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Isolation and Identification of Rhizospheric Bacteria in Sugarcane (*Saccharum* spp. L.) Cultivated on Acrisols and Ferrasols of Dong Nai Province, the Southeast of Vietnam

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Abstract: Nitrogen-fixing and phosphate-solubilizing bacterial diversity and population dynamics in the Acrisol and Ferrasol rhizosphere of sugarcane grown in Dong Nai province, the eastern of South Vietnam was studied. Soil rhizosphere samples were taken in three districts (sites) 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 (27F and 1492R). A total of 31 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 nitrogen-fixing and phosphate-solubilizing bacteria correlated with N total concentration and organic matter content in soil closely ($P<0.05$). The sequences from selected nitrogen-fixing and phosphate-solubilizing bacteria (12 isolates) showed high degrees of similarity to those of the GenBank references strains (between 98% and 99%). From 12 isolates, 7 belonged to Bacilli, while 2 were *Beta-Proteobacteria*, 1 was *Acidobacteria* and 2 were Bacteroides. Based on Pi value (nucleotide diversity), Bacilli group had the highest theta value and Theta values (persequence) from S of SNP for DNA polymorphism were calculated for each group and Bacilli group had the highest values in comparison to three groups. From these results showed that two strains (*Bacillus megaterium* B6 (Ferrasols) and *Sphingomonas* sp. P14 (Acrisols)) revealed promising candidates with multiple beneficial characteristics and they have the potential for application as inoculants adapted to poor soils and sugarcane tree because they are not only famous strains but also are safety strains for agricultural sustainable.

Keywords: Acrisols, 16S rRNA Gene Sequence, Ferrasols, Nitrogen-Fixing Bacteria, Phosphate-Solubilizing Bacteria, Rhizosphere, Sugarcane

1. Introduction

Sugarcane (*Saccharum* sp. L.) is grown in more than 120 countries, mainly in Brazil and India [1] and Vietnam with 283,000 ha in which the eastern of South Vietnam occupied 34,395 ha and Dong Nai province had 9,287 ha (27% among 34,395 ha) in 2012 [2]. It has been a general practice to apply 250 kg N ha^{-1} yr^{-1} or more than in most sugarcane cultivating countries [3] and Vietnamese farmers want to have a high sugarcane yield (over 200 tons/ha), they should apply high quantity of inorganic nitrogen fertilizer (urea)(approx. 200 kg N ha^{-1} yr^{-1}, 100 kg P_2O_5 ha^{-1} (equivalent 400 kg superphosphate 15% P_2O_5 ha^{-1}) and 150 kg K_2O ha^{-1} (equivalent 250 kg KCl 60% K_2O ha^{-1} yr^{-1})[4].

The narrow zone of soil directly surrounding the root systems is referred to as rhizosphere [5], while the term "rhizobacteria" implies a group of rhizosphere bacteria component in colonizing the root environment [6]. Plant growth promoting rhizobacteria are the soil bacteria inhabiting around ≠ or on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere [7]. Over the last two decades, an increasing number of reports have

been published showing that plant growth-promoting rhizobacteria (PGPR) are associated with important crops such as rice, wheat, sorghum, sugarcane and maize [8][9][10][11]; many plant-associated bacteria are well known for their capacity to promote growth [12]. One of the main focuses of PGPR research is on nitrogen-fixing bacteria (diazotrophs), and this research is aimed at decreasing the use of costly chemical fertilizers that can negatively affect the environment [13, 9].

The eastern South Vietnam and the Mekong Delta are two big sugarcane cultivation regions in the South Vietnam; the sugarcane area occupied 12.7% with 34,395 ha, 66.5 ton/ha and productivity was 2,329,435 tons [2]. The eastern of South Vietnam locates from 105o49" to 107o35" E and from 10o20" to 12o17" 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 [14].

In order to make sugarcane cultivation sustainable and less dependent on chemical nitrogen fertilizers, it needs to be found the proportion of plant promoting bacteria, which are PGPR. The aims of this study were (i) isolation of rhizospheric bacteria, (ii) studying characteristic such as nitrogen fixation, phosphate solubilization and IAA production, (iii) the genetic diversity of PGPR isolated from soil was evaluated in order to identify an efficient growth promotion strains that can be also improve the growth of sugarcane plant as biofertilizer.

2. Material and Methods

2.1. Soil Sample and Isolation of Bacteria

The sugarcane plants were sampled at the stage of plant having 6 month-old (July 2013) from the fields of Dinhquan, Trangbom and Vinhcuu districts (Dong Nai province)[map determined the locates which to collect samples were presented in Tam and Diep [15]. Rhizosphere soil around sugarcane plants were collected to moving the soil that adhered to the roots (stem and root of sugarcane plant will be used in further experiment) and they were kept in refrigerator for counting by viable drop plate count [16] and isolation of nitrogen-fixing bacteria in Burk's N free medium [17] and phosphate-solubilizing bacteria in NBRIP medium [18]; cultures were streaked on media to obtain single colonies. To check for phosphate solubilization ability or nitrogen fixation ability, colonies from Burk's N free medium were streaked to NBRIP medium and colonies from NBRIP medium were also cultivated to Burk's N free medium in order to select the colonies which developed on two media (or microbes having N2-fixing and phosphate-solubilizing ability).

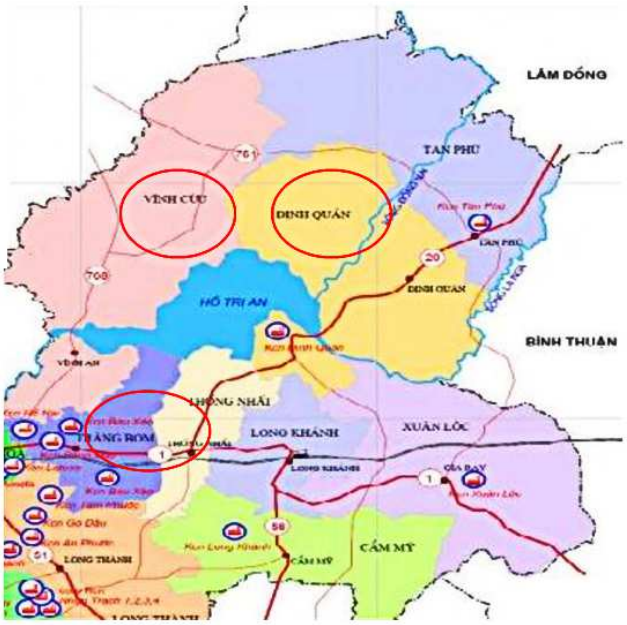

Figure 1. *The locations were examined in this study and sugarcane rhizospheric soil were collected at three sites (Dinhquan, Vinhcuu, Trangbom distrcits) of Dongnai pronvince, the eastern of South Vietnam*

2.2. Screening for Biofertilizer Activities

The ability to fix N_2 was tested on Burk's N-free liquid medium incubating at 30oC and the ammonium concentration in medium was measured by Phenol Nitroprusside method after 2, 4, 6 and 8 days inoculation (DAI) and inorganic phosphate solubilization ability was tested on NBRIP liquid medium and they were incubated at 30oC 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 [19]. Precultures were grown in Burk's N free (100 mL) without tryptophan in 250mL-flask at 30oC 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 [20] and organic carbon measured by Walkley-Black method [21].

2.3. 16S rDNA Gene Amplification and Sequencing

Bacterial DNA was isolated following published protocols [22]; Amplification of 16S rDNA by PCR was carried out using the universal primers 27F and 1492R [23]. The 50 µ L reactionmixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 µ M of each deoxynucleotide triphosphates,

500 nM of each primer (Fermentas) and 20 ng DNA. The

thermocycling profile was carried out with an initial denaturation at 95^0C (5 min) followed by 30 cycles of denaturation at 95^0C (30 s), annealing at ~~55°C~~ 55^0C (30 s), extension at 72^0C (90 s) and a final extension at 72^0C (10 min) in C1000 Thermal Cycler (Bio-Rad).

Aliquots (10 µ l) of PCR products were separated and visualized in 1% agarose gels by using standard electrophoresis procedures. Partial 16S rRNA genes of selected 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 12 isolates of 3 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 [24] and phylogenetic tree were constructed by the neighbor-joining method using the MEGA software version 6.06 based on 1000 bootstraps.

2.4. PCR Amplification of the nif-H Genes

PCR amplification was performed to determine the presence of nif-H gene using specific primers described by Poly et al. [25]. Amplification reaction was performed in a total volume of 25 µ l. The reaction mixture contained: 2.5 µ l 10x PCR buffer, 2.5 µ l of 2 mM each of dATP, dCTP, dTTP and dGTP, 3 µ l of each forward PolF (5"-TGCGYCCSAARGCBGACTC-3") and reverse PolR (5"-ATSGCCATYTCRCCGGA-3") primer (30 ng), 1 µl of template DNA (10 ng) and 0.3 µ l of (3 U/l) Taq polymerase; final volume was made into 25 µ l using mili-Q water. The step-up PCR procedure included denaturation at 95oC for 3 min, 58oC for 1 min, and 72oC for 1 min, followed by 35 cycles of 95oC for 45 sec, 58oC for 45 sec, and 72oC for 1 min, with a final extension at 72oC for 7 min. Amplication products were electrophoreses on 1.5% agarose gel in 1X Tris-borate-EDTA (TBE) buffer.

2.5. 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.

2.6. Nucleotide Diversity (Θ)

Nucleotide diversity (Θ) was calculated by the method described by Halushka et al. [26]

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

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

2.7. 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 and P=0.05 were used to differentiate between statistically different means using SPSS version 16.

3. Results and Discussion

Soil Characteristics

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

Soil sample site	Soil pH	N total (%)	Avail able P_2O_5 (mg/k g)	Organ ic matter (%)	N_2-fixing bacteria populatio CFU log g/soil	Phosphate -solubilizi ng bacteria
Trangbom 1	4.58	0.12	15.6	2.12	6.126	5.175
Trangbom 2	4.62	0.14	12.7	1.51	6.114	5.005
Trangbom 3	5.07	0.13	11.7	1.13	6.078	5.125
Trangbom 4	5.22	0.17	22.1	1.91	6.178	5.182
Trangbom 5	5.49	0.15	15.3	1.62	6.024	5.282
Vinhcuu 1	4.91	0.19	31.8	2.15	5.556	7.326
Vinhcuu 2	5.12	0.11	16.7	1.25	5.531	6.892
Vinhcuu 3	5.58	0.14	17.3	1.68	5.381	6.381
Dinhquan 1	5.39	0.04	10.6	3.25	8.107	8.182
Dinhquan 2	5.49	0.03	6.6	2.25	7.958	7.458
Dinhquan 3	5.66	0.05	7.1	3.01	7.444	6.459
Dinhquan 4	5.37	0.07	8.3	2.78	7.129	7.989

Three sites in Dong Nai province (in eastern of South Vietnam) have large cultivated sugarcane area (Trangbom,

Vinhcuu and Dinhquan) and sugarcane has been cultivated on Acrisols (Trangbom and Vinhcuu) and Ferrasols (Dinhquan) showed that characteristic of acrisol is low soil pH together with low organic matter content while soil pH and organic matter of ferrasols (Dinhquan) are high; however N total and available P_2O_5 are lower than acrisols (Trangbom and Vinhcuu)(Table 1). Interestingly, nitrogen – fixing bacterial population and phosphate-solubilizing bacterial population in ferrasols were high (almost over one million cells per dry soil gram) but population of phosphate-solubilizing bacteria inacrisols was low? (from one hundred to ten million cells per soil gram).

The results from Table 2 showed that there was no significant linear relationship between population of N_2-fixing and phosphate-solubilizing bacteria and soil pH and both of microbes with organic matter content were a linear relationship significantly at $P<0.05$ ($y = 1.0853x + 2.388$, $r=0.778^{**}$; $y = 1.1376x + 4.0335$, $r = 0.645^{*}$, respectively) and there was a significant linear relationship between N_2-fixing bacteria population with N total concentration in soil at $P<0.05$ ($y = 15.854x + 8.2394$, $r = 0.879^{**}$) but there was a significant linear relationship between phosphate-solubilizing bacterial population with N total concentration in soil at $P<0.05$ ($y = -13.166x + 7.841$, $r = 0.879^{**}$) and there was no difference between population of phosphate-solubilizing bacteria with 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 bacterial population rather than phosphate-solubilizing bacteria population in acrisols.

Thirty-one bacterial isolates were isolated from 12 soil samples in two media (Burk's N free and NBRIP medium) (Table 3) with 10, 11 and 10 isolates from Trangbom, Vinhcuu and Dinhquan, 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 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 = 0.418 (ns)	r = 0.427 (ns)
	y = 1.103x + 0.724	y = 1.422x - 1.039
N total	r = 0.879**	r = 0.578*
concentration (%)	y = 15.854x + 8.239	y = - 13.166x + 7.841
Available P	r = 0.689*	r = 0.419 (ns)
(mg/kg soil)	y = - 0.093x + 7.835	y = - 0.025x + 6.739
matter (%)	r = 0.778**	r = 0.645*
	y = 1.085x + 4.238	y = 1.137x + 4.033

ns : not significantly different

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's N free medium	NBRIP medium
Trangbom	10	6	4
Vinhcuu	11	6	5
Dinhquan	10	5	5

Table 4. *Ammonium (NH_4^+), Available P (P_2O_5) and IAA concentration (mg/l) of 31 isolates*

Site	Bacterial name	Ammonium (NH_{4+}) concentration	Available P (P_2O_5) concentration	IAA concentration (mg/L)
Trangbom	B1	0.057	36.13	0.144
	B2	0.231	45.11	0.734
	B4	0.143	41.88	0.467
	B16	0.649	34.01	0.574
	B17	0.375	26.57	0.472
	B19	0.572	27.67	0.595
	P1a	0.231	85.20	0.525
	P2	0.310	116.20	0.035
	P4b	0.108	122.80	0.115
	P19	0.161	156.70	0.533
Vinhcuu	B9	0.985	26.98	1.031
	B10	0.572	15.39	0.735
	B11	0.583	37.09	0.987
	B12	0.407	31.27	0.343
	B13	0.405	39.87	0.675
	B18	0.417	29.84	0.447
	P8a	0.110	30.63	0.268
	P9	0.079	108.05	0.501
	P10	0.489	103.05	1.023
	P14	0.579	132.90	0.913
	P15	0.075	113.58	0.468
Dinhquan	B3	0.293	41.88	0.850
	B5	0.049	21.75	0.883
	B6	0.706	17.82	0.780
	B7	0.044	40.58	1.036
	B8	1.179	25.69	1.302
	P3	0.113	173.38	0.088
	P5a	0.097	102.28	0.381
	P5b	0.275	94.88	0.254
	P6	0.094	118.50	0.996
	P7	0.156	90.40	0.176
	control	0.000	0.00	0.000
	LSD.01	0.053	19.68	0.168
	C.V	8.77%	16.41%	15.88%

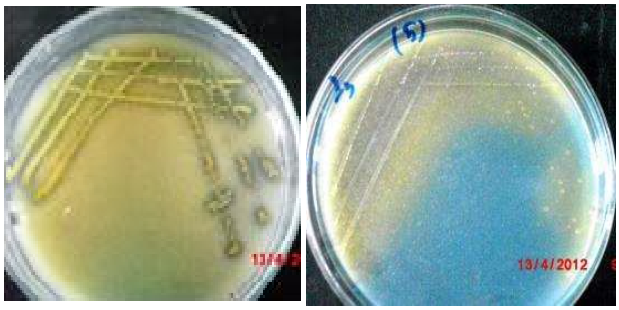

Figure 2. *The colonies of several isolates on NBRIP medium (left) with the halos around the colonies and on Burk's N free (right)*

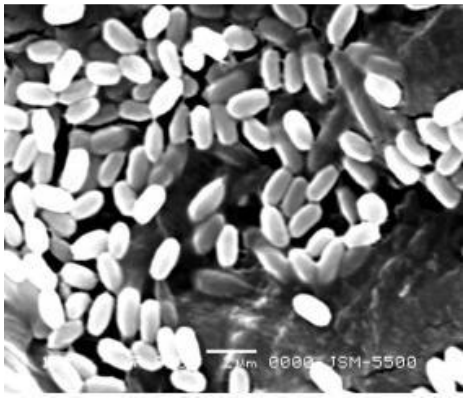

Figure 3. *Electron micrograph of cells*

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 site (district); IAA concentration varied from 0.035 to 1.032 mg/L. Especially P14 isolate solubilized high amount of phosphorus and synthesized high IAA concentration (Table 4).

Almost their colonies have round-shaped; milky (on Burk's medium) and yellow (on NBRIP medium); entire or lobate margin (Figure 2); 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. Especially phosphate-solubilizing bacteria make a halo around colonies in NBRIP medium as described of Thanh and Diep [27](Figure 2, P14).

The cells were observed by SEM and appeared as short rods and most of them have motility (Figure 3).

All 31 isolates have nitrogen fixation (including 14 isolates isolated on NBRIP having nif gene (examined by Pol primers), phosphate solubilization and IAA biosynthesis however 12 isolates having good biofertilizer activity were chosen to study including many isolates from Burk's N free and NBRIP medium.

The fragment of 1485 bp 16S rRNA were obtained from PCR with 27F and 1492R primers and sequencing. Homology searches of 16S rRNA gene sequence of selected strain in GenBank by BLAST revealved that they had similarity to sequences of Bacilli (7/12 isolates), 2 isolates belonged to Betaproteobacteria, 1 was Acidobacteria and 2 were Bacteroides (Figure 4)(Table 5). Especially 7 isolates belonged to Bacilli which isolated on Burk's N free medium while 5 isolates that isolated on NBRIP medium belonged to three other groups.

Table 5. *Phylogenetic affiliation of isolates on the basis of 16S rRNA genes sequences by using BLAST programme in the GenBank database based on sequences similarity*

Taxonomic group and strain	Closest species relative	Similarity (%)	Medium
Bacilli			
B2	*Bacillus subtilis* strain PWK31 (KJ620421)	99	Burk's N free
	Bacillus subtilis strain Bp-1 (KP229430)	99	
B6	*Bacillus megaterium* strain HNS88 (KF933685)	99	Burk's N free
	Bacillus aryabhattai isolate PSB59 (HQ242772)	99	
B8	*Bacillus megaterium* strain HNS68 (KF933665)	99	Burk's N free
	Bacillus megaterium strain HT-Z56 (KJ526880)	99	
B9	*Bacillus subtilis* strain YXA1-23 (JF701929)	99	Burk's N free
	Azospirillum lipoferum, isolate 1:2 (LK3917040	99	
B10	*Bacillus methylotrophicus* strain JF29 (KC172004)	98	Burk's N free
	Geobacillus sp. CRRI-HN-1 (JQ695928)	98	
B11	*Bacillus megaterium* strain B12 (KF010350)	99	Burk's N free
	Bacillus horikoshii strain IARI-HHS2-13 (KF054756)	99	
B17	*Geobacillus stearothermophilus* strain DDKRC4 (JN641292)	99	Burk's N free
	Bacillus tequilensis strain M60 (JF411297)	99	
Acidobacteria			
P2	*Terriglobus roseus* strain KBS 112 (DQ660895)	98	NBRIP
	Terriglobus sp. TAA 43 (AY587228)	98	
Betaproteobacteria			
P6	*Burkholderia acidipaludis* strain NBRC 101816 (NR_113024)	99	NBRIP
	Burkholderia sp. D54 (HM467915)	99	
P8a	*Burkholderia pyrrocinia*, strain: Rai 3 (AB898035) *Burkholderia ambifaria* strain 1835 (KM487704)	99	NBRIP
Bacteroides			
P14	*Sphingomonas* sp. WFR-69 (KC455432)	98	NBRIP
	Sphingomonas sp. 23R (AY741145)	98	
P19	*Sphingomonas* sp. EC-K085 (AB264174)	98	NBRIP
	Novosphingobium subterraneum 23R (AY752914)	98	

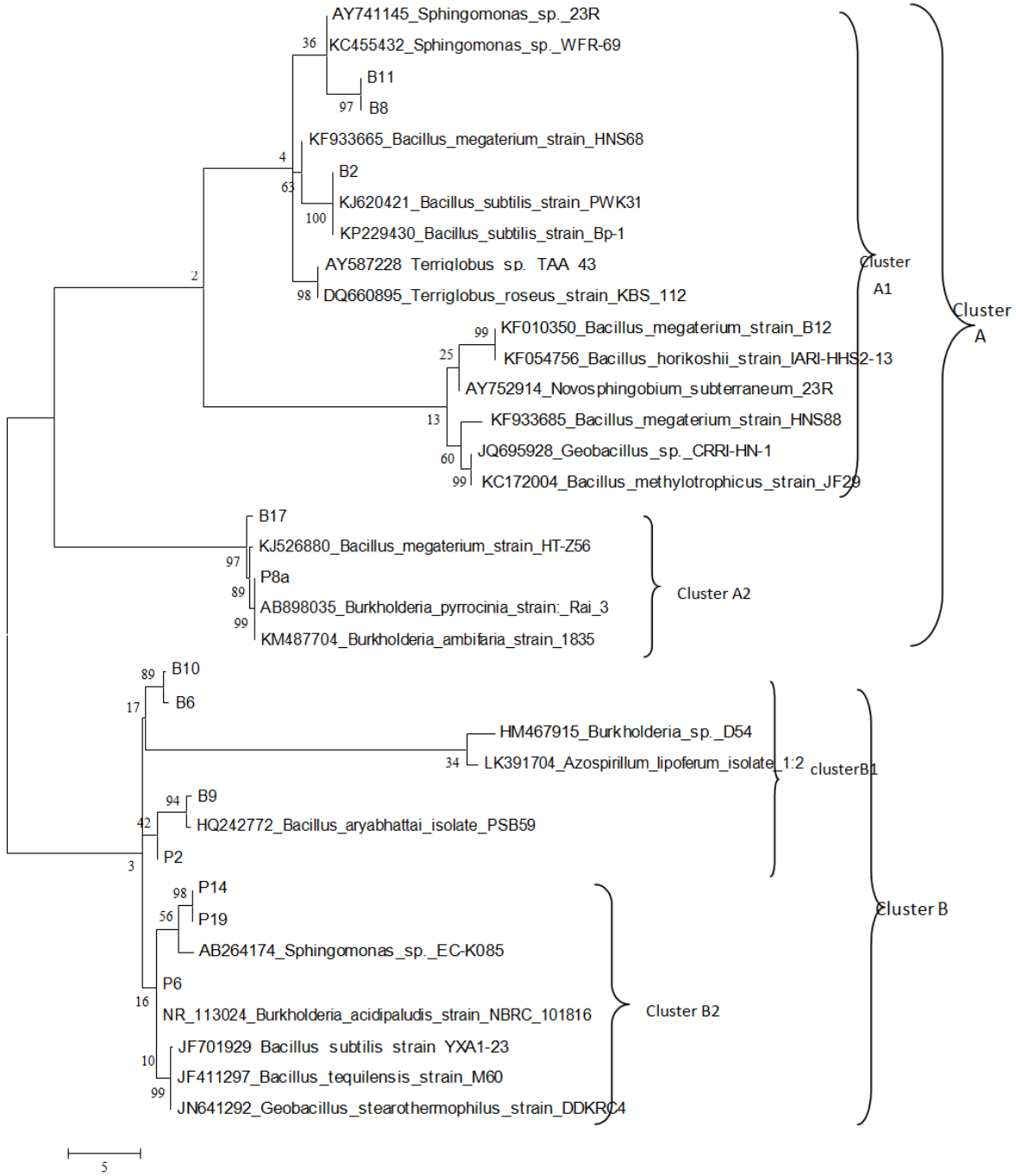

Figure 4. *Phylogenetic tree showing the relative position of rhizobacteria (PGPR) by the maximum-likelihood method of complete 16S rRNA sequences. Bootstrap values of 1000 replicates are shown at the nodes of the trees.*

A maximum-likelihood tree phylogenetic tree in these isolates showing the two clusters: cluster A with 5 isolates including two small clusters as cluster A1 with 3 isolates (B11, B8 and B2) and cluster A2 with B17 and P8a while cluster B with 7 isolates arranged in two small clusters, cluster B1 with B10, B6, B9 and P2 and cluster B2 with P14, P19 and P6, this result showed that the isolates were isolated from other rhizospheric soils as acrisols or ferrasols, they originated from Vinhcuu site, Dinhquan site or Trangbom site (with distance more than 100 km far from) but they have the nearest phylogenetic sequences. This result was also demonstrated in the phylogenetic tree with 12 strains (Figure 5). Cluster A divided two small clusters: cluster A1 with cluster A11 with *Bacillus megaterium* B6, *Bacillus methylotrophicus* B10 and *Burkholderia acidipaludis* P6; cluster A12 with *Bacillus subtilis* B9 and *Terriglobus roseus* P2 while cluster A2 with cluster A21 composed of *Bacillus megaterium* B8 and *Bacillus megaterium* B11 and cluster A22 with *Sphingomonas* P14 and *Spingomonas* P19. Cluster B included with *Bacillus subtilis* B2, *Geobacillus stearothermophilus* B17 and *Burkholderia pyrrocinia* P8a.

Theta values (per sequence) from S of SNP for DNA

polymorphism were calculated for Each group, and Bacilli group had the highest values as comparison with Acidobacteria, Betaproteobacteria and Bacteroides (Table 6).

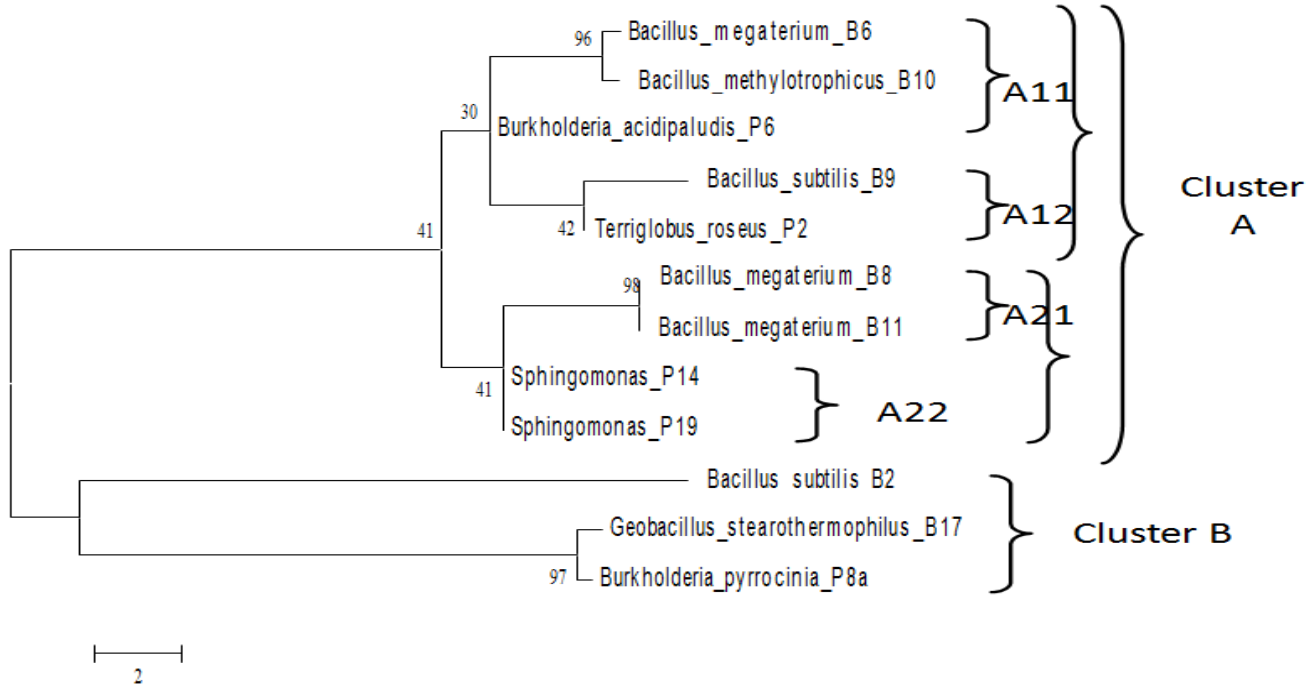

Figure 5. *Phylogenetic tree for partial 16S rRNA gene sequences from 12 strains by using primers (27F-1492R) showing relationships between representative strains along with related sequences retrieved from GenBank. The numbers at the nods indicate the levels of bootstrap support (%) based on Maximum-Likelihood analysis of 100-re-sampled datasets. The scale bar indicates the phylogenetic distance corresponding to 5 changes per 100 bases.*

Table 6. *Genetic diversity of 12 strains*

	Nucleotie diversity	Theta (per site) from Eta	Theta (per site) from S (Θ)
12 strains	0.71432	0.88476±0.126	0.33114±0.016

Primers 27F and 1492R

The rhizospheric bacteria has been studied and described as beneficial bacteria with Gram-positive bacteria presented on Burk"s N free medium and it occupied over 50% among 12 strains in our result (Figure 6).

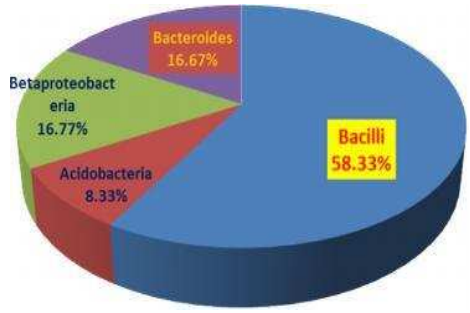

Figure 6. *The proportion of group and they distributed in four clusters*

Nucleotide polymorphism can be measured by many parameters, such as halotype (genes) diversity, nucleotide diversity, Pi, Theta (Θ) (per group) etc… In this study, nucleotide diversity was estimated by Theta, the number of segregating sites [28], and its standard deviation (SΘ). These parameters were estimated by DNA Sequence Polymorphism software version 4.0 [29]. Pi values explained nucleotide diversity of sequences for each gene, the higher values, the more diversity among group. Bacilli group had the highest

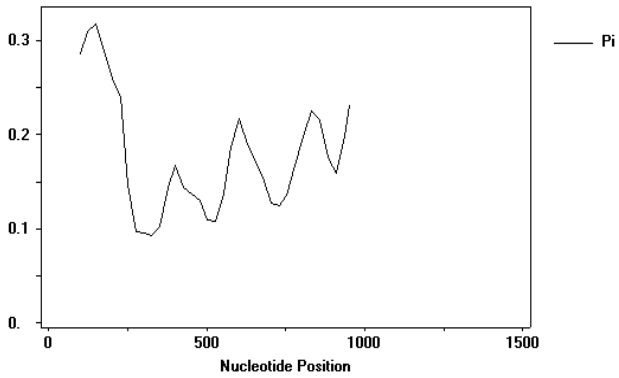

Figure 7. *Variation of nucleotide from position 200 to position 400*

In 7 Bacilli strains had variation of nucleotide from position 200 to position 400 (Figure 7).

The plant-beneficial rhizobacteria may decrease the global dependence on hazardous agricultural chemicals which destabilize the agro-eco-systems [7]. The rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility [30]. The plant growth promoting rhizobacteria (PGPR), are characterized by the following inherent distinctiveness: (i) they must be proficient to colonize the root surface (ii) they must survive, multiply and compete with other microbiota, at

least for the time needed to express their plant growth promotion/protection activities, and (iii) they must promote plant growth [31]. Many plant-associated bacteria are well known for their capacity to promote [32]. Analyses of 16S rRNA gene sequences revealed the presence of plant growth promoting (PGPR) species of *Burkholeria, Pantoea, Pseudomonas* and *Microbacterium* in sugarcane endophytic bacterial communities [33,34]. Furthermore, new species of aerobic endospore forming bacteria (AEFB) have been isolated from sugarcane internal tissues and rhizosphere [35][36][37].

'*Bacilli'* AEFB are a diverse group with wide distribution in agricultural soils that contribute both directly and indirectly to plant development [38]. Numerous *Bacillus* and related genera with plant growth promoting (PGP) activities have been isolated from soybean, corn, sorghum and wheat rhizospheres [39][40][41]. Eichorst et al. [42] discovered a new genus *Terriglobus* with *Terriglobus roseus*, belonged to phylum *Acidobacteria*, its colonies were approximately 1 mm in diameter and either white or pink, the latter due to a carotenoid(s) that was synthesized under 20% instead of 2% oxygen, especially its potential widespread distribution in soil. Genus *Sphingomonas* discovered and presented the physiology and ecology by White et al. [43] and Ali et al. [44] reported genus *Sphingomonas* has a ability of nitrogen fixation, phosphate and potassium solubilization and *Sphingomonas* together with *Burkholderia, Bacillus, Pseudomonas* have been used biofertilizing microbes. Recently Bumunang and Babalota [45] examined the rhizobacteria from field grown GM maize in South Africa as follows species of *Pseudomonas, Aeromonas, Sphingomonas, Burkholderia, Bacillus, Stenotrophomonas, Achromobacter, Ewingella,* they have catalase activity, ammonia production, IAA production, phosphate solubilisation and antifungal activity. Our results also found the rhizospheric bacteria in acrisols and ferrasols with species of bacilli, betaproteobacteria as *Burkholderia, Acidobacteria* and *Sphingomonas.*

4. Conclusion

From 13 soil samples of acrisols and ferrasols of sugarcane regions in three districts (sites) of Dong Nai province, the eastern of South Vietnam, 31 isolates were isolated on two media (Burk's N free and NBRIP) and they were identified as rhizospheric bacteria and 12 isolates having good plant growth promotion were chosen to analyse their relationship. These isolates were identified as Bacilli (more than 50%), Acidobacteria, *Burkholderia*, *Sphingomonas* on both soils (acrisols and ferrasols). Among them, there are two strains will be suggested to produce for sugarcane cultivation on acrisols and ferrasols in the future.

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