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IDENTIFICATION AND BIOACTIVE POTENTIAL OF ENDOPHYTIC FUNGI FROM *MONOCHORIA HASTATA* (L.) SOLMS

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Key words: Endophytes, *Monochoria hastata*, Antimicrobial activity, Antioxidant Activity.

Abstract

Drug resistance in microbes has become a global concern and the search for new antimicrobial agents is urgent and ongoing. Endophytes provide an abundant reservoir of bioactive metabolites for medicinal exploitation, and an increasing number of novel compounds are being isolated from endophytic fungi. This study was conducted to characterize and explore the endophytic fungi from the aquatic plant *Monochoria hastata* (L.) Solms for their bioactive potential. A total of 7 strains of endophytic fungi were isolated and characterized morphologically. Among the isolated strains, 5 strains were identified up to the genus level, of which two belong to *Carvularia*, while the rest of the isolates comprised of *Trichoderma*, *Penicillium* and *Fusarium* sp. Two isolates could not be assigned to a genus as they did not display taxonomically characterized organisms. Several extracts prepared from the PDA culture of these fungi demonstrated strong antibacterial activity and poor antioxidant activities. Further studies on the selected endophytes may lead to the isolation of novel natural products for use in medicine, industry and agriculture.

Introduction

Endophytes are metabolically more active than their free counterparts due to their specific functions in nature and activation of various metabolic pathways to survive in the host tissues (Strobel *et al.* 2004). Aquatic plants have adapted to living in aquatic environments (salt water or fresh water). In Bangladesh about 130 angiospermic, 6 pteridophytic, 3 bryophytic and several hundred algae species have been identified as aquatic plants. The aquatic environment prevailing in the deeply flooded area in Bangladesh has great potential in terms of propagation of aquatic plants, most of which are untouched for investigation of their biological activity (Chong *et al.* 2009). The aquatic plant *Monochoria hastata* (L.) Solms (Bengali name: Boronukha, Family: Pontederiaceae) is a family of 6 genera and 40 species which are widespread in tropical and sub tropical regions (Holscher *et al.* 2006). The entire plant, excepting the roots is eaten as vegetable in Java (Guofang and Hom 2000). The plant is used for treating many ailments such as toothache, asthma, coughs, cold, fever, stomach and liver problem, general debility, hemorrhage, hepatitis, anemia, scurvy, diabetes etc. (Ileperuma *et al.* 2015). Thus, an investigation was carried out to isolate and characterize the endophytic fungi of selected plant growing in the aquatic atmosphere of Bangladesh, with the aim to establish a repository, explore their bioactive potential and isolate new leads for drug discovery, industry and agriculture.

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Materials and Methods

The plant samples were collected from low land of Gazipur district, Dhaka, Bangladesh in June, 2013. The plant material was identified and authenticated by Shardar Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium (BNH). A voucher specimen of this collection is maintained at BNH under the Acc. No. DACB- 38364. About 300 grams of fresh and healthy parts of the plant (leaves, root and flowers) were cut with a sterile scalpel and stored at 4°C in a sterile polythene bag prior to use. Endophytic fungi were isolated from the fresh plant parts following the procedure, suitably modified, Kusari *et al.* (2014).

For the identification of endophytic fungal isolates, slides prepared from cultures were stained with lactophenol cotton blue reagent and examined with a bright-field and phase contrast microscope (Sadananda *et al.* 2014). Identification was based on morphological characteristics such as growth pattern, hyphae, the color of the colony and medium, surface texture, margin character, aerial mycelium, sporulation and production of acervuli, coloration of the medium, and the size and coloration of the conidia using standard identification manuals (Devi and Prabakaran 2014). The fungi were identified using relevant keys and taxonomic notes from various standard manuals (Barnett and Hunter 1998).

After the completion of incubation period for 21 days at 28°C, the culture media were extracted three times with ethyl acetate to obtain the crude extracts. The extracts of the fungi were made concentrated into solid residue by evaporation under rotary evaporator (Alzoreky and Nakahara 2003).

The test microorganisms used in the antimicrobial study included four pathogenic bacterial strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 28739, *Bacillus megaterium* ATCC 18 and *Pseudomonas aeruginosa* ATCC 27833, two fungal strains *Aspergillus niger* and *A. flavus*. All the cultures were obtained from ICDDR,B. The bacterial cultures were sub-cultured every two weeks on fresh nutrient agar (NA) slants and incubated at 37°C whereas the fungal cultures were sub-cultured every four weeks on the fresh potato dextrose agar (PDA) slants and incubated at 28°C.

Spectrum of antibacterial and antifungal activities were studied by using the technique described by Bauer *et al.* (1966). Kanamycin and Ketoconazole sensitivity disc (30µg/disc) were used as a positive control for bacteria and fungi, respectively. Solvents are used as negative control. The zones of growth inhibition around the discs were measured after 18 to 24 hrs of incubation at 37°C for bacteria and 48 to 96 hrs of incubation at 28°C for fungi. The sensitivities of the microorganism species to the fungal extract (100 µg/disc) were determined by measuring the diameter of inhibitory zones in millimeter.

The free radical scavenging activity of the endophytic fungal samples were assayed by the discoloration of methanol solution of DPPH (1,1-diphenyl-2-picryl hydrazyl) radical (violet color) according to Brand-William *et al.* (1995). The scavenging activity of free radical by the fungal extracts was evaluated spectroscopically at 517 nm. The experiment was carried out in triplicate and averaged. The scavenging activity was calculated as follows:

$$\text{Scavenging ability (\%)} = (A_{517} \text{ of control} - A_{517} \text{ of sample} / A_{517} \text{ of control}) \times 100.$$

For each sample, the result was presented as an IC50 (sample concentration that produced 50% scavenging of the DPPH radical). Ascorbic acid, trolox and BHA were used as positive control.

Results and Discussion

Isolation, purification and cultivation of endophytic fungi have been done following the published method, suitably modified. A total of seven endophytic fungi, namely MHLE-1, MHLE-2, MHLE-3, MHFE-1, MHFE-2, MHFE-3 and MHRE-1 were isolated and purified from the plant *Monochoria hastata* (L.) Solms. They exhibited characteristic colony and microscopic morphology that could be used to differentiate them. Fungi were identified taxonomically to the genus level on the basis of macroscopic and microscopic morphological characters in culture medium (Fig. 1A-F, Fig. 2A-F and Fig. 3A-B).

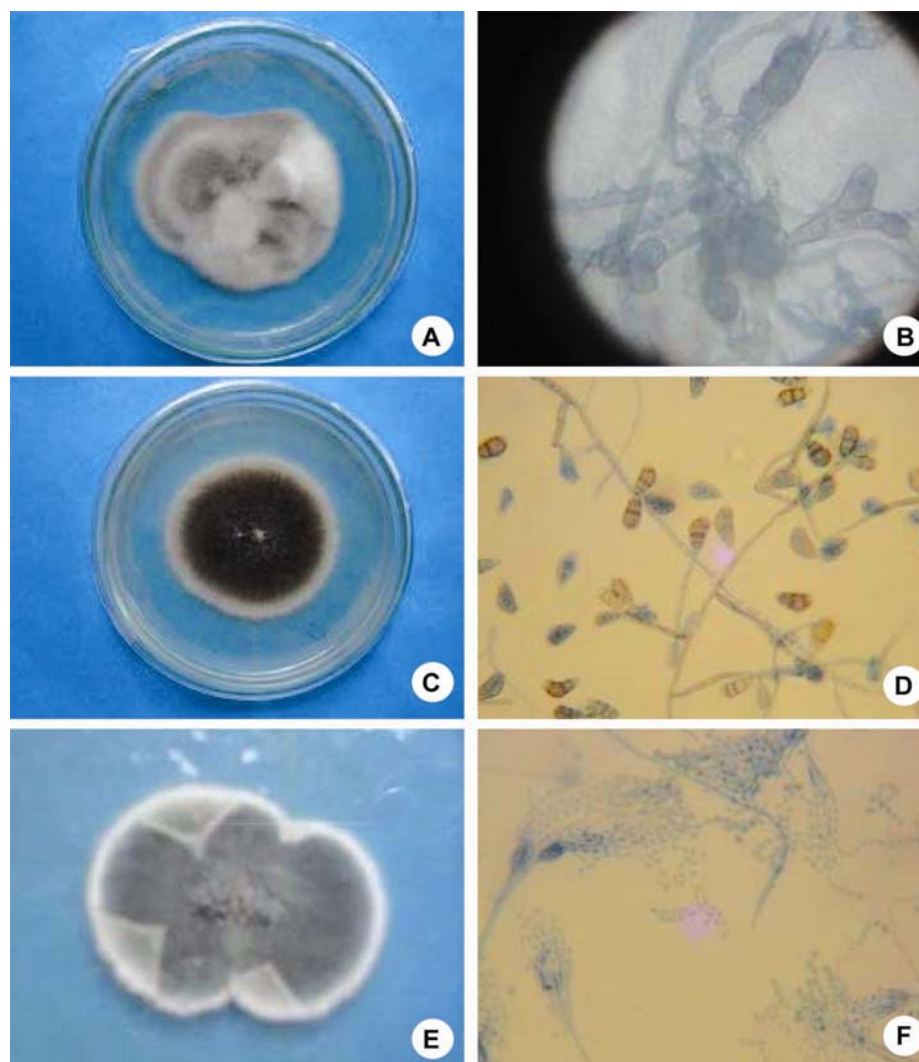


Fig. 1. Macroscopic and microscopic colony morphologies of the endophytic fungi from *Monochoria hastata*. A. MHLE-1 (*Carvularia* sp.), B. MHLE-1 (Microscopic view), C. MHLE-2 (*Carvularia* sp.), D. MHLE-2 (Microscopic view), E. MHLE-3 (*Penicillium* sp.) and F. MHLE-3 (Microscopic view).

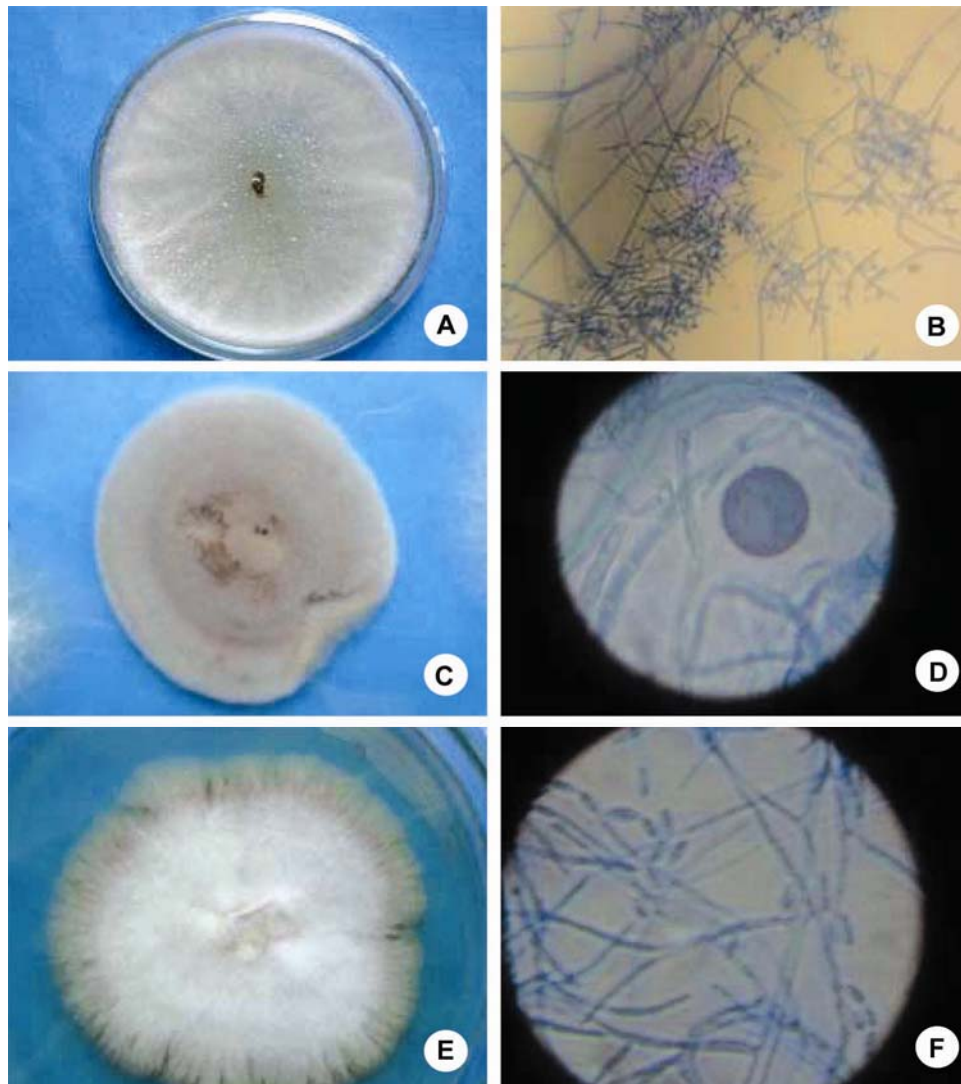


Fig. 2. Macroscopic and microscopic colony morphologies of the endophytic fungi from *Monochoria hastata*. A. MHFE-1 (*Trichoderma* sp.), B. MHFE-1 (Microscopic view), C. MHFE-2 (Macroscopic view), D. MHFE-2 (Microscopic view), E. MHFE-3 (*Fusarium* sp.) and F. MHFE-3 (Microscopic view).

Four fungi were taxonomically identified up to the genus level on the basis of macroscopic and microscopic morphological characters. In case of strain MHLE-I, mycelium was hyaline, profusely branched, septate, conidophores are brown, bearing spore on new sympodial growing point, 3 - 5 celled and fusiform. It was identified as *Carvularia* sp. Strain MHLE-2 showed profusely branched, septate and hyaline mycelium, conidophores are brown and simple, bearing spore apically, conidia dark, end cell lighter, typically bent, with one of the central cell enlarged. It was also identified as *Carvularia* sp. Strain MHLE-3 exhibited mycelium hyaline, pale or brightly colored branched and septate, conidophores are arising from the mycelium singly or less often in

synnemata, branched near the apex, ending in phialides, spores brightly colored mass, one celled, ovoid, in dry basipetal chain. It was identified as *Penicillium* sp. Strains MHFE-1 was identified as *Trichoderma* sp. though its microscopic characters showed mycelium hyaline, pale or brightly colored branched and septate, conidiohores are arising from the mycelium singly or less often in synnemata, branched near the apex, ending in phialides, spores brightly colored mass, one celled, ovoid, in dry basipetal chain. Strain MHFE-3 was identified as *Fusarium* sp. It exhibited conidia or phialo spores hyaline, two kinds of spores are observed. Macroconidia were several celled, slightly curved or bent at the pointed end. Microconidia were one celled, oblong, borne singly or in chain, colony was fluffy, spores cylindrical, septate and aseptate. MHFE-2 and MHRE-1 could not be identified yet as their conidiophores and conidia were absent. Taxonomical identification of these fungal strains is underway. Unidentified endophytes were found to be unique one. Therefore it can be speculated that these unique endophytes might produce unique or potential bioactivities.

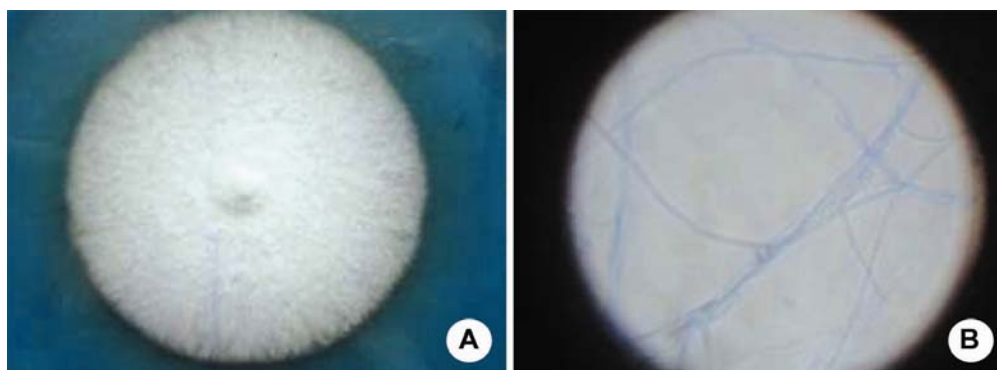


Fig. 3. Macroscopic and microscopic colony morphologies of the endophytic fungi from *Monochoria hastata*. A. MHRE-1 and B. MHRE-1 (Microscopic view).

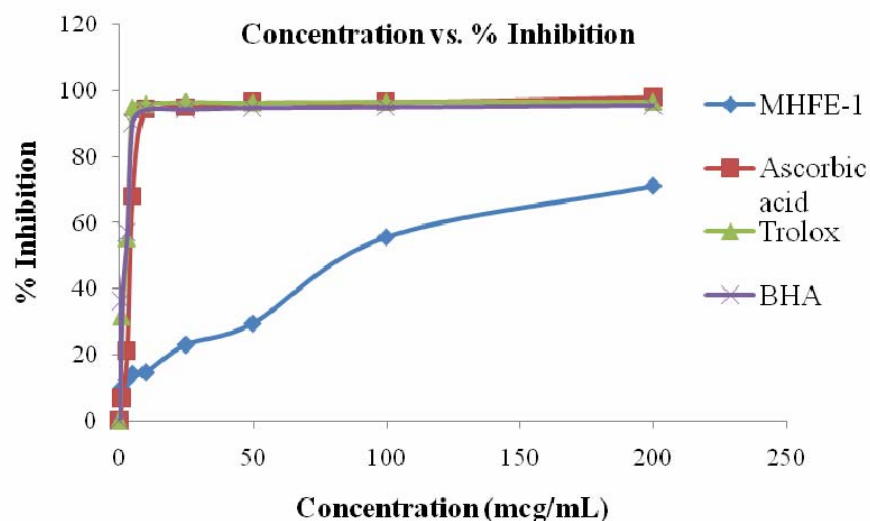
Among 7 endophytic fungi screened for antimicrobial activity, all of them showed activity against the tested pathogenic bacteria. The results of the antimicrobial tests of ethyl acetate extracts of laboratory culture of seven endophytic fungi are listed in Table 1. One isolate (MHLE-3) showed potential inhibition against four pathogenic bacteria and three isolates (MHFE-1, MHFE-2 and MHRE-1) exhibited moderate inhibition against four pathogenic bacteria. One isolate (MHLE-1) inhibited the growth of *S. aureus* as well as *B. megaterium*. None of the endophytes exhibited any activity against the tested pathogenic fungi.

The percentage scavenging of DPPH radical was concentration dependant. The radical scavenging activity of the compounds can be measured by the decolorizing effect following the trapping of the unpaired electron of DPPH. Six endophytic fungi exhibited very insignificant antioxidant activity. Only one endophytic fungus namely, MHFE-1 exhibited moderate antioxidant activity with IC_{50} value 89.7 $\mu\text{g/ml}$ compared to the IC_{50} value of ascorbic acid 4.8 $\mu\text{g/ml}$, trolox 3.3 $\mu\text{g/ml}$ and BHA 3.4 $\mu\text{g/ml}$ (Fig. 4).

This is the first study to describe the isolation and antibacterial activities of endophytic fungi from *Monochoria hastata*, an aquatic plant from the low land of Bangladesh. Present investigation revealed that *M. hastata* harbors several endophytic fungi which are capable of producing antimicrobial substances with selective antibacterial activities. The endophytic *Penicillium* sp. (internal strain no. MHLE-3) displayed significant activities, exhibiting its potential for development as antimicrobial agents and clearly deserving further research. Moreover, *Carvularia*

Table 1. Antimicrobial activity of endophytic fungal strains isolated from *Monochoria hastata* at concentration of 100 µg/disc.

Sample	Diameter of the inhibition zone (mm)			
	<i>B. megaterium</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
MHRE-1	10	13	12	13
MHFE-1	14	15	17	15
MHFE-2	7	8	9	8
MHFE-3	-	-	-	-
MHLE-1	9	9	-	-
MHLE-2	-	-	-	-
MHLE-3	22	23	24	12
Kanamycin (30 µg/disc)	32	32	31	30
Solvent (Methanol)	-	-	-	-

**Fig. 4.** Free radical scavenging activity of different concentrations of endophytic fungus, *Trichoderma* sp. (MHFE-1) and three standards.

and *Trichoderma* species showed moderate antimicrobial activities against four types of bacteria. Now our next aim is to explore the isolation and characterization of lead compounds liable for aforementioned activity from this plant and its associated endophytic fungi.

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