Growth and Aflatoxin Production by Aspergillus parasiticus from Various Carbon Sources

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R. I. Mateles and J. C. Adye (Appl. Microbiol. 13:208, 1965) reported that aflatoxin was produced by Aspergillus flavus from 14 of 17 carbon sources tested. The highest yields were from sucrose, fructose, and glucose. The organism did not grow when acetate and succinate were the sole sources of carbon. It grew, but produced only small amounts of aflatoxin, on lactose, maltose, xylose, sorbose, glycerol, mannose, or glutamate. Starch, mannitol, sorbitol, and galactose yielded intermediate amounts of aflatoxin, whereas malic acid gave none. H. De Iongh et al. (p. 243, in G. N. Wogan [ed.], Mycotoxins in Foodstuffs, M.I.T. Press, Cambridge, Mass., 1965) reported that the largest amount of aflatoxin was produced from glucose, mannose, fructose, and glyceraldehyde. and that some was produced from galactose, xylose, and possibly ribose. Aflatoxin was not produced from gulose, arabinose, or erythrose. Davis et al. (Mycopathol. Mycol. Appl. 31:251, 1967) obtained highest yields of aflatoxin with glucose, sucrose, fructose, and raffinose. Some aflatoxin was produced with mannitol and galactose, but the fungus failed to grow on lactose.

A. parasiticus Speare var. globosum Murikami (A. flavus ATCC 15517) was used throughout this study. The organism was cultured on 2% yeast extract solution to which the carbohydrate source was added. Where possible, 15% carbohydrate was used. When toxicity or solubility problems were encountered at the 15% level, lesser concentrations of carbon source were supplied. In the case of xylose and ribose, the yeast extract and carbohydrate solutions were autoclaved separately and mixed at the time the organism was added, since the organism failed to grow when the two components were autoclaved together. Flasks of medium were inoculated with spore suspensions and incubated for 6 days at 27 \pm 1 C as stationary cultures. Aflatoxins were determined by thin-layer chromatography (W. A. Pons, Jr., and L. A. Goldblatt, J. Am. Oil Chemists Soc. 42:471, 1965). Analyses were performed on duplicate 1-ml samples of medium, which were extracted with two 25-ml portions of chloroform in

a separatory funnel. Results are means of four replications based on 25 ml of medium per 125-ml flask (Table 1).

Glucose, ribose, xylose, and glycerol were excellent carbon sources for both growth of the organism and aflatoxin production. Oleic and fumaric acids supported good growth, but aflatoxins were not produced. Except for succinic and citric acids, other compounds supported some growth but no aflatoxin production. Generally, compounds that are normally oxidized through both the hexose monophosphate shunt and the glycolytic pathway supported both growth and aflatoxin production. Except for fumaric acid, the organism grew poorly or not at all on the Krebs cycle intermediates. It grew only slightly on terminal compounds of glycolysis, such as pyruvate, acetate, and ethyl alcohol, from

TABLE 1. Growth and aflatoxin production by
Aspergillus parasiticus from various carbon
sources in 2% yeast extract solution (25 ml/125-
ml flask)

None 0.04 0 Glucose, 15% 1.33 3.9 Ribose, 15% 1.32 3.7 Xylose, 15% 1.13 3.5 Glycerol, 15% 1.38 3.2 Oleic acid, 10% 1.08 0 Fumaric acid, 15% 0.80 0	Carbon source Mycelium wt (g/flas	dry Aflatoxins ^a (mg/flask)
Glucose, 15% 1.33 3.9 Ribose, 15% 1.32 3.7 Xylose, 15% 1.13 3.5 Glycerol, 15% 1.38 3.2 Oleic acid, 10% 1.08 0 Fumaric acid, $15\%^b$ 0.80 0	0.04	0
Ribose, 15% 1.32 3.7 Xylose, 15% 1.13 3.5 Glycerol, 15% 1.38 3.2 Oleic acid, 10% 1.08 0 Fumaric acid, 15% 0.80 0	1.33	3.9
Xylose, 15% 1.13 3.5 Glycerol, 15% 1.38 3.2 Oleic acid, 10% 1.08 0 Fumaric acid, 15% ^b 0.80 0	se, 15% 1.32	3.7
Glycerol, 15% 1.38 3.2 Oleic acid, 10% 1.08 0 Fumaric acid, 15% ^b 0.80 0	e, 15% 1.13	3.5
Oleic acid, 10% 1.08 0 Fumaric acid, 15% ^b 0.80 0	rol, 15% 1.38	3.2
Fumaric acid. $15\%^{b}$ 0.80 0	acid, 10% 1.08	0
	ric acid, 15% ^b	0
Shikimic acid, 3% 0.33 0	mic acid, 3% 0.33	0
Sodium acetate, 10% 0.29 0	m acetate, 10% 0.29	0
Pyruvic acid, 5% 0.21 0	ic acid, 5%	Ō
Ethyl alcohol, 3% 0.19 0	alcohol, 3% 0.19	0
α -Ketoglutaric acid, 4% 0.13 0	oglutaric acid, 4% 0.13	0
Succinic acid, 6% 0.04 0	nic acid, 6%	0
Citric acid, 8% 0.03 0	acid, 8% 0.03	Ŏ

 a Approximately 50% aflatoxin B_1 and 50% aflatoxin $G_1.$

^b Solubility of acid was exceeded, but all of the precipitate went into solution during the course of the fermentation.

none of which aflatoxin was produced. Oleic acid, which would normally be converted to acetate when metabolized, supported good growth, but no aflatoxin production.

Evidence presented herein supports the hypothesis (J. C. Adye and R. I. Mateles, Biochim. Biophys. Acta 86:418, 1964) that a carbon com-

pound, to support both aflatoxin production and growth, must be metabolized through both the hexose monophosphate and the classical glycolytic pathway.

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