The Induction of Pigmentation Change in a Non-acid-fast Strain of *Mycobacterium phlei* by Ultraviolet Radiation

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ABSTRACT. Five scotochromogenic mutants and 11 achromogenic mutants were induced by UV irradiation of the non-acid-fast photochromogenic PN strain of *Mycobacterium phlei*. Spontaneous scotochromogenic and achromogenic mutants were not obtained. Colonies of the scotochromogenic mutants are orange, except for one mutant which is ochre. Three mutants are resistant to STM. Out of 11 achromogenic mutants 3 were induced by UV treatment of the original photochromogenic strain, 8 were prepared from the scotochromogenic mutant. No significant differences in the sensitivity to UV rays were found among the scotochromogenic mutant, achromogenic mutant and the photochromogenic PN strain of *Mycobacterium phlei* under the given experimental conditions. Scotochromogenic mutants and most achromogenic mutants are stable and suitable for further genetic investigation. Pigmentation changes can be used as genetic marker in mutation studies.

The induction of scotochromogenic and achromogenic mutants of the PN strain of *Mycobacterium phlei* by UV rays was studied in order to find out whether pigmentation changes can be used as genetic marker in the given model microorganism. Pigmentation changes are known but we do not know if they are physiological or genetic. Reasons for the study of mutagenesis in *Mycobacterium phlei* have already been presented (Radochová, Koníček & Málek, 1966). Pigments of mycobacteria are mostly of carotenoid nature (Goodwin, 1952; Penso, 1951), however, their biological role remains unclear.

MATERIALS AND METHODS

A photochromogenic non-acid-fast strain of *Mycobaterium phlei* (PN strain) obtained by Hubáček and Málek (1958) was used in the study. Biochemical and serological characteristics of this strain as well as the contents of bases in deoxyribonucleic acid were given by Rytíř *et al.* (in press 1968).

A liquid Dubos medium containing Tween 80 (0.05% final concentration) and a solid medium with tryptose (Bacto tryptose Difco 15.0, NaCl 0.2, K_2HPO_4 0.2, distilled water ad 1,000 ml, pH adjusted to 7.2, agar 20.0, 10 ml of 50% glucose were added after sterilization) were used for cultivation. The suspension of the bacterial culture used in experiments was prepared by growing cells in the liquid medium at 37° C for 48 h. The cells were then washed with physiological saline containing Tween 80 and concentrated to about 7×10^8 cells per ml prior to UV irradiation. The solid medium was used to assay the viable cells count. Induced mutants were isolated on the solid medium with tryptose and the same medium containing streptomycin (STM) at a concentration of 10 μg/ml (Streptomycin Jenapharm, Jena).

The UV lamp TESLA 30W, 220 V with a power stabiliser was used as a source of

UV radiation. A very thin layer of the suspension was irradiated in a Petri dish from a distance of 25 cm. The dose of UV rays was about 72 erg/sec/mm². The lethal effect of UV radiation on the original PN strain of *Mycobacterium phlei* was studied. Samples of the culture were taken at 15 sec intervals for 90 sec, diluted, sprayed onto the solid medium with tryptose and incubated 3—4 days at 37° C. Various UV doses were used for the induction of scotochromogenic and achromogenic mutants. Survival curves of these mutants depending on time of irradiation were constructed in the same way as it was at the original photochromogenic strain. Plates with grown colonies were exposed to daylight for 3 days when isolating achromogenic mutants.

All procedures following UV irradiation were performed in dark using the insect-repellent lamp TESLA 100W, 220V in order to prevent photoreactivation.

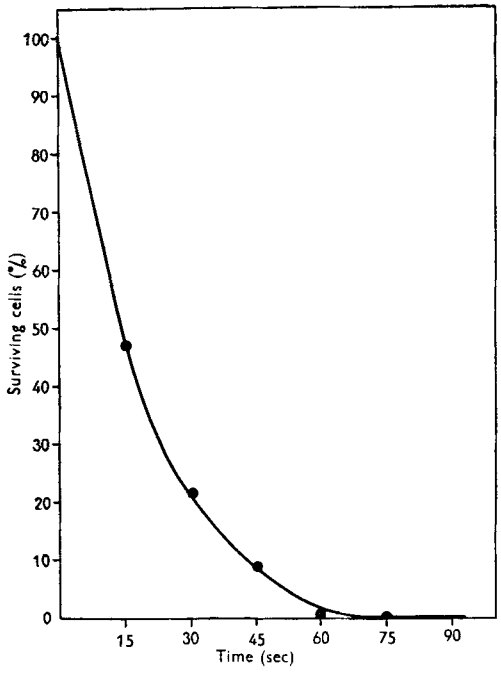

Fig. 1. Survival curve of the photochromogenic PN strain of *Mycobacterium phlei* after irradiation with UV rays.

RESULTS

Survival curve of the cells of the PN strain of *Mycobacterium phlei* depending on time of UV irradiation at given experimental conditions (Fig. 1) shows a considerable lethal effect of the mutagen used. Sixty sec irradiation results in the survival of a very small portion of the original population.

Induction of scotochromogenic mutants.

Five scotochromogenic mutants were induced by UV rays from the original photochromogenic PN strain of *Mycobacterium phlei* (Table 1). All mutants were obtained when using UV doses yielding relatively high survival of the cells of the original suspension. Orange pigmentation of the colonies can be compared with that exhibited by the original photochromogenic PN strain after 2—3 days exposure to day light. The mutants grow well in all media used for the cultivation of the PN strain of *Mycobacterium phlei*. The pigmentation is very expressive in the Davis minimal medium. Morphology of the colonies and rods of cultures in liquid media does not differ from the photochromogenic PN strain. Colonies of the scotochromogenic mutant No. 4 are ochre and as compared with the original strain do not grow into the agar. In this respect they resemble the acid-fast

Table 1. Scotochromogenic mutants induced from the PN strain of *Mycobacterium phlei* by UV radiation

Mutant	% Survival*	Pigmentation of colonies	STM^r 10 μg/ml
1.	28	orange	+
2.	58	orange	+
3.	58	orange	+
4.	25	ochre	—
5.	25	orange	—

* — % survival of the cells from the original suspension after UV treatment.

PA strain of *Mycobacterium phlei*, however they remain non-acid-fast and their other properties do not differ from the other scotochromogenic mutants. Spontaneous reversions to the original photochromogenic state were not observed in any of the scotochromogenic mutants. Generation time of the mutants does not differ from the original strain. Three out of five scotochromogenic mutants are resistant to STM, being selected on a solid medium with STM.

The survival curve of the scotochromogenic mutant No. 1 was constructed after repeated experiments with UV treatment under the given experimental conditions. The curve is similar to that obtained with the photochromogenic PN strain of *Mycobacterium phlei* (Fig. 2). A similar spread of points on the survival curve was also observed when studying the PA strain of *Mycobacterium phlei* under the same experimental conditions (Radochová *et al.*, 1966). It is evident that the scotochromogenic mutant is not more resistant to UV radiation than the photochromogenic PN strain.

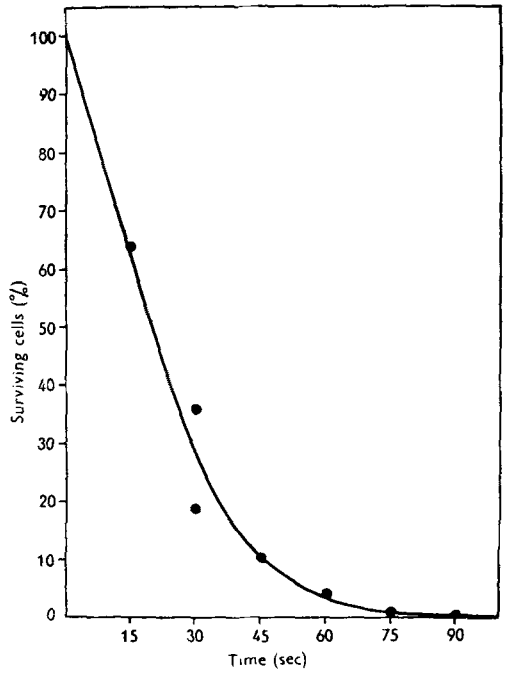

Fig. 2. Survival curve of the scotochromogenic mutant of the PN strain of *Mycobacterium phlei* after irradiation with UV rays.

Induction of achromogenic mutants.

Three achromogenic mutants were obtained from the original photochromogenic PN strain of *Mycobacterium phlei* after UV treatment. Eight achromogenic mutants were prepared from the scotochromogenic mutant No. 1 (Table 2).

Table 2. Achromogenic mutants induced by UV irradiation of the original photochromogenic strain and the scotochromogenic mutant No. 1 of the PN strain of *Mycobacterium phlei*

Mutant	Original strain		% Survival *	Spontaneous** reversions
	Photochromogenic	Scotochromogenic		
1.		+	0.9	frequent
2.		+	64.1	—
3.	+		58.0	—
4.	+		58.0	—
5.		+	0.08	—
6.		+	0.03	—
7.		+	22.3	frequent
8.		+	0.08	rare
9.		+	0.03	—
10.		+	0.03	—
11.	+		58.0	—

* % survival of the cells from the original suspension after UV treatment;
** spontaneous reversions to the original type of pigmentation.

Character of all achromogenic mutants is preserved on subcultures. They do not form pigments even after long-term exposure to daylight, and morphology of the colonies and rods of the cultures in liquid media does not differ from the original PN strain of *Mycobacterium phlei*. All achromogenic mutants induced from the scotochromogenic mutant are STM resistant as the original scotochromogenic mutant used is resistant to 10 μg STM/ml. The mutants were obtained when using UV doses resulting in a low survival

of the original suspension. Also the mutant No. 11 induced from the photochromogenic PN strain is resistant to STM, being selected on a solid medium containing STM. Spontaneous reversions to the original scotochromogenic state occurred in three mutants induced from the scotochromogenic mutant of the PN strain of *Mycobacterium phlei*.

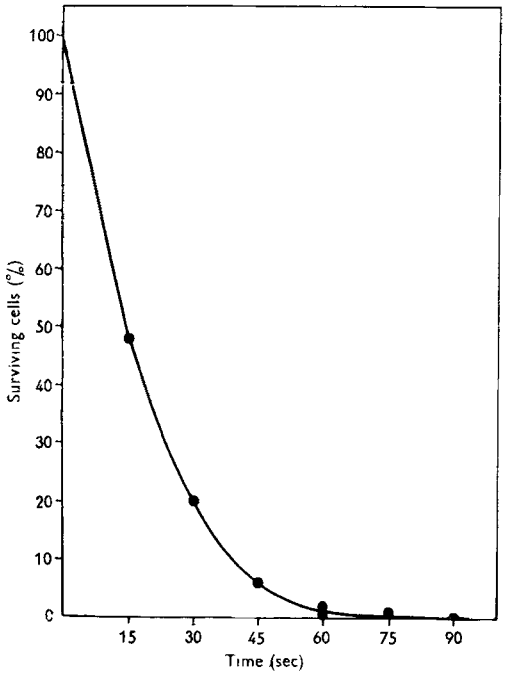

Fig. 3. Survival of the achromogenic mutant of the PN strain of *Mycobacterium phlei* after irradiation with UV rays.

Survival of the cells of the achromogenic mutant plotted against time of UV treatment is shown in Fig. 3. The achromogenic mutant is as sensitive to UV radiation as the original PN strain and the scotochromogenic mutant.

Spontaneous scotochromogenic and achromogenic mutants were not found.

A scotochromogenic reversion was obtained by UV treatment of the achromogenic mutant (obtained by UV treatment of the scotochromogenic mutant). As compared with all other scotochromogenic mutants the reversion does not grow into the medium, and forms a red-orange pigment. The pigmentation disappears reversibly after a certain time due to the exhaustion of nutrients of the medium.

DISCUSSION

It is commonly accepted that carotenoid pigments produced by bacteria have a light-protective role (Jensen, 1965). Biological role of pigments and their relation with virulence in mycobacteria was studied by Tsukamura (1963a, b). The author used photochromogenic strains of *Mycobacterium kansasii* and scotochromogenic strains of non-classified mycobacteria and induced non-photochromogenic strains by UV radiation. He found that the mutation frequency of non-pigmented mutants in the photochromogenic strain is 3—14fold higher than in the scotochromogenic strain. The author also found that the scotochromogenic strains were the most resistant to UV rays, whereas the photochromogenic strains were the most sensitive. He concludes that UV rays can penetrate more easily into the cells of the photochromogenic strains than into the cells of the scotochromogenic strains (this fact explains a higher frequency of induced mutants in the photochromogenic strains) and that protection against UV radiation is one of the biological roles of pigments. An increased resistance of the cells of the scotochromogenic mutants to UV radiation was not observed in our model microorganism used and under the described experimental conditions. No significant differences in the sensibility to UV radiation were found among the scotochromogenic and achromogenic mutants and the photochromogenic PN strain of *Mycobacterium phlei*. We did not induce more achromogenic mutants from the photochromogenic PN strain of *Mycobacterium phlei* by UV radiation. On the contrary, a higher number of these mutants was prepared from the scotochromogenic mutant. It should be point-

ed out that many more colonies of the irradiated photochromogenic strain were tested than those of the scotochromogenic strain. It can be concluded from the results obtained that the protective effect of pigments against UV radiation is not general.

Three out of five scotochromogenic mutants of the PN strain of *Mycobacterium phlei* are resistant to STM, being selected on the solid medium containing STM. Tarshis (1958) found that subcultivation of the H_{37}Rv strain of *Mycobacterium tuberculosis* in media containing antimycobacterial drugs (STM, isoniazide) results in the formation of pigmented colonies.

As scotochromogenic mutants and most achromogenic mutants of the PN strain of *Mycobacterium phlei* are stable and spontaneous mutants of this type were not observed, it can be concluded that they can be used in further genetic studies It can also be deduced from the results obtained that the pigmentation changes can be used as a genetic marker in mutation experiments using the given model microorganism.

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