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Research Paper

Isolation and study of endophytes from leaves of *Ficus racemosa L.*

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Abstract

Endophytes are endosymbionts residing in the internal tissues of host plants without causing any apparent damage or harm to the host. They have been found in virtually every plant studied so far. Microbial endophytes are known to produce a wide range of bioactive compounds which may actually contribute to host plant health such as phyto-hormones or by increasing resistance against the plant pathogens and parasites. Considering that only a few endophytes have been studied, they constitute a poorly investigated group of microorganisms with abundant potential as a source of biologically important and chemically novel compounds with significant pharmaceutical and agricultural applications. *Ficus racemosa* L. is a moderate sized avenue tree found throughout India either wild or cultivated. It has been largely used in indigenous medicinal systems. Phytochemical analysis of this plant has revealed the presence of various medicinally active compounds which may be of endophytic origin. These may be exploited for the large scale production of various associated bioactive compounds. The current study deals with the isolation and study of bacterial endophytes from leaves and nodules, of *Ficus racemosa*. Further, the isolates were screened for production of phenolic compounds. The total phenolic content was estimated by performing a spectrophotometric assay.

Keywords: *Ficus racemosa L*., bioactive compounds, bacterial endophytes, phenolics.

Introduction

Living beings are often found in association. There have been many examples of symbiotic associations between two different kinds of living organisms. In one such kind of symbiotic association, microorganisms, viz., bacteria and fungi are associated with plants. The microorganisms in this association are known as endophytes. “Endo” means inside and “phyta” means plant.

Endophytes are the organisms which reside in the tissues of the host plants without causing any apparent symptoms or harm to the host plant. Both fungi and bacteria are the most common microbes existing as endophytes. Endophytes favour the host plant directly or indirectly by producing some compounds. They produce some secondary metabolites which might be bioactive and hence have a particular effect in the host plant. These bioactive compounds could have a variety of functions, ranging from growth promotion to disease resistance. The research on endophytes has yielded novel antibiotics, anti-cancer compounds, plant growth hormones, stress tolerance factors, resistance to plant pathogenic agents, etc in different plants[1].

The study of endophytic bacteria has evoked great interest due to their beneficial properties like fixing nitrogen, producing auxins and protecting the host plants from plant pathogens. Endophytic bacteria have been isolated from many plants like maize, rice, wheat, sugarcane, marigold, coffee, potatoes, tomatoes, lettuce, fodder plants like alfalfa, sorghum, and trees like yew and pine.[2]

Plants constitute a major component of the medication offered by almost all traditional medicinal systems. Thus, we have examined certain medicinally important plants with a view to discover and develop potential remedies for the ailments [1]. Earlier study of medicinally important plants has revealed that a large number of compounds contributing to their medicinal properties are in fact produced by the endophytes residing in the plants [3].

The plants with ethnobotanical [4] importance are the ones which are more likely to harbour endophytes capable of producing pharmaceutically important bioactive compounds. It is highly possible that various unique bioactive compounds associated with the host tree are in reality being produced by the residing endophytes. Thus the isolated endophytes could be cultured and used for commercial production of the bioactive compounds [5].

The endophytes, thus provide us with eco-friendly drugs which could be harvested directly from the fermentation cultures rather than from host trees which has often lead to deforestation. With the world battling the green house effect and unnatural temperature fluctuations, and only 10-15% of the rainforests remaining, endophytes are boon waiting to be discovered [6].

Some of the earlier workers have uncovered a number of bacterial and fungal endophytes producing bioactive compounds with potential pharmaceutical applications. A few are listed in the following table no.1:[6,7,8]

Table 1: Endophytic compounds and their sources

S. No.	Endophyte	Source	Compounds produced by endophytes	Application
1	*Pestalotiopsis microspora*	*Torreya taxifolia*	Pestaloside, aromatic β glucosides, pestalopyrone and hydroxypestalopyrone	Antifungal agents
2	*Pseudomonas viridiflava*	leaves of many grass species	Ecomycins	Antimycotic agent
3	*Serratia marcescens*	*Rhyncholacis penicillata*	oocydin A	antioomycetous compound
4	*Taxomyces andreanae*	*Taxus brevifolia*	Paclitaxel	Anticancer agent
5	*Streptomyces sp. strain NRRL 30562*	*Kennedia nigricans*	Munumbicins	Anti malarial and antibiotic agents
6	*Nodulisporium sp.*	*Bontia daphnoides*	nodulisporic compounds	Insecticidal compounds

Ficus racemosa L., (family: Moraceae) was selected for the current study because of its medicinal value as reported in numerous treatise on Indian traditional medicines. This plant is a large deciduous tree found all over India from outer Himalayan ranges to south India. It grows throughout the year in moist localities and on the banks of rivers and streams. It is often cultivated for its edible fruits- figs, which are red when ripe and grow in clusters on leafless branches.

Hence this tree is also called cluster fig tree in English, Gular in Hindi and Udumbara in Sanskrit. The tree grows up to a height of 18m and its leaves are ovate, ovate-lanceolate or elliptic, sub acute, entire and petiolate. This tree has been used in traditional systems of Indian medicine for treating various ailments such as dysentery, hiccoughs, asthma, diabetes, epididymis, cancer, scabies, myalgia, haemoptysis, intrinsic haemorrhage, etc[9]

Pharmacological analysis of the plant extracts suggests various additional properties including antioxidant, antitussive, analgesic, antihelminthic, antifilarial, hepato-protective, antibiotic, renal anitcarcinogenic, antidiuretic and also antifungal. A phytochemical analysis of the plant has revealed that it is a rich source of bioactive compounds which explains the variety of pharmacological activities shown by plants. The bark, leaves and fruits show presence of various bioactive compounds including Stigmasterol, β-sitosterol, gluanol acetate, ceryl behenate, lupeol acetate, racemosic acid, etc. The phytochemical profile of this plant being so rich, it has been widely used in the traditional medicinal systems.[10]

Earlier, this plant has been successfully examined for the presence of endophytes which might be responsible for production of one or more of the above mentioned compounds. The possibility of detecting some other bioactive compounds produced by the endophytes also cannot be ruled out. A fungal endophyte has been previously isolated from the plant. However, no significant bioactive property was attributed to it during the study.[11] There has been no report of any other work related to endophytes isolated from *Ficus racemosa*.

Materials and Methods

The leaves of the plant *Ficus racemosa L.* were collected from Thane district (19.2000° N, 72.9700° E). The leaves were cleaned and washed with tap water and were then further analysed. The leaves were sterilised and potential bacterial endophytes were isolated from them as described below.

Isolation of endophytes:

The endophytes were isolated according the method of Ali M. et. al.[2] The procedure is as follows:

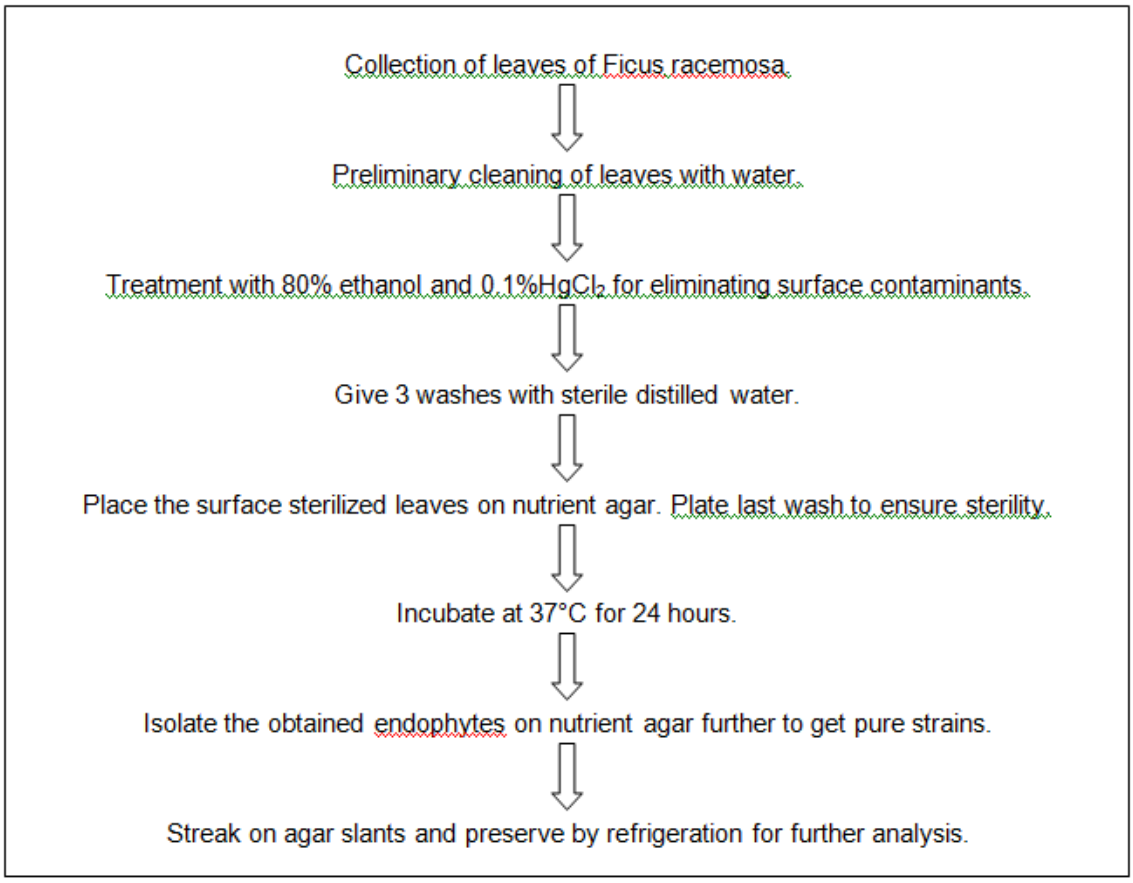

Figure 1: Flowchart for isolation of endophytes

The endophytes were isolated on nutrient agar and were picked from the plate just after 24 hours of incubation. The plates were further incubated for 48 hours to allow growth of fungal endophytes if any. There was no additional growth obtained even after the extended incubation. The isolates are further confirmed to be bacterial by microscopic examination.

These endophytic bacterial isolates were gram stained and subjected to biochemical identification. The isolates were grown in liquid media and were tested for production of phenolic compounds by a lead acetate qualitative test. When 10% lead acetate was added to the supernatant of centrifuged culture media, white precipitate was obtained confirming the presence of phenolic compounds [12]. The phenolic content was estimated using a spectrophotometric assay performed by the Jing Chung Chen method in triplicates. The spectrometric readings were recorded and average total content was calculated [13].

Results and Discussion

Bacterial growth was obtained along the periphery of the surface sterilised leaves. Different colonies were further isolated to obtain pure strains. The last wash that was plated showed no growth, thus it is only the endophytes that were isolated on the test plates. The endophytes isolated from the leaves and nodules on the leaves of *Ficus racemosa* were preserved on a nutrient agar slant for further study.

The cell wall nature of the endophytic strains was determined by performing gram staining procedure on the isolates and the relative frequency for each endophyte was calculated. The results are tabulated below. (Figure 3,4, 5, 6 and 7).

A positive lead acetate qualitative test confirmed the presence of phenolic compounds. The spectrometric assay was performed to estimate the production of phenolic compounds. The assay was performed in triplicates and the average obtained was reported. The results are displayed in table no.3. The isolates showed significant production of phenolic compounds which could be identified further by different analytical procedures.

Table 3: Morphology and relative frequency of isolated endophytes

Isolates	Gram nature	Morphology	Frequency	Relative Frequency (%)
1	Gram positive	Rods in chains and singles	9	52.94
2	Gram positive	Rods in chain	3	17.65
3	Gram positive	Cocci in clusters	2	11.76
4	Gram positive	Short rods	2	11.76
5	Gram positive	Coccobacilli in clusters	1	5.89
Total			17	100

Table 2: Phenolic production by endophytes

Strains	Phenolic content (µg/ml)
1	14.03
2	19.8
3	15.23
4	15.25
5	18.35

Figure 2: Endophytic growth along the leaf on nutrient medium

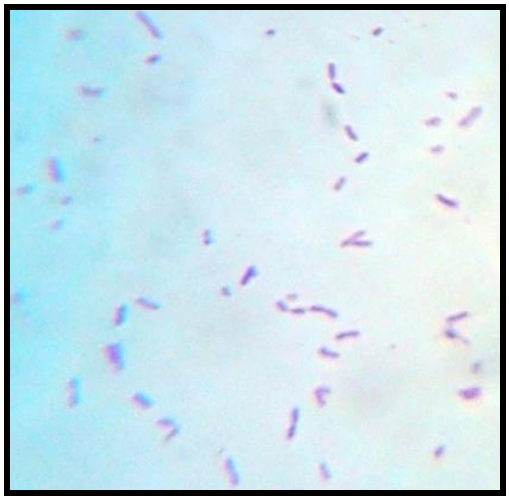

Figure 3: Strain1 showing gram positive rods

Figure 4: Strain 2 showing gram positive rods in chains and single

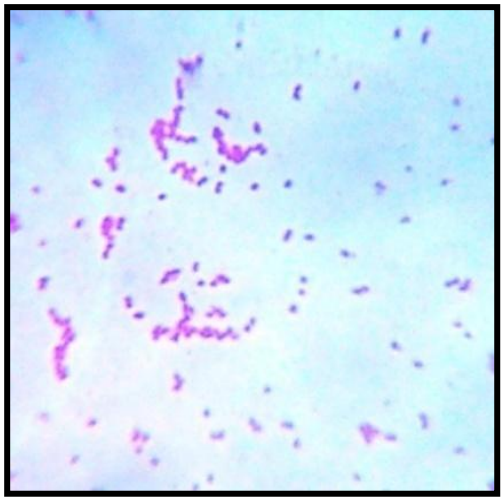

Figure 5: Strain 3 showing gram positive cocci in clusters

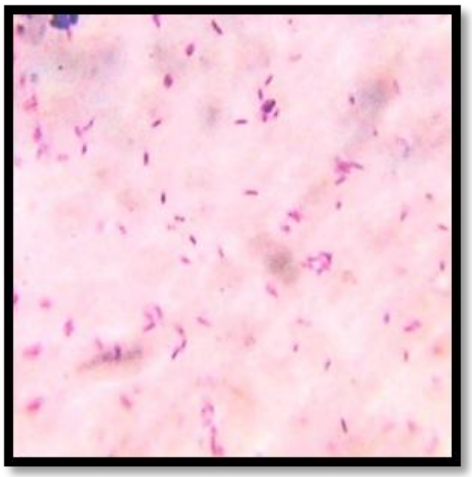

Figure 6: Isolate 4 showing gram positive short rods

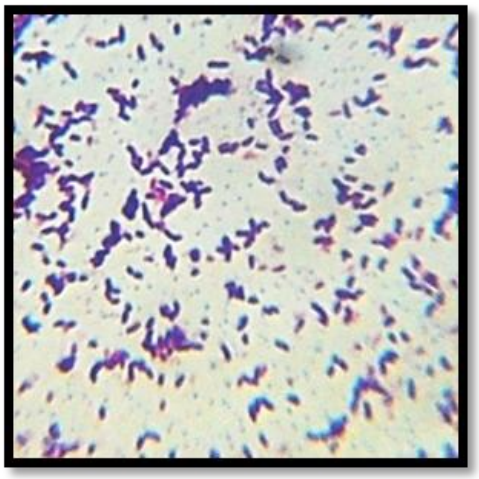

Figure 7: Isolate 5 showing gram positive cocobacilli in clusters

Conclusion

This study demonstrates the occurrence of culturable bacterial endophytes in the plant under study. The presence of bacterial endophytes in the leaf tissues has uncovered a new arena which remains largely unexploited. The endophytic isolates also showed production of some compounds which if further identified can be used for future probable applications. Many such medicinal plants associated with endophytes are an untapped source of novel bioactive compounds which could have significant industrial, pharmaceutical and agricultural applications.

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