Preliminary Extraction of Secondary Metabolites of Arthropod-Borne Fungi.

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Abstract
The potential for secondary metabolite production by some arthropod-borne fungi was investigated. The fungi included Myrothecium verrucaria, Aspergillus niger, Aspergillus flavus, Stachybotrys alternaris, Scytalidium dimidiatum, Conidiobolus sp, Syncephalastrum sp. and Ulocladium chartarum. All indicated potential for production of secondary metabolites. The amount and nature of compounds produced depended on the strain of fungi and other conditions for extraction.

Introduction

Microorganisms produce chemical substances as a result of primary and secondary metabolism. While primary metabolism involves metabolic pathways to assemble biosynthetic precursors into essential molecules of cellular structure and function, secondary metabolism involves metabolic pathways for synthesis of compounds that are the essential for the growth of the organism producing it (1).

The occurrence of large scale secondary metabolites is not common to all living organisms but restricted to certain taxonomic groups. Among animals, arthropods and other marine invertebrates are the most prolific producers of secondary metabolites. Within the plant kingdom, a great abundance of secondary metabolites have been isolated. A rich and extensive secondary metabolism has also been observed in bacteria and fungi (2).

A variety of fungi produce a wide variety of secondary metabolites, some of which are potent toxins called mycotoxins.

The mycoflora of unique ecological niches have some common features and it will be very rewarding to explore fungal diversity in various habitats such as insects and several other natural sources (3). A characteristic feature of the most prolific producers of secondary metabolites among microorganisms is their adaptability to changing environmental conditions such as substrate, pH, temperature, nutrient, oxygen and water availability. The ability to use a wide variety of substrates of different compositions which enter primary metabolism at different points leads to an enlargement of the available pool of secondary metabolites. Microbes confined to extreme environments and those able to use substrates which can not be metabolized by others, do not seem to have important secondary metabolites (4).

The cockroach has been reported to harbour a wide variety of fungi and may represent a dynamic habitat where competition among organisms is intense and, such microorganisms may be a productive source of novel molecules (4).

Materials and Methods

The fungi were isolated from gut-contents of cockroaches picked from house holds within the University of Jos, Jos senior staff quarters. Pure cultures of the fungal isolates were grown on Sabouraud's dextrose agar slants and used for the investigation.

The extraction process was carried out using ethyl acetate. Two (2)ml of the solvent was added to each of the samples and

thoroughly mixed by placing the test tube in a vortex mixer. A homogenous mixture was obtained and filtered using Whatman No.1 filter paper. The extracts were collected into properly labeled conical flasks and then concentrated to near dryness by evaporating in a water bath. This process eliminated the solvent (ethyl acetate) leaving the residue of the extract. This was then subjected to thin layer chromatography (TLC), which indicated the presence of different compounds in the extracts.

The TLC was carried out using three solvent systems; namely; ethyl acetate, ethanol and hexane. The thin layer was made up of a solid support pre-coated with cellulose. The retention factor (RF) value was calculated for the different compounds using the formula:

$$RF = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}$$

The compounds were observed under ultra violet lamp (U.V. 365nm Vmax) and recorded appropriately.

Results

Eight species of fungi were isolated from the cockroaches. They included *Aspergillus niger, Stachybotrys alternaris, Ulocladium chartarum, Myrothecium verrucaria, Aspergillus flavus, Scytalidium dimidiatum Conidiobolus* sp. and *Syncephalastrum* sp. The colour and quantity of each extract varied for each fungus sample after concentration. The results of the TLC are as shown (Tables I – III).

Discussion

The potential of arthropod-borne fungi to produce secondary metabolites was established. Some of the fungi produced more than one metabolite as indicated by the TLC profiles obtained using ethyl acetate: hexane solvent system. This was not observed for other solvent systems used, an indication that the ethyl acetate:

hexane solvent may be more suitable for the isolation of these compounds. It probably allows or enhances proper separation of these chemical compounds. The disparities in the RF values obtained for the different compounds suggests that the compounds differ for the different fungi. These compounds are presently being subjected to further analysis to determine their actual nature and hence identification.

Table I. TLC extracts from the fungal samples using ethyl acetate : hexane : ethanol as solvent system (ratio 1 : 1 : 1).

S/N	Fungus	No. of resolved spots	RF value
1.	*Myrothecium verrucaria*	1	0.973
2.	*Aspergillus niger*	1	0.908
3.	*Stachybotrys alternaris*	1	0.909
4.	*Ulocladium chartarum*	1	0.928
5.	*Syncephalastrum* sp.	1	0.913
6.	*Conidiobolus* sp.	1	0.870
7.	*Aspergillus flavus*	1	0.947
8.	*Scytalidium dimidiatum*	1	0.993

Table II. TLC extracts from the fungal samples using ethyl acetate : hexane as solvent system (ratio 2 : 3).

S/N	Fungus	No. of resolved spots	RF value
1.	*Myrothecium verrucaria*	3	0.53; 0.931; 0.965
2.	*Aspergillus niger*	1	0.444
3.	*Stachybotrys alternaris*	2	0.667; 0.443
4.	*Ulocladium chartarum*	2	0.603; 0.531
5.	*Syncephalastrum* sp.	1	0.714
6.	*Conidiobolus* sp.	1	0.400
7.	*Aspergillus flavus*	1	0.900
8.	*Scytalidium dimidiatum*	2	0.500; 0.400

Table III. TLC extracts from the fungal samples using ethyl acetate as solvent system.

S/N	Fungus	No. of resolved spots	RF value
1.	*Myrothecium verrucaria*	1	0.966
2.	*Aspergillus niger*	1	0.966
3.	*Stachybotrys alternaris*	1	0.910
4.	*Ulocladium chartarum*	1	0.931
5.	*Syncephalastrum* sp.	1	0.966
6.	*Conidiobolus* sp.	1	0.979
7.	*Aspergillus flavus*	1	0.979
8.	*Scytalidium dimidiatum*	1	0.952

Several studies have demonstrated the importance of insect pests as sources or transmitters of fungal infections. The activities of domestic insect pests such as cockroaches may have a profound effect on human subjects in cockroach infested households, especially if the fungi they carry produce toxigenic metabolites. Cockroaches have been known to produce repulsive odour and allergic reactions amongst other toxic effects (5). A link has also been found between inhaled aflatoxin and liver cancer (6). Considering the above, there is little doubt as to the possible transmission of fungal diseases from cockroaches to humans via the secondary metabolites.

This work highlighted the preliminary extraction of secondary metabolites from arthropod – borne fungi. Further work is focusing on the purification and identification of these compounds. Bioassays may also be carried out to determine the bioactivity of the compounds to evaluate their usefulness in biotechnological applications.

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