Toxicity of Rubratoxin B to Fungi

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SUMMARY

The fungi *Aspergillus flavus*, *A. niger*, *Penicillium expansum*, *Neurospora crassa*, *Cladosporium herbarum*, *Mucor spinosus* and *Rhizopus nigricans* were cultivated (i) in malt extract solutions with various concentrations of rubratoxin B (100, 10, 1 µg/ml) in order to study the hyphal morphology and (ii) on filter discs containing different amounts of the same toxin (100, 10, 1 µg/disc) to detect any influence on sporulation. In the presence of 100 µg toxin/ml the hyphal tips of *A. niger* and *A. flavus* were very often swollen and much septate, or were transformed into giant cells. Plasmolysis often occurred. The germ tubes of *R. nigricans* were much deformed. It is assumed that all these toxic effects were caused by weakening of the hyphal tips by interference with wall synthesis. Rubratoxin B (100 µg/disc) completely inhibited the sporulation of all of the fungi. Lower concentrations diminished sporulation of most of the fungi.

INTRODUCTION

The rubratoxins A and B were isolated by Townsend, Moss & Peck (1966) from cultures of *Penicillium rubrum*. The chemical structure of the two compounds was elucidated by Moss, Robinson & Wood (1967, 1968), Moss, Robinson, Wood, Paisley & Feeney (1968) and Moss, Wood & Robinson (1969) and conditions for biosynthesis of rubratoxin B were reported by Hayes & Wilson (1968). Both substances are potent hepatotoxins to mice and rats (Townsend *et al.* 1966; Edwards & Wogan, 1968). According to Natori *et al.* (1970) *P. purpurogenum* can also produce rubratoxin B.

Very little is known about the toxicity of rubratoxin B to fungi. Using the agar streak method with filter discs, Hayes & Wyatt (1970) could not observe any effect of this compound on the development of some fungi. As it is known that the mycotoxin aflatoxin B₁ can affect fungi in many different ways (Lillehoj, Ciegler & Hall, 1967; Ong, 1970; Reiss, 1970*a*, 1971*a*, *b*), a study was undertaken to establish whether rubratoxin B also can be harmful to fungi. This paper presents information on the influence of rubratoxin B on morphology and sporulation of some fungi.

METHODS

The following fungi were used: *Aspergillus flavus* (IMI 89717; Commonwealth Mycological Institute, Kew, Surrey), *A. niger* (strain D3 from the collection of the Institute of Special Botany, University of Mainz, Germany), *Penicillium expansum* (strain D19), *Neurospora crassa* (strain D12), *Cladosporium herbarum* (strain F4), *Mucor spinosus* (strain B5) and *Rhizopus nigricans* (strain B20). Stock cultures were prepared in Petri dishes on malt extract agar (4%, w/v, malt extract; 0.5% peptone; 2.5% agar).

To detect any influence of the toxin on the morphology of hyphae, the fungi were cultured for 24 h on cover glasses in malt extract solution (1.7%, w/v, malt extract; 0.3% peptone). Solutions with different amounts (200, 20, 2 µg/ml) of rubratoxin B (Makor Chemicals Ltd, Jerusalem, Israel) in 0.3% dimethylformamide (DMF) (2 ml) were added to double-strength malt extract solution (2 ml.) so that the final toxin concentrations were 100, 10 and 1 µg/ml. To avoid its destruction the toxin solutions in 0.3% DMF were not sterilized, but all other solutions, the cover glasses and the moist chambers were sterile. Bacterial growth never occurred. Controls were prepared in a similar way except that either 0.3% DMF without toxin or distilled water was used instead of rubratoxin B solutions. In all test solutions the final concentration of the solvent DMF was 0.15% (v/v) and the final pH was 5.6. The cover glass preparations were inoculated with spores removed from stock cultures by means of a sterile needle and cultivated in moist chambers at 20 to 22 °C in darkness to avoid any destruction of the toxin. For microscopic observation the cover glasses were turned over and laid on slides.

Sporulation of the fungi was studied with filter discs containing rubratoxin B as has been described elsewhere for aflatoxin tests (Reiss, 1971*a*). The toxin was dissolved in acetone and filter discs with 100, 10 and 1 µg toxin were prepared. The controls were filters soaked with acetone and subsequently dried (control a) or discs without any pretreatment (control b). The filter discs containing toxin, malt extract solution and spores as well as the controls were incubated in micro-cultivation chambers (Reiss, 1970*b*) in darkness at 20 to 22 °C.

RESULTS

Influence of rubratoxin B on morphology of hyphae

After an incubation period of 24 h many abnormalities could be observed in *Aspergillus niger*, *A. flavus* and *Rhizopus nigricans*, whereas the other test fungi had grown normally. *Aspergillus niger* hyphae showed the greatest damage. Very often the tips were ruptured and the contents extruded (Fig. 1). Usually the hyphae survived the damage and continued to grow with reduced diameter (Fig. 2). Frequently the ends of short hyphal branches were swollen and much septate or had formed spherical bodies with diameters of 18 to 21 µm (Fig. 3, 4). Such malformations occurred especially often after culture in malt extract solution with 100 µg toxin/ml for 24 h and could also be observed in preparations with 10 µg toxin/ml after 48 h. In both controls the hyphae were normal (Fig. 5).

The morphology of the hyphae of *Aspergillus flavus* was affected by rubratoxin B after 24 h. After culture in nutrient with 100 µg and 10 µg toxin/ml many hyphal branches

Fig. 1. Plasmoptysis in *Aspergillus niger* after one day in malt extract solution with 100 µg rubratoxin B/ml.

Fig. 2. Plasmoptysis and newly formed hypha in *Aspergillus niger* after one day in malt extract solution with 100 µg rubratoxin B/ml.

Fig. 3 and 4: Spherical bodies in *Aspergillus niger* after one day in malt extract solution with 100 µg rubratoxin B/ml.

Fig. 5. Normal hyphae of *Aspergillus niger* after one day in malt extract solution (control).

Fig. 6. Swollen and septate hypha of *Aspergillus flavus* after one day in malt extract solution with 100 µg rubratoxin B/ml.

Fig. 7. Abnormal germ tubes of *Rhizopus nigricans* after one day in malt extract solution with 100 µg rubratoxin B/ml.

Fig. 8. Normal germ tubes of *Rhizopus nigricans* after one day in malt extract solution (control).

Table 1. Intensity of sporulation of fungi after 3 days under the influence of different concentrations of rubratoxin B (expressed in $\mu\text{g}/\text{disc}$)

	Rubratoxin B			control a	control b
	100	10	1		
<i>Aspergillus flavus</i>	—	+	+	+++	+++
<i>A. niger</i>	—	+++	+++	+++	+++
<i>Penicillium expansum</i>	—	+++	+++	+++	+++
<i>Neurospora crassa</i>	—	++	+++	+++	+++
<i>Cladosporium herbarum</i>	—	+++	+++	+++	+++
<i>Mucor spinosus</i>	—	—	+	++	++
<i>Rhizopus nigricans</i>	—	—	++	++	++

+++ , Heavy sporulation; ++ , moderate sporulation; + , mature or developing sporangia rare;
— , no sporulation.

were swollen and much septate (Fig. 6), as with *A. niger*. Other damage was not observed. In both controls the hyphae had developed normally.

The malformations of the hyphae of *Rhizopus nigricans* were of a different kind. After culture for 24 h in malt extract solution with 100 μg toxin/ml all spores had germinated; however, all the germ tubes were short and of irregular shape. Their diameter was much greater than that of normal hyphae (Fig. 7, 8). In solutions with 10 μg and 1 μg toxin/ml as well as in both controls normal mycelium had developed (Fig. 8).

Influence of rubratoxin B on sporulation

Rubratoxin B in high concentration inhibited the sporulation of all the fungi tested as is shown in Table 1.

After cultivation in the presence of 10 μg toxin/disc the conidial heads of *Aspergillus niger* were simpler and the conidiophores of *Neurospora crassa* were less branched than they were in the controls.

DISCUSSION

Rubratoxin B damaged the hyphae of *Aspergillus niger*, *A. flavus* and *Rhizopus nigricans* in several different ways. Of special interest was the bursting of the hyphal tips of *A. niger* and the subsequent extrusion of the cytoplasm. Such plasmoptysis phenomena have been observed in different fungi under the influence of hypotonic solutions, e.g. in *A. niger* (Talburtt & Johnson, 1955), in *Neurospora crassa* (Robertson & Rizvi, 1968) and in *Trichothecium roseum* (Dargent, 1969). Rizvi & Robertson (1965) reported that the hyphae of *N. crassa*, whose tips had burst as a result of the addition of L-sorbose to the culture medium, after recovery began to grow again with reduced diameter. A similar observation was made with *A. niger* under the influence of rubratoxin B (Fig. 2). According to Rizvi & Robertson (1965) sorbose weakens the hyphal apex thus causing plasmoptysis. It is possible that rubratoxin B interferes with wall synthesis thus weakening the hyphal tips and leading to plasmoptysis.

'Giant cells' in *Aspergillus flavus* influenced by aflatoxin have been described by Wildman (1966). It must be assumed that these swellings are the results of a high osmotic pressure of the cytoplasm within a weakened and consequently flexible wall.

The damage to the germ tubes of *Rhizopus nigricans* is similar to that produced in *Thamnidium elegans* by aflatoxin B₁ (Reiss, 1971*b*) and in *Fusarium roseum* by toxic fractions of culture filtrates of *Phialophora cinerescens* (Moreau & Moreau, 1968). These alterations also could be due to interference by rubratoxin B with wall synthesis at the hyphal tips.

Rubratoxin B (100 µg/disc) completely inhibited sporulation of all the fungi tested and 10 µg/disc that of *Aspergillus flavus*, *Mucor spinosus* and *Rhizopus nigricans*. When comparing the influence of rubratoxin B and aflatoxin B₁ (Reiss, 1971*a*) on different fungi it is evident that the former is the more toxic. Rubratoxin B is also a more potent inhibitor of the germination of seeds of *Lepidium sativum* than is aflatoxin B₁ (Reiss, 1971*c*).

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