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Isolation and identification of rhizospheric bacteria in Acrisols of maize (*Zea mays* L.) in the eastern of South Vietnam

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Abstract: Rhizobacterial diversity and population dynamics in the Acrisol rhizosphere of maize grown in the eastern of South Vietnam was studied. Soil rhizosphere samples were taken in three provinces of this region. Physical and chemical characteristics of soil samples and total nitrogen-fixing and phosphate-solubilizing bacteria counts were determined by drop plate count method together with 16S rRNA gene fragments amplified from DNA using eubacterial universal primers (8F and 1492R). A total of 149 isolates were isolated on two media (Burk's N-free and NBRIP) and all of them have ability of nitrogen fixation and phosphate solubilization together with IAA biosynthesis. Population of rhizobacteria correlated with soil pH and organic matter content in soil closely ($P<0.05$). The sequences from selected rhizobacteria (24 isolates) showed high degrees of similarity to those of the GenBank references strains (between 97% and 99%). From 24 isolates,13 belonged to *Beta-Proteobacteria*, while 11 were *Firimicutes* and *Actinobacteria*. Based on Pi value (nucleotide diversity), rhizobacteria (PGPR) group in Tay Ninh province had higher than rhizobacteria (PGPR) group in Baria-Vungtau province with the highest Theta value (per site). From these results showed that three strains (*Burkholderia vietnamiensis* VDN6a, VDB6a and VDN7c) revealed promising candidates with multiple beneficial characteristics and they have the potential for application as inoculants adapted to poor soils and local crops because they are not only famous strains but also are safety strains for agricultural sustainable.

Keywords: Acrisols, 16S rRNA Gene Sequence, Maize, Rhizobacteria, Rhizosphere

1. Introduction

Soils are the product of the activities of plants, which supply organic matter and play a pivotal role in weathering rocks and minerals. The rhizosphere is an environment that the plant itself helps create and where pathogenics and beneficial microorganisms constitute a major influential force on plant growth and health [1]. Most plants depend on soil, but plants and their associated microorganisms also play a crucial role in the formation or modification of soil [2][3] and microorganisms present in the rhizosphere play important roles in the growth and in the ecological fitness of their plant host [4]. Microbial interactions with roots may involve either endophytic or free living microorganisms and can be symbiotic, associative or casual in nature; beneficial microorganisms include N_2-fixing bacteria in association with legumes and interaction of roots with mycorrhizal fungi and phosphate-solubilizing microorganisms in relation to plant P uptake, enhancement of root growth (i.e. through plant growth promoting rhizobacteria, or PGPR)[5].

The eastern of South Vietnam locates from 105°49' to 107°35' E and from 10°20' to 12°17' N, it is one of the two regions of South Vietnam situated in the east of part of South Vietnam, covering 2.34 millions ha, occupied approximately 20.3% of total of Vietnam area. The soils are mainly red latosols (from origin of volcanic mountain) and acrisols with a pH range of 4.5 – 5.0. They are considered nutrient poor, with an average organic matter of 2%, a total nitrogen range of 0.14 – 0.19%, and a very low available phosphorus, cation exchange capacity, exchangeable K and contain more sand in their structure [6]. After rice, maize (*Zea mays* L.) is the second crop which provide food for human in Vietnam, it has

been cultivated on this region including three provinces (Dongnai, Tayninh and Baria-Vungtau) with a big area (89,500 ha) however it requires a big amount of chemical fertilizer (400 kg urea – 1000 kg superphosphate – 400 kg KCl/ha) to produce high grain yield (6 – 8 tons/ha) when maize has been planted on acrisols. The fields are not irrigated (mainly rain water), herbicides as well as pesticides therefore high cost of corn cultivation and their income is low.

The application of native, adapted microorganisms might improve yields by direct plant growth promotion and increasing grain yield, decreasing cost in corn cultivation in order to enhance income for the farmers. The aims of this study were (i) study of physical and chemical characteristics of acrisol, (ii) isolation and characterization of rhizospheric bacteria such as nitrogen-fixing and phosphate-solubilizing, (iii) analysis of relationship of beneficial bacterial population with physical and chemical characteristics of acrisol soil, (iv) selection of best bacterial isolates and (v) identification of these bacterial isolates. These bacteria can be considered promising candidates for application in sustainble agricultural management for this region.

2. Material and Methods

2.1. Soil Sample and Isolation of Bacteria

The maize plants were sampled at the stage of flowering during the rainy season (July 2013) from the fields of three provinces (Tayninh, Dongnai and Baria-Vungtau) which are the biggest area in the eastern South Vietnam. Samples were collected on 7 districts of Tayninh province, 3 dictrists of Dongnai province and 3 districts of Baria-Vungtau province because maize has been planted on acrisols. Samples were taken whole plant with stem, root (10-20 cm depth) together with soil which around maize plants; samples were kept in 18^{o}C plastic box before tranferred to laboratory in Can Tho University. Rhizosphere soil around maize plants were collected to moving the soil that adhered to the roots (stem and root of maize plant will be used in further experiment) and they were kept to refrigerator for counting by viable drop plate count [7] and isolation of nitrogen-fixing bacteria in Burk'N free media [8] and phosphate-solubilizing bacteria in NBRIP media [9]; cultures were streaked on media to obtain single colonies. To check for phosphate solubilization ability or nitrogen fixation ability, colonies from Burk'N free media were streaked to NBRIP media and colonies from NBRIP media were also cultivated to Burk's N free media in order to select the colonies which developed on two media (or microbes having N_2-fixing and phosphate-solubilizing ability).

2.2. Screening for Biofertilizer Activities

The ability to fix N_2 was tested on Burk'N-free liquid medium incubating at 30^{o}C and the ammonium concentration in medium was measured by Phenol Nitroprusside method after 2,4,6 and 8 day inoculation (DAI) and inorganic phosphate solubilization ability was tested on NBRIP liquid medium and they were incubated at 30^{o}C and the P_2O_5 concentration was measured by ammonium molypdate method. The qualitative detection of indole-3-acetic acid (IAA) production was carried out based on the colorimetric method [10]. Precultures were grown in Burk's N free (100 ml) without tryptophan in 250mL-flask at 30^{o}C on a roller at 100 rpm and samples were taken from at 2, 4, 6, and 8 DAI, cell free supernatants were mixed 2:1 with Salkowski reagent (0.01 M $FeCl_3$ in 35% perchloric acid) and incubated in the dark for 20 min at RT. IAA-containing solutions were indicated by reddish color with an absorption peak at 530 nm on Thermo Scientific GENESYS 10uv spectrophotometer.

Besides that, the pH of rhizosphere soil was measured in a 1:5 soil to water (w/v) mixture in 20 min and read on a Jenway 3510 pH meter, N total were measured using the micro-Kjeldahl digestion method, the colorimetric P determination was based the method of ammonium molypdate method [11], organic carbon measured by Walkley Black method [12].

2.3. 16S rDNA Gene Amplification and Sequencing

Bacterial DNA was isolated following published protocols [13]; Amplification of 16S rDNA by PCR was carried out using the universal primers 8F and 1492R [14]. The 50 µL reactionmixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 µM of each deooxynucleotide triphosphate, 500 nM of each primer (Fermentas) and 20 ng DNA. The thermocycling profide was carried out with an initial denaturation at 95^{o}C (5 min) followed by 30 cycles of denaturation at 95^{o}C (30 s), annealing at 55^{o}C (30 s), extension at 72^{o}C (90 s) and a final extension at 72^{o}C (10 min) in C1000 Thermal Cycler (Bio-Rad). Aliquots (10 µl) of PCR products were electrophoresed and visualized in 1% agarose gels using standard electrophoresis procedures. Partial 16S rRNA gene of selectived isolates in each group were sequenced by MACROGEN, Republic of Korea (dna.macrogen.com). Finally, 16S rRNA sequence of the isolate was compared with that of other microorganisms by way BLAST (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi); In the best isolate(s) (high nitrogen fixation and phosphate solubilization ability) and 24 isolates of 4 sites were chosen to sequence and the results were compared to sequences of GenBank based on partial 16S rRNA sequences to show relationships between PGPR strains [15] and phylogenetic tree were constructed by the neighbor-joining method using the MEGA software version 6.06 based on 1000 bootstraps.

2.4. SNPs Discovery

The sequence date from 24 root-associated 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.

2.5. Nucleotide Diversity (Θ)

Nucleotide diversity (Θ) was calculated by the method described by Halushka et al. [16].

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

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

2.6. Data Analyses

Relationship between population of nitrogen-fixing and phosphate-solublilizing bacteria and soil pH, N total, available P and organic matter content in acrisols were explored with simple regressions using Exel in Microsoft version 7.0. Data from ammonium, orthophosphate and IAA concentrations in media were analysed in completely randomized design with three replicates and Duncan test at P=0.01 or P=0.05 were used to differentiate between statistically different means using SPSS version 16.

3. Results and Discussion

3.1. Soil Characteristics

Three provinces in eastern of South Vietnam have large cultivated corn area (Dongnai, Tayninh and Baria-VungTau) and corn has been cultivated on Acrisol mainly in comparison to red latosol and the results from 23 soil samples (7 from Tayninh, 7 from Baria-VungTau and 9 from Dongnai province) showed that characteristic of acrisol is low soil pH together with low available P concentration (this is soil characteristic of Acrisol) however N total and organic matter content in Acrisol also are low (Table 1) except soil sample of Dongnai4 (neutral soil pH and high N total level). Interestingly, nitrogen – fixing bacterial population in acrisol was high (almost over one million cells per dry soil gram) but population of phosphate-solubilizing bacteria was low (approx ten thousand and one hundred thousand cells per soil gram).

Table 1. *Soil characteristics and N_2-fixing and Phosphate-solubilizing bacterial population in acrisol rhizosphere.*

Soil sample site	Soil pH	N total (%)	Available P (mg P_2O_5/100 g soil)	Organic matter content (%)	N_2-fixing bacteria population	P-solubilizing bacteria population
					CFU log_{10}/g soil	
TayNinh 1	4.14	0.12	1.43	1.64	6.838	5.999
TayNinh 2	4.59	0.09	3.45	1.03	5.763	4.856
TayNinh 3	4.43	0.07	3.02	0.81	5.547	4.893
TayNinh 4	4.78	0.07	3.55	1.10	5.960	4.574
TayNinh 5	4.54	0.18	3.39	1.59	6.035	4.856
TayNinh 6	4.86	0.08	2.96	1.13	6.074	5.116
TayNinh 7	5.01	0.08	3.49	1.22	6.611	5.238
BaRia-VungTau1	5.40	0.17	3.11	1.54	6.253	5.064
BaRia-VungTau2	4.90	0.06	1.46	1.03	6.166	5.022
BaRia-VungTau3	5.23	0.15	1.81	2.03	6.224	4.945
BaRia-VungTau4	4.97	0.12	3.92	1.69	6.595	4.873
BaRia-VungTau5	4.72	0.10	2.61	1.32	5.722	4.942
BaRia-VungTau6	5.37	0.17	3.48	1.82	6.894	5.926
BaRia-VungTau7	4.42	0.12	3.34	1.72	6.959	5.189
DongNai1	4.58	0.12	1.56	1.72	6.055	5.075
DongNai2	4.62	0.12	1.27	1.31	6.241	5.069
DongNai3	6.25	0.18	1.33	1.38	6.565	5.728
DongNai4	6.19	0.23	1.58	2.06	6.996	5.478
DongNai5	5.95	0.11	1.42	1.43	6.580	5.699
DongNai6	5.07	0.15	1.14	1.33	6.478	5.025
DongNai7	5.22	0.13	2.26	1.84	6.878	5.983
DongNai8	5.49	0.12	2.05	1.52	6.824	5.812
DongNai9	5.08	0.14	1.53	1.29	5.865	4.812

The results from Table 2 showed that there were a significant linear relationship between population of N_2-fixing and phosphate-solubilizing bacteria and soil pH at P<0.05 (y=0.741x + 0.378, r=0.724*; y=0.655x + 1.674, r=0.692*, respectively) and both of microbes with organic matter content were a linear relationship significantly at P<0.05 (y=0.472x – 1.534, r=0.651*; y=0.277x + 0.021, r=0.412, respectively) and there was a significant linear relationship between N_2-fixing bacteria population with N total concentration in soil at P<0.05 (y=0.047x – 0.176, r=0.491*) but there were no difference between population of phosphate-solubilizing bacteria with N total and available phosphorus concentration in soil significantly. These results showed that soil pH and organic matter content in soil are two important factors affecting to populations of nitrogen-fixing bacteria and phosphate-solubilizing bacteria

in soil while N total in soil correlated with nitrogen-fixing bacterila population rather than phosphate-solubilizing bacteria population in acrisols.

Table 2. *The relationship between population of N_2-fixing and phosphate-solubilizing bacteria with pH, N total, available phosphorus and organic matter content in soil.*

Characteristics	Population (cfu/dry soil gramme)	
	N_2- fixing bacteria	Phosphate-solubilizing bacteria
Soil pH	r = 0.724* y = 0.741x + 0.378	r = 0.692* y = 0.655x + 1.674
N total concentration (%)	r = 0.491* y = 0.047x – 0.176	r = 0.396 (ns) y = 0.035x – 0.059
Available P (mg/100 g soil)	r = -0.176 (ns) y = -0.377x – 4.817	r = -0.353 (ns) y = -0.689x – 6.001
Organic matter (%)	r = 0.651* y = 0.472x - 1.534	r = 0.412* y = 0.277x + 0.021

ns: not significantly different

One hundred and forty-nine bacterial isolates were isolated from 23 soil samples in two media (Burk'N free and NBRIP medium)(Table 3) with 67, 43 and 39 isolates from Tayninh, Dongnai and Baria-Vungtau, respectively and all isolates grew well on both of media (they have nitrogen fixation and phosphate solubilization ability) and all of them produced indole-3-acetic acid (IAA) *in vitro*.

Table 3. *Number of bacterial isolates were isolated from Acrisol from three provinces of the eastern of South Vietnam on two media.*

Site	Total of bacterial isolates	Burk'N free medium	NBRIP medium
Tayninh province	67	33	34
Dongnai province	43	19	24
Baria-Vungtau province	39	17	22

The results showed that these bacterial isolates synthesized low ammonium concentration but they solubilized big quantity of phosphorus while the IAA-biosynthesis concentration changed to group of bacterial isolates which isolated from each province; IAA concentration varied from 0.30 to 9.58 mg/L. Especially DDN10b isolate solubilized high amount of phosphorus and IAA concentration (Table 4).

Table 4. *Ammonium (NH_4^+), Available P (P_2O_5) and IAA comcentration (*)(mg/l) of some good isolates in 149 isolates*

Site	Bacterial name	Ammonium (NH_4^+) comcentration	Available P (P_2O_5) concentration (ml/L)	IAA concentration
Dongnai province	DDB7b	0.36	38.65	6.55
	DDN10b	0.46	110.52	9.58
Baria-Vungtau province	VDN1a	0.47	62.97	1.06
	VDN3c	0.29	53.04	1.25
	VDN4a	0.31	55.41	1.12
	VDN6a	0.34	70.15	1.37
	VDN6b	0.32	52.34	1.50
	VDB3a	0.35	43.38	3.05
Tayninh province	VDB6a	0.41	71.24	2.25
	VDN7c	0.42	69.50	1.56
	VDB7b	0.53	70.34	2.52
	TDN2	0.48	48.85	0.34
	TDN4	0.53	59.12	0.32
	TDN6	0.61	53.52	0.32
	TDN7	0.66	49.43	0.31
	TDN9	0.57	64.01	0.30
	TDN11	0.59	89.74	0.33
	TDN19	0.57	43.14	6.14
	TDN24	0.65	43.60	3.78
	TDB1	0.76	34.92	3.67
	TDB3	0.61	33.62	2.67
	TDB8	0.71	32.64	2.68
	TDB9	0.63	56.47	2.51
	TDB13	0.75	25.59	8.77

Almost their colonies have round-shaped; milky (on Burk' medium) and yellow (on NBRIP medium); entire or lobate margin (Figure 1); diameter size of these colonies varied from 0.2 to 2.5 mm and all of them are Gram-positive and Gram-negative by Gram stain. The cells were observed by SEM and appeared as short rods and most of them have motility (Figure 2). Especially phosphate-solubilizing bacteria make a halo around colonies in NBRIP mediun as described of Mai and Diep [17](Figure 2, VDN5c).

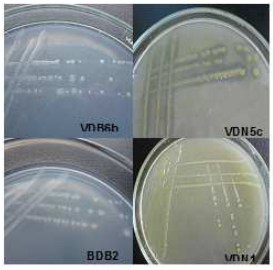
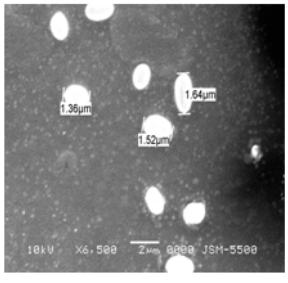


Figure 1. *The colonies of several PGPR isolates.*



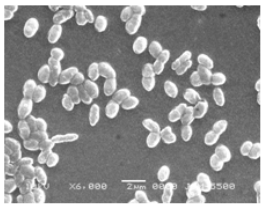


Figure 2. *Eelectron micrographs of cells.*

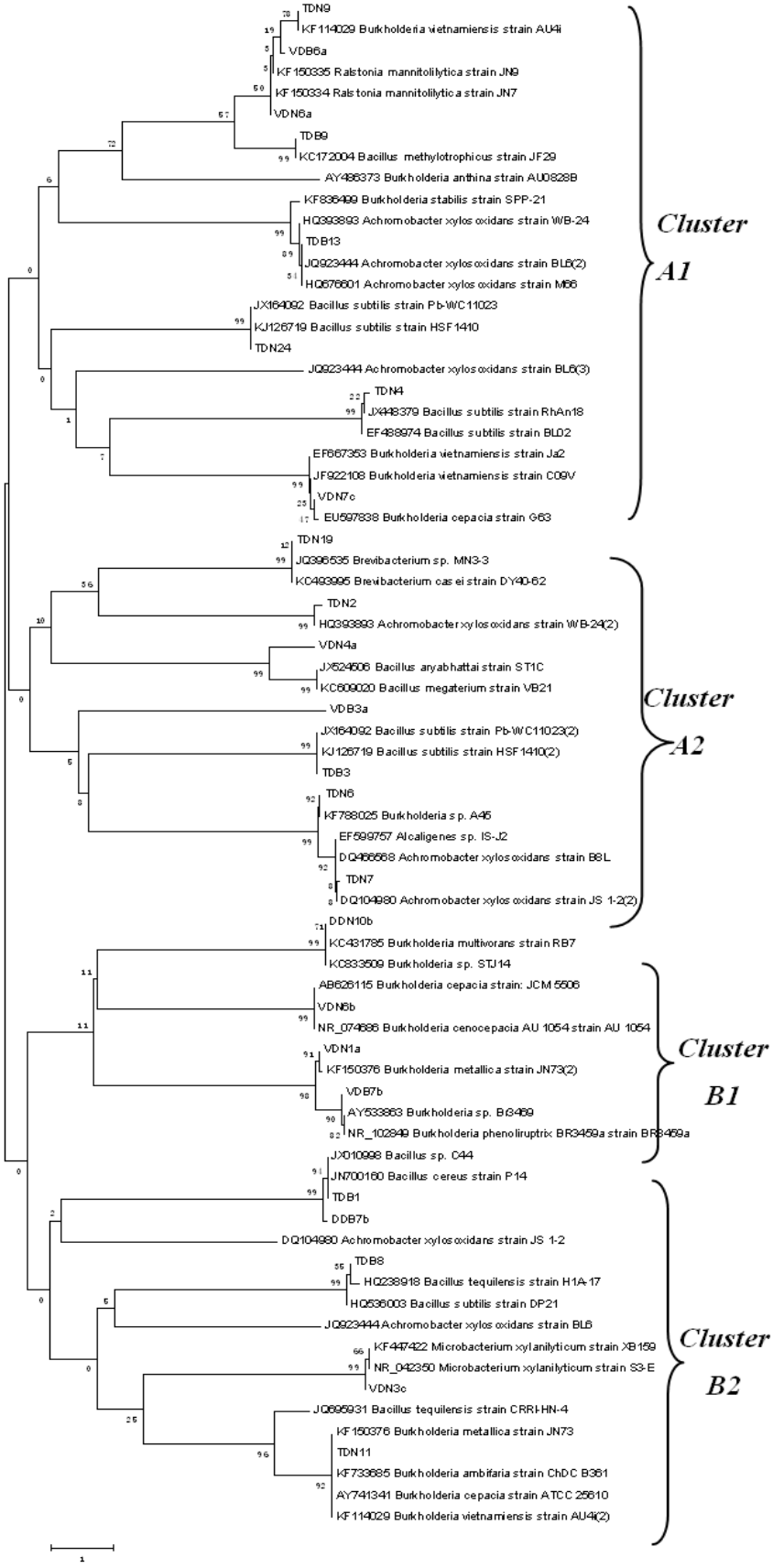


Figure 3. *Phylogenetic tree showing the relative position of rhizobacteria (PGPR) by the neighbor-joining method of complete 16S rRNA sequences. Bootstrap values of 1000 replicates are shown at the nodes of the trees.*

Based on the good characteristics of these isolates (Table 4), 24 isolates were chosen to identify from 3 provinces (Tayninh, Baria-Vungtau and Dongnai) but isolate number from Tayninh and Baria-Vungtau provinces were much more than Dongnai province.

The fragments of 1485 bp 16S rRNA were obtained from PCR and sequencing. Homology searches of the 16S rRNA gene sequence of selected strain in GenBank by BLAST revealved that they had similarity to sequences of *Bacillus* genus and *Burkholderia* genus together with some isolates are belong to *Achromobacter* and *Microbacterium* genus. A neighbor-joining phylogenetic tree in these isolates showing the two clusters: cluster A with 15 isolates including two small clusters as cluster A1 with 8 isolates as TDN9, VDB6a, VDN6a, TDB9, TDB13, TDN24, TDN4, VDN7c, and cluster A2 with 7 isolates as TDN19, TDN2, VDN4a, VDB3a, TDB3, TDN6 and TDN7 while cluster B with 2 small clusters: cluster B1 with 4 isolates as DDN10b, VDN6b, VDN1a, VDB7b and cluster B2 with 5 isolates as TDB1, DDB7b, TDB8, VDN3c, TDN11 (Figure 3).

However, these isolates divided to two branches or two clusters in two provinces (Tayninh and Baria-Vungtau)(Figure 4).

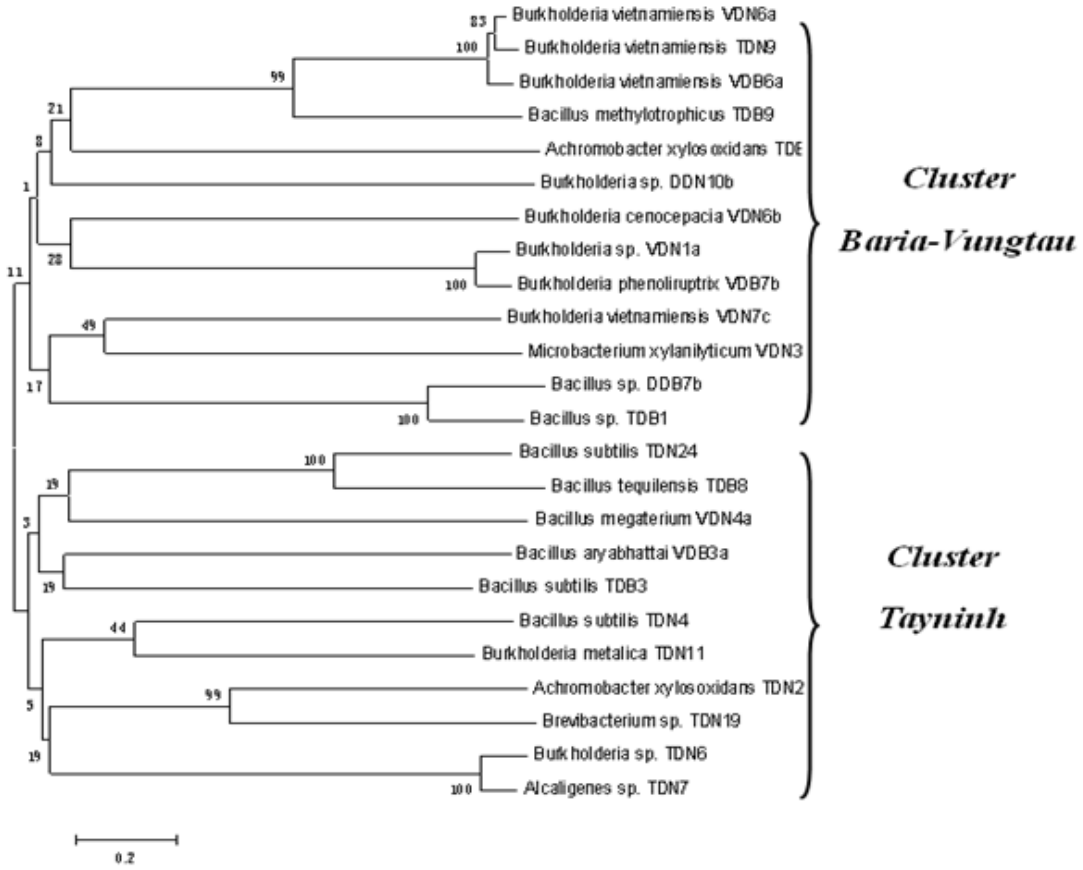


Figure 4. *Phylogenetic tree showing the relative position of rhizobacteria (PGPR) by the neighbor-joining method of complete 16S rRNA sequences. Bootstrap values of 1000 replicates are shown at the nodes of the trees.*

Nucleotide polymorphism can be measured by many methods, for example, haplotype (gene) diversity, nucleotide diversity, (Pi), Theta (Θ)(per site) etc.

In this study, nucleotide diversity was estimated as Theta (Θ), the number of segregating sites[18], and its standard deviation (SΘ). These parameters were estimated by DNA Sequence Polymorphism software version 4.0 [19].

Pi value explained nucleotide diversity of sequences for each gene. The higher values, the more diversity among PGPR group in Tayninh province had highest values and PGPR group in Baria-Vungtau province had the lowest values. Theta values (per sequence) from S of SNP for DNA polymorphism were calculated for each group and PGPR group in Tayninh province had the highest theta values in comparison with PGPR group in Baria-Vungtau province (Table 5).

Table 5. *Necleotide diversity (Θ) value of two ESTs using the programme DNASp 4.0 [18].*

ESTs	PGPR in Tayninh province	PGPR in Baria-Vungtau province
Nucleotide diversity (Pi)	0.73085	0.70492
Theta (per site) from Eta	0.95795	0.89439

Primer 8F 5'-AGATTTGATCCTGGCTCAG-3'
Primer 1492R 5'-TACGGTTACCTTGTTACGACTT-3'

One in many characteristrics of an Acrisol is low soil pH and this leads to low available P in soil. In addition, low moisture of this area limits maize cultivation and maize only plants in rainy season (from April to November in year) and local farmers often applies urea fertilizer instead of P fertilizer and K fertilizer however phosphate-solubilizing bacteria population in soil is low in comparison to nitrogen-fixing bacteria however phosphate-solubilizing bacteria population did not correlate with available P in soil. The results of Picard *et al*. [20] mainly focussed on plant growth-promoting rhizobacteria [PGPR] such as fluorescent pseudomonads but Di Cello *et al*. [21] and Dalmastri *et al*. [22] found that *Burkholderia cepacia* population presented from the maize rhizosphere at different plant growth stages. A higher degree of polymorphism found for *B. cepacia* isolated during the early stages of maize growth was also reported by Di Cello *et al*. [21] and the degree of diversity was significantly higher between *B. cepacia* population recovered from maize planted in different soils than between *B. cepacia* populations from different cultivars [22] and our results also found that high occurrence of *Burkholderia* genus in Beta-Proteobacteria (Gram-negative) compared to Firmicutes and Actinobacteria (gram-positive) especially *Burkholderia vietnamiensis*, this species was found in rice soil in the Mekong Delta, Vietnam [23][24][25][26]), *B. vietnamiensis* is the beneficial bacterial strain which has been used in biofertilizer production for rice cultivation [27]. Recently Gronemeyer et al. [28] showed that phylogenetic analysis revealed the association of the isolates which isolated soil rhizosphere of agricultural crops, among maize, in Kavango region of Namibia with three highly supported main phyla: *Proteobacteria, Firmicutes* and *Actinobacteria*, these results were in agreement with the results on endophytes from maize and sorghum in Nebraska [29] where an almost equal distribution of Gram-positive and Gram-negative isolates was found. Our results also found three these phyla but number of species in Gram-negative was higher than species in Gram-positive and many species of *Bacillus* genus were identified in soil because of forming cysts that are desication resistant [30]. Descriptions of PGPR-relevant capacities include free nitrogen fixation, phosphate solubilization and IAA synthesis, especially *Burkholderia vietnamiensis* strains have been used in biofertilizer for rice (named DASVILA)[27] in the Mekong Delta, Vietnam extensively.

4. Conclusions

Several novel putative hizospheric bacteria were detected in our study, they originated from Acrisols which low soil pH, low available P. The desiccation-resistant isolates may have especially high potential as plant-beneficial inoculants for areas with seasonal drought. *Burkholderia vietnamiensis* strains that are adapted to these conditions and to the low-fertility Acrisoils of the eastern of South Vietnam. These strains should be tested in pot and in the field experiments in order to confirm their capacity to improve maize yields and soil fertility of this region.

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References

[1] J.M Lynch, “Introduction: Some consequences of microbial rhizosphere competence for plant and soil,” In: Lynch JM (ed.) The rhizosphere. Wiley Interscience, New York, pp: 1-10, 1990.

[2] J.S. Pate, and W.H Verboom, “Comtemporary biogenic formation of clay pavements by eucalypts: further support for the phytotarium concept,” Ann. Bot. vol 103, pp:673-685, 2009.

[3] L.L. Taylor, J.R Leake, J. Quirk, K. Hardy, S.A. Banwarts, and D.J. Beerling, “Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm,” Geobiology vol 7, pp:171-191, 2009.

[4] M. Buee, W. De Boer, F. Martin, L. van Overbeek, and E. Jurkevitch, “The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archea, and fungi, and of some of their structuring factors,” Plant Soil, vol 321, pp:189-212, 2009.

[5] J.M. Raaijmakers, C.P. Timothy, S. Christian, A. Claude, and Y. Moenne-Loccoz, “The rhizosphere: aplayground and battlefield for soilborne pathogens and beneficial microorganisms” Plant Soil, vol 321, pp:341-361, 2009.

[6] L.H. Ba, “*Vietnam Soil Resource*,” Education Publishing House, Vietnam, 2009.

[7] H.J. Hoben, and P. Somasegaran, “Comparison of Pour, Spread and Drop Plate Methods for Enumeration of *Rhizobium* spp. in Inoculants made from Presterilized peat,” Appl. Environ. Microbiol., vol. 44, pp. 1246-1247, 1982.

[8] M. Park, C. Kim, J. Yang, H. Lee, W. Shin, S. Kim and T. Sa, “Isolation and characterization of diazotrophic growth promoting bacteria from Gram rhizosphere of agricultural crops of Korea,” Microbiological Research, vol 160, pp: 127-133, 2005.

[9] C.S. Nautiyal, “An efficient microbiological growth medium for screening phosphate-solubilizing microorganisms,” FEMS Microbiology Letters, vol. 170, pp:256-270, 1999.

[10] S.A. Gordon and R.P. Weber., “Colometric estimation of indolacetic acid,” Plant Physiol., vol 26, pp:192-195, 1951.

[11] J. Murphy and J.P. Riley, “A modified single solution for determination of phosphate in natural waters,” Anal. Chim. Acta., vol 27, pp:31-36, 1962.

[12] J.M. Andersen and J.S.I. Ingram, “Tropical Soil Biology and Fertlity: A Handbook on Methods, 2nd edn”, CAB International Wallingford, Oxford, UK. 221, p, 1993.

[13] B. Neumann, A. Pospiech, and H.U. Schairrer, “Rapid isolation of genomic DNA from Gram-negative,” Trends Gent., vol. 8, pp. 332-333, 1992.

[14] S. Turner, K.M. Pryer, V.P.M. Miao, and J.D. Palmer, “Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subnit rRNA sequence analysis,” J. Eukaryotic Microbiol., vol. 46, pp. 327-338, 1999.

[15] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar, “MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likehood, Evolutionary Distance and Maximum Parsimony Methods,” Mol. Biol. Evol., vol. 28, pp. 2731-2739, 2011.

[16] M.K. Halushka, J.B. Fan, K. Bentley, L. Hsie, N. Shen, A. Weder, R. Cooper, R. Lipshutz, and A. Charavarti, “Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homestasis,” Nat. Genet., vol. 22(3), pp: 239-247, 1999.

[17] D.T. H. Mai and C. N. Diep. “Isolation of phosphate solubilizing micro-organisms” Proceeding of the conference on science of Cantho University in 2002, pp: 353 – 358, 2002.

[18] G.A. Watterson, “On the number of segregation sites in general models without recombination,” Theor. Pop. Biol. vol. 7, pp: 256-276, 1975.

[19] J. Rozas and R. Rozas, “DnaSP versiom 4.1: an integrated program for molecular population genetics and molecular evolution analysis,” Bioinformatics, vol. 15, pp: 174-175, 2005.

[20] C. Picard, F. Di Cello, M. Ventura, R. Fami and A. Guckert, “Frequency and biodiversity of 2,4-diacetylphloroglucinol-producing bacteria isolated from maize rhizosphere at different stages of plant growth,” Appl. Environ. Microbiol. vol. 66:948-955, 2000.

[21] F. Di Cello, A. Bevivino, L. Chiarini, R. Fani, D. Paffetti, S. Tabacchioni and C. Damalstri, “Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different stages,” Appl. Environ. Microbiology., vol. 63, pp:4485-4493, 1997.

[22] C. Dalmastri, L. Chiarini, C. Cantale, A. Bevivino, and S. Tabaccioni, “Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* population,” Microb. Ecol., vol. 38, pp:273-284, 1999.

[23] T.V. Van, O. Berge, N. K. Suong, J. Balandreau, T. Heulin, “Repeated benefical effects of rice inoculation with a strain of *Burkholderia vietnamiensis* on early and late yield components in low fertility sulphate acid of Vietnam,” Plant Soil, vol.218, pp:273-284, 2000.

[24] N. T. Phong, N. T. P. Thao and C. N. Diep, “Isolation and identification of nitrogen-fixing bacteria in rice rhizosphere cultivated on alluvial soil of Vinh Long province, Vietnam”. Biotechnology J., Vietnam, vol.9 (4), pp: 521-528, 2011.

[25] C. N. Diep and D. T. Dep, “Effect of DASVILA biofertilizer on high-yielding rice (cv. OM 2514) cultivated on alluvial soil of Song Hau farm, Can Tho city,” Soil Science J., Viet nam, vol.36, pp:47-51, 2011.

[26] C. N. Diep and N. T. M. Tuyen,”Effect of DASVILA biofertilizer on high-yielding rice cultivated on alluvial soil of Tan Hiep district, Kien Giang province and Vinh Thạnh district, Can Tho city,” Soil Science J., Viet nam, vol.38, pp:91-94, 2011.

[27] C. N. Diep,” Effect of biofertilizer on High-yielding Rice Cultivated on Alluvial Soil of Mekong Delta,” Proceedings of JSPS AA International Seminar Can Tho University, Vietnam, September 2010, pp: 18-24. 2010.

[28] J.L. Gronemeyer, C.S. Burbano, T. Hurek and B. Reinhold Hurek, “Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia,” Plant Soil, vol. 356, pp: 67-82, 2012.

[29] K.D. Zinniel, P. Lambercht, N.B. Harris, Z. Feng, D. Kuczmarshki, P. Higley, C.A. Ishimaru, A. Arunakumari, R.G. Barletta, and A.K. Vidaver, “Isolation and charcaterization of endophytic bacteria from agronomic crops and prairie plants,” Appl. Environ. Microbiol., vol. 68, pp: 2198-2208, 2002.

[30] T. Heulin, M. Barakat, R. Christen, M. Lesourd, L. Sutra, G. De Luca, and W. Achouak, “*Ramlibacter tataouiensis* gen. nov., sp. nov., and *Ramlibacter henchirensis* sp. nov., cyst-producing bacteria isolated from subdesert soil in Tunisia,” Int. J. Syst. Evol. Microb., vol. 53, pp:589-594, 2003.