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LOCALIZATION OF UREASE IN *ANABAENA DOLIOLUM*

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THE degradation of urea by micro-organisms is effected by either urease (urea amidolyase, EC 3.5.1.5) or ATP-urea amidolyase (UALase)[1]. The latter enzymatic process, also found in yeast and green algae, involves two distinct enzymatic activities: urea carboxylase (urea: CO_2 ligase (adenosine 5-diphosphate forming), EC 6.3.4.6) and allophanate hydrolase, EC 3.5.1.13. All blue-green algal species studied so far contain urease[2–4]. Urease from a bacterial system is a soluble enzyme located in the cytoplasm[5]. The present paper describes the localization of the enzyme urease in the blue-green alga, *Anabaena doliolum*.

A. doliolum was grown in modified Chu No. 10 medium[6] supplemented with 1 mM urea. The cultures were incubated at 24 ± 1°C and illuminated with daylight fluorescent tubes (intensity 2500 lux on the surface of the vessels) for 14 h per day. For preparation of cell-free extract, exponentially growing algal cultures collected by centrifugation were washed repeatedly with sterile distilled water and finally with 5 mM phosphate buffer, pH 7.3. The cells were ground in a pre-chilled glass mortar and pestle at 4°C in an ice bath with an equal volume of acid-washed sand. Crude enzyme was extracted with 5 mM phosphate buffer, pH 7.3. The supernatant collected after centrifugation was used for estimation of urease activity. For preparation of spheroplasts, algal cells were treated with ethylenediaminetetraacetic acid–lysozyme according to Neu and Heppel[7], with the modification that a 20% glycerol solution was substituted for sucrose solution to protect the spheroplasts[5]. Urease was estimated by determining the amount of urea decomposed within a given period of time at 37°C (pH 7.3). The reaction mixture contained 2 μmoles of urea in a final volume of 2 ml of 5 mM phosphate buffer (pH 7.3). The reaction was initiated by the addition of cell-free enzyme extract and terminated by the addition of 4 ml of mixed reagent used for the colorimetric assay of urea[8]. Protein was determined by the method of Lowry *et al.*[9] using bovine serum albumin as the standard.

Table 1 *Urease activity in whole cell extract and spheroplasts of Anabaena doliolum*

Fraction	Urease activity (nmol urea hydrolysed per mg protein per h)	Activity as percentage of that in cell extract
Whole cell extract	652.0	100.00
Spheroplast lysate	591.0	90.64
Supernatant (glycerol–tris buffer after sedimentation of spheroplasts)	15.0	2.30

Table 1 gives urease activity in whole cell crude extract, spheroplast lysate, and supernatant obtained after sedimentation of spheroplasts. Since most (90%) of the urease activity was found in spheroplast lysate (released from spheroplasts after osmotic shock and lysis), urease of *A. doliolum* is present in spheroplasts as a soluble enzyme.

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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

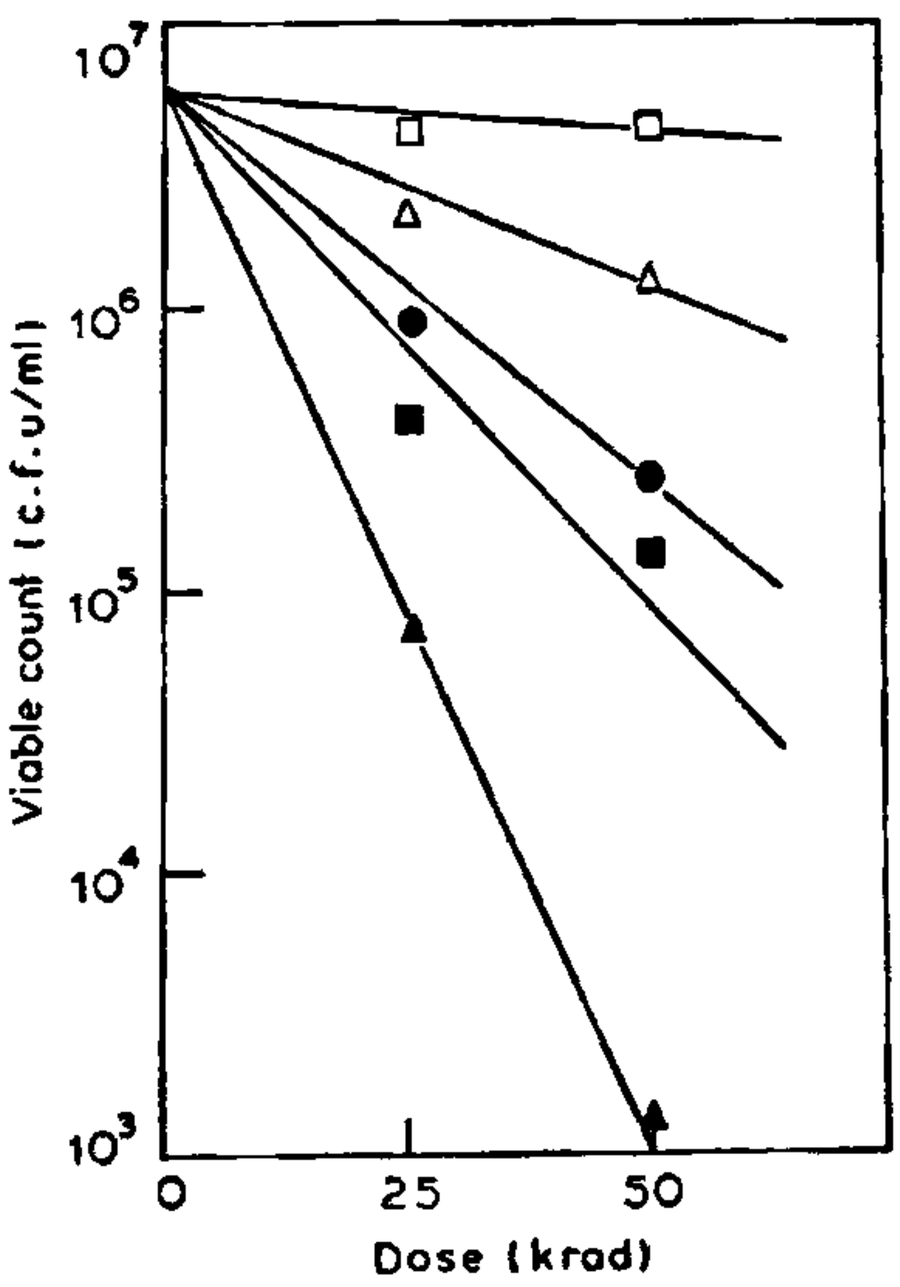

Figure 1. Survival curves for *A. parasiticus* spores irradiated (●——●) in saline, (■——■) 0.1% hydrogen peroxide, (▲——▲) 1% hydrogen peroxide, or incubated without irradiation (□——□) in 0.1% hydrogen peroxide and (△——△) 1% hydrogen peroxide.