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Factors influencing fumonisins (B_1) production by *Fusarium moniliforme*

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Abstract

Influence of different media, pH and different nutrients on growth and fumonisins (B_1) production by *Fusarium moniliforme* was investigated. The toxin production was analyzed by Thin Layer Chromatography method. Among different media studied Nash and Synders medium, pH of 7.5 and malt extract at concentration of 0.5% was found to be optimum for maximum production of fumonisins. The study suggests the significance of these factors hence it is concluded that factors should be optimized for maximum production of fumonisins.

Keywords: Fumonisins (B_1), *Fusarium moniliforme,* mycotoxin.

Introduction

Fumonisins are toxic metabolites of a recently discovered family of mycotoxins produced by *Fusarium moniliforme* (Cawood *et al*., 1991). They were first isolated by Gelderblom *et al*. (1988). The fungus *Fusarium moniliforme* occur world wide on corn (zeamays L.) intended for human and animal consumption (Marasas *et al*., 1984). The incidence of *F. moniliforme* in home-grown corn has been correlated with the incidence of human Esophageal Cancer (EC) in the Transkei, southern Africa (Marasas *et al*., 1981; 1982; 1988; Marasas, 1982). Cultures on corn of *F. moniliforme* strain MRC 826, which was originally isolated from corn in a high Esophageal Cancer (EC) risk area in Transkei, have been shown to be hepatocarcinogenic in rats (Jackiewicz *et al*., 1987). The Isolation of a group of structurally related compounds, the fumonisins, from culture material of *F. moniliforme* strain MRC 826 was reported by Gelderblom *et al*., (1988). Six fumonisins mycotoxins have sub sequently been isolated and characterized (Gelderblom *et al*., 1988; Bezuidenhaout *et al*., 1988; Cawood *et al*., 1991). Three of these fumonisin B_1 (FB_1), Fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) have been reported to be the major fumonisins produced in corn cultures of *F. moniliforme* strain MRC 826, while the others, fumonisin B_4 (FB_4), Fumonisin A_1 (FA_1) and fumonisin A_1 (FA_2) are produced in relatively minor quantities (Cawood *et al*., 1991). However, only limited information is available on the factors influencing fumonisns (B_1) production and its management.

Materials and methods

Fumonisins (B1) (Fig.1) was purchased from Sigma-Aldrich, Mumbai, India. Peptone, yeast extract, beef extract, glucose and all other chemicals of highest available purity were obtained from Himedia, Mumbai, India.

The culture *Fusarium moniliforme* was procured from *Fusarium* research center collection (Pennsylvania State University Park, p.a.). Stock cultures were maintained on potato dextrose agar slants at 4°C and subcultured for every 3 months.

Effect of media

To find a suitable media for maximum production of fumonisins, different media were screened. The compositions (per liter) of media used were given below:

Czapek's: $NaNO_3$ 2 g; KH_2PO_4 0.5 g; KNO_3 3 g; yeast extract 7 g; distilled water 1000 ml.

Richard's medium: KNO_3 10 g; KH_2PO_4 1 g; $MgSO_4.7H_2O$ 2.5 g; sucrose 35 g; $FeCl_2$ traces; distilled water 1000 ml.

SMKY: Sucrose 20 g; $MgSO_4.7H_2O$ 0.5 g; KNO_3 3g; yeast extract 7 g; distilled water 1000ml.

YES medium: Yeast extract 20 g; sucrose 40 g; distilled water 1000 ml.

Maize flour: Maize flour 40 g; sucrose 30 g; yeast extract 1 g; distilled water 1000 ml.

Sorghum flour: Sorghum flour 40 g; sucrose 30 g; yeast extract 1 g; distilled water 1000 ml.

Rice flour: Rice flour 40 g; sucrose 30 g; yeast extract 1 g; distilled water 1000 ml.

Asthana & Hawker's: Glucose 5 g; KNO_3 3.5 g; KH_2PO_4 1.75 g; $MgSO_4.7H_2O$ 0.75 g and distilled water 1000 ml.

Malt extract: Glucose 20 g; malt extract 20 g; peptone 1 g; distilled water 1000 ml.

Nutrient agar: Bacto-beef extract 3 g; peptone 5 g; distilled water 1000 ml.

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Glucose asparagines: Glucose 20 g; aspargine 5 g; KH_2PO_4 3.4 g; $MgSO_4.7H_2O$ 1.9 g; NaCl 0.01 g; distilled water 1000 ml.

Nash & Snyder's medium: Bacto peptone 20 g; KH_2PO_4 1 g; $MgSO_4.7H_2O$ 0.5 g; PCNB (Penta chloronitrobenzene) 1 g; agar agar 1 g; distilled water 1000 ml.

Specified quantities of the media ingredients were dissolved in distilled water and 50 ml of each medium was taken in 250 ml of Erlenmeyer conical flasks separately and pH of the media was adjusted to 6 with 0.1 N NaOH, sterilized by autoclaving. After autoclaving the media was cooled to room temperature and the flasks of different media were inoculated with *F. moniliforme* and incubated at 27^{0}C for 20 days. The experiment was performed in triplicates.

At the end of incubation period the mycelial mat was dried and extracted with ethyl acetate in soxhelet apparatus for 24 hours. Ethyl acetate was evaporated to dryness and redissolved in methanol. A portion of methanol solution was spotted on TLC plates and developed in water: methanol (1:3) mixture. The plates thus developed were sprayed with p-anisaldehyde (0.2% in ethanol) solution and heated at 120^{o}C until colour development. Fumonisins appeared as brown colour spot. Fumonisins were estimated quantitatively as suggested by Sydenham *et al.* (1989).

Effect of pH

Studies were performed to determine the suitable pH for higher production of fumonisins by *F. moniliforme*. The experiment was studied at pH viz., 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5 and 10.5.

Influence of nutrients

In order to find the effect of nutrients on fumonisins production, different nutrients viz., yeast extract, peptone, malt extract, beef extract at concentration of 0.5% and 1.0% was studied individually by adding in to the medium.

Results and discussion

Table 1 reveals that the production of fumonisins by *F. moniliforme* varied with the medium. Nash and Snyders medium was the good substratum for production of fumonisins by *F. moniliforme*. Czapek's medium was next preferred substratum for elaboration of fumonisins. YES medium, sorghum flour, rice flour and maize flour medium were almost same in their nutritive value for fumonisins production by *F. moniliforme.* On the other hand, nutrient broth and Asthana and Hawker's medium A were poor substrates and supported only meager amount of fumonisins production.

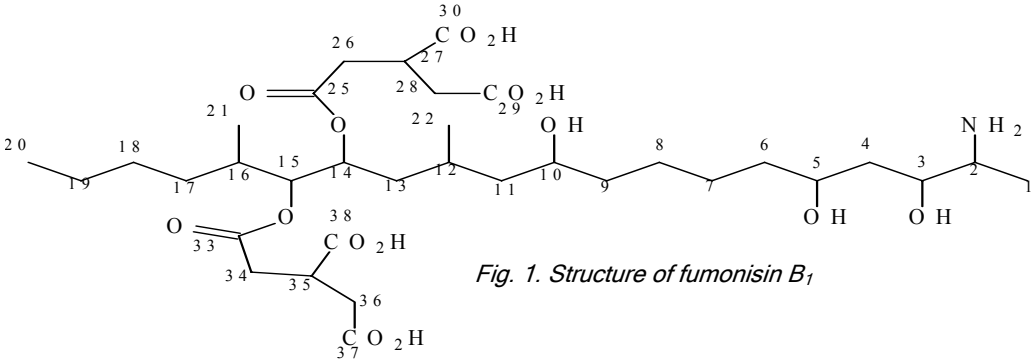

Fig. 1. Structure of fumonisin B_1

Nash and Snyder's followed by Asthana and Hawker's medium A was responsible for good vegetative growth of *F. moniliforme.* Nutrient broth was poor substratum for the vegetative growth and fumonisins production. Czapek's medium, YES medium; sorghum flour, maize flour and rice flour medium were almost same nutrient value for the mycelial growth and fumonisins production by *F. moniliforme.* Glucose asparagine, SMKY medium, Malt extract and Asthana and Hawker's medium A supported moderate amount of mycelial growth. The final pH in SMKY medium was strongly acidic, while in nutrient broth, it was reverse. pH of rest of the media varied and it was in the acidic range. No positive correlation could be observed between fumonisins production and mycelial growth and pH changes. Table 2 reveals that the pH exerted extended significant influence on the growth and fumonisins production by *F. moniliforme.* It could grow in the pH range of 3.5-10.5. No growth of *F. moniliforme* was recorded at pH 2.5. The amount of fumonisins production increased with the increase in pH. Maximum growth was recorded at pH 7.5. The amount of fumonisins production increased with the increase in pH. The mycelial growth decreased with the increase in acidity as well as increase in alkalinity. The fumonisins production was maximum at

Table 1. Influence of different media on growth and fumonisins production of F. moniliforme

Name of the medium	Dry weight (mg/ml)	Fumonisins B_1 (μg/ml)	Final pH
Czapeck's medium	6.12	0.79	5.9
YES medium	6.53	0.72	6.2
Asthana & Hawkers medium A	4.20	0.32	6.0
Malt extract medium	4.32	0.53	5.2
Sorghum flour medium	6.21	0.71	6.6
Rice flour medium	6.31	0.70	5.8
Maize flour medium	6.15	0.73	5.4
SMKY medium	4.20	0.43	4.8
Richard's medium	5.98	0.68	6.1
Glucose asparaginase medium	3.88	0.48	5.8
Nutrient broth medium	2.13	0.21	7.6
Nash & Snyder's medium	6.98	0.85	6.8

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pH 7.5, which decreased both with the increase in acidity or alkalinity. At pH 10.5 only meager amount of fumonisins production could be recorded.

Most of the nutrients added to the medium stimulated fumonisins production (Table 3). The degree of stimulation increased with the increase in their concentration. Malt extract was responsible for maximum production of fumonisins. However, the stimulatory effect of malt extract and beef extract decreased at higher concentration. Interestingly all the microbial nutrients inhibited the mycelium growth of *F. moniliforme*. The inhibitory effect increased with the increase in malt extract and beef extract. On the other hand, yeast extract and peptone stimulated fumonisins production, which increased with the increase in their concentration. The final pH was near neutral. The present observations are in agreement with those of Krishna Reddy and Reddy (1988) who also recorded stimulatory effect of growth substances on CPA production by *P. griseofulvum.* Giridhar and Reddy (1997) have recorded varying effect of growth regulators on citrinin production by *P. citrinum.*

Table 2. Influence of pH on growth and fumonisins production by F. moniliforme

Initial pH	Dry weight (mg/ml)	Fumonisins B_1 (μg/ml)	Final pH
2.5	--	--	2.8
3.5	3.88	0.28	3.8
4.5	3.97	0.33	4.3
5.5	4.28	0.48	5.2
6.5	5.92	0.68	7.0
7.5	6.80	0.94	7.2
8.5	5.30	0.71	8.0
9.5	4.93	0.69	8.4
10.5	1.62	0.11	9.1

Table 3. Influence of microbial nutrients on growth and fumonisins production by F. moniliforme

Nutrient	Conc. (%)	Dry wt. (mg/ml)	Fumonisins B_1 (μg/ml)	Final pH
Yeast extract	0.5	6.20	0.78	7.10
	1.0	6.92	0.81	6.90
Peptone	0.5	5.80	0.83	7.00
	1.0	6.30	0.86	6.80
Malt extract	0.5	8.92	0.91	7.00
	1.0	7.01	0.87	6.90
Beef extract	0.5	7.92	0.89	7.10
	1.0	7.20	0.80	6.70
Control	--	9.20	0.68	6.50

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