Diversity of *Pseudomonas* spp. Isolated from Rice Rhizosphere Populations Grown along a Salinity Gradient

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**ABSTRACT**

Along the coastline of Tamil Nadu, five sites were chosen to assess the diversity of *Pseudomonas* populations isolated from rice (*Oryza sativa*) cultivated along a salinity gradient. One of these sites was under organic farming while the other four were under inorganic farming. A total of 256 *Pseudomonas* strains isolated from these five sites were analyzed using both phenotypic (substrate utilization patterns and antibiotic resistance assay) and genotypic (PCR-RFLP of 16S rDNA) characteristics. The results derived from this study indicate that soil salinity affects rhizosphere *Pseudomonas* populations. It was observed that increasing salinity led to decreasing diversity. Fluorescent pseudomonads were the dominant species found in the non-saline site, while in the saline sites they were replaced by salt-tolerant species, in particular *Pseudomonas alcaligenes* and *P. pseudoalcaligenes*. An interesting observation was the increase in diversity found in the saline site under organic farming. Organic farming was found to be capable of mitigating the harmful effects of saline stress to a large extent, and restoring the *Pseudomonas* diversity, thereby making it comparable with the diversity encountered in the non-saline site.

**Introduction**

The coastal region is a distinct ecological zone with its own niche-specific bacteria. The issue of salinity assumes great proportions here due to frequent seawater intrusions. Salt overloading in soils, as encountered in the coastal niche, is a major hindrance for plant growth and a crucial problem in agriculture. In fact, one of the major manifestations of the process of land degradation is in the increase of soluble salt concentrations due to improper irrigation practices and/or application of chemical fertilizers. However, microorganisms have learned to adapt themselves to adverse environments, making them highly flexible.

Among the different microbial populations, the rhizobacteria residing in the rhizosphere region of plants are most beneficial. This is a zone of great microbial activity with a bacterial population density 10–200 times greater than that of the adjacent bulk soil. These organisms are more tolerant than their counterparts residing in the soil as the ionic strength and salinity of the rhizosphere is...
elevated because of depletion of essential nutrients and water and exclusion of nonessential or toxic solutes by plant roots [27].

Knowledge about the genetic structure of bacterial populations in the rhizosphere can help in relating its changes to environmental variations over time [25, 31]. They play a key role in agricultural environments and are promising for their potential use in sustainable agriculture [8]. However, any microbial utilization in agriculture requires an evaluation of the environmental risks associated with the introduction of indigenous or nonindigenous microorganisms into the plant rhizosphere as well as an assessment of the most suitable conditions for the effective and successful establishment of the PGPR as inoculants in the rhizosphere of the host plant [7]. The approach to both problems is based on the accurate characterization of bacterial populations naturally associated with the roots. Therefore, an analysis of the genetic structure of a microbial population has practical importance: the results can be used to assess the fate of the released strains and their impact on resident microbial communities [9]. The most judicious way of obtaining conclusive information would be to follow a polyphasic approach, in which one could assess the information obtained from all avenues. This would also help in elucidating the species or genetic information with greater precision. The present analysis, as discussed in the subsequent sections, precisely intends to do so.

The main aim of this study was to assess the influence of soil salinity and farming practices on the rhizobacterial populations, in particular the genus *Pseudomonas*. This genus is a common member of the plant growth-promoting microflora present in the rhizosphere of plants [16]. They have received tremendous attention mainly due to their widespread distribution in the soil, ability to colonize the rhizosphere of host plants and capacity to produce a large number of compounds antagonistic to various serious plant pathogens [2]. They are particularly sensitive indicators of soil perturbations that affect microbial communities [19]. To accomplish this, the diversity of five different rice (*Oryza sativa*) rhizosphere populations of *Pseudomonas*, isolated from the coastline of Tamil Nadu, was studied.

### Materials and Methods

#### Site Description and Sample Collection

The study sites were chosen along the coastline of Tamil Nadu in Southern India. They were located at a longitude of 11°00′N to 12°00′N, 79°45′E. The main selection criteria were cultivation of rice, salinity levels in the sites, farming practices and consent of the farmers who owned the sites. The area of study was approximately 1.5 to 11 km from the sea and possessed a clayey soil. The elevation and size of each site, weather conditions, irrigation practices, and pH values were similar. The main difference was in their salinity levels, which is reflected in their respective electrical conductivity (E.C.) values recorded (Table 1). Canal irrigation and inorganic farming were the most common practices in this region. Only site IV practiced the traditional system of organic farming. Entire, healthy, mature plants were collected by sampling at intervals, taking care to cover the entire area of cultivation, and then pooled together before bacterial isolation. The top 5 cm of the soil adjacent to the plants was also collected from 20 places in each field, pooled, and mixed thoroughly. Two replicate samples from each site were air dried at 30°C overnight, sieved though a 2 mm pore size, and analyzed for their electrical conductivity (E.C.) and pH values.

#### Isolation of Bacterial Strains

The intact root systems were collected and shaken gently to remove all but the soil closely adhering to the roots. These root portions with just a layer of closely adhering rhizosphere soil were then transferred to 100 mL sterile water and shaken at 120 rpm for 30 min. Suspensions from all five samples were serially diluted up to 10^-4 with three replications for each sample. From 10^-3 and 10^-4 dilutions, 1 mL was poured plated on *Pseudomonas*

<table>
<thead>
<tr>
<th>Site</th>
<th>Acc. No. of the strains</th>
<th>Distance from the sea (km)</th>
<th>Soil pH</th>
<th>E.C.</th>
<th>Available nutrients (kg/acre)</th>
<th>Farming practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>MSP330-388</td>
<td>11.18</td>
<td>7.45</td>
<td>0.78</td>
<td>N 67, P 5.0, K 105</td>
<td>Inorganic</td>
</tr>
<tr>
<td>II</td>
<td>MSP389-434</td>
<td>1.54</td>
<td>7.74</td>
<td>2.31</td>
<td>N 90, P 2.5, K 100</td>
<td>Inorganic</td>
</tr>
<tr>
<td>III</td>
<td>MSP435-482</td>
<td>1.54</td>
<td>6.33</td>
<td>2.93</td>
<td>N 69, P 2.5, K 90</td>
<td>Inorganic</td>
</tr>
<tr>
<td>IV</td>
<td>MSP483-534</td>
<td>2.63</td>
<td>6.45</td>
<td>4.28</td>
<td>N 104, P 7.5, K 95</td>
<td>Organic</td>
</tr>
<tr>
<td>V</td>
<td>MSP535-585</td>
<td>2.33</td>
<td>6.48</td>
<td>3.8</td>
<td>N 74, P 2.5, K 105</td>
<td>Inorganic</td>
</tr>
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