

*Short communications***Effect of 2-deoxyglucose, α -methylglucoside, and glucosamine on aflatoxin production by *Aspergillus parasiticus***

Abstract. The effects of 2-deoxyglucose (2-DOG), α -methylglucoside (α -MG), and glucosamine (GA) on aflatoxin production by *Aspergillus parasiticus* were studied using conidia-initiated and replacement cultures. In conidia-initiated, 2-DOG, α -MG, and GA supported varying amounts of growth when employed as sole carbon sources. In both conidia-initiated and replacement cultures, 2-DOG, but not α -MG nor GA, as sole carbon sources support toxin formation. None of the compounds inhibited aflatoxin production when used in combination with glucose. It appears that neither 2-DOG, α -MG, nor GA can be considered nonmetabolizable analogs of glucose in *A. parasiticus*.

Key words: Aflatoxins — *Aspergillus parasiticus* — Glucose analogs

It has been well established that the synthesis of aflatoxins is dependent on the identity and concentration of available carbohydrate sources; these compounds supplying the two-carbon units comprising the polyketide skeleton of the toxins and influencing cellular bioregulatory processes to direct synthesis of this class of secondary metabolites (Buchanan and Stahl 1984; and the references cited therein). Recent work in our laboratory on the inhibition of aflatoxin synthesis by caffeine suggested that toxin production is influenced strongly by the rate of carbohydrate transport into the cell (Buchanan and Lewis 1984a). In order to evaluate this hypothesis, work has been initiated to characterize carbohydrate transport in *Aspergillus parasiticus*. As an initial step, efforts have been directed towards identifying suitable nonmetabolizable analogs of glucose. The present study reports on the evaluation of three glucose analogs, 2-deoxyglucose (2-DOG), α -methylglucoside (α -MG), and glucosamine (GA), for their effects on growth and aflatoxin production by *A. parasiticus*.

Materials and methods*Microorganism*

Aspergillus parasiticus NRRL 2999 was employed throughout the study. The mold was maintained on potato

Abbreviations. YES, yeast extract sucrose; PMS, peptone-mineral salts; 2-DOG, L-deoxyglucose; α -MG, α -methylglucoside; GA, glucosamine

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dextrose agar (Difco¹) slants, and spore suspensions prepared as previously described (Buchanan and Lewis 1984b).

Media

Yeast extract sucrose medium (YES) (Davis et al. 1966) and peptone-mineral salts medium (PMS) (Buchanan and Lewis 1984b) were prepared as previously described. The mineral salts mixture of PMS [which per liter consisted of KH_2PO_4 , 10 g; $(\text{NH}_4)_2\text{SO}_4$, 4 g; MgSO_4 , 2 g; trace mineral mix, 1 ml] was employed in conjunction with glucose and the analogs to assess their effects on growth and aflatoxin production. The mineral salts mixture was prepared at double strength, adjusted to the appropriate pH (5.5 or 4.5), and sterilized by autoclaving. Stock solutions (200 mg/ml) of glucose and 2-DOG, α -MG, or GA were prepared and sterilized by filtration. Appropriate amounts of mineral salts mixture, glucose, glucose analogs, and sterile water were then combined to yield desired concentrations of glucose and/or glucose analog.

Culture techniques

The effects of 2-DOG, α -MG, and GA on aflatoxin synthesis were studied using both culture directly initiated from conidia and pregrown mycelia. The conidia-initiated cultures consisted of 50-ml Erlenmeyer flasks containing 10 ml of medium. Each flask was inoculated with 0.2 ml of spore suspension to achieve an inoculum of approximately 2×10^4 conidia/ml, and incubated without agitation at 28°C. After 3, 7, 10, and 14 days, replicate cultures were analyzed for pH, aflatoxin production, and mycelial dry weight.

Pregrown mycelia were studied using the sequential culturing technique of Buchanan and Lewis (1984b). This entailed initially culturing the mold in YES (72 h), disrupting the mycelial pellets, and reculturing in PMS (24 h). The mycelia were then transferred in 2.0 g portions to 50 ml flasks containing 10 ml of basal medium with the desired concentrations of glucose and/or 2-DOG, α -MG, or GA. After being mixed, the cultures were incubated without agitation at 28°C for 72 h, and then analyzed for pH, aflatoxin production, and mycelial dry weight.

Analyses

The pH of the cultures was determined using a pH meter equipped with a combination electrode. The cultures

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Table 1. Growth and aflatoxin production by conidia-initiated cultures of *Aspergillus parasiticus* grown on glucose, 2-deoxyglucose, α -methylglucoside, and glucosamine as sole carbon source

Carbon source ^a	Incubation time (days)	Mycelium dry weight (mg)	pH ^b	Aflatoxin	
				Culture (μ g)	Mycelium (ng/mg)
Glucose	3	86 (10) ^c	2.5 ^c	17.8 (2.6) ^c	207
	7	93 (3)	2.5	20.4 (0.3)	219
	10	90 (6)	2.3	26.8 (0.7)	298
	14	73 (3)	2.4	18.9 (2.1)	258
α -Methylglucoside	3	43 (4)	3.9	ND ^d	0
	7	80 (0)	2.6	ND	0
	10	77 (3)	2.4	ND	0
	14	63 (3)	2.4	ND	0
2-Deoxyglucose	3	40 (2)	4.2	ND	0
	7	47 (3)	3.7	ND	0
	10	67 (3)	2.9	0.6 (0.3)	9
	14	67 (3)	2.3	0.2 (0.1)	3
Glucosamine	3	47 (1)	4.1	ND	0
	7	50 (0)	3.6	ND	0
	10	53 (3)	3.2	ND	0
	14	43 (3)	3.0	ND	0

^a 20 mg/ml

^b Initial pH = 4.5

^c \bar{x} (\pm SEM), $n = 3$ replicate cultures

^d None detected

Table 2. Effect of α -methylglucoside, 2-deoxyglucose, and glucosamine on growth and aflatoxin production by conidia-initiated cultures of *Aspergillus parasiticus* grown in glucose-containing medium

Supplement ^a	Incubation time (days)	Mycelium dry weight (mg)	pH ^b	Aflatoxin	
				Culture (μ g)	Mycelium (ng/mg)
None	4	172 (13) ^c	2.4 ^c	3.4 (1.7) ^c	20
	7	183 (18)	2.0	5.6 (3.9)	40
	10	116 (37)	1.7	4.7 (2.8)	33
α -Methylglucoside	4	191 (49)	2.5	8.5 (1.3)	42
	7	316 (22)	1.9	9.5 (3.5)	31
	10	199 (10)	1.9	3.6 (1.6)	18
Glucosamine	4	161 (56)	2.2	6.2 (2.8)	59
	7	285 (12)	1.9	11.8 (0.7)	41
	10	175 (6)	1.9	5.6 (2.2)	32
2-Deoxyglucose	4	80 (4)	2.8	0.4 (0.1)	5
	7	237 (19)	1.9	8.0 (2.9)	32
	10	188 (5)	1.8	3.2 (0.5)	17

^a Medium contained 30 mg/ml glucose. Analogs added at concentration of 30 mg/ml

^b Initial pH = 5.5

^c \bar{x} (\pm SEM), $n = 3$ replicate cultures

(mycelia + medium) were then extracted and concentrated as previously described (Buchanan and Stahl 1984). The extracts were then analyzed for aflatoxins B₁, B₂, G₁, and G₂ by thin-layer chromatography (Buchanan and Ayres 1976), and quantitated with a fluorodensitometer (model CS-930, Shimadzu, Corporation). The lower limit of detection was approximately 0.01 μ g aflatoxin/culture. Samples were also examined by thin-layer chromatography to determine if there was significant production of interfering compounds (Buchanan and Houston 1982). After extraction the mycelia were collected on individual predried and

weighed filter paper, rinsed with distilled water, and dried for 24 h at 85°C. Mycelial dry weights were then determined gravimetrically.

Results

Conidia-initiated cultures

The dry weight, pH, and aflatoxin production of cultures inoculated with washed conidia, and containing glucose, 2-DOG, α -MG, or GA as sole carbon source is presented in

Table 3. Aflatoxin production by replacement cultures containing various combinations of glucose with α -methylglucoside, glucosamine, and 2-deoxyglucose

Analog	Glucose conc. (mg/ml)	Analog conc. (mg/ml)	Mycelium dry weight (mg)	pH ^a	Aflatoxin/culture (μ g)	
α -Methylglucoside	0	0	170 (20) ^b	7.0 ^b	3 (1) ^b	
		20	160 (20)	6.3	3 (2)	
		40	210 (20)	5.4	2 (1)	
	20	0	320 (80)	6.4	44 (9)	
		20	330 (60)	5.2	69 (2)	
		40	370 (70)	3.8	71 (13)	
	40	0	450 (80)	4.6	127 (7)	
		20	440 (60)	3.0	104 (7)	
		40	440 (60)	3.0	119 (10)	
	Glucosamine	0	0	170 (20)	7.0	3 (1)
			20	260 (70)	5.8	7 (4)
			40	230 (50)	5.1	6 (3)
20		0	290 (40)	7.3	96 (17)	
		20	270 (40)	5.4	152 (7)	
		40	200 (20)	3.8	166 (4)	
40		0	150 (10)	6.1	213 (43)	
		20	250 (60)	2.8	278 (27)	
		40	260 (10)	2.3	288 (16)	
2-Deoxyglucose		0	0	170 (20)	7.0	3 (1)
			20	330 (70)	6.7	102 (6)
			40	290 (30)	3.2	58 (8)
	20	0	330 (40)	6.5	104 (6)	
		20	310 (30)	5.8	126 (30)	
		40	370 (20)	3.5	103 (43)	
	40	0	330 (30)	5.0	197 (15)	
		20	400 (20)	3.3	214 (16)	
		40	400 (20)	2.8	135 (31)	

^a Initial pH = 5.5

^b Each analog examined in a separate set of duplicate experiments. Values are \bar{x} (\pm SEM) of four replicate cultures

Table 1. Growth and acid production were observed with all of the compounds, though differences in the rate and extent of these responses were apparent among the carbon sources. Only glucose supported abundant aflatoxin production, though small amounts of toxin were detected in 10- and 14-day 2-DOG cultures. Neither α -MG nor GA supported detectable levels of aflatoxin synthesis.

When conidia-initiated cultures containing glucose were supplemented with the analogs, neither α -MG nor GA had any inhibitory effect on growth or aflatoxin production (Table 2). Growth and toxin production were depressed in the 4-day 2-DOG supplemented cultures, but this effect was overcome in the 7-day cultures.

Replacement cultures

The glucose analogs were studied further by examining their effects on pregrown mycelia of *Aspergillus parasiticus*. A sequential replacement culture protocol was employed to evaluate various combinations of glucose (0, 10, 20, 30, 40, and 60 mg/ml) and glucose analog (0, 10, 20, 30, and 40, mg/ml). The results observed with the 0, 20, and 40 mg/ml combinations of glucose and analog are presented in Table 3, and are representative of the results obtained with the other concentration combinations.

Mycelial dry weights were largely unchanged over the range of concentrations tested, which is consistent with previous observations that employing high densities of pregrown mycelia results in minimal differentials in mycelial

mass upon transfer and further incubation (Buchanan and Lewis 1984 b). Supplementation with the analogs produced a concentration dependent decrease in the pH of the cultures, which is indicative of increased acid production. However, it should be noted that the pH values observed with the lower levels of carbon source combinations actually represents an increase over the initial pH. Typically in this culturing system, there is a pH decrease as available carbohydrates are utilized, followed by a rise in pH after depletion of the carbohydrate source (Buchanan and Lewis 1984 a).

No significant do novo aflatoxin production occurred in the replacement cultures with α -MG and GA as sole carbon sources. The low levels of toxin detected could be accounted for by carryover from the initial growth in YES (Buchanan and Lewis 1984 b; Buchanan and Stahl 1984). Aflatoxin production was observed in the cultures with 2-DOG as sole carbon source, with maximal production occurring in conjunction with 2-DOG concentrations of 20 mg/ml. In replacement cultures containing both glucose and analog, aflatoxin production was directly related to glucose concentration, and neither 2-DOG, α -MG, nor GA had any inhibitory effect on toxin formation.

Discussion

In establishing that a compound is a nonmetabolizable analog of glucose, one of the major criteria is the inability of the compound to support growth when employed as a sole source of carbon. Using this criterion, it would appear that

neither 2-DOG, α -MG, nor GA can be classified as non-metabolizable. All supported growth to varying degrees, though there was a substantial depression of growth rate as compared to glucose controls. These results were surprising since in the related species, *Aspergillus nidulans*, it was reported that 2-DOG did not support growth and α -MG was not taken up in any significant amounts (Romano and Kornberg 1969).

Due to the dependency of aflatoxin synthesis on the availability of an appropriate catabolizable carbohydrate, it would be expected that the presence of a nonmetabolizable analog of glucose would suppress toxin production. While none of the compounds tested could be classified as nonmetabolizable based on ability to support growth, neither α -MG nor GA supported significant de novo aflatoxin synthesis when employed as the sole source of carbon in conidia-initiated or replacement cultures. This suggests that changes in the mechanism or rate of carbohydrate utilization can strongly affect the synthesis of aflatoxins, and that insights into how carbohydrate catabolism influences the regulation of aflatoxin synthesis may be gained by comparing the means by which the mold utilizes α -MG and glucose.

While α -MG and GA did not support toxin production as sole carbon sources, neither had any inhibitory effect on aflatoxin production when employed in combination with glucose. This lack of inhibition suggests that either glucose and the analogs are transported and/or catabolized by separate systems, or that there is a strong preferential uptake of glucose by a shared transport system. In the latter case, it is possible that the relative concentration of analog to glucose was insufficient to elicit an inhibitory response. For example, Romano and Kornberg (1969) reported that 2-DOG was competitively taken up by *Anacystis nidulans* at only 1/20 the rate of glucose. However, in the present case it is experimentally difficult to increase the ratio of analog to glucose much above that employed in the current study. A

minimal glucose concentration of 10–20 mg/ml is needed to achieve reasonable aflatoxin production by *A. parasiticus*, while total carbohydrate levels above 100 mg/ml would be expected to impede the mold due to depression of water activity.

Determination of the specific effects of 2-DOG, α -MG, and GA on glucose transport in *A. parasiticus* awaits further research, and such studies are currently underway in our laboratory. However, the results of the present investigation indicate that regardless of their effect on glucose transport, these compounds cannot be considered nonmetabolizable in this species. Identification of such compounds will require evaluation of other potential analogs.

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