Special Conference Edition, November, 2017
http://dx.doi.org/10.4314/bajopas.v10i1.19S

Bayero Journal of Pure and Applied Sciences, 10(1): 95 - 98
ISSN 2006 – 6996

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

Inuwa, A.B.*[1], Maryam, Y.A.[1], Arzai, A.H.[1], Hafsat, Y.B.[1], Kawo, A.H. [1], Usman, A.U[1], Ama, S.J [1], and Ibrahim, K.H[2],

[1]Department of Microbiology Bayero University Kano Nigeria
[2]Department of Biology, Kano State College of Arts, Science and Remedial Studies
*Correspondence author: abinuwa.mcb@buk.edu.ng, Phone: +23480-95153202

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 pnuemoniae (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 Kloepper 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.*

Bacterial isolates	Root	Leaves
Bacillus	2	1
Escherichia	1	0
Klebsiella	2	1
Micrococcus	2	1
Pseudomonas	0	1
Rhizobium	2	0
Staphylococcus	1	2
Total	**10(62.5%)**	**6(37.5%)**

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

Gram reaction/Morphology	Root	Leaves
Gram positive rods	2 (20%)	1(16.7%)
Gram positive cocci	3(30%)	**3(50%)**
Gram negative rods	**5(50%)**	2(33.3%)
Gram negative cocci	**0**	**0**
Total	**10(100%)**	**6(100%)**

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.

Acknowledgement

This work was supported by the Department of Microbiology, Bayero University, Kano.

REFERENCES

Barthomelow, J.W. (1962).Variables influencing results and precise definition of steps in Gram staining as a means of standardizing the results obtained. *Stain Technology* **37(3):** 139-155.

Bent, E. and Chanway CP. (1998). The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. *Can J Microbiol*. **44(10):** 980–988.

Cappuccino G and Sherman N. (2000). Microbiology: A laboratory manual, 6th Edition, Pearson education inc. San Francisco, California. VOL. (2): 15-224.

Chanway CP. (1997). Inoculation of tree roots with plant growth promoting soil bacteria: an emerging technology for reforestation: an emerging technology for reforestation. *Forest Science* **43(1):** 99–112.

Deshmukh SK, Kolet, MJ and Verekar SA. (2010). Distribution of endophytic fungi in lemon grass (*Cymbopogon citratus* (DC.) Stapf.). *Journal of Cell and Tissue Research* **10(2):** 2263.

Dimock MB, Beach RM, and Carlson PS. (1988). Endophytic bacteria for the delivery of crop protection agents. In: Roberts DW. and Granados RR. (eds). Biotechnology, biological pesticides and novel plant-pest resistance for insect pest management. Boyce Thompson Institute for Plant Research, Ithaca,N.Y. pp. 88-92.

Hallmann J, Kloepper J, Rodriguez-Kabana R, and Sikora RA. (1995). Endophytic rhizobacteria as antagonists of *Meloidogyne incognita* on cucumber. *Phytopathology* **85(1):**136.

Hallmann J, Hallmann QA, Mahaffee WF and Kloepper JW. (1997). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* **43(10):** 895-914.

Ingham, SC, Fanslau MA, Engel RA, Breuer JR, Breuer JE, Wright TH, Reith-Rozelle JK and Zhu J. (2005). Evaluation of fertilization-to-planting and fertilization-to-harvest intervals for safe use of non-composted bovine manure in Wisconsin vegetable production. *Journal of Food Protection* **68(6):**1134-1142.

Kobayashi DY and Palumbo JD. (2000). Bacterial endophytes and their effects on plants and uses in agriculture. *Microbial endophytes*. New. York. Basel: Mercel Dekker **(19):** 199–236.

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani- Kleiner AA and Azevedo JL. (2004). Isolation and characterization of soybean associated bacteria and their potential for plant growth promotion. *Environmental Microbiology* **6(12):**1244-1251.

McInroy JA and Kloepper JW. (1995). Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant and Soil* **173(2):**337-342.

Parrotta JA. (2001). Healing Plants of Peninsular India. CABI publishing, New York.

Rosenblueth M and Martinez Romero E. (2004). *Rhizobium etli* maize population and their competitiveness for root colonization. *Archives of Microbiology* **181(15):** 337-344.

Ryan RP, Germaine K, Franks A, Ryan DJ and Dowling DN. (2007). Bacterial endophytes: recent developments and applications. *FEMS Microbiology letters* **278(1):** 1-9.

Sheng XF, Xia JJ, Jiang CY, He LY and Qian M. (2008). Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environmental Pollution* **156(3):** 1164-1170.

Strobel G, Daisy B, Castillo U and Harper J. (2004). Natural products from endophytic microorganisms. *Journal of Natural Products* **67(2):** 257-268.

Surette MA, Sturz AV, Lada RR. and Nowak J. (2003). Bacterial endophytes in processing carrots *(Daucuscarota L. var. sativus)* their localization, population density, biodiversity and their effects on plant growth. *Plant and Soil* **253(2):** 381-390.

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip- Hollingsworth S, Orgambide G, De Bruijn, F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK and Dazzo FB. (1997). Natural endophytic association between *Rhizobium leguminosarum bv. trifolii* and rice roots and assessment of potential to promote rice growth. *Plant and Soil* **194(1-2):**99-114.