Types of endophytic bacteria associated with traditional medicinal plant *Lantana camara* Linn.

Bhore Subhash Janardhan and Komathi Vijayan

Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Bedong-Semeling Road, Semeling 08100, Kedah, Malaysia

Submission Date: 7-7-2012; Review Completed: 22-7-2012

ABSTRACT

Background: Traditionally, *Lantana camara* plant (Family: Verbenaceae) is used in herbal medicine as an antiseptic for wounds, in the treatment of skin itches, and externally for leprosy and scabies. The leaves of this plant possess adulticidal activity against different mosquito species. Endophytic bacteria (EB) can produce bioactive compounds found in their host; hence, investigation to find out what types of EB are associated with *L. camara* is necessary. **Objectives:** The main objective of this study was to isolate and identify EB associated with *L. camara*. **Materials and methods:** *Lantana camara* twigs along with the leaves and fruits samples were collected; and EB were isolated from surface-sterilized tissue samples. The 16S rRNA gene fragments were amplified using PCR method; and endophytic bacterial isolates (EBIs) were identified based on 16S rRNA gene sequence similarity method. **Results:** Cultivable, 50 EBIs were analyzed; and analysis of their 16S rRNA gene sequences suggests that varied 40 types of EB are associated with *L. camara*. Majority (24%) of EBIs were from *Bacillus* genus. **Conclusion:** Thus, we conclude that *Lantana camara* plants harbour a wide array of cultivable endophytic bacteria.

Keywords: 16S rRNA, diversity, endophytes, herbal medicine, Malaysia, natural products

INTRODUCTION

Use of medicinal plants in the treatment of various health ailments is as old as mankind. Plant genus, *Lantana* contains about 150 species and some of the species are used as antibacterial, antirheumatic, biological control, stimulant and as ornamental plant.[1–2] *Lantana camara* is one of the species and used in traditional medicinal system of various countries. *Lantana camara* is a shrub type of plant which belongs to family Verbenaceae. This plant is a native of Africa and America; and it is extensively used as an ornamental plant in some other countries. However, in Malaysia and India, this plant is found in the forests as well as along the roadsides and on the waste land. In some countries like Brazil, this plant is found in most of the regions.[2–3]

The leaves of *L. camara* are used in the treatment of skin injuries, as an antiseptic for wounds, and externally for leprosy and scabies.[4] Leaves are also used in the treatment of various other health ailments such as biliary fever, bronquitis, rheumatism, scratching, stomachache, and toothache.[2,5] In addition to this, the leaves of this plant are also used in the treatment of pulmonary diseases and rheumatism;[6] and plant is also explored for some other potential pharmaceutical application.[7]

The essential oil of *L. camara* leaves contains a high amount of sesquiterpenes, and oil is known to inhibit the growth of *Pseudomonas aeruginosa*, *Aspergillus niger*, *Fusarium solani* and *Candida albicans*.[5] The oil obtained from *L. camara* also possesses insecticidal and repellent activities; and as stated by Sousa et al. (2010), the essential oil also shows repellent effect against *Aedes* mosquitoes.[8] The research findings reported by Sousa et al. (2010) suggest that the essential oil of *L. camara* could be used as a source of plant-derived natural products with resistance-modifying activity.[8]

***Corresponding author.**
Dr. Subhash J. Bhore,
Department of Biotechnology, Faculty of Applied Sciences,
AIMST University, Bedong-Semeling Road, 08100 Bedong,
Kedah, Malaysia
Tel: 604-429 8176; Fax: 604- 429 8009 / 8109
E-mail: subhashbhore@gmail.com

DOI: 10.5530/pj.2012.32.4

In year 2010, Mehanni and Safwat has reported that endophytic microorganisms isolated from medicinal plants do produce the same metabolites as their hosts.[9] Hence, endophytes can be considered as one of the potential sources of the therapeutic compounds. *Lantana camara* is an important medicinal plant; but, despite its various medicinal applications we do not know what types of endophytes are associated with it.[4,10]

The main aim of this study was to understand what types of EB are associated with *L. camara*; and the specific-objective of this study was to isolate and identify the EB from *L. camara*.

MATERIALS AND METHODS

The leaves, twigs and fruits from 15 individual plants of *L. camara* were collected from Taman Tasek Semeling, Kedah, Malaysia. The stem pieces, leaves, and fruits were carefully washed under plenty of running tap water. Surface-sterilization of leaves (along with petioles), stem pieces and fruits samples were carried out as described in earlier publication.[11]

The leaves, petiole and stem tissue pieces, and fruits (by making injuries) were inoculated aseptically in the Petri plates containing nutrient agar. Petri plates containing inoculated tissue samples were incubated at 37ºC (± 3ºC) for 18 to 20 h in the dark in an incubator.

Isolation and cultivation of endophytes, amplification of 16S rRNA gene fragments, sequencing of 16S rRNA gene fragments and identification of EB was carried out as described in previous publication.[11]

RESULTS

Incubation of Petri plates containing the inoculated tissues sample on nutrient agar enabled cultivable EB to grow; and the grown EB colonies were visible on the margins of the inoculated tissues. In total, fifty (50) EBIs were examined (12, 10, 23, and 5 from stem, petiole, leaf, and fruit tissues, respectively).

All EBIs were identified based on their sequenced 16S rRNA gene sequence BLAST (megablast) hits analysis. The annotated 16S rRNA gene fragment's nucleotide sequences of all 50 EBIs have been submitted to the international DNA database (GenBank/DDBJ/EMBL) under accession numbers: JN835522 – JN835571.

The analysis of the identified EBIs revealed that there were 40 different types of species (Table 1) of the bacteria in isolates; and the majority (24%) of EBIs were from *Bacillus* genus. Results clearly indicate that *L. camara* harbours diverse types of endophytic bacteria.

DISCUSSION

It is strongly believed that almost every plant on the earth harbours EB.[12] The EB do have various potential applications not only in pharmaceutical industry but in other

Table 1. The types of endophytic bacteria associated with *Lantana camara* as revealed by identification of isolates based on their 16S rRNA gene sequence.

No.	Species	GBN#
1	*Bacillus aerophilus*	JN835561
2	*Bacillus amyloliquefaciens*	JN835555
3	*Bacillus aryabhattai*	JN835568
4	*Bacillus axarquiensis*	JN835538
5	*Bacillus cereus*	JN835564
6	*Bacillus megaterium*	JN835567
7	*Bacillus methylotrophicus*	JN835539
8	*Bacillus pumilus*	JN835565
9	*Bacillus subtilis*	JN835563
10	*Bacillus tequilensis*	JN835548
11	*Bacillus vallismortis*	JN835547
12	*Chryseobacterium daejeonense*	JN835522
13	*Chryseobacterium taeanense*	JN835560
14	*Chryseobacterium taichungense*	JN835557
15	*Cronobacter dublinensis*	JN835541
16	*Cronobacter malonaticus*	JN835544
17	*Cronobacter muytjensii*	JN835546
18	*Cronobacter sakazakii*	JN835534
19	*Cronobacter turicensis*	JN835537
20	*Edwardsiella tarda*	JN835528
21	*Enterobacter cancerogenus*	JN835527
22	*Enterobacter cloacae*	JN835530
23	*Enterobacter cowanii*	JN835536
24	*Enterobacter hormaechei*	JN835523
25	*Enterobacter pyrinus*	JN835525
26	*Erwinia amylovora*	JN835559
27	*Escherichia hermannii*	JN835532
28	*Escherichia senegalensis*	JN835533
29	*Klebsiella oxytoca*	JN835535
30	*Klebsiella pneumonia*	JN835542
31	*Pantoea agglomerans*	JN835554
32	*Pantoea ananatis*	JN835524
33	*Pantoea dispersa*	JN835550
34	*Pantoea eucalypti*	JN835569
35	*Pantoea stewartii*	JN835570
36	*Pseudomonas argentinensis*	JN835549
37	*Pseudomonas flavescens*	JN835553
38	*Pseudomonas fulva*	JN835543
39	*Pseudomonas straminea*	JN835529
40	*Raoultella planticola*	JN835545

#GenBank accession numbers of deposited 16S rRNA gene sequence fragment from respective endophytic bacterial isolate (EBI).

sectors also. We isolated and identified 50 EBIs from *L. camara*. Similarly, endophytic bacteria has been reported from various medicinal plants; for instance, *Gynura procumbens*,[11] *Piper nigrum*,[13] *Trifolium repens*[14] and *Artemisia annua*.[15] However, to our knowledge, this study is the first to illustrate diverse types of EB in *L. camara*. The similarity comparison between the 16S rRNA gene fragment sequences from 50 EBIs and the sequences from GenBank/DDBJ/EMBL database using the BLASTN program revealed identity of each isolate.

Bacterial 16S rRNA gene sequence do provide species-specific signature and can be used in bacterial identification.[16] In fact, this approach is commonly used in identification of bacteria.[17] Therefore, we amplified DNA of 16S rRNA encoding gene for the rapid and accurate identification of EBIs.

Soil bacteria such as *Bacillus spp*., *Pseudomonas spp*. and *Azospirillum spp*. are commonly associated with plants as endophytes. But, we did not find any *Azospirillum spp*. in 50 isolates. Perhaps, the growth medium used might be directly affecting the number and type of EB that can be isolated from the plant tissues. It has been reported that a seasonal fluctuation of the endophytes does occur in plants;[18,19] hence, it is likely that various other types of EB might be colonizing the *L. camara*. We have used 4 different types of tissues (leaf, petiole, stem and fruit) from different plants of *L. camara*; and diverse types of EB were found in analysed 50 EBIs. Most recently, it has been reported that soil type is a major factor that determines the diversity of EB in plants.[20] Plants are also known to harbour endophytic fungi; and most probably, *L. camara* might be harbouring some unique entophytic fungal community like other medicinal plants.[21]

The EB can produce the same metabolites as their hosts;[9] and therefore, EB may serve as one of the potential sources of the natural products and novel antibiotics. The antibacterial, antifungal and antiviral activities of some EB are reported by other researchers.[22,23,24,25,26,27,28] The EB from plants with antimicrobial activities are likely to serve as the potential candidates that may produce novel antibiotics and could help in combating drug resistant microorganisms and pathogens.[29,30,31]

Based on the results, we conclude that *L. camara* does contain diverse types of cultivable EB. This study is the first of its kind to report the endophytic bacterial community associated with *L. camara*. However, the benefits derived by *L. camara* from these EB are not clearly understood yet. We hypothesize that in *L. camara*, these EB might be playing an important role in producing medicinally important bioactive compounds. Nevertheless, our research findings could serve as foundation for further research on *L. camara* and role of its EB in producing therapeutic compounds.

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