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INVESTIGATION OF SALT-TOLERANT RHIZOSPHERE MICROBIOME FROM SEAWATER-INTRUDING RICE PADDY FIELD IN VIETNAM

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ABSTRACT

Salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) are known as potential tools to improve rice salinity tolerance. In this study, we aimed to investigate the PGPR community richness from the rice paddy fields in the Soc Trang and Ben Tre provinces, which is seriously affected by sea level rise. The salinity in the sampling sites ranged from 0.14 to 2.17 ‰ in the November 2018, in the rainy season. The microbial diversity of samples were evaluated by spreading the samples in TSA medium with the different concentration of NaCl. With the increase of salt concentration up to 10% NaCl, the total number of bacteria decreased for all the samples, ranging from 10^4 to 10^6 CFU/gr but no bacterial colonies were observed at 30% of NaCl. Out of 48 salt-resisting bacteria in total were isolated from the rice paddy field mud, 22 isolates were able to produce IAA (phytohormone for the plant growth). Six of them which possessed the high activity of IAA, nitrogen fixation and phosphate solubilization were identified as *Bacillus* (DT6, LT16, and LHT8), *Halobacillus* (DT8), *Aeromonas* (LHT1), and *Klebsiella* (LHT7) genus, respectively. All the sequences were registered in the gene banks with the numbers MK335670, MK335671, MK335672, MK335673, MK335674, and MK335675.

Keywords: PGPR, seawater intrusion, salinity tolerance, Mekong delta, rhizosphere microbiome.

1. INTRODUCTION

Vietnam is a leading country for rice (*Oryza sativa)* export, half of production and 70% of exported rice come from the Mekong Delta [1]. Recently, the production of rice in this region is affected by the salt intrusion and draught. In 2013, 500 ha among 1,158 ha of rice field in Binh Dien district (Ben Tre province) have been suffered from the draught, lack of water, and high salinity in the soil. The productivity of the crop reduced by 70%. In addition to Ben Tre province, Soc Trang province in the Mekong delta lost 600 ha of rice due to the salt intrusion. In 2016, the 11/13 provinces (including Ben Tre and Soc Trang) in the Mekong delta were reported to suffer natural disasters (draught and salinity). Developing salt-tolerant crops has been a much desired scientific goal but still little success to date [2]. An alternative an improved methodology may be to introduce salt-tolerant microbes that enhance crop growth.

Plant Growth Promoting Rhizobacteria (PGPR) play an important role in sustainable agricultural systems. PGP microbes can promote plant growth because of its ability for non-symbiotic nitrogen fixation, Phosphate solubilization, increased iron uptake, suppression of plant pathogenic microorganisms, or regulation of different plant hormone levels, which includes developing resistance to drought and salinity stress. PGPR also have been shown to enhance plant growth in a wide range of root-zone salinities, and this strategy can be developed into crops to manage with climate change induced abiotic stresses [3, 4]. Thus, this research focused on the

diversity of salt-tolerant PGPR present in the salinity regions of rice culture in Mekong river delta, can then be exploited to maintain crops in the current difficult conditions.

2. MATERIAL AND METHODS

Sampling and Bacteria Isolation: Water samples were collected from six different sites (Dinh Trung, Thanh Phuoc, An Hiep, Dai An 2, Lieu Tu, Lich Hoi Thuong) along the coastal areas of the Mekong delta (Figure 1). Plastic containers used for collection of samples were pre-treated by washing with 0.05 M HCl and then rinsed with distilled water [3]. After collection, samples were measured various physicochemical parameter (pH, temperature, salinity, conductivity, etc.) by using Horiba U-52 Multiparameter Meter (Horiba, Japan).

The rhizospheres of plants and soils plants from paddy field in the sampling area (Figure 1) were collected for selection and isolation of PGPR microbes. Salt-tolerant PGPR microbiomes were characterized by spreading soil samples in the Tryptic Soy Agar (TSA) media with different NaCl concentrations (0.5; 1; 1.5; 2; 2,5; 3 % w/v) three times. The total number of colonies were counted and compared in the different samples. Then, colonies were randomly selected and spread on the original medium for three times to avoid contamination risks. Pure strains were frozen in 25% glycerol at −80°C [4-6].

Resistance to Abiotic Stresses: Resistance to salt stress was assessed by growing the isolates at 30°C in growth medium supplemented by different sodium chloride concentrations, ranging from 0 to 30% w/v. The ability to grow under osmotic stress was tested at 30°C by adding 5-20% of polyethylene glycol (PEG) to growth medium. Finally, the capability to growth in a wide range of temperatures was verified by incubating the growth medium plate at 4°, 37°, and 45°C.

Screening of Bacterial Isolates for their Plant Growth Promoting (PGP) Activities: All isolates were first screened on Pikovskaya's agar plates for phosphate solubilization as described by [7]. The production of indole-3-acetic acid was detected by the method described by [8]. The ability of NH_3 production was estimated according to [9].

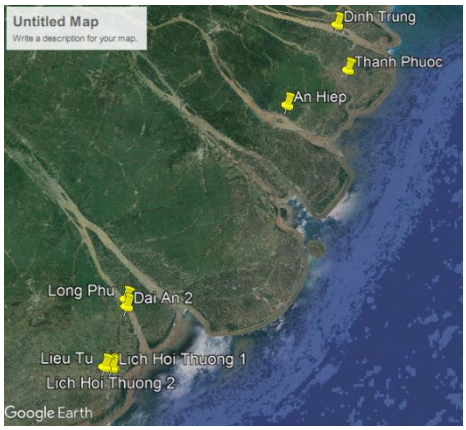

Figure 1. The sampling sites in the Ben Tre and Soc Trang Provinces.

Sequencing of 16S rDNA: The isolated bacteria were identified by using 16S rDNA sequences. The total DNA of the isolated bacteria was used for the multiplication of 16S rDNA by the PCR with the 16S rDNA universe primers. The PCR products were sequenced by Macrogen (Seoul, Korea). The sequence of 16S rDNA was blasted in NCBI for the identification of the isolate.

3. RESULT

3.1. Environmental factors in the sampling site.

Table 1 showed the average results of salinity, temperature, pH, TDS, conductivity, dissolved oxygen, reduction potential of the water environment in the sampling rice paddy fields. The salinity, pH, turbidity, DO, and conductivity of the water were the highest values at Thanh Phuoc sampling site, approximately 2.2 ^{o}/oo, 8.1, 2.7, 12.2 mg/L, and 4700 µS/m, respectively. Meanwhile, water samples from Dai An 2 showed the lowest value of salinity, turbidity, DO, and conductivity. The temperature of the sampling sites were ranging from 29 to 34^{o}C, and pH values were in the range of the base from 7.4 to 8.1 (Table 1). The data confirmed that there was the salt intrusion in the several water environment of rice paddy fields.

Table 1. Showing mean results of physico-chemical analyses of samples

Sampling Sites	Sal (^{o}/oo)	T(^{o}C)	pH	TDS	Conductivity (µS/m)	DO (%)	DO (mg/L)	ORP
Dinh Trung	0.68	34.15	7.4	0.9	1630.5	134.1	7.52	-38.15
Thanh Phuoc	2.17	32.64	8.15	2.68	4735	171	12.22	-31.8
An Hiep	1.29	29.12	7.89	1.64	2722.5	114.6	8.74	-27.7
Dai An 2	0.14	30.19	7.85	0.19	335	27.8	2.09	-56.2
Lieu Tu	0.68	30.59	7.61	0.89	1505	78.65	5.74	-41.55
Lich Hoi Thuong	1.07	32.66	7.4	1.38	2425	118.3	8.49	-13.15

3.2. Microbial diversity and abundance of the rhizospherical soil muds of rice plant.

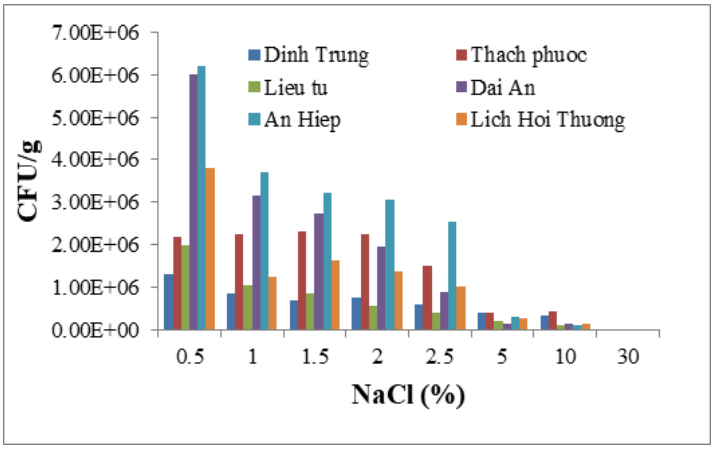

Figure 2. The abundance of the bacteria in the samples from sites cultured in TSA supplemented with different concentration of NaCl.

Figure 2. described the total number of bacteria counted in the TSA medium supplemented different concentration of NaCl. With the increase of NaCl concentration, the total number of bacteria decreased for all the samples, ranging from 10^4 to 10^6 CFU/gr. The density of bacteria was the lowest at NaCl concentration of 5 and 10%, and no bacterial colonies were observed at 30% of NaCl. The density of bacteria was different at different sites. The number of bacteria was highest at Dai An 2 and An Hiep sites, and the lowest density of bacteria was recorded at Dinh Trung and Lieu Tu at the medium with 0.5% of NaCl. However, increasing the NaCl in the TSA, the number of bacteria were reduced significantly all the sites (for examples, Dinh Trung reduced 36%, Lieu Tu and Dai An: approximately 48%, An Hiep: 40%) except from Thanh Phuoc sample. Samples from Thanh Phuoc had a consistant density at various concentration of NaCl, but the density changed at 5 and 10 % of NaCl.

3.3. Production of IAA, dissolution of Phosphate and nitrogen fixation by isolates

Isolates were purified from the plates of TSA with NaCl concentration of higher 2.5%. Total 46 isolates were purified from the TSA of the high concentration of NaCl. The purified isolates were tested for the IAA production, phosphate dissolution, and nitrogen fixation. The result of the tests was shown in the Table 2.

Table 2. IAA production, Phosphate dissolution and Nitrogen fixation properties of the isolates

No	Reaction / Isolates	IAA	Phosphate dissolution	Nitrogen Fixation	No	Reaction / Isolates	IAA	Phosphate dissolution	Nitrogen fixation
1	DT.MR1_1	-	-	-	25	LT. MR1_7	-	-	-
2	DT.MR1_2	+	-	G+	26	LT.MR1_16	+	G+	G+
3	DT.MR1_3	-	-	G+	27	ĐA2. MR_1	-	G++	G+
4	DT.MR1_4	+	G+	-	28	ĐA2. MR_3	-	G+	G+N+
5	DT.MR1_5	+	-	-	29	ĐA2. MR_4	+	-	-
6	DT.MR1_6	++	G++	G+	30	ĐA2. MR_5	+	-	G+
7	TP. MR1_1	+	-	G+	31	ĐA2. MR_6	+	G+	-
8	TP. MR1_2	-	-	G+	32	ĐA2. MR_7	+	-	-
9	TP. MR1_5	-	-	G+	33	AH. MR1_1	++	G++	-
10	TP. MR1_6	+	G+	G+	34	AH. MR1_2	-	-	G+
11	TP. MR1_7	-	-	G+	35	AH. MR1_3	+	G+	-
12	TP. MR1_8	+	-	-	36	AH. MR1_4	-	-	-
13	TP.MR1_10	+	G++	G++	37	AH. MR1_5	-	-	-
14	LT. MR_1	-	-	G+	38	AH. MR1_6	-	-	-
15	LT. MR_2	-	-	G+	39	LHT. MR1_1	+	G+	G+
16	LT. MR_3	++	G+	G+	40	LHT. MR1_2	+	G++	G+
17	LT. MR_4	-	-	-	41	LHT. MR1_3	+	-	-
18	LT. MR_5	-	G++	G+	42	LHT. MR1_4	-	G+	-
19	LT. MR1_1	+	-	-	43	LHT. MR1_5	-	-	-
20	LT. MR1_2	-	-	-	44	LHT. MR1_6	-	G++	G+
21	LT. MR1_3	-	G+	G+	45	LHT. MR1_7	-	G+P+	G+N+
22	LT. MR1_4	-	G++	G+	46	LHT. MR1_8	+	G+	G+
23	LT. MR1_5	-	-	G+	47	LHT. MR1_16	+	G+	G+
24	LT. MR1_6	-	-	-	48	DT.MR1_8	+	G+	G+
Total						**48**	**23**	**22**	**28**

G++: strong growth, G+: weak growth; P+ or N+: positive for P solubilizing or N fixing

Results from the tests showed that 23 out of 48 isolates produced the plant hormone, IAA; 22 isolates showed the growth on the phosphate medium with/without clear zone of phosphate dissolution; and 25 isolates grew on the medium without nitrogen supplement and some of them produced ammonium. From the tests, seven isolates showed high activities either IAA production such as DT.MR1_6 or LT.MR1_16 or phosphate dissolution and nitrogen fixation such as TP.MR1_10, LHT.MR1_2, LHT_MR1_7, LHT.MR1_8, DT.MR1_8 (from now on for short abbreviation, they were relabeled as DT6, LT16, TP10, LHT1, LHT7, LHT8, and DT8, respectively). Three out of them (LT16, LHT8, and DT8) were isolated from the medium with 10% of NaCl.

3.4. Identification of the selected isolates by 16S rDNA

The results obtained identified six PCR products to belong to *Bacillus* (DT6, LT16, and LHT8), *Halobacillus* (DT8), *Aeromonas* (LHT1), and *Klebsiella* (LHT7) genus. All the sequences were registered in the gene banks with the numbers MK335670, MK335671, MK335672, MK335673, MK335674, and MK335675. The phylogenetic trees showed that the 16S rRNA sequences (~1400 bp) of LHT8 was closely related to KY928104.1 *Bacillus marisflavi* strain R3 (99.8%). The 16S rRNA sequence (1500 bp) was found to relate with AY505519.1 *Halobacillus* sp. GSP34 và AY505518.1 *Halobacillus* sp. GSP15 (99.8%). Comparing the sequence of 16S rRNA gene (~1500bp) of the isolate LHT1 to other bacteria sequences, the result confirmed the LHT1 was similar to AP019195.1:86381-87921 *Aeromonas caviae* GSH8M-1 (99.9%). Lastly, the minimum evolution method confirm the isolate LHT7 was CP030857.1:249514-251063 *Klebsiella pneumoniae* subsp. pneumoniae strain JNM8C2 with the similarity of 99.9%.

4. CONCLUSION

In conclusions, moderate halophile bacteria were isolated from rice paddy fields. Total 48 isolates of salt-resisting bacteria were isolated from the rice paddy field mud via the TSA supplemented with high concentration of NaCl. Among these isolates, 22 isolates were able to produce IAA. Several isolates were found to possess the capability of nitrogen fixation and phosphate solubilization. Six of them which possessed high activity of IAA, nitrogen fixation and phosphate solubilization were identified as *Bacillus* (DT6, LT16, and LHT8), *Halobacillus* (DT8), *Aeromonas* (LHT1), and *Klebsiella* (LHT7) genus. Several physic-chemical properties of six isolates were described such as the activity of urease, sugar fermentation, motility, and Gram types. These information were necessary for the further investigation in the application of these isolates. It is suggested that the investigation in the co-fermentation of the isolates and antagonistic properties will be essential for the application of the isolates for promoting the rice growth in the high saline conditions.

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REFERENCES

[1]. Nguyen, H., T. Minh, and T. Kawaguchi, *Overview of rice production system in the Mekong Delta-Vietnam.* 2002.

[2]. Munns, R. and M. Tester. (2008). Mechanisms of salinity tolerance. A*nnu. Rev. Plant Biol.*, 59, p. 651-681.

[3]. APHA, *Standard Methods for the Examination of Water and Wastewater.* America Public Health Association,18th Edition, New York, 1992.

[4]. Mapelli, F., et al. (2013), Potential for plant growth promotion of rhizobacteria associated with Salicornia growing in Tunisian hypersaline soils. *BioMed research international*, 2013.

[5]. Soussi, A., et al. (2016), Plant-associated microbiomes in arid lands: diversity, ecology and biotechnological potential. *Plant and Soil*, 405(1-2). p. 357-370.

[6]. Ferjani, R., et al., The date palm tree rhizosphere is a niche for plant growth promoting bacteria in the oasis ecosystem. BioMed research international, 2015. **2015**.

[7]. Gaur, A., *Physiological functions of phosphate solubilizing micro-organisms.* Phosphate solubilizing micro-organisms as biofertilizers. Omega Scientific Publishers, New Delhi, 1990: p. 16-72.

[8]. Patten, C.L. and B.R. Glick. (2002). Regulation of indoleacetic acid production in Pseudomonas putida GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. *Canadian Journal of Microbiology*, 2002. 48(7), p. 635-642.

[9]. Cappuccino, J.G. and N. Sherman, Microbiology: a laboratory manual. 2005: Pearson/Benjamin Cummings San Francisco.