EFFECTS OF VISIBLE LIGHT UPON UV-TREATED CONIDIA OF *ASPERGILLUS SOJAE*

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INTRODUCTION

There are three phases in the life cycle of *Aspergillus oryzae* and *Asp. sojae*; i.e. haploid, heterocaryon, and heterozygous diploid. It was discovered by the present author (1) that the heterocaryons irradiated by ultraviolet light (UV) gave rise to heterozygous diploids and the heterozygous diploids irradiated by UV gave somatic segregants in higher frequencies. The present work concerns the relationship between the above mentioned two UV effects and the so-called inactivation (killing) effect. Haploid, heterocaryotic, and diploid conidia of *Asp. sojae* were exposed to visible light immediately after UV irradiation. As to the inactivation effect, the phenomenon of photorecovery (2) is known to occur among many microorganisms.

METHODS AND MATERIALS

1. *Strains*

The following seven mutants of *Asp. sojae* K.S. were employed. L-120 yellow leucineless (*y leu*), K-271 yellow thiamineless (*y thi*), K-94 yellow p-aminobenzoic acidless (*y pab*), K-146 yellow nicotinic acidless (*y nic*), K-7 yellow methionineless (*y meth*), H-158 albino histidineless (*al his*) K-1012 albino arginineless (*al arg*).

2. *Media*

Media employed in this work are the same as those described in a previous paper (3); minimal medium (MM) is CZAPEK's agar and complete medium (CM), koji-extract agar.

3. *Light Sources*

Ultraviolet light: A 15-watt germicidal lamp (Matsuda, GL-form) was used, of which 95 per cent of the emitting light is of 2,537Å wave length, where the light intensity at a distance of 37 cm and at a current of 0.30A stands for 22.5 ergs/mm²/sec on the surface of conidial suspension.

Visible lights: (i) Black light fluorescence lamp (Matsuda, FL-20 BLB), emitting waves of 3,200–4,000Å, with a peak at 3,600Å. (ii) Day light type fluorescence lamp (Matsuda, FL-20D), emitting waves longer than 4,000Å,

with peaks at 4,600Å and 5,800Å.

Filters: (i) Glass filter (Matsuda, V-Y3C) which transmits waves above 3,950Å. (ii) Interference filter (Shonan Optical Film) which cuts off radiation at 4,360Å.

For convenience, the following symbols are employed. BL, black light fluorescence lamp; D, day light fluorescence lamp; IF, interference filter; VY, glass filter; and +, with filter.

4. Definition

Percentage of survival: Conidial suspension containing about 5×10^5 conidia in one ml sterile water was exposed to ultraviolet light in a dark room, and aliquots were withdrawn at intervals. Plating was made under a yellow lamp onto CM in quadruplicate. Survival fractions were determined by counting colonies appearing on CM after 40–48 hours' incubation.

Percentage of photoreactivation: Fractions of reactivated conidia by visible light was estimated from the formula, $100 \times (N_L - N_D / N_0 - N_D)$. N_0 is the number of survivors before UV irradiation, N_D (dark survival) the number of survivors after UV irradiation, and N_L (light survival) the number of survivors after treatment with visible light. Degree of relative recovery by visible light is to be expressed as N_L / N_D .

Percentage of diploids: When heterocaryotic conidia are plated on MM with or without UV irradiation, there appear three kinds of colonies; (i) colonies covered with green conidia (whole green colonies), (ii) colonies with green sectors or spots (sectorized green colonies), and (iii) ordinary heterocaryotic colonies with yellow and white sectors, but with no green sectors. Among the three, the first and the second types were considered to be diploids, and their percentages are recorded. Criteria of a diploid strain were given previously (4).

Percentage of segregants: When conidia arising from a heterozygous diploid are plated on CM, there appear the following five kinds of colonies; (i) green colonies with small yellow spots or sectors (G+(Y)), (ii) green colonies with small white spots or sectors (G+(Al)), (iii) yellow colonies (Y), (iv) white colonies (Al), and (v) whole green colonies (G). Considering that the first four types are somatic segregants, their percentages are recorded.

RESULTS

1. Effects of Visible Light upon Survival of UV-treated Conidia.

Haploid conidia of strain L-120 *yleu* were exposed to UV for various periods, and subsequently treated by visible light (BL and D lamps) for 30 minutes at a distance of 37 cm. Fig. 1 shows the survival curve and the degree of relative recovery by visible light. Generally speaking, the degree of relative recovery increased with UV dosage, attaining to the maximum at the dosage of five minutes' irradiation (6750 ergs/mm²), where the percentage of survival was about 0.5.

In the second experiment, the effect of visible light upon UV-inactivated conidia was examined under the same conditions as in the first experiment, that is, five minutes' UV irradiation and 30 minutes' visible light illumination. Table 1 shows results of five independent experiments, where, as visible light sources, BL and D lamps were employed with or without filters. The data were examined statistically, and it was concluded that visible light could reactivate UV-damaged conidia significantly. The two filters, VY and IF,

Fig. 1. Effects of Visible Light (Black-and Day-light lamps) upon Survival and Degree of Relative Recovery of UV-treated Conidia (Strain L-120 *y leu*).

were used to cut off waves below $4,000\text{\AA}$, but no remarkable differences were found in their employment. Similar experiments were carried out by the use of haploid conidia of strain K-7 *y meth*, and the same conclusion was drawn.

2. Effects of Visible Light upon Diploidization of UV-treated Conidia.

Preliminarily, frequencies of spontaneous diploidization were examined by using seven kinds of heterocaryons (Table 2). As seen in the last column,

Table 1. Effects of Visible Light upon Survival of UV-treated Conidia. (Strain L-120 *y leu*)

Exp. No.	Survival (%) after Treatment with Visible Lights of UV-treated Conidia					
	None	BL*	BL+VY	D	E+VY	D+IF
I	0.40	0.43	0.44	0.47	0.50	0.43
II	0.49	0.72	0.61	0.63	0.58	0.61
III	0.50	0.70	0.79	0.80	0.79	0.80
IV	0.46	0.51	0.57	0.78	0.60	0.68
V	0.44	0.65	0.57	0.74	0.62	0.72
Average	0.46	0.60	0.60	0.68	0.62	0.65
NL/ND*		1.30	1.30	1.48	1.34	1.41
% PR*		0.14	0.14	0.22	0.16	0.19
A**		0.05	0.05	0.055	0.045	0.058
B**		0.14	0.14	0.22	0.16	0.19
B/A		2.8	2.8	4	3.5	3.2

* See text.

** A: Variance of differences in average compared with group "None".

B: Difference of average compared with group "None".

Table 2. Frequencies of Spontaneous Diploidization in Seven Heterocaryons.

Heterocaryons	No. of Diploid Colonies		No. of Colonies on MM Examined	% of Diploids*
	Whole Green Colonies	Sected Green Colonies		
L-120 <i>y leu</i> + H-158 <i>al his</i>	8 16	2 1	4438 2594	0.22 0.65
L-120 <i>y leu</i> + K-1012 <i>al arg</i>	3	1	2674	0.15
K-271 <i>y thi</i> + K-1012 <i>al arg</i>	18 20	1 2	2600 2560	0.73 0.86
K-94 <i>y Pab</i> + K-1012 <i>al arg</i>	0 0	1 0	2906 2530	0.034 0
K-146 <i>y nic</i> + K-1012 <i>al arg</i>	1 0	0 1	2880 3005	0.034 0.033
K-7 <i>y meth</i> + K-1012 <i>al arg</i>	12	7	2718	0.7
K-7 <i>y meth</i> + H-158 <i>al his</i>	1 0	0 1	3560 2715	0.028 0.037

* See text.

percentage of diploids differed according to the combinations of the strains, showing the maximal value in the combination between K-271 *y thi* and K-1012 *al arg*.

Two heterocaryons, (L-120 *y leu* + H-158 *al his*) and (K-7 *y meth* + H-158 *al his*), effects of UV irradiation were studied. Conidia harvested from these heterocaryons were exposed to UV for five minutes under conditions described in the foregoing section. As seen in the third column of Table 3, the UV irradiation enhanced about ten times the frequencies of diploidization.

Next, the UV-irradiated heterocaryotic conidia were exposed to visible light for 30 minutes and plated onto MM under a yellow lamp. As seen

Table 3. Effects of Visible Light upon Diploidization Induced by Ultraviolet Light.

Heterocaryons	% of Diploids (Spontaneous)	% of Diploids after UV-treatment		
		Without Visible Light Treatment	With Visible Light Treatment	
			BL	D
L-120 <i>y leu</i> + H-158 <i>al his</i>	0.22*	1.2*	1.3*	1.8*
	0.65	3.2	3.9	3.5
	0.15	2.5	2.3	1.9
	0.35	4.3	5.0	4.0
K-7 <i>y meth</i> + H-158 <i>al his</i>	0.028	0.29	0.33	0.31
	0.037	0.45	0.42	0.48
	0.030	0.50	0.52	0.64
	0.010	0.70	0.60	0.65

* See text.

Table 4. Effects of Visible Light upon Segregation Induced by Ultraviolet Light.

L-120 *y leu*/H-158 *al his*.

Segregants	Spontaneous Segregation			Segregation after UV Treatment								
				Without Visible Light Treatment			With Visible Light Treatment					
							BL			D		
	Exp. I	II	III	Exp. I	II	III	Exp. I	II	III	Exp. I	II	III
G*	603	529	827	630	444	843	787	299	768	—	266	770
G+(Y)	5	4	11	34	24	52	41	18	48	—	28	55
G+(Al)	8	14	26	46	42	65	50	34	63	—	29	80
Y	0	0	0	0	2	0	1	4	1	—	2	0
Al	1	1	1	1	2	0	0	0	0	—	0	0
Totals	617	548	865	711	514	950	879	355	880	—	325	905
% of Segregants	2.3	3.5	4.4	11.2	13.6	12.3	10.5	15.8	13.7	—	18.1	14.9

K-7y meth/H-158 al his.

Segregants	Spontaneous Segregation			Segregation after UV Treatment								
				Without Visible Light Treatment			With Visible Light Treatment					
							BL			D		
	Exp. I	II	III	Exp. I	II	III	Exp. I	II	III	Exp. I	II	III
G*	703	873	807	643	808	995	688	786	953	—	630	950
G+(Y)	12	16	15	60	82	112	44	74	123	—	66	118
G+(Al)	22	29	23	48	63	89	37	49	104	—	44	104
Y	0	0	4	2	2	3	1	4	0	—	8	2
Al	0	0	2	0	3	1	1	0	0	—	1	1
Totals	737	918	851	753	958	1200	771	913	1180	—	749	1175
% of Segregants	4.7	4.9	5.2	14.5	15.7	17.1	10.8	13.9	19.2	—	16.0	19.1

* See text and *J. Gen. and Appl. Microbiol.* **2**, 401-430 (1956).

in the fourth and the fifth columns, no significant increase or decrease of diploidization was observed.

3. Effects of Visible Light upon Segregation of UV-treated Conidia.

Two heterozygous diploids, L-120 *y leu/H-158 al his* and K-7 *y meth/H-158 al his*, were employed. As seen in the second column of Table 4, the two diploids gave rise to somatic segregants in percents of 2-5 under natural condition. Meanwhile, when they were irradiated by UV, the percentages increased to 10-17.

Next, the UV-treated conidia were exposed to visible light for 30 minutes immediately after UV irradiation. However, percentage of segregants was neither enhanced nor reduced; results are shown in Table 4.

DISCUSSION

Ever since the phenomenon of photorecovery was discovered by KELNER (2), it has been studied extensively with fungi (5, 6), yeast (7, 8), bacteria (9-13), and bacteriophages (14, 15). In this paper, evidences that visible light could restore UV-induced damage occasioned in haploid conidia of *Asp. sojae* were presented. In the light of latest knowledge, the finding is not particularly new.

It was demonstrated again that ultraviolet light could enhance frequencies of diploidization and segregation significantly. However, visible light illumination immediately after UV irradiation gave no significance effects upon the frequencies. The only conclusion that the author can draw at the present time is, therefore, that the function of UV resulting in inactivation

of conidia is different from those resulting in enhancement of diploidization and segregation. Different mechanism may be at work.

At the outset of this work, it was hoped to discern the UV effect on diploidization from the UV effect on segregation by the employment of visible light illumination technique, but, as a matter of fact, no difference was found. Whether or not the mechanism of UV function on diploidization is the same as that on segregation is not yet known. The subject will be studied in future.

SUMMARY

By using mutants of *Aspergillus sojae*, it was demonstrated again that the heterocaryons and the heterozygous diploids irradiated by ultraviolet light gave rise to diploids and somatic segregants in higher frequencies. These two effects of ultraviolet light were compared with the so-called inactivation (killing) effect of UV. The haploid conidia inactivated by ultraviolet light were reactivated by visible light, while visible light gave no significant effect on diploidization and segregation. It was concluded, therefore, that the mechanism of UV function on inactivation is different from that on diploidization and segregation. Differences between two functions of UV, on diploidization and on segregation, are not known.

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