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COMBINED EFFECTS OF SALINITY, RICE VARIETY AND RICE GROWTH STAGE ON THE DIVERSITY OF BACTERIAL COMMUNITIES ASSOCIATED WITH RICE (*Oryza sativa L.*)

Nolissa Delmo-Organo[1,3], Evelyn F. Delfin[2], Glenn B. Gregorio[3], Nacita B. Lantican[4], Jessica F. Simbahan[5] and Erlinda S. Paterno[1]

[1]Agricultural Systems Institute, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), Philippines

[2] Institute of Plant Breeding, College of Agriculture and Food Science, UPLB, Philippines

[3] Plant Breeding Genetics and Biotechnology Division, International Rice Research Institute Headquarters, Philippines

[4] Institute of Biological Sciences, College of Arts and Sciences, UPLB, Philippines

[5] Institute of Biology, College of Science, University of the Philippines Diliman, Philippines

Corresponding author: ndorgano@up.edu.ph

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ABSTRACT

The combined effects of salinity, rice variety, and rice growth stage on the diversity of bacterial communities associated with rice were analyzed using a molecular approach. Samples were taken from a field experiment that was set-up from January to May 2011 at the International Rice Research Institute (IRRI). Culture-independent isolation of total rhizosphere DNA was performed using a commercially available soil DNA extraction kit while the modified CTAB method was used to isolate the cultivable microbial community DNA from culture enrichments from bulk soil, rhizosphere, and sterilized root samples. The V6 to V8 region of the bacterial 16s rRNA gene was amplified through Polymerase Chain Reaction (PCR) and the amplicons were separated using Denaturing Gradient Gel Electrophoresis (DGGE). Rhizosphere bacterial diversity, measured by the Shannon index, was significantly higher under saline conditions compared to normal conditions. Diversity at the reproductive stage was also significantly higher than the vegetative stage. Combined DGGE profile and sequence analysis of the cultivable bacterial community revealed that salinity has a strong influence on the bacterial diversity in the bulk soil. The influence of crop growth stage on the bacterial community was more evident in the rhizosphere while the effect of salinity on specific microbe-plant interactions were observed in surface-sterilized roots. Salt-tolerant varieties were able to maintain association with bacteria that are reported to have plant-growth promoting properties even under saline conditions.

Key words: DGGE, bacterial diversity, salt tolerant variety

INTRODUCTION

Soil salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increased rice production worldwide (Gregorio et al. 1997). Millions of hectares in the humid regions of South and Southeast Asia are technically suited for rice production but are left uncultivated or are grown with very low yields because of salinity and abiotic stress (Gregorio et al. 2002). Saline-prone areas in the Philippines are small compared with other countries in the South and Southeast Asia, but are still a potential and important resource base for the production of rice and other related staple food. Although other crops are more tolerant to salinity, for most farmers, there is often no alternative to growing rice in these regions because it is the only crop that can tolerate flooding during the wet season (Yeo et al. 1990; Flores, 2004).

To combat problems of salinity, plant breeders in the International Rice Research Institute (IRRI) developed rice varieties that can tolerate salinity. Aside from this, studies were also performed to reveal the underlying mechanisms of salt-tolerance. One view point that has not been looked into closely is the fact that plants have the ability to interact with soil microorganisms, some of which may have the ability to provide the plant with beneficial substances that enhance growth and tolerance to stresses. The relationship between plants and soil microbial community is very complex and occurs in many ways, resulting in beneficial effects such as stimulation of plant hormones synthesis and enhancement of biological nitrogen fixation and phosphate solubilization, or in negative effects like occurrence of diseases (Ferreira et al. 2008). The rice rhizosphere represents the soil area under the direct influence of roots and serves as a favorable aerobic-anaerobic interface suitable for diverse groups of microorganisms. Plants are capable of increasing soil microbial population through root exudates, which are used as nutrient source for their growth (Sung et al. 2006). Plant rhizodeposition in the rhizosphere results in increased microbial population size and community structures distinct from that in bulk soil (Bais et al. 2006). There is a need to understand the microbial community that is associated with the rice root and its response to salinity. Salinity is the major environmental determinant of microbial community composition rather than extremes of temperature, pH, or other physical and chemical factors (Lozupone and Knight 2007). Unfortunately, very few researches worked on the combined effects of salinity, plant type or growth stage on the microbial community associated with the rhizosphere of salt-tolerant and susceptible rice varieties. Molecular biology analysis of complex microbial communities can be done through genetic fingerprinting. The most common technique is the use of denaturing gradient gel electrophoresis (DGGE) which separates PCR-generated DNA products based on sequence differences (Nakatsu 2007). Changes in the rice rhizosphere microbial communities can be traced using PCR-DGGE (Organo et al. 2013) making it a very useful tool in comparing samples from different environments.

This paper analyzes the effects of salinity, rice variety, and growth stages on the diversity of soil bacterial communities associated with rice (*Oryza sativa L.*) using the PCR-DGGE fingerprinting technique as well as determines which factor has the major contribution in shaping the bacterial community in the rhizosphere. This information will be useful in determining if soil microorganisms can definitely contribute to the resistance and productivity of crops grown on salt-affected soils. This can also serve as a guide in the selection and utilization of beneficial soil microorganisms that can be applied in such conditions.

MATERIALS AND METHODS

Field experiment

A field experiment using four rice varieties (IR29, PSB Rc 82, FL478, and Salinas1) with various responses to salinity was set-up for this study. IR29 is an improved *indica* cultivar that is used as a salt-sensitive standard while PSB Rc 82, a popular variety among farmers, is categorized as moderately tolerant to salt stress. FL478, a recombinant inbred line derived from a population developed for salinity tolerance studies, has high tolerance to salinity stress, particularly at the vegetative stage of growth (Gregorio et al. 1997). Salinas1 is a salt-tolerant rice variety released by IRRI in the Philippines in 2010. The rice plants were transplanted in concrete plots that are specifically designed for experiments on salinity tolerance. All plots were initially flooded with normal irrigation water prior to transplanting. Three plots were salinized using sea water with three plots remaining as control at two weeks after transplanting. Electrical conductivity (EC) was initially maintained at 6 to 7 dS m^{-1} during the vegetative stage and was raised to 9 dS m^{-1} during the reproductive stage. The experiment was laid-out in Split Plot Arrangement and Randomized Complete Block Design (RCBD) with salinity level as the main plot and rice variety as the subplot. Thirty-six rice seedlings were transplanted for each treatment.

Sample collection

Three replicated bulk soil and rice plant samples (with intact roots) were randomly collected for each variety on each plot during the vegetative (30 days after transplanting, DAT) and reproductive stage (50 DAT) of rice. Sterile metal core samplers, measuring approximately 10 cm in height, were used to collect bulk soil samples from the plots. Rhizosphere and plant root samples, on the other hand, were gathered from randomly selected rice plants. The plants were uprooted using a spade to ensure that the root system was still intact. Bulk soil and plant samples were placed in sterile polypropylene bags and sealed in a container with ice while being transported to the laboratory.

***Bulk soil samples*.** The contents of the core samplers were transferred to a sterile polypropylene bag and mixed thoroughly. Once mixed, a five-gram portion was placed in 45 mL sterile saline solution (NaCl 0.85%) to create a 10^{-1} dilution.

***Plant samples*.** Separation of the rhizosphere from the plant roots was performed as described previously (Organo et al. 2013). The obtained root samples were gently washed with sterile distilled water to remove the remaining clay particles. It was then cut to approximately 1.5 inches, enough to fit a 2 mL collection tube. Four to five root slices were placed in 2 mL microcentrifuge tubes. Root-surface sterilization was performed by immersing the roots in 70% ethanol for 3 min, washing with fresh sodium hypochlorite solution (2.5% available Cl^{-}) for 5 min, rinsing with 70% ethanol for 30 seconds, and finally, washing five times with sterile distilled water. To confirm root surface sterility, 100 µL of the sterile distilled water used in the last rinse was set on Tryptic Soy Agar (TSA) plates and the plates were incubated at room temperature for 2 days. Rice root samples that were not contaminated were used as source for culture enrichments for endophytic microorganisms. The rhizosphere sample was transferred to sterile 50 mL tubes and served as the source of samples for direct DNA extraction and culture enrichments from the rhizosphere.

Preparation for direct soil DNA extraction and culture enrichments

To obtain samples for direct rhizosphere DNA extraction, 2 mL of the rhizosphere suspension was transferred to a collection tube and centrifuged at 10,000 x g for 1 minute. The supernatant was removed, and the soil pellet was washed with TE Buffer then stored at -20 °C prior to DNA Extraction.

To prepare culture enrichments from the bulk soil and rhizosphere suspensions, serial dilutions of up to 10^{-6} were prepared using sterile water as diluent. One hundred µL samples from dilutions 10^{-4} to 10^{-6} were inoculated in 10 mL vials of Nutrient Broth (NB) and Tryptic Soy Broth (TSB). To prepare endophytic culture enrichments, two tubes containing surface-sterilized roots were used. The roots were macerated while inside the tubes using a flame-sterilized rod. One mL of sterile distilled water was mixed to the macerated samples and 100 µL of the suspension were inoculated in 10 mL vials of NB and TSB. All the inoculated vials were placed in a rotary shaker for 5 days at ambient temperature. After 5 days, 2 mL of cultures were transferred to a collection tube and centrifuged at 10,000 x g for 1 minute. The supernatant was removed, and the cell pellet was washed with TE buffer then stored at -20 °C prior to DNA extraction.

DNA extraction

Total rhizosphere DNA was extracted as previously described (Organo et al. 2013) using a DNA Isolation Kit (MoBio Ultraclean™) following the manufacturer's instructions with some modifications. The modified CTAB method was used to extract DNA from the pellets obtained from culture enrichments (William et al. 2004). The quality and quantity of extracted DNA was assessed by both agarose gel electrophoresis and spectrophotometric analysis using Nanodrop ®. Samples with poor quality DNA extracts were not used as template for PCR.

PCR Amplification

A 17-mer forward primer, designated 968f (5'AA CGC GAA GAA CCT TAC 3'), to which a 40-mer GC clamp (5'-CGCCCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G 3') was attached at the 5' end, was paired with 1378r (5'GCG TGT GTA CAA GGC CCG GGA ACG 3') to amplify the bacterial 16S rRNA gene fragments (Brons and van Elsas 2008). PCR reaction was performed as previously reported (Organo et al. 2013).

Denaturing Gradient Gel Electrophoresis

DGGE was performed using DCode™ (Bio-Rad, Hercules, Calif., USA) universal mutation detection system using 8% polyacrylamide gels with a gradient of 30% to 60% denaturing conditions. Electrophoresis was initially started at 100V for 10 minutes and was then lowered to 60V and allowed to run for 15 hours. The gel was stained with ethidium bromide for 5 minutes then destained with deionized water for 20 minutes. The gel was viewed and photographed using QuantityOne™ 1-D Gel Analysis Software (Bio-Rad).

DGGE Profile Analysis.

The indices of diversity and dominance of bacterial populations were calculated using the images of DGGE profiles. To determine the diversity and evenness of the bacterial communities, the Shannon index of diversity (H') and Simpson Index of Dominance (D) was calculated for each of the gel lane using the trace quantities generated by Quantity One™ 1-D Gel Analysis Software.

H' is defined as:

$$H' = -\sum_{i=1}^{S}(p_i \ln p_i)$$

where S is the species richness, P*i* is the importance probability of the bands in the lane. P*i* was calculated using the formula P*i* =n*i*/N, where n*i* is the trace quantity (peak intensity x width of the band) of the *i*th band and N is the total trace quantities of all the detected bands in the lane (Shannon and Weaver 1963).

The obtained Shannon index of diversity (H') for each of the gel lane were subjected to ANOVA and treatment means were compared using Duncan's Multiple Range Test at 5% level of significance. Statistical analysis was performed using the Statistical Analysis Software System®.

16S rDNA sequence analysis

Selected bands in the DGGE gel were excised using sterile plastic forceps and were carefully transferred to labelled collection tubes containing 50 µL HPLC water. The excised gel was macerated using a sterile yellow tip, centrifuged for 30 seconds at 5,000 x g, then incubated at 37 °C for 30 minutes. The resulting DNA was stored at -20 °C prior to re-amplification using primers without GC-clamp. The amplified DGGE bacterial DNA fragments were submitted to Macrogen, Inc., Korea for further purification and sequencing using the 1378r primer. The quality of the sequences obtained was assessed using Finch TV Version 1.4.0 (Geospiza Inc.) Chimera check with Decipher was the program used to check for chimeric sequences (http://decipher.cee.wisc.edu/index.html). The obtained sequences were processed and compared to those available in GenBank using the BLASTn tool for the 16s ribosomal DNA database for bacteria and archaea. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 5 (Tamura et al. 2011). Multiple sequence alignment was carried out using the ClustalW alignment function of the MEGA software. The phylogenetic tree was constructed based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) using Maximum Composite Likelihood as nucleotide substitution model. *Methanobacterium oryzae* was used as the out-group and the tree topology was evaluated by 1,000 replications of bootstrap analysis.

RESULTS AND DISCUSSION

Soil chemical analysis

The $EC_{1:5}$ values were measured and were converted to the ECe based on the recommendation of Watling (2007). In addition, sodium adsorption ratio (SAR) was used to assess soil salinity. SAR indicates the extent to which sodium contributes to the total salinity. Soils can be categorized into non-saline, saline, sodic and saline-sodic based on ECe, SAR and pH (Table 1). Soil chemical analysis confirmed that salinity has been maintained in the saline plots (Table 2). The EC_e levels in the saline plots were greater than 4 dS/m. In addition, very high concentrations of Na^+ and Cl^- ions in the saline plots were measured. Although high amounts of Na were measured, the saline plots will not be considered as saline-sodic because the SAR values are still below the limit of 13. All the other parameters were not significantly different between the normal and saline plots.

A few days after salinization, symptoms of salt stress were already seen on IR29 and PSB Rc82 plants in the salinized plots. The plants are smaller compared to those planted in normal plots and the tips of the leaves initially turned white, and this later on progressed as tip burns. While the first two varieties showed serious negative response to salinity at the vegetative stage, FL478 and Salinas 1 remained green and healthy just like the plants under normal conditions. During the reproductive stage, all plants showed symptoms of salt stress such as reduced tillering and spikelet sterility, but it was noted that Salinas 1 performed better than the other varieties during the reproductive stage.

Table 1. Classification of salt-affected soils and their distinguishing properties.

Class	ECe	SAR	pH
Non-saline	<4	<13	<8.5
Saline	>4	<13	<8.5
Sodic	<4	>13	<8.5
Saline-Sodic	>4	>13	<8.5

ECe: Electrical Conductivity (dS/m) of extract of saturated soil paste
SAR: Sodium Adsorption Ratio
pH: pH of saturated soil paste

Analysis of the DGGE profiles of cultivable bacterial communities

Figure 1 shows the representative DGGE profiles of cultivable bacterial communities from bulk soil, rhizosphere and surface-sterilized roots. Plants are capable of increasing soil microbial population through root exudates, which are used by microorganisms as nutrient source for their growth (Sung et al. 2006). The resulting DGGE profiles of the culture enrichments from this study is in accordance with this statement, as the DGGE profiles from bulk soil clearly differs from that of the rhizosphere. The effect of salinity on the bacterial community is very evident on the banding patterns of bulk soil samples at both vegetative and reproductive stages. Some bands were very intense at normal condition, but were no longer present at saline condition such as the lane represented by bands N10 and N12. On the other hand, some bands are more prominent in samples under saline conditions. Band N7, for example, is very intense during saline condition, but although still visible at normal salinity levels, it is very faint and difficult to recognize.

It can be observed that salinity also plays a role in the bacterial community in the rice rhizosphere as indicated by band N16, which is present in all varieties during normal EC levels, but is no longer observed under saline conditions. N20, a band found under Variety B (PSB Rc82), is very intense during normal EC level, but is very faint under saline condition. Interestingly, some bands are specific to a rice variety and salinity level during the reproductive stage, regardless of the enrichment media. These bands are: N26 and T20, which are all observed only in Variety C (FL478) under saline condition.

The DGGE profiles from the culture enrichment of surface-sterilized roots showed specific bands for each rice variety, especially during saline conditions. These include: N38 and T29 for IR 29, N32 and T30 for PSB Rc82, and N35 and T32 for Salinas 1. This is an indication that each variety may have interaction with particular groups of endophytic bacteria that may have helped illicit their response to salinity. These bands need to be identified to determine if the corresponding bacteria were those that have PGPR properties. Based on the DGGE profiles alone, it can be concluded that salinity plays a great role in the bacterial community in the bulk soil and rice rhizosphere. On the other hand, the bacterial profiles inside the plant roots tend to be highly dependent on the rice variety.

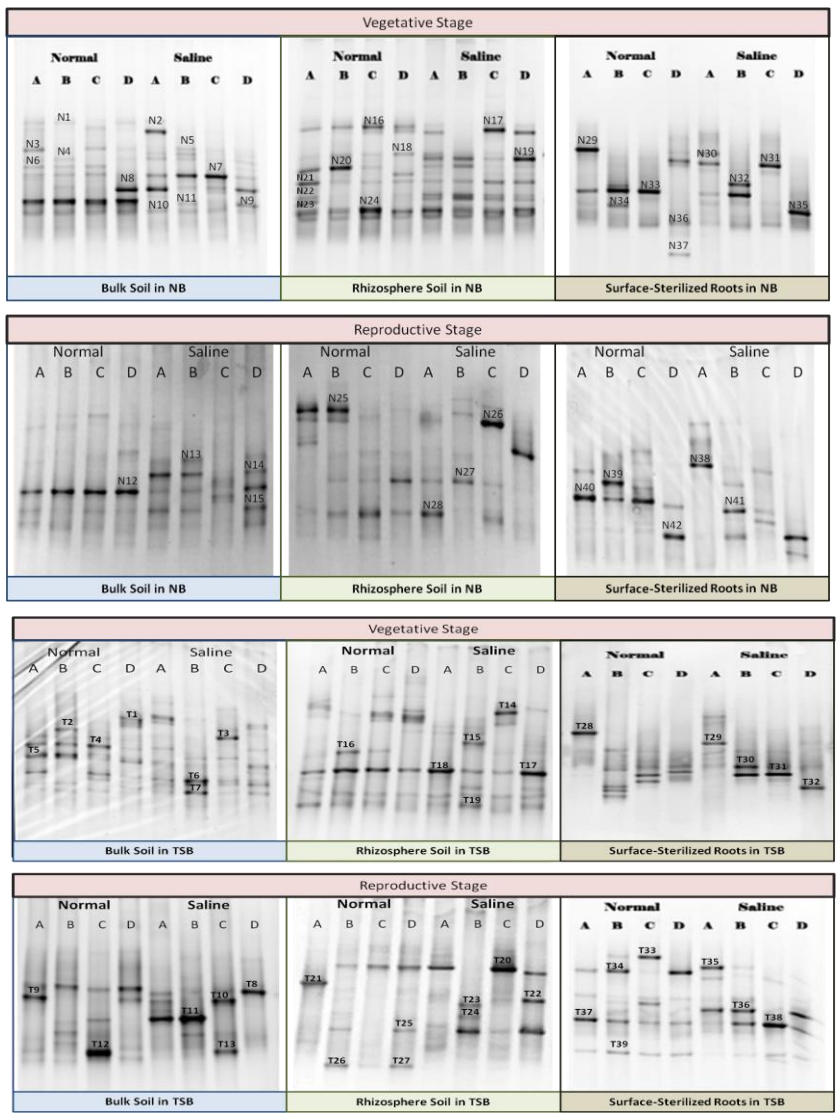

Fig. 1. DGGE profiles generated from culture enrichments using the primer pair 968fGC-1401r. (A) IR29; (B) PSB Rc 82; (C) FL478; (D) Salinas 1.

Table 2. Soil chemical properties of the soil samples from the IRRI B14 demo plot at the end of the experiment.

Result of Analysis	Normal 1	Normal 2	Normal 3	Saline 1	Saline 2	Saline 3
pH	6.10	6.20	6.20	6.10	6.00	6.00
OM%	2.45	2.36	2.54	2.60	2.59	2.51
N%	0.11	0.06	0.11	0.11	0.13	0.11
P (ppm, Olsen)	75	67	79	82	89	89
K (me/100g soil)	0.94	2.40	1.03	1.07	0.87	0.84
Na (me/100g soil)	4.24	8.81	6.67	15.01	13.47	13.27
Ca (me/100g soil)	12.28	12.96	12.01	10.66	11.88	10.53
Mg (me/100g soil)	7.83	7.96	8.09	7.19	6.81	8.60
Cl (ppm)	324.00	324.00	417.00	1390.00	1205.00	1298.00
Fe (ppm)	463.00	362.00	386.00	448.00	411.00	368.00
Zn (ppm)	5.00	3.00	4.00	4.00	4.00	3.00
$EC_{1:5}$ (µS/cm)	150.50	145.70	225.45	981.50	772.50	936.50
Ece (dS/m)	1.35	1.31	2.03	8.83	6.95	8.43
SAR	1.34	2.72	2.10	5.02	4.41	4.29

Bacterial diversity analysis

Statistical analysis revealed that the Shannon index of diversity in the rhizosphere is significantly affected by salinity and rice growth stages (Table 3). The bacterial diversity in the rhizosphere is higher under saline conditions compared to normal conditions, especially in the reproductive stage. For both rice growth stages, the increase in bacterial diversity were higher for the salt-tolerant varieties, FL478 and Salinas 1. However, the effect of rice variety on the rhizosphere bacterial diversity was not significant. These observations are consistent with findings demonstrating the dynamic nature of microbial communities in the rhizosphere which varies during the life cycle and with the seasonal response of plants (Hussain et al. 2012).

Table 3. Statistical analysis of the Shannon diversity (H') values of the four rice varieties grown at normal and salinized conditions during vegetative and reproductive growth stages.

Salinity Level	**Mean Diversity Index***
Normal	2.26193 b
Saline	2.43282 a
Rice Growth Stage	**Mean Diversity Index**
Vegetative	2.27883 b
Reproductive	2.42262 a
Variety	**Mean Diversity Index**
IR 29	2.33932 a
PSB Rc82	2.28611 a
FL 478	2.36668 a
Salinas1	2.40972 a
Variety	**Mean Difference Between Saline And Normal Levels**
IR 29	0.0615 a
PSB Rc82	0.1053 a
FL 478	0.2460 a
Salinas1	0.2656 a

* Means followed by similar letters within the same variable are not significantly different at 6% level DMRT.

Comprehensive analysis of the DGGE profile and band identity

A total of forty-two bands were excised for the DGGE profiles from NB culture enrichments, thirty-nine were selected from the TSB culture enrichment, and seven bands were excised from the DGGE profile of the total rhizosphere DNA. Nine out of the 88 excised bands did not meet the required 97% level of similarity through BLASTn. Majority of the sequence matches from enriched samples have high level of similarity to the well-studied members of the Gammaproteobacteria.

Aside from the overall microbial diversity, it is important to fully understand the combined effects of salinity, rice growth stage, and rice variety on specific bacterial populations. The matrix combining the band identities and the DGGE banding patterns indicates that salinity has a strong effect on the bacterial diversity in the bulk soil (Fig. 2). Bands identified to belong to genus *Providencia* was found only in normal plots. The genus *Aeromonas*, *Pseudomonas, Serratia, Shewanella* and *Vibrio*, on the other hand, represent those that are present in both normal and saline conditions.

While results from the bulk soil profiles revealed the impact of soil salinity on the soil bacterial community, DGGE profiles from rhizosphere enrichments showed the "rhizosphere effect" on the bacterial community. The genus *Providencia,* which was found only in normal EC levels of the bulk soil, was observed in the rhizosphere of all rice varieties under both normal and saline conditions. Members of the genus *Providencia* have been reported to promote rice growth in wheat and rice. On

the other hand, the genus *Serratia* and *Shewanella* which were both observed at both normal and saline in the bulk soil, were observed only under normal conditions in the rhizosphere. It appears that salinity, somehow, has an influence on the ability of the plant to interact with microorganisms.

Genus	Bulk Soil								Rhizosphere								Roots							
	Normal				Saline				Normal				Saline				Normal				Saline			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Aeromonas sp.	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Pseudomonas sp.	B	B	B	B	B	B	B	B																B
Serratia sp.	V	V	V	V	V	V	V	V	R	R							R	R	R	R				
Shewanella sp.	B	B	B	B	B	B	B	B	V	V	V	V												
Vibrio fluvialis	B	B	B	B	B	B	B	B													R	R		
Providencia sp.	B	B	B	B					V	V	V	V	V	V	B	V								
Bacillus sp.										V							V	V	V	V	B	V	V	V
Cedecea sp.		V																						
Azomonas macrocytogenes																						V		
Brevibacterium halotolerans																			V				V	
Enterobacter asburiae							V										V							
Klebsiella variicola																			R					
Lysinibacillus boronitolerans																					V		V	
Methylobacterium populi																				V				
Morganella morganii									R															

Legend:

V	Bacteria was observed during the vegetative stage
R	Bacteria was observed during the reproductive stage
B	Bacteria was observed on both stages

Fig. 2. Consolidated 16s rDNA analysis and DGGE profile observation.

The effect of salinity was visible on the bacterial community in surface-sterilized rice roots. Several root-bacterial interactions were observed only under normal conditions. These were *Enterobacter* for IR29, *Klebsiella* for FL 478 and *Methylobacterium* for Salinas 1. In addition, *Azomonas macrocytogenes* was detected only in the roots of PSB Rc82 under saline condition during the vegetative stage. *Vibrio* sp was observed for both IR 29 and PSB Rc82 under saline condition. Interestingly, members of the genus *Aeromonas* were found in almost all regions and conditions. An iron reducing bacterial strain was identified as a member of the *Aeromonas* group by 16S rRNA gene sequence analysis with a very wide range of tolerance to salinity (Wang et al. 2009).

Serratia sp. was observed in both normal and saline conditions in the bulk soil, but only during the vegetative stage. During the reproductive stage, *Serratia* sp. were found in the rhizosphere of IR29 and PSB Rc82 under normal conditions, and inside the roots of all varieties under normal conditions. These results suggest the possible role of *Serratia* sp. during the reproductive stage of rice. This interaction, however, was not observed during saline conditions. This is an indication that salinity has an influence on several plant-microbe associations. A strain of *Serratia marcecens*, IRBG500, was reported to significantly increase the root length and root dry weight, but not the total N content of rice variety IR72. Its initial entry was at the points of lateral root emergence and root tip (Gyaneshwar et al. 2001).

Although the effect of salinity was very evident, several plant-specific interactions were still observed. FL478, a salt- tolerant variety, was observed to be associated with *Klebsiella sp.* and *Brevibacterium halotolerans. Klebsiella* is a genus widely known to have the ability to fix nitrogen

(Mano and Morisaki 2008) while *Brevibacterium* species are known to exist in a number of different habitats, especially in those having a high salt concentration. Most members of this family grow well in the presence of 8% NaCl, and many strains also grow in 15% NaCl (Collins 2006). *B. halotolerans* has been isolated from the halophyte, *Prosopis strombulifera*, which was grown under extreme salinity. It was found to have ACC-deaminase activity, nitrogen fixation and even possible biocontrol activity (Sgroy et al. 2009). In this study, FL478 was the only variety that was shown to interact with *B. halotolerans*. This plant-microbe interaction may have contributed to FL478's tolerance at the vegetative stage.

In this study, the presence of *Pseudomonas* species was observed in the DGGE profiles of enriched bulk soil samples at both salinity levels and reproductive stage. No *Pseudomonas* species were found in the rhizosphere. The only other *Pseudomonas* present was found inside the roots of Salinas 1, which was a released tolerant variety. It is highly possible that the rice plant is in a mutualistic interaction with a Pseudomonas species, which could have already been present during the seedling stage, and could have established itself inside the roots, even prior to salinization. Under saline conditions, *Pseudomonas sp.* was only observed inside the roots of Salinas 1 at both stages. This interaction was not observed in other rice varieties. Members of the genus *Pseudomonas* is widely known to promote plant growth via different mechanisms, and is reported to promote salinity tolerance in corn (Mano and Morisaki 2008). Several pseudomonads are used as plant growth promoting bacteria. Specifically, fluorescent pseudomonads were found to have catabolic versatility, excellent root colonizing ability and their capacity to produce a wide range of enzymes and metabolites that enable the plant to withstand varied biotic and abiotic stress conditions (Mayak et al. 2004). A *Pseudomonas fluorescens* strain possessing ACC deaminase activity was reported to enhance the saline resistance in groundnut plants (Saravanakumar and Samiyappan 2007) and a P-solubilizing *Pseudomonas* strain was also found to have a positive influence on plant nutrition under salt-stressed conditions when co-inoculated with *Rhizobium* sp (Bano and Fatima 2009).

Some endophytic bacteria have beneficial effects on the host plant, such as plant growth promotion, the induction of increased resistance to pathogens, as well as the supply of fixed nitrogen to the host plant (Mano and Morisaki 2008). In this study, several known plant growth promoting bacteria were found, not only in rice rhizosphere, but inside the roots as well. The combined result of DGGE profile analysis and band identification revealed that salt-tolerant varieties FL478 and Salinas1 interact with bacteria that have PGPR-like properties inside their roots. This indicates possible interaction of rice roots with endophytic PGPR. Further analysis should be done to determine these possibilities. Isolation of DNA directly from rice roots and analysis through PCR-DGGE is strongly recommended to confirm these findings and to check for the presence other microorganisms with plant-growth promoting properties.

CONCLUSION AND RECOMMENDATIONS

The results of this study suggest the interplay of salinity level and rice variety as factors affecting the characteristics of the microbial community in the rhizosphere. A detailed physiological analysis of the four rice varieties is highly recommended. An analysis of the exudates that the rice varieties release under salt stress can provide a great insight into how the microbial community in the rhizosphere is affected by salinity. Based on the results of this study, it is possible that under salt stress, the plants may have released substances which tend to enhance the bacterial diversity. Salt-tolerant varieties may also have greater capability to produce more variety of root exudates that can promote the growth of a more diverse and, possibly, beneficial microorganisms.

The dominance of several Deltaproteobacterial bacteria in the rhizosphere is also interesting. Since salinity is associated with increased amounts of solutes and ions in the rhizosphere, it appears that *Geobacter* species play an important role in saline environments. It is therefore necessary to further

analyze the community, focusing on Deltaproteobacterial species as well as the archaeal community in the rhizosphere. The presence of several plant-microbe interactions in the rhizosphere and in surface-sterilized roots of salt-tolerant varieties is noteworthy. Further research needs to be done to determine if these specific organisms can contribute to the crop's ability to tolerate salinity.

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