Isolation and Identification of Endophytic Bacteria from *Neolamarckia cadamba* (Roxb.) Bosser (Ma-U-Lat-Tan-She)

Kyi Kyi Khine

Abstract

In this investigation, the isolation and identification of endophytic bacteria from different parts of *Neolamarckia cadamba* (Roxb.) Bosser (Ma-U-Lat-Tan-She) was studied. These plant samples were collected from Yae-Lal Street, Maubin Township, Ayeyarwady Region. The experiments were carried out at the Microbiology Laboratory, Department of Botany, Maubin University from 2018 to 2019. Three endophytic bacterial strain 1, strain 2 and strain 3 were isolated from leaf, petiole and bark of *Neolamarckia cadamba* (Roxb.) Bosser respectively. The identification of isolated bacterial strains was carried out by their morphological characters, microscopic characters and biochemical tests. According to the result, the isolated bacterial strain1 was identified *Bacillus* sp., strain 2 was *Micrococcus* sp. and strain 3 was *Neisseria* sp.

Key words: Endophytic bacteria, Biochemical tests, *Bacillus*, *Micrococcus* and *Neisseria*

Introduction

Microorganisms living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence are defined as endophytes (Saikkonen ,et al., 2004). The term "Endophyte" is derived from the Greek words "endo" meaning within, and "Phyte" meaning plant. Previously, endophytes were defined as microorganisms such as bacteria and fungi that inhabit the plant endosphere during all or part of their life cycle without any apparent the plant.

Endophytic bacteria that promote host growth and resistance to environmental stress (Saikkonen, 2010) and also serve as potential sources of natural products for exploitation in medicine, agriculture and industry (Strobel and daisy, 2003).

Endophytes are the part of the microbial community found in all species of plant (Amold, 2000) that include bacteria, fungi actinomycetes (Bandara, 2006), which live inside plant tissue for at least a part of their life cycle without any disease symptoms in the host (Petrini ,1991).

Neolamarckia cadamba (Roxb.) Bosser also known as kadam, is a tropical tree species that is native to South Asia and Southeast Asia. It belongs to the family of Rubiaceae. Kadam is a large tree with a broad umbrella-shaped crown and straight cylindered bole. The tree usually reaches a height of 45m with a stem diameter of 100-116 cm.

Bacillus species are ubiquitous in nature but found in higher concentrations in soil, water, and food products that have a plant origin. *Bacillus* is a genus of Gram-positive, rod-shaped bacteria, a member of the phylum Firmicutes, with 266 named species. *Bacillus* species can be either obligate aerobes, oxygen dependent, or facultative anaerobes, having the ability to be anaerobic in the absence of oxygen.

Micrococcus sp. is gram-positive, oxidase-positive, and strictly aerobic cocci belonging to the family Micrococcaceae. They usually occur in irregular clusters, tetrads, and pairs, where individual cells are about 1 to 1.8 mm in diameter and are usually non-motile and non spore-forming. They are found on the skin of humans and other animals and in soil, marine and fresh water, plants, dust, and air (Doddamani and Ninnekar, 2001).

Associate professor, Department of Botany, Dagon University

Neisseria are aerobic gram-negative diplococci belonging to the family *Neisseriaceae*, which mainly colonize the human oral cavity and nasopharynx. Most *Neisseria* are members of the normal microflora of the human body and are usually nonpathogenic (Knapp and Hook. 1988).

The aim and objectives of the research were to isolate the endophytic microorganisms from *Neolamarckia cadamba* (Roxb.) Bosser, to study morphological and microscopic characters of isolated bacterial strains and to identify the isolated endophytic microorganisms.

Materials and methods

Collection of plant sample

The plant samples were collected from Yae-lae street, Maubin area. Ayeyarwady Region. The collected plants were shown in Table 1.

Table 1. Plant samples for isolation of endophytes

No.	Scientific Name	Family	Myanmar Name
1.	*Neolamarckia cadamba* (Roxb.) Bosser	Rubiaceae	Ma-U- Lat –Tan- She

Figure 1. Habit

Figure 2. Leaf and petiole

Figure 3. Bark

Isolation of endophytic microorganisms

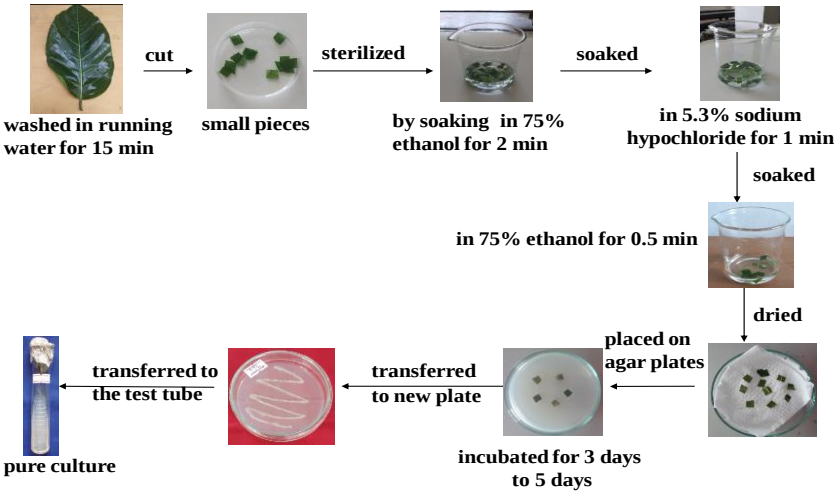


Fig. 4. Isolation procedure of endophytic strains (Lee, et al., 1996 and Phay, 1997)

Morphological and microscopic characters of isolated endophytic bacterial strains

The isolated bacterial strains grown on slant culture were transferred onto the plates containing sucrose yeast (SY) medium. Medium composition of sucrose yeast (SY) was sucrose 1.0 g, yeast extract 0.3 g, NaCl 0.1 g, $CaCO_3$ 0.01 g, agar 1.8 g, distilled water 100 ml and pH 7. Then, these plates were incubated at 30°C for 24 hrs. After 24 hours, isolated strains were checked under microscope by gram staining method for their morphological characters.

Results

Isolation of endophytic bacterial strains

Three bacterial strains were isolated from three different plant parts. These bacterial strains were named as strain 1, strain 2 and strain 3 respectively (Table 2).

Table 2. Isolated strains from different plant parts

Scientific name	Myanmar name	Plant parts	Isolated strains
Neolamarckia cadamba (Roxb.) Bosser	Ma-U-Lat-Tan-She	Leaf	Strain 1
Neolamarckia cadamba (Roxb.) Bosser	Ma-U-Lat-Tan-She	Petiole	Strain 2
Neolamarckia cadamba (Roxb.) Bosser	Ma-U-Lat-Tan-She	Bark	Strain 3

Table 3. Morphological and microscopic characters of isolated strains

Bacterial strains	Gram stain	Motility	Aerobic/ Anaerobic	Cell morphology	Cultural Character (colour)
Strain 1	+	motile	Aerobic	Bacilli	Yellowish white
Strain 2	+	non-motile	Aerobic	Cocci	Pale yellow
Strain 3	_	motile	Aerobic	Cocci	Cream

+ = Positive − = Negative

Table 4. Biochemical characteristics of isolated strains

No.	Biochemical Test	Strain 1	Strain 2	Strain 3
1	Catalase test	+	+	+
2	Nitrate reduction test	+	-	+
3	Citrate test	-	+	+
4	Methyl red test	-	+	+
5	Urease test	+	+	-
6	Starch hydrolysis test	+	+	+
7	Sugar fermentation test	+	-	+

+ = Positive − = Negative

Strain 1 **Strain 2** **Strain 3**

Figure 5. Catalase test

Fig. 6 Cell morphology and cultural character of Strain 1 and 2

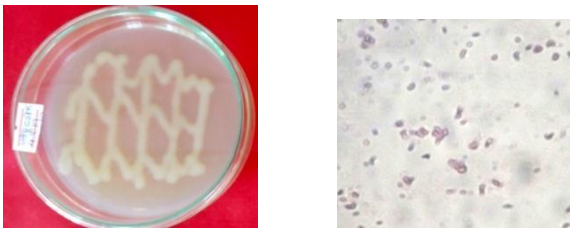
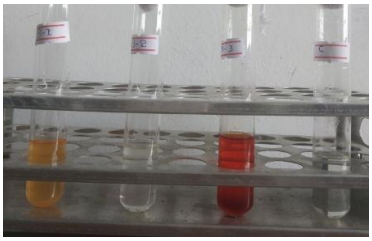


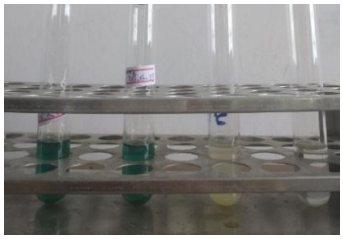
Fig. 7 Cell morphology and cultural character of Strain 3

Table 4. Biochemical characteristics of isolated strains



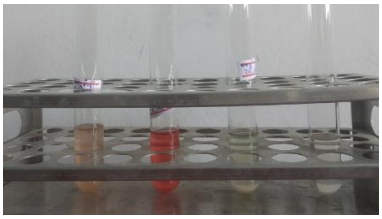
3 2 1 Control

Figure 8. Nitrate reduction test



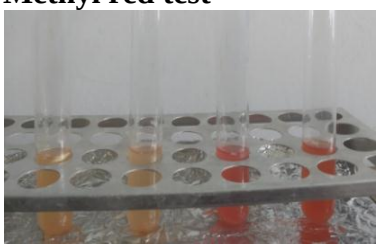
3 2 1 Control

Figure 9. Citrate test



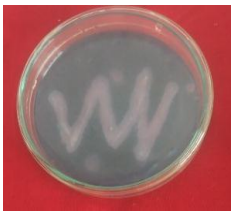
3 2 1 Control

Figure 10. Methyl red test



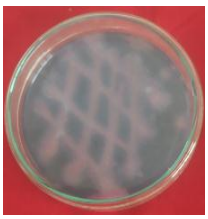
Control 3 2 1

Figure 11. Urease test



Strain 1

Strain 2



Strain 3

Figure 12. Starch hydrolysis test

Identification of Isolated Bacterial Strains

According to the morphology, microscopic characters and biochemical tests, isolated bacterial strain 1 was identified as *Bacillus,* strain 2 was member of *Micrococcus* and strain 3 was members of *Neisseria.*

Discussion and conclusion

In this study, three endophytic bacterial strains were isolated from the three different plant parts of *Neolamarckia cadamba* (Roxb.) Bosser. Hallmann and Antoun (1997) reported that endophytic bacteria were isolated from various plant tissues since 1940. Strain 1 was rod shaped and strain 2 and strain 3 were spherical shaped in this research. These shapes were similar to the previous researchers' investigation (Yardley et al., 1961). The surface color of strain 1 was yellowish white, strain 2 was pale yellow and the surface color of strain 3 was cream colour.

According to the result of biochemical test, catalase test, nitrate reduction test, citrate test, methyl red test, urease test, starch hydrolysis test and sugar fermentation test indicated positive and negative reactions. Catalase test, isolated bacterial strains show bubbles, thus strain 1, strain 2 and strain 3 were positive. Nitrate reduction test, isolated bacterial strain showed red color and colorless, thus strain1 and strain 3were positive and strain 2 was negative. Citrate test, isolated bacterial strains 1 showed blue color, thus negative and strain 2 and 3 showed green color, it is positive. Methyl red test, isolated bacterial strain showed red color and colorless, thus strain 2 and 3 were positive and strain 1 was negative.

Among these three isolated endophytic bacterial strains, strain 1 and strain 2 were Gram positive and strain 3 was Gram negative. All isolated bacterial strains were aerobic bacteria. According to the morphology, microscopic characters and biochemical characteristics of isolated bacterial strain 1 was identified as *Bacillus,* strains 2 and 3 were members of *Micrococcus* and *Neisseria.* These strains were supposed to the 8th edition of Bergey's Manual of Determinative Bacteriology (1974).

In conclusion, all isolated strains: *Bacillus*, *Micrococcus* and *Neisseria* were plant growth promoting bacteria according to literatures. Therefore, these endophytic strains should be utilized as bio-fertilizers to promote the growth and yielding of some crops such as peas, beans, pepper, egg plants, etc. for further research.

Acknowledgements

I would like to express my deepest gratitude to Dr. Myat Myat Moe (Professor and Head, Department of Botany, Dagon University) and I am thankful to Dr. Mar Mar Aye, Professor and Head, Department of Botany, Maubin University, for allowing me to use research facilities in Botany Department and also encouragement given to me.

I also thank Professor Dr. Yee Yee Thu, Department of Botany, Panglong University, for her kind help, suggestions and supports of my research work. I am thankful to Dr. Thet Thet Mar Win, Associate professor, Department of Botany, Maubin University, for her kindly advice and suggestions based on her experiences.

References

Amold, A. E.; Z. Maynard; G. GFilbert; P. D. Coley and T. A. kursar. 2000. Are tropical fungal endophytes byperdiverse. Ecol Lett 3: 267-274.

Bandara, W. M. M. S., S. A. Senevitratne , G. Kulasoariya. 2006. Interaction among endotphytic bacteria and fungi effects and potentials. J. Bioscience 31(s):645-650.

Bergey, 1974. Bergey's Manual of Determinative Bacteriology (8th edition)

Doddamani H, H. Ninnekar. 2001. "Biodegradation of carbaryl by a Micrococcus species". Curr Microbiol. 43 (1): 69–73. doi:10.1007/s002840010262. PMID 11375667.

Knapp, J. S.; E.W. Hook. 1988. "Prevalence and persistence of *Neisseria cinerea* and other *Neisseria* spp. in adults". Journal of Clinical Microbiology. 26.5: 896–900.

Lee, K. D, J. Kim and H. Kim. 1996. Isolation and characterization of Bacilllus sp. KD.1014 producing carboxyl methyl- cellulose. J. Microbiology 34: 305-310.

Petrini O. 1991. Fungal endophytes of tree leaves. In: Andrewa JH, Hirano SS, eds.Microbial Ecology of Leaves. New York: Spring Verlag; 179- 197.

Phay N. 1997. Studies on Selective Antibiotics. Doctoral Thesis. Faculty of Agriculture, Hokkaido University, Japan.

Saikkonen K., M. Helander and S. H. Faeth. 2004. Fungal endophytes: hitchhikers of the green world; in *Plant microbiology* (eds) M Gillings and A Holmes (Oxford: BIOS Scientific Publishers) pp 77–95.

Saikkonen, K., S. Saari and M. Helander. 2010. Defensive mutualism between plants and endophytic fungi. Fungal divers. 41:101-113.

Stroble, G. A. and B. Daisy. 2003. Bioprospecting for microbial Endophytes and their natural products. Microbiology a Molecular Biology Review. 67: 491-502.

Yardley, J. H. and T. R. Hendrix. 1961. Combined electron and light microscopy in Whipples's disease: demonstration of "bacillary bodies" in the intestine. Bull Johns Hopkins Hosp; 109: 80-98.