Bull. Environm. Contam. Toxicol. 28, 637–639 (1982)

Toxicity of Metabolites from *Rhizopus* Spp.

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THOMPSON & SMALLEY (1980) isolated several fluorescent compounds from the crude extracts obtained from seven fungi of the genus Rhizopus. THOMPSON et al. (1981) identified one of these compounds, a green-fluorescent metabolite, as 1-(2,6,10,14-tetramethyl-17-carbomethyl heptadecyl)-2-(2,6,10,14-tetramethyl-17-methanoyl heptadecyl)-benzene. This report describes the antimicrobial activities of the green-fluorescent metabolite and of the crude extracts.

MATERIALS AND METHODS

Fungi and bacteria were obtained from the American Type Culture Collection (Rockville, MD), the Department of National Health and Welfare (Ottawa, Canada), and Mrs. G. Jackson (Southern University, Baton Rouge, LA). Stock cultures of fungi were maintained at 5°C on slants of potato-dextrose agar (PDA) enriched with 2% yeast extract. Nutrient agar (NA) slants were used to maintain the bacteria cultures.

Fungi were grown by using the cultural procedures described by VAN WALBEEK et al. (1968). Procedures for the extraction, separation and detection of the metabolites were described by THOMPSON & SMALLEY (1980). The purification of the isolated metabolite was described by THOMPSON et al. (1981).

The paper disc assay method described by GHOSH et al. (1978) was used to obtain the antimicrobial spectrum of the crude chloroform fungal extracts and the isolated fluorescent metabolite. Bacteria were subcultured in Trypticase Soy Broth (TSB) for 24 h. Trypticase Soy Agar (TSA) plates were prepared and seeded with 0.1 mL of each bacterial suspension and allowed to solidify. Disks (7 mm in diameter) were touched to the surface of the crude chloroform extract (in chloroform at 1 mg/mL) and the isolated metabolite (in chloroform at 0.2, 0.4, 0.8, and 1.0 ug/mL) so that the absorption was only by capillary action. The disks were then placed on the agar surface. Each sample was assayed in triplicate and incubated

0007-4861/82/0028-0637 $01.00

at 37°C for 24 to 48 h. Appropriate controls were performed with chloroform to determine any effects of this reagent upon growth. A pure sample of aflatoxin G_2, obtained from Sigma Chemical Company, was used as a reference standard. The diameters of the clear zones were measured.

RESULTS AND DISCUSSION

The crude fungal extracts, which contained numerous fluorescent compounds, were observed to affect the growth of Bacillus megaterium. The diameters of the zones of inhibition were not significantly different among the crude extracts (18 mm). The zones of inhibition appeared as halos instead of a clear ring which suggested a bacteriostatic action rather than a bactericidal action. The control disk showed no effect on the organisms. In an effort to determine the nature of inhibition on B. megaterium, cells from the zone inhibition were observed with a phase contrast microscope. Filamentous cells were present after 24 and 48 h, respectively. These filamentous cells reverted to normal growth and division when placed on Trypticase Soy Agar which lacked the crude extract. A similar finding was reported by BEUCHAT & LECHOWICH (1971) when B. megaterium was grown in the presence of aflatoxin B_1. They suggested that aflatoxin B_1 could be binding to the particular locus on the DNA molecule responsible, in part, for the initiation of mesosome development or mesosomal control of cross plate or septum formation, or both. Inhibition of mesosomal function in this manner might still leave the cell capable of synthesizing other cellular components and thus to form filaments.

The green fluorescent metabolite, 1-(2,6,10,14-tetramethyl-17-carbomethyl heptadecyl)-2-(2,6,10,14-tetramethyl-17-methanoyl heptadecyl)-benzene, inhibited the growth of Micrococcus roseus and Candida sp. The metabolite as well as aflatoxin G_2 inhibited the growth of the microorganisms (Figure 1). In each case, the minimum inhibitory concentration was 1.0 ug/mL. The diameters of the zones of inhibition were 24.1 mm and 25.1 mm for M. roseus and Candida, respectively. The action of the green fluorescent metabolite may be somewhat similar to the action reported for benzoates and their derivatives which were used extensively as antimicrobial agents (CHIPLEY & URAIH 1980). The mechanism of action of the green metabolite, like the action of benzoates and their derivatives, is not known at this time and merits additional studies. It is however interesting to note here that aflatoxin G_2, which also showed antimicrobial activity against M. roseus and Candida sp., has been reported to be toxic to ducklings (CARNAGHAN et al. 1963). Therefore final evaluation of the toxigenic potential of the green fluorescent metabolite is yet to be ascertained.

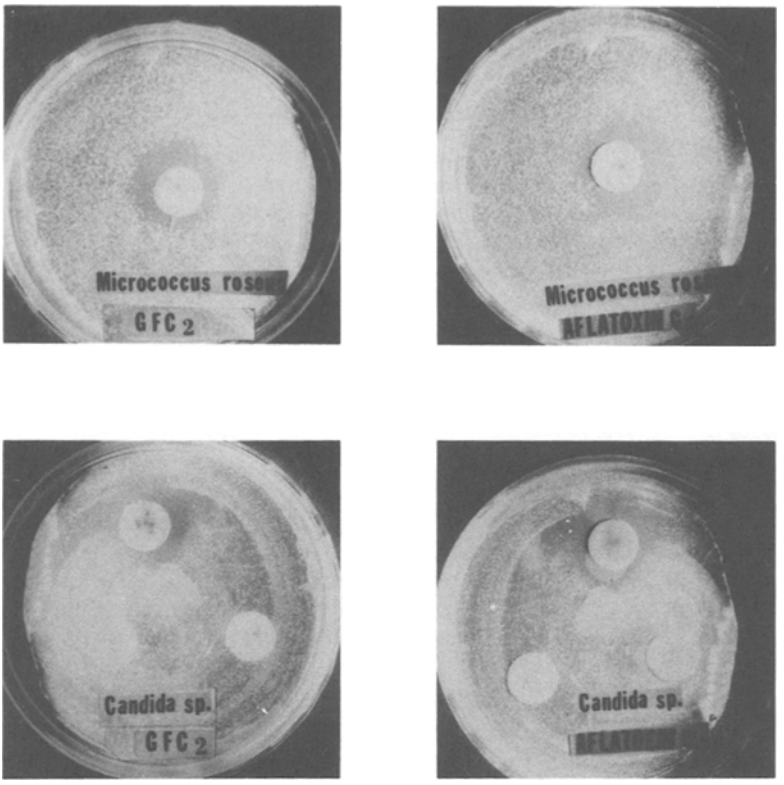

Figure 1. Inhibition of growth of Micrococcus roseus and Candida sp. by an isolated green fluorescent metabolite, 1-(2,6,10,14-tetramethyl-17-carbomethyl heptadecyl)-2-(2,6,10,14-tetramethyl-17-methanoyl heptadecyl)-benzene (GFC_2) and aflatoxin G_2(G_2).

Acknowledgement. Work was supported by Grant RR08025-11 from the National Institutes of Health.

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