LONGEVITY IN SPORES OF ASPERGILLUS ORYZAE AND RHIZOPUS NIGRICANS

RATHER frequent reference may be found in literature dealing with the cryptogams to the belief that spores will "live for years," but when actual foundation for the truth of such statement is sought for, it is surprisingly scarce. Little seems to have been done in the way of definite, properly controlled investigation of this point, under conditions which would preclude all possibility of error in the final conclusions. It was, therefore, felt that an account of the study herein reported might be worth while if for nothing more than to bring out similar reports possibly known to other workers.

Wehmer[1] has recorded germinating dried spores of Aspergillus species as follows: *A. Wentii,* after more than a year; *A. niger,* about three years; *A. oryzae,* more than four years. Brefeld[2] found viability in *A. flavus* after six years; Eidam[2] in *A. fumigatus* after ten years; and Hansen[2] in *A. glaucus* after sixteen years. But in these cases the data are not given clearly enough to entirely exclude possibility of chance growth.

The organism considered in this study is a well-known species of the Aspergillaceae, *Aspergillus oryzae.* The conidial material in question was collected in 1897 from cultures of this mold—sifted out with the dust from a container in which it had been grown upon a bran base, and had been allowed to become thoroughly air dry. It was placed in a tube 10 x 100 mm, which was sealed at that time and kept in the dark under ordinary laboratory range of temperature. No attempt was made to secure a vacuum, but the tube was two thirds full of spore-dust and was sealed in the flame, hence there would be, at the most, only a very small amount of air left within.

In November, 1919, after a lapse of twenty-two years, the tube was opened for testing. Since it was considered doubtful whether the spores would show any signs of life after such prolonged desiccation, inoculations were made upon a great variety of media. Plantings were made (a) direct from the tube, (b) from dextrose-bullion suspension, after "soaking" several hours. Transferring was done, with every precaution against accidental contamination, under a glass cubicle two feet high, 36′ x 24′ at the bottom, 36′ x 12′ at the top, the sloping front plate being adjusted to any height desired above the worker's hands. The base of the cubicle is also of glass; hence the whole structure may be readily disinfected. As a further precaution, half a dozen Petri plates of nutrient agar were exposed on the floor of the cubicle while the material was being removed from the tube. All plates remained sterile but one, upon which appeared a colony of "hay" bacillus.

The following media were inoculated in duplicate, one series being incubated at 37° C., the other at laboratory temperature. In general, it may be said the higher temperature is rather more favorable or at least hastens development.

(1) Sabouraud's agar (American ingredients)
(2) Czapek's agar (Formula as modified by Dox. Cane sugar the source of carbon)
(3) Plain gelatin
(4) Sugar gelatin
(5) Dextrose-bouillon
(6) Potato agar
(7) Rice flour agar
(8) Wheat bran
(9) White rice
(10) Brown rice
(11) Cracked corn
(12) Cracked beans

Cultures of varying degrees of vigor were obtained, the organism developing upon every medium tried, much more luxuriantly, however, where there was abundant starch or sugar content, 4 per cent. or more, readily available. Considerable range in height of upright hyphae, color and size of conidial heads, etc., was found upon the various substrata; but these proved to be interchangeable according to the food, *e.g.,* when transferred from No. 3 above to No. 8, growth was comparable to that upon other cultures of No. 8 and vice versa.

On the whole, this strain can not be seen to have lost ground either in the development of typical herbage or in physiological activity. It is a vigorous diastase producer, and exhibits considerable proteolytic power as well. Since 1919 it has been maintained in pure culture upon three of the above media: Czapek agar—Dox modification; Sabouraud's formula prepared from American ingredients; and rice flour agar. It is still a thrifty strain.

The tube of spore-dust was resealed, to be kept indefinitely.

Rhizopus nigricans. In the course of the above work, although quite unintentionally, longevity was also demonstrated in another organism—the common *Rhizopus nigricans,* cultures of which were several times obtained from the tube of spore-dust where it had doubtless found entry as an invader of the original culture. Only one strain was preserved and has since been kept in culture. This, tested a year or two ago against Blakeslee's minus strain, proved to be of the plus type.

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[1] Centralblatt für Bakt., 2 Abt., 1897.

[2] Cited by Lafar: "Technical Mycology," Vol. II, Part I.