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SENSITIZATION OF *ASPERGILLUS PARASITICUS* SPORES TO GAMMA RADIATION IN PRESENCE OF HYDROGEN PEROXIDE

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AFLATOXINS, produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*, are considered to be the most carcinogenic natural substances. Contamination of different crops by these fungi and aflatoxins is a serious problem all over the world, and no method for an effective aflatoxin control is known to date[1]. Various methods for detoxification of aflatoxin have been described[2]. The effect of gamma radiation on spores of *A. parasiticus* and aflatoxin production has been studied earlier[3,4]. Recently we have reported an effective detoxification system for aflatoxin by the synergistic effect of hydrogen peroxide and gamma radiation[5]. In this communication we report sensitization of *A. parasiticus* spores to gamma radiation in presence of hydrogen peroxide.

A. parasiticus NRRL 3240 was grown on agar containing glucose, 4%, and peptone, 1% (pH 4.5). Spores were harvested after 7 days of growth and the spore suspension was adjusted to 10^7 spores/ml in saline. Irradiation was carried out in a gamma chamber 900 (provided by Bhabha Atomic Research Centre, Bombay, India) at 28 ± 2°C and a dose rate of 6.6 krad/min. The irradiation source was ^{60}Co. Spore suspension, in saline or in 0.1, 1 or 5% hydrogen peroxide, was irradiated at different doses. Small portions of suspension were removed and the surviving population was determined using the standard plate count method. Spores were also incubated in 0.1, 1 and 5% hydrogen peroxide for different times and their viability checked as above. The surviving population was grown on agar plates and screened for mutants unable to produce aflatoxin as described by Aybe *et al.*[6]

The survival curve for spores of *A. parasiticus* irradiated in saline (figure 1) gives a D_{10} value (90% killing) of 38.75 krad. Similar results were also obtained by Sharma *et al.*[3] In our detoxification system[5] we had used 5% hydrogen peroxide and gamma radiation. When irradiation was carried out in presence of 5% hydrogen peroxide, at 50 krad the surviving population was found to be less than 10% (data not presented). Hydrogen peroxide is a widely used surface-sterilizer and disinfectant. Its killing effect on spore-forming and non-spore forming bacteria is well documented[7].

It was shown that 3% hydrogen peroxide could bring down the population to 0.001% in 15 min. The present results (table 1) suggest that hydrogen peroxide is effective against spores of *A. parasiticus*. The surviving fraction of spores after a 10-min incubation in the presence of 5% hydrogen peroxide

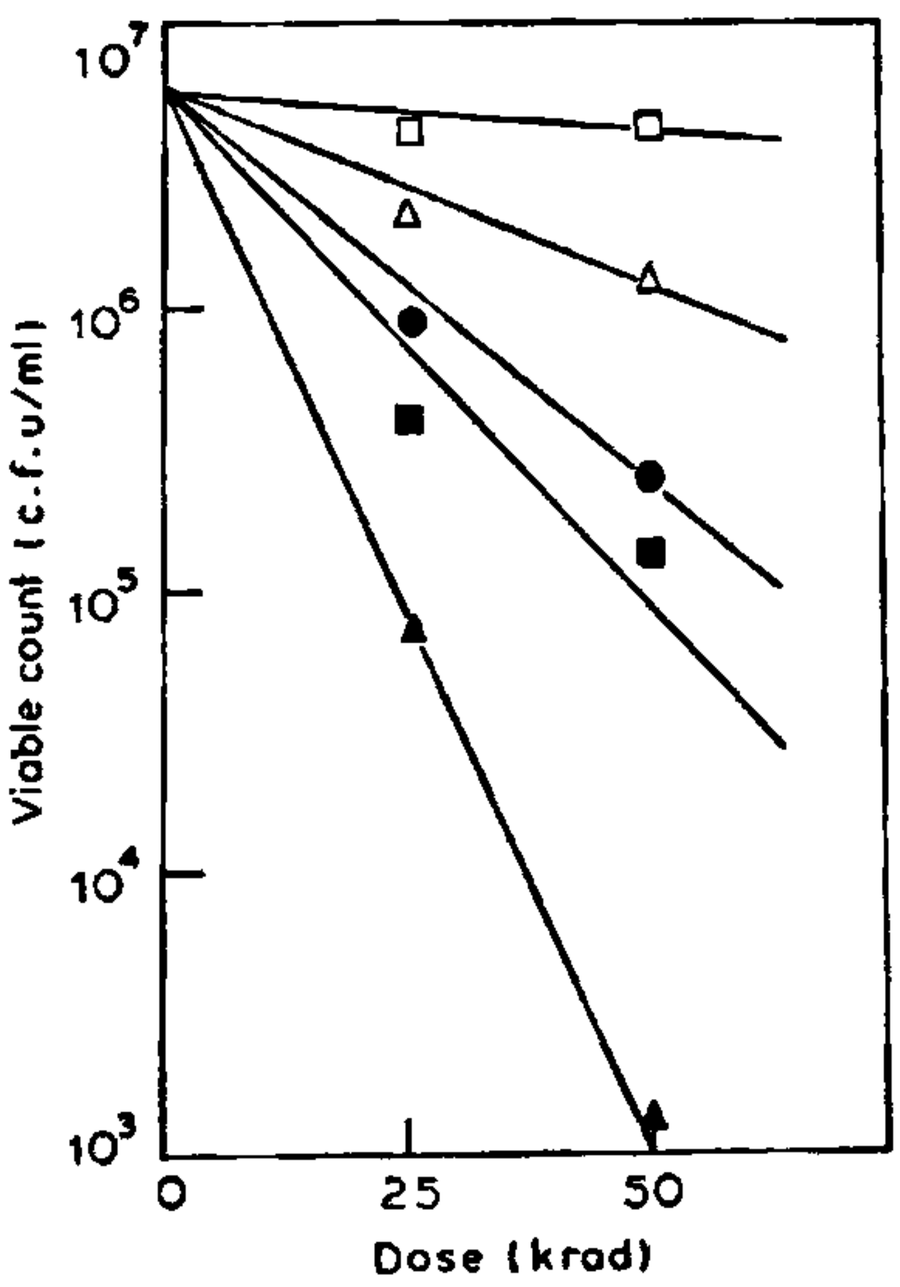

Figure 1. Survival curves for *A. parasiticus* spores irradiated (●——●) in saline, (■——■) 0.1% hydrogen peroxide, (▲——▲) 1% hydrogen peroxide, or incubated without irradiation (□——□) in 0.1% hydrogen peroxide and (△——△) 1% hydrogen peroxide.

Table 1 *Effect of hydrogen peroxide on viability of spores of A. parasiticus*

H_2O_2 (%)*	Viable count	Per cent killing
0	2.5×10^6	0
0.1	1.5×10^6	40
1.0	1×10^6	60
5	1×10^5	96

*Spores were incubated in saline or hydrogen peroxide for 10 min. Viable counts shown are mean of triplicates.

was 4%; in presence of 1 and 0.1% hydrogen peroxide it was 40 and 60% respectively. Spores were also irradiated in presence of 1 and 0.1% hydrogen peroxide and the survival curves were obtained (figure 1). The D_{10} values are 10.6 krad and 30 krad for 1 and 0.1% hydrogen peroxide respectively. Surviving spores were grown on agar and the colonies were observed under UV light for the presence of aflatoxinless mutants. Several colonies were checked but none of them showed the aflatoxinless phenotype. Similar results were also obtained by Moreno *et al.*[8] who nonetheless obtained a few mutants that showed partial reduction or altered aflatoxin production.

The results obtained suggest that hydrogen peroxide alone can act as an effective killing agent in the case of *A. parasiticus* spores. The spores also showed very low resistance to gamma radiation and a low D_{10} value in saline. Hydrogen peroxide is one of the radiolytic products during radiation and was shown to function as a spore radiation sensitizer in the case of bacteria[9]. In the present experiments irradiation and hydrogen peroxide showed additive effects (figure 1). This suggests that hydrogen peroxide might be one of the intermediates during irradiation, and addition of hydrogen peroxide enhances sensitization of the spores to gamma radiation. Earlier observations[6], showed that hydrogen peroxide along with gamma radiation is an effective system for detoxification of aflatoxin in contaminated food. Thus, the use of hydrogen peroxide and gamma radiation for treating contaminated foodstuffs would be economical and effective, as it results in killing of *A. parasiticus* spores and in degradation of aflatoxin.

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EFFECT OF DIFFERENT MEDIA ON THE MORPHOLOGY AND CULTURAL CHARACTERISTICS OF *CANDIDA ALBICANS*

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THE morphology of various species of fungi is significantly affected by different physical and nutrient media[1-7]. In the present study morphology and cultural characters of *Candida albicans* were studied in five nutrient media. The aim of the investigation was to identify those morphological characters that are stable and can be used conveniently for the identification and differentiation of species of *Candida*.

Two male individuals, aged 42 and 20 years, were brought to the laboratory complaining of severe itching on the toes. The interdigital portions were erethematous, and in severe cases the skin had peeled off leaving pink spots. Scrapings were taken from the area and cultured, and pure cultures prepared. The KOH test gave positive results. The isolate was identified on the basis of the criteria suggested by Frey *et al.*[8] The test isolate was grown on five different agar media, viz. malt agar, potato dextrose agar, glucose yeast extract agar, rice agar and Asthana and Hawkers agar, and incubated at