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The Effect of Caffeine and Light on Killing of the Blue-green Alga *Anabaena doliolum* by Ultraviolet Radiation

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Summary. Pretreatment of spores of the blue-green alga *Anabaena doliolum* with caffeine is antagonistic to UV lethality and posttreatment with caffeine is synergistic to UV lethality and mutagenicity. The results of photoreactivation experiments suggest that photoreactivation is independent of photosynthesis in blue-green algae.

Recent studies on UV survival characteristics of various blue-green algae under different conditions indicate the presence in them of reactivation phenomena analogous to the dark repair (excision repair) and photoreactivation found in bacteria and other microorganisms (Wu *et al.*, 1967; Singh, 1968; Van Baalen, 1968; Singh *et al.*, 1969; Asato and Folsome, 1969). Werbin and Rupert (1968) have provided biochemical evidence suggesting the presence of photorepair enzyme system in blue-green algal cells.

While these studies do demonstrate the involvement of environmental factors in the control of UV killing in blue-green algae, virtually nothing is known about the nature of UV photoproducts, its targets in general and the biochemical events leading to fixation of UV effects. Nevertheless, a multiplicity of factors affecting UV damage and its repair systems has to be considered and studied in detail before a broad picture of the general effects of UV radiation in blue-green algae can be envisaged.

In this paper, we report the results obtained following pre-and/or post-irradiation treatment of the spores of *A. doliolum* with caffeine and blue, red and white light.

Material and Methods

The general methods of culture and maintenance of *A. doliolum* and of UV irradiation including its source and dose were the same as described previously (Srivastava and Kumar, 1969). The stock solution of 0.1 % caffeine was prepared in sterilized distilled water.

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The experimental samples consisted of washed suspensions of spores derived from axenic and clonal cultures and the caffeine dose-effect curve was determined by colony count method.

Blue and red lights were procured by covering the experimental cultures with a single layer of blue and red cellophane respectively and exposing the cultures to tungsten lamps emitting a radiation dose of 3×10^4 ergs/cm^2/sec at a distance of 40 cm from the source. The transmission spectra of the coloured cellophanes used were similar to those described by Wu *et al.* (1967).

In caffeine pretreatments, the caffeine sensitivity of spores was determined by incubating them in the aqueous stock solution of caffeine for different time intervals upto 5 hours. The results obtained suggested even a maximal dose of 5 hours to be practically nonlethal to the spores. Nevertheless, in order to ensure a sufficiently high survival, spores were routinely treated with caffeine for only three hours.

The postirradiation study was conducted by inoculating irradiated spores on culture medium supplemented with up to 220 μg caffeine/ml. That this dose of caffeine is also practically nonlethal was ascertained from the study of the caffeine dose-effect curve.

The post-irradiation experiments with different spectral regions of white light (= tungsten light) were performed by inoculating irradiated samples onto culture plates covered with blue or red cellophane. The controls run were of two kinds, one series after 24 hours of incubation in the dark was transferred to white light while the other was directly transferred to white light. Blue and red light cultures were also transferred to white light following their incubation in the dark for 24 hours.

The size of inoculum was nearly 10^4 spores per plate. The intensity of different lights was kept nearly uniform by adjusting the distance of cultures from the light source.

Results

Caffeine Sensitivity. Fig. 1 shows the sensitivity curve of spores plated on different concentrations of caffeine. Evidently the alga tolerates fairly high concentrations of caffeine. There is virtually no killing in upto 200 μg caffeine/ml. But this dose of caffeine did induce a significant delay in the timing (onset) of sporulation. However, none of the caffeine doses used delayed heterocyst formation or affected heterocyst frequency. No non-sporulating mutants could be detected following caffeine treatment.

UV Sensitivity. The alga is apparently very resistant to UV killing as its D_{37} (dose for 37 % survival) is nearly 7632 ergs/mm^2/sec (Fig. 2). The survival curve is of the sigmoidal type with a prominent shoulder at lower UV doses. This clearly suggests a threshold action of UV on spores. No nonsporulating mutants could be detected following a single exposure of the spores to UV radiation. The surviving colonies did not show any significant change in their morphology except size; most of the colonies were macrocolonies but about 10 % of them turned out to be microcolonies. Heterocyst differentiation was significantly delayed and the extent of this delay depended upon the UV dose used.

Post-treatment with Light. The D_{37} of spores post-treated with white, blue and red light were 15840, 13684 and 8640 ergs/mm^2/sec respectively.

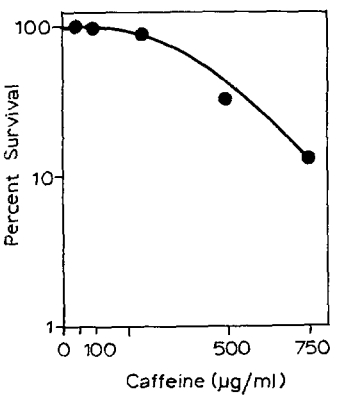

Fig. 1

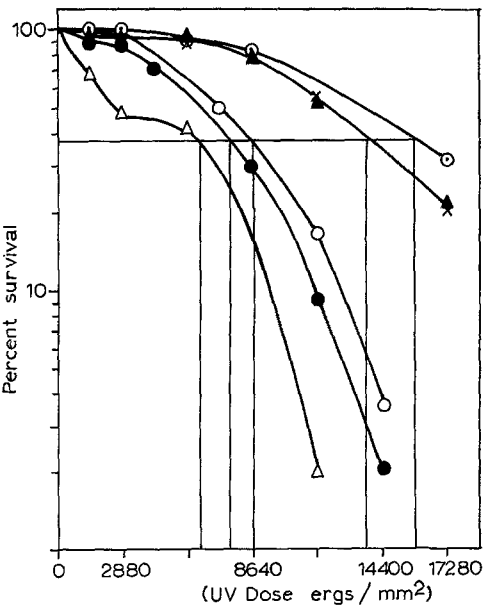

Fig. 2

Fig. 1. Caffeine survival of spores of *Anabaena doliolum*

Fig. 2. Pre- and post-irradiation factors affecting spore survival of *Anabaena doliolum*; irradiated dark control (•——•); pretreatment with caffeine (▲——▲); post-treatment with caffeine (△——△), blue (⊙——⊙) and red (○——○) light

This indicates that post-treatment with white light causes considerable increase in per cent UV survival over its irradiated dark control (Fig. 2). Blue light is more or less similar to white light in this respect. Red light also promotes UV survival but its action is feeble in comparison to white or blue light.

Pretreatment with Caffeine. Caffeine pretreated spores show significant increase in their resistance to UV killing and the extent of this increase is comparable to that observed with post-treatment with white or blue light (Fig. 2). All the surviving colonies differentiated heterocysts and spores. There appeared no significant change in their morphology except for that observed with UV alone.

Posttreatment with Caffeine. The posttreatment of spores with caffeine enhances their UV sensitivity (Fig. 2). Clearly a kind of lethal synergism between UV and caffeine cannot be overlooked. In the course of this study, it was observed that a few colonies on various plates did not sporulate when the majority of them had undergone sporulation. The frequency of such nonsporulating colonies could tentatively be estimated

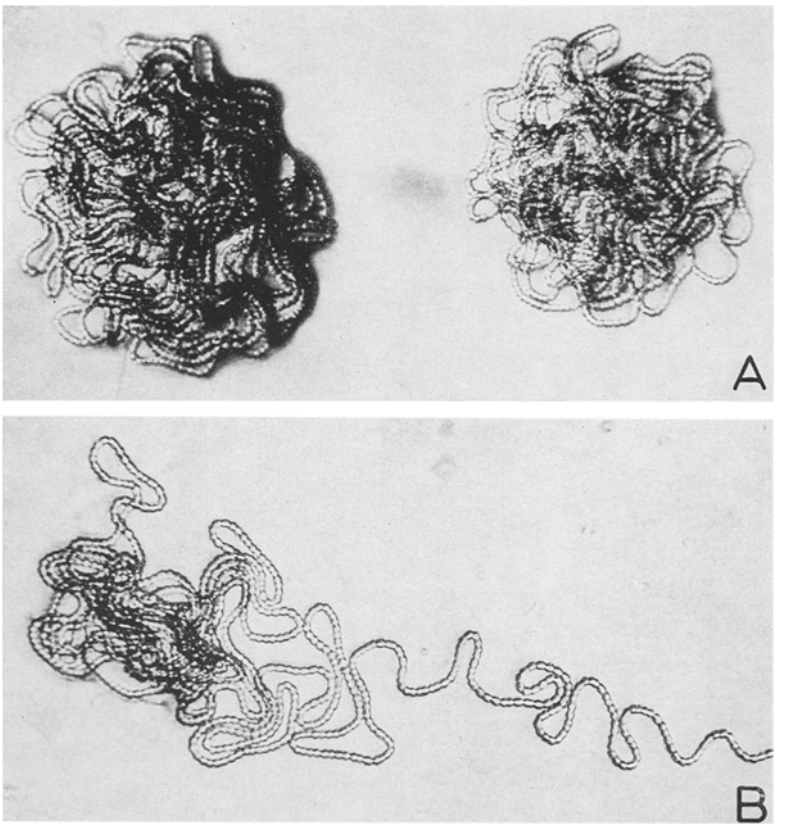

Fig. 3. A The circular colonies characteristic of unirradiated parent; B The "spreading" colonies as observed in irradiated population

to be 0.4% on the basis of survivors. These colonies retained the non-sporulation character despite several subcultures in the basal culture medium. A few of the irradiated spores were observed to form "spreading" type of colonies rather than the circular or oval colonies characteristic of the parent (Fig. 3). Such variant colonies when regrown individually on fresh medium reverted back to the parental type. The per cent frequency of the variant (spreading) colony was nearly 0.9.

Discussion

Pyrimidine dimers produced following UV irradiation are the principal cause of UV lethality in microorganisms. Photoreactivation and dark or excision repair promotes UV survival by eliminating these dimers

from the DNA. The post replication repair reduces UV lethality through a recombinational process in which the single strand gaps induced by unrepaired pyrimidine dimers in replicating DNA are eliminated (see Witkin, 1969).

Caffeine seems to act as an inhibitor of dark repair and/or post-replication repair in *Escherichia coli* since its application in the post-irradiation medium causes considerable increase in UV lethality and mutagenesis. The survival characteristics of spores post-treated with caffeine clearly indicate that caffeine is an inhibitor of repair systems in blue-green algae as well. This view is supported by the observation that only post-treatment of spores with caffeine could lead to the production of nonsporulating mutants. Thus the observed lethal and mutational synergism between caffeine and UV radiation in *A. doliolum* seems consistent with the known role of caffeine as an inhibitor of dark repair system in other procaryotic organisms. Curiously, pretreatment with caffeine is antagonistic to UV killing. The basis underlying the protection of UV-sensitive targets in spores of *A. doliolum* pretreated with caffeine is obscure.

Blue light does include certain spectral bands absorbed by photosynthetic pigments. The spores incubated in blue light germinated but did not grow and divide into mature filaments. Perhaps the blue light is not photosynthetically sufficient to support the further growth and development of *A. doliolum*. On the contrary, red and white light both support its normal growth and development (Kaushik and Kumar, 1970). The maximum photorecovery observed with green or white light and not with red light indicates the involvement of nonphotosynthetic light in photoreactivation. Further, Fay (1969) has reported the absence of photosynthetic pigments and photosynthetic carbon metabolism in the spores of *Anabaena cylindrica*. *A. doliolum* spores under normal photosynthetic conditions usually take 36—48 hours for the formation of photosynthetic pigments (Kaushik and Kumar, 1970). Since the post-treatment with blue or red light in our experiments was only of 24 hours duration, it would not be likely for the spores to have formed photosynthetic pigments by then. Thus despite the presence of photosynthetic spectral regions in photoreactivating blue light, one may reasonably conclude that photoreactivation in *A. doliolum* is independent of its photosynthetic metabolism. Moreover the basis underlying the blue light and not red light treated spores showing a percent photorecovery more or less identical with that shown by white (photosynthetic) light cannot be explained in any other way except that photoreactivation in *A. doliolum* does not depend upon photosynthesis. In this respect our results agree with the view of Wu *et al.* (1967) that photoreactivation in blue-green algae is independent of photosynthesis.

References

Asato, Y., Folsome, C. E.: Mutagenesis of *Anacystis nidulans* by N-methyl-N′-nitro-N-nitrosoguanidine and UV irradiation. Mutation Res. **8**, 531—536 (1969).

Fay, P.: Metabolic activities of isolated spores of *Anabaena cylindrica*. J. exp. Bot. **20**, 100—109 (1969).

Kaushik, M., Kumar, H. D.: The effect of light on growth and development of two nitrogen fixing blue-green algae. Arch. Mikrobiol. **74**, 52—57 (1970).

Singh, H. N.: Effect of acriflavine on ultraviolet sensitivity of normal, ultraviolet-sensitive and ultraviolet-resistant strains of a blue-green alga, *Anacystis nidulans*. Radiat. Bot. **8**, 355—361 (1968).

— Kumar, H. D., Prakash, G.: Post-irradiation modification of ultraviolet sensitivity of normal and nitrofurozone-resistant strains of the blue-green alga, *Anacystis nidulans*. Radiat. Bot. **9**, 105—110 (1969).

Srivastava, B. S., Kumar, H. D.: Inactivation and killing of the blue-green alga *Anabaena doliolum* grown on ultraviolet irradiated culture medium. Radiat. Bot. **9**, 291—295 (1969).

Van Baalen, C.: The effects of ultraviolet-irradiation on a coccoid blue-green alga: Survival, photosynthesis, and photoreactivation. Plant Physiol. **43**, 1689—1695 (1968).

Werbin, H., Rupert, C. S.: Presence of photoreactivating enzymes in blue-green algal cells. Photochem. Photobiol. **7**, 225—230 (1968).

Witkin, E. M.: Ultraviolet induced mutation and DNA repair. Ann. Rev. Microbiol. **23**, 487—514 (1969).

Wu, J. H., Lewin, R. A., Werbin, H.: Photoreactivation of UV-irradiated blue-green algal virus LPP-1. Virology **31**, 657—664 (1967).

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