POST UV IRRRADIATION EFFECT ON *ASPERGILUS TERREUS*,[1] [2]

A Morphological and Biochemical Study of the Aged UV-induced Variants

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In a previous investigation, molasses was fermented with *Aspergillus terreus*, NRRL 1960, for the production of itaconic acid under various conditions by Tsao and Su (1963). In order to improve the yield, Tsao, Huang, and Su (1962) had induced some variations of *Aspergillus terreus* by ultraviolet irradiation of the spores with wave length 2537 Å and intensity 7.53×10^5 ergs min^{-1} cm^{-2}. They found that within 60 to 120 minutes exposure, 95% of the spores were killed or delayed in germination, that the rest 5% were morphologically changed and that the variants, in general, produced more itaconic acid from molasses than the parent strain.

The variants were not called mutants because, in the first place, genetic analysis was not made, and in the second place, further studies must be done before true mutants could be determined. Also, lethal effect was found rather erratic, or somowhat sigmoidal. A great number of references has been supplied by Hollaender (1955) to offer explanations for the genetic cause, sensitivity, and recovery of the immediate lethal and mutagenic effect of ultraviolet radiation. But very few works have been devoted to the study of the post effect of the aged variants which have survived of the stimulating and damaging irradiation of ultraviolet light. This kind of study might furnish some idea from a different point of view to the understanding of the morphological and biochemical effect of the aged, or long stored, ultraviolet induced variants of *Aspergillus terreus*.

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[2] This paper was read by the author at the second International Congress of Radiation Research, August 5-11, 1962 at Harrogate, Yorkshire, England.

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The Effect of Aging on the Morphological Characteristics of the Ultraviolet-Induced Variants:

Experimentation and Results

Sixteen 1 year old variants of *Aspergillvs terreus,* NRRL, 1960, namely, A_1, A_2, D_1, C_1, E_2, F_1, F_2, G_2, B'_3, C'_1, C'_3, D'_2, F'_1, F'_3, G'_1, & G'_2, were used in this study. These variants had been ultraviolet induced in the previous investigation by Tsao, Huang, and Su (1962). The wavelength used was 2537 Å, intensity 7.53×10^5 ergs min^{-1} cm^{-2}, and the time of exposure ranged from 60 to 120 minutes. These aged culture slants in Underkofler (1955) medium, after six generations, were left stored and untouched for a year over the winter of 1961–62 in Taiwan when the coldest weather was 14°C for two months. The rest of the year had temperatures between 20-30°C. Although moisture was limitedly supplied all the time, these cultures were mature but dry and shrunken when they were twice inoculated and subcultured to new agar slants. Two control parent strains (H_1 & H_2) both unirradiated, (H_2 was also stored for a year, but H_1 was kept vigorous by monthly inoculation), were now also inoculated at the same time for the purpose of comparison. They were incubated at 32±1°C. Daily observations were made and recorded for their colors and morphological changes to the naked eyes as well as to the microscopic magnifications. Photomicrographs were also made. Care was always taken each time to avoid contamination. The results are reported in Tables 1 and 2. Representative photomicrographs are included in a plate.

Discussion:

From the results of Tables 1 and 2, and of the microscopic examinations, it is seen that the parent strains, H_1 and H_2, have always been very stable, always woolly white when young, later developing into tan or cinnamon colored columnar masses of typical globose conidial heads with smooth stalks and very little secondary sterigmata. They never have green colors and their substrata are always bright yellow. Even after a year's storage at unfavorable temperature, drying condition, and deficient nutrient, they can quickly recover and restore to vigorous growth at favorable media.

As a rule, all the ultraviolet-induced variants develop colors. After a year's unfavorable storage, some of them still survived when transferred to satisfactory condition without much change in color. These are the strains E_2, F_2, G_2, B'_3, and D'_2. They are stable variants growing unchanged for more than a year.

On the other hand, four variants, A_2, F_1, G'_1 and F'_1, the first three had very late germination and poor growth and quickly died out and the last one, F'_1, suffered no growth at all. These poor variants had changeable colors, and F_1 and F'_1 had pink or red colors. The latter colors (pink and red) seem to be the most unsatisfactory deadly colors which would lead the fungi intole-

Table 1. *Morphological changes of the 1 year old UV-induced variants of* ***A. terreus.*** *Wavelength 2537 Å. Intensity 7.53 × 10^5 ergs min^{-1} cm^{-2} . Concentration 5.2×10^5 spores per ml.. incubated at 32±1°C.*

Variant no.	Exposure time (min.)	Energy in ergs per spore	Type of colony and subcultures			
			isolated & subcultured from irradiated spores	1st subcultured after 1 year storage, observed at 10th day incubation	2nd subcultured after 1year storage, observed at 10th day incubation	microscopic appearance
H_1 control	0	0	normal white woolly	kept monthly inoculation without change	no change	normal globes conidial heads in columnar masses, smooth stalks
H_2 control toreds	0	0	normal white wooly	no change	no change	similar to H_1
A_1	60	44.4	light green, abundant spores	late germination, light-bluish green, heavy spores	no change	spores in chains, very little fine stalks, no heads found
A_2	60	44.4	white layered flower; like, changed to light yellow, no spores	very late germination	no growth	no conidial heades, few spores
C_1	80	59.2	chalk white colonies, no spores after 20 days	late germination, little green colonies	green changed to black, poor growth	chains of spores as sec. sterigmata from the loose heads
D_1	90	66.6	white pinkish, changing colors to dark purple, dark red & yellowish pink with dark spores	tan purple spores, substratum tan colored	purple spores tan colored substratum	pri.& sec. sterigmata, plenty of spores
E_2	100	74.0	yellow spored green colonies	dirty green, abundant spores	no change	abundant clear spores, very little fine stalks & heads
F_1	110	81.4	white creamish woolly layered, big colonies, changing to beautiful red purple color, some reversion to white	very late germination to small green colonies, quickly died out	no growth	few fine chains of spores
F_2	110	81.4	black green short colonies, changing to dark purple spores	dark purple spores, substratum brown	no change	well formed conidial heads with abundant spores
G_2	120	88.8	dark green layered colonies, bluish black substratum	vigorous, abundant dark green spores, bluish black substratum	no change	fiine stalks with secondary sterigmata

Table 2. *Morphological changes of the 1 year old UV-inducea variants of* ***A. terreus.*** *Wavelength 2537 A. Intensity 7.53×10^5 ergs min^{-1}, cm^{-2}. Concentration 2.0×10^5 spores per ml. Incubated at 32±1°C.*

Variant No.	Exposure time (min.)	Energy in ergs per spore	Type of colony, and Subcultures			
			isolated & subcultured from irradiated spores	1st subcultured after 1 year storage, observed at 10th day incubation	2nd subcultured after 1 year storage, observed at 10th day incubation	microscopic appearance
B'_3	70	99.3	light pretty green, abundant spores	light pretty green	no change	well formed conidial heads with sec. sterigmata
C'_1	80	113.4	yellow spored, green colonies	changed to dirty green, brown exodus	color fades to white green, reversion, abundant spores	spores mixed with fine structures of fibrous mycelia
C'_3	80	113.4	light green to tan spores	green, white yellow spores, brown exodus	green, yellew white spores abundant spores	abundant unicellular spores, fine short stalks
D'_2	90	127.8	dark green colony, carly black spored	vigorous growth, heavy black spored, colorless substratum	no change	well formed heads with secondary sterigmata
F'_1	110	156.0	white pinkish layered woolly changed to dirty green and brown spores, black substratum	no growth	no growth	dead cells, not viable
F'_3	110	156.0	white thin layered big colonies changed to scalelike hard colonies, no spores	revived to dark brown spores	black spored	abundant brown black spores, conidial heads, some sec. sterigmata
G'_1	120	170.1	black green layered colonies, some wite layered, abundant spores	very late germination to small green and white colonies	no growth	drawfed unhealthy fine short mycelia no conidial heads
G'_2	120	170.1	white creamish woolly layered big colonies, changed to tan color, very little spores	light green to pretty green spores	abundant spores	occasional heads, abundant small spores attached on the short stalks

rable to unfavorable environment of temperature, moisture, and nutrients. The cause and effect of decoloration might be due to the change in the macromolecule DNA which will be discussed in the later section.

To determine the vitality by colors is, of course, dangerous and is undependable. But variants with changeable colors are at least unstable and it might be predicted that they would eventually lead to poor growth and death finally occurs. According to this prediction, the suspicious ones, like, A_1, C_1, D_1, C'_3, G'_2, C'_1, & F'_3, now grow fairly well after a year's storage might not live too long. Further study is, therefore, necessary to verify.

The well-formed conidial heads, from E_2, F_2, G_2, B'_3, D'_2 and, of course, the parent strains H_1 and H_2 with or without secondary sterigmata, show healthy reproductive organs and these strains are strong enough to have suffered the damaging energy of ultraviolet irradiation and are now resistant to a year's unfavorable storage. They ought to be able to survive indefinitely like the parent strain. This may also be used to predict whether a stable variant has been induced by the 2537 Å ultraviolet irradiation.

Many workers, as reported by Hollaender (1955), try to explain the radiation-induced mutations by genetics studies. The genes and chromosomes are responsible for the heredity. Change must be done on the genes by the absorption of ultraviolet irradiation before a mutation can be induced.

From a recent chemical stand point of view by Benzer (1962) and Hurwitz and Furth (1962), a giant molecule of deoxyribonucleic acid, or DNA, is the fundamental carrier of genetic information, and ribonucleic acid, or RNA, acts as a messenger to carry instruction from the genes to the particles in the cell where proteins are manufactured. Therefore, if mutation should be induced by any outside radiation including ultraviolet light, the fundamental reason must be that the progeny must have a DNA molecule not an exact copy of the molecule of either parent. Again, to synthesize a new protein its structure is dictated by a sequence of different bases in the RNA. So the irradiation problem turns out to be a complicated chemical problem. When an ultraviolet-induced variant continues to change, for instance, change in colors, on storage or aging, that means unstable compounds of DNA or RNA have been induced, which are able to undergo continuous modifications, capable of either long existence, or finally drying out of the whole microorganism. All these are just reasonings. But the chemical proof has been demonstrated according to Deering (1962), who states that ultraviolet can change DNA in specific ways and can partially reverse those changes. There is considerable evidence that much of the damage that ultraviolet radiation inflicts on cell and viruses is caused directly by its effects on DNA. There only remains the task of identifying all the ultraviolet reactions in living organisms.

The Effect of Aging on the Biochemical Properties of the Ultraviolet-Induced Variants—Their Production of Itaconic Acid from Molasses:

It is of great interest to know the effect of storage, or aging, of the ultraviolet-induced variants toward the production of itaconic acid from molasses. All the variants together with the control parent strains, H_1 and H_2, except the four strains, A_2, F_1, F'_1 and G'_1 whose activity had ceased, were subject to fermentation in molasses according to the method described by Tsao, Huang, and Su (1962) for the production of itaconic acid in the previous investigation. The results are condensed in Table 3.

The resulting % yields are compared with those produced under the similar conditions by the same variants before storage, whether increased (inc.) or decreased (dec.) are also recorded.

Discussions:

From Table 3, it is seen that while the control H_1 does not have much difference in its ability to produce itaconic acid from molasses, the stored H_2 shows a slight increase. All the ultraviolet induced variants, after 1 year's unfavorable storage, produce either increased, or decreased, amounts of itaconic acid from molasses. The variants A_1, D_1 and C_1 which were the leaders in the producing of itaconic acid in the previous investigation, now after being stored for a year, all decreased in their biochemical activity of producing itaconic acid from molasses. This is perhaps due to the decreased vitality on aging because A_1 and C_1 show late germination, and D_1 is not a stable variant. The other variants which have decrease acid producing activities are F_2, G_2, and C'_1, F_2 and G_2 are good stable variants and their decrease in acid producing abilities after aging are but small and insignificant. However, all the rest variants, namely, E_2, B'_3, C'_3, D'_2, F'_3 and G'_2, all produce increased amount of itaconic acid even after one year long resting period at unfavorable condition. Among them, E_2, D'_2 and B'_3 are by far the best strains in their itaconic acid producing properties. They are also stable strains and are probably the desired stable variants. Without this aging studies, their usefulness may not be so definitely found. In the last investigation, a question was raised about the relationship between formation of spores and production of acid. Now affirmation can be answered. Whenever spores are abundantly formed in a stable strain, it will be able to produce more acid than could be otherwise. This can be explained that abundant spore formation of a stable variant means its high vigor, and, therefore, high biochemical property in the production of itaconic acid from molasses can be expected.

Explanation of the figures:

H_1 The parent strain *A. terreus* H_1 The parent strain showing

Table 3. *Post effect of UV-induced variants of* ***A. terreus.*** *on itaconic acid production from molasses. Variants stored one year, molasses 200 ml/l. Other nutrients,* $MgSO_4 \cdot 7H_2O$ *2g/1,* $(NH_4)_2SO_4$ *2.67 g/1 Temperature, 32±1°C, pH=2.5, fermentation time, 5 days*

Variant No.	Colony type	glucose consumed g/1	itaocnic acid produced g/1	% yield			mycelium wt. g.
				based on glucose consumed *(1)	based on glucose supplied *(2)	compared with the yield before storage in *(1)	
H_1 control unstored	normal white woolly	57.9	18.25	45.2	31.6	no difference	1.054
H_2 control stored	normal white woolly	53.4	17.75	46.0	33.24	inc. (increased) 0.8	1.123
A_1	light green	52.9	19.90	46.7	32.06	dec. (decreased) 3.0	1.144
C_1	chalk white	56.7	17.00	41.5	30.00	dec. 8.9	1.567
D_1	white pinkish layered woolly	55.65	15.35	38.2	27.60	dec. 12.2	1.659
E_2	yellow spored	53.65	17.75	45.8	33.03	inc. 8.4	0.959
F_2	dark green	52.15	17.25	46.7	33.03	inc. 0.4	1.108
G_2	white creamish woolly layered	53.4	16.75	43.5	31.4	dec. 2.6	2.155
B'_3	light pretty green	55.15	17.60	44.2	31.90	inc. 3.9	1.004
C'_1	yellow spored	57.15	17.25	41.7	30.17	dec. 3.9	0.904
C'_3	light green	55.4	17.25	43.1	31.20	inc. 1.8	0.373
D'_2	dark green	50.9	18.00	49.0	35.40	inc. 9.4	1.003
F'_3	white layered flowerlike	55.65	17.90	44.60	32.20	inc. 1.8	0.927
G'_2	white creamish woolly layered	53.9	17.75	45.6	32.95	inc. 5.4	0.735

* (1) Based on glucose consumed (assming 1 mole glucose yields 1 mole itaconic acid)
* (2) Based on glucose supplied (g. itaconic acid produced per g. glucose supplied)

NRRL, 1960 without irradiation and had the normal conidial heads and smooth stalks.

D_1 Head with pri. and sec. sterigmata, plenty of spores, smooth stalk.

D'_2 Well-formed conidial heads with short sec. sterigmata, most easily found.

conidial heads in columnar masses.

B'_3 Well-formed conidial heads with sec. sterigmata.

F'_3 Abundant brown black heads with sec. sterigmata.

Summary

This work was a continuation of the author's previous investigation on the ultraviolet-induced variations in *Aspergillus terreus.*

Sixteen 1 year old morphologically different variants, which were induced by 2537 Å UV dosages ranging from 4.518×10^5 to 9.036×10^5 ergs mm^{-2}, and which were left stored under unfavorable environment, were now twice resubcultured on new Underkofler's agar slants and incubated at 32±1°C for 10 days each.

A morphological study of these subcultures by naked eyes as well as under microscope was able to screen out five stable variants.

The stable variants grew with undiminished vigor and had well-formed conidial heads.

The poor variants had pink, red, or changeable colors and died out after a year's stroage.

The intermediate variants were not stable strains because they had changeable colors and did not have good conidial heads for reproduction.

A comparative study of the biochemical activities in the production of itaconic acid from molasses also revealed the fact that the morphologically determined stable variants were also the good itaconic acid producers.

The poor acid producers were the ones which could not resist the long storage.

It is suggested that a fundamental clue for determining the radiation induced mutations must rely on studying the messenger RNA and genetic information carrier DNA.

紫外光照射Aspergillus Terreus之後效果

(對紫外光誘導體放置一年後在形態上及生化上之研究)

曹 簡 禹

此一研究，爲本人以前所作紫外光誘導 Aspergillus terreus 變異之繼續研究。

十六種形態上不同之變異體，曾經紫外光波長 2547Å 及能量每平方毫米 4.518×10^5 至 9.036×10^5 爾格之照射，並經放置於不良之環境下一年後，重行接種，保溫 (32±1°C), 孵育兩次，每次十天。

用肉眼及顯微鏡觀察其形態，發見其中五種爲穩定之變異體，四種爲甚不穩定之變異體，及七種中間之變異體。

穩定之變異體生長旺盛，且有良好之球果頭 (Conidial heads)。最壞之變異體具有紅，粉紅或其他可變之顏色，經保藏一年後，卽行死去。中間之變異體亦非穩定之種，因亦有可變之顏色，且無可供繁殖之良好球果頭。

由糖蜜產生分解烏頭酸 (Itaconic Acid) 之生物化學活性之比較研究，亦發見凡形態上決定之穩定變異體，亦爲分解烏頭酸之良好產生者。凡不良於產生分解烏頭酸者，亦爲不耐長久貯藏者。

作者以爲欲決定一放射線導致之變種，基本上必須賴研究其傳信之核酸 (Messenger RNA)，及傳達基因消息之去氧核酸 (Genetic information carrier DNA)，此事目前已大有了解矣。

Literature Cited

BENZER, SEYMOUR, The fine structure of the gene, Scientific American, 206, No. 1. 70-84, 1962.

DEERING, R. A., ultraviolet Radiation and Nucleic Acid, Scientifiic American, 207, No. 6: 135-144, 1962.

HOLLAENDER, ALEYANDER. Radiation biology, Mcgraw-Hill Book Company, Inc. Velume II. 1955.

HURWITZ, JERARD, and J. J. FURTH. Messenger RNA, Scientific American 206. No. 2; 41-49, 1962.

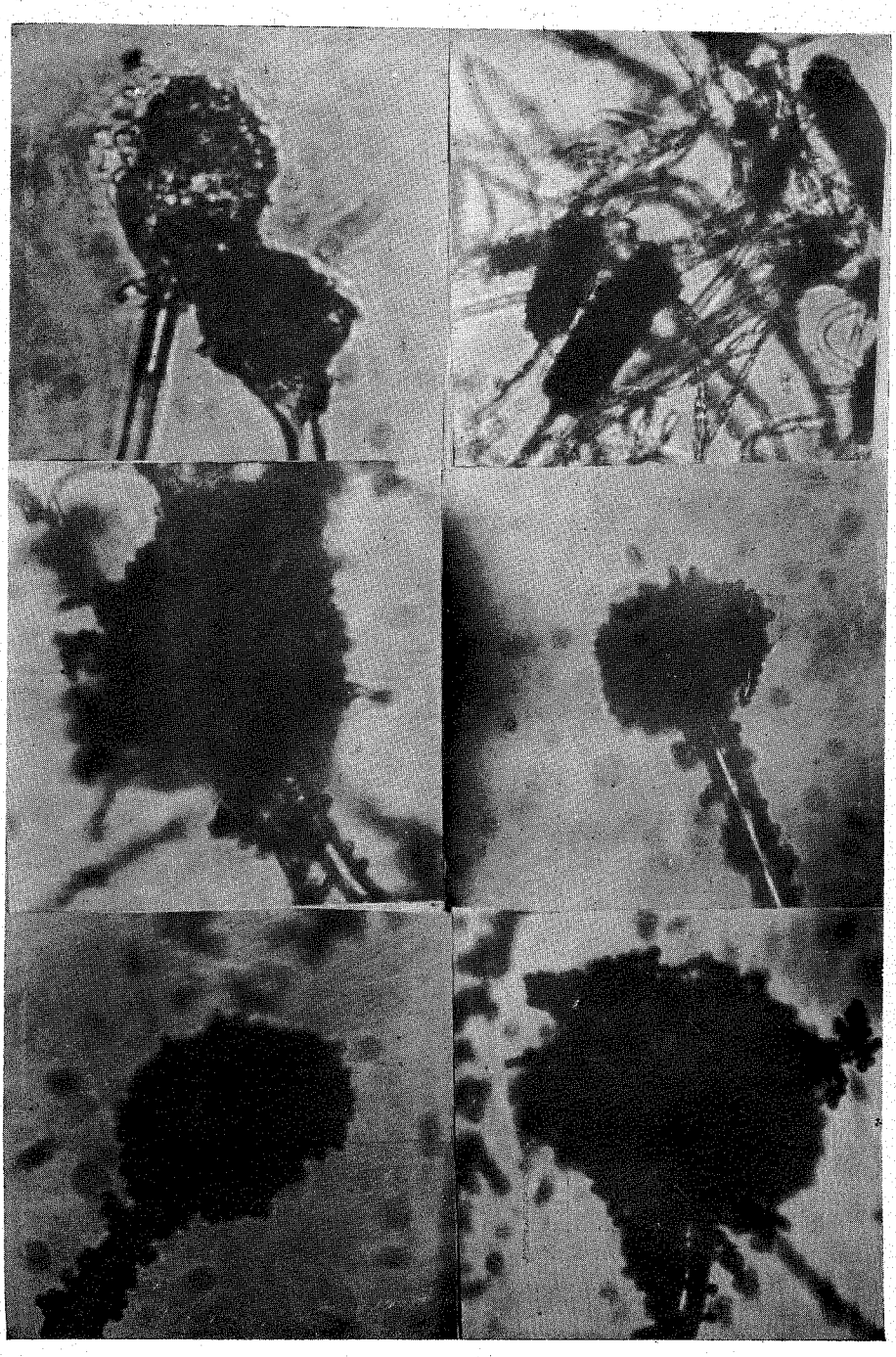
TSAO, JUNE C. Y. and SHIRLEY CHUNG-HUA SU. Fermantation of molasses by *Aspergillus terreus* for the production of itaconic acid. Sugar by Azucar, 1963.

TSAO, JUNE C. Y., TING-CHIA HUANG, and SHIRLEY CHUNG- HUA SU. The ultraviolet-induced variations in *Aspergillus terreus,* their morphological changes, their production of itaconic acid from molasses. Bot. Bull. of Academia Cinica 3: 1-17, 1962.

UNDERKOFLER, L. A. and R. J. HICKLY. Ind. Fermentation, C. A. 5761 g. 1955.

Plate

Representative photomicrographs of 1 year old UV-induced variants of *Aspergillus terreus*



$H_1 \times 850$ $H_1 \times 200$

D1 × 300 $B'_3 \times 300$

$D'^2 \times 300$ $F'_3 \times 300$