



STUDIES ON ISOLATION AND CHARACTERIZATION OF PINK *CATHARANTHUS ROSEUS* ENDOPHYTES FOR THEIR BIOCHEMICAL ACTIVITIES

Mr. Vidyadhar Tupe

Department of Life science, Ramnarain Ruia College, affiliated to the University of Mumbai, Matunga, Mumbai, Maharashtra, India

Abstract

Total 4 different endophytes were isolated from *Catharanthus roseus* (pink) plant; two from Leaf, two from Stem. From the leaves and stem the endophytic bacterial isolates were obtained mainly of bacillus sp. Isolation of bacterial endophytes is done on Nutrient agar and Luria Bertanii agar. Culturing of endophytic bacteria, detection of different biochemical activities and decomposition of biopolymers activities etc. were carried out. Recently this plant has been studied to improve the content of terpenoid indole alkaloids like serpentine, ajmalacine, vindoline and vinblastine. The angiosperms or flowering plants are all plants with flowers and fruit and are the foremost recently evolved of all plant groups. This study aims to study the biochemical activities of endophytic bacteria from *Catharanthus roseus* (pink) angiosperm medicinal plants. Plant was selected to isolate and characterize diversity of activities of endophytic bacteria of *vinka rosea*. The biochemical activities were characterized using culture dependant methods.

Key words: Isolates, Endophytes, Biopolymers, biopolymers.

Introduction

Catharanthus roseus, Family -Apocynaceae is an important anngiosperm medicinal plant and commonly known as Madagascar periwinkle because it has its origin from Madagascar. In India, it is commonly found in gardens and in warm conditions, it is found all over the world. It synthesizes useful secondary metabolites like-vendolin, vincristine and vinblastine, which were prevalently used in the treatment of cancer. Endophytes, the microbes that colonize the internal tissues of the plants without causing any overt negative effects, could thus be the potential sources of these valuable bioactive metabolites [1]. The endophytes are different types of microorganisms due to the fact that the vascular plants are examined up till now have been found to colonize the endophytic microorganisms [2], [3]. Due to varied ecological niches of *Catharanthus roseus*, there may be the variety of endophytic bacteria localized inside the plant tissues. Endophytes isolated from *Catharanthus roseus* (pink) not only yield these valuable therapeutic molecules but have recently been studied to improve the in planta content of terpenoid indole alkaloids like serpentine, ajmalacine, vindoline and vinblastine which has great influence in medicins

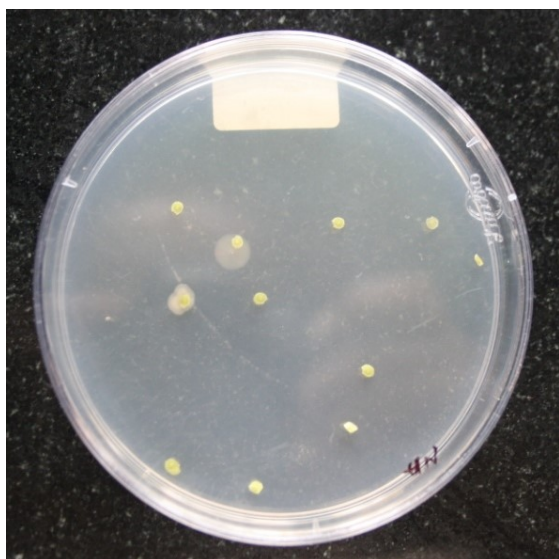


Fig.1. Inoculation thin cross section of plant leaf on nutrient agar plate for isolation of endophytes



Leaf section Inoculation

Fig.2 Inoculation thin cross section of plant stem on nutrient agar plate for isolation of endophytes



Stem section Inoculation

Materials and Methods

A) Collection of Plant Sample:

Pink *Vinca rosea*, *Catharanthus roseus* (pink) plant parts were collected from Panvel, location in Raigad district, Maharashtra, India in the sterile polythene bag and used for the further study within four hours.



B) Surface sterilization of plant material:

The healthy parts of plant was washed with running tap water then surface sterilized with 0.1 % teepol, and rinsed with sterile distilled water until the residues of teepol removes. Then the plant leaf and stem was cut with 70% sterilized scalpel .This explants were treated with 70 % alcohol for 5 mins followed by mercuric chloride (0.5%,and 1%) for leaf and Stem respectively for 5 minutes, and finally rinsed with sterile distilled water for four times to remove the traces of chemical surface sterilants and used for further study of isolation .

B) Isolation of endophytic bacteria:

The leaf portion and stem is used to take thin cross sections in the laminar air flow unit and these sections are placed on Sterile Nutrient agar and Luria Bertanii agar plates, incubated at 28⁰c for 2 day's .The bacterial growth was observed on the culture medium after incubation. After isolation well isolated colonies were used for further studies

C) Morphological characteristics of isolated four endophytic bacterial isolates:

Isolates are characterized on the basis of its Macroscopic and Microscopic characteristics. Total 4 different isolates obtained are studied with its different characteristics is given in table 1.

Table 1: Morphological characteristics of the isolated bacterial endophytes.

Characteristics used	Isolates			
	1 (leaf)	2 (leaf)	3 (Stem)	4 (Stem)
Colour	Whitish cream	cream	White	Yellowish cream
Size(mm)	2	2.7	2.5	3
Shape	Circular	Circular	Circular	Circular
Margin	Regular	Regular	Regular	Regular
Elevation	Convex	Convex	Flat	Convex
Consistency	Moist	Moist	Moist	Moist
Opacity	Opaque	Opaque	Semi translucent	Opaque
Gram's reaction	+ve bacilli single	+ve long bacilli	+ve bacilli	+ve bacilli spidle shape
Motility	+ve	+ve	+ve	+ve
Endospore (position)	Sub terminal	Sub terminal	Sub terminal	Sub terminal
Capsule	+ve	+ve	+ve	+ve



D) Decomposition of biopolymers activities:

1. Determination by Casease activity:

Cultures were spot inoculated on St. Milk agar plat and incubated at 28⁰c. After incubation zone of clearance around the spot indicates positive activity and No zone of clearance is negative activity

2. Determination of decomposition of biopolymers by alpha amylase activity:

After incubation of cultures on st. Starch agar plate, plate was treated with Iodine solution and drained excess amounts of iodine and observations are made within 10 mins. Transparent zone around the spot of growth indicates positive results and no transparent zone is negative activity

3. Determination of decomposition of biopolymers by Lipolytic activity:

By using St. Goradkova's agar plate cultures are spot inoculated and after incubation plates were observed for zone of clearance around growth showed positive results for Lipolytic activity and no zone of clearance is negative activity was observed

E) Determination of Bile-esculin activity:

Bile-esculin test is basically used to differentiate Enterococci and nonenterococcus group of D-streptococci, which are bile tolerating and can hydrolyzes the esculin to esculetin, from non-group D viridans group of streptococci, which grow poorly on bile.

Results of other activities are given in the table2

Table 2: The activities of decomposition of biopolymers by isolated bacterial endophytes:-

Activity Checked	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Casease activity	-	+	+	-
Alpha amylase activity	+	+	+	+
Lipolytic	+ve	+ve	+ve	-ve
DNase	+ve	No growth	No growth	-ve
Esculin hydrolysis	+ve (nonenterococcus which are bile tolerant)	No growth (Bile-Inhibits most G+ve bact.)	+ve (nonenterococcus which are bile tolerant)	+ve (nonenterococcus which are bile tolerant)
Phosphatase	-ve	-ve	No growth	-ve

Key: (+ve) positive activity (-ve) negative activity



F) Determination of Biochemical activities

Results and Discussion

Isolation and characterization of endophytes for their biochemical activities:

Total 4 different endophytes were isolated from *Catharanthus roseus* (pink) plant; two from Leaf, two from Stem. Table 1 shows the number of isolates, isolated from Leaf, Stem of pink *Catharanthus roseus* plants and Morphological characteristics of the isolated bacterial endophytes. The decomposition of biopolymers by isolated bacterial endophytes, i.e. Casease activity, Alpha amylase activity, Lipolytic etc. along with biochemical activities listed in Table 2 were analyzed in endophytic bacteria of *Catharanthus roseus* (pink). All the endophytic bacteria of *Catharanthus roseus* (pink) showed the presence of these different activities. Endophytic bacteria are found in virtually every plant on earth [4]. Different plant parts such as root, stem and nodule [5], leaves, stems and root [6] can also be used for isolation of endophytic bacteria.

The endophytic bacterial isolates indicate that the plant *Vinca rosea* is enriched with

Activity Checked	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Hugh-Liefson's test	fermentative	Non Saccharolytic	Non Saccharolytic	Non Saccharolytic
Lipolytic activity	+	+	+	-
Phosphatase activity	-	-	-	-
Esculine activity	+	-	+	+
Effect of Temperature on growth	Optimum growth (Room Temp)	Optimum growth (Room Temp)	Optimum growth (Room Temp)	Optimum growth (Room Temp)
Effect of heavy metal on growth	-	+	+	+

various bacterial populations. Endophytes are reported as novel source of bioactive compounds. [7] The identification of bacterial isolates was done based on different morphological characteristics. The colony characteristics of endophytic bacteria isolated from *Catharanthus roseus* are having irregular in shape while flat elevation on Petri plate, margin of the colonies were undulated; the surface of the growth was rough, opaque and white in color. The microscopic examination of endophytic bacteria has seen through compound microscope

Conclusion

Catharanthus roseus (pink) is an important medicinal plant with a wide range of uses. The leaves of this plant have been used extensively in medicines. The pharmacological activities



of this plant are attributed to its leaves. Endophytes could thus be exploited as the sources of the valuable secondary metabolites of medicinal, agricultural and industrial importance. The research findings of this study could be used as fundamentals for further studies of this plant to determine the production of any new secondary metabolites with its therapeutic importance. Owing to the huge microbial biodiversity of endophytes, bacterial endophytes are still the less investigated group of microorganisms that need to be explored for their huge potential being used as the source of pharmacologically active therapeutic compounds.

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