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RESEARCH ARTICLE

EVALUATION OF LETHAL POTENCY OF A CORAL REEF INHABITING FUNGUS ON MAMMALIAN SYSTEM

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ABSTRACT

A fungus was always found to be associated with the necrotic patches of the corals in the Gulf of Mannar Biosphere Reserve. It was nonsporulating under both natural and culture conditions. Hyphae of the fungus were septate, highly melanised and presented cottony appearance in the colony. Lethal potency of the acidified ethanol extract of this fungus was tested by the mouse bioassay method and the LD_{50} is determined as 75mg per mouse.

INTRODUCTION

Marine organisms are potential sources for a variety of toxins. Almost all the groups of the marine animals have been well explored for the toxins than the plants (Hashimoto, 1979). Among the marine plants, phytoplankton (bluegreens and dinoflagellates) and seaweeds have been studied for the toxin production. But, fungi, an important group of organisms in the marine environment have not been much studied for such aspects though their occurrence (as saprophytes and/or parasites) and importance in the marine environment have been stressed by the marine mycologists since 1950.

MATERIALS AND METHODS

Isolation of fungus

The fungus was isolated from the necrotic patches of corals collected from the Gulf of Mannar Biosphere Reserve by the following procedures. Coral pieces having necrotic patches were surface sterilized with sodium hypochloride solution, cut in to smaller pieces and treated with antibiotic solution (1% streptopenicillin) for 12 hrs. Then the slabs were placed in the petriplates containing 2% Malt Extract Agar (MEA) medium (18g – Agar; 20g – Malt Extract; 1000ml – Aged filtered seawater; 8ml – 1% Streptopenicillin solution; pH – 7) (Nadimuthu,1998). Invariably, highly melanised, nonsporulating septate mycelial colony appeared from the coral pieces..

Culture for mycelial mass

Growing tip of the culture colony was cut in to pieces (5cm dia.) using a sterilized cork borer and cultured in 500 ml conical flasks containing 250 ml of 2% ME broth at $23 \pm 2^{o}C$. After 15 days of growth, the mycelial mat was harvested, washed with distilled water and shade- dried.

Extraction of toxin

Dry weight of 25 g of mycelial sample was ground well with acidified water (pH 3.0) using a pestle and mortar in 100 ml of water. The cellular extract was centrifuged at 5000 g for 30 minutes. The supernatant was collected and reduced to 5 ml.and 40 ml of the acidified ethanol was added to this and again centrifuged at 5000 g. The supernatant was collected and ultra-filtered using Millipore filtering system through a 0.45uM membrane filter. Then the filtrate was allowed to evaporate water and ethanol molecules to get the crude toxin in the form of powder.

Mouse bioassay

For initial screening and LD_{50} determination, male Swiss mice weighing 20 ± 5 g were used. Doses of fungal toxic extract were suspended in 0.5 ml of 0.1% Tween 60 in 0.15M NaCl and administered by i. p. injection. Mice were observed for a period of 48 hours.

RESULTS AND DISCUSSION

A 5 cm dia. inoculum of the fungus yielded an average weight of 7.8 g of mycelium (wet weight) during the 15 days of the culture period in 500 ml conical flasks containing 250 of medium. This gave an average of 1.8 g of dry mycelial biomass. The yield of crude toxin from the fungus was 1.63 mg g^{-1}dry wt. When the mice were administered with 50, 60, 70, 80, 90 and 100 concentrations in 0.5 ml dose during the initial screening, no mortality was observed up to 24 hours. But, notable behavioural changes were found in the animals injected with 70 mg and above. The changes were ataxia, inactivity, phyloerection followed by cyanosis of the tail and

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feet with concurrent hypothermia, impairment hind limbs motor ability followed shortly by complete paralysis and respiratary distress. When the observations were continued up to 48 hours, first mortality was noticed in 26 hours in the 100 mg injected mouse followed by 30:35, 35.05 and 38.55 hours respectively at 90, 80 and 70 mg injected animals. The animals administered with 60 mg and below returned to normalcy from the initial shock.

For determining the LD_{50} also, the fungal cellular extract with toxin was retained in the crude state. The LD_{50} of the crude toxin of the coral reef inhabiting fungus was 75mg $mouse^{-1}$. This was comparatively much less than that of the toxic levels reported for the other marine plants viz. dinoflagellates (Dickey, 1984) and blue-greens (Hasimotto, 1979). Tepsic *et al.* (1997) have observed that 45% of the strains of *Aspergillus fumigatus* isolated from the salty soils and hyper-saline water samples were able to produce different kinds of mycotoxins. Further, they opined that the prevailing salty environmental conditions would inhibit or lower the production of mycotoxins. Thus, the present study suggests that there is some toxin production in the fungus inhabiting the corals and there is an imperative need for the study of mycotoxin production in the marine fungi as they play a key role in the marine food-web process, along with other floral and faunal organisms.

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