Mutagenicity of Stemphyllotoxin III, a Metabolite of Alternaria alternata

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Some common decay organisms of vegetables and ripened fruits are Alternaria species. Even fruits and vegetables kept under refrigeration can be spoiled by Alternaria species because the mold grows at low temperatures. Alternaria alternata is commonly found in grain in areas with a high incidence of esophageal cancer. Three metabolites, altetroxins I, II, and III, have been isolated from A. alternata and have hydroxyperylenquinone structures. Although other perylenquinone metabolites, such as stemphyllerylend and stemphyllotoxins I, II, III, and IV, have been isolated from Stemphyllotous var. lactucum, a plant pathogen and mold, we isolated and identified stemphyllotoxin III from A. alternata. This metabolite was tested for mutagenicity in the Ames Salmonella typhimurium plate incorporation assay with and without Aroclor 1254-induced rat S-9 metabolic activation. A positive response was noted with and without metabolic activation in S. typhimurium TA98 and TA1537, and there was a marginal response in strain TA100.

Molds of the genus Alternaria are widely distributed plant pathogens and decay organisms of fruits and vegetables (7). Alternaria propagules were found in 184 of 230 test samples of grain from the United States (4). Because the mold grows at low temperatures, Alternaria spoilage may also occur in fruits and vegetables kept under refrigeration (12). Thus, small amounts of some toxic metabolites of Alternaria species may be found in food. In less-developed nations, repeated consumption of food heavily contaminated with mold may have chronic toxic effects (7). There is a high incidence of esophageal cancer in areas such as Linxian County, China, where Alternaria contamination of grains is heavier than in areas with a low incidence (2). Dong et al. (5) reported that an extract of an Alternaria culture induced mutations and transformations in mammalian cells and suggested that contamination of grain with metabolites of Alternaria species may contribute to the high incidence of esophageal cancer in China.

Alternaria species produce a wide variety of primary and secondary metabolites, e.g., phytotoxins and mycotoxins (6-9). Among the mycotoxins are several perylenquinone metabolites, designated altetroxins I, II, and III, that have been shown to be mutagenic in the Ames Salmonella typhimurium assay (11). Recently, another perylenquinone metabolite was isolated from A. alternata and identified as stemphyllotoxin III (10). Previously, stemphyllerylend and stemphyllotoxins I, II, III, and IV were isolated from the mold and plant pathogen Stemphyllotous var. lactucum (3). Stemphyllotoxin II is identical to altetroxin II (3, 11). This paper reports on the mutagenicity of stemphyllotoxin III with S. typhimurium TA98, TA100, TA1535, and TA1537.

MATERIALS AND METHODS

Culture conditions and isolation procedure. A. alternata 42 (isolated from cherries) was cultured on autoclaved rice and water and extracted with chloroform as described by Stack and Prival (11). Stemphyllotoxin III occurs as a minor metabolite that is midway in polarity between altetroxin I and altetroxin II in thin-layer chromatographic and liquid chromatographic separations. Isolation by silica gel column chromatography and crystallization from chloroform-hexane yielded 154 mg of a brown microcrystalline solid. The purity of the compound was checked by silica gel thin-layer chromatograph with benzene-methanol-acetic acid (90:5:5) as the mobile phase. After the plate was viewed under UV light, it was dried and exposed to iodine fumes. Stemphyllotoxin III appeared as a single dark spot at an Rf of 0.46 when 10 μg was applied to the plate. No other spots appeared, indicating that the stemphyllotoxin III was free from other mutagenic compounds, including the altetroxins. The other physical properties of the same lot of stemphyllotoxin III were previously described by Stack and Mazzola (10) and are consistent with the structure shown in Fig. 1. This lot of stemphyllotoxin III was used for all mutagenicity tests.

Mutagenicity tests. S. typhimurium TA98, TA100, TA1537, and TA1535 were used to identify reverse mutations from histidine dependence to histidine independence by the plate incorporation method of Ames et al. (1). Bacteria from an overnight culture (0.1 ml), the test substance in dimethyl sulfoxide (0.05 ml), and S-9 mix (0.5 ml when used) were added to 2 ml of molten top agar. The base agar contained 0.5% glucose instead of the 2% glucose used by Ames et al. (1). A total of 50 μl of S-9 derived from the livers of Aroclor 1254-induced male Sprague-Dawley rats was used per plate. Stemphyllotoxin III was unstable in dimethyl sulfoxide, and dark brown decomposition products were formed. Nitrogen was bubbled into the solutions during the test procedures to reduce the amount of oxygen present (oxygen may have been responsible for the decomposition), and the assay was completed as rapidly as possible in a further attempt to reduce decomposition. The results for all tests, which were run in triplicate with concurrent positive (known mutagen) and solvent controls (Table 1), were within 10% of each other. Plates were incubated for 2 days at 37°C, and all colonies were counted manually. A revertant count equal to twice the background count was considered positive. Bacterial cultures were routinely tested for viable counts and sensitivity to ampicillin and crystal violet.

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RESULTS AND DISCUSSION

The results of the mutagenicity assays of stemphytolxin III are presented in Fig. 2 and 3, and the concurrent positive control data for these tests are given in Table 1. Stemphytolxin III was tested at twofold dose intervals from 0.6 to 38.4 μg per plate. Doses of 0.02 to 0.06 μg per plate were also tested and caused no mutagenic response in any of the four strains examined. Stemphytolxin III was highly mutagenic for S. typhimurium TA98 and TA1537 both with and without metabolic activation (Fig. 2 and 3). The mutagenic response to stemphytolxin III (38.4 μg per plate in the presence of S-9) increased 19-fold in strain TA1537 and 7-fold in strain TA98 over that of the dimethyl sulfoxide control. In tests of stemphytolxin III in the absence of S-9, the number of revertants increased 16-fold with TA1537 and 6-fold with TA98 over that with the solvent control. The compound was negative for TA1535 and marginally mutagenic for TA100 with and without activation. Table 2 lists the minimum doses at which the number of revertants per plate doubled with stemphytolxin III and presents, for comparison, the results obtained with altertoxins I, II, and III (11). At stemphytolxin III doses greater than 38.4 µg per plate, toxicity was observed in all S. typhimurium strains, as shown by a decrease in revertant counts. The effect of rat liver S-9 activation is probably due to the ability of the enzymes contained in S-9 to increase the mutagenicity of the epoxide-containing compounds.

The data clearly show that stemphytolxin III is mutagenic in both the presence and the absence of metabolic activation. The doubling dose of stemphytolxin III is, in general, higher than those of altertoxins II and III but is about the same as that of altertoxin I (Table 2). Stemphytolxin III differs from altertoxin II only by a double bond (Fig. 1). The double bond may decrease the mutagenicity of stemphytolxin III by providing an additional reactive site for metabolism by the bacteria to a less mutagenic derivative. Stemphytolxins I, II, and IV are likely to be mutagenic as well, although to our knowledge these mycotoxins have not been tested.

Altertoxins I, II, and III and stemphytolxin III are probably the Alternaria metabolites responsible for the mutations and transformations in mammalian cells reported by Dong et al. (5). Alternariol methyl ether has been reported to be weakly mutagenic (2). Because mutagens are often carcino-

![FIG. 1. Structures of stemphytolxin III and altertoxins I, II, and III.](image)

![FIG. 2. Mutagenicity of stemphytolxin III with S-9. Symbols: ○, TA98; △, TA100; ○, TA1537; ■, TA1535.](image)

![FIG. 3. Mutagenicity of stemphytolxin III without S-9. Symbols: ●, TA98; △, TA100; ●, TA1537; ■, TA1535.](image)
genic, these perylenequinone mycotoxins may increase cancer risk if they are present in the food supply. Further study of these perylenequinone mycotoxins is planned.

REFERENCES


