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# Identification of "Water-Soluble" Toxins Produced by a *Stachybotrys atra* Strain from Finland

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Toxins of a *Stachybotrys atra* strain from Finland proved to be soluble in a simulated gastrointestinal system. They were purified and characterized as satratoxin H, satratoxin G, and an unknown macrocyclic trichothecene with a molecular ion of 528.

The mycotoxicosis in animals and humans (1) called stachybotryotoxicosis is caused by the toxic compounds of the fungus *Stachybotrys atra*. However, all of the *Stachybotrys* toxins identified so far have been isolated by use of organic solvents, which does not reflect the natural situation. The only *Stachybotrys* toxins that have proved to be soluble under physiological conditions (in swine stomach and intestinal fluid) are the "water-soluble" toxins extracted from grain artificially contaminated with a strain of *S. atra* isolated in Finland (6, 7). We attempted to identify the two major toxic fractions of these toxins to determine whether the water-soluble toxins are also macrocyclic trichothecenes, as were the earlier identified *Stachybotrys* toxins, and whether the *S. atra* strain from Finland produces the same toxins as those produced by strains from the United States (2, 3) and Hungary (4).

The toxins were extracted from a laboratory-contaminated oat-wheat-barley mixture with ethyl acetate and purified by silica gel 60 column chromatography as described by Niku-Paavola et al. (5) and Nummi and Niku-Paavola (7). Preparative thin-layer chromatography (TLC) with chloroform-isopropanol (98:2 [vol/vol]) was used in purifying the toxic fractions. Brine shrimp (*Artemia salina*) were used in a bioassay to detect the toxic bands resolved by TLC (4). Two toxic components were found in the less polar toxic fraction; methylene chloride-acetone (8:2 [vol/vol]) gave superior resolution of the two. This system was also used to purify the other water-soluble toxic fraction.

The three toxins were determined to be pure by TLC, high-performance TLC, and high-pressure liquid chromatography (4). The toxins did not fluoresce under UV light but did fluoresce blue when exposed to $H_2SO_4$ and heat. Each toxin quenched fluorescence on Silica Gel 60 $F_{254}$ TLC plates, was positive in the 4-(*p*-nitrobenzyl)pyridine color reaction test, and had an absorption maximum in the range of 255 to 262 nm. Upon hydrolysis with a base, all three toxins yielded verrucarol, as detected by gas chromatography-mass spectrometry for which the selected ion monitoring mode was used (4).

The most polar toxin had a molecular ion of 528 (determined with direct probe) and a mass spectrum and $R_f$ value identical to those of satratoxin H (2), as determined by TLC, high-performance TLC, and high-pressure liquid chromatography. A standard of satratoxin H was supplied by R. M. Eppley, Food and Drug Administration, Washington, D.C. The two other toxins were identified by mass spectrometry, TLC, high-performance TLC, and high-pressure liquid chromatography as satratoxin G (3) and a newly described macrocyclic trichothecene reported by Harrach et al. (4).

It appears that there is no difference between the water-soluble toxins and the most polar macrocyclic trichothecene *Stachybotrys* toxins found earlier. The toxins produced by the *S. atra* strain from Finland were the same compounds isolated from United States and Hungarian strains.

The evidence that the macrocyclic trichothecene *Stachybotrys* toxins can be dissolved under physiological conditions and that strains from different parts of the world can produce the same dangerous toxins supports the causal importance of macrocyclic trichothecenes in stachybotryotoxicosis.

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