Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2015 Vol. 5 (4) October-December, pp.6-10/Kumari et al.
Research Article

ISOLATION OF ENDOPHYTIC MICROORGANISM FROM *KIGELIA PINNATA* (JACQ.) DC AND *BARLERIA PRIONITIS* LINN.

Purnima Kumari, Sandeep Tripathi and *Sandeep Kumar
Department of Biotechnology, NIET, NIMS University Jaipur, Rajasthan, India
**Author for Correspondence*

ABSTRACT
The present study was done to isolate the endophytic microorganisms from two selected ethno-medicinal plants *Kigelia pinnata* (Bignoniaceae) and *Barleria prionitis* (Acanthaceae). These are ethno-pharmacologically important plant species used in traditional medicine to treat the microbial infection. A total of 43 endophytic fungi, 58 endophytic bacteria from *K pinnata* and 26 endophytic fungi, 34 endophytic bacterial from *B. prionitis* isolates were obtained from different symptomless tissue fragments of bark, leaves, and fruits of *K. pinnata* and from stem and leaves of *B. prionitis* respectively.

Keywords: *Endophytes, Kigelia Pinnata, Barleria Prionitis, Traditional Medicine, Colonization Frequency*

INTRODUCTION
Endophytes are the microorganisms which are associated with plants and live within the living tissues of their host plants without causing any harm to them (Jena and Tayung, 2013). Almost all groups of endophytes have been found in association with plants it may be bacteria, fungi or actinomycetes. They stimulate the production of secondary metabolites which are also used as bioactive compounds. Some of the compounds can be exploited for human health and welfare. Some of the endophytes can produce the similar secondary metabolites as that of the plant thus making them a promising source of novel compounds.

Ethno-medicinal plants provide valuable therapeutic agents in traditional medicines which are used for a wide variety of human health issues. These are gaining global attention due to the fact that the herbal drugs are money-making, easily available and with negligible side effects. World Health Organization reported over 80% of the world's population or 4.3 billion people rely upon such traditional herbal medicine to provide them with primary health care (Bannerman *et al*., 1983). On the other hand, indiscriminate exploitation of this plant resource has rapidly declined their population making some of them critically endangered. It is important to note that homeopathy and modern medicine have their bases in medicinal plants. But now it is known that medicinal plants harbour some specific fungal endophytes which are allied with the production of pharmaceutical yield (Zhang *et al*., 2006).

Kigelia pinnata and *Barleria prionitis* are two traditionally important ethanomedicinal plants. *Kigelia pinnata* (Jacq.) DC is the indigenous medicinal plant belongs to family Bignoniaceae. It has more potent inhibition for carrageenan induced paw edema in rat and possess higher anti-inflammatory activity (Kumari *et al*., 2012). Indian traditional healers have used various parts of this plant to treat a wide range of skin ailments, from relatively mild complaints, for example fungal infections, psoriasis, and boils to the more serious diseases like syphilis, leprosy, and skin cancer. Other herbal applications include the treatment of malaria, dysentery, ringworm, post-partum hemorrhaging, tapeworm, diabetes, pneumonia etc.

Barleria prionitis belonging to the family Acanthaceae, is a gifted medicinal herb which mentioned in earliest ayurvedic literature having great economic potential. The plant is native to the Indian subcontinent which is reported to contain a major chemical constituent like alkaloids, carbohydrates, flavonoids, terpenoids, lignin and glycosides (Kumari *et al*., 2013). In traditional medicine, *Barleria* is used mainly for the treatment of tooth ache, dental caries (Kumari *et al*., 2013), catarrhal affections, inflammations, boils, urinary infection, gastrointestinal disorders, jaundice, fever, cough, bronchial asthma, whooping cough and glandular swellings (Khare, 2008). Many essential phytochemicals such as

primary and secondary metabolites like alkaloids, flavonoids, steroids, saponins, tannins, carbohydrate, glycosides/cardiac glycoside and phenolics compounds are isolated from the whole plant including leaf, stem and root which have antitumor, antibacterial, anti-diabetic, anti-inflammatory, antioxidant, antipyretic and hepato-protective activity.

The endeavor of the study was to isolate the endophytic microorganisms from these ethano-medicinal plants.

MATERIALS AND METHODS

Sterilization of Selected Plant Parts

Plant samples were treated by triple surface sterilization technique (Bussaban *et al*., 2001). Along with this technique the plant parts or tissues were washed in running tap water for one hour. Each sample of plant was cut into small pieces. Bark, leaves and fruits of *Kigelia pinnata* and stems and leaves of *Barleria prionitis* were cut into 5 mm pieces. Final sterilization of explant was performed individually using 0.1% $HgCl_2$ for 3 min and rinsed thoroughly with sterile distilled water inside laminar hood.

Inoculation of Selected Plant Parts Segments

Each surface sterilized plant samples segment was crushed with the help of motor pestle then squeezed out their sap. The sample was diluted four times and then placed on PDA for endophytic fungi and NA media for endophytic bacterial isolation. After incubation, the fungal and bacterial colonies are isolated and then performed using tenfold serial dilution and spread on potato dextrose agar media with streptomycin 100 mg/l and nutrient agar media with Clotrimazole 50mg/l and purified by several streaking and incubation at 26 ± 2°C for fungus and 37^0C for bacteria for 24 hours (Oses *et al*., 2008; Rungjindamai *et al*., 2008; Theantana *et al*., 2009).

Isolation of Endophytic Microorganisms

After incubation of serially diluted sap samples, individual endophytic colonies were picked from the edge of an advancing colony with a sterile fine tipped needle under binocular microscope and transferred endophytic fungi onto potato dextrose agar (PDA) and endophytic bacteria onto nutrient agar (NA) and also stored separately at 4^0C.

Identification of Endophytic Microorganisms

Fungal isolates were identified by the study of colony and hyphal morphology of the fungal cultures and characteristics of the spores (Ellis, 1971; Barnett and Hunter, 1972) under observation of microscope. Bacterial isolates were characterized on the basis of morphological analysis, Gram's reaction and biochemical tests. Biochemical tests such as Catalase, Indole test, Methyl-red, Voges Proskauer tests, TSI, gas production, Citrate utilization tests and Lactic acid test were performed.

RESULTS AND DISCUSSION

The presence of endophytic fungi in plant tissues was discovered more than 75 years ago when Sampson (1935) reported such fungi from Lolium grass. Endophytes are known to be ubiquitous and many plant species examined to date have been found colonized with endophytes (Arnold *et al*., 2001). Endophytes are one of the major potential sources for new, useful metabolites (Dreyfuss and Chapela, 1994). There has been a great interest in endophytes as potential producers of novel, biologically active products (Schulz *et al*., 2002; Strobel and Daisy, 2003; Urairaj *et al*., 2003).

In the present study a total no. of endophytic fungi were isolated 23, 14 and 06 from bark, leaves and fruits respectively a total no. of bacteria were isolated 17, 21 and 20 from barks, leaves and fruits tissues of symptomless *Kigelia pinnata* respectively. In other hand, a total no. of endophytic fungi 27 from leaves and 09 from stem and a total no. of endophytic bacteria 14 from leaf and 20 from stem tissues of symptomless *Barleria prionitis*. It has been found that a single plant species may harbour hundreds of endophytes and may inhabit all available tissues, including leaves, petioles, stems, twigs, bark, xylem, roots, fruit, flowers, and seeds (Saikkonen *et al*., 1998; Chapela and Boddy, 1988; Fisher *et al*.,1993).

The endophytic fungal communities of *K. pinnata* and *B. prionitis* comprises of fungi belonging to genera *Aspergillus , Curvularia, Trichoderma, Alternaria, Rhizopus and some unidentified species* (Table 1). The

tissue of *K. pinnata* and *B. prionitis* were colonized with endophytic bacteria of the genera Flavobacterium, Staphylococcus, *Micrococcus, Bacillus, Pseudomonas, Enterobacter, Microbacterium* and some unidentified species (Table 2). Both the plant species showed colonization of similar endophytic fungi and endophytic bacteria but in different tissues.

Among the endophytic fungi *Aspergillus niger* showed highest colonization in both the plant tissues. Maximum endophytic fungi isolates of genus *Aspergillus* were obtained from bark segments followed by leaves and fruit tissues of *K. pinnata*.

Four species were successfully identified as *Aspergillus flavus, Aspergillus sp., Curvularia lunata* and *Cladosporium sp*. from different tissues of *K. pinnata* (Al-mahi *et al*., 2013).

The study showed that bark tissues were richer in *Aspergillus niger* than leaf and fruit tissues. The leaf of *K. pinnata* was with more isolates of *Aspergillus niger* and *Rhizopus oryzae* whereas *A. niger* is completely absent in its bark tissues (Maheswari and Rajagopal, 2013).

Maheswari1 and Rajagopal in 2013 was also reported that the endophytic fungal population is more prevalent during winter on bark and leaf tissues and the colonization frequency and isolation frequency were greater in the leaves than bark tissue of *K. pinnata*.

In case of *B. prionitis* maximum endophytic fungi of *Aspergillus niger* were also shown in leaf tissues than stem. This finding concurs with earlier reports that colonization of endophytic fungi is more prevalent in leaf than other tissues of *Morinda pubescence* (Suryanarayan *et al*., 1998; Rajagopal and Suryanarayanan, 2000). Endophytic fungi colonize very special and often very hostile habitats and were increasingly recognized as a group of organisms that are likely to be sources of new metabolites (Rajagopal, 2012).

The rich diversity of *Aspergillus* species as endophytes in different tissues of both the plants may be due to germination of more number of spores of these fungi due to favorable environmental condition such as humidity and precipitation which are normally high during monsoon season.

Seasonal variation plays a major role in endophyte harvesting where environmental conditions have the way for the symbiotic microbes to survive and explore; precipitation may be one of the major factors that influences the infection of endophytes. It has been reported that precipitation may influence the infection of endophytic fungi as well (Shashi *et al*., 2000).

In both the plants *Aspergillus niger* were dominant over other fungal strain. Several studies have shown that the frequency of colonization increases with age of the tissue colonized (Espinosa-Gracia and Langenheim, 1990; Rajagopal, 1998). Similarly, the endophyte community of a plant is affected by the quality of air (Petrini, 1991) and leaf chemistry (Rajagopal *et al.*, 2010).

The endophytic bacteria *Flavobacterium sp. and Microbacterium sp.* were found only in tissues of *K. pinnata*. *Enterobacter sp*. showed maximum colonization frequency in *K. pinnata* and its bark also contained maximum frequency and *Bacillus cereus* and *Bacillus pumilus* were only present in leaves and fruits of *K. pinnata*.

But in case of *B. prionitis Staphylococcus aureus, Micrococcus sp.* and *Pseudomonas fluorescens showed* same no. of colony but its leaves contained maximum isolates of *Staphylococcus aureus. Bacillus cereus* and *Bacillus pumilus* were only present in stem of *B. prionitis*. *Bacillus, Pseudomonas, Staphylococcus, Micrococcus* and *Acidomonas* bacterial species were isolated from surface sterilized leaf stem and root tissues of *Hygrophila spinosa* T. Anders of Acanthaceae family (Pal and Paul, 2013).

A diverse array of bacterial species have been reported to be endophytic including *Acetobacter, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Herbasspirillum and Pseudomonas* from the tissues of *Pongamia glabra* belonging to family Fabaceae (Jalgaonwala and Mahajan, 2011).

Maheswari and Rajagopal (2013) were reported that high colonization of endophytes in leaf tissue may be due to their anatomical structure and supply of nutrient elements on which the endophyte depends. More number of endophytic isolates in leaf tissues may be due to the fact that sampling were done in wet season and in many instances leaves sampled during the wet season harboured more endophytes than those screened during the dry season (Rodrigues, 1994; Wilson and Caroll, 1994).

Table 1: Isolation of endophytic fungi from *Kigelia pinnata* and *Barleria prionitis* on PDA media (CFU × 10^4/ ml of dilution)

	No. of colony				
	Kigelia pinnata			*Barleria prionitis*	
Endophytic fungi	**Bark**	**Leaf**	**Fruit**	**Leaf**	**Stem**
Aspergillus niger	4	3	1	3	2
Aspergillus flavus	2	1	1	1	1
Curvularia lunata	3	2	2	2	1
Trichoderma sp.	3	1	1	2	1
Alternaria alternate	2	2	1	1	-
Rhizopus oryzae	2	1	-	2	1
Unknown	7	4	-	6	3
Total no. of isolates recovered	**23**	**14**	**06**	**17**	**09**

Table 2: Isolation of endophytic bacteria from *Kigelia pinnata* and *Barleria prionitis* on NA media (CFU × 10^4/ ml of dilution)

Endophytic bacteria	No. of colony				
	Kigelia pinnata			*Barleria prionitis*	
	Bark	**Leaf**	**Fruit**	**Leaf**	**Stem**
Flavobacterium sp.	-	2	2	-	-
Staphylococcus aureus	1	2	-	3	2
Micrococcus sp.	2	1	2	2	3
Bacillus megatarium	1	2	3	2	3
Bacillus cereus	-	3	1	-	2
Bacillus pumilus	-	3	3	-	2
Pseudomonas fluorescens	2	2	3	2	2
Enterobacter sp.	4	3	3	1	2
Microbacterium sp.	2	-	-	-	-
Unknown	5	3	3	3	4
Total no. of isolates recovered	**17**	**21**	**20**	**14**	**20**

REFERENCES

Al-mahi Idris, Ietidal Al-tahir and Eihab Idris (2013). Antibacterial activity of endophytic fungi extracts from the medicinal plant, *Kigelia africana*. *Egyptian Academic Journal of Biological Sciences* **5**(1) 1-9.

Dreyfuss MM and Chapela IH (1994). Potential of fungi in discovery of novel low molecular weight pharmaceuticals. In: *The Discovery of Natural Products with Therapeutic Potential*, edited by Gullo VP (Butterworth-Heinemann) London, UK 49-80.

Fisher PJ and Petrini O (1987). Location of fungal endophytes in tissue of Suaeda fruticosa: A preliminary study. *Transactions of the British Mycological Society* **89** 246–249.

Jalgaonwala RE and Mahajan RT (2011). Isolation and Characterization of Endophytic Bacteria From roots of *pogamia glabra* vent. *International Journal of Pharma and Bio Sciences* **2**(1).

Jena SK and Tayung K (2013). Endophytic fungal communities associated with two ethno-medicinal plants of Similipal Biosphere Reserve, India and their antimicrobial prospective. *Journal of Applied Pharmaceutical Science* **3** 7-12.

Kumaresan V and Suryanarayanan TS (2002). Endophyte assemblages in young, mature and senescent of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove litter degradation. *Fungal Diversity* **9** 81–91.

Maheshwari S and Rajagopal K (2013). Biodiversity of endophytic fungi in *Kigelia pinnata* during two different Seasons. *Current Science* **104** 515-518.

Pal A and Paul AK (2013). Bacterial endophytes of the medicinal herb *Hygrophila spinosa* T. Anders and their antimicrobial activity. *British Journal of Pharmaceutical Research* **3**(4) 795-806.
Rajagopal K, Maheswari S and Kathiravan G (2012). Diversity of endophytic fungi in some tropical medicinal plants – A report. *African Journal of Microbiology Research* **6**(12) 2822-2827.
Rodrigues KF (1994). The foliar endophytes of the Amazonian palm *Eutrepe oleracea*. Mycologia. *Mycological society of America* **86** 376–385.
Sampson K (1935). The presence and absence of endophytic fungi in *Lolium temulentum* and *L. perenne. Transactions of the British Mycological Society* **19** 337-343.
Schutz B, Boyle C, Draeger S, Rommert AK and Krohn K (2002). Endophytic fungi; a source of novel biologically active secondary metabolites. *Mycological Research* **106** 996-1004.
Strobel GA and Daisy B (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* **67** 491-502.
Urairaj C, Khanongnuch C and Lumyong S (2003). Ligninolytic enzymes from tropical endophytic xylariaceae. *Fungal Divers* **13** 209-219.
Wilson D and Carroll GC (1994). Infection studies of *Discula quercina*, an endophyte of *Quercus garryana*. *Mycologia* **86** 635–647.