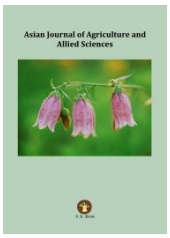

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Eco-Physiological and Biological Characterization of Phosphate Solubilize Bacteria Isolated from Low pH Soils of Tigray Region

Aklil Gebremedhin Meressa [a*] and Haile Alene Tareke [b]

[a] Tigray Agricultural Research Institute, Mekelle Soil Research Center, Soil Microbiology Researcher, Tigray, Ethiopia.
[b] Tigray Agricultural Research Institute, Humera Agricultural Research Center; Soil Fertility Researcher, Tigray, Ethiopia.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Phosphate solubilize bacteria plays a key role on an increasing bioavailability of the insoluble phosphates through the action of releasing organic acids. The research was conducted on soil microbiology laboratory to evaluate the selected physiological and biochemical characteristics of the isolated strains. Series of research methodology was used to evaluate eco-physiological, morphological and biochemical tests. The nominated isolates show gram negative, mucous texture and all of them grown well at pH 6 and 7, 15-35℃ temperature and 1-1.5% salt concentration (Na Cl2). Promising result was observed from 1.5mg/l and 3.0mg/l of MnSO4 and 0.25mg/l ZnSO4. Regarding to carbohydrate utilization, all of the tested isolates were utilized the tested carbon source. Promising result was obtained from phosphate solubilization, the highest solubilization

**Corresponding author: E-mail: aklilg3@gmail.com;*

index was recorded from IS-B (2.21) followed by IS-E (2.18). The pull-down of pH from 7.0 to 4.75 shows that the ability of the isolated strain to release phosphates. Therefore, the authors concluded that, effective phosphate solubilize bacteria recommended for acidic soils particularly for the study sites to enhance phosphorus uptake to plant growth.

Keywords: Phosphate solubilization; antibiotics; biochemical; morphological; Pikovaskay's.

1. INTRODUCTION

"One of the major limiting nutrients next to nitrogen is phosphorus for plant growth, which is abundant in the form of organic and inorganic in soil" [1]. "Unlike nitrogen, phosphorus can acquire from chemical industries nor from biochemical fixation to meet the crop production. Majority (75-90%) of the applied synthetic plant available P fertilizer is fixed by chemical complexes that exist in the soil system" [2]. Moreover, in acidic soils the efficiency of applied chemical fertilizers rarely exceed 30% due to phosphorus (P) fixation either in the form of iron phosphate or aluminium phosphate [3] and in neutral to alkaline soils that can fix in the form of calcium phosphate [4].

"Majority (70-75%) of the Ethiopian soils are categorised as phosphorus deficiency" [5]. "the deficiency is very severed in the acidic soils of southern, south-western and western regions. Areas with high in aluminium atoms (Al^{+3}) and iron (Fe^{+3}) are totally incriminated with high phosphorus fixation" [6]. "95 to 99% of the insoluble phosphates of organic and inorganic phosphorus easily bound by the presence of Al and Fe in acidic soils and Ca and Mg in alkaline soils" [7]. "To achieve the sustainability of agricultural production conversion of insoluble phosphates to available form is important. Several reports show that, the ability of diverse beneficial soil bacteria that converts inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate had a promising result" [8]. "Hence, in agricultural lands huge amount of chemical fertilizer especially, phosphorus has been used to alleviate agricultural production for more than three decades, and causes a negative impact on the agricultural farm lands as well as climate change" [9]. Resent literatures indicated that, use of microbial inoculants (strains) which is environmentally suitable, economically visible, and socially acceptable bioinoculants that possess P-solubilization in soils particularly in acidic soils are important [9].

"Among the beneficial microbial inoculants that possess phosphate solubilization are Pseudomonas, Bacillus, rhizobia, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium and Erwinia have been isolated and globally Known" [10]. The above-mentioned phosphate solubilize bacteria can grow in specific media supplemented by calcium phosphates, which is used as source of phosphorus in soils. The capability of microbial inoculants that solubilizes the insoluble phosphates can determine by the presence of halo zone formation around the colony [11]. Certainly, the selection of possible phosphate solubilize bacteria from acidic soils might be best way of releasing huge amount of P from the fixed soils to increase the availability of P. Therefore, this research was determined and characterizations of phosphate solubilize bacteria as an alternative source of fertilizers for acidic soils.

2. METHODOLOGY

2.1 Stady Site and Sample Collection

The study site is located in Western Zone of Tigray Region between 37° 16'0'' to 37°30'0'' East longitude and 13° 18' 40'' to 13°22' 0'' North latitude and in the range of 2319 to 2939 masl with highland Agro-ecological zones. The study site has characterized by varied features such as high and Rocky Mountains, flat topped plateau, deep gorges and rolling plains.

Prior to sampling the sampling site were categorised based on their pH level in to moderately acidic and strongly acidic. Accordingly, a total of sixty (60) soil samples were collected following the standard sapling method from an oxen plough depth (0-20cm) by inserting sterilized auger to prevent contamination and polyethylene plastic bags. Indaslasie site covers 30 soil samples, 15 samples from Chegwar Gudo and the remaining 15 samples from Inta bila site. Off the collected samples ten (10) composite samples (two from Chegwar Gudo, three from Inta bila and five from Indaslasie) were collected by quartering the

composite sample. After aseptically drying and ground using pestle and mortar each soil samples were coded, labelled, backed and stored at 4^{O}c refrigerator for farther eco-physiological and biochemical characteristics, the remain soil samples were used for physical and chemical properties as indicated in Table 1.

2.2 Isolating Phosphate Solubilising Bacteria

To determine capability of phosphate, solubilize bacteria 10-fold serial dilution method was used. One gram (1gm) of sieved soil were weighed using sensitive balance and dispersed in to sterilized test tubes containing 9 ml of di-ionized water and then soaked overnight. Each test tube was vortexed thoroughly to homogenised the suspension and a series of 10 -fold dilutions was prepared starting from 10^{-1} down to 10^{-9}. 0.1ml of the serially diluted (10^{-5}, 10^{-6}, 10^{-7}) suspensions were plated on *Pikovaskya's* agar medium containing tricalcium phosphate (TCP) as phosphate source. Prior to plating the required growth media was weighed, added to volumetric flask, filled with di-ionised water and adjusted its pH 7.0 using digital pH meter, sterilized in autoclave (121℃ at 15pci) and poured to sterilized petri plates. After overnight cooling of each petri plates isolation was conducted using spread plate method and incubated at heated incubator (27-30 ^{0}C) for 5-7 days. After 7 days of incubation period pure colonies were picked and preserved at 4℃ refrigerators by inserting them in to a slant test tube for farther biochemical characterization [12].

2.3 Biological Properties

The ability of the isolated phosphate solubilizes bacteria to utilize various physiological characteristics (carbohydrate and amino acid utilization), each rhizobial isolates were farther characterized. Well preserved isolates were inoculated on newly prepared yeast extract mannitol agar supplemented with different carbon and nitrogen levels by triplicate. Yeast extract mannitol agar containing Congo red were used as control check and the presence or absence of the isolates were nominated by plus (+) to indicate the presence or minus (-) for the absence of bacteria on the growth media by comparing with the positive control after 5-7 days of incubation period [13].

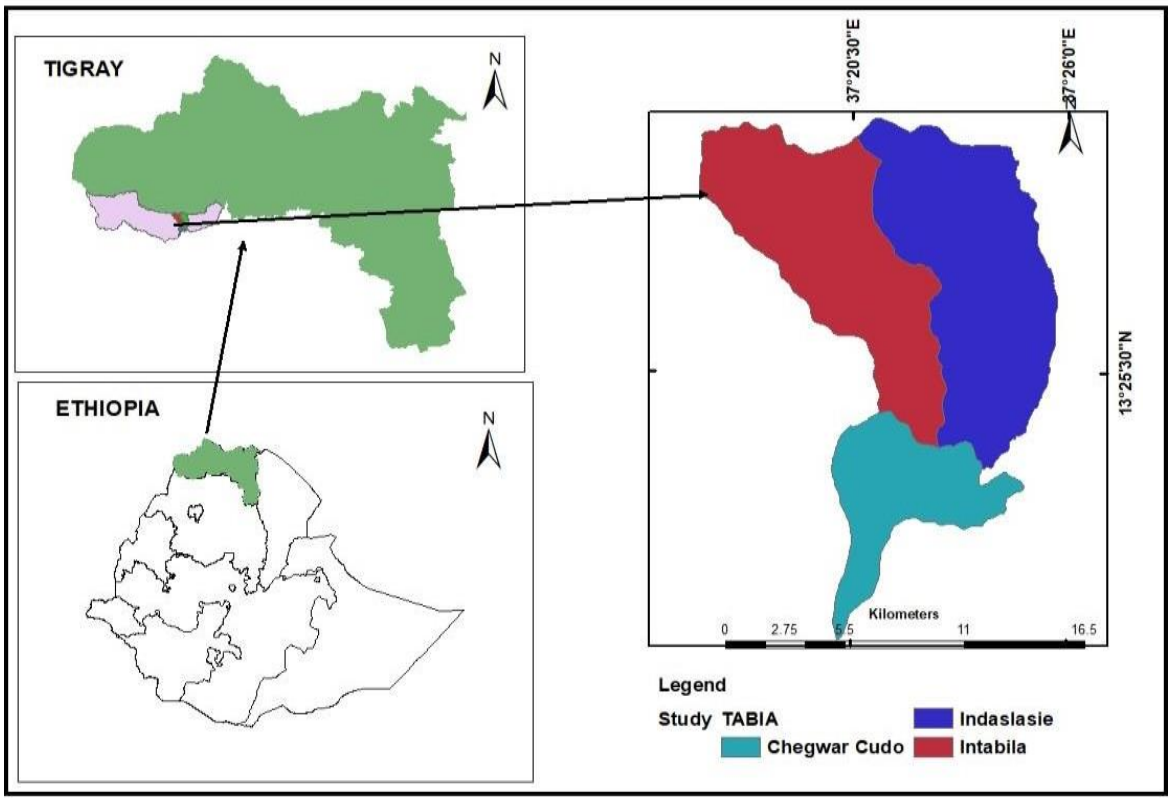

Fig. 1. Map of the study site and soil sample collection (map using GIS software by Haile Alene, 2019)

2.4 Eco-Physiological Characteristics

Each the isolated and preserved phosphate solubilize bacteria were tested for various eco-physiological characteristics following the standard procedures at Mekelle soil microbiology laboratory. The listed candidate (PSB isolates) was verified on different temperature levels (5,10, 15, 20, 25, 30, 35, 40 and 45 ℃), pH levels (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9 and 9.5) with 0.5 interval by adjusting using 1 normal HCl and $NaHO_2$. Ten levels of sodium chloride concentrations starting from 0.5 to 5% of NaCl with 0.5% interval were used for farther morphological and physiological characteristics. All the selected isolates were tested in triplicates to each physiological properties, and the growth of the colony was recorded as positive (+ve) for the presence or negative (-ve) for the absence after the recommended incubation period indicated by Howieson and Dilworth, [14] Somasegran and Hoben, 1994 working manual.

2.5 Biochemical Test

The capability of the nominated bacterial isolates for various biochemical tests, four levels of $MnSO_4$ $7H_2O$, four levels of $ZnSO_4$ $7H_2O$ and four types of antibiotics were used to test their resistance or sensitivity. Freshly prepared YEM agar plates were supplemented with 1.5, 3.0, 6 and 12 mg/l $MnSO_4$ $7H_2O$, 0.25, 0.5, 1 and 2mg/l $ZnSO_4$ $7H_2O$ and Ampicillin at 5 and 10 mg/l, Erythromycin at 5 and 10mg/l, Rifampicin at 5 and 10mg/l and Streptomycin at 5 and 10mg/l) [15] were used. All except Erythromycin others were dissolved in sterilized di-ionized water and added to autoclaved YEMA media cooled approximately to 45ºC [13].

2.6 Qualitative Measurement

For quantitative measurement 0.1 ml^{-1} of the preserved tested isolates were confirmed on *Pikovskaya's* agar plates supplemented with insoluble tricalcium phosphate. A loop full of a 72 h old isolates with 10^{-8} viable cells ml^{-1} were streaked on newly prepared and sterilized the required agar media, and incubated at the recommended temperature (28 ± 2^0C) for 7 days. After seven (7 days) of incubation period, the clear halo zone, halo zone diameter, colony diameter, phosphate solubilisation index (SI) and solubilization efficiency were measured and calculated following the standard formula indicated in Edi-Premono and his colleagues [16].

$$SI = \frac{Colony\ Diameter + Hallo\ zone\ diameter}{\text{Colony Diameter}}$$

2.7 Quantitative Measurement

Quantitative measurement is another option to verify either the selected isolates have the capability to convert insoluble phosphates in to soluble form. Accordingly, 100 ml of stock solution of *Pikovskaya* broth was prepared without phosphate source and dispensed in to 250 ml Erlenmeyer flasks. To each ready-made flask, about 0.5g of tri-calcium phosphate ($Ca_3(PO_4)_2$) was added and autoclaved at 121^0C at 15 psi for 15 minutes to eliminate contamination. Then after 1ml of culture containing about 10^8 cells ml^{-1} suspensions of each isolate was inoculated into the medium and kept at 28 ± 2^0C in shaker incubator for about 12 days. 10 ml of each isolate was withdrawn at 3 days regular intervals to measure the release pattern of soluble phosphates and pH change. Each test was examined in triplicates in each step following the standard method cited in Subba Rao, [17].

3. RESULTS AND DISCUSSION

3.1 Selection, Screening and Determination of Phosphate Solubilize Bacteria

In the present study the collected soil samples were subjected to phosphate solubilizing bacteria in *Pikovaskaya's* solid and liquid media. Eight (8) out of ten phosphate solubilize bacterial strains were evaluated on *the* agar medium having tri-calcium phosphate (TCP). One soil sample from Chegwar Gudo (CH-A) and one from Indaslasie (IS-D) were failed during the process, this might be due to unproper storage and codding, while the remain Eight samples collected from the study sites were pass to different Eco-physiological and biochemical characteristics. Having this, the soil pH of the study sites was categorised in the range of moderately acidic to strongly acidic according to Tekalign classification [18]. All the tested isolates (PSB) as the characteristics of rhizo-bacteria, they are gram negative, colour less and yellowish colour after they grow on yeast extract mannitol agar having Congo-red (CR) and Bromothymol blue (BTB) respectively. Regarding to the morphological characteristics, 75% of the tested isolates were dominated by large mucoid (LM) (Table 1). The key point for phosphate solubilizes

bacteria is its hallo zone formation, literatures indicated that, different rhizobacteria has different capability of hallo zone formation, as indicated in the current research the highest hallo zone formation was observed from IS-A and IS-B (5.56mm) followed by IS-E (4.33mm) in diameter. The highest and smallest solubilization index was obtained from IS-B (2.18) followed by IS-E (2.21) and CH- B, IT-A and IS-C (2.21) respectively (Table 1).

Regardless of the collected sites, the developed phosphate solubilize bacteria from Indaslasie site has more performance in both hallo zone formation as well as solubility index, this indicates that the release pattern of soluble phosphates through the action of acid production in the mobilization process and significantly correlated with a drop-down pH culture. Variations of the halo zone formation and solubility index (SI) could be the possibly difference in; natural environment, isolate type, soil pH, soil management and agricultural practices. The possibility of isolated strains from diverse environment could avoid the competition with indigenous microbes [19]. Supriya T. *et al*, [20] reported that, bacterial colonies screening solubilization index ≤ 7 mm were nominated for phosphorus (P) quantification and the current research was agreed to this research findings. Microbial colonies forming a clear hallo zone around the colony were assumed as phosphate solubilize bacteria. Various research findings stated that, hallo zone formation around the selected microbial colonies has created due to the production of organic acids, polysaccharides or due to the activities of phosphate enzymes [21].

3.2 Taxonomic Characteristics

Morphological and cultural properties are among the taxonomic classification of the growth characteristics of the tested isolates. Soil pH, gram stain and colony colour are some of the cultural properties and colony diameter and morphology are among the morphological properties. Having this in to consideration, majority of the tested isolates were conquered by moderately acidic soil pH, gram negative, yellowish colour and color-less after they grow on BTB and YEMA having Congo-red respectively (Table 1). Mideksa *et al*, [22] and Mulata, [23] indicated that, most agricultural soils had dominated by gram negative compare with gram positive which is the characteristics of rhizo-bacteria. With regard to morphological properties, almost all the tested isolates were large mucoid (LM) (Table 1). Colony diameter is in between 3.67 and 5.33 mm, the highest colony diameter (5.33 mm) was recorded from soils with 4.92 pH value which is categorised as strongly acidic IS-A obtained from Indaslasie (Table 1). The lowest colony diameter (3.67mm) were obtained from soils with 5.26 soil pH moderately acidic of the same area, this indicates that P solubilization increases with the mechanism of lowering soil pH. As soil pH drops from 5.76 to 4.92 the mechanism of P solubility increases from 1.67 to 2.06. Similar research findings were obtained by Kumar *et al*., [24] and Satyaprakash *et al*., [25]. In acidic soils, the presence of microbial enzymes has the capacity to produce organic acids that releases acidic substances, which is the lowering mechanism of soil pH and enhances phosphorus solubilization. Many research findings reported that, direct correlation between P solubilization and organic acid production has been recorded Alam et al., [26] and Selvi et al., [27].

3.3 Physiological Resistance

Tolerance to various physiological characteristics (salt concentrations, pH and temperature) may be important in the survival, multiplication and distribution of P solubilize bacteria as source of bio-inoculant to elevate crop production. All the selected and nominated isolates were subjected to various physiological characteristics as indicated in Table 2 in triplicates. Accordingly, impressing results were obtained, 100% of the tested isolates were resistance to 0.5, 1 and 1.5% $NaCl_2$ concentration comparing to the others (Table 2). In contrary to this no one of the tested isolates were grown on 4.5 and 5% $NaCl_2$ concentration. Regarding to the collected sites IS-A from Indaslasiea tabia is highly resistance to concentrated $NaCl_2$ concentration (4%). As the salt concentration rises resistance of the tested isolates were decreased; this implies that, the capablity of P solubilize bacteria is highly influenced by salinity stressed soils. This condition may result poor growth and survival of PSB and limits P availability to the host plant and plays a key role in yield reduction. Isolates also showed variation in tolerance during culturing to various pH levels. All the tested isolates were survived at pH level of 6.5 to 7.5 (Table 2), which could be considered as the optimum pH for the bacterial growth. Similar results were reported by Girmaye Kenasa *et al*., [28]. None of the isolated strains were grown at pH 4.0 and 4.5. With regard to the collected sites IT-B and

IT-C PSB strains obtained from Inta liba and IS-A from Indaslasiea were more resistance to pH level of 5.0 to 9.5 comparing to the other strains (Table 2). Regardless of the pH level most of the isolated strains were more resistance at pH level greater than 8.0. This could be related to the additional extracellular organic acid release by the fast grower isolates which can aggravated their tolerance at low pH growth media [29]. 100% of the isolated strains was grew very well at a temperature range of 15-35 ^{0}C (Table 2). None of the collected P solubilize bacterial isolates were grew at 5, 40 and 45 ^{0}C.

3.4 Biochemical Test

The collected and screened isolates were required for farther investigations on various biochemical testes with different levels, some of the candidate biochemical tests are $MnSO_4$, $ZnSO_4$, carbon and nitrogen sources. Accordingly, all the tested phosphate solubilize bacteria isolates were more resistance to 1.5 mg/l and 3.0 mg/l Mn SO_4 comparing to the other levels of Mn SO_4 (Table 3). Regardless of the collected sites, bacterial strains originated from Chegwar Gudo (CH-B) and IT-B from Inta liba were survived 100% of Mn SO_4. Except one isolate obtained from Indaslasie (IT-B) the remain all the tested isolates were sensitive to zinc sulphate ($ZnSO_4$) (Table 3). The intrinsic antibiotic resistance of PSB isolates were tested to determine their diversity. Hence, except IT-A obtained from Indaslasie most (>50%) of the tested isolates have resistance to intrinsic antibiotics. Moreover, two isolates (IS-C and IS-E) from Indaslasie site were resist 100% of the tested antibiotics, these inoculants might be important for future research. Differences in tolerance to different kinds of intrinsic antibiotics was reported among strains belongs to same species of rhizobia [30,31]. The capability of phosphate solubilizes bacterial isolates had extra characteristics to carbohydrate and amino acid utilization, consequently all the candidates showed visible colony development. Hundred present (100%) of the nominated isolates were utilized all the tested carbon sources, however, L-arginine as nitrogen sources utilized by the collected isolates and none of the isolates were utilizes L-alanine and urea (Table 3).

3.5 Quantitative Measurement of Phosphate Solubilize Bacteria

Quantitatively phosphate solubilize bacteria was measured their capability of each selected isolates to solubilize phosphorus using *Pikovaskaya* broth media containing tri-calcium phosphate ($Ca\ PO_4)_2$ at different incubation period. Accordingly, as Table 4 and Fig. 1 indicated that, P solubility (mg L^{-1}) increases at the first three days of incubation period and pends down at the next day (day 9). Similarly, Amit and his colleagues also reported that, P solubility gradually increases with time increasing [32-36]. The highest phosphate solubility record was obtained by ES-A (30.70mg L^{-1}) at day 6 followed by IT-C (29.96 mg L^{-1}) and the changes in pH (Table 4 and Fig. 2). This implies that, the selected rhizobia isolates can convert insoluble phosphates in to soluble form. The lowest P solubility was recorded at day 0, this is implying that, day 0 is the inoculating period,

Table 1. Soil pH, colony morphology and solubilization characteristic of phosphate solubilize bacteria isolates from acidic soils

PSB isolate	Soil pH	pH (Tekalign, 1991)	G. CR	G. BTB	Gram stain	C. Morphology	CD. (mm)		
							C	H	SI
CH-A	ND	ND	ND	ND	ND	ND	ND	ND	ND
CH-B	5.30	MA	CL	Y	-ve	LM	5.33	3.67	1.67
IT-A	4.98	SA	CL	Y	-ve	LM	4.67	4.00	1.86
IT-B	5.32	MA	CL	Y	-ve	LW	5.00	3.33	1.67
IT-C	5.70	MA	CL	Y	-ve	LM	4.00	3.67	1.92
IS-A	4.92	SA	CL	Y	-ve	LM	5.33	5.67	2.06
IS-B	5.26	MA	CL	Y	-ve	LM	4.67	5.67	2.21
IS-C	5.76	MA	CL	Y	-ve	LW	4.00	2.67	1.67
IS-D	ND	ND	ND	ND	ND	ND	ND	ND	ND
IS-E	5.26	MA	CL	Y	-ve	LM	3.67	4.33	2.18

Where; ND not determined, MA moderately acidic, SA strongly acidic, CL color less, Y Yellow, LM Large mucoid, LW Large watery, CD colony diameter, C colony, H, halo zone, SI solubilisation index.

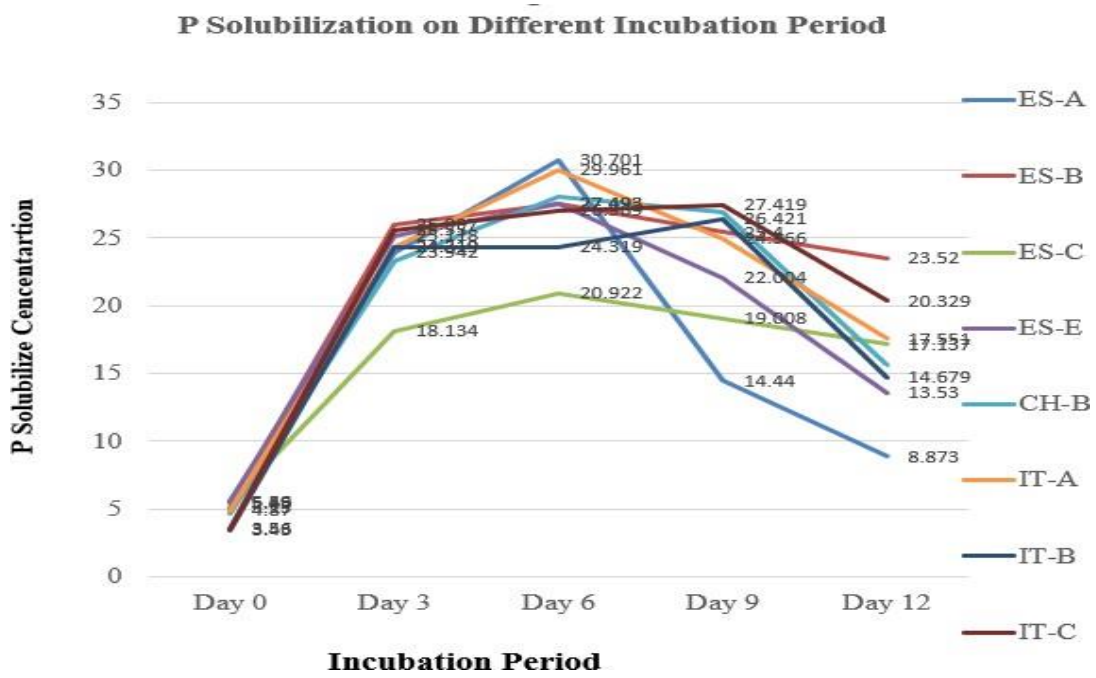

Fig. 2. Growth period vs phosphate solubilization

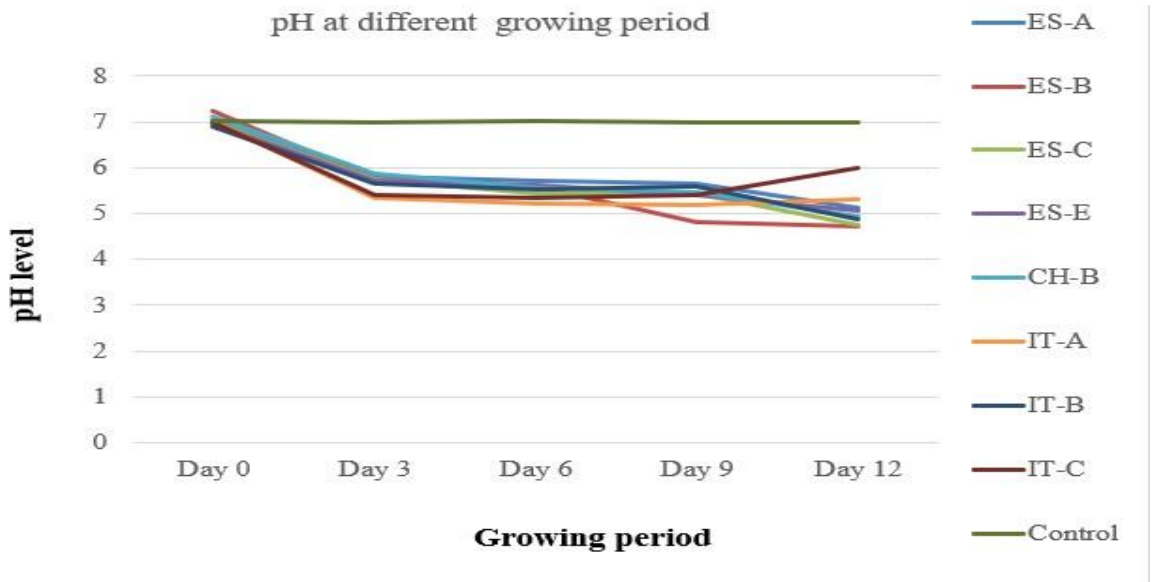

Fig. 3. Growth period vs pH change

no incubation and no bacterial multiplication. The amount of phosphate solubilizes released by the isolates exhibited wide variation ranging from 3.43 to 30.701mg L^{-1} with a significant pH drop from 7.00 to 4.75 (Table 4, Fig.1). Figure one shows that, phosphate solubilization is slow in the first two days (day 0 and day 3) and exponentially increases in the next day (day 6). Fig. 2 indicated that, as the incubation period increases pH in the culture declined to 4.75 from an initial pH of 7.00. Minimum pH was observed at day 12 by IS-B and IS-C (4.75), followed by day 9 IS-B (4.80). The mechanisms of solubilisation were indicated lowering of pH values by production of organic acids or releasing protons. Organic acids, like gluconic acid, formic acid, oxalic acid, and citric acid secreted by PSB can directly solubilize mineral phosphate as a result of anion exchange [37-39].

Table 2. Physiological characteristics of phosphate solubilize bacteria on solid media

Physiological Characters		Phosphate Solubilize Bacterial Isolates										Resistance (%)
		CH-A	CH-B	IT-A	IT-B	IT-C	IS-A	IS-B	IS-C	IS-D	IS-E	
NaCl2 levels (%)	0.5	NT	+	+	+	+	+	+	+	NT	+	100
	1	NT	+	+	+	+	+	+	+	NT	+	100
	1.5	NT	+	+	+	+	+	+	+	NT	+	100
	2	NT	-	+	-	-	+	+	+	NT	-	50
	2.5	NT	-	+	-	-	+	-	-	NT	+	37.5
	3.0	NT	-	-	+	-	+	-	-	NT	-	25
	3.5	NT	-	-	+	-	+	-	-	NT	-	25
	4.0	NT	-	-	+	-	+	-	-	NT	-	25
	4.5	NT	-	-	-	-	-	-	-	NT	-	0
	5	NT	-	-	-	-	-	-	-	NT	-	0
pH levels	4.0	NT	-	-	-	-	-	-	-	NT	-	0
	4.5	NT	-	-	-	-	-	-	-	NT	-	0
	5.0	NT	-	-	+	+	+	-	-	NT	+	50
	5.5	NT	-	+	+	+	+	-	-	NT	+	62.5
	6.0	NT	-	+	+	+	+	-	-	NT	+	62.5
	6.5	NT	+	+	+	+	+	+	+	NT	+	100
	7.0	NT	+	+	+	+	+	+	+	NT	+	100
	7.5	NT	+	+	+	+	+	+	+	NT	+	100
	8.0	NT	+	+	+	+	+	+	-	NT	+	87.5
	8.5	NT	-	+	+	+	+	+	-	NT	-	62.5
	9.5	NT	-	+	+	+	+	-	-	NT	-	50
Temperature (^{O}c)	5	NT	-	-	-	-	-	-	-	NT	-	0
	10	NT	-	+	-	+	+	-	-	NT	-	37.5
	15	NT	+	+	+	+	+	+	+	NT	+	100
	20	NT	+	+	+	+	+	+	+	NT	+	100
	25	NT	+	+	+	+	+	+	+	NT	+	100
	30	NT	+	+	+	+	+	+	+	NT	+	100
	35	NT	+	-	+	+	+	-	-	NT	-	50
	40	NT	-	-	-	-	-	-	-	NT	-	0
	45	NT	-	-	-	-	-	-	-	NT	-	0

Where; NT no test

Table 3. The efficacy of the isolated PSB on different biochemical test

BiochemicalCharacters		Phosphate Solubilize Bacterial Isolates									
		CH-	**CH-B**	**IT-A**	**IT-B**	**IT-C**	**IS-A**	**IS-B**	**IS-C**	**IS-D**	**IS-E**
Mn SO4 levels (mg/l)	1.5	ND	+	+	+	+	+	**+**	**+**	ND	**+**
	3.0	ND	+	+	+	+	+	**+**	**+**	ND	**+**
	6.0	ND	+	**+**	**+**	-	-	**+**	**-**	ND	**+**
	12.0	ND	+	-	**+**	-	+	**-**	**-**	ND	**-**
Resistance (%)			100	75	100	50	75	75	50		75
Zn SO4 level (mg/l)	0.25	ND	+	+	+	+	-	-	-	ND	+
	0.5	ND	-	+	**+**	+	-	-	-	ND	+
	1.0	ND	-	-	**+**	-	-	**-**	**-**	ND	**+**
	2.0	ND	-	+	+	-	-	-	+	ND	-
Resistance (%)			10	75	100	50	0	0	10		75
Antibiotics levels (mg/l)	E (5)	ND	+	-	+	-	+	+	+	ND	+
	E (10)	ND	+	-	+	-	+	+	+	ND	+
	S (5)	ND	-	-	+	-	-	+	+	ND	+
	S (10)	ND	-	-	+	-	-	-	+	ND	+
	R (5)	ND	+	-	-	+	-	+	+	ND	+
	R (10)	ND	+	-	+	+	+	-	+	ND	+
	A (5)	ND	-	+	+	+	+	+	+	ND	+
	A (10)	ND	+	-	-	+	-	+	+	ND	+
Resistance (%)			62.5	10	75	50	50	75	100		100
N sources	Glycine	ND	-	-	+	-	-	-	-	ND	+
	Alanine	ND	-	-	-	-	-	-	-	ND	-
	Arginine	ND	+	+	+	+	+	+	+	ND	+
	Urea	ND	-	-	-	-	-	-	-	ND	-
Resistance (%)			10	10	50	10	10	10	10		50
C source	Glucose	ND	+	+	+	+	+	+	+	ND	+
	Fructose	ND	+	+	+	+	+	+	+	ND	+
	Lactose	ND	+	+	+	+	+	+	+	ND	+
	Galactose	ND	+	+	+	+	+	+	+	ND	+
	Dextrose	ND	+	+	+	+	+	+	+	ND	+
	Sorbitol	ND	+	+	+	+	+	+	+	ND	+
Resistance (%)		ND	100	100	100	100	100	100	100	ND	100

Where: ND not determine, E Erythromycin, S Streptomycin, R Rifampicin, A Ampicillin

Table 4. The ability of phosphate solubilization due incubation period

	Day 0		Day 3		Day 6		Day 9		Day 12	
PSB	**PS (mg/l)**	**pH**	**PS (mg/l)**	**pH**	**PS (mg/l)**	**pH**	**PS (mg/l)**	**pH**	**PS (mg/l)**	**pH**
IS-A	5.56	7.01	23.942	5.82	30.701	5.71	14.44	5.66	8.873	5.12
IS-B	5.13	7.23	25.997	5.75	27.493	5.60	25.4	4.80	23.52	4.75
IS-C	5.49	6.98	18.134	5.75	10.922	5.43	19.008	5.44	17.137	4.75
IS-E	5.45	6.96	25.118	5.73	27.493	5.62	22.004	5.42	13.53	5.06
CH-B	4.67	7.12	23.236	5.87	28.052	5.57	26.908	5.48	15.582	4.93
IT-A	4.87	7.03	24.319	5.34	29.961	5.21	24.966	5.20	17.551	5.31
IT-B	3.43	6.89	24.319	5.66	24.319	5.52	26.421	5.60	14.679	4.89
IT-C	3.56	7.00	25.55	5.40	26.969	5.33	27.419	5.40	20.329	5.00
Control		7.01		7.0		7.02		7.0		7.0

4. CONCLUSION

It can be concluded that the phosphate solubilizing rhizobia exhibited a broad range of ability of solubilising tri-calcium phosphate (TCP) in *vitro*. The selected and nominated rhizobial isolates had the ability to tolerate a wide range of pH, sodium chloride, temperature, intrinsic antibiotics as well as utilized different biochemical testes. The current research indicated that, an inverse correlation between phosphate solubilization and its pH value. Isolated strains which are effective in nitrogen (N_2) fixation and an able to solubilize tri-calcium phosphates (TCP) are found to be effective in improving nodulation and plant growth. Further research is recommended to investigate its efficacy under field trials in diverse soil types having different amount of soil P.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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