THE ULTRAVIOLET-INDUCED VARIATIONS IN *ASPERGILLUS TERREUS*, THEIR MORPHOLOGICAL CHANGES, AND THEIR PRODUCTION OF ITACONIC ACID FROM MOLASSES[1]

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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 of pH values, sizes of inoculum, time of fermentation, nitrogen and magnesium supplies (Tsao and Su, 1961). It was reported that when all were under the optimum conditions for *Aspergillus terreus,* molasses was an excellent carbohydrate raw material, much superior to the widely used pure glucose.

It occurred to the investigators that an ultraviolet-induced variant of *Aspergillus terreus* might change the chemical make up of the fungus and thus, other things being equal, might increase the yield of itaconic acid production from the molasses.

It has long been known that variants can be induced in either bacteria or fungi by suitable exposure to ultraviolet or X-ray radiations. Hollaender and Claus (1936), Hollaender and Emmons (1939 and 1941), Emmons and Hollaender (1939), Hollaender, Raper and Coghill (1945), Lockwood, Raper, Mayer, and Coghill (1945), Hollaender Sansome and Demeree (1945), and Hollander and Zimmer (1945) have all confirmed this statement.

Hollaender and many of his coworkers, studying on *Escherichia coli* (1936), Dermatophytes (1939), *Penicillium notatum* (1945), and *Aspergillus terreus* No. 265 (1945) have well established the fact that monochromatic ultraviolet radiation of 2280 to 2962 Å is an efficient means of producing mutation. Hollaender *et al*

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have also described the cultural and morphological characteristics of the mutations, and the physiological and biochemical characteristics of the mutants of *Aspergillus terreus* No. 265. Itaconic acid production from glucose and other necessary inorganic ingredients was found in varying quantities from increased to nothing by the mutants which Hollaender and his coworkers had isolated from irradiated *Aspergillus terreus.*

Although genetical analysis can not be determined here, it is certain that different types of variation can be induced by the ultraviolet radiation on different strain of *Aspergillus terreus,* and that these variants will have varying degrees of morphological changes and biochemical changes in the production of itaconic acid.

The present paper deals with the results of the authors' investigation on the production of ultraviolet-induced variations in *Aspergillus terreus,* NRRL, 1960, their morphological changes, and their production of itaconic acid from molasses.

Experimentation and Results

The microorganism:

The *Aspergillus terreus* used in this investigation was the strain No. 1960 indirectly obtained from the Northern Reginal Research Laboratory, Peoria, Illinois, U. S. A. and was used by Lockwood and Ward (1954), as well as by the authors in the previous report (Tsao and Su, 1961) for the production of itaconic acid from molasses. This *A. terreus* NRRL, 1960, seeded upon Underkofler's (1955) agar slants was incubated for 12 days at 30°C. The inoculated cultures were white woolly at first and became yellow brown in about eight days from the production of abundant conidial heads. After ten days, the spore production was heavy, and the cultures became cinnamon brown.

The spores were washed off in sterile water in the proportion of about 1 to 100 by volume in a bacteria-free box; the suspension was shaken in a culture tube vigorously by hand, and filtered by sterilized filter paper. The resulting uniform suspensions were used for irradiation. The number of spores was determined in a blood counting chamber. For the first irradiation experiment, it was 5.2×10^5 spores per ml, and for the second, it was 2×10^5 spores per ml.

Source of Ultraviolet light:

An ultraviolet lamp was used for irradiation purpose. It was of short wave, wavelength 2537 Å, mercury quartz type, Model V-41 mineralight, manufactured

by Ultra-Violet Products, Inc. For this wavelength, each photon has an energy of 7.81×10^{-12} ergs. According to the catalog the intensity of this lamp is 217 μW (microwatts) per square centimeter at a distance of 1 foot. When asked, the manufacturer wrote back that the intensity was also 106.2 microwatts per square centimeter at a distance of 18 inches. The distance we used in this investigation was 7.75 cm (3.05 in). The variation in the intensity with the distance of the ultraviolet light can be estimated by a diagram given by Hollaender (1956) in his Radiation Biology. When this is done it should be 1.35 uv watts per square foot, or 1.255×10^{3} μW which is equal to 7.53×10^{5} ergs min^{-1} cm^{-2} at a distance of 7.75 cm.

Irradiation technique:

The experiment was carried out in a bacteria-free box. The emerging monochromatic beam was concentrated on an open exposure cell (diameter 9 cm.) which contained the carefully prepared suspensions of spores in a depth of 1.2 cm. The distance perpendicular to the liquid surface from the source was an average of 7.75 cm. (9 cm to 6.5 cm) The spores in sterile water were stirred by magnetic stirrer during irradiation, so that on the average each spore was exposed to an equivalent amount of light energy. Before and during the process of irradiation equal amounts of samples (0.01 ml for the first irradiation experiment and 0.05 ml for the second experiment, measured and diluted with a blood pipette) were removed at intervals of 10 minutes beginning from 60 minutes to 120 minutes for plating on Underkofler's medium. Preliminary experiments carried out under the same conditions had found that the spores of the strain of *A. terreus* used here were quite resistant to the ultraviolet radiation within one hour of exposure. The temperature in the spore suspension during irradiation was found to be 28°C initially. Magnetic stirrer generated some heat. So at the end of the experiment the temperature rose to 31°C. Practically no ultraviolet light can be lost by transmission through ordinary glass. But whether the energy put in was completely absorbed by the spores is diffecult to say. Stable or unstable substance (Hollaender, 1956) such as hydrogen peroxide or other mutagenic substances might have been formed in the medium and thus play a role in the variations we found later. However, it might not make too big a mistake by assuming that the total incident energy, all concentrated on the exposure cell, divided by the total number of spores, should give the average energy acted on each spore.

To calculate the energy, ε, required to inactivate a single spore, it should follow an equation (Hollaender 1936):

$$-dN/dE=1/\varepsilon\cdot N\mu \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where $-dN$ is the decrease in the number, N, of viable spores in the suspension and μ is the average fraction of the total energy, E, absorbed by each spore.

If we represent the total number of spores by N_T, then for a suspension in which practically no energy is lost by transmission,

$$\mu N_T = 1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The survival ratio, N/N_0, can be obtained by integrating (1) after $\mu = 1/N_T$ is substituted. Thus

$$\frac{N}{N_0} = e^{-\frac{E}{N_T} \cdot \frac{1}{\varepsilon}} \quad \ldots\ldots\ldots\ldots (3)$$

Where N_0 is the number of viable spores at $E = O$.

The calculation is made as follows:

Total energy of the lamp at a distance of 7.75 cm $= 7.53 \times 10^5$ ergs min^{-1} cm^{-2} × area of the lamp window ($6 \times 6.5 = 39\ cm^2$) $= 2.936 \times 10^7$ ergs min^{-1}.

The total volume in the exposure cell was $(\frac{9}{2})^2 \times \pi \times 1.2 = 76.354$ ml. It contained 5.2×10^5 spores per ml in the first experiment, and 2.0×10^5 spores per ml in the second experiment. Therefore, the total numbers of spores in the cell were $5.2 \times 10^5 \times 76.354 = 3.97 \times 10^7$ spores, and $2.0 \times 10^5 \times 76.354 = 1.53 \times 10^7$ spores respectively. The energy received per spore per minute $= \frac{2.936 \times 10^7}{3.97 \times 10^7} = 0.740$ ergs, and $\frac{2.936 \times 10^7}{1.53 \times 10^7} = 1.418$ ergs in the first and second experiments respectively. To calculate the energy received per spore in 60 minutes, would only need to multiply the above numbers by 60. So $0.740 \times 60 = 44.4$ ergs received per spore in 60 minutes. This gives E/N_T in equation (3) for A_1 in Table 1. The rest were calculated in the same manner.

Treatment and results after incubation:

The colonies were examined and counted after an incubation period of 3 to 5 days at 30°C. The number of colonies in control gives N_0, and those of all others give N. After counting, certain sections of the control and irradiated colonies were isolated and transferred to agar slants for further observation and inoculation. The percentage of survival, $N/N_0 \times 100$, were calculated, and the types of variations were determined by examination and comparison of their morphological appearances. White woolly is considered as normal; others are morphologically different variants. The latter when divided by N_0 and multiplied by 100 gives the percentage of variation. The inactivation energy ε is also calculated according to equation (3). The results of the two experiments are reported in Tables 1 and 2.

Table 1. First Experiment. Number and types of variations induced by the ultraviolet irradiation, wavelength 2537 Å, concentration 5.2×10^5 spores per ml, incubated at 30°C.

Exp. marks	Exposure time, (min.)	No. of colonies (5 days)	Colony type	$(N/N_o \times 100)\%$ survival (5 days)	% variation (5 days)	Energy in ergs/spore (E/N_T)	Energy of inactivation (ε) ergs/spore
control H_1	0	26	Normal abundant white woolly	100.00	0	0	0
A_1	60	3	1 three layered flower like 2 greenish with white surrounding	11.52	11.52	44.4	20.5
A_2	60	2	1 big white 1 small red exudate	7.69	7.69	44.4	17.3
B_1	70	2	all white woolly	7.69	0	51.8	20.2
B_2	70	3	1 big white long woolly 2 small white woolly	11.52	3.84	51.8	24.0
C_1	80	1	chalk white woolly	3.84	3.84	59.2	18.2
C_2	80	3	1 white-yellow woolly 2 small dark ones	11.52	7.69	59.2	27.3
D_1	90	3	1 big dark 1 small greenish 1 white pinkish woolly layered flower-like	11.52	11.52	66.6	30.8
D_2	90	3	all greenish	11.52	11.52	66.6	30.8
E_1	100	2	1 small greenish 1 small white woolly	7.69	3.84	74.0	28.8
E_2	100	2	1 small greenish 1 small white woolly	7.69	3.84	74.0	28.8
F_1	110	1	greenish	3.84	3.84	81.4	25.0
F_2	110	1	black green	3.84	3.84	81.4	25.0
G_1	120	1	1 big black	3.84	3.84	88.8	27.2
G_2	120	1	1 creamish exudate	3.84	3.84	88.8	27.2

Table 2. Second Experiment. Number and types of variations induced by the ultraviolet irradiation, wavelength 2537 Å, spore concentration 2.0×10^5 spores per ml., incubated at 30°C.

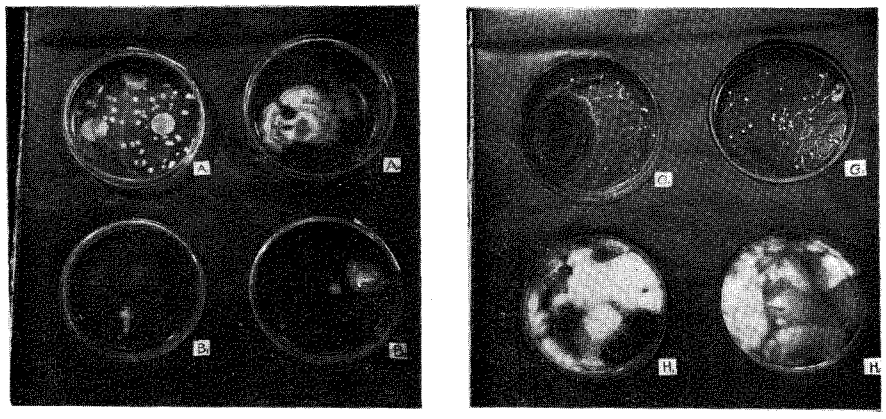
Exp. marks	Exposure time, (min.)	No. of colonies (3 days)	Colony type	$(N/N_0 \times 100\%)$ survival (3 days)	% variation (3 days)	Energy in ergs/spore (E/N_T)	Energy of inactivation (ε) ergs/spore
H_1	control	193	All white woolly	100	0	0	0
A_1'	60	9	1 big green inner layered 3 exudate 5 small green	4.66	4.66	85.1	27.7
A_2'	60	7	All small green	3.63	3.63	85.1	25.8
A_3'	60	10	All small green	5.18	5.18	85.1	28.8
B_1'	70	11	1 big white long woolly 10 small green	5.70	5.70	99.3	34.6
B_2'	70	20	1 big light green 19 small green ones	10.35	10.35	99.3	43.2
B_3'	70	8	6 small white ones 1 white woolly 1 yellowish	4.14	3.63	99.3	31.2
C_1'	80	4	All small light green	2.07	2.07	113.4	29.3
C_2'	80	14	1 three-layered inside green, outside white 1 two-layered, black inside, white outside 12 small green ones	7.25	7.25	113.4	43.2
C_3'	80	17	3 two layered inner green and white outside 14 small light green ones	8.80	8.80	113.4	46.7
D_1'	90	9	All small green	4.66	4.66	127.8	42.4
D'_2	90	5	1 big white long woolly with black spores inside 4 small green	2.59	2.59	127.8	34.9

D_3'	90	10	1 big white long woolly 5 small green 4 small white (3 exudate)	5.18	5.18	127.8	43.2
E_1'	100	6	All small green	3.11	3.11	141.8	41.0
E_2'	100	17	1 big green 2 white woolly 2 white exudate 12 small green	8.80	7.77	141.8	58.3
E_3'	100	27	2 big white long woolly 25 small green	1.40	1.40	141.8	33.2
F_1'	110	9	7 small green 2 big white pinkish woolly flower-like	4.66	4.66	156.0	50.8
F_2'	110	10	1 big with white color in outside layer 9 small green	5.18	5.18	156.0	52.7
F_3'	110	2	1 white layered flower-like 1 exudate	1.035	1.035	156.0	34.2
G_1'	120	48	45 minute small exudate 3 dark green layered	24.86	24.86	170.1	125.0
G_2'	120	13	1 big white creamish woolly layered 12 small green	6.74	6.74	170.1	63.0
G_3'	120	8	6 small green 1 big white woolly 1 very big with inner black outside white	4.14	4.14	170.1	53.3

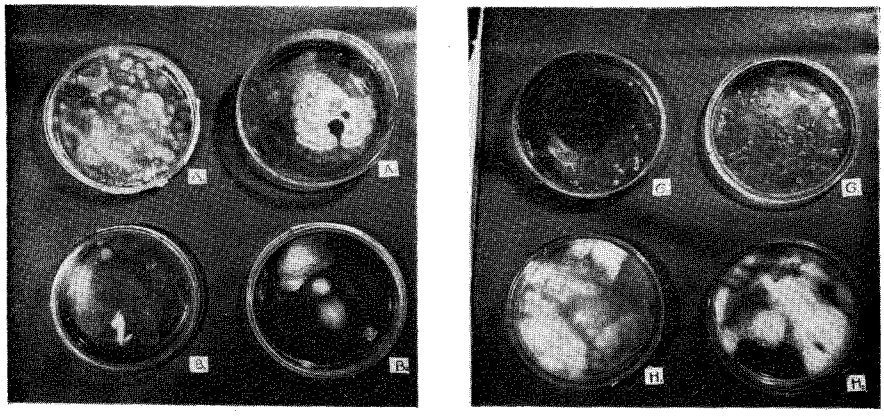
The following Plate I is representative figures of the variants and the control of the first experiment showing the growing appearance after 7 to 12 days:

Plate I. The representative figures of ultraviolet-induced variations of *A. terreus* and control.

*** 7 days old.



*** 12 days old.



Explanation of the figures:

Fig. A_1, A_2, both received 60 min. exposure of the ultraviolet irradiation, 2537 Å.

Fig. B_1, B_2, both received 70 min. exposure of the same radiation.

Fig. G_1, G_2, both received 120 min. exposure of the ultraviolet irradiation.

Fig. H_1, H_2, These are the controls of the parent strain which did not receive any radiation and which appeared normal and white woolly.

*** These photographs were taken at 7 th and 12 th day.

Notice the same corresponding ones grew bigger in sizes, and new small ones developed after 5 days and within 12 days' incubation, showing delayed germination of many spores.

Discussions:

The ultraviolet energy used in these expriments showed an average killing and depressing rate of over 95% within 3 to 5 days. The lethal effect seems to be rather erratic instead of a linear function of the exposure time. It might be due to the insufficient number of runs so that no statistical result could be evaluated. So is the rate of survival, which is only less than 5% as a total in 3 to 5 days.

However, one thing is almost certain that those which survived were mostly induced variants. The morphological studies revealed the fact that generally after incubated at 30°C for 12 days, nearly all colonies developed pigments, mostly greenish, some yellowish, and some reddish. Some showed delayed germination, others differed in rate of growth. Some bore heavy spores, while still others seemed to be sterile, producing no or very little spores.

From Table 1 it is seen that the least amount of the energy of inactivation, ε, is 17.3 ergs per spore for A_2. Since each photon contains 7.81×10^{-12} ergs then $\frac{17.3 \text{ ergs/spore}}{7.81\times10^{-12} \text{ ergs/photon}}=2.215\times10^{12}$ photons per spore. That means if all the energies are completely absorbed, each spore requires at least 2.215×10^{12} photons to inactivate. In another words, each photon can only inactivate $\frac{1}{2.215\times10^{12}}$ spore. Here it shows that the irradiated energy may not all be absorbed by the spores. Part of it may be absorbed by the surrounding medium.

Morphological changes of the variants:

Many of the ultraviolet-induced variants of *A. terreus* morphologically appeared similar, so only a few representative types, namely, A_1, A_2, C_1, D_1, E_2, F_1, F_2, and G_2 from the first experiment, and B_3', C_1', C_3', D_2', F_1' F_3', G_1', and G_2' from the second experiment together with control H_1 were each isolated out and transferred or subcultured to agar slants of Underkofler's medium. These were examined daily while incubated at 30°C. After 12 to 20 days they were second time subcultured to agar slants and incubated at the same temperature. The results of their characteristics and morphological changes are reported in Tables 3 and 4. Microscopic examinations were also done to the first subcultures. Care was taken each time during transferring to avoid contamination.

Their representative photomicrographs showing their growing condition with their conidial heads, if any, are shown in plate II.

Table 3. Characteristics and morphological changes of ultraviolet induced variants of *A. terreus*

(from first experiment)

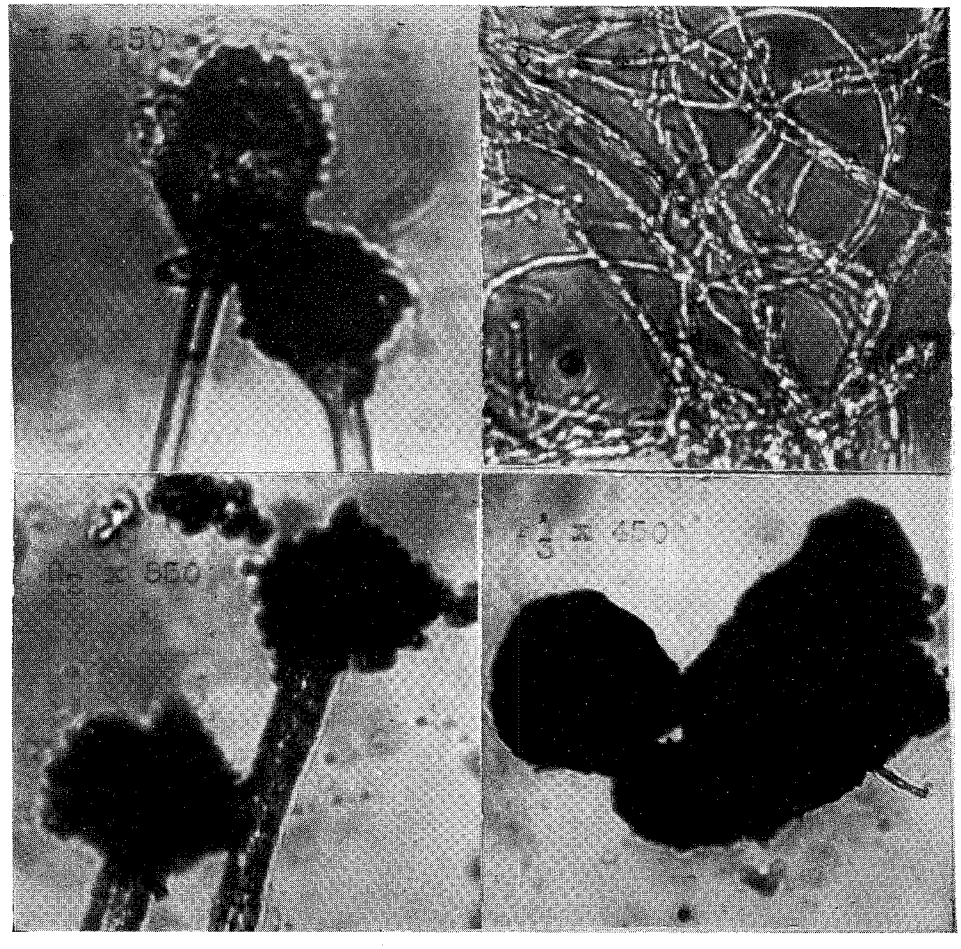
Exp. marks	Exposure time (min.)	Type of colony, isolated and subcultured		
		isolated from 16 days old irradiated spores	1'st subcultured from 16 days old spores (after 20 days incubation)	2'nd subcultured from 20 days old agar slant (observed after 12 days incubation)
control H_1	0	white normal woolly, after 6-8 days, turned yellow with spores	normal and no change	no change
A_1	60	light green abundant spored colonies	light green colonies abundant spores	no change
A_2	60	white layered big flower-like colonies	white color changed to light yellow. no spores after 20 days	light yellow feathery, still no spores
C_1	80	chalk white colonies	chalk white changed to yellow feathery, but no spores after 20 days	no change
D_1	90	white pinkish, layered woolly flower-like colonies	pinkish color changed to dark purple with dark red spores	yellowish pink mycelia, dark base with dark spores, almost no change
E_2	100	yellow spored green colonies	mycelia became light green with yellow spores	no change
F_1	110	white creamish woolly layered big colonies	creamish color changed into dark yellow and red, with abundant spores	beautiful red purple color with some yellow white mycelia, some reversion to parent type
F_2	110	black green short colonies	black green became purple green with black spots at the base	dark purple mycelia and spores
G_2	120	dark green layered colonies with black base	still dark green with abundant green spores, black base	no change

Table 4. Characteristics and morphological changes of ultraviolet induced variants of *A. terreus*

(from second experiment)

Exp. marks	Exposure time (min.)	Type of colony. isolated and subcultured		
		isolated from 20 days irradiated spores	1'st subcultured from 20 days old (after 12 days incubation)	2'nd subcultured from 16 days old agar slant (observed after 12 days incubation)
control H_1	0	white normal woolly turned yellow with spores	normal and no change	no change
B_3'	70	light pretty green, abundant spores	light pretty green abundant spores	no change
C_1'	80	yellow spored green colonies	still yellow spored	no change
C_3'	80	light green abundant spored colonies	light green with green spores, some tan colored spores	no change
D_2'	90	dark green and black spored colonies	dark green and black spored	no change
F_1'	110	white pinkish layered woolly flower-like colonies	dirty brown pigment green spores, black base	all dark dirty green spores some black base
F_3'	110	white thin layered flower-like big colonies	dirty white scale-like thin colonies with no spores	scale-like hard colonies, no spores
G_1'	120	black green layered colonies	black green colonies abundant spores	some white layered black green colonies, aboundant spores
G_2'	120	white creamish woolly layered big colonies	white creamish woolly layered	hyphae in tan color, very little spores

Plate II. Representative photomicrographs of the ultraviolet-induced variants produced in the two experiments:



Explanation of the figures:

Fig. H, H was the parent strain, Aspergillus terreus NRRL, 1960 and had the normal hyphae, conidial heads and spores.

Fig, C_1, C_1 was irradiated for 80 min. and became chalk white, but no heads or spores could be found.

Fig. G_2, G_2 was exposed 120 min. and had well formed abundant conidial heads and spores.

Fig. F_3', F_3' received 110 min. exposure, and had scale-like hard colonies showing no spores.

Discussions:

It is seen from the Table 3 and 4 and Plate II that although the spores were ultraviolet irradiated with the same amount of energies, the variants could be widely different in characteristics and morphological appearances. They also differed in developing pigments. Sufficient and equal amounts of energies make them changed, but not necessarily to the same degrees or to the same

types. Both A_1 and A_2 have been irradiated 60 minutes but A_1 is a light green strain with abundant spores, while A_2 is a white layered flower-like strain with fine feathery yellowish hyphae, but bears no conidial heads and no spores. F_1, 110 minutes irradiated, is a white creamish woolly layered fast growing strain, later developed into dark yellow and purplish hyphae with abundant spores, while F_2, irradiated at the same length of time, is a black green short strain, later developed purplish green with black spots at the base. C_1' and C_3', 80 minutes irradiated, both seem quite stable, but the former is yellow spored and the latter is green spored. F_1' and F_3' have received the same 110 minutes ultraviolet radiation, but the former has dark dirty green spores, while the latter becomes scale-like hard colonies with no spores at all.

On the other hand, even though the time of irradiation was different, similar strains of variants could be obtained. For instance, C_3' irradiated 80 minutes and A_1 only 60 minutes are both light green with heavy spores and are both quite stable.

Also, some ultraviolet-induced variants have growth rate much faster than the parents, resulting in unusually big size colonies from single spores, such as A_2, F_1 and G_2'. Microscopic examinations revealed that F_1 and G_2' grew so fast that their conidial heads broke into loose spores almost as quickly as they were formed and that A_2 grew almost entirely by cell division into fine structures without spore formation.

Pigments may even fade after incubated for some time like F_1, and F_3' even became scale-like hard colonies without sporulation. Reversion to parent strain seems to occur after second time subculturing like F_1, and second cultures may change to the more stable type like F_1' to G_2.

All these points show that variations are ultraviolet-induced some what by certain probability. Photons must be absorbed in order to produce a change. They may be caused to hit and be absorbed at different points of the genes so that a variety of mutagenetic changes can occur. Also certain particular points of the genes may be more susceptible to the ultraviolet photons of the wavelength 2537 Å than other points, so that similar variants can be produced. In another words, the probability of energy absorption at different points in the make up of the genes can be very different but also may be the same. Of course, there must also be a possibility of injury of some parts of the gene by the 2537 Å photons so that a complete sporulation is made impossible, while the fungus may still survive for a certain length of time.

The biochemical properties of the ultraviolet-induced variants—their production of itaconic acid from molasses

The chief theme of this investigation is to develop a strain of *Aspergillus*

terreus NRRL, 1960, so that a better production of itaconic acid can be obtained from molasses. Therefore, after a number of the ultraviolet-induced variants have been produced, and their characteristics and morphological changes studied, the important work is to find out their ability for itaconic acid production.

The optimum conditions of fermentation found by previous investigation (Tsao and Su) were followed so that a comparison could be made.

A series of cultural solutions containing molasses, 200 ml/1 and other necessary nutrients, $MgSO_4 \cdot 7H_2O$ 2 g/1, and $(NH_4)_2SO_4$ 2.67 g/1 were made. pH value was adjusted to 1.8. The solution then was divided into 100 ml per culture in 500 ml special Erlenmeyer flasks. After sterilization in the usual manner, these cultural solutions were inoculated with controls as well as with the few typical ultraviolet induced variants. Then they were subjected to submerged aerial fermentation on an electric shaker at 26±3°C. After ten days, the mycelia from each culture were filtered out, dried in an oven, and weighed. The fermentation liquors were analyzed as (Tsao and Su) for itaconic acid by the method of Friedkin (1945) and for the glucose by a modified method of Shaffer and Somogyi (1933).

The results are reported in Tables 5 and 6.

Table 5. Effect of variants on itaconic acid production from molasses.

variant No.	colony type	glucose consumed g/1	itaconic acid produced g/1	% yield		mycelium wt. g/1
				based on glucose consumed *(a)	based on glucose supplied *(b)	
control H_1	normal white woolly	50.4	16.6	45.2	32.9	4.375
A_1	light green	51.2	18.5	49.7	36.2	4.762
A_2	white layered flower-like	50.2	12.8	35.1	26.5	3.758
C_1	chalk white	48.3	17.7	50.4	36.6	6.499
D_1	white pinkish layered woolly flower-like	49.7	18.2	50.4	36.6	7.419
E_2	yellow spored	51.8	14.1	37.4	27.2	7.295
F_1	white creamish woolly layered	49.5	17.0	47.2	34.4	4.493
F_2	black green	50.5	17.3	47.1	34.3	5.026
G_2	dark green layered with black base	50.0	16.8	46.1	33.6	5.835

* (a) Based on glucose consumed (assuming 1 mole glucose yields one mole itaconic acid)
* (b) Based on glucose supplied (g. itaconic acid produced per g. glucose supplied)

Table 6. Effect of variants on itaconic acid production from molasses.

variant No.	colony type	glucose consumed g/1	itaconic acid produced g/1	% yield based on glucose consumed *(a)	% yield based on glucose supplied *(b)	mycelium wt. g/1
control H_1'	normal white woolly	54.5	15.8	39.8	29.2	3.795
B_3'	light pretty green	54.5	16.0	40.3	29.4	3.247
C_1'	yellow spored	55.5	17.6	43.6	31.7	3.694
C_3'	light green	53.5	16.1	41.3	30.5	4.125
D_2'	dark green black ground	54.2	15.6	39.6	28.8	3.705
F_1'	white pinkish layered woolly flower-like	54.0	16.2	41.2	30.0	3.595
F_3'	white thin layered flower-like	54.0	16.8	42.8	31.1	4.091
G_1'	dark green layered	54.3	15.9	40.2	29.3	3.930
G_2'	white creamish woolly layered	54.4	15.9	40.2	29.3	4.074

* (a) Based on glucose consumed (assuming 1 mole glucose yields 1 mole itaconic acid)
* (b) Based on glucose supplied (g. itaconic acid produced per g. glucose supplied)

Discussions:

From the results reported in Tables 5 and 6, we understand that all the 17 types of ultraviolet-induced variants produced itaconic acid from molasses. In general, most variants prouced more acids than the unirradiated parent strain of *Aspergillus terreus* NRRL, 1960, with only two strains, A_2, and E_2, definitely less acid than that from the parent strain, and with A_1, D_1, and C_1 as leaders in the producing of itaconic acid. The question, whether the fast growing types would produce more acid, is difficult to say. It is very likely that way but there is not without exception. Also whether the morphologically similar types from the two experiments would produce the same amount of acid, it is very probable. Even the strains F_3' and C_1 which did not seem to sporulate, produced more acid than the parent. So as long as the ultraviolet-induced variants are growing in the molasses, they seem to produce itaconic acid. There is no relationship between formation of spores and production of acid in their biochemical property. Further investigation perhaps would be able to reveal more truths on these lines.

Summary

The method for the production of variations in *Aspergillus terreus,* NRRL, 1960 by the ultraviolet radiation is described.

With the energy of the short wave 2537 Å ultraviolet radiation in a period of one hour to two hours exposure over 95% of the spores from the parent strain are found killed or depressed and only less than 5% in the average survived within 3–5 days.

Nearly all the spores survived after the irradiation are changed.

Seventeen morphologically different types of variants are isolated, incubated, subcultured, and microscopically studied.

The probability of how these variants are induced by the energy produced by the short wave ultraviolet radiation is discussed.

In general, the ulturaviolet-induced variants produce biochemically more itaconic acid from molasses than the unirradiated parent strain.

紫外光誘導 *ASPERGILLUS TERREUS* 之變異與其形態上之變化及其由糖蜜產生 Itaconic 酸之研究

曹簡禹　黃定加　蘇瓊華

本文詳述用紫外光誘導 *Aspergillus terreus,* NRRL, 1960 而生變異之方法。

由母系而來之孢子凡經紫外光波長 2537 Å 之光能照射一至二小時後在三至五天內百分之九十五以上被殺死或抑制，能生存者平均不到百分之五。

經照射後尚能生存者，幾皆成變異型。

十七種形態之變異型，被分離後，曾加以保溫孵育，接種，以及在顯微鏡下研究。

用紫外短波光能照射而生變異之或然性，本文曾略加討論。

大體說來，凡由紫外光誘導而生之變異型，皆比母系能自糖蜜產生更多之 Itaconic 酸。（摘要）

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