

## Sterigmatocystin production by *Aspergillus nidulans*

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Sterigmatocystin (ST), a mycotoxin with the molecular formula C<sub>18</sub>H<sub>12</sub>O<sub>6</sub>, is reported from seven genera of fungi, including 22 species of *Aspergillus* (Cole and Cox 1981 Handbook of Toxic Fungal Metabolites, Academic Press pp 67-93). Sterigmatocystin possesses a bisdihydrofuran moiety like its more potent and biogenetically related mycotoxin, aflatoxin (Fig. 1). This moiety is believed to be the focus of the toxicity, mutagenicity and carcinogenicity displayed by these two secondary metabolites (Pachter and Steyn 1985 Mutation Res. 143:87-91). When grown on complex substrates such as wheat and rice, ST is produced by both agricultural (Schroeder and Kelton 1975 Appl. Microbiol. 30:589-591) and laboratory (Hajjar et al. 1989 Mycol. Res. 93:548-551) strains of *A. nidulans*. In this study, we report ST production on commonly used laboratory media.

Wild type transfers of the Glasgow strain of *A. nidulans* (Pontecorvo et al. 1953 Adv. Genetics 5:141-238) were obtained from Dr. E. Mullaney, Southern Regional Research Center, New Orleans LA (SRRC 273) and Dr. W. Timberlake, Univ. of Georgia, Athens GA (FGSC A4). Other transfers of Glasgow wild type are maintained at the Northern Regional Research Lab. (Peoria IL) as NRRL 194; and at the American Type Culture Collection as ATCC 12996, 26451, and 38163; and at fungal genetics laboratories throughout the world under many other "unofficial stock culture designations. Strain A42355 (NRRL 11440; ATCC 20600), a cilofungin producing wild type, was obtained from Dr. W. Nakatsukasa, Lilly Research Labs, Indianapolis IN.

Triplicate liquid cultures were grown on *Aspergillus* Defined Minimal Nitrate Medium (ADM) (Kafer 1977 Adv. Genet. 19:33-131) or yeast extract (5%)-glucose(20%) medium (YAG) for one week. Petri plates with potato dextrose agar + 5% yeast extract (PDA+YE) were incubated for one month. Control cultures were grown in rice (20 gm Water Maid Enriched + 8 ml deionized water) for one week. All cultures were incubated at 27° or 37°C in the dark without agitation.

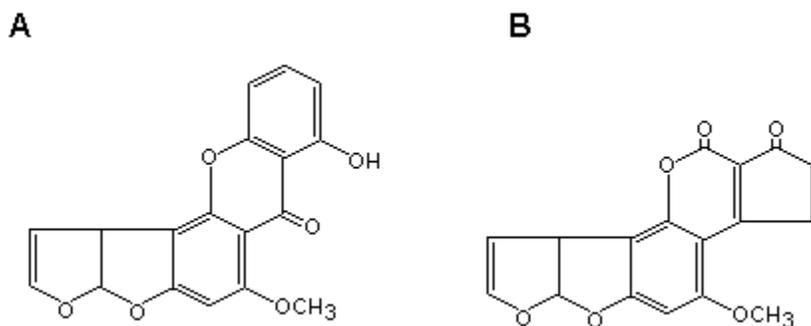


Figure 1: Chemical structures of (A) sterigmatocystin and (B) aflatoxin B1

Sterigmatocystin was extracted by blending total cultures with aqueous acetone, vacuum filtering the resultant slurry, partitioning the extract into methylene chloride, drying under N<sub>2</sub> and resuspending in 1.0 ml of methylene chloride for analyses. For extracts from Petri plates, the entire culture was heated in a microwave until the agar melted prior to extraction. Appropriately diluted extracts in methylene chloride were subjected to thin layer chromatography on Silica Gel G (250  $\mu$ m, EM Science, Merck #5763-7) and developed in methylene chloride:acetone (20:1), sprayed with 20% aluminum chloride in ethanol (vol:vol), and heated at 80°C for approximately 5 minutes to visualize ST. Plates were scanned for fluorescent materials with a Shimadzu dual wavelength LC scanner (CS-930) at 360 nm against authentic standards of ST and O-methylsterigmatocystin (Henderberg et al. 1988 J. Gen. Microbiol. 134:661-667). Sterigmatocystin production is shown in Table I.

**Table I.** Sterigmatocystin production on defined and complex laboratory media.

A. nidulans Strain	Incubation Temperature (°C)	Sterigmatocystin (ug/g)		Rice
		ADM (a)	YAG (b)	
SRRC 273	27	22	ND (c)	301
	37	174	293	180
FGSC A4	27	4	ND	67
	37	72	537	68
Lilly A423255	27	78	ND	46
	37	96	352	28

a. ADM = Aspergillus Defined Minimal Nitrate Medium

b. YAG = Yeast Extract-Glucose Medium

c. ND = None Detected

Sterigmatocystin production was detected on both defined and complex media as well as in control rice cultures. In general, more was produced at 27° C than at 37° C, and on YAG or rice than on ADM. Petri plate cultures of PDA+YE were extracted over a one month period at one week intervals. No ST was detected in the first three weeks from any of the three strains tested. After four weeks, ST was detected from all three strains: SRRC 273 (595  $\mu$ g/g), FGSC A4 (346  $\mu$ g/g), and Lilly A423255 (226  $\mu$ g/g). Occasionally, in our initial experiments, no ST was detected in some replicates of trials in which production had been previously observed. Altering our water supply from a single deionizing column to a five column Modulab Bioscience Research Water Grade System removed this observed anomaly.

The production of ST by these strains has health implications for scientists who routinely handle *A. nidulans* in biotechnological studies. Although the majority of toxicology research has focused on ingestion as the primary mode of contact, ST is also able to act as a proximal carcinogen producing squamous cell carcinomas when applied to the shaved backs of Wistar rats (van der Watt 1977 in Mycotoxins, Purchase, ed., pp. 369-382). Laboratory workers, should, therefore, use caution when handling mycelia of these fungi, and safe disposal of spent cultures should be practiced, similar to the procedures used for other toxigenic or pathogenic fungi.

In summary, these studies confirm widespread suspicions that the Glasgow strain of *A. nidulans* produces toxins in the laboratory, even on defined media.