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ULTRA-VIOLET IRRADIATION OF SPORES OF CERTAIN MOLDS COLLECTED FROM BREAD

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Since the discovery of the anti-biotic effects of the blue-violet end of the spectrum, (Ward, 1894) this light has been widely applied to practical advantage. The bactericidal properties of these rays have been extensively utilized in the sterilization of air. Fulton (1929), Welch (1930), Luyet (1932), Duggar and Dimond (1940), and others, have shown the deleterious effect of ultra-violet light on certain fungal spores.

Problems of food spoilage due to contamination with common molds have recently become more significant than heretofore. Spoilage of bakery products has become especially serious due to delays in deliveries and prolonged storage occasioned by curtailment of transportation. The well-known anti-biotic properties of ultra-violet radiation upon microorganisms seemed to offer possibilities of a simple method of controlling the common molds involved in bread spoilage. As an approach to such a solution of spoilage in bakery goods, it seemed desirable first to test the sensitivity of the molds most commonly causing damage. Data are given on the inactivating effects of varying dosages of continuous and intermittent ultra-violet energy applied directly to the mold spores.

MATERIALS AND METHODS

The source of ultra-violet light used was similar to one of the lamps used by Koller (1939) in his work with bacteria. It is a low-pressure mercury vapor lamp manufactured by the General Electric Company under the trade name of "Germicidal Lamp." It is a 15-watt, T-8 lamp made of a special glass having some of the characteristics of fused quartz. The voltage, vapor pressure and current density are so regulated that sixteen per cent of the energy input is emitted at a single line 2537 A° (Buttolph).

In order to establish the relative resistivity of the various mold spores, the amount of the ultra-violet energy reaching the irradiated surface was measured with a Luchiesh-Taylor ultra-violet meter. With the use of a conversion factor obtained by calibration with a known source, the amount of irradiation of ultra-violet quality may be expressed in terms of micro-watts per square centimeter (uw/cm^2) of surface area.

Several loaves of wrapped bread were purchased at random from various bakeries. These were brought into the laboratory and incubated unopened at room temperature for ten days. At the end of this period the wrappers were opened and the molds found therein transferred to potato dextrose agar in Syracuse petri dishes. Seven spore-bearing forms were isolated in this manner: *Aspergillus flavus, A. wentii, A. niger, A. repens, A. ruber, Rhizopus nigricans* and *peni-*

cillium sp. Subcultures of each were prepared in tubes containing 10 ml. of 20% sucrose Czapek's solution to which 0.5% peptone was added. These subcultures were incubated at room temperature for six days. Each tube was then shaken thoroughly and the contents filtered aseptically to remove clumps of spores, pieces of mycelium, sporangia, etc. One ml. of the filtrate was diluted with 99 ml. of sterile distilled water and immediately used as the inoculant.

A De Villois atomizer was used to apply the inoculant uniformly to the surface of potato dextrose agar in Syracuse petri dishes. The atomizer was fixed in a rack with the nozzle in an upright position to preclude flooding and uneven "seeding." The cover of each petri dish was removed and half of the area was protected from the rays by a piece of enameled metal. Thus one-half of each plate served as a control for the irradiated half. The plates were exposed at 25 cm. from the lamp for from one to eight minutes. There was no detectable increase in temperature during exposure. After 48 hours of incubation at 30° C the number of colonies on either half of the dish was determined and recorded. The plates devoid of colonies on the exposed half were stored at room temperature for ten days. In the case of *R. nigricans* individual colonies could not readily be discerned because of its growth habit.

Three plates were exposed continuously for three hours prior to atomizing with a spore suspension. During this time the agar surface received 252,000 uW min/cm^2 of ultra-violet energy or 31 times the lethal dose of the most resistant form.

RESULTS

The amount of continuous ultra-violet light necessary to render the spores inactive is shown in table I. In an attempt to determine the effects of intermittent irradiation and possible economies of power consumption, mold colonies on agar were also exposed to repeated dosages at varying intervals of time. Coblentz and Fulton (1924) demonstrated that intermittent radiation of *E. coli* is cumulative. Fulton (1929) showed that discontinuous exposure was very slightly less effective on fungal spores than when applied in one steady radiation. Table II shows the results of intermittent exposures equivalent to one steady lethal dose of each of the seven kinds of spores.

After ten days of storage at room temperature the halves of the plates which showed no colonies at the end of 48 hours were still free from mycelium originating on the exposed side. The plates which were irradiated with many times the lethal dose before inoculation produced normal colonies when atomized with a spore suspension. Irradiation of *E. coli* indicated that an exposure of five seconds or 5175 uW/cm^2 was sufficient to prevent the formation of colonies on peptonized beef agar.

Table I. Effect of ultra-violet irradiation on mold spores atomized on agar surface.

Mold	Length of irradiation in minutes	Length of irradiation uW min/cm²	No. of Colonies: Control half	No. of Colonies: Irradiated half	Percent Survival
A. flavus	1	1350	92	29	31.5
	2	2700	89	12	13.5
	3	4050	96	0	0
A. wentii	1	1350	107	44	41.1
	2	2700	127	23	18.1
	3	4050	132	20	15.1
	4	5400	120	0	0
A. niger	1	1350	230	138	60.0
	2	2700	210	53	25.2
	3	4050	234	69	29.5
	4	5400	194	26	13.4
	5	6750	249	30	12.0
	6	8100	228	2	0
A. repens	1	1350	217	54	24.8
	2	2700	194	25	12.9
	3	4050	209	0	0
A. ruber	1	1350	84	13	15.5
	1.5	2025	94	0	0
Penicillium sp.	1	1350	1406	407	28.9
	2	2700	1504	119	7.9
	3	4050	1664	0	0
R. nigricans	1	1350	growth	growth	
	2	2700	growth	complete inactivation	

Table II. Effect of intermittent exposure on mold spores

Molds	Irradiation Exposures: No. of	Irradiation Exposures: Min. ea.	Irradiation Exposures: Total uW min/cm²	No. Colonies: Control half	No. Colonies: Irradiated half	Percent Survival
A. flavus	3	1	4050	197	1	0.5
A. wentii	4	1	5400	234	0	0
A. niger	3	2	8100	356	2	0.6
A. repens	3	1	4050	253	0	0
A. ruber	3	½	2025	127	0	0
Penicillium sp.	3	1	4050	1043	2	0
R. nigricans	2	1	2700	growth	no growth	0

DISCUSSION

Gates (1933) has suggested that the failure of irradiated bacteria to multiply has been unjustifiably accepted as an indication of death; that actually, though cell division is arrested by ultra-violet light, growth continues producing forms that "look like spaghetti." In this paper the inability of spores to produce colonies has been taken as evidence of their inactivation. For practical purposes the failure of a spore to send mycelium into the substrate seems to be the critical characteristics of mold action.

The destructive force of ultra-violet light has been correlated with the selective absorption of nuclear derivatives (Harris and Hoyt, 1917, Gates, 1928, Oster, 1934). Swan and del Rosario (1932) demonstrated a high absorption of the 2536 A° line by the nucleus of Euglena cells. Differences in susceptibility of microorganisms to the effects of ultra-violet light have been attributed to the size of cell, quality of pigment in the cell wall (Tanner and Ryder, 1923) and secretion of a fatty or waxy coat which offers protection from the rays (Ellis and Wells, 1925, Welch, 1930). The relative resistivity and the characteristics of the spores used in this investigation seem to coincide with the foregoing observations.

Conflicting reports appear in the literature regarding the formation of toxic substances in the medium, caused by the ionizing power of ultra-violet light. The results of the present experiments show that even after irradiation of the medium with many times the lethal dose it still supported normal growth.

SUMMARY

Experiments were conducted to determine the effect of ultra-violet light on the spores of certain molds found growing on bread. The source of the light was a 15-watt General Electric mercury vapor lamp high in 2537 A° radiation. All forms collected were rendered inactive by exposures of from 1½ to 6 minutes. The thick-walled, heavily pigmented spores of *A. niger* were the most resistant, requiring from two to four times the irradiation of some of the other forms. The inability of the spores to send out mycelium was taken as a criterion of their inactivation.

Mold spores subjected to intermittent radiation were also inactivated when the total exposure equalled in amount the continuous lethal dose. The occurrence of inhibitory substances in the medium after prolonged irradiation was not demonstrable.

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