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ISOLATION AND CHARACTERIZATION OF RHIZOSPHERIC BACTERIA IN SOYBEAN (*Glycine max* (L.) Merrill) CULTIVATED ON FERRALSOLS OF CUJUT DISTRICT, DAKNONG PROVINCE, VIETNAM

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ABSTRACT

Rhizobacteria in the ferralsols rhizosphere of soybean grown in CuJut district, DakNong province, highland of Vietnam was studied. A total of 63 isolates were isolated on two media (Burk's N-free and NBRIP) from 24 soil rhizosphere samples were taken in 24 sites of this region and all of them have ability of nitrogen fixation and phosphate solubilization. The sequences from selected rhizobacteria (11 isolates) were analysed 16S rRNA gene fragments amplified from DNA using eubacterial universal primers (8F and 1492R) and sequenced. The results showed they had high degrees of similarity to those of the GenBank references strains (between 97% and 99%). From 11 isolates, 6 belonged to Bacilli while Proteobacteria having 2, and 3 were Alpha, and Gamma-Proteobacteria, respectively. Based on Pi value (nucleotide diversity), Bacilli group had highest Theta value and Thete values (per sequence) from S of SNP for DNA polymorphism were calculated from each group and Bacilli group had the highest values in comparison to others. There were 18/63 and 22/63 isolates producing IAA and siderophores, respectively. From these results showed that there are five strains as *Bacillus subtilis* CJ27, *Acinetobacter lwoffii* CJ28, *Agrobacterium tumefaciens* CJ32, *Bacillus subtilis* CJ33 and *Bacillus subtilis* CJL18 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, especially for soybean cultivation on ferralsols in the future.

KEYWORDS: 16S rRNA, Ferralsols, Gene Sequence, Rhizobacteria, Rhizosphere, Soybean.

INTRODUCTION

Soil is replete with microscopic life forms including bacteria, fungi, actinomycetes, protozoa and algae. Of these different microprganisms, bacteria are by far the most common (i.e., 95%). It has been known for some time that the soil hosts a large number of bacteria (often around 10^8 to 10^9 cells per gram of soil) and that the number of culturable bacterial cells in soils is generally only about 1% of the total number of cells present (Schoenborn et al. 2004). They are involved in various biotic activities of the soil ecosystem to meke it dynamic for nutrient turn over and sustainable for crop production (Ahemad et al., 2009). Based on their experiments on radishes, Kloepper and Schroth (1978) introduced the term "rhizobacteria" to the soil bacterial community that competitively colonized plant roots and stimulated growth and thereby reducing the indidence of plant diseases. They stimulating plant growth regulators, protecting plants from phytopatogens by controlling or inhibiting them, improving soil struture and bioremediating the polluted soils by sequesting toxic heavy metal species and degrading xenobiotic compounds (like pesticides)(Ahemad, 2012). Indeed, the bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilizing the nutrients compared to those from soils (Hayat et al. 2010). Therefore, the plant growth promoting rhizobacteria (PGPR), are characterized by the following inherent distinctiveness's: (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 protion/protection activities, and (iii) they must promote plant growth (Kloepper, 1994).

DakNong province is situated in the highland of Vietnam, it locates from $107^o42'03"$to $107^o44'44"$ E and from $11^o59'01"$ to $12^o40'56"$ N and CuJut is a district of DakNong province, it locates the north of province (Figure 1). The soils are mainly red latosols (from origin of volcanic mountain) or ferralsols (FAO classification) with a pH range of 4.61 - 4.91. They are considered a good nutrient, with an average organic matter of 2.75 – 4.06%, a total nitrogen range of 0.11 – 0.13%, but it has concentrations of low available phosphorus, cation exchange capacity, exchangeable K (WASI, 2014). Many kinds of crop such as rubber, coffee, pepper, upland-rice, corn and soybean have been cultivated on ferralsols permenantly. Soybean [*Glycine max* (L.) Merr.], is an important protein and oil source and is one of the

most important grain legumes in the world (Li et al., 2011); soybean is also planted on ferralsols during wet season (April to November in year) but low grain yield (1 – 2 ton(s)/ha).

Figure:1 The location was examined in this study and Cujut district (DakNong province)[with dark blue] and ferralsols were presented soils with reddish brown latosols and red & brown latosols

The application of native, adapted microorganisms might improve yields by direct plant growth promotion and increasing grain yield, decreasing cost in soybean cultivation in order to enhance income for the farmers. The aims of this study were (i) isolation and characterization of rhizopheric bacteria, (ii) selection of best bacterial isolates and (iii) identification of these bacterial isolates. These bacteria can be considered promising candidates for application in sustainable agricultural management for this region.

MATERIALS AND METHODS

Soil sample and isolation of bacteria

The soybean plants were sampled at the stage of flowering during the rainy season (April to July 2014) from the fields of CuJut district, DakNong province which is one of the biggest soybean cultivation areas in highland of Vietnam. Samples were collected on 8 sites (3 samples in each site) of this town because soybean has been planted on ferralsols. Samples were taken whole plant with stem, root (10-20 cm depth) together with soil which around soybean plants; samples were kept in 18^{o}C plastic box before transferred to laboratory in Can Tho University. Rhizosphere soil around soybean plants were collected to moving the soil that adherered to the roots (stem and root of soybean plant will be used in further experiment)

and isolation of nitrogen-fixing bacteria in Burk'N free media (Park et al., 2005) and phosphate-solubilizing bacteria in NBRIP media (Nautiyal, 1999); 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).

Screening for biofertilizer activities

The ability to fix N_2 was tested on Burk'N-free liquid medium incubating at 30^oC and the ammonium concentration in medium was measured by Phenol Nitro-prusside method after 2,4,6 and 8 day after inoculation (DAI) and inorganic phosphate solubilization ability was tested on NBRIP liquid medium and they were incubated at 30^oC 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 (Gordon and Weber 1951). Precultures were grown in Burk's N free (100 ml) without tryptophan in 250mL-flask at 30^oC 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 Salkowki 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 Genesys 10uv Thermo Scientific spectrophotometer. The isolates were spot inoculated onto Chrome azurol S agar plates divided into equal sectors, and the plates were incubated at 28^oC for 48 h. Development of a yellow, orange or violet halo around the bacterial colony was considered to be positive for siderophore production (Schwyn and Neilands, 1987).

16S rDNA gene amplification and sequencing

Bacterial DNA was isolated following published protocols (Neumann et al., 1992); Amplification of 16S rDNA by PCR was carried out using the universal primers 8F and 1492R (Turner et al., 1999). The 50 µL reactionmixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 µM of each desoxynecleotide triphosphate, 500 nM of each primer (Fermentas) and 20 ng DNA. The thermocycling profide was carried out with an initial denaturation at 95^oC (5 min) followed by 30 cycles of denaturation at 95^oC (30 s), annealing at 55^oC (30 s), extension at 72^oC (90 s) and a final extension at 72^oC (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. 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 11/63 isolates of 24 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 (Tamura et al., 2011) and phylogenetic tree were constructed by the neighbor-joining method using the MEGA software version 6.06 based on 1000 bootstraps.

SNPs Discovery

The sequence date from 11 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.

Nucleotide Diversity (Θ)

Nucleotide diversity (Θ) was calculated by the method described by Halushka et al. (1999)

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

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

Data analyses

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.

RESULTS AND DISCUSSIONS

Isolation of bacteria and Screening for biofertilizer activities

Sixty-three bacterial isolates were isolated from 24 soil samples in two media (Burk'N free and NBRIP medium)(Table 1) and all isolates grew well on both of media (they have nitrogen fixation and phosphate solubilization ability)(Figure 2a and Figure 2b).

The results showed that these bacterial isolates synthesized high ammonium concentration but they solubilized big quantity of phosphorus. Especially CJ09, CJ27 and CJL01a isolates synthesized the highest ammonium comcentration and BHN8 isolate solubilized high amount of phosphorus (Table 1).

Figure: 2a The colonies of isolate CJL08 on Burk's N free

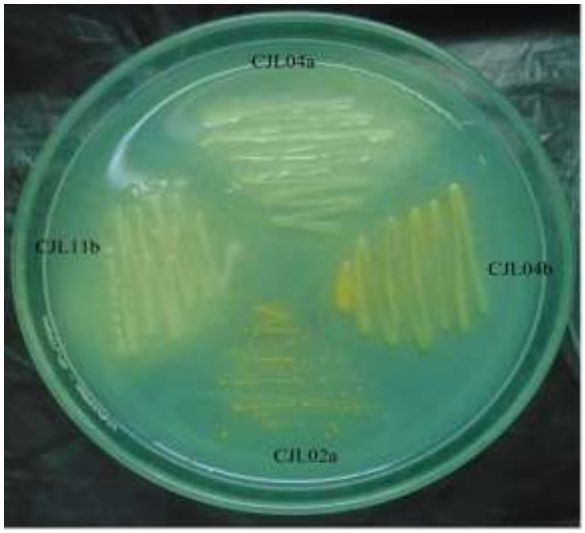

Figure: 2b The colonies of four isolates in NBRIP medium with the halos around the colonies and changed the color of medium because of organic acids

Table 1. Ammonium (NH_4^+) and available P (P_2O_5)(mg/L) of 63 bacterial isolates

No	Bacterial name	Ammonium (NH_4^+) conc. (ml/L)*	Available P (P_2O_5) conc. (mg/L)**	No	Bacterial name	Ammonium (NH_4^+) conc. (ml/L)*	Available P (P_2O_5) conc. (mg/L)**
0	**Control**	0.000 t	0.00 r	32	CJ32	0.648 k	530.70 h
1	CJ01	0.000 t	198.60 n	33	**CJ33**	**1.176 e**	**768.48 d**
2	CJ02	0.000 t	646.57 f	34	CJ34	3.861 a	363.71 l
3	CJ03	0.000 t	261.55 n	35	CJ35	0.616 l	438.95 j
4	CJ04	0.000 t	295.78 m	36	**CJL01a**	0.000 t	**896.67 a**
5	CJ05	0.000 t	709.75 e	37	CJL01b	1.051 g	203.21 n
6	CJ06	0.000 t	152.43 p	38	CJL02a	0.637 k	628.50 f
7	CJ07	0.000 t	182.95 o	39	CJL02b	0.862 i	6.33 r
8	CJ08	0.000 t	599.59 g	40	CJL03a	0.000	311.67 m
9	**CJ09**	0.000 t	**861.81 b**	41	CJL03b	0.943 h	181.28 n

10	CJ10	0.000 t	109.08 q	42	CJL04a	0.000 t	583.17 g
11	CJ11	0.000 t	738.63 d	43	CJL04b	0.172 o	372.83 l
12	CJ12	0.000 t	377.68 l	44	CJL05a	1.190 d	276.15 m
13	CJ13	0.000 t	169.10 o	45	CJL05b	0.000 t	325.17 m
14	CJ14	0.000 t	172.25 o	46	CJL06	0.964 h	190.13 n
15	CJ15	0.000 t	144.55 p	47	CJL07a	0.010 t	97.67 q
16	CJ16	0.043 s	732.92 d	48	CJL07b	1.205 c	98.21 q
17	CJ17	0.000 t	206.57 n	49	CJL08	0.990 h	27.41 s
18	CJ18	0.000 t	532.92 h	50	CJL09	1.205 c	360.17 l
19	CJ19	0.000 t	706.89 e	51	CJL10	**1.282 b**	113.28 q
20	CJ20	0.110 r	649.11 f	52	CJL11a	0.000 t	206.33 n
21	CJ21	0.040 s	560.86 g	53	CJL11b	0.142 p	298.00 m
22	CJ22	0.060 s	557.68 g	54	CJL12	1.143 f	140.72 p
23	CJ23	0.171 o	623.40 f	55	CJL13	0.118 q	212.83 n
24	CJ24	0.129 q	338.95 l	56	CJL14	0.309 m	196.67 n
25	CJ25	0.102 r	432.60 j	57	CJL15a	1.026 g	194.49 n
26	CJ26	0.120 q	308.79 m	58	CJL15b	0.122 q	482.17 i
27	**CJ27**	0.260 n	**803.08 c**	59	CJL16	1.046 g	12.87 r
28	CJ28	0.634 k	486.25 k	60	CJL17	0.157 f	623.50 f
29	CJ29	0.634 k	270.70 m	61	CJL18	0.078 s	**746.17 d**
30	CJ30	0.435 l	326.89 l	62	CJL19a	**1.154 f**	**209.50 n**
31	CJ31	0.769 j	718.63 e	63	CJL19b	1.057 g	85.85 q
C.V (%)		**1.93**	**0.45**	**C.V (%)**		**1.93**	**0.45**

*data were recorded at 2 DAI and ** data were recorded at 15 DAI

Means within a column followed by the same letter/s are not significantly different at p<0.01

However, all isolates were chosen to test IAA concentration in vitro in NBRIP medium adding with 100 mg tryptophan/L. After 4 days incubation, there were twenty isolates synthesed high IAA concentration (>1 mg/L)(Table 2).

Table 2. IAA concentration (ml/L) of 18 isolates

No	Bacterial name	IAA (mg/L)	No	Bacterial name	IAA (mg/L)	No	Bacterial name	IAA (mg/L)
01	CJ02	0.703 g	07	**CJ27**	**0.572** h	13	CJL08	1.133 e
02	CJ08	0.815 fg	08	**CJ28**	**0.679** h	14	**CJL06**	**2.267** c
03	CJ17	0.903 f	09	**CJ32**	**0.739** g	15	**CJL10**	**2.800** b
04	CJ24	0.679 h	10	CJL01a	**1.600 d**	16	**CJL18**	**3.467** a
05	CJ25	0.542 h	11	**CJL02b**	**3.067** b	17	**CJL15a**	**2.333** a
06	**CJ33**	**0.909** f	12	**CJL05a**	1.000 ef	18	**CJL19a**	**3.400** a

CV= 10.7%

Means within a column followed by the same letter/s are not significantly different at p<0.01

Almost their colonies have round-shaped; milky, white clear (on Burk's medium) and yellow, reddish yellow (on NBRIP medium); entire or loabate margin (Figure 2a and 2b); diameter

size of these colonies varied from 0.2 to 3.0 mm and all of them are Gram-positve and Gram-negative by Gram stain. Especially phosphate-solubilizing bacteria make a halo around colonies in NBRIP medium as described of Thanh and Diep (2014), Tam and Diep (2014)(Figure 2b).

16S rDNA gene amplification and sequencing

The fragments of 1495 bp 16S rRNA were obtained from PCR with 8F 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 (6/11 isolates), 3 isolates belonged to Gammaproteobacteria, and 2 were Alphaproteobacteria with 6 strains grew on Burk's N free and 5 strains on NBRIP medium (Table 3).

Table 3. Phylogenetic affiliation of isolates on the basis of 16S rRNA genes sequences by using BLAST programmes in the GenBank database based on sequences similarity

Taxonomic Group and Strain	Closest species relative	Similarity (%)	Medium
Bacilli			
CJL5a	*Bacillus megaterium* strain AceR-2 (FJ605385)	99	Burk's N free
	Bacillus aryabhattai strain SMT49 (KF962977)	99	
CJL2b	*Bacillus aryabhattai* strain fwzb8 (KF208490)	99	Burk's N free
	Bacillus megaterium strain IARI-BC-13 (JX312585)	99	
CJL6	*Bacillus tequilensis* strain M53-1 (JF411295)	97	Burk's N free
	Bacillus amyloliquefaciens strain BF10 (KJ524508)	97	
CJ33	*Bacillus subtilis* strain CanL-28 (KT580556)	98	NBRIP
	Bacillus methylotrophicus strain KD3 (KR855693)	98	
CJL18	*Bacillus subtilis* strain SH23 (KP735610)	99	Burk's N free
	Bacillus tequilensis strain CRRI-HN-4 (JQ695931)	99	
CJ27	*Bacillus subtilis* strain D3 (KC441752)	99	NBRIP
	Bacillus subtilis strain GD3b (HM055602)	99	
Alphaproteobacteria			
CJ32	*Agrobacterium tumefaciens* strain WSP165 (KR827434)	99	NBRIP

	Rhizobium pusense strain B38 (KT380510)	99	
CJL19a	*Rhizobium* sp. BAB-3360 (KF984469)	99	Burk's N free
	Pantoea agglomerans strain A9 (KC434965)	99	
Gammaproteobacteria			
CJL10	*Enterobacter hormaechei* strain RB18 (KC431796)	99	Burk's N free
	Enterobacter cloacae strain RCB973 (KT261185)	99	
CJ15a	*Enterobacter cloacae* strain 1Bg-24 (KR061390)	99	NBRIP
	Enterobacter ludwigii strain KAR11 (KR054973)	99	
CJ28	*Acinetobacter lwoffii* strain AL-24 (KF817639)	98	NBRIP
	Acinetobacter lwoffii strain MTB-0-3 (KJ401076)	98	

A neighbor-joining tree phylogenetic tree in these isolates showing the two clusters: cluster A divided into two cluster A1 and A2. Cluster A1with cluster A11 had 3 isolates as *Bacillus arybhattai* CJL2b, *Agrobacterium tumefaciens* CJ32 and *Pantoea agglomerans* CJL19a correlated very closely while cluster A12 had *Bacillus subtilis* CJ33 and *Enterobacter cloacae* CJL15a.

Cluster A2 composed of two strains: *Bacillus megaterium* CJL5a and *Bacillus tequilensi* CLJ6 and these strains had relationship very closely. Cluster B had cluster B1 with two strains: *Acinetobacter lwoffii* CJ28 and *Bacillus subtilis* CJL1817 while cluster B2 composed of two strains: *Bacillus subtilis* CJ27 and *Enterobacter homaechei* CJL10.

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 Proteobacteria (Table 4).

Table 4. Nucleotide diversity (Θ) values of two EST's using the programme DNASp 4.0 (Watterson, 1975)

ESTs	11 isolates
Ncleotide diversity (Pi)	0.74448 ± 0.00001
Theta (per sequence) from Eta	0.97786 ± 0.13418

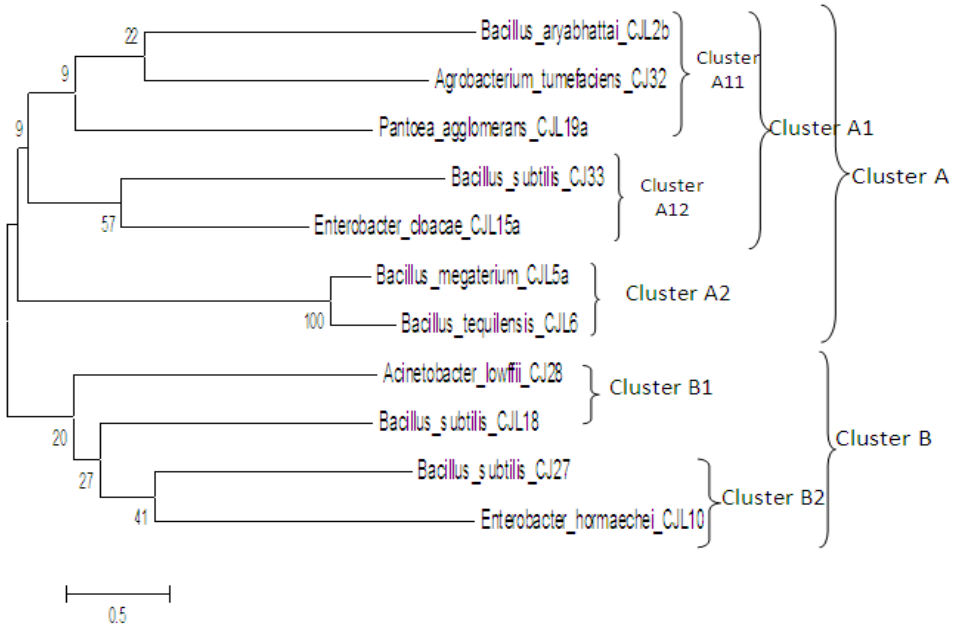

Figure: 3 Phylogenetic tree showing the relative position of rhizobia (PGPR) by the neighbor-joining method of complete 16S rRNA sequence.

Bootstrap values of 1000 replicates are shown at the nodes of the trees.

The rhizospheric bacteria has been studied and described as beneficial bacteria with Gram-positive bacteria presented on both of media and its occupied over 50% among 11 strains in our result (Figure 4).

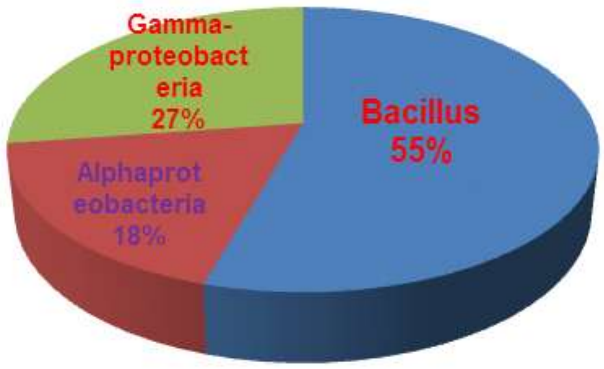

Figure 4. The proportion of group and they distributed in three clusters

Nucleotide polymorphism can be measured by many parameters, such as halotype (genes) diversity, nucleotide diversity, Pi, Theta (θ)(per gourp) etc...In this study, nucleotide diversity was estimated by Theta, the number of segregating site (Watterson, 1975), and its standard deviation (Sθ). These parameters were estimated by DNA Sequences Polymorphism sofware version 4.0 (Rozas and Rozas, 2005). Pi values explained nucleotide diversity of sequences

for each gene, the higher values, the more diversity among group, Bacilli group had the highest.

There were 22/63 bacterial isolates having siderophores after 2 days incubation on CAS medium (Table 4) and (Figure 5).

Table: 4 Siderophores were made by 22 rhizopheric bacterial isolates

No	Bacterial name	Sidero-phores	No	Bacterial name	Sidero-phores	No	Bacterial name	Sidero-phores
1	CJ01	-	22	CJ22	-	43	CJL04b	+
2	CJ02	-	23	CJ23	-	44	CJL05a	-
3	CJ03	+	24	CJ24	-	45	CJL05b	+
4	CJ04	-	25	CJ25	-	46	CJL06	+
5	CJ05	-	26	CJ26	-	47	CJL07a	+
6	CJ06	-	27	CJ27	-	48	CJL07b	-
7	CJ07	-	28	CJ28	-	49	CJL08	-
8	CJ08	-	29	CJ29	-	50	CJL09	+
9	CJ09	-	30	CJ30	-	51	CJL10	+
10	CJ10	-	31	CJ31	+	52	CJL11a	+
11	CJ11	-	32	CJ32	-	53	CJL11b	+
12	CJ12	-	33	CJ33	-	54	CJL12	+
13	CJ13	-	34	CJ34	-	55	CJL13	+
14	CJ14	-	35	CJ35	-	56	CJL14	+
15	CJ15	-	36	CJL01a	+	57	CJL15a	+
16	CJ16	-	37	CJL01b	+	58	CJL15b	+
17	CJ17	-	38	CJL02a	+	59	CJL16	+
18	CJ18	-	39	CJL02b	+	60	CJL17	-
19	CJ19	-	40	CJL03a	-	61	CJL18	-
20	CJ20	-	41	CJL03b	-	62	CJL19a	+
21	CJ21	-	42	CJL04a	+	63	CJL19b	-

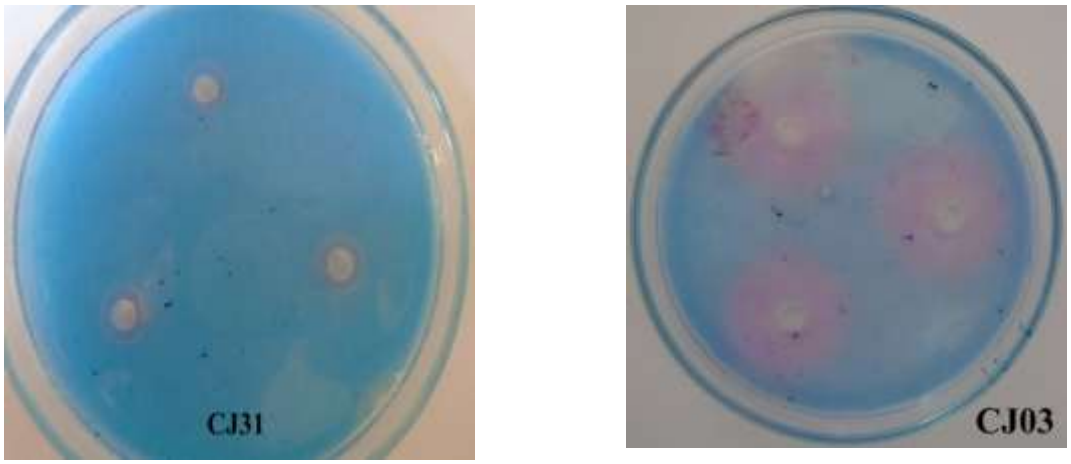

Figure: 5 Two bacterial isolates made a yellow, orange halo round well containing bacterial liquid on CAS agar after 48 h incubation

The narrow zone of soil directly surrounding the root system is referred to as rhizosphere (Walker et al. 2003), while the term "rhizobacteria" implies a group of rhizosphere bacteria competent in colonizing the root environment (Kloepper et al. 1991). When rhizobacteria exert a beneficial effect on plant growth and are termed as plant growth promoting rhizobacteria (PGPR)(Kloepper and Schroth, 1978). Plant growth promoting rhizobacteria can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (Viveros et al. 2010). The ePGPR may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex while iPGPR locates generally inside the specialized nodular structures of the root cells. The bacterial genera such as *Agrobacterium, Arthobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas* and *Serratia* belongs to ePGPR (Ahemad and Kilbert, 2014) and two mechanisms of plant growth promotion of these bacteria as direct mechanism and indirect mechanism have been presented more clearly in review of Ahemad and Kilbert (2014). However direct mechanism is an important mechanism that facilitate nutrient uptake or increase nutrient availability by nitrogen, solubilization of mineral nutrients, mineralize organic compounds and production of phytophomones (Bhardwaj et al. 2014; Arora et al. 2012). Furthermore, siderophore production has been regconized as an important factor of PGPR because microorganisms have evolved specialized mechanisms for the assimilation of iron, includind the production of low molecular weight iron-chelating compounds known as siderophores, which transport this element into their cells (Arora et al., 2013; Schwyn and Neilands, 1987). The our results on rhizospheric bacteria in acrisols of maize with *Bacillus* genus and *Burkholderia* genus together with some isolates are belong to *Achromobacter* and *Microbacterium* genus (Thanh and Diep, 2014), on rhizospheric bacteria in acrisols and ferralsols of sugarcane are *Bacillus* genus, *Burkholderia* genus, *Terriglobus* genus and *Sphingomonas* genus (Tam and Diep, 2015). This result identified rhizospheric bacteria in ferralsols of soybean are genera as *Bacillus, Acinetobacter, Agrobacterium, Enterobacter, Pantoea*. In general, rhizospheric bacteria in acrisols and ferralsols of many kinds of crop have identified as previous genera (Ahemad and Kilbert, 2014) among Bacili always presented and occupied maximally.

'*Bacilli'* AEFB are a diverse group with wide distribution in agricultural soils that contribute both directly and indirectly to plant development (McSadden, 2004). Numerous *Bacillus* and related genera with plant growth promoting (PGP) activities have been isolated from soybean, corn, sorghum and wheat rhizospheres (Cohelo et al., 2007; Beneduzi et al, 2008a;

Beneduzi et al., 2008b), Bacilli are bacteria having endospore and this support their survival in drought condition of ferralsols in dry season (from November to April).

CONCLUSION

From 24 ferralsols samples of soybean regions in Cujut district, DakNong province, the highland of Vietnam, 63 isolates were isolated on two media (Burk's N free and NBRIP) and they were identified as rhizospheric bacteria and 11 isolates having good plant growth promotion were chosen to analyse their relationship. These isolates were identified as Bacilli (more than 50%), Alphaproteobacteria with *Rhizobium* sp., *Agrobacterium tumefaciens*, *Pantoea agglomerans* and Gammaproteobacteria with two genera are *Enterobacter*, *Acinetobacter* on ferralsols. Among them, there are five strains as *Bacillus subtilis* CJ27, *Acinetobacter lwoffii* CJ28, *Agrobacterium tumefaciens* CJ32, *Bacillus subtilis* CJ33 and *Bacillus subtilis* CJL18 will be suggested to produce for soybean cultivation on ferralsols in the future.

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