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Materials and methods

Media

The solid media employed included Czapek-Dox agar (CZA), glucose ammonium chloride agar (GAA), malt extract agar (MA), Sabouraud dextrose agar (SDA), bean dextrose agar (BA), potato dextrose agar (PA), rice dextrose agar (RA) and yam dextrose agar (YA). To prepare bean dextrose agar, 25 ml of distilled water was added to 20 g of bean seeds and boiled till well cooked. The cooked bean was filtered through muslin cloth. The filtrate was made up to 100 ml with distilled water. To this, 0.1 g of D-glucose and 1.2 g agar were added (Ricker & Ricker, 1936). The same process was used to prepare the other local foodstuff-agar media.

Liquid media were prepared as above but agar was not added, and all the media were sterilized by autoclaving at 1.0 kg/cm^2 for 15 min.

Growth studies

Effect of temperature on growth. Malt extract broth was employed. One millilitre of the spore suspension of the fungus was transferred to 250 ml Erlenmeyer flasks containing 50 ml of the medium. The inoculated flasks were then incubated at 15, 20, 25, 30, 35, 40 and 45° C for 10 days. The contents of three flasks from each temperature were separately filtered by suction through previously dried and weighed glass papers and dried at 80° C for 15 h. The average of at least three results is usually given in the results.

Effect of different media on growth. The effect of different media on linear growth of the fungus was carried out using the media mentioned above. Two lines intersecting were drawn at the bottom of each Petri dish and the media contained inoculated at the point of intersection, with a 5 mm diameter disc inoculum taken from the advancing margin of 3-day-old culture. The cultures were incubated at 30° C and the linear growth of the fungus was determined daily for 5 days. The average of three readings was recorded.

Effect of glucose concentration. Glucose ammonium chloride broth was used and the concentration of the glucose content was varied to give 1, 2 and 3% glucose constituent. The flasks were inoculated with 1 ml of spore suspension and incubated at 30° C for 10 days before determining the dry weight as described earlier.

Effects of fungicides on growth. Seven fungicides were tested for their effect on growth of the fungus in culture. For each fungicide, 500 and 1000 ppm concentration were prepared. One millilitre of each concentration was withdrawn into each Petri dish and 9 ml of molten medium added. The agar medium and the fungicide suspension were mixed thoroughly to obtain the final concentration of 50 and 100 ppm active ingredient of each fungicide. Each dish was inoculated at the centre with a 5 mm agar disc of the fungus and treated as described for linear growth.

Effects of some antibiotics on growth. The antibiotics employed were amphotericin B, sulfamycin, nalidixic acid and kanamycin. Four millilitres of heart infusion broth was added to each antibiotic stock solution to give 5 ml of antibiotic solution containing 200 μg or U/ml.

The antibiotics were separately incorporated into growth medium contained in 250 ml Erlenmeyer flasks and dry-weight determinations of the fungus were carried out after 10 days incubation at 30° C.

Effect of ultraviolet light on growth. Freshly inoculated dishes were exposed to the u.v. light and withdrawn after 1, 2 and 5 h. All the exposed dishes, and a set of unexposed plates which served as controls, were then incubated at 30° C in the dark for 5 days and the average colony diameter growth measured.

Spore suspensions of the fungus were also variously exposed to u.v. light to determine its effect of the germination of the spores. Germination was observed with a light microscope at intervals for a period of 2 days.

Results

Growth studies

The growth of the fungus in malt-extract broth showed that the fungus grew over a wide range of temperatures (20–35° C, Fig. 1). The fungus did not grow at 15 and 40° C. The best growth occurred at 30° C.

On the effect of different media on growth of

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A possible new pathogenic Aspergillus isolation and general mycological properties of the fungus

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Summary

A species of Aspergillus was isolated from vomitus and scrapings of the tongue of a patient with a form of respiratory illness. The fungus has since been identified as *Aspergillus aculeatus*, Iizuka.

The fungus grew over a wide range of temperatures, the spores appeared to be thermophilic. Many local foodstuffs supported the growth of the fungus in culture. Ultraviolet light inhibited mycelia growth and sporulation of *A. aculeatus*.

The fungicides brestan, benlate, fundazole and kocide 101 inhibited the growth of the fungus more than daconil, demosen and dithane M-45. Amphotericin B and sulfadiazine completely arrested the growth of the fungus while sulfamycin, nalidixic acid and kanamycin had no detectable effects.

Résumé

Une espèce d'Aspergillus a été isolée du vomitus et des extrails de la langue d'un malade qui souffrait d'une maladie respiratoire. On a identifié le mycète par la suite comme *Aspergillus aculeatus*, Iizuka.

Le mycète a poussé dans des températures variées, les spores avaient l'air thermophiliques. Le mycète a pu pousser sur beaucoup de nourritures locales dans le laboratoire. La lumière ultraviolette a empêché la croissance et la sporulation de *A. aculeatus*.

Les mycèticides brestane, benlate, fundazole et kocide 101 ont empêché la croissance du mycete plus que daconile, demosène et dithane M-45. Amphotericine B et sulfadiazine ont totalement éliminé la croissance du mycète tandis que sulfamycine, l'acide nalicidixique et kanamycine n'ont manifesté aucun effet visible.

Introduction

Aspergillus species are highly ubiquitous (Emmons, 1962) and are commonly found in spoiled food and open medications among other environments. Although there are many species of the genus Aspergillus, only about eight species (Rippon, 1974) have been consistently involved in human infectious diseases. *A. aculeatus* was first isolated from a patient suffering from a disease whose symptoms are very similar to diphtheria. In man, diphtheria is usually found spreading along the throat up to the base of the tongue. The first indication of the disease from which *A. aculeatus* has been isolated is marked with the saliva being bitter when swallowed. This is accompanied by a slight rise in temperature. About 3–5 days later, the surface of the tongue, up to and as far down as the back of the tongue, becomes coated white. The coating later turns black. Anorexia, especially for spicy foods, and polydypsia follows.

Several investigations have been carried out during the past decade (Williams, unpublished data) to elucidate the connection of any microorganism with the disease with no success. In 1976 however, Williams (the first author) isolated a fungal agent from the scrapings of the inner part of the tongue of a patient at the John F. Kennedy Hospital, in Monrovia, Liberia, West Africa. This fungus has since been identified as *A. aculeatus* by the Commonwealth Mycological Institute in London, U.K. The investigation reported here is part of our observations on the properties of this likely pathogen of man.

Table 1. The effect of some fungicides on growth of *A. aculeatus* growing on malt-extract agar

Fungicide	Diameter of mycelium colony (mm) after the indicated periods of incubation (days)				
(ppm)	1	2	3	4	5
Benlate 50	0	0	0	0	0
100	0	0	0	0	0
Brestan 50	0	0	0	0	0
100	0	0	0	0	0
Daconil 50	8·5	10	10	10	11
100	8·0	10	10	10	10
Demosan 50	3	6	7	10	11
100	3	7	10	14	15
Dithane-M45 50	13	23	36	48	60
100	12	23	35	48	61
Fundazol 50	0	0	0	0	0
100	0	0	0	0	0
Kocide 101 50	0	0	0	0	0
100	0	0	0	0	0
Control	17	36	57	80	*

*Indicates where the fungus has grown to fill the Petri dish

light did not prevent germination of spores.

Discussion

Growth experiments show rather interesting observations with regard to the ability of the fungus to flourish in local foodstuff materials. If conclusive evidence is obtained about the pathogenicity of the fungus to man, it is conceivable that the organism may infect man through its contamination of foodstuffs like beans, yam, rice and potato. From the mycological point of view, it is evident that these foodstuffs can serve basic constituents of media for the cultivation of the pathogen rather than the expensive synthetic media. It is also possible that other fungi species may grow well in different forms of media with these foodstuffs serving as a base, or an important component of the media.

The disease which is closest to the disease complained about by the patient from which the fungus has been isolated is similar to the one called 'Opa-aro' in some parts of Ondo State of Nigeria. It is commonly treated with a concoction prepared by boiling the bark of a tree *Bridelia ferrugena*, with lime and gypsum.

In the present studies, we have shown that benlate, brestan, fundazol and kocide 101 effectively prevented the growth of the fungus in culture at low, safe-for-human-consumption concentrations (Preiser *et al.*, 1970). Although these fungicides would not be used directly on patients, similar drugs could be developed to treat the infection in man if the pathogenic role of the fungus in human disease is confirmed. Amphotericin B completely inhibited the growth of the fungus. This is not surprising since the use of this drug in the treatment of aspergillosis in man is well known (Laustela, 1969; Colp & Cook, 1975; Mohr, Mickown & Muchmore, 1971). This study, in addition, shows that sulfadiazine also inhibits growth of

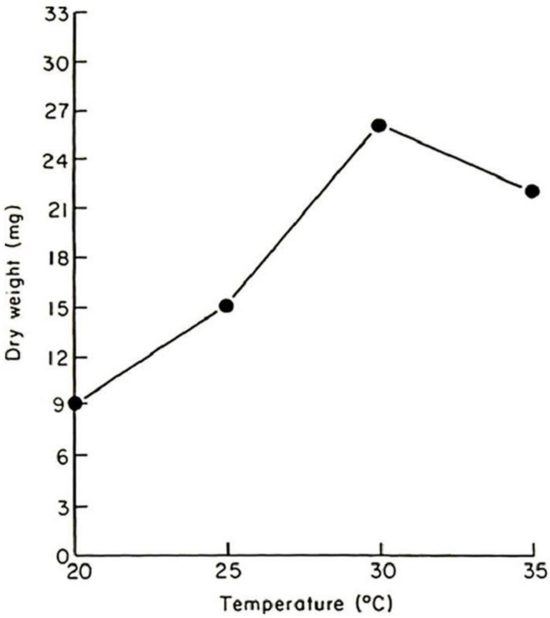

Fig. 1. Effect of temperature on the growth of *A. aculeatus* in malt extract.

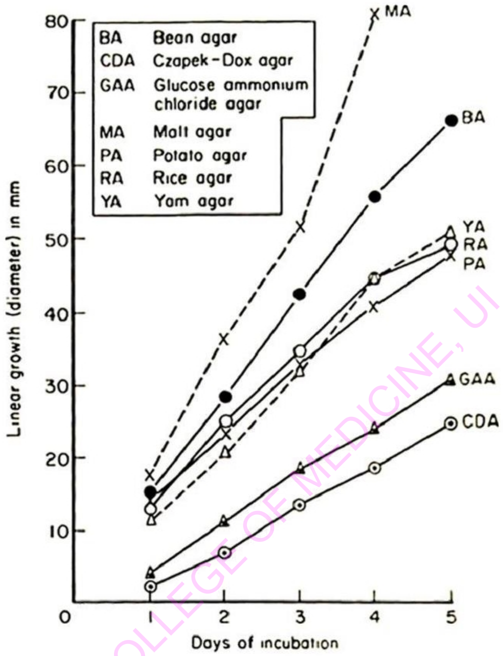

Fig. 2. Effect of different media on the growth of *A. aculeatus* at 30° C.

A. aculeatus shown in Fig. 2, malt extract agar has the best linear growth followed by bean dextrose agar. Yam dextrose agar, rice dextrose agar and potato dextrose agar supported a good growth of the fungus and there was virtually no difference in the rate of growth on the three media. Growth was poorest on glucose ammonium chloride agar Czapek-Dox agar.

The results of the effects of fungicides on growth of *A. aculeatus* is shown in Table 1. Altogether seven fungicides were tested and four of them inhibited/prevented the growth of the organism at the concentration of 50 ppm. These are benlate, brestan, fundazol and kocide 101. Dithane M-45, daconil, and demosan were effective even at 100 ppm concentration.

On the effect of antibiotics and some chemicals on growth, it was observed that amphotericin B and sulfadiazine completely prevented the growth of the fungus.

Treatment with u.v. light

The result of the effect of u.v. light on growth of the fungus presented in Fig. 4 shows that growth occurred in all the plates inoculated but there was progressive decrease in growth with increase in exposure time. Exposure to u.v.

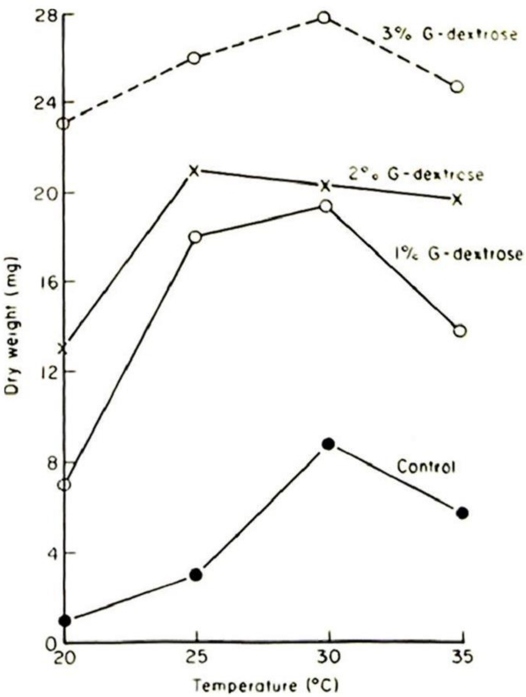

Fig. 3. Effect of increasing glucose concentration at different temperatures on the growth of *A. aculeatus*.

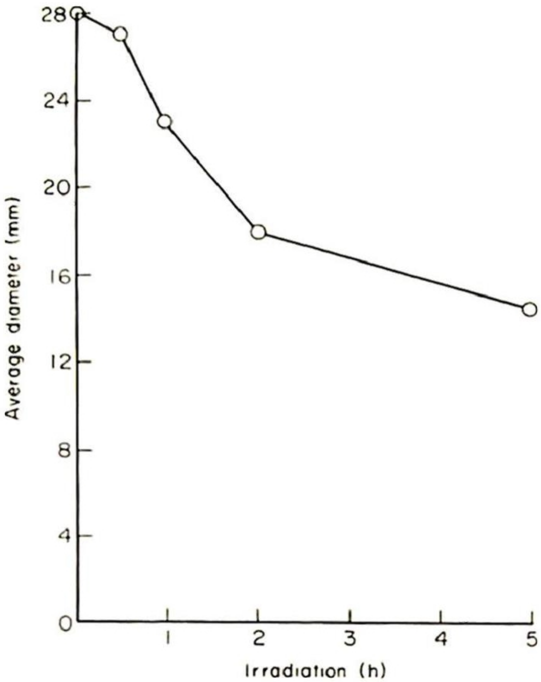

Fig. 4. Kinetic effect of ultraviolet light on the growth of *A. aculeatus*.

A. aculeatus. These may therefore be useful in the treatment of infection resulting from the fungus.

In a preliminary experiment (Williams, unpublished), it appeared as if the preparation from *Bridelia ferrugena* showed some inhibitory effect on the growth of the fungus. The poor growth or total inability of the fungus to grow at lower (below 15° C) and higher (40° C and above) temperatures is considered significant and may be relevant in its role as a pathogen. A few other pathogenic fungi have a similar characteristic (Christensen, 1961; Reppon, 1974; Emmons, Binford & Utz, 1974).

Exposure to u.v. light appeared only to slow down the rate of mycelial growth but did not prevent germination. Some metabolic processes may have been temporarily affected by the treatment and so a period of re-adjustment may have been needed before the fungus could start growing. The maximum growth rate was not attained.

There was no appreciable difference in the rate or time of growth of the spores whether treated with u.v. light or not. This may indicate that the spores do not become dormant at any stage in their lives. Ultraviolet light treatment of spores is often used to break dormancy of some fungal and other spores. The observation on the effect of u.v. light appears to support the contention that the fungus may be hardy or 'resistant' to adverse treatments.

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