Diversity and Phosphate Solubilization Efficiency of Phosphate Solubilizing Bacteria Isolated from Semi-Arid Agroecosystems of Eastern Kenya

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ABSTRACT: Phosphorus (P) is a major nutrient required for plant growth but it forms complexes with other elements in soil upon application. A cost-effective way of availing P to plants is by use of Phosphate Solubilizing Bacteria (PSB). There is a wide range of PSB suited for diverse agro-ecologies. A large part of Eastern Kenya especially the lower altitude regions are semi-arid with nutrient depleted soils and predominated by low-income smallholders farmers who do not afford costly inorganic fertilizers. To alleviate poor soil nutrition in this agroecosystem, we sought to study the diversity of phosphate solubilizing bacteria and their phosphate solubilization efficiency. The bacteria were selectively isolated in Pikovskaya's agar media. Bacterial colonies were enumerated as Colony Forming Units and morphological characterization determined by analyzing morphological characteristics. Genetic characterization was determined based on sequencing of 16S rRNA. A total of 71 PSB were isolated and they were placed into 23 morphological groups. Correlation analysis showed a negative correlation between phosphate solubilizing bacteria and the levels of phosphorus, iron, calcium, magnesium and soil pH. Analysis of 16S rRNA sequences revealed that the genetic sequences of the isolates matched the strains from the genera *Burkholderia, Pseudomonas*, *Bacillus, Enterobacter, Pantoea, Paraburkholderia, Cronobacter, Ralstonia, Curtobacterium, and Massilia* deposited in NCBI Database. Analysis of Molecular Variance showed that variation within populations was higher than that of among populations. Phosphate solubilization index values ranged between 1.143 and 5.883. Findings on biodiversity of phosphate solubilizing bacteria led to identification of 10 candidate isolates for plant growth improvement and subsequently, bio-fertilizer development.

KEYWORDS: Phosphate solubilizing bacteria, diversity, phosphate solubilization index

Introduction

Phosphorus (P) is the second most essential nutrient after nitrogen that is required by plants for growth and development.[1] It plays a vital role in various physiological and biochemical activities including respiration, photosynthesis, transduction, cell division, biosynthesis of macromolecules and tissue development.[2] Lack of Phosphorus is characterized by formation of brown leaves, and its deficiency leads to poor plant development and delayed maturity.[3] P is found as mineral deposits in the earth's crust as a finite supply.[2] Most of it is found as apatite, oxyapatite or hydroxyapatite which is insoluble.[4] It occurs in the soil in 2 forms; organic and inorganic. The proportions of these forms found in the soil differ in different places due to soil fertility management methods, soil types and soil use.[3] Soluble organic phosphate is in form of orthophosphate and organic polyphosphates. Plants mainly absorb P in the form of phosphate anions, most of which are HPO_4^{2-} and $H_2PO_4^-$.[5]

In most soil, P is deficient making it one of the plant growth limiting nutrient.[6] This is because it forms complexes with other elements upon application to the soil hence becoming unavailable for plant use.[7] Formation of the complexes depends on soil type and the pH.[8] In acidic soil, P form complexes with Al and Fe oxides, while in alkaline soil, it forms complexes with calcium.[9] Since phosphorus supplies are finite, ways of reclaiming this chemically bound P in the soil are continuously being exploited.[4,10] This promotes sustainable agriculture which in the long run will lead to mitigation of negative climate change effects.

Soil microorganisms have been shown to have abilities of utilizing the natural reservoir to siphon out scarce nutrients and thereby enriching the soil with important nutrients.[11] Plant Growth Promoting Rhizobacteria (PGPR) is a group of soil bacteria associated with the plant rhizosphere that have the ability of promoting growth in plants.[12,13] Plant growth promotion is mainly by production of important metabolites required by plants, including phytohormones and nutrients.[10] A number of bacteria, fungi, actinomycetes and algae have the capabilities of improving plant growth through various mechanisms.[14] Particularly, bacteria have proven to be the most effective and their population is higher in the plant rhizosphere.[13] These bacteria enhance plant growth through the provision of phosphorus and other important plant growth-promoting metabolites.[15] Several mechanisms in which micro-organisms solubilize phosphates have been reported.[16] PSB are believed to solubilize P through secretion of organic acid which lowers the pH, chelation reaction of ions bound to P and by competing with P for adsorption sites in the soil.[17]

Soil bacteria which have been demonstrated to be powerful phosphate solubilizers are from genera *Bacillus*, *Pseudomonas*, *Rhizobium*, *Enterobacter*, and *Burkholderia*.[18-22] Other reported phosphate solubilizers include species from genera *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Xanthomonas*, *Klebsiella*, *Agrobacterium*, *Azotobacter*, *Erwinia*, *Kushneria*, and *Pantoea*.[12,19,23-26] The occurrence, abundance, diversity and bioactivity of PSB vary in different soils. The variation is attributed to the different soil properties including the nutritional conditions and physiochemical properties.[27] Phosphate solubilization is influenced by several factors including interactions with other microorganisms, agronomic activities, ecological conditions, and soil types.[28]

Plant growth promoting microorganisms including phosphate solubilizers have been harnessed and used to develop biofertilizers. Biofertilizers are microbial inoculants applied to soil to improve fertility and enhance crop growth.[29] Their use is recommended because they are non-toxic, cost-effective and eco-friendly. Microbial inoculants influence soil fertility through mineralization, decomposition and release of plant growth-promoting metabolites.[30] To develop efficient microbial inoculants, continuous screening of natural biodiversity of soil microorganisms is undertaken and potential organisms screened for growth improvement potential. Development of effective molecular techniques has aided in identification and classification of a wide variety of PSB.[31,32] This study was aimed at determining the biodiversity of phosphate solubilizing bacteria in smallholder agroecosystems in Eastern Kenya and to assay their solubilization efficiency.

Materials and Methods

Study site and sample collection

Soil samples were collected from the dry regions of Tharaka-Nithi (S 0°9′42″ E 37°50′44″), Embu (S 0°29′8″ E 37°41′19″) and Kitui (S 1°11′36″ E37°51′29″) counties in Eastern Kenya from smallholder farmers. Tharaka-Nithi lies at 882 m above sea level and receives annual rainfall of 860 mm per year with average temperature of 22.3°C. Embu lies at 1137 m above sea level with annual rainfall of 1120 mm and average temperature of 20.2°C. Kitui is 1141 m above sea level with an annual rainfall of 1068 mm and average temperature of 21.4°C (Source: http//www.en.climate-data.org/Africa/Kenya). These regions experience 2 rainy seasons per year in the months of March to June and Ocober to December.[33] The selected farms in which the samples were taken were under cultivation and had no history of bioaugmentation with PSB or other biofertilizers. Sampling was done after crop harvesting at the end of planting season on maize, cowpea, beans, millet, and green-gram fields. The roots of plants and the immediate rhizospheric soil were collected in a clean sterile khaki bag at various sampling points from the chosen farms. Samples from each farm were air-dried then mixed to obtain homogenous composite sample which was then sieved through 2 mm diameter sieve. Soil Physio-chemical analysis were performed for total C, total N, exchangeable cations (Mg, K, Ca), micronutrients (Fe, Cu, Zn, and Mn) and soil pH using established protocols.[34]

Selective isolation of Phosphate solubilizing bacteria

Ten grams of the soil sample was suspended in 90 ml of sterile distilled water and continuously agitated for 1 hour in a shaker. Serial dilution was prepared up to 10^{-5} and aliquots of 100 µl were plated evenly on Pikovskaya's agar (0.5 g yeast extract, 10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$, 0.002 g $MnSO_4.2H_2O$, 0.2 g KCl, 0.002 g $FeSO_4.7H_2O$, and 15 g agar per L).[35] The samples were incubated for 6 days at 28°C. The bacterial colonies which formed a clear halo zone in the plates were selected and purified by streaking single colonies in fresh Pikovskaya;s agar medium. PSB and total bacteria population were enumerated and expressed as colony-forming units (CFU)[24]:

$$CFU / g\ soil = \frac{number\ of\ colonies \times dilution\ factor}{Volume\ of\ the\ aliquot}$$

Phosphate solubilization index assay

Pure single colonies were streaked into the middle of the Pikovskaya's agar plate and then incubated at 28°C for 7 days. The diameter of the bacteria and the cleared zone was measured on the fourth day and used to calculate the Phosphate Solubilization Index (PSI) using the equation below[36]:

$$Phosphate\ solubilising\ index\ (PSI) = \frac{halozone\ diameter\ including\ colony\ diameter}{colony\ diameter}$$

Morphological characterization

The isolates were morphologically characterized by streaking on agar plates and observing the colony characteristics based on Bergey's Manual of Systemic Bacteriology.[37] The observable characteristics used to distinguish them included the colony shape, color, opacity, size, elevation surface texture, and surface form. Gram staining was also done to confirm gram reaction of the bacterial isolates. Bacterial isolates with similar gram reaction and morphological characteristics were grouped together.

Molecular characterization

Pure colonies of isolated bacterial cells were grown on PVK agar media for 2 days and used for DNA extraction. They were transferred to 400 µl of sterile normal saline and mixed thoroughly. The mixture was centrifuged at 13 000 rpm for 10 minutes to obtain a pellet. DNA was extracted using Zymo Research Quick-DNA™ miniprep kit as per the manufacturer's protocol. The quality of the isolated DNA was checked by running gel

electrophoresis using agarose gel and visualizing on UV trans-illuminator.

PCR was performed using universal primers, 27f (5′AGAGTTTGATCCTGGCTCAG 3′) and 1492r (5′GGTTACCTTGTTACGACTT 3′) which are complimentary to the highly conserved regions of the bacterial 16S rRNA gene.[32] PCR master mix was prepared by mixing 1 μl of 10 μM dNTPs, 0.5 μl of both 27f and 1492r primers, 0.5 μl of taq polymerase, 2.5 μl of 10X dream taq buffer and DNase, RNase free PCR water for top up to a total of 24 μl. 1 μl DNA template was then added.

Amplification was performed on Techgene Thermal Cycler (Techne) programed as follows: an initial denaturation step of 3 minutes at 94°C followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing for 45 seconds at 51.8°C and extension for 2 minutes at 72°C. The final extension was at 72°C for 5 minutes. PCR products were stained with SYBR green stain and resolved in 1.4% agarose gel in 0.5X TBE buffer at 80 V for 30 minutes and then visualized on a UV trans-illuminator. Thereafter, PCR products were sequenced from both ends using both forward (27f) and reverse (1492r) primers.

Data analysis

Morphological diversity indices were calculated using PAST software version 3. Redundancy analysis (RDA) was used to show relationship between soil properties and colony-forming units using Canoco software version 5. The phosphate solubilization index was analyzed using non-parametric Kruskal Wallis test *H* using the Minitab software version 17. Consensus sequences were prepared from the sequenced data using BioEdit software version 7.2.5. Basic Local Alignment Test (BLAST) was used to draw comparison from the available bacterial standard sequences in NCBI GeneBank (http://www.ncbi.nlm.nih.gov/) for bacterial strain identities. Genetic Phylogenetic tree was constructed using MEGA X software. Sequences were aligned using ClustalW and the evolutionary history was inferred using the Neighbor-Joining method computed with the p-distances. Nucleotide diversity was calculated using DnaSP 6 software. Sequenced data was converted to haplotypes and used to calculate Analysis of Molecular variance (AMOVA) and genetic differentiation using Arlequin software version 3.5.2.2.

Results

Morphological characterization

A total of 71 isolates that formed a clear halozone in Pikovskaya's agar media were isolated. Based on their morphological characteristic, they were placed into 23 groups (Table 1). The isolates exhibited varied morphological characteristics. Colony sizes varied from medium to large while their texture was either glistening, dull or mucoid. Colony shape was either circular, spindle or punctiform while their elevation varied

from convex and flat to raised. The color of the isolates varied from yellow to white. All the isolates except those in group I and K were Gram negative.

Based on morphological diversity Embu had the highest number of individuals at 30 while Kitui had the lowest at 17, (Table 2, Figure 1). According to Shannon *H* diversity and Dominance *D*, the variation among the 3 regions was low. Kitui had the highest diversity evenness while Embu had the lowest.

Relationship between population of phosphate solubilizing bacteria and soil properties

In all the regions, the proportion of total microorganisms that were able to grow in the plates were higher than the phosphate solubilizing bacteria (Table 3). PSB formed colony units ranging from 1.3×10^4 to 3.63×10^4 per gram of soil. On the other hand, total microorganism colony forming units ranged from 2.327×10^5 to 3.507×10^5 per gram of soil. Sample T4 had the lowest percentage of PSB to total microorganism at 3.45% while sample E1 had the highest at 12.93%.

Redundancy analysis (RDA) between colony forming units and select soil properties are displayed in Figure 2. There was a positive correlation between PSB colony forming unit and K, Zn and Na. On the hand, there was a negative correlation between PSB colony forming unit and the available P, soil pH, Ca, Mg, and Fe. Total bacteria colony forming unit was positively correlated to available P but was negatively correlated to nitrogen, calcium, carbon, and magnesium.

Molecular characterization

Amplification of 16S rRNA gene of the isolates resulted in single bands of approximately 1550 base pairs (Figure 3). Genetic sequencing of the amplified 16S rRNA gene revealed 94% to 100% similarity of the isolates to other DNA sequences deposited at NCBI database (Table 4). These isolates belong to the genera *Burkholderia*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Enterobacter*, *Cronobacter*, *Massilia*, *Curtobacterium*, *Caballeronia*, *Paraburkholderia*, *Ralstonia*, *Erwinia* and *Citrobacter*. Sequences of all the isolates were deposited in NCBI GenBank and were assigned Accession numbers as indicated in Table 4.

Phylogenetic analysis

The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary distances were computed using the p-distance method (Figure 4). The sum of branch length in the optimal tree shown is =0.776. Bootstrap test of 1000 replicates were used to cluster associated taxa and the percentage of replicate trees are shown next to the branches. Based on the sequence data, phylogenetic analysis clustered the isolates into 2 main clusters (I and II) (Figure 4). The first cluster (cluster I) grouped together isolate 9 and isolate 41 with strains from the genera *Bacillus* and

Table 1. Morphological characteristics of the isolates.

GROUP	SHAPE/ FORM	COLOR	COLONY SIZE	OPACITY	TEXTURE	ELEVATION	GRAM STAIN
A	Circular	Cream Yellow	Medium	Opaque	Glistening	Convex	**Neg**
B	Circular	Cream white	Small	Opaque	Glistening	Flat	**Neg**
C	Circular	White	Medium	Opaque	Dull	Convex	**Neg**
D	Spindle	White	Small	Opaque	Dull	Raised	**Neg**
E	Circular	Cream white	Small	Opaque	Glistening	Convex	**Neg**
F	Circular	Yellow	Medium	Opaque	Glistening	Convex	**Neg**
G	Circular	Cream white	Medium	Opaque	Glistening	Convex	**Neg**
H	Punctiform	White	Small	Opaque	Glistening	Flat	**Neg**
I	Circular	White	medium	Transluscent	Glistening	Flat	**Pos**
J	Circular	White	Large	Opaque	Mucoid	Convex	**Neg**
K	Circular	Cream white	Medium	Opaque	Glistening mucoid	Convex	**Pos**
L	Spindle	White	Small	Transluscent, Opaque center	Glistening	Convex	**Neg**
M	Spindle	White	Medium	Opaque	Glistening	raised	**Neg**
N	Circular	White	Medium	Transluscent	Glistening	Convex	**Neg**
O	Circular	White	Large	Transluscent	Glistening	Convex	**Neg**
P	Circular	Yellow	Medium	Transluscent	Glistening	Convex	**Neg**
Q	Circular	White	Small	Opaque	Dull	Flat	**Neg**
R	Circular	White	Small	Transluscent	Glistening	Flat	**Neg**
S	Circular	White	Medium	Transluscent	Glistening	Convex	**Neg**
T	Punctiform	Cream Yellow	Small	Opaque	Glistening	Convex	**Neg**
U	Circular	White	Medium	Transluscent, Opaque center	Glistening mucoid	Convex	**Neg**
V	Circular	White	Small	Opaque	Glistening	Convex	**Neg**
W	Circular	White	Small	Opaque	Dull	Convex	**Neg**

Table 2. Diversity indices of isolates based on morphological characteristics.

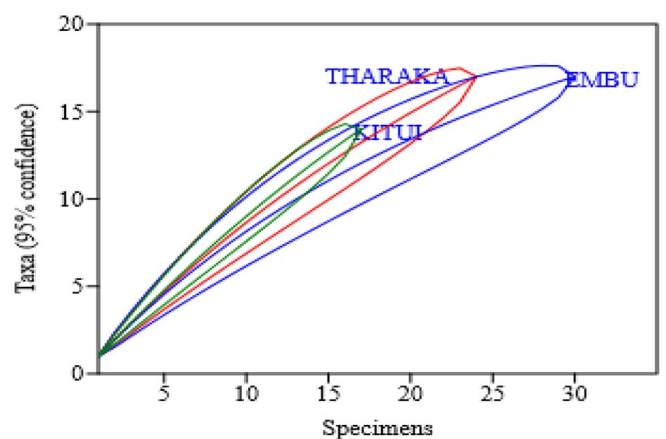|	THARAKA-NITHI	EMBU	KITUI
Taxa_S	17	17	14
Individuals	24	30	17
Dominance_D	0.07292	0.08	0.07958
Simpson_1-D	0.9271	0.92	0.9204
Shannon_*H*	2.73	2.682	2.589
Evenness_e$^{H/S}$	0.902	0.8598	0.9508
Brillouin	2.047	2.094	1.849
Equitability_J	0.9636	0.9467	0.9809

Figure 1. Individual rarefaction diagram showing isolates per region and taxa at 95% confidence.

Table 3. Tukey's Studentized Range (HSD) Test for CFU. %PSB is the proportion of Phosphate solubilizers to the total bacteria. T1-T5; Tharaka-Nithi samples, K1-K5; Kitui samples, E1-E5; Embu samples.

SAMPLE	PSB ×10⁴ CFU/G SOIL	TOTAL 10⁴ CFU/G SOIL	% PSB TO TOTAL
T1	1.67 ± 0.067^e	35.07 ± 0.706^b	4.75
T2	3.30 ± 0.058^{ab}	28.60 ± 0.173^{de}	11.54
T3	3.63 ± 0.088^a	25.70 ± 0.252^g	14.14
T4	1.30 ± 0.058^e	37.63 ± 0.186^a	3.45
T5	1.60 ± 0.058^e	25.83 ± 0.273^g	6.19
K1	1.43 ± 0.033^e	29.60 ± 0.100^d	4.84
K2	1.33 ± 0.067^e	34.90 ± 0.265^b	3.82
K3	3.50 ± 0.058^a	27.17 ± 0.555^{efg}	12.88
K4	2.47 ± 0.088^d	23.47 ± 0.203^h	10.51
K5	2.77 ± 0.088^{cd}	32.70 ± 0.208^c	8.46
E1	3.50 ± 0.058^a	27.07 ± 0.176^{efg}	12.93
E2	3.40 ± 0.115^a	29.33 ± 0.240^d	11.59
E3	3.53 ± 0.067^a	27.50 ± 0.321^{ef}	12.85
E4	2.90 ± 0.058^c	25.97 ± 0.203^{fg}	11.17
E5	2.93 ± 0.088^{bc}	23.27 ± 0.145^h	12.61

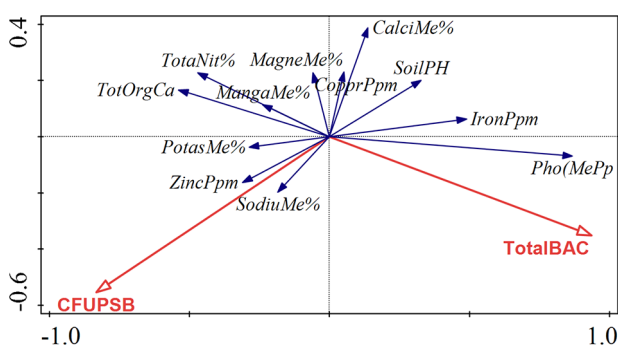*Means with the same superscript letter are not significantly different. $P < .05$

Figure 2. Redundancy analysis (RDA) showing relationship between Colony Forming Units (CFU) and soil properties. CFU PSB- Phosphate solubilizing bacteria, Total BAC- CFU of total bacteria.

Curtobacterium supported by bootstrap values of 100. Isolate 9 had 98% match with *Bacillus amyloliquefaciens* strain while isolate 41 had 99.57% match with *Curtobacterium citreum* (Table 4). Cluster II had the highest number of isolates and formed 2 main sub-clusters (A and B). The first main sub-cluster (sub-cluster A) had 2 sub-clusters; sub-clusters A1 and A2. Sub-cluster A2 grouped isolate 15 with strains from the genus *Massilia* with 99.93% match and supported by

bootstrap value of 100. Sub-cluster A1 formed 2 minor sub-clusters; A1.1 and A1.2. Sub-cluster A1.1 grouped together Isolates 5, 12, 33, 22, 37, and 50 with strains from the genera *Burkholderia*, *Caballeronia* and *Paraburkholderia* with sequence similarity of between 99.64% and 100%. Sub-cluster A1.2 grouped Isolate 15 with strains from the genus *Ralstonia* with sequence match of 94.9% supported by bootstrap value of 100. The second main sub-cluster B formed 2 sub-clusters; B1 and B2. Sub-cluster B1 grouped together Isolates 17, 36, and 42 with isolates from the genus *Pseudomonas* having sequence matches of 99.86%to 99.95%. Sub-cluster B2 further formed 2 minor sub-clusters; B2.1 and B2.2. Sub-cluster B2.1 grouped isolates 29, 47, and 46 with isolates from the genus *Cronobacter* with sequence similarity of 99.86% to 99.93%. Sub-cluster B2.2 grouped Isolates 55, 60, 7, 58, 18, 57, and 6 with isolates from the genera *Pantoea*, *Enterobacter*, *Erwinia* and *Citrobacter* with sequence homology of between 99.79% and 100%.

Genetic diversity

Molecular diversity of the isolates is displayed in Table 5. Generally, isolates from Tharaka-Nithi were more diverse compared to those from other regions. Isolates from Tharaka-Nithi had the highest number of Segregating sites- *S* at 1266 followed by Kitui at 1219 while Embu had the lowest at 880. The number of haplotypes- *h* ranged from 5 (Tharaka-Nithi) to 11 (Embu). Isolates from all the region had high haplotype diversity- *Hd* of 1. In contrast, nucleotide diversity was relatively lower in all the regions, with isolates from Tharaka-Nithi having nucleotide diversity- *Pi* of 0.651 while those from Kitui and Embu had 0.456to 0.329 respectively. The highest values of nucleotide diversity based on Juke's Cantor- *PiJC* was obtained from Tharaka-Nithi isolates at 2.47 while the lowest was from Embu (0.48).

Genetic differentiation

Analysis of variance showed that there was significant variation ($P < .05$) between isolates from various populations. The variation was high within population (92.05%) but low among population (7.95%) (Table 6).

Based on distance method, a significant population pairwise differentiation was observed between Tharaka-Nithi and Embu population at $p < .05$ (Table 7). However, pairwise differentiation analysis demonstrated that there was no significant differentiation ($p < .05$) between Tharaka-Nithi and Embu populations and Kitui and Embu populations.

Screening for Phosphate solubilization

The isolates had different phosphate solubilization index (PSI) (Table 8). Non-parametric Kruskal-Wallis test was used to analyze Phosphate Solubilization Index (PSI) because the values did not fulfil the assumptions of ANOVA. Bigger clearance zone in relation to colony size is an indication of greater

Table 4. Isolates reference matches based on 16s rRNA sequencing.

ISOLATE	GROUP	TOTAL NO. OF ISOLATES	MATCH	% GENE SIMILARITY	ACCESSION NO.
12	A	8	*Burkholderia cenocepacia*	99.86	MW013466
22	B	5	*Burkholderia cepacia*	100	MW013470
51	C	7	*Massilia* sp.	99.93	MW013481
15	D	5	*Ralstonia pickettii*	94.90	MW013467
5	E	4	*Burkholderia ambifaria*	100	MW013462
42	F	4	*Pseudomonas oryzihabitans*	99.93	MW013476
55	G	3	*Erwinia* sp.	99.72	MW013482
60	H	4	*Pantoea eucrina*	100	MW013485
41	I	3	*Curtobacterium citreum*	99.57	MW013475
29	J	3	*Cronobacter sakazakii*	99.86	MW013471
9	K	3	*Bacillus amyloliquefaciens*	98.39	MW013465
7	L	3	*Citrobacter* sp.	97.95	MW013464
18	M	3	*Enterobacter* sp.	99.86	MW013469
58	M	1	*Enterobacter* sp.	99.93	MW013484
47	N	2	*Cronobacter turicensis*	99.93	MW013478
57	O	1	*Pantoea stewartii*	99.79	MW013483
36	P	2	*Pseudomonas psychrotolerans*	99.95	MW013473
33	Q	2	*Burkholderia contaminans*	100	MW013472
17	R	2	*Pseudomonas putida*	99.86	MW013468
46	S	1	*Cronobacter dublinensis*	99.93	MW013477
50	T	2	*Caballeronia calidae*	99.64	MW013480
6	U	1	*Pantoea septica*	99.63	MW013463
37	V	1	*Burkholderia territorii*	99.93	MW013474
49	W	1	*Paraburkholderia phenoliruptrix*	99.93	MW013479

solubilization. In this study, only the bacteria that were able to retain their ability to solubilize P were characterized. The lowest PSI value was 1.143 (isolate 7) while the highest was 5.883 (isolate 22). A total of 15 isolates had PSI values greater than 4 representing 21.1% of all the isolates. Fourteen isolates had PSI values of between 3 and 4 making up 19.7% of the total isolates. Twenty-eight isolates had PSI value of between 2 and 3 representing 39.5% of the total isolates. The number of isolates with PSI value of less than 2 was 14 which represents 19.7% of the total.

Discussion

We sought to determine the diversity and efficiency of phosphate solubilizing microorganisms with the goal of identifying suitable strains that can be used to improve soil fertility and productivity in the semi-arid regions of Eastern Kenya. As the world population grows, so does the demand for food and in order to meet

this demand, there is need to develop eco-friendly and sustainable farming techniques. Plant growth nutrients are getting depleted from the soil due to a number of factors including finite supply and soil erosion.[38] To guarantee crop yields, farmers have to replenish the soil with fertilizers and this increases the production cost. Nitrogen, phosphorus and potassium are the most important nutrients needed by plants for growth.[1] Use of phosphate solubilizing microorganisms as a way of providing P to plants is gaining momentum since it is safe and effective.

The population and diversity of phosphate solubilizers were determined. All the isolates were able to solubilize insoluble phosphate and are therefore potential plant growth promoters. It is imperative to note that only those bacteria which are culturable in selective media were characterized in this study. Using morphological data, Diversity indices were calculated to check the diversity of the isolates. Results on diversity analysis indicated that Embu had higher number of isolates than the other region.
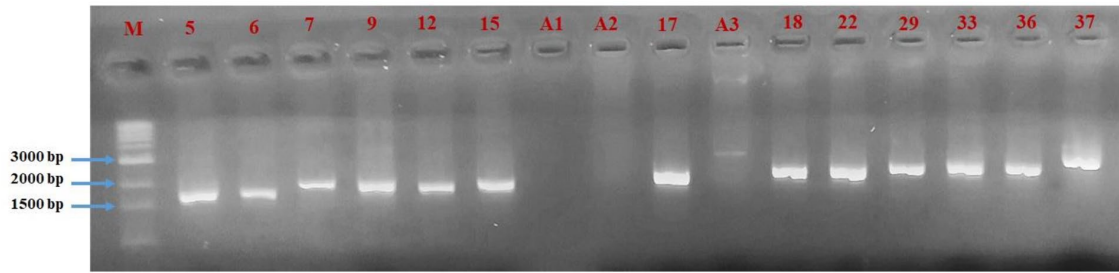
Figure 3. Gel electrophoresis of amplified 16S rRNA on 1.4% agarose gel. Lane M – Quick-load® 2-log DNA ladder (0.1-10 kb), Lanes 5 to 37- amplified samples of the isolates, Lanes A1 to A3- non-amplified samples.
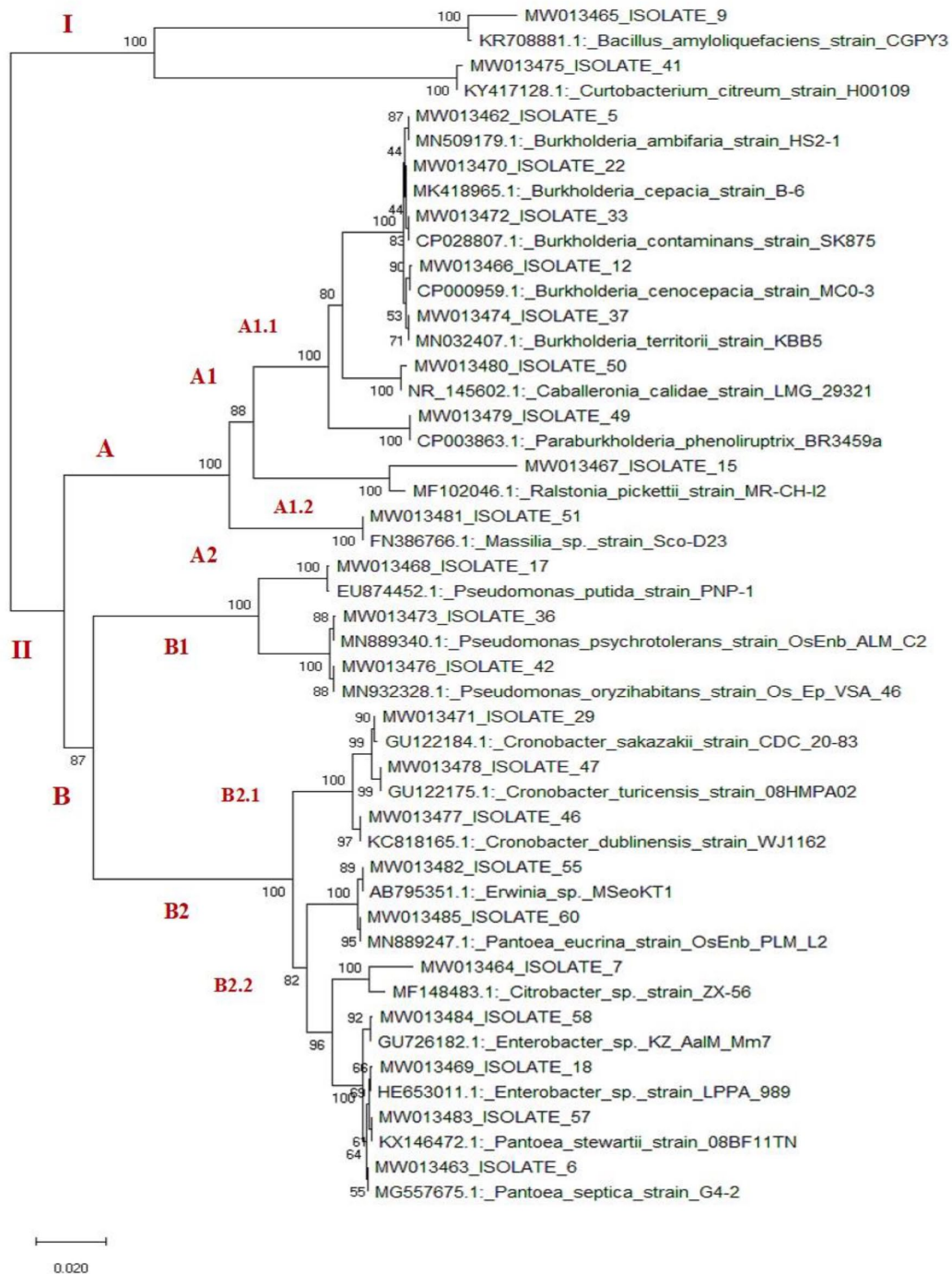

Figure 4. Phylogenetic tree showing genetic relationship between isolates based on the Neighbor-Joining method. Evolutionary distances were computed by p-distance method and are in the units of the number of base differences per site. Cluster levels are indicated by labels in red at the branches.

Table 5. Molecular diversity of PSB isolates from the 3 regions.

	NUMBER OF SEGREGATING SITES, S	NUMBER OF HAPLOTYPES, H	HAPLOTYPE DIVERSITY, HD	AVERAGE NUMBER OF DIFFERENCES, K	NUCLEOTIDE DIVERSITY, PI	NUCLEOTIDE DIVERSITY WITH JUKE'S CANTOR, PIJC
THARAKA-NITHI	1266	5	1.00	854.90	0.651	2.47
EMBU	880	11	1.00	432.84	0.329	0.48
KITUI	1219	8	1.00	598.93	0.456	0.81

Table 6. Analysis of Molecular Variance (AMOVA) for 23 isolates from 3 populations based on 16S rDNA sequences.

SOURCE	DF	SS	VC	% MOL VAR.	P-VALUE
Among populations	2	947.83	24.67 Va	7.95	.039
Within populations	21	6002.04	285.81 Vb	92.05	$<$.001
Total	23	6949.88	310.48		
Fixation index	F_{ST} :	0.07946			

Abbreviations: Df, Degrees of freedom; SS, sum of squares; VC, variance components; % Mol var., percentage molecular variance.

Table 7. Population pairwise F_{ST} difference based on distance method.

	THARAKA-NITHI	EMBU	KITUI
THARAKA-NITHI	0.000		
	*		
EMBU	+0.1699	0.000	
	0.00488 ± 0.0020	*	
KITUI	−0.025	−0.0434	0.000
	0.23438 ± 0.0131	0.15723 ± 0.0120	*

Matrix of significant F_{ST} P values, Significance Level = 0.0500.

This can be attributed to the fact that the region receives higher amount of the rainfall compared to others and therefore there is increased farming activities.[33] Increased land tillage would probably favor proliferation of soil microorganisms.[39]

The population of culturable PSB was in the range 1.3×10^4 and 3.63×10^4 per gram of soil. These values was within the ranges of a similar study conducted in Morocco which reported values of between 0.0021 and 7.24×10^5 CFU g-1 soil.[40] However, the percentage of culturable PSB to total culturable bacteria was lower than the finding of the same study. In their study, Nannipieri et al,[41] showed that soil properties and land use affect the population of soil bacteria. The isolated PSB were negatively correlated to P level in the current study. Soil pH have also been shown to have an impact on the abundance of PSB.[40] This finding is in agreement with the finding of Ndung'u-Magiroi et al,[24] who showed that the population of PSB is higher in soils with low phosphorus levels. One mechanism in which PSB work is believed to be through production of organic acid which lowers the pH.[42]

Al, Ca, Fe, and Mg are the main ions which adsorb the phosphate ions and therefore their level in the soil is correlated to PSB population. The action of PSB will lead to liberation of these elements from phosphate complex. In the current study, the levels of these elements were negatively correlated to the population of PSB as was also demonstrated by Zheng et al,[42] in their study.

Genetic sequencing using 16S rRNA is a popular and reliable method of identifying bacteria according to Yang et al.[43] In this study, all the isolates were identified using the highly conserved 16S rRNA gene. Majority of the isolates had sequences that matched strains from the genus *Burkholderia*. Several members which showed great genetic similarity to *Burkholderia cepacia* complex were isolated and this is in agreement with similar study by Draghi et al,[44] who isolated several members of the genus *Burkholderia* in Argentinean soil. The genus *Paraburkholderia* is a group of *Burkholderia* with nitrogen fixing ability.[45] The family *Burkholderiaceae* also consists of the genus *Caballeronia* and *Ralstonia* which were isolated in this

Table 8. Kruskal-Wallis test on Phosphate Solubilization Index (PSI).

ISOLATE	MEDIAN	AVE RANK	ISOLATE	MEDIAN	AVE RANK
1	3.125	136.8	37	4.833	204.2
2	4.500	188.5	38	1.857	35.3
3	3.333	148.0	39	4.286	180.5
4	4.000	170.0	40	2.200	65.0
5	3.375	152.3	41	3.200	139.2
6	2.500	86.5	42	3.200	142.0
7	1.143	2.0	43	4.600	193.2
8	1.545	9.7	44	1.800	26.5
9	2.500	86.2	45	4.800	199.7
10	2.571	101.8	46	4.167	177.5
11	2.667	108.8	47	2.500	89.3
12	3.333	150.3	48	2.000	48.0
13	3.000	128.3	49	1.800	34.2
14	1.714	19.7	50	1.667	17.3
15	2.000	45.2	51	1.500	7.5
16	2.167	63.2	52	3.000	126.0
17	2.500	89.3	53	3.143	137.8
18	2.714	112.7	54	3.750	162.8
19	1.857	41.7	55	3.750	162.8
20	1.833	31.7	56	2.857	125.3
21	2.500	89.0	57	3.333	149.5
22	5.833	212.0	58	4.200	179.0
23	4.800	199.7	59	2.500	84.8
24	2.333	73.5	60	3.750	162.8
25	3.714	161.5	61	2.250	66.0
26	4.833	204.2	62	1.667	16.2
27	2.000	45.2	63	1.500	7.5
28	2.600	108.3	64	4.167	176.3
29	4.600	192.0	65	2.000	43.0
30	1.833	37.7	66	2.600	101.5
31	2.500	89.3	67	2.750	113.2
32	2.833	123.5	68	4.286	181.7
33	4.857	206.7	69	2.000	45.2
34	2.833	120.0	70	2.333	73.5
35	2.143	59.8	71	2.500	90.0
36	1.833	37.7	Overall		107.0

$H=210.29$ DF$=70$ $P=.000.$

$H=210.43$ DF$=70$ $P=.000$ (adjusted for ties).

study. In their similar study, Kailasan and Vamanrao[46] reported isolation of *Ralstonia pickettii* which was an effective phosphate solubilizer from Pomegranate Rhizosphere. To the best of our knowledge, there is little literature about the isolated species of *Caballeronia calidae* being a plant growth promoter and therefore its isolation in this study provides an area of interest for further characterization. The genus *Massilia* belong to the family *Oxalobacteraceae* and can inhabit broad range of niches. Several studies have classified *Massilia* as rhizosperic and endorhizal colonizers and they have also been shown to have plant growth promoting properties as demonstrated by Kong et al.[47]

The genera *Pantoea, Enterobacter, Citrobacter,* and *Cronobacter* belong to the family *Enterobacteriaceae*.[48] They were reported as plant growth promoters by Büyükcam et al.[49] In most instances, *Pantoea* have been isolated from the environment as demonstrated by Chakdar et al,[50] in their study. *Cronobacter* have been isolated from food, environment and clinical samples and a study by Zeng et al,[51] revealed that they can cause rare ailments in immune compromised individuals. *Citrobacter* are found in water, soil and human intestines and it utilizes citrate as sole carbon source.[52] The genus *Pseudomonas* is one of the diverse groups of bacteria found in water, soil, plants and animal tissues. It belongs to the family *Pseudomonadaceae* and can tolerate a range of physical environment. Isolates with great gene match to *Pseudomonas oryzihabitans* and *Pseudomonas putida* from this study had the ability to solubilize phosphate as was also demonstrated by Leontidou et al,[53] in their study.

Bacillus is one of the most studied and diverse bacteria of the family Bacillaceae. Some members from this genus have been proven to be among the best phosphate solubilizers. In this study, an isolate with a sequence match to *Bacillus amyloliquefaciens* was isolated and Fan et al,.[54] in their study showed that it has the ability of improving plant growth. *Curtobacterium* belongs to the family Microbacteriaceae and their primary habitat is soil and water.[55] *Curtobacterium citreum* was isolated in the current study and it was shown to be a great phosphate solubilizer.

Analysis of molecular variance showed a low variability among the populations but the variability within the populations was high. This suggests that there is limited physical barriers to gene flow in the populations as shown by Muthini[56] in his study. Nucleotide and haplotype diversity are commonly used in populations as a measure of genetic diversity. Haplotype diversity is the representation of probability that 2 alleles which are randomly sampled from genetic sequence are different while nucleotide diversity is the number of nucleotide differences for very site in pairwise comparisons of genetic sequence. Isolates from the 3 population displayed the maximum Haplotype diversity which is an indication that their haplotypes were highly diverse. Low nucleotide diversity shown by the population is an indication that they share common haplotypes with relatively small divergence. The nucleotide diversity

based on Juke's Cantor varied across the population. This confirms the fact that the isolates were highly diverse. This large variation is probably due to a wide range of bacteria families that have the ability of solubilizing the phosphate.

Burkholderia cepacia (Isolate 22) had the highest solubilization index in the current study. This solubilization index was slightly higher than the value obtained by Pande et al,[57] in their similar study. This isolate form a good potential plant growth promoter and further analysis are ongoing on the same. A total of 15 isolates representing 21.1% of total isolates had PSI value greater than 4. These isolates are from the following groups; *Burkholderia cenocepacia, Burkholderia cepacia, Burkholderia ambifaria, Curtobacterium citreum, Cronobacter sakazakii, Bacillus amyloliquefaciens, Enterobacter sp., Burkholderia contaminans, Cronobacter dublinensis, Caballeronia calidae,* and *Burkholderia territorii*. It is worth noting however that some of the isolates from the above groups had PSI values less than 4. This could be due to the different soil properties and also different microbial interactions at their isolation points. Availability of adequate P in the soil may make the P solubilizers less active and therefore reduce their solubilization potentials. All the isolates belonging to *Pseudomonas* had PSI values less than 4. However, their efficiency in improving plant growth may be better due to production of other growth improving metabolites.[58]

The ability of microorganisms to solubilize P ensures that there is adequate P in the soil for plant uptake. Once the ideal effective phosphate solubilizing bacteria have been isolated, they can be inoculated into the soil where they will convert insoluble P to a form that can be utilized by plants. This will lead to establishment of sustainable cropping systems with reduced application of costly chemical fertilizers.

Conclusion

Based on morphological analysis, 71 isolated PSB bacteria were placed into 23 groups. Representative of these groups were identified by genetic sequencing and they showed high genetic variability. This variability provides a good gene-pool of capturing organisms that are able to solubilize phosphates. Ten isolates which showed effectiveness in solubilizing the phosphate were identified and further analysis are on-going to assay their effectiveness in plant growth improvement. The end goal of this process is the development of effective PSB that will be used in the development of low-cost biofertilizers.

Authors' Contributions
The concept of this work was developed by E.M.N. C.K.K did the laboratory analysis and drafted the manuscript. E.M.N. and S.R revised the manuscript.

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REFERENCES

1. Medici A, Szponarski W, Dangeville P, et al. Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. *Plant Cell*. 2019;31:1171-1184.

2. Koch M, Kruse J, Eichler-Löbermann B, et al. Phosphorus stocks and speciation in soil profiles of a long-term fertilizer experiment: Evidence from sequential fractionation, P K -edge XANES, and 31 P NMR spectroscopy. *Geoderma*. 2018;316:115-126.

3. Bai Z, Li H, Yang X, et al. The critical soil P levels for crop yield, soil fertility and environmental safety in different soil types. *Plant Soil*. 2013;372:27-37.

4. Menezes-Blackburn D, Giles C, Darch T, et al. Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. *Plant Soil*. 2018;427:5-16.

5. Kalayu G. Phosphate solubilizing microorganisms: promising approach as biofertilizers. *Int J Agron*. 2019;2019:1-7.

6. Lu C, Tian H. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth Syst Sci Data*. 2017;9:181-192.

7. Hajjam Y, Cherkaoui S. The influence of phosphate solubilizing microorganisms on symbiotic nitrogen fixation: Perspectives for sustainable agriculture. *J Mater Environ Sci*. 2017;8:801-808.

8. Yadav AN, Verma P, Singh B, Chauhan VS, Suman A, Saxena AK. Plant growth promoting bacteria: Biodiversity and multifunctional attributes for Sustainable Agriculture. *Adv Biotechnol Microbiol*. 2017;5: 1-16. doi:10.19080/aibm.2017.05.555671

9. Aliyat FZ, Maldani M, Guilli ME, Nassiri L, Ibijbijen J. Isolation and characterization of phosphate solubilizing bacteria from phosphate solid sludge of the moroccan phosphate mines. *Open Agr J*. 2020;14:16-24.

10. Kafle A, Cope K, Raths R, et al. Harnessing soil microbes to improve plant phosphate efficiency in cropping systems. *Agronomy*. 2019;9:127.

11. Goswami D, Thakker JN, Dhandhukia PC. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food Agric*. 2016;2:1-19.

12. Kuan KB, Othman R, Abdul Rahim K, Shamsuddin ZH. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. *PLoS One*. 2016;11:e0152478.

13. Santos MS, Nogueira MA, Hungria M. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express*. 2019;9:205.

14. Kumar VV. Plant growth-promoting microorganisms: interaction with plants and soil. In: Khalid RH, Mohd SA, Akmar AS, eds. *Plant, Soil and Microbes: Volume 1: Implications in Crop Science*. Springer International Publishing; 2016;1-16.

15. Stephen J, Shabanamol S, Rishad KS, Jisha MS. Growth enhancement of rice (*Oryza sativa*) by phosphate solubilizing *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) under greenhouse conditions. *3 Biotech*. 2015;5:831-837.

16. Oteino N, Lally RD, Kiwanuka S, et al. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front Microbiol*. 2015;6:745.

17. mma E, devaswamy M, Rao S, Ramesh YM, Naik NM. Isolation and screening of phosphate solubilizing bacteria from paddy rhizosphere soil. *Int J Curr Microbiol Appl Sci*. 2020;9:477-485.

18. Shintu PV, Jayaram KM. Phosphate solubilising bacteria (*Bacillus polymyxa*) – an effective approach to mitigate drought in tomato (*Lycopersicon esculentum* Mill.). *Tropical Plant Research*. 2015;2:2349-9265.

19. Wang J, Li R, Zhang H, Wei G, Li Z. Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. *BMC Microbiol*. 2020;20:38-12.

20. Yadav A, Yadav K, Vashistha A. Phosphate solubilizing activity of *Pseudomonas fluorescens* PSM1 isolated from wheat rhizosphere. *J Appl Nat Sci*. 2016;8:93-96.

21. Safirzadeh S, Chorom M, Enayatizamir N. Effect of phosphate solubilising bacteria (Enterobacter cloacae) on phosphorus uptake efficiency in sugarcane (*Saccharum officinarum* L. *Soil Res*. 2019;57:333-341.

22. Martins da Costa E, de Lima W, Oliveira-Longatti SM, de Souza FM. Phosphate-solubilising bacteria enhance *Oryza sativa* growth and nutrient accumulation in an oxisol fertilized with rock phosphate. *Ecol Eng*. 2015;83:380-385.

23. Long XE, Yao H, Huang Y, Wei R, Zhu YG. Phosphate levels influence the utilisation of rice rhizodeposition carbon and the phosphate-solubilising microbial community in a paddy soil. *Soil Biol Biochem*. 2018;118:103-114.

24. Ndung'u-Magiroi KW, Herrmann L, Okalebo JR, Othieno CO, Pypers P, Lesueur D. Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya. *Ann Microbiol*. 2012;62:897-904.

25. Javadi Nobandegani MB, Saud HM, Yun WM. Phylogenetic relationship of phosphate solubilizing bacteria according to 16S rRNA genes. *Biomed Res Int*. 2015;2015:201379.

26. Mohamed EAH, Farag AG, Youssef SA. Phosphate solubilization by *Bacillus subtilis* and *Serratia marcescens* isolated from tomato plant rhizosphere. *J Environ Prot*. 2018;09:266-277.

27. T. Nikitha MS, B. Sadhana EUR, Vani SS. Phosphorous and phosphate solubilising bacteria and their role in Plant Nutrition. *Int J Curr Microbiol Appl Sci*. 2017;6:2133-2144.

28. Ceulemans T, Bodé S, Bollyn J, et al. Phosphorus resource partitioning shapes phosphorus acquisition and plant species abundance in grasslands. *Nat Plants*. 2017;3:16224.

29. Soumare A, Boubekri K, Lyamlouli K, Hafidi M, Ouhdouch Y, Kouisni L. From isolation of phosphate solubilizing microbes to their formulation and use as biofertilizers: status and needs. *Front Bioeng Biotechnol*. 2020;7:425-514.

30. Suyal DC, Soni R, Sai S, Goel R. Microbial inoculants as biofertilizer. In: Pratap Singh D, Bahadur Singh H, Prabha R, eds. *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*. Springer India; 2016;311-318, 10.1007/978-81-322-2647-5_18.

31. Sinclair L, Osman OA, Bertilsson S, Eiler A. Microbial community composition and diversity via 16S rRNA gene amplicons: evaluating the illumina platform. *PLoS One*. 2015;10:e0116955.

32. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*. 1991;173:697-703.

33. King JA, Richard W, Sebastian E. Representation of the Indian Ocean Walker circulation in climate models and links to Kenyan rainfall. *Int J Climatol*. 2021;41:E616-E643.

34. Jones J. *Soil Analysis Handbook of Reference Methods*. Routledge; 2018.

35. Pikovskaya RI. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiologia*. 1948;17:362-370.

36. Pathak R, Paudel V, Shrestha A, Lamichhane J, Gauchan DP. Isolation of phosphate solubilizing bacteria and their use for plant growth promotion in tomato seedling and Plant. *Kathmandu Univ J Sci Eng Technol*. 2018;13:61-70.

37. Brenner DJ, Staley JT, Krieg NR. Classification of procaryotic organisms and the concept of bacterial speciation. In: Garrity GM, ed. *Bergey's Manual® of Systematic Bacteriology*. Springer-Verlag. 2005;27-32.

38. Classen AT, Sundqvist MK, Henning JA, et al. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*. 2015;6:art130.

39. Bossio DA, Girvan MS, Verchot L, et al. Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microb Ecol*. 2005;49:50-62.

40. Rfaki A, Zennouhi O, Nassiri L, Ibijbijen J. Soil properties related to the occurrence of rock phosphate-solubilizing bacteria in the rhizosphere soil of faba bean (*Vicia faba* L.) in Morocco. *Soil Syst*. 2018;2:31.

41. Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. Microbial diversity and soil functions. *Eur J Soil Sci*. 2017;68:12-26.

42. Zheng BX, Zhang DP, Wang Y, et al. Responses to soil pH gradients of inorganic phosphate solubilizing bacteria community. *Sci Rep*. 2019;9:25-38.

43. Yang B, Wang Y, Qian PY. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics*. 2016;17:135.

44. Draghi WO, Degrossi J, Bialer M, et al. Biodiversity of cultivable *Burkholderia* species in Argentinean soils under no-till agricultural practices. *PLoS One*. 2018;13:e0200651-NaN18.

45. Dall'Agnol RF, Bournaud C, de Faria SM, Béna G, Moulin L, Hungria M. Genetic diversity of symbiotic *Paraburkholderia* species isolated from nodules of *Mimosa pudica* (L.) and *Phaseolus vulgaris* (L.) grown in soils of the Brazilian Atlantic Forest (Mata Atlântica). *FEMS Microbiol Ecol*. 2017;93:1-15.

46. Kailasan NS, Vamanrao VB. Isolation and characterization of *Ralstonia pickettii* - A novel phosphate solubilizing bacterium from Pomegranate rhizosphere from Western India. *Int J Life Sci Biotechnol Pharma Res*. 2015;4:1-9.

47. Kong BH, Li YH, Liu M, et al. *Massilia namucuonensis* sp. Nov., isolated from a soil sample. *Int J Syst Evol Microbiol*. 2013;63:352-357.

48. Cunningham DJ, Amy L. Chapter 140: Enterobacter Cronobacter, and Pantoea species. In Long, SS, Charles GP, Marc F, eds. *Principles and practice of pediatric infectious diseases*. 5th ed. Elsevier Health Sciences; 2017;824-827.

49. Büyükcam A, Tuncer Ö, Gür D, et al. Clinical and microbiological characteristics of *Pantoea agglomerans* infection in children. *J Infect Public Health*. 2018;11:304-309.

50. Chakdar H, Dastager SG, Khire JM, Rane D, Dharne MS. Characterization of mineral phosphate solubilizing and plant growth promoting bacteria from termite soil of arid region. *3 Biotech*. 2018;8:463-511.

51. Zeng H, Li C, He W, et al. *Cronobacter sakazakii*, *Cronobacter malonaticus*, and *Cronobacter dublinensis* genotyping based on CRISPR locus diversity. *Front Microbiol*. 2019;10:1989-2010.

52. Sadiq HM, Jahangir GZ, Nasir IA, Iqtidar M, Iqbal M. Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere soil. *Biotechnol Biotechnol Equip*. 2013;27:4248-4255.

53. Leontidou K, Genitsaris S, Papadopoulou A, et al. Plant growth promoting rhizobacteria isolated from halophytes and drought-tolerant plants: genomic characterisation and exploration of phyto-beneficial traits. *Sci Rep*. 2020;10:14857-14915.

54. Fan B, Blom J, Klenk HP, Borriss R. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "Operational Group B. amyloliquefaciens" within the *B. subtilis* species complex. *Front Microbiol*. 2017;8:1-15.

55. Chase AB, Arevalo P, Polz MF, Berlemont R, Martiny JB. Evidence for ecological flexibility in the cosmopolitan genus *Curtobacterium*. *Front Microbiol*. 2016;7:1874-1911.

56. Muthini M. Morphological assessment and effectiveness of indigenous Rhizobia isolates that nodulate *P vulgaris* in water hyacinth compost testing field in Lake Victoria Basin. *Br J Appl Sci Technol*. 2014;4:718-738.

57. Pande A, Pandey P, Mehra S, Singh M, Kaushik S. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *J Genet Eng Biotechnol*. 2017;15:379-391.

58. Anand K, Kumari B, Mallick MA. Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. *Int J Pharm Pharm Sci*. 2016;8: 37-40.