Isolation of Indole Acetic Acid Producing Endophytic Bacteria from *Lantana Camara*, An Invasive Weed

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Abstract : "Endophytic bacteria are microorganisms that live within plant tissue without causing harm to the host. They produce a variety of natural products, including indole acetic acid (IAA), a plant growth regulator. The current study isolated endophytic bacteria from Lantana camara, an invasive weed, and evaluated their ability to produce IAA. A total of twenty-three bacterial strains were isolated, twelve from the root, eight from the leaves, and three from the stem of the plant. Four isolates, designated as LCL1, LCL4, LCR7, and LCR9, showed positive results for IAA production. Maximum IAA production (43.81 ± 0.12 μg/ml) was obtained from LCR7. The identification of the isolates by 16srRNA gene sequencing is still ongoing. However, the results of this study suggest that endophytic bacteria from Lantana camara have the potential to produce IAA, which could be used for a variety of applications in biotechnology.
Key Words : Bacterial Endophyte, Biochemical characterization, IAA production, *Lantana camara*, Morphological characterization

Received Date : 01/05/2023 Revised Date : 19/05/2023 Acceptance Date : 24/05/2023

1. Introduction

Endophytes and plant growth-promoting rhizobacteria (PGPR) are gaining popularity as supplements or alternatives to the use of pesticides to boost crop yield in agriculture. These bacteria have been demonstrated to benefit plants in a variety of ways, including :

- Boosting growth and yield
- Increasing stress tolerance
- Promoting disease resistance

The use of endophytes and PGPR in agriculture is still in its early stages, but there is a lot of promise for these bacteria to help improve crop productivity and sustainability (1). Endophytes are microorganisms that survive and colonize internal tissues of host plants and do not cause visible harm. They may be transferred directly from parent to progeny through seeds or plant to plant by entering the plant tissue through root zone or aerial portions such as flower stems and cotyledons(2). Lantana camara is a flowering plant that is native to the Americas and the Caribbean. It is a member of the Verbenaceae family and is known for its attractive flowers. Lantana camara can grow in a variety of climates, but it thrives in the humid heat of the tropics and subtropics. Lantana camara is an invasive plant, meaning that it spreads rapidly and can displace native plants. It is listed as a top ten global noxious weed, and it is a problem in many parts of the world, including India (3).

2. Materials And Methods

2.1. Collection of plant sample

Healthy plant samples of *Lantana camara*(Figure 1) were collected at the flowering stage from Vesu, region of Surat, Gujarat. At the flowering stage, the plants were gathered in a bag, brought to the laboratory, and gently cleaned under running water to remove any adhering dirt and debris.

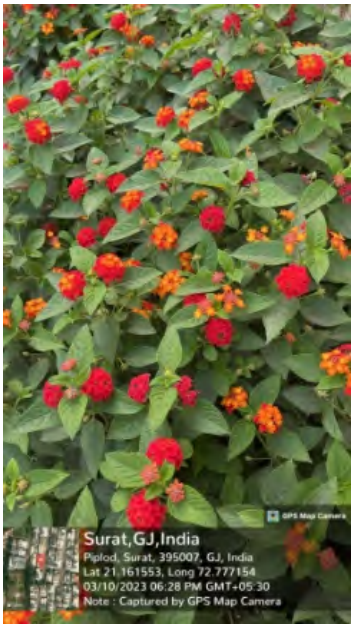

Figure 1 – Lantana Camara

2.2. Isolation of Endophytes

The flower and the leaves were divided into pieces that were two to three centimeters in length. After placing the parts in a beaker and soaking them in distilled water for a period of time, the beaker was afterwards drained of its contents. This piece of the leaf and flower was first sterilized on the surface with 0.1 % $HgCl_2$ for three minutes, after which it was disinfected with 70% ethanol for thirty seconds. After that, the tissue was sterilized by being washed multiple times in sterile water. Homogenizers were used to perform an aseptic maceration on portions that had been surface-disinfected. By adding 9 volumes of sterile distilled water, tissue that had been macerated was made into dilutions with a ratio of 10-1. A serial dilution was performed up to a factor of 10 - 6, and 0.1 milliliters of the appropriate dilution was spread out and plated on Nutrient Agar (4). Plates were then sealed with paraffin to prevent contamination and to ensure that the greatest number of endophyte colonies were recovered. The plates were then incubated at a temperature of 28 degrees Celsius, and observations were made between 24 and 48 hours afterward.

2.3. Estimation of IAA

The colorimetric approach developed by Gordon and Weber (5) was utilized for the purpose of performing a quick quantitative estimate of IAA in broth culture. The cultures were cultivated in the dark for seven days, and daily samples were taken during the process. After being centrifuged at 13,000 rpm for ten minutes, the supernatants were analyzed to determine how much IAA was produced. The amount of IAA that was present in each supernatant was evaluated using a colorimetric method after Salkowski's reagent was added. It is combined in a ratio of 2 :1, with two parts of 0.01 M $FeCl_3$ in 35% $HClO_4$ to one part of supernatant, and then the optical density is measured at 530 nm after 25 minutes. After reading off the recorded absorbance, a standard curve was created from pure IAA (Hi-Media), and the amount of IAA was measured with the help of the IAA standard curve.

2.4. Morphological characterization

Following the purification process of the colony, a comprehensive analysis was conducted on each individual isolate in order to ascertain its morphological resemblances to other colonies. The observed similarities encompassed various characteristics of the colonies, such as their size, shape, margin, elevation, pigmentation, and opacity. Following the established guidelines for standard microbiological testing, the Gram staining procedure was employed to assess the characteristics of each individual isolate. This involved the application of crystal violet and safranin staining techniques (6).

2.5. Metabolic activities of the isolates

In order to measure the metabolic activity of bacteria, a number of the regular biochemical tests were carried out. These tests were carried out in accordance with a standard technique (7), and the names of the biochemical assays are as follows : Uses of carbohydrates as well as organic acids The Methyl-Red (M-R) test, the Voges-Proskauer (V-P) test, and the Citrate Utilization Test were all utilized during the testing process. The indole synthesis test, the urea hydrolysis test, and the nitrate reduction test were used in the carrying out of the examination of the utilization of nitrogenous compounds. The Catalase test was carried out so that a variety of other tests might be identified. The triple sugar iron agar test was performed as part of an effort to identify integrated tests by making use of composite test media.

3. Results and Discussions

A total of 23 endophytic bacterial isolates were obtained from *Lantana camara.* Twelve from the root, eight from the leaves, and three from the stem of the plant. These isolates were further screened for IAA production.

Plant part	Isolates	Number of isolates
Roots	LCR1, LCR2, LCR3, LCR4, LCR5, LCR6, LCR7, LCR8, LCR9, LCR10, LCR11, LCR12	12
Leaves	LCL1, LCL2, LCL3, LCL4, LCL5, LCL6, LCL7, LCL8	08
Stem	LCS1, LCS2, LCS3	03
Total		23

Table 1 – Distribution of bacterial endophytes obtained from different parts of the plant *Lantana camara*

3.1. IAA production :

Only four of the twenty-three endophytic bacterial isolates exhibited positive results for IAA synthesis by forming a pink-colored ring. These isolates were the only ones to produce this outcome. Using the Salkowsaki reagent, a spectrophotometric analysis was carried out in order to obtain a quantitative determination of IAA at 530 nm. The highest IAA production (43.81±0.12µg/ml) was observed in endophytic isolate LCR7 (Figure 2, Table 2). The least IAA production (30.12±0.41 μg/ml) was recorded from isolate LCR9(Figure 2, Table 2). Comparable results of IAA production in a range from 10.96 to 37.78 μg/ml by plant growth-promoting rhizobacteria (PGPR) isolates were reported by previous work7. Another study8 reported IAA production in a range from 32.56 μg/ml to 56.12 μg/ml by PGPR isolates in the presence of L-tryptophan.

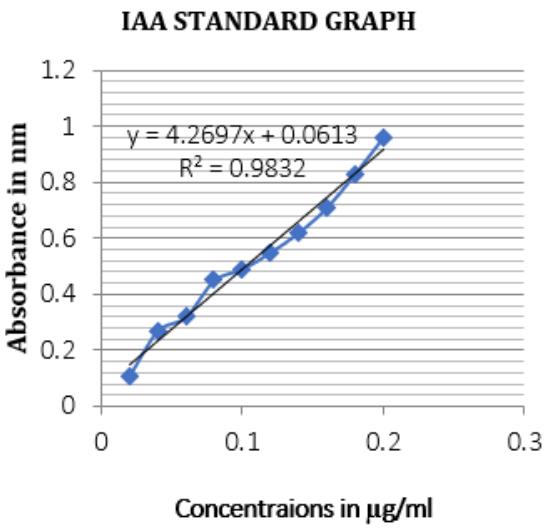

Figure 2 – Standard graph of IAA

Isolates	Concentration μg/ml
LCL1	37.59±0.23
LCL4	34.48 ± 0.37
LCR7	43.81 ± 0.12
LCR9	30.12 ± 0.41

Table 2 – IAA production by the bacterial endophytes

3.2. Morphological & Biochemical characterization

Characterization of the isolates in terms of their morphology and their biochemistry may be found in Tables 3 and 4, respectively. The morphological analysis of the IAA-producing isolates revealed that Gram-negative bacteria, which made up 55% of the total, were more prevalent in the root of the plants than Gram-positive bacteria, which made up 45% of the total. These findings are consistent with those that were established in other papers (9).

Morphological features	Isolates			
	LCL1	LCL4	LCR7	LCR9
Size	Medium	Small	Small	Small
Shape	Round	Round	Round	Round
Margin	Entire	Entire	Entire	Entire
Elevation	Flat	Raised	Convex	Flat
Pigmentation	White	Yellow	White	Yellow
Opacity	Translucent	Opaque	Transparent	Opaque
Gram's Reaction	+ve	-ve	+ve	-ve

Table 3 – Morphological traits of the isolates

Test	Isolates			
	LCL1	LCL4	LCR7	LCR9
M-r test	-	-	-	-
V-p test	-	+	-	+
Citrate utilization	-	-	+	+
Urea hydrolysis	-	-	+	-
TSI	+	+	+	+
Catalase	+	+	+	+
H2s production	-	-	-	-
Nitrate reduction	+	+	+	-

Table 4 – Biochemical traits of the isolates

4. Conclusions

IAA is a commercially valuable product having widespread application in agriculture and horticulture sector. IAA is naturally produced by microorganisms, mostly fungi, and bacteria. In the the current study, IAA was found to be produced by four endophytic bacterial isolates designated as LCR7, LCR9,LC1, and LCL4 isolated from root and leaf of the invasive weed *Lantana camara*.Further the study is required for the identification of the IAA producing strains and efficiency of the IAA produced under field conditions.

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