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Isolation and characterization of endophytic fungi from *Crinum asiaticum* Lin Kodiyakarai, Tamilnadu, India

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ABSTRACT

The aim of the research is isolate and identify the fungal endophytes from the leaves of Crinum asiaticum L. Totally thirteen isolates of fungal endophytes were isolated from the leaf of Crinum asiaticum L. The endophytic fungi such as Acremonium rutilum, Alternaria sp, Alternaria tenuis, Chaetomium globosum, Chrysosporium tropicum, Curvularia sp, Cylindrocarpon candidum, Fusarium solani, Fusarium chlamydosporum, Fusarium sporotrichoides, Fusarium javanium, Humicola sp, Helminthosporium sativum, Nigrospora sphaerica and Verticillium terrestre were isolated. The isolated endophytic fungal characters were conformed.

Key words: Endophytic fungi, Kodiyakarai, Mangrove, *Crinum asiaticum*

INTRODUCTION

Mangrove forests are fascinating and complex ecosystems [1]. Mangrove plants are salt-tolerant plants which act as primary producers in the estuarine food chain and they produce novel metabolites unique to the environment with various important economic and environmental functions [2]. Most mangrove plants do not consider the fact that plants in natural ecosystems have symbiotic associations with fungi.

Endophytic fungi that live inside the tissues of living plants are under-explored group of microorganisms. Recently they have been received considerable attention after they were found to protect their host against insect, pests, pathogens and even domestic herbivores. Almost all the plant species harbour one or more endophytic microorganisms. Endophytes are hidden within healthy host plants are a poorly investigated group of microorganisms, but they represented an abundant and dependable source of novel bioactive compounds with huge potential for exploitation in a wide variety of medical, agricultural, and industrial areas [3]. These fungi are important to the structure, function, and health of plant communities and may be responsible for the adaptation of plants to environmental stresses [4]. In addition, mutualistic symbiosis with mycorrhizal and endophytic fungi can confer salt tolerance to plants and decrease yield losses in cultivated crops grown in saline soils [5].

Crinum asiaticum (poison bulb, giant crinum lily, grand crinum lily, spider lily) is a plant species widely planted in many warmer regions as an ornamental. It is a bulb-forming perennial producing an umbel of large, showy flowers that are prized by gardeners. All parts of the plant are poisonous if ingested. Some reports indicate exposure to the sap may cause skin irritation.

Endophytic fungi have been recognized as possible sources of bioactive secondary metabolites [6] [7]. There is a need to search new ecological niches for potential sources of natural bioactive agents for different pharmaceutical, agriculture, and industrial applications.

MATERIALS AND METHOD

Collection of plant samples

The leaves of *Crinum asiaticum* were collected from the Kodiyakarai, Nagappattinam District. Healthy and mature plants were carefully chosen for sampling. The plants randomly collected and brought to the laboratory in sterile bags for further investigation.

Isolation of endophytic fungi

Asymptomatic healthy leaf materials were thoroughly washed in running tap water, then surface sterilized by a modified method of Raviraja [8]. The selected leaf segments were immersed in 95% ethanol for 30 sec, 45% sodium hypochlorite solution for 15sec and 95% ethanol for 30sec followed by rinsing with sterile distilled water three times for 10sec and allowed to surface dry under sterile conditions. After drying, each leaf segment was cut into approximately 0.5cm squares and placed on Petri Plates containing potato dextrose agar medium (PDA). The Streptomycin sulphate (100mg/L) was added to prevent the growth of bacteria. Then it was monitored every day for growth of endophytic fungal colonies. Fungi growing out from the samples were subsequently transferred to fresh PDA plates.

$$\textbf{Colonization frequency of endophytes} = \frac{\textbf{Number of segments colonized by endophytic fungi}}{\textbf{Total number of leaf segments}} \times \mathbf{100}$$

Identification of endophytic fungi

The identification of fungi was done using cultural and microscopic characteristics such as shape, color, pattern arrangement of mycelium, conidial arrangement, and types of spores by using standard manuals such as, a manual of Penicillia by Kenneth [9]. Fungi in Agricultural Soils by Domsch and Gams [10] and manual of soil fungi by Gilman [11]. All the isolated fungi were identified up to genus level on the basis of detailed culture and microscopic study and by consulting relevant literature. The pure culture of isolated fungal strains was maintained in PDA slants at 28° C.

Lacto phenol cotton blue mounting

A loopful culture was picked up with the help of a sterile inoculation loop and semi-permanent slides were prepared using lacto phenol cotton blue. The slides were gently heated in a spirit lamp so as to release the air bubbles, if any present inside the cover glass. The excess stain was removed by using tissue paper and the cover glass was sealed with white nail polish.

RESULTS

This result in association of endophytic fungi with *Crinum asiaticum* was selected and its leaves only taken for isolation of endophytic fungi. Each isolates were sub-cultured into a PDA agar plates to remove the adherent plant metabolite from the mycelia and stored at 4°C for further studies.

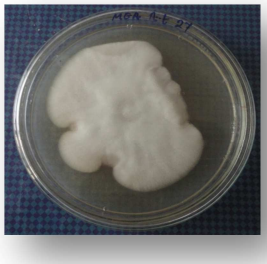
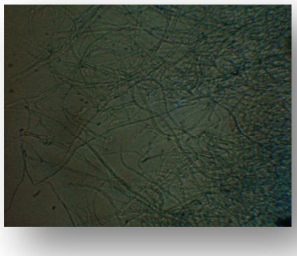
Totally thirteen endophytic fungi have been isolated from the leaves of *Crinum asiaticum.* The isolated endophytic fungi such as *Acremonium rutilum, Acrophilaophora fusisphora, Alternaria* sp, *Chaetomium globosum, Chrysosporium tropicum, Curvularia* sp, *Cylindrocarpon candidum, Fusarium chlamydosporum, Fusarium solani, Humicola* sp, *Helminthosporium sativum, Nigrospora sphaerica* and *Verticillium terrestre. Fusarium* genera are most predominant endophytic isolates (Table 1 and fig 1) conformed.

Table 1 Isolation and characterization of endophytic fungi from *Crinum asiaticum* leaves sample

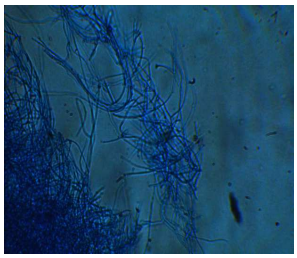
S.no	Isolated fungi	Characteristics of endophytic fungi
1	*Acremonium rutilum*	Colonies are usually slowed growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in color. Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides. Conidia are usually one-celled (ameroconidia), hyaline or pigmented, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide.
2	*Acrophilaophora fusisphora*	Colonies fast growing, greyish-brown with a black reverse. Conidiophores arising singly, terminally and laterally from the hyphae, erect, straight or slightly flexuose, tapering towards the apex, pale brown, rough-walled with whorls of phialides on the upper part. Phialides flask-shaped with a swollen base and a long, narrow neck, hyaline, smooth-walled or echinulate.
3	*Alternaria* sp.	Colonies are fast growing, black to olivaceous-black or greyish, and are suede-like to floccose. Microscopically, branched acropetal chains (blastocatenate) of multicellular conidia (dictyoconidia) are produced sympodially from simple, sometimes branched, short or elongate conidiophores. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak, pale

		brown, smooth-walled or verrucose.
4	*Chaetomium globosum*	Members of this genus typically have superficial, ostiolar perithecia, covered in hairs. Asci are often clavate and evanescent, bearing eight spores. Ascospores are usually lemon-shaped, commonly colored olive-brown. Mycelia often grows in conglomerate masses that resemble rope.
5	*Chrysosporium tropicum*	Colonies are moderately fast growing, flat, white to tan to beige in colour, often with a powdery or granular surface texture. Reverse pigment absent or pale brownish-yellow with age. Hyaline, one-celled (ameroconidia) are produced directly on vegetative hyphae by non-specialized conidiogenous cells.
6	*Curvularia* sp.	Colonies are fast growing, suede-like to downy, brown to blackish brown with a black reverse. Conidia are pale brown, with three or more transverse septa (phragmoconidia) and are formed apically through a pore (poroconidia) in a sympodially elongating geniculate conidiophore similar to
7	*Cylindrocarpon candidum*	Species of the genus cylindrocarpon candidum produce chains of hyaline, smooth, one-celled, subglobose to cylindrical, slimy arthroconidia (ameroconidia) by the holoarthric fragmentation of undifferentiated hyphae. The arthroconidia, which are quite variable in size, may germinate at one end giving the appearance of a bud.
8	*Fusarium solani*	Colonies are usually fast growing, pale or brightly colored (depending on the species) and may or may not have a cottony aerial mycelium. The color of the thallus varies from whitish to yellow, brownish, pink, reddish or lilac shades. Species of Fusarium typically produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform- to sickle-shaped, hyaline, pyriform, fusiform to ovoid, straight or curved. Chlamydoconidia may be present or absent.
9	*F.chlamydosporum*	Colonies growing rapidly, with abundant aerial mycelium, deep pink, red or ochraceous to brownish; reverse carmine red or tan to brown. Sporodochia orange, flesh-coloured or ochraceous. Conidiophores scattered over the aerial mycelium, branched; numerous polyblastic conidiogenous cells are present. Macroconidia rarely produced and appearing only on sporodochial phialides, usually three-five, septate, slightly curved. Microconidia and blastoconidia fusiform, rounded apically and tapered towards the base, single-celled to one- three, septate. Chlamydospores abundant, intercalary, often roughened.
10	*Humicola sp*	Colonies has slow growth rate. Colony texture is cottony and the colony color is golden brown. The conidiophores are undistinguished and the spores (aleuriospores) are born directly off the vegetative hyphae or conidiophores. The spores are large, globose or subglobose. slow growth rate. Colony texture is cottony and the colony color is golden brown.
11	*Helminthosporium sativum*	The mycelium deep olive-brown. Conidia develop laterally from pores beneath each conidiophore setpum. Conidia are olive-brown and ovate to oblong, with rounded ends and a prominent basal scar.
12	*Nigrospora sphaerica*	The mycelium is immersed within the outer tissues of the host and is composed of hyaline, branched, septate hyphae. The hyphae penetrate the epidermis and produce on its surface clusters of short branched, pale-brown swollen conidiophores, bearing singly at their apices depressed globose shining black aleuriospores.
13	*Verticilliu terrestre*	Colonies are fast growing, suede-like to downy, white to pale yellow in colour, becoming pinkish brown, red, green or yellow with a colourless, yellow or reddish brown reverse. Conidiophores are usually well differentiated and erect, verticillately branched over most of their length, bearing whorls of slender awl-shaped divergent phialides. Conidia are hyaline or brightly colored, mostly one-celled, and are usually borne in slimy heads

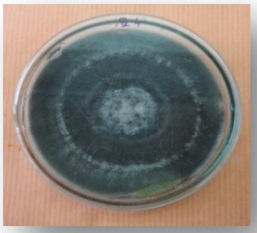
Fig 1 Macro and micro view endophytic fungi isolated from *Crinum asiaticum*

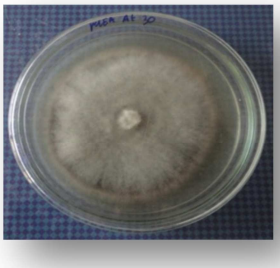
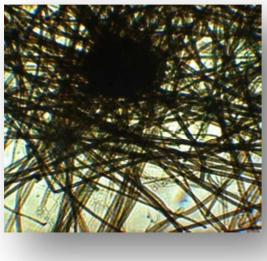
Acremonium rutilum

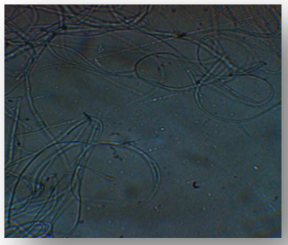
Acrophialophora fusispora



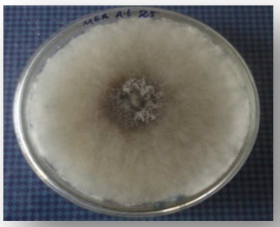

***Alternaria* sp**

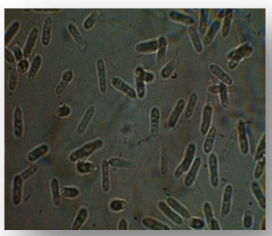
Chaetomium globosum

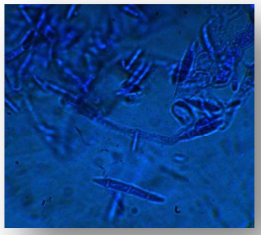
Chrysosporium pannorum



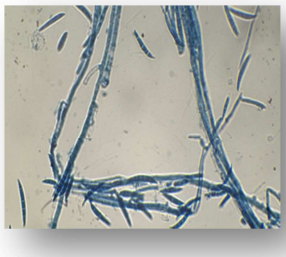

***Curvularia* sp**

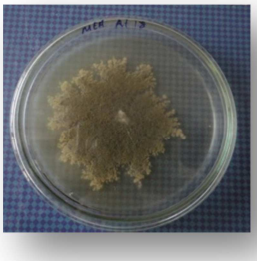
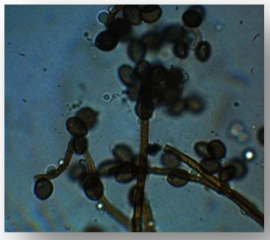
Cylindrocarpom candidum

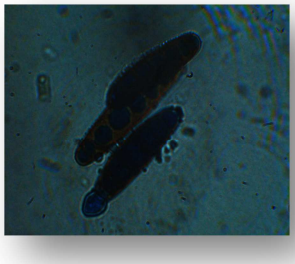
Fusarium chlamydosporum

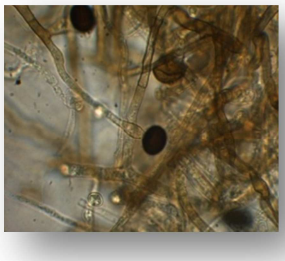
Fusarium solani

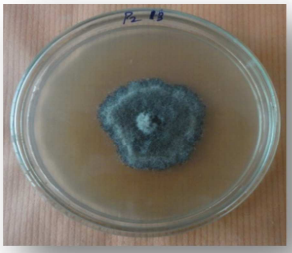
***Humicola* sp**

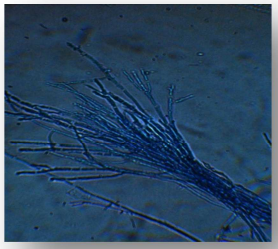



Helminthosporium sativum

Nigrospora sphaerica





Verticillium terrestre

DISCUSSION

The endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and may produce beneficial substance for host [12] [13]. Endophytic organisms have received considerable attention after they were found to protect their host against insect pests, pathogens and even domestic herbivorous [12]. Fungi have been widely investigated as a source of bioactive compounds an excellent responsibility for anticancer drug taxol, which had been previously to occur only in the plants [7].

In the present investigation totally thirteen fungi have been isolated from the leaves of *Crinum asiaticum.* Similarly, Rajendran *et al*., [14] Victoria [15] and Sandhu *et al*., [16] reported that the colonization rate in the leaves was found to be significantly higher than other parts of the plant.

Ten genera of endophytic fungi were isolated from root system of palm trees [17] and cocoa plant [18]. *Fusarium* sp. had also been isolated from root systems of tomato and banana [19] [20]. Amin *et al*., [17] pointed out the fungal colony of endophytic fungi influenced by biotic and abiotic components. Rubini *et al*., [21] claim that endophytic fungal colony is very specific whiten the crop layer and may depend on the interaction between other crop pathogens. Moreover, Pirttilla and Frank [22] contend that diverse organism is needed to have long term stabilization of biogeochemical cycle in ecosystem.

CONCLUSION

Focusing on the investigation of endophytic fungal diversity. The endophytes isolated during this study offer an excellent platform for the discovery of natural product-transforming fungus novel drugs.

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