Book Chapter

Isolation, Characterization and Identification of Phosphate and Potassium- Solubilizing Bacteria from Weathered Materials of Granite Rock Mountain, in the Mekong Delta Vietnam

Cao Ngoc Diep* and Nguyen Thi Don*

Can Tho University, Can Tho City, Vietnam

***Corresponding Authors:** Cao Ngoc Diep, Can Tho University, Can Tho City, Vietnam

Nguyen Thi Don, Can Tho University, Can Tho City, Vietnam

Published **May 11, 2020**

How to cite this book chapter: Cao Ngoc Diep, Nguyen Thi Don. Isolation, Characterization and Identification of Phosphate and Potassium- Solubilizing Bacteria from Weathered Materials of Granite Rock Mountain, in the Mekong Delta Vietnam. In: Malik Badshah, editor. Prime Archives in Biosciences. Hyderabad, India: Vide Leaf. 2020.

© The Author(s) 2020. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License(<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Summary

The weathering of rocks plays a role in a number of important environmental processes [1] to clay, silt and sand and these minerals are important components of soil in earth. Many types of microorganisms are known to inhabit soil, especially

rhizosphere and they play an important role in plant growth and (plant) development.

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 [2]. The Mekong Delta occupies 2.9 M (million) ha (12% of the Vietnam's total land area) and this delta 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 [3].

In Vietnam, granitoid distributed very popularly, they occupied 90% in magma rock. They distributed mainly at (in/across) East-Northern region (Song Chay, Biooc, Cho Chu, Nui La), at West-Northern region (Ca Vinh, Po Sen, Dien Bien Phu, Fanxipan), Middle region (Hai Van, Kong Tum, Song Chu – Ban Chieng), Southern region and South Middle region (Dai Loc, Chu Lai, Deo Ca, Dinh Quan, Ankroet), West Southern region (That son, Nui Sam, Hon Dat) and (granitoid in West Southern region) (this) is a valuable resource for farmers at the Mekong Delta, Vietnam, With 3 rock granite mountains (That son, Sap mountain, Ba Hon), they only have been exploited for tourism and temples and they have not used in agricultural production.

In three sites (in Mekong Delta), 137 weathered material/rock samples were collected to isolated on Alexandrov medium and 392 P-K solubilizing bacterial isolates were isolated and stored at -20°C. There are 43 among 392 isolates had high ability of phosphate and potassium solubilization (they consisted of including 13 strains of Bacilli and 30 strains were Proteobacteria.

Some good phosphate- and potassium- solubilizing bacteria strains were evaluated their effects on many kinds of crop, (the result) showed that they saved 25% amount of PK chemical fertilizers in comparison to (the) control.

Twelve P-K solubilizing bacterial strains are PGPR which they have characteristics as nitrogen fixation, phosphate and potassium solubilization, increasing concentration of nitrogen chemical fertilizer applied to vegetable led to increase nitrate concentration in leaf of *Brassica juncea* and *Acinetobacter calcoaceticus* NT30 strain suggest to use in biofertilizer production for leaf-eating vegetable nearly in the future.

Keywords

Bacilli; Kinds of Crop; Rock Granite Mountains; P-K Solubilizing Bacterial Strains; Proteobacteria; Weathered Rock

Introduction

Deficiency in plant-available phosphorus and potassium is considered to be a major limiting factor to food production in many agricultural soils [4]. 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 [5].

The P content in average soils is about 0.05% (w/w), but only 0.1% of the total P is available to plants [6]. 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 [7]. Soil has rich reserves of K, among which only 1 - 2% can be directly absorbed by plants [8]. Consequently, about 90 - 98% of the soil K exists in silicate minerals such as K-feldspars and mica, which only release K slowly [9]. 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 [9]. The weathering of rocks plays a role in a number of important environmental processes [1] 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 [10,11].

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 [2]. The Mekong Delta occupies 2.9 M (million) ha (12% of the Vietnam's total land area) and this delta 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 [3]. 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 available 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 [13-16]. In addition, researchers have discovered 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. [2] 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.

P-K solubilizing bacteria used as PGPR in crop cultivation because PGPR are a heterogeneous group of bacteria that can be found in the rhizosphere, which can improve the quality of the plant growth directly and or indirectly [18] as (i) their ability to produce plant growth regulators like indoleacetic acid, gibberellic acid and cytokinins [19], (ii) asymbiotic nitrogen fixation [20], (iii) antagonism against phytopathogenic microorganisms by production of siderophores [21], antibiotics [22] and cyanide [23], (iv) solubilization of mineral phosphates and other nutrients [24] and (v) active removal and bioaccumulation of heavy metals and their capacity to assist the root growth [25] to valuate in the cultivation of many kinds of crops.

Material and Methods

Earth formed approximately 4.6 billion years ago from a disk of dust and gas orbiting the newly formed Sun. It formed via accretion, where planetesimals and other smaller rocky bodies collided and stuck, gradually growing into a planet. This process generated an enormous amount of heat, which caused early Earth to melt completely. As planetary accretion slowed, Earth began to cool, forming its first crust, called a primary or primordial crust [2].

All soils initially come from rocks, this is termed the 'parent material'. The Parent Material may be directly below the soil, or great distances away if wind, water or glaciers have transported the soil. The formation of soils can be seen as a combination of the products of weathering, of structural development of the soil, of differentiation of that structure into horizons or layers, and lastly of its movement or translocation. In fact there are many ways in which soil may be transported away from the location where it was first formed. Soil is the result of the process of the gradual breakdown of rock - the solid geology that makes up the earth. As rock becomes broken down through a variety of processes, such as weathering and erosion, the particles become ground smaller and smaller.

Granite rock is calling the parent-materials there have 3 kinds of parent-materials: granite rock, sedimentary rocks, metamorphic rocks [26].

Magmatic rock, or Igneous rock is formed through the cooling and solidification of magma or lava. The magma can be derived from partial melts of existing rocks in either a planet's mantle or crust. Typically, the melting is caused by one or more of three processes: an increase in temperature, a decrease in pressure, or a change in composition. Solidification into rock occurs either below the surface as intrusive rocks or on the surface as extrusive rocks. Igneous rock may form with crystallization to form granular, crystalline rocks, or without crystallization to form natural glasses. Igneous rocks occur in a wide range of geological settings: shields, platforms, orogens, basins, large igneous provinces, extended crust and oceanic crust [27].

According to La Thi Chich [28], granite can be classified 4 types as follows: Granite type M: plagiogranite, tonalite.

- Granite type I: granodiorit, granite (poor Al, rich Na, Ca).
- Granite type S: light, neutralied Al
- Granite type A: base granite and fews sienit.

In Vietnam, granitoit distrubuted very popularly, they occupied 90% in magma rock. They distributed mainly at East-Northern region (Song Chay, Biooc, Cho Chu, Nui La), at West-Northern region (Ca Vinh, Po Sen, Dien Bien Phu, Fanxipan), Middle region (Hai Van, Kong Tum, Song Chu – Ban Chieng), Southern region and South Middle region (Dai Loc, Chu Lai, Deo Ca, Dinh Quan, Ankroet), West Southern region (That son, Nui Sam, Hon Dat) (Figure 1), and Hon Khoai island. They have different year-old from ancient (Pre-Cambrien) to young (mezozoi) and they distributed in varirous stages, different components from granitoit to granite alaskit, with many different types as granite type I, S or M [28].

Three Sites have Granite Mountains That Son Site

That Son site has seven mountains (Cam, Dai, Dai Nam-Gieng, To, Tuong, Ket, Nuoc) with high altitude from 54 m to 705 m in comparison to sea level, That Son belongs to An Giang province, Vietnam, situated 10°22'52" north latitude and 105°52'12" east longitude. Many mountains have the beautiful pagodas that are famous tourism, granite rock of mountain has been used as material for way construction.

Sap Mountain Site

Sap mountain site with high altitude from >105 m in comparison to sea level. Sap mountain located at Thoai Son district, An Giang province, situated 10°15'35.9" north latitude 105°15'49.5" east longitude (Figure 1).

Ba Hon site

Ba Hon site is comprised of Hon Dat, Hon Me and Hon Queo; Ba Hon have high altitude >150 m in comparison to sea level, they located at Hon Dat district, Kien Giang province (at the seaside of Thailand Bay); situated 10°06'05.3" north altitude 104°53'44.9" east longitude (Figure 1).

Mineral Component in Granite Rock

Mineral component in granite rock been classified to per cent of the minerals containing in the granite rock at New Brunswick (Table 1). From the result of Table 1 showed that K₂O element in granite rock contains K element very rich, and it is an important element for crop cultivation.

Figure 1: Three regions have granite in the Mekong Deltas, Vietnam (West Southern region (That son, Nui Sam, Hon Dat)).

Table 1: Representattion chemical composition (wt%dspar in granitoids from southwestern New Brunswick.

Intrusions	Mount Pleasant	Beach hill	Kedron	Pleasant Ridge	Sorrel Ridge	Mount Douglas	Lake George	Tower Hill	Magaguadavie
Sample	AM96-3-1233	AM96-3-1233	BH01-111	BH01-111	BR84-4-87	BR84-4-87	C82-4-14	C82-4-14	C81-9-258
Mineral	AM96-3-1233	AM96-3-1233	BH01-111	BH01-111	BR84-4-87	BR84-4-87	C82-4-14	C82-4-14	C81-9-258
No. of Grains	K-feldspar	Plagioclase	K-feldspar	Plagioclase	K-feldspar	Plagioclase	K-feldspar	Plagioclase	K-feldspar
n	2	3	2	1	2	2	2	2	6
SiO ₂	5	5	3	3	6	6	6	6	11
TiO ₂	0.01	0.00	0.00	0.00	0.01	0.01	0.03	0.00	0.01
Al ₂ O ₃	19.37	21.39	19.66	21.48	19.67	20.89	19.41	21.20	18.89
Cr ₂ O ₃	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.05	0.01
Fe ₂ O ₃	0.06	0.19	0.05	0.23	0.00	0.02	0.00	0.02	0.05
MnO	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.03	0.00
MgO	0.00	0.01	0.00	0.00	0.00	0.00		0.00	0.00
CaO	0.01	0.23	0.01	0.92	0.01	0.05	0.07	0.20	0.09
Na ₂ O	0.16	12.25	0.44	11.90	0.18	12.88	0.29	13.01	0.83
K ₂ O	16.12	0.25	15.71	0.24	16.02	0.12	15.57	15.32	15.52
Total	101.6	100.9	101.2	101.2	100.0	101.0	101.2	99.6	100.9

Intrusions	Mount Pleasant	Beach hill	Kedron	Pleasant Ridge	Sorrel Ridge	Mount Douglas	Lake George	Tower Hill	Magaguadavie
Sample	AM96-3-1233	AM96-3-1233	BH01-111	BH01-111	BR84-4-87	BR84-4-87	C82-4-14	C82-4-14	C81-9-258
Mineral	AM96-3-1233	AM96-3-1233	BH01-111	BH01-111	BR84-4-87	BR84-4-87	C82-4-14	C82-4-14	C81-9-258
No. of Grains	K-feldspar	Plagioclase	K-feldspar	Plagioclase	K-feldspar	Plagioclase	K-feldspar	Plagioclase	K-feldspar
n	2	3	2	1	2	2	2	2	6
SiO ₂	5	5	3	3	6	6	6	6	11
TiO ₂	0.01	0.00	0.00	0.00	0.01	0.01	0.03	0.00	0.01
Al ₂ O ₃	19.37	21.39	19.66	21.48	19.67	20.89	19.41	21.20	18.89
Cr ₂ O ₃	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.05	0.01
Fe ₂ O ₃	0.06	0.19	0.05	0.23	0.00	0.02	0.00	0.02	0.05
MnO	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.03	0.00
MgO	0.00	0.01	0.00	0.00	0.00	0.00		0.00	0.00
CaO	0.01	0.23	0.01	0.92	0.01	0.05	0.07	0.20	0.09
Na ₂ O	0.16	12.25	0.44	11.90	0.18	12.88	0.29	13.01	0.83
K ₂ O	16.12	0.25	15.71	0.24	16.02	0.12	15.57	15.32	15.52
Total	101.6	100.9	101.2	101.2	100.0	101.0	101.2	99.6	100.9

Origin: Table 3 Representative chemical composition (wt%) of feldspar in granitoids from southwestern New Brunswick and end-member moleratios (XAb, XOr, and XAn) from Xue-Ming Yang & David R. Lentz Chemical composition of rock-forming minerals in gold-related granitoid intrusions, southwestern New Brunswick, Canada: implications for crystallization conditions, volatile exsolution, and fluorine-chlorine activity Contrib Mineral Petrol (2005) 150: 287–305 DOI 10.1007/s00410-005-0018-7

Bacteria Isolation and Growth Conditions

Bacterial isolates were isolated from weathering materials of granite rock mountain, That Son, An Giang Province, Vietnam (Figure 2), by virtue of their abilities to solubilize mineral P- and K. The samples were stored at 10°C during transit and processed immediately.

Figure 2: Plants and herbs developed on the granite rock of That son site (right) and weathering material and soil samples were collected from weathered materials and soil along from rock (left).

Soil samples or weathered 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 [2] with 0.2% apatite and 0.2% kaolinite and shaken for 24 h on orbital shaker at 50 rev min⁻¹ at temperature room.

At Sap mountain site, weathered materials and soils were also collected as described above (Figure 3)

Figure 3: Plants and herbs developed on the granite rock of Sap mountain site (right) and weathered material and soil samples were collected from one plant grew on the this material (left).

Similarly, at Ba Hon site, plants and herbs developed on the granite rock on weathered material and soil (Figure 4)

Figure 4: Plants and herbs developed on the granite rock of Hon Me (right) and weathered material and soil samples of Hon Dat (left) (Ba Hon site).

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 3×10^7 cfu ml⁻¹). Autoclaved, uninoculated medium served as controls. The falcon tubes were incubated for 10 days on orbital 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 centrifuged 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 [29]. 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.

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.

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.

Phylogenetic Analysis of 16S rRNA Gene Sequence

Bacteria universal primers, the forward primer fD1 and the reverse primer rP [2] 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 [30]. The 16S rRNA gene sequence was amplified in a PCR mixture, composed with 1 $\mu\text{mol l}^{-1}$ of each primer. 200 $\mu\text{mol l}^{-1}$ of each dNTP, 50 $\mu\text{mol l}^{-1}$ KCl and 1.5 mol l^{-1} MgCl_2 in 10 mol l^{-1} Tris/HCl (pH 8.3) buffer. DNA (0.1 μg) and 2.5U 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 [31] and the phylogenetic analysis was constructed by the Maximum Likelihood method based on 1.000 bootstraps.

SNPs Discovery

The sequence data 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.

Nucleotide Diversity (Θ)

Nucleotide diversity (Θ) was calculated by the method described by Halushka et al. [32]

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

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

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

Results and Discussion

Bacteria Isolation and Colony Characteristics

That Son Site

In That son site, physical and chemical characteristics of weathered material from granite rock of these mountains showed that neutral pH, low N total but high organic matter, P_2O_5 and K_2O content and high sand ratio compared to silt and clay.

Table 2: Physical and chemical characteristics of weathered material from granite rock of these mountains of That Son, An Giang province, Vietnam (That Son site)

Site	pH	N _{total} (%)	Available P ₂ O ₅ (mg/kg)	Exchangeable K ₂ O (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 5 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

Origin: Advanced Lab., Can Tho University, Vietnam

From 118 soil samples/weathering materials, 237 isolates were isolated on Aleksandrov medium (Table 3). They developed very well on this medium from 48 - 72 h at 30°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 5 and 6). This result was the same Xiufang's experiment [2] 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°C, these results were the same of previous our experiments in denatured rock mountan, Ha Tien, Viet nam [33], from calcacerous mountain, Ha Tien [34].

Table 3: 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 5: Characteristic of colonies of bacterial isolates

Figure 6 Electromicrographs of cell after grown on Aleksandrov medium

Sap Mountain Site

In SAP mountain site, high pH and sand ratio but low P₂O₅ and organic matter perhaps people's action in the tourism rather than agricultural action (Table 4).

Table 4: Physical and chemical characteristics of weathered material from granite rock of SAP mountain, An Giang province, Vietnam (Sap mountain site).

pH	N _{total} (%)	Available P ₂ O ₅ (mg/kg)	Exchangeable K ₂ O (mg/kg)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
7.19	0.19	15.34	133.61	3.02	81.24	5.16	13.60

Analysed: Advanced Lab., Can Tho University, Vietnam

From 9 weathered material samples of granite rock of Sap mountain An Giang province, twenty-six P- and K-solubilizing bacteria were isolated. Their colonies were round or irregular,

white or pale yellow (Figure 7) and their cell were rod shape or the short chain and nonmotile or motile (Figure 8). Twenty-six isolates had ability of solubilized of K and P and all of them had their solubilization higher than control, sixteen isolates were chosen to study next step (Table 7).

Ba Hon site

On the contrary with Sap mountain, physical and chemical characteristics of weathered material from granite rock of Ba Hon mountains are similar with That Son site because people planted many kinds of fruit on the mountains (Hon Soc, Hon Queo and Hon Dat), this led high N total, exchangeable K and organic matter concentration in weathered material and soil but low available P₂O₅ level (Table 5).

Table 5: Physical and chemical characteristics of weathered material from granite rock of Ba Hon mountains, Kien Giang province, Vietnam (Ba Hon site).

pH	N _{total} (%)	Available P ₂ O ₅ (mg/kg)	Exchangeable K (mg/kg)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
6.45	0.21	7.42	310.1	5.02	65.25	18.50	16.25

Analysed: Advanced Lab., Can Tho University, Vietnam

Twenty-nine bacterial isolates were isolated from weathered material/soils of Hon Queo, Hon Me, Hon Dat, their colonies were round or irregular, both white (Figure 9) and their shape was rod, motile (Figure 10). Especially, all of bacterial isolates isolated at three sites have characteristic producing slime (high viscosity) covering isolates.

Figure 7: Characteristic of colonies of bacterial isolates

Figure 8: Electromicrographs of cells at Sap mountain site

Figure 9: Characteristic of colonies of bacterial isolates

Figure10: Electromicrographs of cells at Ba Hon mountains

The Ability of P and K Solubilizing Bacterial Isolates That Son Site

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 6) in 34 good isolates which isolated at weathered material/soil of rock granite at That son site.

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 K₂O (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 6). 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.

Table 6: Phosphate and potassium solubilization of 34 isolates.

Isolate	Solubilization of phosphate (mg P_2O_5 l ⁻¹)	Solubilization of potassium (mg K_2O l ⁻¹)	Isolate	Solubilization of phosphate (mg P_2O_5 l ⁻¹)	Solubilization of potassium (mg K_2O 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

P solubilizing bacteria and silicate bacteria play an important role in plant nutrition through the increase in P and K uptake by the plant [1]. Application of phosphate solubilizing bacteria have been used as P-biofertilizer for crop cultivation [35]. 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 [5,36].

Sap Mountain Site

Table 7: Phosphate and potassium solubilization of 16 isolates.

Isolate	Solubilization of phosphate (mg P_2O_5 l ⁻¹)	Solubilization of potassium (mg K_2O l ⁻¹)	Isolate	Solubilization of phosphate (mg P_2O_5 l ⁻¹)	Solubilization of potassium (mg K_2O l ⁻¹)
NS1	17.51 g	25.66 e	NS10	45.50 c	19.90 f
NS2	33.71 d	32.19 c	NS11	70.32 b	27.49 d
NS3	22.17 f	28.62 d	NS12	16.14 g	36.04 b
NS4	23.27 f	26.07 e	NS13	12.67 k	33.94 c
NS5	30.67 e	31.10 c	NS14	10.95 k	21.04 f
NS6	17.98 g	48.76 a	NS15	16.87 g	21.01 f
NS7	14.37 h	28.59 d	NS16	17.74 g	21.54 f
NS8	85.66 a	28.59 d	Control	02.22 i	05.18 g
NS9	21.21 f	25.11 e	C.V.	5.87%	3.11%

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

Ba Hon Site

From 29 isolated isolates, 12/29 isolates had high solubilization of P and K was presented in Table 8.

Table 8: Phosphate and potassium solubilization of 12 isolates.

Isolate	Solubilization of phosphate (mg P ₂ O ₅ l ⁻¹)	Solubilization of potassium (mg K ₂ O l ⁻¹)	Isolate	Solubilization of phosphate (mg P ₂ O ₅ l ⁻¹)	Solubilization of potassium (mg K ₂ O l ⁻¹)
HĐ1C	15.33 b	52.64 c	HQ8A	10.89 e	6.71 g
HĐ1D	20.34 a	59.28 a	HQ9A	11.97 d	5.34 h
HĐ2B	14.09 b	58.04 a	HQ9B	10.21 e	11.71 f
HĐ2C	14.09 b	52.22 c	HQ9C	21.33 a	15.51 e
HĐ2E	14.62 b	50.27 d	HQ10A	20.44 a	14.64 e
HM5C	13.00 c	55.35 b	Control	01.43 h	00.69 o
HM6A	15.04 b	58.79 a	C.V.	4.10%	1.38%

In general, the isolates had the highest phosphate solubilization isolated at weathered material/soils of Sap mountain site (NS8 and NS11 isolates) and the isolates had the highest potassium solubilization isolated at Ba Hon site (HD1D, HD2B, HM6A).

Identification of Bacterial Isolates

That Son Site

All of them (34 isolates) were chosen to identify and the fragment of 1500 bp 16S rDNA were obtained from PCR and sequencing (Table 9).

Table 9: 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	<i>Bacillus subtilis</i> A2-9 (JF496331)	99
DG12	<i>Bacillus subtilis</i> ANctcri3 (HQ286641)	99
D7B	<i>Bacillus cereus</i> strain DZ4 (HQ143564)	99
DG4B	<i>Bacillus aryabhatai</i> PSB59 (HQ242772)	99
DG6	<i>Bacillus pumilus</i> strain PJ-3 (KJ195695)	99
Tu15	<i>Paenibacillus edaphicus</i> EA3-10 (JF496414)	100
N24	<i>Paenibacillus polymyxa</i> CE42 (JN084141)	99
Tu40	<i>Paenibacillus polymyxa</i> M1 (FR727737)	100
Alphaproteobacteria		
CA09	<i>Agrobacterium tumefaciens</i> M5 (EF443163)	99
CA25	<i>Agrobacterium tumefaciens</i> AN17 (KF439828)	99
CA28	<i>Agrobacterium tumefaciens</i> B228 (GQ169811)	99
D9	<i>Agrobacterium tumefaciens</i> NGB-SR16 (AB825997)	99
D15B	<i>Agrobacterium tumefaciens</i> B8S (AY850392)	99
DG10	<i>Agrobacterium tumefaciens</i> T912-2 (KF463142)	99
NT1	<i>Agrobacterium tumefaciens</i> BLN4 (GQ181060)	99
D15A	<i>Agrobacterium</i> sp. EC080527_02 (FJ593843)	99
K16	<i>Agrobacterium</i> sp. P29 (KF465838)	99
CA21	<i>Azotobacter tropicalis</i> KBS (AB236160)	99
K16B	<i>Azotobacter tropicalis</i> BKK.2 (AB236162)	99
K30	<i>Azotobacter tropicalis</i> IARI-THW-22 (KF054975)	99
CA10	<i>Rhizobium tropici</i> CIAT 899 (NR_102511)	99
CA29	<i>Rhizobium tropici</i> strain CAF439 (FJ405380)	99
N18	<i>Rhizobium tropici</i> Br859 (HQ394213)	99
DG1	<i>Rhizobium leguminosarum</i> NGB-FR-137 (AB749224)	99
K35	<i>Rhizobium leguminosarum</i> J-7HPT1 (KF468790)	99
N4	<i>Rhizobium leguminosarum</i> 37-2 (JN105996)	99
D10A2	<i>Rhizobium multihospitium</i> strain CC-13H (JN896359)	99
Tu39	<i>Rhizobium multihospitium</i> CCBAU 83364 (EF490014)	99
Tu09	<i>Rhizobium</i> sp. ICB456 (HM486519)	99
Gammaproteobacteria		

NT3	<i>Acinetobacter calcoaceticus</i> LCR59 (FJ976567)	99
NT4	<i>Acinetobacter calcoaceticus</i> LCR100 (FJ976609)	99
NT11	<i>Acinetobacter calcoaceticus</i> LCR102 (FJ976611)	99
NT21	<i>Acinetobacter calcoaceticus</i> EU99 (FJ681294)	99
NT30	<i>Acinetobacter calcoaceticus</i> LCR17 (FJ976526)	99

Using the Maximum-Likelihood method analyzed the relationship of 34 strains through the sequences for 16S rRNA gene sequence by the BLAST search programme, the results showed that they can be grouped into two clusters (Figure 11).

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 in That son site 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 bacteria was higher than that of Gram-positive bacteria.

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 11).

Figure 11: 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 nodes 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 12: 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, (P_i), Theta (Θ) (per group) etc... In this study, nucleotide diversity was estimated by Theta (Θ), the number of segregating sites [37], and its standard deviation ($S\Theta$). These parameters were estimated by DNA Sequence Polymorphism software version 4.0 [38]. P_i values explained nucleotide diversity of sequences for each gene, the higher values, and the more diversity among groups. Bacilli group had the highest P_i 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 12). However Bacilli group with 8 strains had P_i 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 12).

Theta value in nucleotide position (Figure 12) showed that nucleotide position varied from 200 position to 232 position, from 252 position to 576 position and 579 position to 755 position.

Table 10: Nucleotide diversity (Θ) values of two EST's using the programme DNAsp 5.0 [31].

ESTs	Alpha Proteobacteria	Bacili	Gama Proteobacteria
Nucleotide diversity (Pi)	0.09261	0.13005	0.10966
Theta (per sequence) from S	51.421 $\pm$ 3.780	84.463 $\pm$ 5.707	89.862 $\pm$ 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 13: Variation of Theta value from 200 position to 755 position of 34 strains.

Haplotype analysis of 34 strains was presented in Figure 13. This figure revealed that there was genetic diversity between the strains of *Agrobacterium* group, the strains of *Azotobacter tropicalis*, strain of genus *Rhizobium leguminosarum*, 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 [39] and Matinez-Romero et al. [40] 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 they are symbiotically now stable. Based on the phenotypic characterization and *nodD*, *nifH* 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* [41].

Agrobacterium tumefaciens, updated scientific name: *Rhizobium radiobacter* [42], 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 (Kass et al, 1977) and *Azotobacter tropicalis* is a species in tropical soil.

Sap Mountain

All of them (26 isolates) were chosen to identify and the fragment of 1500 bp 16S rDNA were obtained from PCR however 4 isolates had high P and K solubilization to sequence (Table 10).

Among 4 atrains divided to two 2 groups:cluster A cmposed of three strains: *Bacillus* sp. NS7, *Acinetobacter soli* NS12 and *Bacillus altitus* NS3 and cluster B only had 1 strain *Brevibacillus nitrificans* NSs.

Table 10: 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 (%)
NS3	<i>Bacillus altitus</i> strain 391 (EM_PROKT260603)	100
NS7	<i>Bacillus</i> sp. CSGSY4 (EM_PROKJ 184936)	99.4
NS8	<i>Brevibacillus nitrificans</i> HBUM07084 (EM_PROMF882510)	99.4
NS12	<i>Acinetobacter soli</i> strain 25M (EM_PROMK874924)	99.8

Figure 15: Phylogenetic tree for partial 16S rRNA gene sequences from 5 isolates by using primers (fD1, rP) showing relationships between representative strains along with related sequences retrieved from GenBank. The numbers at the nodes indicate the levels of bootstrap support (%) based on a UPGMA analysis of 100 re-sampled datasets. The scale bar indicates the phylogenetic distance corresponding to 5 changes per 100 bases.

Ba Hon site

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

Cluster A composed of three strains: *Bacillus* sp. HD1c, *Pseudomonas* sp. HM6a and *Klebsiella aerogenes* HQ10a, three these strains originated from three different islands (Hon Dat, Hon Me and Hon Queo). Cluster B had 2 strains were Bacilli (*Bacillus* sp. HD1D and *Bacillus subtilis* HQ9c) and they originated from 2 islands (Hon Dat and Hon Queo)

Table 11: 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 (%)
HD1c	<i>Bacillus</i> sp. ESC203 (EM_PROHQ024491)	99.9
HD1D	<i>Bacillus</i> sp. ESC203 (EM_PROHQ024491)	99.8
HQ9c	<i>Bacillus subtilis</i> strain H19 (EM_PRO MG383451)	99.9
HM6a	<i>Pseudomonas</i> sp. strain LJL11 (EM_PRO MG8333337)	99.6
HQ10a	<i>Klebsiella aerogenes</i> strain 1103 (EM_PRO MG031163)	99.8

Figure 16: Phylogenetic tree for partial 16S rRNA gene sequences from 5 isolates by using primers (fD1, rP) showing relationships between representative strains along with related sequences retrieved from GenBank. The numbers at the nodes 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.

All strains described above are beneficial bacteria which have been used in biofertilizer production. 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.

Effect of P-K Solubilizing Bacterial Strains on the Various Crops

Effect of P-K Solubilizing Bacterial Strains on White Radish, Peanut, and High Yielding Rice

The effects of three effective phosphate and potassium - solubilizing bacterial strains (*Agrobacterium tumefaciens* CA09, *Rhizobium tropici* CA29, *Azotobacter tropicalis* K16B) on the growth and yield of white radish, peanut, and high-yielding rice cultivated on sandy soil of TriTon district, An Giang province.

The experiment was conducted with 4 levels of potassium and phosphorus fertilizers (0% PK, 25% PK, 50% PK and 75% PK) combined with three isolates. The results showed that there was no significant difference in growth and component of yields of white radish, (Table 12) peanut, and high yielding rice between the treatment of 75% PK + potassium – solubilizing bacteria with the positive control (100% PK). It is therefore concluded that three phosphate and potassium solubilizing bacterial strains had ability of solubilization of phosphate and potassium and provided 25% amount of P and K for the growth of white radish, peanut and high yielding rice. On the other hands, three isolates made increasing in the concentrations of available phosphate, total of nitrogen and organic matter in soil.

White Raddish

Application of 150 kg N/ha + 120 kg P + 90 kg K/ha in white raddish cultivation in sandy soil to help enhancing high yield component and tuber yield (Table 12) however applying P-K solubilizing bacterial strain *Azotobacter tropicalis* K16B plus 75% amount of P-K: 120 kg P + 90 kg K/ha had the highest yield component, tuber yield and biomass yield (Figure 17). Therefore we can save 25% amount of P-K and applying *Azotobacter tropicalis* K16B plus 75% amount of P-K had biomass yield higher than white raddish only applied 25%, 50% and 75% amount of P-K without *Azotobacter tropicalis* K16B. If compared to biomass yield, application of *Azotobacter tropicalis* K16B plus 75% amount of P-K had biomass yield did not differ

from applying 100% P-K 150 kg N/ha + 120 kg P + 90 kg K/ha in white raddish cultivation significantly (Figure 17).

Table 12: Effects of P-K solubilizing bacterial strain (*Azotobacter tropicalis* K16B) and phosphate and potassium fertilizers on the growth and yield of white raddish cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

Treatment	Plant height (cm)	Root length (cm)	Leaf number/plant	Tuber Length (cm)	Tuber Diameter (cm)	Tuber Weight (gr)
Control	19.5 c	20.8 d	14. c	20.1 e	3.31 d	95.3 f
100% P-K	23.4 a	23.7 a	15.8 a	23.5 a	4.32 a	149.5 b
K16B + 0% P-K	22.2 b	21.2 d	14.5 c	21.2 c	3.54 cd	105.3 ef
K16B + 25% P-K	22.9 ab	22.6 abc	15.1 abc	21.7 c	3.81 bc	107.0 ef
K16B + 50% P-K	22.3 b	23.0 abc	15.9 a	22.3 b	4.03 b	128.8 d
K16B + 75% P-K	23.2 a	23.5 abc	16.0 a	23.0 ab	4.52 a	156.1 a
25% P-K	21.2 c	21.7 cd	14.7 bc	20.9 d	3.61 cd	102.8 ef
50% P-K	22.5 ab	23.5 abc	15.6 ab	21.9 cd	3.65 bc	116.8 e
75% P-K	23.4 a	23.2 ab	15.6 ab	22.6 b	3.91 bc	136.0 c
C.V%	5.03	4.13	4.12	5.03	7.03	14.3

Numbers following the same word not difference at 1% level
All treatments applied 150 kg N/ha , P-K: 120kg P + 90kg K (ha)

Figure 17: Effects of P-K solubilizing bacterial strain *Azotobacter tropicalis* K16B) and phosphate and potassium fertilizers on biomass yield (ton/ha) of white raddish cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

The result from table showed that pH soil after harvesting white raddish reduced in comparison to initial, this can explain the

action of bacteria as excretion of organic acid to soluble Ca-P or complex-K in soil and the result led to pH soil reduced as oxalic acid, citric acid, butyric acid malonic acid, adipic acid and 2 – ketogluconic acid and by the bacteria as *Pseudomonas* [43], *Enterobacter* [44,45], *Burkholderia* [46] and the led to reduce soil pH [47]. however available P and organic matter concentration in soil increasing (Table 13).

Available P concentration in soil at initial stage at positive control 9100 P-K treatment) reached to 9.92 mg/kg soil (Bảng 6) was higher than and did not differ from K16B + 75% PK (9.32 mg/kg soil treatment. Treatment of application of *Azotobacter tropicalis* strain K16B had available P concentration in soil higher than available P concentration of control (>2 times). This showed that *Azotobacter tropicalis* strain K16B had the ability of P solubilization in soil to provide for the growth of white radish and it also enhanced organic matter content in soil, this result contributed to improve soil fertility and indirectly for soil fertility in next cropping-season.

Peanut

In this experiment, *Rhizobium tropici* strain CA29 used as P-K solubilizing bacterial strain together with different chemical P-K fertilizer levels

The highest plant height and root length of treatment of applying P-K solubilizing strain *Phizobium tropici* strain CA29 plus 75% PK chemical P-K fertilizer and this treatment did not differ from treatment positive control (100% PK = 150P - 100K) significantly (Table 13). On the other hand, inoculated peanut without PK fertilizer had plant height and root length equivalent with peanut only applied 25% PK without inoculation therefore rhizobia also supported nutrients to develop plant height and root length of peanut cultivated on sandy soil.

Table 13: Effects of P-K solubilizing bacterial strain *Azotobacter tropicalis* K16B) and phosphate and potassium fertilizers on pH and chemical characteristics of sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015 before and after white raddish cultivation.

Treatment	pH	N total (%)	Available P (mg/kg)	Organic matter (%)
Initial	5.65	0.48	0.44	2.53
Control	5.12 a	0.31 d	3.42 e	2.52 d
100% P-K	4.20 c	0.41 a	10.73 a	3.12 ab
K16B + 0% P-K	4.80 b	0.35 c	7.26 c	2.92 bc
K16B + 25% P-K	4.44 cd	0.36 bc	7.42 c	3.08 ab
K16B + 50% P-K	4.35 d	0.37 b	7.77 c	3.15 a
K16B + 75% P-K	4.21 e	0.40 ab	10.09 ab	3.21 a
25% P-K	4.69 b	0.31 d	6.39 d	2.75 c
50% P-K	4.50 c	0.32 d	7.34 c	2.78 c
75% P-K	4.32 de	0.35 c	8.43 b	2.92 c
C.V	6.71%	7.12%	5.09%	8.49%

All treatments applied 150 kg N/ha , P-K: 120kg P + 90kg K (ha)

Table 14: Effects of P-K solubilizing bacterial strain (*Rhizobium tropici* strain CA29) and phosphate and potassium fertilizers on the growth and yield of peanut cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

Treatment	Plant height (cm)	Root length (cm)	2-seed pod /plant	3-seed pod /plant	4-seed pod /plant
Control	67.1 c	16.3 d	7.42 d	4.91 e	0.91 e
100% P-K	80.7 ab	19.9 ab	9.71 a	8.02 a	4.51 a
CA29 + 0% P-K	73.6 bc	16.6 cd	7.43 cd	6.13 cd	2.05 d
CA29 + 25% P-K	81.3 a	18.6 bc	8.61 b	6.34 cd	2.40 cd
CA29 + 50% P-K	80.9 ab	18.4 bc	8.19 bc	6.91 bc	3.22 b
CA29 + 75% P-K	82.5 a	20.8 a	9.41 a	7.45 ab	3.90 ab
25% P-K	75.2 ab	16.8 cd	7.92 bcd	5.83 de	2.21 cd
50% P-K	73.3 bc	16.3 d	7.81 bcd	6.11 de	2.31 cd
75% P-K	81.3 a	18.6 bc	8.09 bcd	6.31 cd	2.82 bc
C.V (%)	3.45	8.12	7.02	13.1	13.4

Numbers following the same word not difference at 1% level

Application of *Rhizobium tropici* strain CA29 plus 75% PK (150-75-50) in peanut cultivation had the highest number of 2-seed pod/plant, 3-seed pod/plant and 4-seed pod/plant and this

result did not differ from positive control treatment (150-150-100). Inoculated peanut plus 75% PK had the highest number of pod/plant and seed-pod/plant and this led it had the highest yield-pod (Table 14) and this result also showed that there was no significant difference in yield pod of peanut of positive control (150-150-100).

Inoculation of peanut with *Rhizobium tropici* strain CA29 plus 25%, 50% and 75% PK had grain yield increasing but peanut plus 25%, 50% and 75% peanut cultivation without inoculation also had grain yield increasing but grain yield of these treatments were lower than inoculated peanut and applying the same PK levels, this demonstrated that the effectiveness of *Rhizobium tropici* strain CA29 enhanced more grain yield because it can fix nitrogen biologically to provide for the growth of peanut.

Application of rhizobia and PK enhanced lipid content in seed in comparison to negative control (0 inoculation – 0 PK); applying 100 N-P-K (150-150-100) [positive control] and inoculation and 75% PK had the highest lipid content in seed and they did not differ from significantly. When combination of seed-yield and lipid content in seed to the highest total lipid in seed compared to others. Inoculation of *Bradyrhizobium* or/plus *Pseudomonas* increased protein content in seed from 25.13% and 25.94% and lipid content in seed from 47.76% and 48.82 (Rizk et al., 2012); Inoculation of *Badyrhizobium* + *Pseudomonas* enhanced protein content in seed from 23.97% and 24.90 and lipid content in seed 47.03% and 48.06% [48].

Peanut cultivation applied NPK chemical fertilizers and inoculation which improved soil fertility as N total in soil, available P and organic matter in comparison to initial (Table 16).

Table 15: Effects of P-K solubilizing bacterial strain (*Rhizobium tropici* strain CA29) and phosphate and potassium fertilizers on the growth and yield of peanut cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

Treatment	Number of pod/plant	Number of seed-pod/ plant	No seed/pod ratio (%)	Pod-yield (ton/ha)	100-seed Weigh (gr)
Control	20.2 d	16.5 e	19.9 a	1.46 f	53.0 d
100% P-K	29.2 a	24.9 a	24.6 c	2.96 a	57.3 ab
CA29 + 0% P-K	21.8 bc	18.4 c	16.1 c	2.06 e	54.1 cd
CA29 + 25% P-K	24.5 c	20.6 c	15.6 de	2.49 bc	53.4 d
CA29 + 50% P-K	25.3 bc	21.4 b	15.4 de	2.74 ab	55.5 bc
CA29 + 75% P-K	28.2 a	24.1 ab	14.5 ef	2.94 a	57.5 a
25% P-K	22.1 bc	18.6 d	15.9 b	2.12 de	54.7 cd
50% P-K	22.3 bc	19.1 d	14.5 c	2.21 de	55.3 c
75% P-K	24.2 ab	20.8 bc	14.4 bc	2.35 cd	55.2 c
C.V (%)	10.02	6.12	7.1	2.35	4.21

Numbers following the same word not difference at 1% level

Figure 18: Effects of P-K solubilizing bacterial strain (*Rhizobium tropici* strain CA29) and phosphate and potassium fertilizers on lipid content in seed (%) and total of lipid concentration (seed yield (kg/ha) x lipid content in seed) of peanut cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

Table 16: Effects of P-K solubilizing bacterial strain (*Rhizobium tropici* strain CA29) and phosphate and potassium fertilizers on pH and chemical characteristics of sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015 before and after peanut cultivation.

Treatment	pH	N total (%)	Available P (mg/kg)	Organic matter (%)
Initial	5.44	0.32	0.42	2.69
Control	6.37 a	0.35 cd	0.39 f	2.55 c
100% P-K	5.22 d	0.46 a	9.92 a	3.00 ab
CA29 + 0% P-K	5.97 b	0.36 c	6.73 d	2.81 c
CA29 + 25% P-K	5.53 cd	0.38 c	6.88 d	2.97 ab
CA29 + 50% P-K	5.41 d	0.39 c	7.20 c	3.03 a
CA29 + 75% P-K	5.24 d	0.42 ab	9.32 b	3.08 a
25% P-K	5.84 c	0.34 d	5.76 c	2.68 d
50% P-K	5.60 c	0.35 cd	6.61 d	2.72 cd
75% P-K	5.37 d	0.41 abc	7.59 c	2.85 c
C.V	4.05%	3.27%	12.17%	9.78%

All treatments applied 150 kg N/ha , P-K: 120kg P + 90kg K (ha)
Numbers following the same word not difference at 1% level

Table 17: Effects of P-K solubilizing bacterial strain (*Agrobacterium tumefaciens* strain CA09) and phosphate and potassium fertilizers on the growth and yield of high-yielding rice cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

Treatment	Plant height (cm)	Flower Length (cm)	Flower Number/ m ²	Grain number/ flower	No-seed grain/ flower (%)	1000-grain weigh (gr)
Control	77.1 c	17.95 c	475 c	66.8 cd	18.11 ab	25.39 b
100% P-K	82.7 a	18.68 b	551 b	70.0 b	15.44 bc	26.22 ab
CA09 + 0% P-K	81.2 ab	18.62 b	490 c	67.4 bcd	15.83 bc	26.47 ab
CA09 + 25% P-K	82.0 ab	19.18 b	580 b	67.7 bcd	15.15 c	26.26 ab
CA09 + 50% P-K	79.8 b	19.47 b	595 ab	73.3 abc	16.99 abc	26.00 ab
CA09 + 75% P-K	81.3 ab	20.48 a	632 a	75.2 ab	15.39 bc	26.76 a
25% P-K	79.7 b	18.91 b	475 c	60.9 d	19.46 a	26.57 ab
50% P-K	80.4 ab	19.46 b	603 ab	76.3 a	15.91 bc	26.36 ab
75% P-K	80.3 ab	19.17 b	571 ab	73.6 abc	16.05 bc	26.02 ab
C.V (%)	2.23	4.57	12.8	10.7	10.3	2.68

Numbers following the same word not difference at 1% level

Plant height and flower length of high-yielding rice of inoculated rice plus 75% PK fertilizers treatment did not differ from positive control treatment (100% PK) significantly (Table 17). Inoculation of *Agrobacterium tumefaciens* strain CA09 into rice grain before sowing without PK fertilizers helped flower number/ m² no difference from treatment of rice applying 25% PK fertilizers without inoculation and grain yield of inoculated rice plus 25%, 50% and 75% PK fertilizers had always higher than rice applying 25%, 50% and 75% PK fertilizers without inoculation, this result demonstrated that the effectiveness of *Agrobacterium tumefaciens* strain CA09 to the rice growth. (Figure 19).

Figure 19: Effects of P-K solubilizing bacterial strain (*Agrobacterium tumefaciens* strain CA09) and phosphate and potassium fertilizers on grain yield (ton/ha) of high-yielding rice cultivated on sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015.

Applying 50% PK or inoculated rice plus 25% PK fertilizers for high-yielding rice cultivation on sandy soils reduced no-seed grain/plant ratio, this result showed that *Agrobacterium tumefaciens* strain CA09 helped roots of rice absorbing better PK nutrients in soils to improve no-seed grain/plant ratio and this contributed for improvement of grain yield.

No variation of soil pH, high soil N total and organic matter in soil were low, especially available P in soil was low and available P was improved after rice cultivation in treatments having PK fertilizers and using *Agrobacterium tumefaciens* strain CA09 (Table 18).

Table 18: Effects of P-K solubilizing bacterial strain (*Agrobacterium tumefaciens* strain CA09) and phosphate and potassium fertilizers on pH and chemical characteristics of sandy soils on Tri Ton district, An Giang province in cropping-season winter-spring 2015 before and after high-yielding rice cultivation.

Treatment	pH	N total (%)	Available P (mg/kg)	Organic matter (%)
Initial	5.52	0.48	0.39	2.23
Control	5.29	0.35 bc	3.89 e	2.46 d
100% P-K	5.34	0.38 a	10.76 a	2.66 abc
CA09 + 0% P-K	5.29	0.39 a	7.74 cd	2.78 ab
CA09 + 25% P-K	5.24	0.38 ab	8.01 bc	2.81 a
CA09 + 50% P-K	5.41	0.37 abc	8.03 bc	2.73 abc
CA09 + 75% P-K	5.21	0.38 ab	8.52 bc	2.65 abc
25% P-K	5.31	0.34 c	5.89 d	2.60 bcd
50% P-K	5.36	0.35 bc	7.34 cd	2.56 cd
75% P-K	5.24	0.35 bc	9.59 ab	2.55 cd
C.V	6.21	5.71	5.09	15.97

All treatments applied 150 kg N/ha , P-K: 120kg P + 90kg K (ha)

Numbers following the same word not difference at 1% level

Effects of P-K Solubilizing Bacteria and Nitrogen Chemical Fertilizer on Growth, Yield and Nitrate Leaf of *Brassica Juncea* L. Cultivated on Acid Sulphate Soils

A field study was conducted to determine the effect of twelve P-K solubilizing bacterial strains plus nitrogen chemical fertilizer (0N, 25N and 50N), on *Brassica juncea* L cultivated on acid sulphate soils in 24 days.

Twelve P-K solubilizing bacterial strains originated from That son site with the characteristics as follows: (Table 19). All of them had the high ability of phosphate and potassium solubilization were used in this study, the experiment was conducted on acid sulphate soils with characteristics as pH: 4.013; CEC: 10.5 meq/100 g soil; organic matter: 1.25%; available P: 950 mg/kg; exchangeable K: 1952 mg/kg; N total: 4.22%; P total: 0.29%

Therefore, unless pH and organic matter were low, other characters were high in acid sulphate soils.

Table 19: Twelve P-K solubilizing strains had ability of solubilization of phosphate and solubilization of potassium in Aleksandrov medium.

Bacterial name	(mg P ₂ O ₅ ⁻¹)	(mg K ₂ O ⁻¹)	Bacterial name	(mg P ₂ O ₅ ⁻¹)	(mg K ₂ O ⁻¹)
<i>Agrobacterium tumefaciens</i> CA09	23.16 a	42.12 e	<i>Acinetobacter calcoaceticus</i> NT1	18.02 bc	43.07 e
<i>Bacillus subtilis</i> CA18	12.31 cd	47.61 b	<i>Acinetobacter calcoaceticus</i> NT4	17.15 bc	31.61 k
<i>Azotobacter tropicalis</i> CA21	14.98 c	43.08 e	<i>Acinetobacter calcoaceticus</i> NT30	22.67 ab	36.78 g
<i>Rhizobium tropici</i> CA29	15.33 bc	48.32 b	<i>Rhizobium</i> sp Tu09	18.85 bc	30.20 l
<i>Rhizobium tropici</i> D9	14.17 cd	34.09 gh	<i>Rhi. tropici</i> N18	12.45 cd	40.17 f
<i>Rhi. leguminosarum</i> DG1	17.59 bc	08.70 q	<i>Rhi. leguminosarum</i> K35	11.30 cd	31.06 k
Control	00.63 e	00.00 r	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

Table 20: Effect of 12 P-K solubilizing bacterial strains and 100 kg/N on plant height and plant component of *Brassica junca* L cultivated on acid sulphate soils.

Treatment	Plant height (cm)	Leaf number /plant	Weight Plant (gr)	pH soil	N total soil (%)	Organic matter (%)
Initial				4.01 d	4.22 ef	1.26 f
No-Inoculated – 0NPK	6.61 g	6.16 e	5.75 l	4.18 c	4.32 d	1.30 d
100 N – no-inoculated	10.31 d	7.11 d	13.54 g	4.28 b	8.10 a	2.43 a
CA09 strain – 0N	10.12 d	7.04 d	15.79 f	4.42 a	4.31 d	1.29 d
CA18 strain – 0N	9.93 de	6.94 d	15.79 f	4.16 c	4.43 c	1.33 c
CA21 strain – 0N	9.36 e	6.88 d	8.96 i	4.31 ab	4.55 b	1.36 b
CA29 strain – 0N	10.10 de	6.76 de	15.62 f	4.38 a	4.46 c	1.34 bc
D9 strain – 0N	8.74 f	6.43 e	10.12 h	4.06 cd	4.23 e	1.28 d
DG1 strain – 0N	8.42 f	6.21 e	6.20 l	4.13 c	4.54 b	1.35 bc
NT1 strain – 0N	11.21 c	8.55 b	24.15 c	4.08 c	4.18 f	1.26 f
NT4 strain – 0N	13.33 a	9.35 a	32.86 b	4.09 c	4.11 f	1.24 f
NT30 strain – 0N	13.45 a	9.81 a	38.07 a	4.26 b	4.43 c	1.33 c
Tu09 strain – 0N	11.22 c	7.86 c	19.34 e	4.43 a	4.10 f	1.23 fg
N18 strain – 0N	13.54 a	8.63 b	32.72 b	4.08 cd	4.11 f	1.23 fg
K35 strain – 0N	12.31 b	8.48 bc	23.42 d	4.13 c	3.98 f	1.21 g
Calculated F	**	**	**	**	**	**
C.V (%)	2.59	3.14	1.51	1.57	0.82	0.72

Basal fertilizer: 80 P₂O₅ – 40 K₂O/ha

*The numbers followed by the same letter do not differ at 1% level significantly

Application of 12 P-K solubilizing bacteria strains for vegetable cultivation supported plant height and plant component clearly especially strains as follows: NT4, NT30, NT1, Tu09, N18 and K35. Applied 100 kg N/ha increased plant height and plant component of *Brassica junca* slightly but N chemical fertilizer induced pH soil, N total and organic matter after harvesting (Table 4). However, several P-K solubilizing bacterial strains not only enhanced plant height, plant component of *Brassica junca* but also improved soil fertility slightly in comparison to initial as CA29, DG1 and NT30 strains. This showed that the effectiveness of these strains as nitrogen-fixing bacteria strains provided nitrogen as nutrient for plant growth and soil fertility, they can be the potential strains for biofertilizer production.

Control and inoculated strains treatments without nitrogen chemical fertilizer did not increase nitrate concentration in leaf of *Brassica juncea* while application of 100 kg N/ha without inoculation enhanced nitrate concentration in leaf of *Brassica juncea* and nitrate concentration exceeded the allowed threshold (>500 mg/kg TCVN7373/2004) (Figure 20). Inoculation of NT4, NT30 and N18 strains supported the highest biomass of *Brassica juncea* significantly but nitrate concentration in leaf of *Brassica juncea* L. of these treatments were low, this results demonstrated the these potential strains.

Figure 20: Effects of 12 P-K solubilizing bacterial strains and 100 kg N on nitrate concentration in leaf of *Brassica juncea* L. cultivated on acid sulphate soil (biomass: g/m²; nitrate in leaf: mg/kg)

Conclusion

From 137 weathered material/soil samples of granite rock at three sites as That Son site, Sap site, An Giang province, and Ba Hon site, Kien Giang province, Vietnam, 392 isolates were isolated and identified as phosphate- and potassium- solubilizing bacteria. Based on the phylogenetic analysis and PCR amplification of 16S rRNA gene, there are 43 out of 392 isolates had high ability of phosphate and potassium solubilization, they are including 13 strains of Bacilli and 30 strains were Proteobacteria.

Some good phosphae- and potassium- solubilizing bacteria strains were evaluated their effects on many kinds of crop the result showed that they saved 25% amount of PK chemical fertilizers in comparison to the control.

Twelve P-K solubilizing bacterial strains are PGPR which they have characteristics as nitrogen fixation, phosphate and potassium solubilization, increasing concentration of nitrogen chemical fertilizer applied to vegetable led to increase nitrate concentration in leaf of *Brassica juncea* and *Acinetobacter calcoaceticus* NT30 strain suggest to use in biofertilizer production for leaf-eating vegetable nearly in the future.

References

1. Cockell CS, Pybus D, Olsson-Francis K, Kelly L, Petley D, et al. Molecular Characterization and Geological Microenvironment of a Microbial Community Inhabiting Weathering Receding Shale Cliffs, Microb. Ecol. 2011; 61: 166-181.
2. Xiufang H, Jishuang C, Jiangfeng G. Two phosphate- and potassium- solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World Journal of Microbiology and Biotechnology. 2006; 22: 983 - 990.
3. Thanh NV, Xuan VT, Tri LQ, Minh VQ. Studies on main crop systems cultivated on sulfaquept soils in Mekong Delta. MSc. Thesis. Can Tho University. Vietnam. 1997.
4. Xie JC. Present situation and prospects for the world's fertilizer use. Plant Nutr. Fert. Sci. 1998; 4: 321–330.

5. Abou-el-Seoud II, Abdel-Megeed A. Impact of rock materials and biofertilizations on P and K availability for maize *Zea mays* under calcareous soil conditions, Saudi J. of Biological Sciences. 2012; 19: 55–63.
6. Scheffer F, Schachtchabel P. Lehrbuch der Bodenkunde. Ferdinand Enke Verlag, Stuttgart. 1992.
7. Zord C, Senbayram M, Peiter E. Potassium in agriculture – Status and perspective, J. Plant Physiol. 2014; 171: 656-659.
8. Malinovskaya M, Kosenko LV, Votselko SK, Podgorskii VS. Role of *Bacillus mucilaginosus* polysaccharide in degradation of silicate minerals. Microbiology. 1990; 59: 49-55.
9. Goldstein AH. Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphate by gram-negative bacteria. In: Torriani-Gorini A, E Yagil, S Silver, editors. Phosphate in Microorganisms: Cellular and Molecular Biology. Washington, DC: ASM Press. 1994; 197 - 203.
10. Setiawati A, Handayanto A. Role of phosphate solubilizing bacteria on availability phosphorus in Oxisols and tracing of phosphate in corn by using ³²P. In: 19th World Congress of Soil Science. Soil Solutions for a Changing World. Brisbane, Australia. 2010.
11. Ekin Z. Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower *Helianthus annuus* L. in the presence of phosphorus fertilizer. Afr. J. Biotech. 2010; 9(23): 3794-3800.
12. Perez E, Sulbaran M, Ball MM, Yarzabal LA. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south eastern Venezuelan region, Soil Biol. Biochem. 2007; 39: 2905-2914.
13. Parmar SP, Sindhu S. Potassium Solubilization by Rhizosphere Bacteria: Influence of Nutritional and Environmental Conditions. J. Microb. Res. 2013; 31: 25-31.
14. Gundala PB, Chinthala P, Sreenivasulu B. A new facultative alkaliphilic, potassium solubilizing *Bacillus* sp. SVUNM9 isolated from mica cores of Nellore District, Andhra Pradesh, India: Research and Reviews, J. Microbiol. Biotechnol. 2013; 12: 76-180.

15. Archana DS, Nandish MS, Savalagi VP, Alagawadi AR. Screening of potassium solubilizing bacteria KSB for plant growth promotional activity. *Bioinfolet*. 2012; 94: 627-630,
16. Archana DS, Nandish MS, Savalagi VP, Alagawadi AR. Characterization of potassium solubilizing bacteria KSB from rhizosphere soil. *Bioinfolet*. 2013; 10: 248-257.
17. Lian B, Fu PQ, Mo DM, Liu CQ. A comprehensive review of the mechanism of potassium release by silicate bacteria. *Acta Mineral Simica*. 2002; 22: 179.
18. Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res*. 2008; 63: 173-181.
19. Mathiyazhagan S, Kavitha K. PGPR mediated management of stem blight of *Phyllanthus amarus* Schumand thonn caused by *Corynespora cassicolca* Berk & Curt. *Arch. Phytopathol. Plant Prot*. 2004; 37: 183-199.
20. Dave BP, Dube TE. Regulation of siderophore production by iron Fe III is certain fungi and fluorescent *Pseudomonads*. *Indian J. Exp. Biol*. 2000; 38: 297-299.
21. Scher FM, Baker R. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. *Phytopathology*. 1982; 720: 1567–1573.
22. Zhender GW, Yao C, Murphy JF, Sikora ER, Kloepper JW, et al. Microbe-induced resistance against pathogens and herbivores: evidence of effectiveness in agriculture. In: Agarwal AA, Tuzun S, Bent E, editors. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology and Agriculture*. St Paul: APS Press. 1999; 33-37.
23. Van Loon LC, Bakker PAHM. Root Associated bacteria inducing systemic resistance. In: Gnanamanickam SS, editor. *Plant Associated Bacteria*. Dordrecht: Springer. 2006; 269–316.
24. Rodriguez H, Frag R, Gonzalez T, Bashan Y. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil*. 2006; 287: 15-21.
25. Carlot M, Giacomini A, Casella S. Aspects of plant-microbe interactions in heavy metal polluted soil. *Acta Biotechnol*. 2002; 22: 13-20.

26. Vũ Quang Mạnh. Sinh thái học đất, NXB Đại học Sư phạm, TPHCM. Vietnamese.
27. Tổng Duy Thanh, Vũ Xuân Đô, Trịnh Hân, Lê Văn Mạnh, Tạ Hòa Phương, Tạ Trọng Thắng, Nguyễn Văn Vinh. 2004. Giáo trình địa chất cơ sở. NXB Đại học quốc gia, Hà Nội Vietnamese. 2003.
28. Chlch LT. Thạc học. NXB Đại học quốc gia. TPHCM Vietnamese. 2001.
29. Manib M, Zahra MK, Abdel AL, Heggo A. Role of solicate bacteria in releasing K and Si from biotite and orthoclase. *In*: Szegi J, editor. Soil biology and Conservation of the Biosphere. Budapest: Akademiai Kiado. 1986; 733-743.
30. Neumann B, Pospiech A, Schairrer HU. Rapid isolation of genomic DNA from Gram – negative bacteria, Trends Genet. 1992; 8: 332 – 333.
31. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. 2011; 2810: 2731–2739.
32. Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, et al. Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. Nat. Genet. 1999; 223: 239-247.
33. Diep CN, Hieu TN. Phosphate and potassium solubilizing bacteria from weathered materials of denatured roch mountain, Ha Tien, Kien Giang province, Vietnam, American Journal of Life Sciences. 2013; 13: 88-92.
34. Diep CN, Don NT, Nhung NTH, Thanh LDT. Isolation and identification of phosphate- and potassium-solubilizing bacteria in weathered materilas from calcacerous mountain, Kien Giang, Vietnam. Proceeding of the 2012 International Symposium on Biology Science and Environmental Engineering ISBSEE 2012 organized at China, Malaysia. 2012.
35. Diep CN, Phong TT, Giang TT. Isolation and Selection of phosphate-solubilizing and IAA-biosynthesis Pseudomonas sp. Science and technology J. Agriculture and Rural Development. Ministry of Agriculture and Rural Development. Vietnam. 2009; 9: 32-35.

36. Basak BB, Biswas DR. Influence of potassium solubilizing microorganism *Bacillus mucilaginosus* and waste mica on potassium uptake dynamics by sudan grass *Sorghum vulgare* Pers. grown under two Alfisols. *Plant Soil*. 2009; 317: 235-255.
37. Watterson GA. On the number of segregation sites in general models without recombination, *Theor. Pop. Biol.* 1975; 7: 256-276.
38. Rozas J, Rozas R. DnaSP version 4.1: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics*. 2005; 15: 174-175.
39. Jordan TD. Familiu III. Rhizobiaceae Conn 1938, 321, 234-254, In: NR Krieg, JG Hodt, editors. *Bergey's mannual of systematic bacteriology*, vol. 1. Baltimore: The William and Wikkins, Co. 1938.
40. Martinez-Romero E, Segovia L, Mercante EM, Franco AA, Graham P, et al. *Rhizobium tropici*, a Novel Species Nodulating *Phaseolus vulgaris* L. Beans and *Leucaena* sp. Trees. *Int. J. Syst. Bacteriol.* 1991; 413: 417-426.
41. Han TX, Wang ET, Wu LJ, Chen WF, Gu JG, et al. *Rhizobium multihospitium* sp. nov. isolated from multiple legume species native of Xinjiang, China, *Int. J. Syst. Evol. Microb.* 2008; 58: 1693-1699.
42. Young JM, Kuykendall LD, Martinez-Romero E, Kerr R, Sawada H. A revision of *Rhizobium* Frank 1889, with an amended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et. 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*, *Int. J. Syst. Evol. Microb.* 2001; 51: 89-103.
43. Park KH, Lee CY, Son HJ. Mechanism of insoluble phosphate solubilization by *Pseudomonas fluorescens* RAF15 isolated from ginseng rhizosphere and its plant growth promoting activities. *Letters in Applied Microbiology*. 2009; 49: 222 - 228.
44. Hwangbo H, Park RD, Kim YW, Rim YS, Park KY, et al. 2 - Ketogluconic acid production and phosphate solubilization by *Enterobacter intermedium*. *Current Microbiology*. 2003; 47: 87- 92.

45. Kumar A, Bharagava P, Rai LC. Isolation and molecular characterization of phosphate solubilizing *Enterobacter* and *Exiguobacterium* species from paddy fields of Eastern Uttar Pradesh, India. *African Journal of Microbiology Research*. 2010; 4: 820-829.
46. Lin TF, Huang HI, Shen FT, Young CC. The protons of gluconic acid are the major factor responsible for the dissolution of tricalcium phosphate by *Burkholderia cepacia* CC - A174. *Bioresource Technology*. 2006; 97: 957 - 960.
47. Nahid EA. Phenotypic and genetic variability among three *Bacillus megatherium* isolates in vitro evaluation of tricalcium phosphate solubilizing potential and growth pattern. Botany Department Faculty. of Science. Benha University Egypt. 2010; 285: 465 – 477.
48. El-Sawi WA, Mekhemar GAA, Kandil BAA. Comparative assessment of growth and yield responses to two peanut genotypes to inoculation with *Bradyrhizobium* conjugated with cyanobacteria or rhizobacteria. *Minufiya. Journal of Agricultural Research*. 2006; 31: 1031-1049.