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Isolation, characterization and identification of phosphate- and potassium- solubilizing bacteria from weathered materials of granite rock mountain, That Son, an Giang province, Vietnam

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Abstract: Two hundred and thirty-seven bacterial isolates from 118 sample soils/weathered granite rock of That Son Mountain, An Giang province, Vietnam were isolated on Aleksandrov medium. Their colonies were round or irregular, white to yellow and their shape was rod, motile. Thirty four of two hundred and thirty-seven bacterial isolates were capable of dissolving both phosphate and potassium and seven strains had high phosphate and potassium dissolution capacity (>15 mg l^{-1} P_2O_5 and >10 mg l^{-1} K_2O). These thirty four strains were identified by using PCR technique with specific primers fd1 and rP and DNA sequencing. The results of DNAsequencing were compared with GenBank database of NCBI by BLAST N software. The sequences from selected phosphate and potassium isolates (34 isolates) showed high degrees of similarity to those of the GenBank references (between 99% and 100%). Among these sequenced isolates, 8 isolates belong to Bacilli (23.53%) and 26 isolates belong to Proteobacteria (76.47%) including 21 alpha-proteobacteria (61.76%) and 5 gamma-proteobacteria (14.71%). Based on Pi value (nucleotide diversity), Proteobacteria group had the highest Theta values and Theta values (per sequence) from S of SNP for DNA polymorphism were calculated for each group and AlphaProteobacteria group had the highest values in comparistion with two groups. These results showed that 3 strains including CA09 (*Agrobacterium tumefaciens*), CA29 (*Rhizobium tropici*) and K16B (*Azotobacter tropicalis*) proposed as potential microbial inoculants or biofertilizers for sustainable crop production in sandy acid soil in Vietnam because of their benefits and biosafety.

Keywords: *Acinetobacter*, *Agrobacterium, Azotobacter, Bacillus*, Identification, Phosphate and Potassium Solubilization, *Rhizobium,* Weathered Materials of Granite Rock

1. Introduction

Deficiency in plant-available phosphorus and potassium is considered to be a major limiting factor to food production in many agricultural soils [1]. Phosphate and potassium are major essential macronutrients for plant growth and development and soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield [2]. The P content in average soils is about 0.05% (w/w), but only 0.1% of the total P is available to plants [3]. Therefore, P is often a limiting nutrient in agricultural soils. Most of the K in soil is in the structural form, mainly comprised of K-bearing primary minerals such as muscovite, biotite and feldspars [4]. Soil has rich reserves of K, among which only 1-2% can be directly absorbed by plants [5]. Consequently, about 90-98% of the soil K exits in silicate minerals such as K-feldspars and mica, which only release K slowly [6]. However, the concentration of soluble P and K in soil are usually very low, and the biggest proportion of P and K in soil are insoluble rocks, minerals and deposits [6]. The weathering of rocks plays a role in a number of important environmental processes [7] to clay, silt and sand and these minerals are important component of soil in earth. Soil-plant-microbe interaction has got much important in recent decades. Many types of microorganisms are known to inhabit soil, especially rhizosphere and play an important role in plant growth and

development. Plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB), were suggested as a sustainable solution to improve plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors [8][9]. In fact, these sources constitute the biggest reservoirs of P and K in soil under appropriate conditions, they can be solubilized and become available for plants [10]. The Mekong Delta occupies 2.9 M (million) ha (12% of the Vietnam's total land area) and this dekta is one of the two principal areas of rice production of Vietnam. About 35% of the Mekong Delta is alluvial soil, covering 1.1 million ha along the rivers with most of the remainder acid sulfate clay soil (1.6 million ha). Both the acid and alluvial soils are deficient in phosphorus since P generally reacts with aluminium and iron under low pH conditions and forms insoluble compounds [11]. Besides that, K is also an important macronutrient for plant growth but potassium fertilizer, as potassium chloride, has been imported with big quantity every year because Vietnam has no mineral resource to produce potassium fertilizer. K fertilizer cost has not to stop enhance every year, leading to increase cost of rice production and farmer's income should reduce.

Phosphate-solubilizing bacteria (PSB) play a significant role in marking phosphorus availble to plants bring favourable changes in soil reaction and in the soil microenvironment leading to solubilization of inorganic phosphate sources [12]. K-solubilizing bacteria are able to release potassium from insoluble minerals [13][14][15][16]. In addition, researchers have discovers that K-solubilizing bacteria can provide beneficial effects on plant growth through suppressing pathogens and improving soil nutrients and structure. Moreover these bacteria can weather silicate minerals to release potassium, silicon and aluminum and secrete bio-active materials to enhance plant growth and they are widely used in biological K-fertilizers and biological leaching [17]. Recently Xiufang *et al.* [10] found the community of microorganisms in soil of Tianmu Mountain, Zhejiang, China with herbal plants flourished were able to dissolve both P- and K-containing minerals. The aims of this study were (i) selection and (ii) identification the isolated P- and K-solubilizing bacteria from weathered materials of granite rock mountains of That Son, An Giang province, Vietnam.

2. Material and Methods

2.1. Bacteria Isolation and Growth Conditions

Bacterial isolates were isolated from weathering materials of granite rock mountain, That Son, An Giang Province, Vietnam (Figure 1), by virtue of their abilities to solubilize mineral P- and K. The samples were stored at 10^{o}C during transit and processed immediately. That Son has seven mountains (Cam, Dai, Dai Nam-Gieng, To, Tuong, Ket, Nuoc) with high altitute from 54 m to 705 m in comparion to sea level, That Son belongs to An Giang province, Vietnam, situated 10^{o}22'52" north latitude and 105^{o}52'12" east longtitude. Many mountains have beautiful pagodas that are famous tourism, granite rock of mountain has been used as material for way construction.

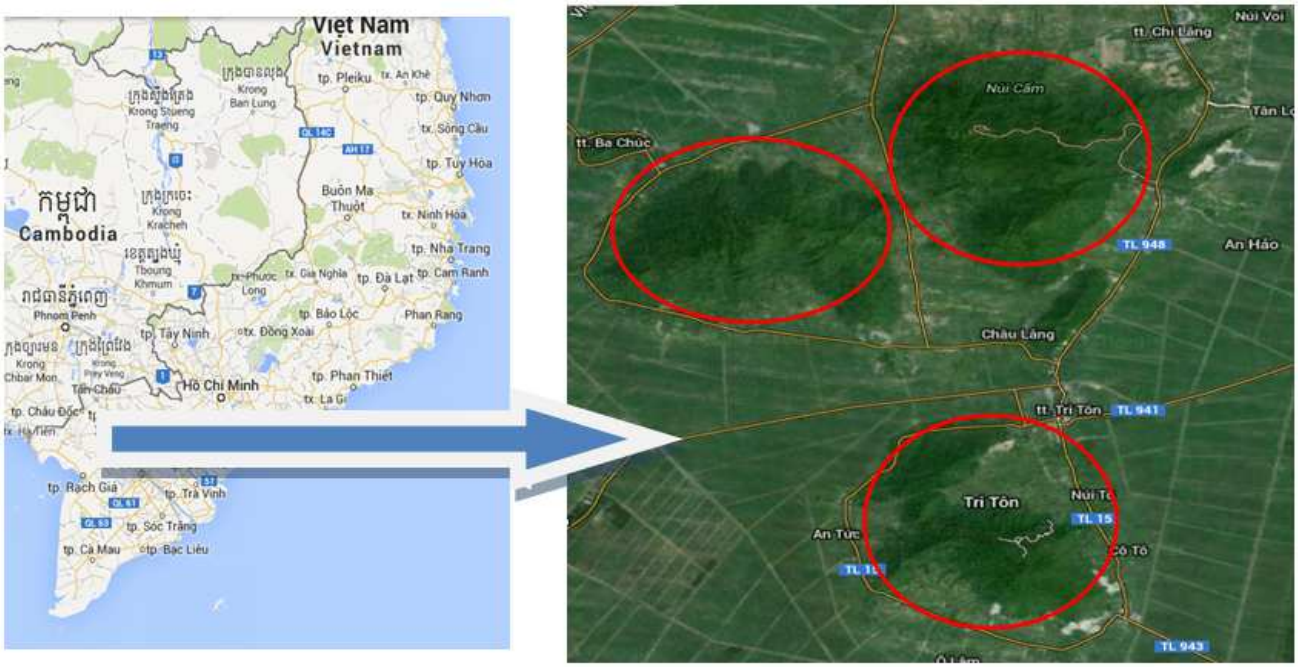

Figure 1. *Location of That Son, An Giang, Vietnam examined in this study and red circles are seven mountain sites*

Soil samples or weathering materials were collected from the areas where herbal plants flourished (Figure 2). Each sample (2 g) was added to 25 ml of liquid Aleksandrov medium [10] with 0.2% apatite and 0.2% kaolinite and shaken for 24 h on orbital shaker at 50 rev min-1 at temperature room.

Figure 2. *Soil samples or weathering materials were collected to isolate bacteria*

2.2. Mineral Dissolution

Apatite from Lao Cai factory, North Vietnam and Kaolinite mineral (commercial) were added to liquid Aleksandrov medium as the sole P and K source to test the ability of the isolates to solubilize these minerals and the isolates with the highest solubilization capacity were kept on Aleksandrov medium for further study.

Quantitative estimation of P and K solubilization was carried out in Falcon tubes (50-mL) containing 30 ml of Aleksandrov medium, and inoculated in triplicate with tested isolates (1 ml inoculum with approximately 3 x 10^7 cfu ml^{-1}). Autoclaved, uninoculated medium served as controls. The falcon tubes were incubated for 10 days on orbicutal shaker with 10 rev min -1 at temperature room. Samples were taken at two times: 5 and 10 day after incubation, pH value was measured with a pH meter, after that samples were centifuged at 8000 g for 10 min. The supernatants were used to assay the solubilized P (5 and 10 day after incubation) and K (only at 10 day after incubation). P was estimated using the molybdo-vanado- method (Oniani method) and K was examined using atomic absorption spectrometry [18]. Values are recorded and presented with average value for each sample, and differences were considered to be significant at the $P<0.05$ level with LSD or Duncan test.

2.3. Isolation of Bacteria

Cultivation-based techniques were used to gain insight into the abundance and species composition of bacterial communities, and to reveal the poly-P accumulation of bacteria. Serial dilutions (10^{-2} to 10^{-4}) of composite samples were prepared. Dilutions (0.05 ml) were aseptically plated on the agar-based culture medium. Plates were incubated at 30°C for 5 days. Bacterial colonies were differentiated on the basis of colony morphology and pigmentation. Colonies were subculture on the agar-based subculture medium plates by striking technique and re-incubated at 30°C for 5 days. This isolation process carries out in shifts of the agar-based culture medium to the agar-based subculture medium until monocultures were obtained. Monocultures were culture on the agar-based culture medium slant in the test-tube (12 ml) and incubated at 30°C for 4 days following by stored 10°C in refrigerator.

2.4. Colony Characteristic and Microscopic Examination

The characteristics of colony such as size, color, shap were presented in each group. Cell morphologies of the isolates were observed using an optical microscope and they were also observed on scanning electron microscope.

2.5. Phylogenetic Analysis of 16S rRNA Gene Sequence

Table 1. *Physical and chemical characteristics of weathered material from granite rock of these mountains of That Son, An Giang province, Vietnam*

Site	pH	N_{total} (%)	Available P_2O_5 (mg/kg)	Exchangeable K_2O (mg/kg)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
Nui Cam	6.41	0.78	44.25	116.80	4.50	60.11	18.11	21.78
Nui To	6.24	0.79	42.10	102.50	4.72	61.25	17.12	21.63
Nui Dai	6.57	0.82	50.20	127.50	5.10	63.14	15.17	21.69
Nui Dai Nam Gieng	6.32	0.69	72.50	82.50	4.89	60.89	20.01	19.10
Nui Ket	6.50	0.78	44.25	116.80	4.51	65.12	18.55	16.33
Nui Tuong	6.34	0.84	53.40	90.68	4.84	64.15	17.11	18.74
Nui Nuoc	6.42	0.99	63.20	82.71	4.73	62.25	16.68	21.07

Bacteria universal primers, the forward primer fD1 and the reverse primer rP [10] were used to amplified partial length of 16S rRNA gene sequence.

Genomic DNA was extracted from the cultures grown in Aleksandrov medium 30°C for 24 h [19]. The 16S rRNA gene sequence was amplified in a PCR mixture, composed with 1 µmol l-1 of each primer. 200 µmol l-1 of each dNTP, 50 µm mol l-1 KCl and 1.5 mol l-1 $MgCl_2$ in 10 m mol l-1 Tris/HCl (pH 8.3) buffer. DNA (0.1 µg) and 2.5 U Taq DNA polymerase [Fermentas] were added in 100 µl PCR mixture. PCR amplications were performed at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 50 s and extension at 72°C for 105 s; and a final extension at 72°C for 10 min. Partial 16S rRNA genes of some good bacterial strains was sequenced by MACROGEN, Republic of Korea (dna.macrogen.com) and they were chosen to sequence, the results were compared to sequences of GenBank based on partial 16S rRNA sequence to show relationships between other P&K-solubilizing bacterial strains [20] and the phylogenetic analysis was constructed by the Maximum Likelihood method based on 1.000 bootstraps.

2.6. SNPs Discovery

The sequence date from 34 lipid-degrading bacterial isolates were analysed with SeqScape@Software (Applied Biosystem, Foster City, CA, USA). SeqScape is a sequence comparison tool for variant identification, SNP discovery and validation. It considers alignment depth, the base calls in each of the sequences and the associated base quality values. Putative SNPs were accepted as true sequence variants if the quality value exceeded 20. It means a 1% chance basecall is incorrect.

2.7. Nucleatide Diversity (Θ)

Nucleotide diversity (Θ) was calculated by the method described by Halushka et al. [21]

$$\Theta = K/aL = \sum_{i=2}^{n} 1/(i-1)$$

where K is the number of SNPs identified in an alignment length, n is alleles and L is the total length of sequence (bp).

2.8. Data Analyses

Data from ammonium and orthophosphate concentrations in media were analysed in completely randomized design with three replicates and LSD test at P=0.01 were used to differentiate between statistically different means using SPSS version 16.

3. Results and Discussion

3.1. Bacteria Isolation and Colony Characteristics

From 118 soil samples/weathering materials, 237 isolates were isolated on Aleksandrov medium (Table 2).

They developed very well on this medium from 48 - 72 h at 30^{o}C, this showed that these isolates had P and K-solubilizing capacity. Their colonies had round-shape, spreading, climy, smooth, transparency, colourless or milk-color, white or yellow. Colonies of all strains were small (0.1 - 1 mm in diameter) and some strains were very large (2.5 – 3 mm in diameter)(Figure 3). This result was the same Xiufang's experiment [10] which was done at Tianmu Mountain, China however these colonies developed faster in Aleksandrov medium in 2- 3 days instead of 4 days as Xiufang's experiment at 30^{o}C, these results were the same of previous our experiments in denatured rock mountan, Ha Tien, Viet nam [22], from calcacerous mountain, Ha Tien [23] and from granite rock, Nui Sap, An Giang [24] and granite rock of Ba Hon, Kien Giang, Vietnam [25].

Table 2. *Total of isolates were isolated from weathered materials and soils of seven sites (That son, An Giang, Vietnam)*

Site	Weathered material and soil number	Isolate number
Nui Cam	20	28
Nui To	20	30
Nui Tuong	15	40
Nui Dai	18	40
Nui Dai Nam Gieng	15	27
Nui Nuoc	15	24
Nui Ket	15	48
Total	118	237

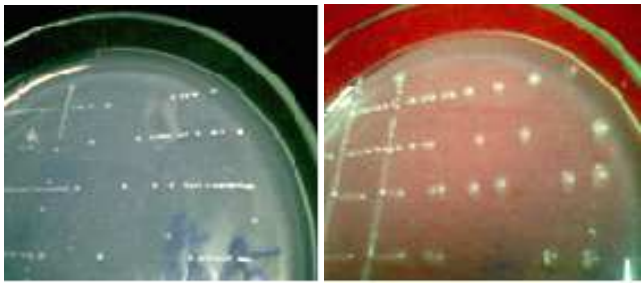

Figure 3. *Characteristic of colonies of bacterial isolates after grown on Aleksandrov medium*

3.2. Microscopic Examination

Microscopic observations showed that the cells of bacterial isolates were motile, short-rod (0.8x1.4 µm) and long-rods (0.8x1.8 µm), Gram-positve and Gram-negative (Figure 4).

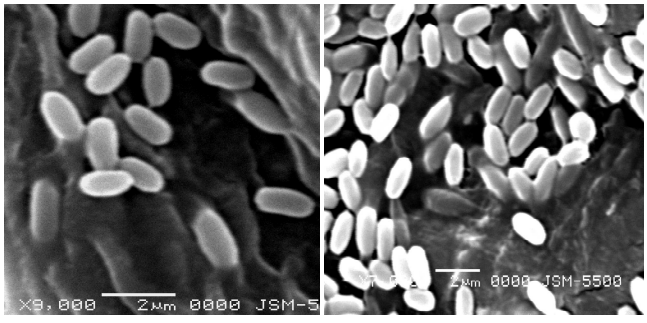

Figure 4. *Electromicrographs of cell of bacterial isolates grown on Aleksandrov medium*

***Table 3**. Phosphate and potassium solubilization of 34 isolates*

Isolate	Solubilization of phosphate (mg P2O5 l-1)	Solubilization of potassium (mg K2O l-1)	Isolate	Solubilization of phosphate (mg P2O5 l-1)	Solubilization of potassium (mg K2O l-1)
CA09	23.16 a	42.12 e	D10A2	17.73 bc	33.59 h
CA10	14.22 c	44.60 d	D15A	18.36 bc	34.50 gh
C18	12.31 cd	47.61 b	D15B	11.01 d	50.32 a
CA21	14.98 c	43.08 e	DG1	17.59 bc	08.70 q
CA25	12.07 cd	48.24 b	DG4B	19.40 ab	09.03 pq
CA28	15.50 bc	40.03 f	DG6	12.24 cd	12.11 n
CA29	15.33 bc	48.32 b	DG10	12.08 cd	12.30 n
D7B	17.15 bc	36.02 g	DG12	18.62 bc	10.21 p
D9	14.17 cd	34.09 gh	K16	12.43 cd	43.04 e
K16B	15.53 bc	45.50 c	N4	16.67 bc	36.46 g
K30	11.19 d	32.38 k	N18	12.45 cd	40.17 f
K35	11.30 cd	31.06 k	N24	10.31 d	33.06 h
NT1	18.02 bc	43.07 e	Tu09	18.85 bc	30.20 l
NT3	17.29 bc	35.02 g	Tu15	09.12 d	21.02 m
NT4	17.15 bc	31.61 k	Tu39	12.25 cd	33.14 h
NT11	15.64 bc	40.05 f	Tu40	12.39 cd	29.01 i
NT21	15.29 bc	40.67 f	Control	00.63 e	00.00 r
NT30	22.67 ab	36.78 g	C.V	8.50%	1.18%

Data were recorded at 10 days after incubation, the means of 3 replications
Numbers following the same word not difference at 1% level

In the modified medium with apatite (Lao Cai, Vietnam) and potassium mineral (kaolinite), the ability of the isolates to solubilized different P and K minerals was also investigated (Table 3).

In 237 isolates, 34 isolates showed significantly higher solubilization of potassium mineral (kaolinite) than the control from 8.70 mg l^{-1} (isolate BG1) to 50.32 mg l^{-1} K_2O (isolate D15B). Isolate D15B was the most efficient isolate in solubilizing mineral K and this isolate also had P-solubilizing ability after 10 days of incubation (Table 3). Isolate CA09 and CA29 have high ability of solubilization of phosphate and potassium. Interestingly, all isolates (high phosphate and potassium) grew in medium having pH value above 6.2.

P solubilizing bacteria and silicate bacteria play an important role in plant nutrition through the increase in P and K uptake by the plant [7]. Application of phosphate solubilizing bacteria have been used as P-biofertilizer for crop cultivation [26]. Some studies have shown that the application of K-solubilizing bacteria and K bearing minerals increases in the amount of available K in the soil and promotes plant uptake of K [27][2].

All of them (34 isolates) were chosen to identify and the fragment of 1500 bp 16S rDNA were obtained from PCR and sequencing (Table 4).

***Table 4.** Phylogenetic affiliation of isolate on the basis of 16S rDNA genes sequences by using BLAST programme in the GenBank database on sequence similarity*

Taxonomic group strain	Closest species relative	Similarity (%)
Bacilli		
CA18	Bacillus subtilis A2-9 (JF496331)	99
DG12	Bacillus subtilis ANctcri3 (HQ286641)	99
D7B	Bacillus cereus strain DZ4 (HQ143564)	99
DG4B	Bacillus aryabhattai PSB59 (HQ242772)	99
DG6	Bacillus pumilus strain PJ-3 (KJ195695)	99
Tu15	Paenibacillus edaphicus EA3-10 (JF496414)	100
N24	Paenibacillus polymyxa CE42 (JN084141)	99
Tu40	Paenibacillus polymyxa M1 (FR727737)	100
Alphaproteobacteria		

Taxonomic group strain	Closest species relative	Similarity (%)
CA09	Agrobacterium tumefaciens M5 (EF443163)	99
CA25	Agrobacterium tumefaciens AN17 (KF439828)	99
CA28	Agrobacterium tumefaciens B228 (GQ169811)	99
D9	Agrobacterium tumefaciens NGB-SR16 (AB825997)	99
D15B	Agrobacterium tumefaciens B8S (AY850392)	99
DG10	Agrobacterium tumefaciens T912-2 (KF463142)	99
NT1	Agrobacterium tumefaciens BLN4 (GQ181060)	99
D15A	Agrobacterium sp. EC080527_02 (FJ593843)	99
K16	Agrobacterium sp. P29 (KF465838)	99
CA21	Azotobacter tropicalis KBS (AB236160)	99
K16B	Azotobacter tropicalis BKK.2 (AB236162)	99
K30	Azotobacter tropicalis IARI-THW-22 (KF054975)	99
CA10	Rhizobium tropici CIAT 899 (NR_102511)	99
CA29	Rhizobium tropici strain CAF439 (FJ405380)	99
N18	Rhizobium tropici Br859 (HQ394213)	99
DG1	Rhizobium leguminosarum NGB-FR-137 (AB749224)	99
K35	Rhizobium leguminosarum J-7HPT1 (KF468790)	99
N4	Rhizobium leguminosarum 37-2 (JN105996)	99
D10A2	Rhizobium multihospitium strain CC-13H (JN896359)	99
Tu39	Rhizobium multihospitium CCBAU 83364 (EF490014)	99
Tu09	Rhizobium sp. ICB456 (HM486519)	99
Gammaproteobacteria		
NT3	Acinetobacter calcoaceticus LCR59 (FJ976567)	99
NT4	Acinetobacter calcoaceticus LCR100 (FJ976609)	99
NT11	Acinetobacter calcoaceticus LCR102 (FJ976611)	99
NT21	Acinetobacter calcoaceticus EU99 (FJ681294)	99
NT30	Acinetobacter calcoaceticus LCR17 (FJ976526)	99

Using the Maximum-Likilihood method analyzed the relationship of 34 strains through the sequnces for 16S rRNA gene sequence by the BLAST search programme, the results showed that they can be grouped into two clusters (Figure 5).

Cluster A was divided to two small clusters: cluster A1 with *Agrobacterium tumefaciens* CA25, *Acinetobacter calcoaceticus* NT21, *Agrobacterium tumefaciens* CA09, *Azotobacter tropicalis* CA21, *Acinetobacter calcoaceticus* NT4 and *Agrobacterium tumefaciens* D15B (Gram-negative bacteria) together with *Bacillus subtilis* CA18 and *Bacillus pumilus* DG6 (Gram-positive bacteria). Cluster A2 included 2 small groups: cluster A21 with *Azotobacter tropicalis* K16B, *Acinetobacter calcoaceticus* NT3, *Agrobacterium* sp. D15A, *Agrobacterium tumefaciens* DG10 and cluster A22 with *Rhizobium tropici* CA10, *Agrobacterium* sp. K16, *Rhizobium* sp. Tu09, *Bacillus aryabhattai* DG4B, *Rhizobium tropici* N18.

Cluster B composed of two clusters: cluster B1 with *Agrobacterium tumefaciens* D9, *Azotobacter tropicalis* K30, *Acinetobacter calcoaceticus* NT30, *Rhizobium tropici* CA29 (Gram-negative bacteria) together with *Paenibacillus edaphicus* TU15. Cluster B2 with 2 small groups: cluster B21 with *Agrobacterium tumefaciens* CA28, *Rhizobium leguminosarum* N4, *Bacillus cereus* D7B, *Agrobacterium tumefaciens* NT1, *Bacillus subtilis* DG12, *Rhizobium leguminosarum* K35. Cluster B22 with *Acinetobacter calcoaceticus* NT11, *Paenibacillus polymyxa* N24, *Rhizobium multihospitium* Tu39, *Paenibacillus polymyxa* Tu40. Two clusters B21 and B22 together with *Rhizobium multihospitium* D10A2.

Although 34 strains distributed into 6 clusters and *Rhizobium multihospitium* D10A2, all of them were isolated from weathered materials/soils of 7 mountains and these seven sites have a distance very far (from 15 to 25 km) but they had a close genetic relationship. Interestingly, all of them are beneficial bacteria but they did not separate Gram-positive or Gram-negative. However number (ratio) of Gram-negative bacter was higher than that of Gram-positive bacteria. While Gram-positive bacteria (Bacilli) occupied the high ratio of the isolated strains from weathered materials/soils of granite rock of Sap Mountain, An Giang [24] and Ba Hon mountain, Kien Giang, Vietnam [25]. Moreover, these bacterial strains also were classified to Bacilli (23.53%) and Proteobacteria (76.47%) and the Proteobacteria group composed of Alpha-Proteobacteria (61.76%), and Gamma-Proteobacteria (14.71%)(Figure 6).

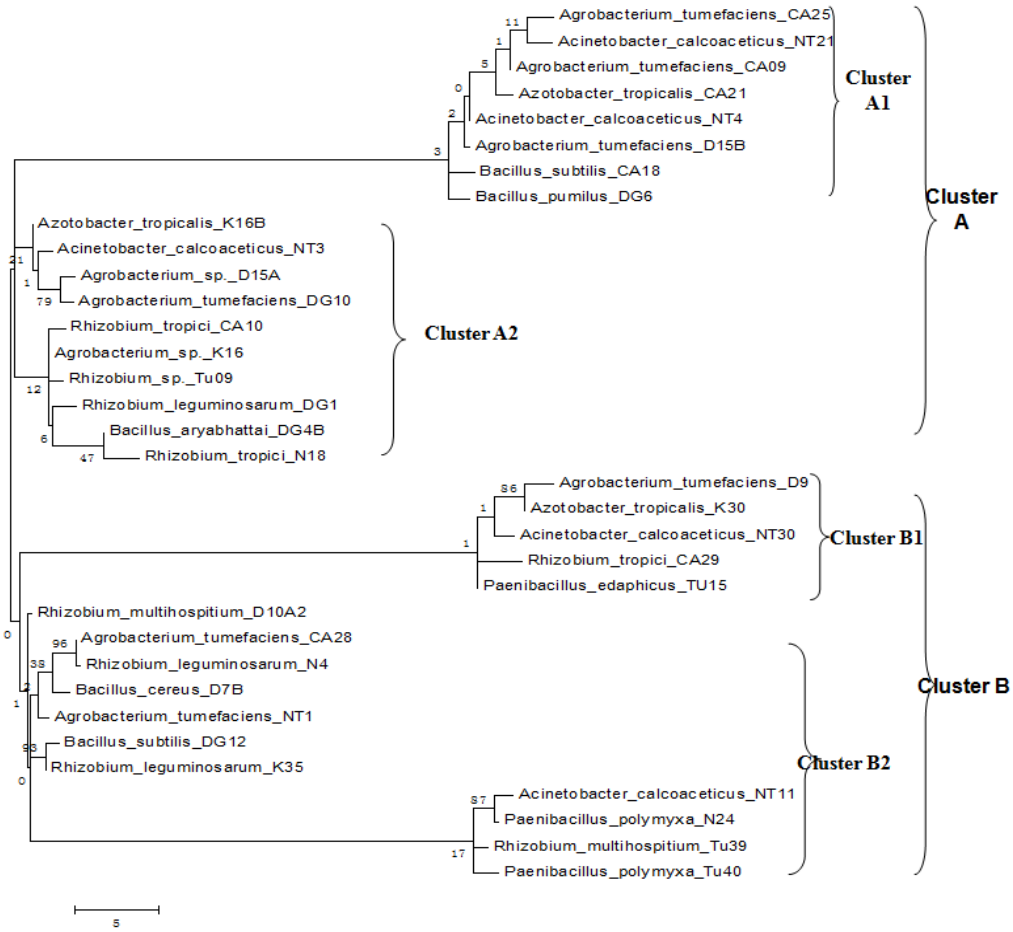

Figure 5. *Phylogenetic tree for partial 16S rRNA gene sequences from 34 isolates by using primers (fD1, rP) showing relationships between representative strains along with related sequences retrieved from GenBank. The numbers at the nods indicate the levels of bootstrap support (%) based on a Maximum - Likelihood analysis of 100 re-sampled datasets. The scale bar indicates the phylogenetic distance corresponding to 5 changes per 100 bases.*

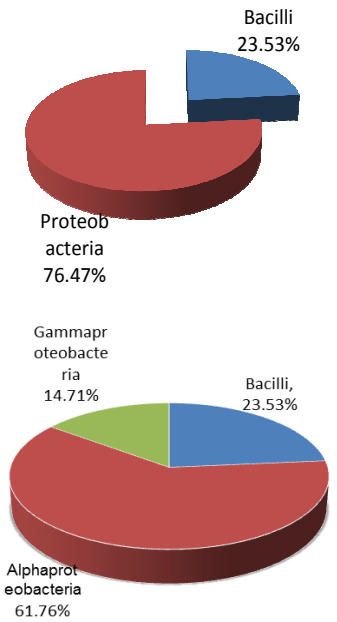

Figure 6. *The proportion of group and they distributed in two clusters*

Nucleotide polymorphism can be measured by many parameters such as halotypes (genes) diversity, nucleotide diversity, (Pi), Theta (Θ) (per group) etc… In this study, nucleotide diversity was estimated by Theta (Θ), the number of segregating sites [28], and its standard deviation (SΘ). These parameters were estimated by DNA Sequence Polymorphism software version 4.0 [29]. Pi values explained nucleotide diversity of sequences for each gene, the higher values, and the more diversity among groups. Bacilli group had the highest Pi values. Theta values (per sequence) from S of SNP for DNA polymorphism were calculated for each group, and Proteobacteria group had the highest Theta values in comparison with Bacilli group (Table 5). However Bacilli group with 8 strains had Pi value and Theta value (from S and from Eta) higher than two groups of Proteobacteria (Alpha and Gammaproteobacteria), this showed that high genetic diversity of Bacilli group presented in nucleotide position (Figure 7).

Theta value in nucleotide position (Figure 6) showed that nucleotide position varied from 200 position to 232 position, from 252 position to 576 position and 579 position to 755 position.

Table 5. *Nucleotide diversity (Θ) values of two EST's using the programme DNASp 5.0 [29]*

ESTs	Alpha Proteobacteria	Bacili	Gama Proteobacteria
Nucleotide diversity (Pi)	0.09261	0.13005	0.10966
Theta (per sequence) from S	51.421± 3.780	84.463± 5.707	89.862 ± 5.887
Theta (per site) from S	0.08585	0.12366	0.11226
Theta (per site) from Eta	0.11090	0.15416	0.11226

Primer fD1: 5'- AAGAGTTGATC(CA)TGGCTCAG – 3'
Primer rP: 5' –TACGG(TC)TACCTTGTTACGACTT-3'

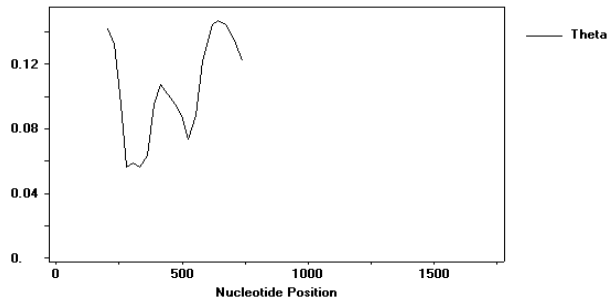

Figure *7. Variation of Theta value from 200 position to 755 position of 34 strains*

Haplotype analysis of 34 strains was presented in Figure 8. This figure revealed that there was genetic diversity between the strains of *Agrobacterium* group, the strains of *Azotobacter tropicalis*, strain of genus *Rhizoleguminosarum*, genus *Rhizobium multihospitium, Rhizobium tropici,* strains of *Bacillus* group and *Paenibacillus* group and strains of *Acinetobacter calcoaceticus*.

Members of the genus *Rhizobium* nodulate the roots of leguminous plants. The rhizobia that infect peas, clovers, and beans (*Phaseolus vulgaris* L.) are clustered in a single species *Rhizobium leguminosarum*, which has three biovars (*Rhizobium leguminosarum* biovar viciae, *Rhizobium leguminosarum* biovar trifolii and *Rhizobium leguminosarum* biovar phaseoli [30] and Matinez-Romero et al. [31] identified *Rhizobium leguminosarum* biovar phaseoli as a new species, *Rhizobium tropici* that nodulates *Phaseolus vulgaris* and *Leucaenae* spp. *Rhizobium tropici* strains tolerate high temperatures and high levels of acidity in culture and thay are symbiotically now stable. Based on the phenotypic characterization and *nod*D, *nif*H genes of *Rhizobium tropici*, a novel species with the name *Rhizobium multihospitium* which nodulates *Robicia pseudoacacia*, but not *Leucaenae leucocephala, Phaseolus vulgaris, Pisum sativum* or *Medicago sativa* [32]

Agrobacterium tumefaciens, updated scientific name: *Rhizobium radiobacter* [33], is an alphaproteobacterium of the family Rhizobiaceae, which includes the nitrogen-fixing the legume symbionts. *Azotobacter* species are free-living bacteria, nitrogen-fixing bacteria without symbiotic relations with plant although some *Azotobacter* species are associated with plants [34] and *Azotobacter tropicalis* is a species in tropical soil.

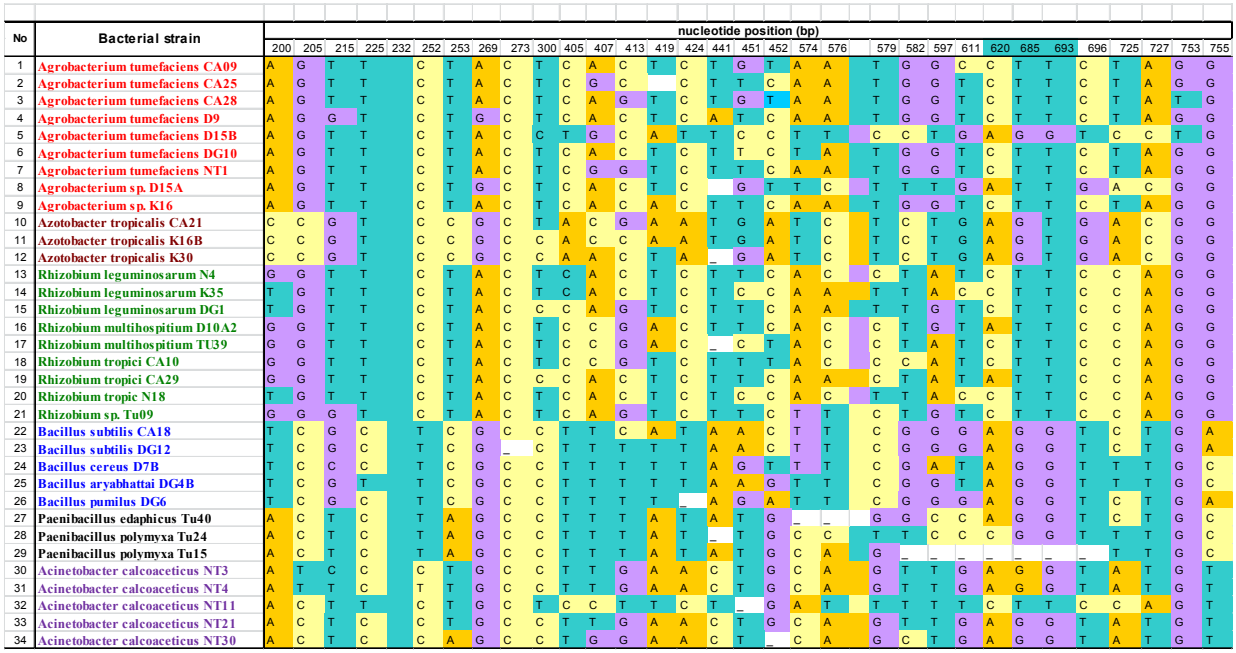

Figure 8. *Halotypes of 34 strains*

Bacillus subtilis is a gram-positive, aerobic, spore-forming soil bacterium ubiquitous in the environment and it is used as a probiotic [35]. Bottone and Peluso [36] discovered that an antifungal compound producing by *Bacillus pumilus* is active against Mucoraceae and *Aspergillus* species.

Siddikee et al. [37] identified *Bacillus aryabhattai* as plant growth promoting rhizobacteria (PGPR) that isolates from coastal soil of Incheon city, Korea. It can produce IAA, nitrogen fixation, P and Zn solubilization. *Paenibacillus polymyxa* (prevoiously *Bacillus polymyxa*) is one of many plant growth-promoting rhizobacteria and it was known to have a broad host plant range [38].

All strains described above are beneficial bacteria which have been used in biofertilizer production. However the strains of *Acinetobacter calcoaceticus* is a species of bacterium the genus *Acinetobacter* and part of the human body normal flora. It can be a pathogen for opportunistic infection on patients which have multiple underlying diseases [39].

Based on bio-safety and good characteristics, this study selected 3 strains as *Agrobacterium tumefaciens* CA09, *Rhizobium tropici* CA29 and *Azotobacter tropicalis* K16B to evaluate their effects on many kinds of crop cultivated on sandy acid soil of Tri Ton district, An Giang province, Vietnam with pot-experiment and the field trial.

4. Conclusion

From 118 weathered material/soil samples of granite rock from seven mountains (That Son), An Giang province, Vietnam, 237 isolates were isolated and identified as phosphate- and potassium- solubilizing bacteria. Based on the phylogenetic analysis and PCR amplification of 16S rRNA gene, 34/237 isolates had high ability of phosphate and potassium solubilization including 21 strains of Alphaproteobacteria, 8 strains of Bacilli and 5 strains of *Acinetobacteria calcoaceticus*. Among them, 3 strains will be evaluated their effects on many kinds of crop because of their biosafety and high ability of phosphate- and potassium-solubilization

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