DISTRIBUTION OF CULTURABLE ENDOPHYTIC BACTERIA IN LEMON GRASS (Cymbopogon citratus)

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ABSTRACT
Endophytic bacteria are currently being harnessed as potential sources of bioactive compounds, potential biofertilizers, and as tools for bioremediation. This therefore stresses the importance of searching for these noble bacteria in various plants. In the present study, fresh and apparently healthy leaves and roots of lemon grass were collected and surface-sterilized using 70% (v/v) Ethanol, 3% sodium hypochlorite solution and sterile distilled water. Isolation of endophytic bacteria was achieved using culture technique, while identification was done based on morphological, biochemical and microscopic characteristics. A total of 16 endophytic bacteria were isolated and identified as Bacillus spp. (3 isolates), Escherichia coli (1 isolate), Klebsiella pneumoniae (3 isolates), Micrococcus spp. (3 isolates), Pseudomonas spp. (1 isolate), Rhizobium (2 isolates) and Staphylococcus aureus (3 isolates). The root portions of the plant harbour 10 (62.5%) of the entire endophytic bacteria isolated, while the leaves harbour the remaining 6 (37.5%). Gram negative rod-shaped bacteria are the dominant of all the bacteria in the roots (50%), whereas, in the leaves, Gram positive cocci are the dominant (50% of all). No Gram negative cocci were isolated from the plant. In conclusion, Lemon grass harbours diverse genera of endophytic bacteria present both in the roots and leaves of the plant, but the roots harbour higher populations of the bacteria.

Keywords: Endophytic bacteria, Lemon grass, Root, Leaf, Isolation

INTRODUCTION
Microorganisms have established such a close association with higher plants that many live inside the internal tissues of the plants without causing any noticeable immunologic response. These microorganisms referred to as endophytes, do not harm the host plant, but rather benefit it in a number of ways. According to Hallmann et al. (1997), endophytic bacteria are those that can be detected inside surface-sterilized plant tissues or extracted from inside plants and having no visibly harmful effect on the host plants. Endophytic bacteria are found in a variety of plants, ranging from herbaceous plants such as maize and beet to woody plants (Ryan et al. 2007).

Several bacteria that differ in many respects have been frequently encountered as endophytes. These include bacteria from the genus Bacillus as endophytes of maize kernel (Surette et al., 2003), Enterobacter as an endophyte of maize (McInroy and Kloeper 1995), Klebsiella pneumoniae in Soybean (Kuklinsky-Sobral et al. 2004), Rhizobium leguminosarum in Rice (Yanni et al. 1997), and Escherichia coli in Lettuce (Ingham et al. 2005). A number of reports have shown that endophytic microorganisms can have the capacity to contribute to the control of plant-parasitic nematodes (Hallmann et al. 1995) and insects (Dimock et al. 1988). In some cases, they can also accelerate seedling emergence, promote plant establishment under adverse conditions (Chanway, 1997) and enhance plant growth (Bent and Chanway 1998).

Cymbopogon citratus, commonly known as the Lemon grass is a tropical herb that is popular in south east Asia and Africa. The plant has lots of medicinal applications as antihelmintic, aphrodisiac, appetizer, laxative (Parrotta, 2001) etc. It is used in Ayurvedic medicine in the treatment of epilepsy, leprosy and bronchitis (Parrotta, 2001).

Strobel et al. (2004) reported that, close to 300,000 different plant species exist on the earth each of which hosts one or more endophytes. Only a fraction of these plants have been fully explored relative to their endophytic biology. In view of the medicinal and other uses of C. citratus, a study on its endophytic microorganisms would be of invaluable benefits to public health and agriculture. In an earlier study, Deshmukh et al. (2010) reported 24 different fungal species belonging to 21 genera isolated from the leaves and rhizomes of C. citratus.
To the best of our knowledge, no previous study has been made regarding the endophytic bacteria of the same plant from the study area (Kano, Nigeria), hence the need for this study. The current study therefore aimed at revealing the endophytic bacteria of *C. citratus*, and their distributions in the roots and leaves of the plant.

**MATERIALS AND METHODS**

**Sample Collection**

For the isolation of endophytic bacteria, fresh and apparently healthy leaves and roots of *C. citratus* were collected from the Botanical Garden of the Department of Biological Sciences Bayero University Kano Nigeria.

**Sample Pre-treatment and Surface Sterilization**

The leaves and roots of the plant were washed separately under running tap water to remove adhering soil particles, and the majority of microbial surface epiphytes. The samples were then subjected to surface sterilization procedure as follows: An initial wash in sterile distilled water to remove adhering soil particles, 1 minute wash in 70% ethanol, followed by a 2 minute wash in 3% sodium hypochlorite and finally, a three times rinse in sterile distilled water (Hallman, *et al.* 1997).

**Isolation of Endophytic Bacteria**

Five different isolation media were used for the isolation i.e., Yeast extract sucrose agar (YESA) which is selective for the isolation of *Rhizobium* species, Nutrient agar, Mac Conkey agar, Nutrient broth yeast extract agar (NBY) and Brain heart infusion agar. The procedure followed the protocol of Sheng *et al.* (2008) with some modifications. In the current study, homogenization of the plant materials was done in a blender as an alternative to the mechanical grinding using pestle and mortar as done in the original protocol. Additionally, pre-formed inoculation media were used in the current work in place of minimal salt media in the original protocol. Each of the collected *C. citratus* samples was aseptically homogenized in a sterile blender and a three-fold (up to 10^3) serial dilution was carried out. One milliliter (1ml) from each dilution was inoculated in triplicates on the various culture media using pour plate technique. Control cultures of the surface-sterilized but un-homogenized leaves of the plant were also prepared the same way. All cultures were incubated at room temperature for 48 hours. Individual colonies were picked and streaked on fresh culture media for purification to generate pure cultures.

**Morphological and Biochemical Characterization of the Bacterial Isolates**

Pure cultures obtained were first characterized using Gram staining method (Barthomeow, 1962). Biochemical tests such as Catalase, coagulase, oxidase, indole, methyl red, Voges-Proskauer urease activity, citrate utilization, cellulose hydrolysis, starch hydrolysis, triple sugar iron tests were done according to the procedures described by Cappuccino and Sherman (2000). Endospore staining and capsule staining were also carried out.

**RESULTS**

*Table 1. Distribution of Bacterial Genera in the Roots and Leaves of C. citratus.*

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Root</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Micrococcus</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10 (62.5%)</strong></td>
<td><strong>6 (37.5%)</strong></td>
</tr>
</tbody>
</table>

*Table 2. Distribution of Different Morphologic Groups of Bacteria in the Roots and Leaves of C. citratus.*

<table>
<thead>
<tr>
<th>Gram reaction/Morphology</th>
<th>Root</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive rods</td>
<td>2 (20%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>3 (30%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Gram negative rods</td>
<td>5 (50%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Gram negative cocci</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10 (100%)</strong></td>
<td><strong>6 (100%)</strong></td>
</tr>
</tbody>
</table>
DISCUSSION
The isolates obtained in this study are similar to the common endophytic bacteria isolated from other plants, by different workers at different times, the list of which was compiled by Ryan et al. (2007) and Rosenblueth and Martinez-Romero (2004). The result showed that, the roots of *C. citratus* contain higher population of endophytic bacteria relative to the leaves. This is most probably due to the fact that, the roots are the primary sites of infection as opined by Kobayashi and Palumbo (2000) and Hallmann et al. (1997). Similarly, Rosenblueth and Martinez-Romero (2004) found that, in most plants, the number of bacterial endophytes is higher in the roots than the above-ground tissues. Moreover, most endophytic bacteria are soil-borne and therefore colonize the roots region first and subsequently spread to other parts of the plants. Interestingly, opposite pattern of distribution was observed among the endophytic fungi that colonize same plant as reported by Deshmukh et al. (2010) who in a study of fungal endophytes of *C. citratus* in two sites in India reported 53% and 50% compared with 25% and 23% of fungi isolated from the leaves and rhizomes of the two sites, respectively. Furthermore, different morphological groups of bacteria were encountered as endophytes of *C. citratus*. However, while Gram negative rods are the dominant in the roots (50% of the total), Gram positive rods are the dominant in the leaves (50% of the total). No Gram negative cocci were isolated. Overall, Gram negative rods are the dominant (31.25% of all) endophytes of *C. citratus*. The Gram negative isolates belong to the family Enterobacteriaceae, Psuedomonaceae and Rhizobiaceae which are all well distributed in the soil. This presence in the soil, can facilitate their entrance into roots of plants resulting in their establishment in other parts of the host plant.

CONCLUSION
This research has shown that, the internal tissues of *C. citratus* harbour a diverse collection of endophytic bacteria that are more dominant in the roots than the leaves.

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REFERENCES


