

### Photodynamic Inactivation and Reversion in *Fusarium* Species with Acriflavine

Acriflavine (AF), a member of acridine dyes, is known to act with DNA molecule<sup>1</sup>, stretching its helical structure by intercalation between the bases<sup>2</sup>. BRENNER et al.<sup>3</sup> proposed that acridines induce mutations by causing deletions or additions of a single base pair during replication. Inactivation and mutagenesis due to photodynamic action of acridines and related dyes on extracellular bacteriophage T4B<sup>4</sup> and in bacterial systems have already been demonstrated. The use of AF to select resistant mutants in fungi and respiratory mutants in yeasts has been quite common; however, its effect on the members of the genus *Fusarium* has not been demonstrated so far. In the present study AF has been used against an auxotrophic mutant of *F. redolens* Wr. isolated through UV-irradiation<sup>5</sup> and characterized by adenine deficiency (R79, aden<sup>-</sup>), widespread and purple-red colonies.

AF solution was prepared in sterile distilled water and stored in complete darkness. A measured quantity of the solution was added to the autoclaved complete medium (NaNO<sub>3</sub>, 2 g; KH<sub>2</sub>PO<sub>4</sub>, 1 g; KCl, 0.5 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g; FeSO<sub>4</sub>, in trace; glucose, 30 g; agar agar, 20 g; and distilled water, 1000 ml) and plated in the petri-dishes. Spore suspension of the above mutant was prepared in sterile distilled water. A known concentration of spores, after measuring with haemocytometer slide, was spread on the complete agar medium already impregnated with

acriflavine and incubated at 27 ± 2°C in continuous light for 24 h with a light intensity of 120 lux. A similar set of petri-dishes was incubated in complete darkness at the same temperature. A control without AF was run for each experiment separately for comparison. Table I shows the comparative observations on the survival of the spores in different conditions.

The results indicated that the action of AF was more lethal in the presence of light as against darkness. Similarly several sets of plates with different concentrations of AF were exposed to light at different periodicity at a light intensity of 1200 lux. The treated spores germinated and formed visible colonies after 3–4 days after inoculation, whereas untreated spores did so after 2 days. During such treatment, a number of revertants were recurrently observed and designated as follows: (1) white fluffy and non-sporulating (WFa); (2) white fluffy with abundant sporulation (WFb); (3) white ropy with the loss of purple-red colour (WR); (4) red mycelial (RM).

The results presented in Table II show the survival and reversion at different AF concentrations. The frequency of revertant type 3 was quite high at all 3 AF concentrations, whereas other revertant types were comparatively low. These revertants differed from the wild type in the morphology and nutritional behaviour of the colonies. Reversion to true wild type (back mutation) was not discernible. On the basis of the present observations it was not possible to conclude the qualitative nature of the revertants. Partial reversion at the adenine locus or reversion at a different locus causing variability in the colony cannot be explained in the organism under study as due to lack of a sexual phase.

**Zusammenfassung.** Es wird erstmals an einem Pilz gezeigt, dass auch bei *Fusarium* infolge der inaktivierenden und mutagenen Wirkung von Acridinen zwischen einer photodynamischen Wirkung im Licht und einer schwächeren, auf anderen Mechanismen beruhenden Wirkung im Dunkeln unterschieden werden kann.

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Table I. Effect of AF on R79 aden<sup>-</sup>, widespread and purple-red mutant of *Fusarium redolens* wr. in light and darkness

In light AF (μg/ml)	Survival (%)	In darkness AF (μg/ml)	Survival (%)
1	100	1	100
5	14	5	60
10	0.5	10	36
15	0.1	15	2.4
20	0.06	20	1.1

Table II. Effect of acriflavine showing survival and reversion in *Fusarium redolens* wr. in total darkness

AF concentration (μg/ml)	No. of spores treated	Survival (%)	Reversion among survivors (%)			
			Revertant types			
			1	2	3	4
10	2000*	36	0.13	0.13	37.5	~
15	2000*	2.4	—	—	33.3	~
20	2000*	1.1	—	—	22.7	4.5

\* Represents the total number of 5 replicates.

<sup>1</sup> E. CHARGAFF and J. N. DAVIDSON, *The Nucleic Acids* (Academic Press, New York 1955), vol. 2.

<sup>2</sup> L. S. LERMAN, *Natn. Acad. Sci., USA*, 49, 94 (1963).

<sup>3</sup> S. BRENNER, L. BARNETT, F. H. C. CRICK and A. ORGEL, *J. molec. Biol.* 3, 121 (1961).

<sup>4</sup> C. M. CALBERG-BACQ, M. DELMELLE and J. DUCHESNE, *Mutation Res.* 6, 15 (1968).

<sup>5</sup> U. P. SINGH and G. M. HOFFMANN, *Arch. Mikrobiol.* 67, 293 (1969).

### Esterase Alterations in the Liver of Dystrophic Mice

We wish to describe a number of changes which occur in esterase zymograms of livers from mice with muscular dystrophy. Hereditary muscular dystrophy in the mouse is caused by an autosomal recessive mutation, designated

by the gene symbol, *dy*<sup>1</sup>. We and many others are extensively studying various aspects of this disorder<sup>2-6</sup>.

In an ongoing investigation of the esterase activity and isozyme patterns of organs from a number of hereditary