Photosensitization of Paramecia by aflatoxin

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Cultures of Paramecium caudatum or P. multimicronucleatum incubated with aflatoxin were killed when irradiated with light of wavelength 366 nm. Cultures incubated either with aflatoxin in the dark or in the light with no aflatoxin were not affected.


Des cultures de Paramecium caudatum ou de P. multimicronucleatum incubées en présence d’aflatoxine sont détruites lorsqu’irradiées par la lumière ayant une longueur d’onde de 366 nm. Les cultures incubées, soit à la noirceur en présence d’aflatoxine ou à la lumière mais sans aflatoxine ne sont pas affectées.

Aflatoxin is a potent hepatic carcinogen produced by the mold Aspergillus flavus (1). Several methods using chicken embryos, ducklings, trout, laboratory animals, and bacteria have been used to detect aflatoxins (4). Doniach and Mottram reported a very strong correlation between photodynamic toxicity and carcinogenicity of hydrocarbons (2). This work was extended by Epstein et al. to cover other classes of compounds (3). Any strong carcinogen may therefore at least be suspected of being phototoxic. More importantly, one of the primary mechanisms of photodynamic action involves the transfer of excitation energy from the photosensitizer to the biological substrate (8). Such a transfer has been shown by Neely et al. to occur between aflatoxin B1 and deoxyribonucleic acid (DNA) (5). Accordingly, we have investigated the effect of aflatoxin B1 on paramecia and bacteria in the light and in the dark. It was found that aflatoxin has a photosensitizing effect on Paramecium caudatum and P. multimicronucleatum, but not on Escherichia coli.

Paramecium caudatum and P. multimicronucleatum were obtained from Carolina Biological Supply Co., Burlington, North Carolina, and grown in Carolina’s paramecium medium at room temperature. The aflatoxin B1 was obtained from Calbiochem, Los Angeles, California, and prepared in ethanol at a concentration of 8 x 10^{-4} M. Because of the lability of aflatoxin to room light (5), all procedures were carried out in a darkened room. The aflatoxin solution or an ethanol blank was evaporated to dryness in a quadrant culture dish. Three milliliters of an active culture of paramecia was added to each quadrant of duplicate culture dishes. One dish was placed under a Blak-ray ultraviolet lamp (Ultraviolet Products Inc., San Gabriel, California) which has its principal radiation at 366 nm. A water-filled heat filter was used to eliminate any heating effect by the lamp. The light intensity was monitored with a Yellow Springs Instrument model 65 Radiometer. The other dish was incubated in the dark. The cultures were observed under a microscope at intervals and changes in the movement, shape, and relative numbers of paramecia were recorded. Escherichia coli K12 was grown in a tris(hydroxymethyl)-aminomethane salts medium containing 0.2% glucose (7). Growth was followed by optical density readings (450 nm, 1-cm light path) against

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a water blank in a Beckman DU spectrophotometer. The bacteria were incubated in a water bath (37°C). Exponential growth was reached and maintained for at least three generations before an experiment was begun. The bacteria were harvested by centrifugation and resuspended in Tris medium without glucose. Three milliliters of the suspension was added to each quadrant of a plastic culture dish and incubated as described for the paramecia. At intervals samples of the culture were removed and diluted in saline. Portions of each dilution were mixed with 5 ml of nutrient agar and the mixture was poured over a layer of nutrient agar, incubated at 37°C for 24 h, and the colonies were counted.

Because of the toxicity of ethanol to the paramecia and the limited solubility of aflatoxin in water, it was difficult to prepare cultures of paramecia with high levels of aflatoxin. The most suitable procedure found was to add the paramecia in the culture medium to the dried residue of aflatoxin in the culture dish and incubate the culture in the dark for 1 h. With this procedure aflatoxin dissolved to a concentration of about $2 \times 10^{-5} M$. This level of aflatoxin did not affect the paramecia in the dark. However, when the paramecia were put in the light, they showed photosensitization as evidenced by slowing of their movement after 30 min. After 75 min exposure to the light some of the paramecia were dead and many were swollen and misshapen (Fig. 1). At 135 min of exposure all of the paramecia were dead and had lysed. Paramecia incubated in the light with no aflatoxin were not affected if the light intensity was less than $4.4 \times 10^4$ ergs/cm$^2$ per second. At higher intensities the paramecia were killed by the light. When the light intensity was less than $2.4 \times 10^4$ ergs/cm$^2$ per second, there was no photosensitizing effect. Since the range of irradiation over which the photosensitization was observed was very narrow ($2.4$ to $4.4 \times 10^4$ ergs/cm$^2$ per second), it was possible that the added stress of aflatoxin might have killed the paramecia rather than the photosensitization. This was probably not the case because (1) we have added much higher levels of other compounds such as acrolein, monuron, and rubratoxin B under these same conditions without any effect and (2) with concentrations of dichlone that were one-half the lethal dose ($0.1 \mu g/ml$) there was no killing of paramecia upon irradiation. Increasing the concentration of aflatoxin added to the culture dish did not decrease the time required to kill the paramecia. Once the solution was saturated ($2 \times 10^{-5} M$) the addition of more compound was not effective. This was probably because the additional aflatoxin exists as a suspension, not a true solution, and thus was ineffective. When the concentration of aflatoxin added was lowered to half of saturation value, the photosensitizing effect was not observed. Both P. caudatum and P. multiformicinucleatum were affected to the same extent by aflatoxin. To be certain that the effect observed was not due to the formation of a toxic photoproduct produced as the result of irradiation, 3 ml of the paramecium medium or 3 ml of ethanol was added to the aflatoxin. This solution was irradiated for 3 h and evaporated to dryness. Three milliliters of paramecia was added to the aflatoxin and one culture was reincubated in the light and one in the dark. The cultures in the light were killed at about the same time as those described above. The dark cultures were not affected. These results support the conclusion that aflatoxin can kill paramecia by photosensitization and not by formation of a toxic photoproduct. In summary, to observe the photodynamic effect of aflatoxin on paramecia, it was necessary that (1) the aflatoxin concentration be close to saturation in the medium used and (2) that the light intensity be between $2.4$ and $4.4 \times 10^4$ ergs/cm$^2$ per second. However, with E. coli there was no phototoxic effect of aflatoxin even after 6 h of irradiation.

As a check on our methods we repeated the experiments with E. coli using a suspension of 3,4-benzpyrene which has a well-established photodynamic effect on various organisms. Under the same conditions benzpyrene killed the paramecia in less than 2 min (Fig. 2) and E. coli within a period of 30 min. Because our experiments do not evaluate the effect of ambient oxygen, we are unable to determine whether or not the phototoxicity of aflatoxin B$_1$ is the result of photodynamic action. Reiss has recently reported that P. caudatum was killed within 24 h by $3.2 \times 10^{-5} M$ of aflatoxin under anaerobic conditions (6). Under aerobic conditions the paramecia were not affected by $3.2 \times 10^{-4} M$ of aflatoxin. Under the experimental conditions used in the present study (aerobic), P. caudatum was not affected by aflatoxin B$_1$ unless the culture was irradiated.
Fig. 1. Photomicrograph of *Paramecium caudatum* treated with aflatoxin 90 min after it was incubated in the light. The photomicrograph was taken with a Zeiss photomicroscope.
Fig. 2. Photomicrograph of Paramecium caudatum treated with benzpyrene 5 min after it was incubated in the light.
Notations 1967

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Preservation of Entomophthora protoplasts in liquid nitrogen

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Protoplasts of Entomophthora spp. were satisfactorily preserved in liquid nitrogen after controlled-rate cooling to -40°C. 5% Dimethyl sulfoxide was the preferred cryoprotectant.


Préservation de cultures protoplasmatiques d’Entomophthora spp. dans du nitrogène liquide, après refroidissement contrôlé à -40°C. Excellence du dimethyl-sulfoxide à 5% comme moyen de protection contre le froid.

The recent isolation of a viable protoplast culture from a species of Entomophthora (2) has opened up interesting possibilities in the field of insect pathology. Because of the need for frequent subculturing of the protoplast culture, with the attendant risk of loss through contamination, we sought a method for reliable storage of the organism which would allow ready access to the culture while maintaining cultural and pathogenic characteristics of the organism over long periods of time.

The successful preservation of a variety of materials, including tissue-culture cells, spermozoa, bacteria, and fungi in liquid nitrogen, prompted us to examine the applicability of this method to the protoplast culture.

The protoplasts were grown for 2 days in Grace’s tissue-culture medium as described previously (2) before cryoprotectant was added to the culture flasks. The cultures were then dispensed in 1-ml amounts in 1.2 ml sterile ampoules, the ampoules flame sealed, and either cooled at a controlled rate of about 1° per minute to -40°C by the method described previously (1), or placed directly in liquid nitrogen. Cultures for viability tests were taken from liquid nitrogen after 3, 19, and 40 days, thawed rapidly in warm (30°C) water, and subcultured, using four to five drops of the ampoule contents to inoculate

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