten and Glick (1996) [43] reported bacteria belonging to the genera *Pseudomonas* to produce auxin which helps in stimulating plant growth. Production of HCN, IAA and Phosphate solubilization has been implicated in the plant growth promotion by PGPR Sullivan and Gara (1992) [56]. Similar result was accomplished by Ahmed and Shahab (2010) [3] a

remarkable change in colour from yellow to Reddish – Brown indicates HCN production and which could be used for the IAA production. Study conducted by Dalal and Kulkarni (2013) [13] is in agreement with present investigation. He found that *Pseudomonas sp*. was able to produce Gibberalic acid. Shivakumar *et al.* (2013) [53] reported strong HCN production by isolate *P. aeruginosa* FP6, as evidenced by change in color of filter paper from yellow to reddish- brown aftr 2-3 days of incubation. Higher amount of HCN was produced by the isolate (0.09 ± 0.01) at 625 nm absorbance.
Hydrogen cyanide is a secondry metabolite produced by microorganism possess importer antifungal features that eventually control root fungi pathogen. Cyanide acts as general metabolic inhibition the host plant are generally not harmfully affected by enoculation with HCN producing bacteria.

PQQ independent activity by *Pseudomonas aeruginosa* MCCB 00186

Pseudomonas aeruginosa was examined for plant growth promoting traits. *Pseudomonas aeruginosa* showed positive PQQ independent activity.
In a study conducted by Ahmed and Shahab (2010) [3] PQQ *i.e.* Pyrroloquinoline (4, 5 – dihydro -4, 5 – dioxa 1H – prrolo –(2, 3 -4) quinoline -2, 7, 9-Tricarboxylic acid is an aromatic, Tricyclic orthoquinine that serve as redox cofactor for several bacterial Dehydrogenase. The redox cofactor PQQ links the oxidation of many different compounds to the respiratory chain in gram negative bacteria and acts as reactive oxygen scavenger (ROS) to neutralize free radicals. It also functions as potent growth facter by enhancing DNA synthesis. Thus it has been regarded as plant growth promating facter. The rhizospheric bacteria are thought to produce HCN towards soil borne pathogens and *Pseudomonas* is found to predominantly acquire the rhizosphere of plant. Study conducted by Suresh *et al.* (2010) [57] are in accordance to the present investigation in which 10 *fluorescent Pseudomonas* were found positive for IAA and HCN. Raychaudhuri *et al.* (2013) [48] Enter Doudoroff (E.D) pathway is a branch metabolic gluconate catalyzed by PQQ dependent enzyme pathway of oxidative glucose metabolism, where oxidative glucose converted to gluconate catalyzed by PQQ dependent enzyme. Kuenen *et al.* (1988) [34] *Pseudomonas aeruginosa* were also grown oxygen limited on acetate/glucose mixture in the presence of 100mM PQQ. Bacterial metabolism of sugares may precede *vi* a defferent catabolic roules. *Pseudomonas spp*. Can catalyse enitail oxidation of phosphorylated or no phosphorylated sugar to the corresponding aldonic acid which transiently accumulats in medium the accumulation of aldanic acid by bacteria is associated with the presence of a membrane bound aldose dehydrogenase (Known as glucose dehydrogenase, GDH) which contains pyrrolo- quinolone quinine (PQQ) as a prosthetic group. The enzyme is located on the periplasmic side of cell membrane and donates its electrons to the electron transport chain at level of cytochrome b.

Table 8: IAA, HCN, Auxin production and PQQ independent activity by *Pseudomonas aeruginosa* MCCB 00186

PGP traits	*Pseudomonas aeruginosa* MCCB00186
IAA production	+
HCN production	+
Auxin production	+
PQQ independent activity	+

Conclusions

Pseudomonas aeruginosa was characterized as a Gram negative, rod shaped that possessed PGPR trait *viz* IAA and HCN production, Phosphate, Potassium and Zinc solubilization efficiency and PQQ independent activity. Strains showed high solubilization value of phosphate, potassium at 10th day of incubation quantified as 3.44±0.19, 2.79±0.41 respectively by *Pseudomonas aeruginosa* for phosphate and potassium.

References

1. Adetula O, Denton, L. Performance of vegetative and yield accessions of cucumber (*Cucumis sativa* L.). Horticultural Society of Nigeria (HORTSON) Proceedings of 21st Annual Conference 2003,10-13.
2. Ahmad H, Ahmad I, Khan MS. Indole Acetic Acid production by the Indigenous Isolates of Azotobacter and fluorescent Pseudomonas in the Presence and Absence of Tryptophan. Turkish Journal of Biology 2005;29:29-34.
3. Ahmed, Shahab K. Involvement of Bacterial Pyrroloquinoline in Plant Growth Promotion: A Novel Discovery. World Applied Sciences Journal 2010,57-61.
4. Archana DS, Savalgi VP. Alagawadi AR. Effect of potassium solubilizing bacteria on growth and yield of maize. Journal of Soil Biology and Ecology 2008;28(2):9-18.
5. Audipudi AV, Allu S, Kumar PN. Isolation, Biochemical and PGP characterization of endophytic *Pseudomonas aeruginosa* isolated from chilli red fruit antagonistic against chilli anthracnose disease. International Journal of Current Microbiology and Applied Science 2014;3(2):318-329.
6. Babana AH, Antoun H. Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field- grown wheat (*Triticum aestivum* L.) in Mali. In: Handbook of Plant Soil 2006;287:51-58.
7. Bapiri A, Asgharzadeh A, Mujjallali H, Khavazi K, Pazirae E. Evaluation of Zinc solubilization potential by different strains of fluorescent *Pseudomonas*. Journal of Applied Sciences and Environmental Management 2012;16(3):295-298.
8. Bhakthavatchal, Shivakumar S, Sullia SB. Characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* FP6, a potential stress tolerant biocontrol agent. Annals of Biological Research 2013;4(2):214-223.
9. Boruah HPD, Roy AS, Yenn R, Singh AK, Saikia N, Deka M. Bioremediation of crude oil contaminated tea plantation soil using two *Pseudomonas aeruginosa* strains AS 03 and NA 108. African Journal of Biotechnology 2013;12(19):2600-2610.
10. Berthelin J, Belgy G. Microbial degradation of phyllosilicates during simulated podzolization. Geoderma 1979;21(4):297-310.
11. Champs CC, Henquell D, Gueron D, Sirot D. Clinical and bacteriological study of Nosocomial infections due to

E. aerogene resistance to imipenem. Journal of Clinical Microbiology 1993;31(1):123-7.
12. Cozzi D, Desidevi PG, Lepri L. The mechanism of ion exchange with alginic acid. Journal of Chromatography 1969;40:130-135.
13. Dalal J, Kulkarni N. Antagonistic and Plant Growth Promoting Potentials of indigenous. Endophytic bacteria of Soybean. Current Research in Microbiology and Biotechnology 2013;1(2):62-69.
14. Daneshvar MH. A study of the effect of biofertilizers containing amino acid on the yield and growth traits of cucumber (*Cucumis sativus*). Shahid Chamran University, Ahwaz 461.
15. Desai S, Praveen GK, Sultana U. Potential microbial candidate strains for management of nutrient requirements of crops. African Journal of Microbiology Research 2012;6(17):3924-3931.
16. Deshwal VK, Kumar P. Plant growth promoting activity of *Pseudomonas* in rice crop. International Journal of Current Microbiology and Applied Sciences 2013;2(11):152-157.
17. Dey R, Pal KK, Bhatt DM. Chauhan SM. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promotig rhizobacteria. Microbiological Research 2004;159:371-394.
18. Dias GB, Gomes VM, Moraes TMS, Zottich UP. Characterization of *Capsicu* species using anatomical and molecular Genetics. Journal of Molecular Research 2013;28:12.
19. El-Yazeid AA, Abou-Aly HA, Mady MA, Moussa SAM. Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. Research Journal of Agriculture and Biological Sciences 2007;3(4):274-286.
20. Enujeke EC. Growth and yield responses of cucumber to five different rates of poultry manure in Asaba area of Delta state, Nigeria. International Research Journal of Agricultural Science and Soil Science 2013;3(11):369-375.
21. Farhan NH, Abdullah HB, Hameed TA. The biological activity of bacterial vaccine of *Pseudomonas* putida2 and *Pseudomonas fluorescens* 3 isolates to protect sesame crop (*Sesamum indicum*) from Fusarium fungi under field conditions. Agriculture and Biology Journal of North America 2010;1(5):803-811.
22. Franco W, Diaz, IMPR. Microbial interactions associated with secondary cucumber Fermentation. Journal of Applied Microbiology 2012;114:161-172.
23. Goldstein AH. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram negative bacteria. Journal of Biology Agriculture and Horticulture 1995;12:185-193.
24. Grayston SJ, Vaughan D, Jones D. Rhizosphere carbon flow in trees, in comparison with annual plants the importance of root exudation and its impact on microbial activity and nutrient availability. Journal of Applied Soil Ecology 1996;5:29-56.
25. Halder AK, Mishra AK, Bhattacharyya P, Chakrabartty PK. Solubilization of rock phosphate by Rhizolium and Bradyrhizobium. Journal of Genetic and Applied Microbiology 1990;36:81-92.
26. Hazen TC, Jimenez L, de Victoria GL. Comparison of bacteria from deep subsurface sediment and adjacent ground water. Microbial Ecology 1991;22:293–304.
27. Holt JG, Krieg NR. Gram Negative Aerobic Rods and Cocci. In: Bergey's Manual of Systematic Bacteriology, Williams and Wikins publishers, USA 1984;1:141-198.
28. Islam R, Trivedi P, Madhaiyan M, Seshadri S, Lee G, Yang J *et al*. Isolation, enumeration, and characterization of diazotrophic bacteria from paddy soil sample under long-term fertilizer management experiment. Journal of Biology of Fertile Soils 2011;46:261-269.
29. Isfahani FM, Besharati H. Effect of biofertilizers on yield and yield components of cucumber. Journal of Biology and Earth Sciences 2012;2(2):83-92.
30. Kannaprinan E, Ramakumar VS. Inoculation effect of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth of black gram (*Phaseolus mungo* Roxb, Eng). Annals of Biological Research 2011;2(5):615-621.
31. Karnwal A. Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L-tryptophan and rice root exudates. Journal of Plant Pathology 2009;91(1):61-63.
32. Karpgam T, Nagalakshmi K. Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural Soil. International Journal of Current Microbiology and Apploed Sciences 2013;3:601-604.
33. Kim KY, Jordan D, Mc Donald GA. Effect of phosphate solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Journal of Biological and Fertilizer Soil 1998;26:79-87.
34. Kuenen JG, Vanschie BJ. Vandijken JP. Effects of growth rate and oxygen tension on glucose dehydrogenase activity in *Acinetobacter calcoaceticus* LMD 1988;55:53-65.
35. Lee KE, Pankhurst CE. Soil organisms and sustainable productivity. Australian Journal of Soil Research 1992;30:855-92.
36. Lugtenberg B, Kamilova F. Plant growth promoting rhizobacteria. Annual Review of Microbiology 2009;63:541-556.
37. Mardad L, Serrano A, Soukri A. Solubilization of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit. African Journal of Microbiology Research 2013;7(8):626-635.
38. Misko AL, Germida JJ. Taxonomic and functional diversity of pseudomonads isolated from the roots of field-grown canola. Journal of FEMS Microbiology and Ecology 2002;42:399-407.
39. Moreira H, Marques APGC, Franco AR, Rangel AOSS, Castro PML. Phytomanagement of Cd-contaminated soils using maize (*Zea mays* L.) assisted by plant growth-promoting rhizobacteria. Journal of Environment Science and Pollution Research 2014;21:9742-9753.
40. Nailwal S, Anwar SM, Budhani, KK, Verma A, Nailwal KT. *Burkholderia* sp. from rhizosphere of Rhododendron arboretum: Isolation, identification and plant growth promotory (PGP) activities. Journal of Applied and Natural Science 2014;6(2):473-479.
41. Okonmah LU. Effects of different types of staking and their cost effectiveness on the growth, yield and yield components of cucumber (*Cumumis sativa* L). International Journal of Agriculture and Science

2011;5:290-295.
42. Parani K, Saha BK. Prospects of Using Phosphate Solubilizing *Pseudomonas* as Bio Fertilizer. European Journal of Biological Sciences 2012;2:40-44.
43. Patten C, Glick BR. Bacterial biosynthesis of indole acid. Canadian Journal of Microbiology 1996;42:207-220.
44. Pawar A, Ismail S, Mundhe S, Patil VD. Solubilization of insoluble zinc compounds by different microbial isolates *in vitro* condition. Journal of National Academy of Agricultural Sciences 2015;33(2):865-869.
45. Peyvast GHA. Vegetable production, Danesh pazir publication, Rasht. International Journal of Farming and Allied Sciences 2009;2(20):857-860.
46. Prajapati KB, Modi HA. Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. Journal of Microbiology 2012;1:2-3.
47. Prasad MP. Production of Lipase enzyme from *Pseudomonas aeruginosa* isolated from lipid rich soil. International Journal of Pure and Applied Bioscience 2014;2(1):77-81.
48. Raychaudhuri U, Ghosh S, Chakraborty R. Pyrroloquinoline Quinone a redox cofactor and its involvement in biological system. International Journal of Science and Nature 2013;4(3):371-380.
49. Rodriguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Journal of Biotechnology 1999;17:319-339.
50. Rokhzadi A, Asgharzadeh A, Darvish F, Nourmohammadi G, Majidi E. Influence of plant growth-promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field condition. American-Eurasian Journal of Agricultural and Environmental Sciences 2008;3(2):253-257.
51. Santhaguru K, Saravanan TS. Funtional diversity of plant growth promoting rhizobacteria from rhizospphere soil of some tropical grasses in southern Tamil Nadu. Asian Journal of Pharmaceutical and Biological Research 2012;4:2231-2218.
52. Servanan VS, Subramoniam SR, Raj SA. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Brazilian Journal of Microbiology 2003;34:121-125.
53. Shivakumar S, Bhakthavatchalu S, Sullia SB. Characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* FP6, a potential stress tolerant biocontrol agent. Annals of Biological Research 2013;4(2):214-223.
54. Simine CDD, Sayer JA, Gadd GM. Solubilization of Zinc phosphate by a strain of *Pseudomonas fluorescens* isolated form a forest soil. Biological Fertilizer Soils 1998;28:87-94.
55. Staub JE, Box J, Meglic V, Horejsi TF, McCreigh JD. Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in Cucumis. Journal of Genetic Resources and Crop 1997;44:257-269.
56. Sullivan DJO, Gara F. Traits of fluorescent *Pseudomonas* spp. Involved in suppression of plant root pathogens. Review of Microbiology Molecular Biology 1992;56:662-676.
57. Suresh A, Pallavi P, Srinivas P, Kumar SP, Chandra SJ, Reddy SR. Plant growth promoting activities of fluorescent Pseudomonas associated with some crop plants. African Journal of Microbiology Research 2010;4:1491-1494.
58. Tambe RS. Assessment of Physicochemical Parameters from Vermicompost of *Eudrilus eugenae* and *Esienia foetida*. Recent Research in Science and Technology 2011;3(3):138-139.
59. Tambekar DH, Gulhane DO, Somkuwar KB, Ingle KB, Kanchalwar SP, Upadhye MA *et al*. Potential Rhizobium and phosphate solubilizer as a Biofertilizer from Saline belt of Akola and Buldhana district (India). Research Journal of Agriculture and Biological Sciences 2009;5(4):578-582.
60. Thomshow LS, Weller D. Current concept in the use of introduced bacteria for biological disease control mechanism and antifungal metabolites. Plant Microbe Interactions. Chapman and Hall, New York 1995;1:187-235.
61. Venkatashwarlu B. Role of bio-fertilizers in organic farming: Organic farming in rain fed agriculture: Central institute for dry land agriculture, Hyderabad 2008,85-95.
62. Vimala P, Ting CC, Salbiah H, Ibrahim B, Ismail L. Biomass production and nutrient yields of four green manures and their effects on the yield of cucumber. Journal of Tropical Agriculture and Food Science 1999;27:47-55.
63. Wani SP, Rego TG, Rajeshwari S, Lee KK. Effect of legume–based cropping systems on nitrogen mineralization potential of Vertisol. Journal of Plant Soil 1995;175(2):265-274.
64. Yadav B, Bajaj A, Saxena M, Paliwal G. Influence by physical properties of coal combustion residues (CCRs) on dry root productivity of *Withania somnifera* grown in black cotton soil. Journal of Pharmacognosy and Phytochemistry 2013;2(1):130-136.
65. Yadav J, Verma JP, Tiwari KN. 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 2010;2(2):15-18.
66. Young CC, Lai WA, Shen FT, Hung MH, Hung WS, Arun AB. Exploring the microbial potentially to augment soil fertility in Taiwan. In: Proceedings of the 6th ESAFS international conference: soil management technology on low productivity and degraded soils, Taipei, Taiwan, 2003, 25-27.