Isolation and Identification of an endophytic fungi associated with mangroves of Kutch, Gujarat, India

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Abstract: Mangroves is one of the chief ecosystem which thrives in tropical and subtropical intertidal zones. It plays an important role in safeguarding beaches and rivers from prefunding sea waves, air pollutants and storms. Since these plants can withstand high biotic and abiotic stress, they can be a source of novel biotechnologically useful products. Mangroves are considered as the most productive ecosystem harboring a variety of flora and fauna. It is a home to a huge number of unique fungal communities known as manglicolous fungi. In the current study mangroves of unexplored location of Kutch, Gujarat, India was investigated for the presence of endophytic fungi. Total 13 different fungi were isolated from various root samples. The fungi were identified based on morphological characteristics. Where, *Penicillium sp, Aspergillus sp. and Alternaria sp.* was the most commonly found genera.

Keywords: *Avicennia marina*, Endophytic fungi, Mangroves

1. Introduction: Mangroves are also known as halophytes, found in estuarine habitat, protecting shorelines and streams from harsh waves and storms. Mangrove vegetation covers 18 million hectors of coastline globally. It is said to be the most productive ecosystem [1]. In comparison to worldwide mangrove cover, overall mangrove cover in India is estimated to be at 2.7% [2]. Sundarbans has the greatest mangrove cover in India, whereas Gujarat, with a coastline of around 1650 km (and 1140 Sq.km of mangroves), has India's second biggest tidal forest [3]. The soil of the mangrove ecosystem has such unique characteristics that it favors the growth of microorganisms [4]. An endophytic microorganisms are the one which reside asymptomatically in the plant tissue without causing any harm to the host [5]. The extensive communication between endophytes and their host plants is however hidden and their roles towards those plants are fascinating. They assist their plants to grow healthy by creating growth hormones. They also participate in phytoremediation, biodegradation, and nutrient cycling, which reduces debris load in our environment [6]. Most of the mangrove fungi are marine and some of these are terrestrial in nature. Generally, mangrove roots, twigs or stems when submerged in water often houses marine fungus into it. While the terrestrial fungi are often found in the branches and upper section of the roots [7]. Mangroves have natural ability to withstand difficult environmental conditions like high salt concentration, anaerobic habitat, high temperature and strong wind velocity. Mangrove forests create a significant quantity of detritus in the form of leaf litter, twigs, bark, timber, inflorescence, and other detritus [8]. Most of the mangrove associated fungi decompose the plant material which serve as organic matter to favor the growth of other microorganisms [9].

Mangrove endophytic fungi represent the second largest ecological group of the marine- derived fungi [10]. Being the inhabitant of such a harsh environment, these fungi have proven to be potent sources of unique bioactive metabolites like antibacterial, anticancer, anti-inflamatory, antidiabetic, antioxidant and antiviral compounds [11-15]. Apart from these, mangrove endophytes have significant contributions in the production of industrially useful extracellular enzymes [16-18]. The current study aims to explore the diversity of endophytic fungal communities associated from the unexplored habitat of gulf of Kutch, Gujarat, India. Isolated fungi can serve as a reservoir of biotechnologically important products.

2. Materials and Methods:

Sample collection site:

The samples were collected from three different location of Gulf of Kutch, Gujarat, India. Mundra, Tuna and Kandla region were chosen for the study. All the three regions are dominated by *Avicennia marina*(Fig.1).

Fig.1 Sample collection site of Kutch, Gujarat, India

Sample collection and Preparation:

Healthy and mature roots of *Avicennia marina* were collected from three mangrove sites Mundra, Tuna and Kandla from Kutch region, Western India. The trees were randomly chosen and two healthy root samples were collected from four different trees at each location. The samples were transported to the laboratory in a sterile plastic container. The sections were stored at 4°C in the refrigerator.

The collected root samples were properly washed with tap water to remove the dirt. The samples were subjected to surface sterilization by treating with 70% alcohol and 0.5% Sodium hypochlorite solution and sterile distilled water repeatedly. Each sample was cut into 0.5 cm long sections with the help of a sterile blade [19].

Isolation of Fungal Endophytes:

Sterilized root sections were inoculated in Czapek dox agar plates. The plates were incubated at 30°C for 7 days. The plates were monitored for the presence of the fungal growth. The observed growth was subcultured into fresh media. The pure culture isolates were stored at 4°C in sterile glycerol solution.

Morphological Characterization of endophytic fungi:

Cultural characteristics such as colour of the isolate, texture of the growth, reverse media colour and growth rate of the selected pure isolates were recorded. The isolates were even characterized microscopically by staining with lacto phenol cotton blue. The morphological features like hyphal structure, type of mycelia, type of spore, characteristics of conidia and sporangia as well as arrangement of sporangiospores and conidiophore. The fungal isolates were identified by an identification key [20].

3. Results and Discussion

About 13 different endophytic fungal isolates were obtained from the root sections of *Avicennia marina*. The samples contrasted with the negative controls kept during sample preparation, confirming the lack of contamination while performing an experiment. Out of 13 isolates, 39% were derived from the Tuna region, 15% were derived from the Mundra region and 46% were derived from the Kandla region (Fig.2). The results indicated the species richness in the Kutch region of Gujarat. Fungal isolates were characterized based on the color, texture and growth characteristics on media. Whereas they were even characterized based on microscopic features like hyphal structure, mycelia, types of spores, sporangia, arrangement of conidiophores and sporangiospores. Based on the morphological examination, total 13 isolates were majorly grouped into 3 different genera including *Aspergillus sp., Penicillium sp.* and *Alternaria sp.* (Table-1).

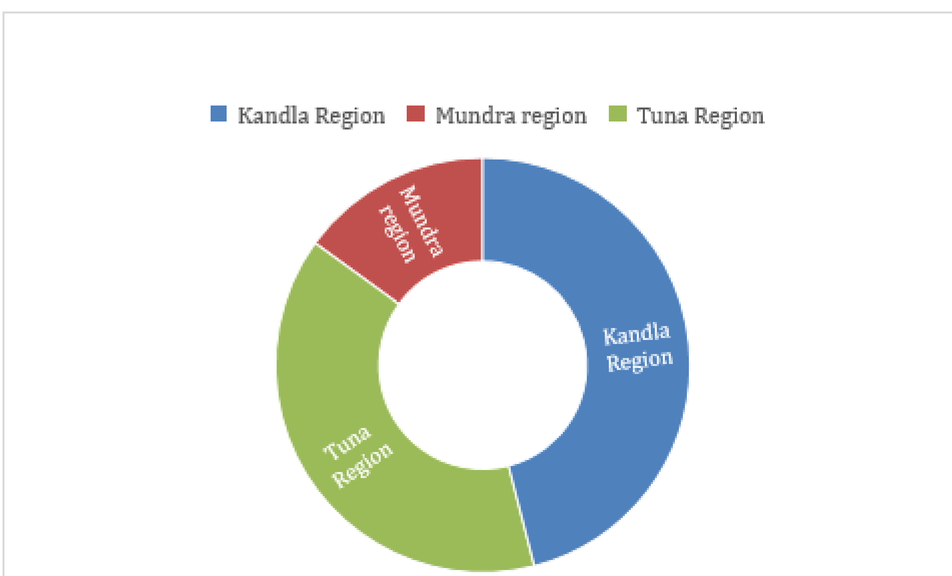

Fig.2 Number of isolates derived from different locations of Kutch,Gujarat,India

Sr. No. of endophytic fungi	Size	Shape	Colony morphology	Colony colour	Type of hyphae
1	Medium	Circular	Powdery	Green	Septate
2	Medium	Circular	Fluffy and Woolly	Brown	Septate
3	Medium	Circular	Dry and Woolly	Black	Septate
4	Punctiform	Circular	Smooth	Black	Septate
5	Medium	Circular	Velvety	Green	Septate
6	Medium	Filamentous	Powdery	green	Septate
7	Medium	Circular	Velvety	Green	Septate
8	Medium	Circular	Velvety	Grey	Septate
9	Medium	Circular	Dry and Woolly	Brownish black	Septate
10	Medium	Irregular	Velvety	Dark brown	Septate
11	Large	Circular	Powdery	Light yellow	Septate
12	Medium	Circular	Powdery	Green	Septate
13	Small	Circular	Velvety	Grey	Septate

Table-1 Morphological characteristics of fungi

Based on the cultural and microscopic characterization, 4 of the isolates were identified as *Aspergillus Sp.* Majority of these isolates were derived from Kandla region of Kutch, Gujarat India. The fungal growth ranged from White to green in colour with powdery texture. The growth was most rapid at 25°C. The colonies showed white colour from the reverse. Filamentous hyphae with blue conidiophores were observed under microscope. Endophytic *Aspergillus sp.* has effectively been known to produce antibacterial compounds [22]. It is equally important reservoir of extracellular enzymes like cellulases [23]. About 3 isolates were identified as *Penicillium sp.* The fungi showed distinct cultural features with white to green in coloration with velvety appearance. Some of the isolates were even found to produce yellow exudates. The development of folds and white coloured border was found around the colony. Endophytic *Penicillium sp.* has been widely known producer of bioactive metabolites [24-25]. About 5 *Alternaria* isolates were derived from the sample with white to brown colouration. Brownish coniophores were observed microscopically. Mangrove endophytic fungi has been potential source of novel secondary metabolites with unusual properties [26]. One of isolate was identified as halotolerant yeast with blackish growth and microscopic examination revealed the yeast like appearance of the cell with septate hyphal structures.

4. Conclusion

Mangrove endophytic fungi are the most extensive group of organisms that have excellent symbiotic relationships with their companions and other higher forms of life. The findings show that *A. marina* fungal endophytes from rarely investigated habitat of the Gulf of Kutch (Western India) might emerge as key sources of new and much more effective bioactive compounds. Morphological examination revealed that the fungi belongs to three different genera of *Aspergillus sp.*, *Penicillium sp.* and *Alternaria sp.* The finding of the current study can open new opportunities for researchers to investigate more endophytes associated with mangroves.

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