STUDIES ON THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS OF A NEW SPECIES OF THE GENUS PAECILOMYCES

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STUDIES ON THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS OF A NEW SPECIES OF THE GENUS PAECILOMYCES

By

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INTRODUCTION

This organism was obtained from the stock rats by Dr. Margaret A. Ohlson and Mrs. Annanell C. Jubb of the Department of Home Economics of Michigan State College in East Lansing, Michigan. They had made the following observations:

"This organism was observed in blood smears from the stock rats. A later check of all stock and experimental animals revealed that 50-75 % of these animals were infected. Ingredients in the diet were examined microscopically with negative results. Old and non-fertile stock animals were anesthetized with chloroform and organs and blood cultured on maltose agar medium. The organs were sectioned (1-6 microns) and stained with Wright's stain. Sections made of tissues from other animals were negative. A variety of organisms showed on the culture plates, and those from the kidneys and spleen were the only ones to take the stain as did the organisms in the blood. The growth on the plates was transferred several times with no results. Maltose agar medium was used throughout. The tubes were left for nearly two months, and were examined before they were discarded. Subcultures of organisms from kidneys were of the size, shape and color of those from the blood. These were

transferred to sugar broth tubes and from these to cornmeal agar medium. Broth tubes produced growth of organisms with the color and shape of those found in the blood, but they were slightly larger. Also, a mycelial mass, which was tough and difficult to break, was found on the surface of broth tube.

"Four rats from another laboratory (Sprague-Dawley) were inoculated intraperitoneally with blood containing the organism from one of our rats. These rats were kept separately, and the food, dishes, etc. were sterilized. When the four injected rats failed to show the organism in their blood they were reinjected with the pure culture. After four weeks the organism was found in the blood. Five months later, it was not found in two of the injected rats. The control rat in the stock colony room had the organism when examined at the end of four weeks. None of the rats manifested pathological manifestations."

The stock culture grown on cornmeal agar medium received from Dr. Ohlson and Mrs. Jubb was transferred to various media for morphological and physiological studies. According to its colony characteristics, microscopical features and physiological manifestations this organism is classified as a new species of the genus Paecilomyces.

REVIEW OF LITERATURE

A review of the literature reveals that a large number of organisms with the characteristics of Paecilomyces have been found, among which 14 species have been identified. These vary in shade of color of colony, color in the substratum; in floccosity; in the arrangement of the sterigmata and the size of the conidia. No one at present claims to know this genus well enough to establish scientific lines of relationship among them. In regard to the descriptions and figures which appear in the literature, this structural type seems to be cosmopolitan and is described under the different generic names Corolium, Spicaria, Penicillium, Paecilomyces, and Eidamia.

In 1907 Bainier obtained an organism, Paecilomyces varioti, and described the genus, translated and amended by Thom, as follows: "Genus related to Penicillium and Aspergillus, distinguished by sterigmata, short-tubular or more or less enlarged, tapering into long conidium bearing tubes, mostly curved or bent slightly away from the axes of the main sterigmatic cells; sterigmata variously arranged, partly in verticils and branching systems suggesting Penicillium, partly irregularly arranged upon short branchlets, partly arising singly

along the fertile hyphae; conidia in chains, elliptical, never green."

In 1910 Thom recorded the cultural data of the Penicillium divaricatum which should be classified as a species of Paecilomyces as it was pointed out later by the same author and summarized as follows: "Cultivated in gelatin or bean agar, yellowish brown (avellaneous) never green, broadly spreading in the substratum; superficial growth consisting mostly of trailing fertile hyphae, becoming powdery in appearance when mature; reverse of colony not discolored; fertile hyphae septate, usually short, mostly creeping; conidial fructification either terminal or on short branches of creeping or partially erected hyphae, consisting of separate sterigmatic cells, of verticils, or of series of verticils of branchlets and sterigmata irregularly distributed along the fertile hyphae; sterigmata 15 to 20 by 3 microns, with long acuminate tubes usually bent from the axis of the cell and broadly divergent at the apices, bearing long chains of conidia; conidia elliptical or fusiform, 5 to 7 by 2.5 to 3 microns, yellowish to brownish, swelling in germination to 10 microns and producing 2 or more tubes; does not liquefy gelatin; litmus reaction alkaline."

In 1912 Sopp reported an organism, Corollium dermatophagum, found first upon rotten leather and later common upon miscellaneous substrata in the laboratory; liquefies gelatin; colonies at first veil-like, suggestive of Penicillium aromaticum, show yellowish brown or greenish yellow colors, later becoming dense and wrinkled, with reverse dirty green; optimum temperature for growth 38 to 40 degrees C., maximum above 45 degrees C. Sopp also reported extensive experimentation upon different substrata in which it produced malic and citric acids, catalase, chymosin, pepsin, and diastase, as well as small amounts of alcohol, ether and other aromatic substances.

In 1913 Bainier and Sartory reported an organism which colonises on licorice sticks, fruiting about 6 days, at first pale yellow then in deeper shades to dark orgnge yellow; the penicillus is rarely terminal, usually borne upon irregularly produced short branches of creeping or ascending hyphae enlarging slightly from base to apex and bearing single verticil of sterigmata with their conidial chains, or more or less biverticillate by the development of part or all of the sterigmata into secondary branches with their verticils of sterigmata; sterigmata 5 to 6 or more in the verticil, 15 to 20 by 7 to 9 microns; conidia variable in size mostly 6 to 8 by 3.5 to 4 microns, smooth, in chains, yellowish,

swelling to double size inggermination and sending out several tubes. They named it Penicillium repandum, but according to Thom's opinion that the description mentioned above would place it among the Paecilomyces strains rather than the strictly penicillate species.

In 1921 Saito reported an organism, Penicillium mandshuricum, appearing on wine yeast generally as a mass of olive green or olive yellow spores, occasionally as aerial hyphae having a threadlike appearance, in artificial media developing a thick membrane which bears a large number of spherical and smooth chlamydospores; stalks very tiny, 170 to 180 microns, irregular; spore bearing branches one to three, irregular, each beating a chain of spores; conidia smooth, elliptical to ovate, generally 6.5 by 4.5 microns, some as great as 10 microns; optimum temperature 36 degrees C.; gradually liquefies gelatin and starch media; secretes as enzymes maltase, protease, peroxidase and catalase. According to Saito this fungus bears a striking resemblance to Penicillium olivaceum, but may be separated by the difference in their optimum temperature, the latter preferring 23 to 25 degrees C. In the opinion of Thom, his strain No. 4279 received as Penicillium mandshuricum in 1918 from Saito is a strain near Paecilomyces varioti Bainier.

In the same year, Moesz reported an organism, Spicaria fimetaria, found in horse dung in the field at Teteny near Budapest. The Latin diagnosis translated by Thom is as follows: "Effused, rosy, powdery; sterile hyphae creeping, branched, septate, about 5 microns in diameter; fertile hyphae suberect, septate, irregularly branched; branches frequently bearing dichotomous branchlets; ultimate branchlets (sterigmata) with apex acute producing conidia ellipsoid, hyaline, smooth, 6.5 to 10 by 5 to 6 microns and in long chains."

Marchals (1921) reported an organism, Penicillium flavum, found upon fruit, apples, pears, cherries, etc., in Belgium: colonies flavous rarely in age verging toward ochraceous; conidiophores erect, simple or funiculose, septate, 145 to 190 by 4 to 5 microns, branched above in 3 to 4 series; sterigmata frequently in 3', scarcely attenuate at the apex, 13 to 19 by 2.3 to 3 microns; conidia ovoid hyaline, smooth, frequently one to several, guttulate, 4.2 to 6 by 3 to 4 microns.

Pollacci (1921) reported an organism, Paecilomyces burci, isolated by G. Burti from an experimentally provoked nodule. In Sabouraud's broth and the same broth with peptone and glucose at 18 degrees C., colonies cottony in 2 to 3 days with delicate, short sterigmata bearing yellowish white, little conidia in chains, avellaneous; mycelium, septate, creeping or ascending, 6 to 7 microns in diameters; conidiophores erect, sep-

tate, hyaline, 50 to 110 microns in length, bearing small branches; conidia globose, rarely elliptical, pale fuliginous, 4 to 5 microns in diameter.

In 1923 Biourge described an organism, Paecilomyces aureo-cinnamomeum, colonies on wort gelatin yellowish to fulvous, cinnamon, reverse at first yellow then cinnamon brown; odor none; conidiophores 3 to 4 microns in diameter; penicillus up to 60 microns long, with walls smooth; branches irregularly produced; metulae 16 to 20 by 2 to 4 microns, irregular in number; sterigmata single or paired, 13 to 30 by 1.5 to 2.5 microns, producing long slender tubes bent from the median line; conidia elliptical varying greatly in size 2.4 to 4.5 by 2.4, to 6.4 by 3.6, and also 5.8 microns.

Shaposhnikow and Manteifel (1923) reported a laboratory contaminant growing at 40 degrees C. under reduced oxygen tension to which the name Penicillium arenarium was given on account of its sandy color. In artificial media as undiluted wort, glucose with 3 to 5 % of peptone and media containing a total nitrogen up to 0.2 % in contrast with the usual 0.05 to 0.08 %, mycelium white, 15 microns in diameter, twining, surface of colony olive sandy or sometimes greenish, even rose, light yellow, dark olive green to blackish, dark blue green in depressions between buckled areas; reverse characterless; conidiophores 150 by 5 to 15 microns,

on short side branches of mycelium, having an indefinite tree-like branching; metulae when present varying in number, and bearing 2 to 4 sterigmata, separated by septa from metulae, or entire structure non-septate(?); sterigmata few sometimes borne directly on the stalk, 25 by 5 to 6 microns, or even 75 to 100 microns in length; conidia in extremely long chains, lemon-shaped with distinctly pointed ends, large, 10 to 11 microns in diameter. No perithecia were found. The optimum temperature was 35 to 40 degrees C. In cultural experiments this organism tolerated 0.5 % acetic acid, 12 % citric acid and 20 % of tannin.

Horne and Williamson (1923) reported an organism, Eidamia catenulata, isolated from a seasoned specimen of the heartwood of Quercus robus. The mycelium consists of colorless septate branched hyphae, varying from 3 to 11 microns in width. These hyphae are usually thin-walled, but thick-walled hyphae do occur when the fungus is grown on certain media, such as potato glucose agar or pure agar. Two kinds of spores are produced, hyaline macrospores and conidia. The macrospores are borne directly on the hyphae or at the end of a short branch, or may be intercalery. They vary in size from 7.5 by 8.5 to 14 by 10 microns, or may be 18 microns in diameter. These hyaline spores occur on all the culture media employed when kept at 20 or 25 degrees C.; they are less

numerous or absent at 30 degrees C. The optimum temperature for growth proved to be 30 degrees C., moderate growth occurred at 25 degrees C., and least at 20 degrees C. This organism inoculated on Bramley's seedling apples at 20 degrees C. for eight days showed a surface growth of mycelium and conidiophores at the place of inoculation, but no parasitism at the living cell occurred. Growth on seasoned pine, chestnut, beech and oak was prolific, and penetration soon took place. In view of their descriptions and the figures this species is better classified under the genus Paecilomyces rather than Eidamia.

In 1927 Gilman and Abbot transferred some species of Paecilomyces such as P. divaricata to the genus Spicaria and gave the following generic descriptions: "Conidiophores erect, septate, usually freely branched, branching often in whorls but also irregular; each branchlet bears a terminal fructification composed of a verticil of divergent metulae on which are borne a verticil of divergent phialides (sterigmata); heads divergent and seldom penicillate; conidial chains usually long; conidia hyaline, round ovoid, elliptical, or elongate."

In 1930 Kennelly and Grimes reported a new species, Paecilomyces hibernicum, found during the examination of moulds from butter. The characteristics are as follows:

The conidial fructifications are either terminal or on short side branches of the main hyphae, and consist of sterigmata variously arranged, either separate, in verticils, or branching systems resembling a Penicillium head. Sterigmata vary very much in the same culture; they may be of the shape characteristic of the genus Paecilomyces, or may be scarcely distinguishable from conidiophores; again they may show a series of swellings and constrictions. Conidia are elliptical, 4 by 2.6 microns, at first hyaline, later pink and densely powdering over the mycelium. It partially liquefies gelatin, with evolution of gas and an acid reaction.

In 1941 Szilvinyi reported 157 species of Mucoraceae and Fungi Imperfecti isolated from soil 1930-1932 near Lunz, Austria, and cultivated for 8 years. Among these species two new species of Paecilomyces, namely Paecilomyces austriacus and Paecilomyces sulflavus were listed with descriptive notes and illustrated figures. The conidiophores of the former are 15-20 microns in length; sterigmata, 12-13 by 2-3 microns; conidia, 3 by 2 microns. The size of the sterigmata of the latter are 11.3-12.5 by 1-1.5 microns; the conidia, 1.1 by 2.5-3.5 microns.

MATERIALS AND PREPARATIONS

Modified Sabouraud's Medium.

Sixty-five grams of Difco Bacto-Sabouraud Dextrose agar was suspended in 1000 ml. of cold distilled water. The medium was boiled for a minute in order to dissolve it. The solution was then distributed into tubes (for making slants) or flasks (for making plates), and sterilized in the autoclave for 20 minutes at 15 pounds pressure (121 degrees C.). The final reaction of the medium was about pH 5.6.

Sabouraud's Broth

Difco Bacto-Dextrose	40gms.
Difco Bacto-Peptone	10 "
Distilled water	1000ml.

Ten ml. of this solution was dispensed to each tube. It was then autoclaved for 20 minutes at 15 pounds pressure (121 degrees C.). The final reaction of this broth was about pH 5.6.

Czapek's Medium (modified by Dox & Thom)

Sucrose	30.00 gms.
Sodium nitrate	2.00 "
Dibasic potassium phosphate	1.00 "
Magnesium sulfate	0.50 "
Potassium chloride	0.50 "
Ferrous sulfate	0.01 "

Agar ---------- 15 gms.

Distilled water ----------1000 ml.

The ingredients were melted in the autoclave and then filtered through cotton. The solution was distributed into tubes (for making slants) and flasks (for making plates), and sterilized in the autoclave for 20 minutes at 15 pounds (121 degrees C.).

Plain Nurient Broth.

Difco Bacto-peptone ---------- 5.0 gms.

Difco Beef Extract ---------- 3.0 "

Sodium chloride ---------- 5.0 "

Distilled water ---------- 1000 ml.

The ingredients were dissolved by heating, filtered, adjusted to pH 7.4, tubed and then autoclaved for 20 minutes at 15 pounds (121 degrees C.).

Beef Extract Agar Medium

Difco Bacto-Beef Extract ---------- 3.0 gms.

Difco Bacto-Peptone ---------- 5.0 "

Sodium chloride ---------- 5.0 "

Agar ---------- 15.0 "

Distilled water ---------- 1000 ml.

The ingredients were dissolved in the autoclave, filtered through absorbent cotton and brought back to volume, 1000 ml. It was then adjusted to pH 7.2 and sterilized in the autoclave for 20 minutes at 15 pounds (121 degrees C.).

Carbohydrate Fermentation Medium

Difco Bacto-Peptone	10.0 gms.
Sodium chloride	5.0 "
Distilled water	1000 ml.

The solution was boiled for five minutes, adjusted to pH 7.6 to 7.8, cooled, and filtered through paper. Andrade's indicator was added. Five tenths of the various carbohydrates which are not easily decomposed by autoclaving was added. In the case of heat labile carbohydrates such as maltose, the carbohydrate solution was separately sterilized by filtration with Seitz filter, and then added to the sterile solution in Durham's fermentation tubes to which Andrade's indicator had been previously added. Ten ml. of the solution was dispensed to each of Durham's fermentation tubes, and sterilized by autoclaving.

Starch Agar Medium

Soluble starch	5.0 gms.
Difco Bacto-Beef Extract	3.0 "
Peptone	2.0 "
Dibasic potassium phosphate	1.0 "
Sodium Chloride	5.0 "
Distilled water	1000 ml.

The ingredients were dissolved in autoclave, filtered through absorbent cotton, adjusted to pH 7.2 and then 15

ml. dispensed into each of the large test tubes. They were sterilized in autoclave for 20 minutes at 15 pounds (121 degrees C.).

Nutrient Gelatin Medium.

Difco Bacto-Nutrient Gelatin ---------- 128.0 gms.

Distilled water ---------- 1000. ml.

It was dissolved by warming to about 50 degrees C. and then sterilized by autoclaving. The final pH of the medium was about 6.8.

Carrot Slant Medium.

A cork borer of the same diameter as that of the test tube was used to pierce the fresh carrot for making carrot cylinders. Each of the carrot cylinders was obliquely cut to make two carrot slants. A piece of cotton was put at the bottom of the test tube and wetted with distilled water. Then the carrot slant was put into the test tube, plugged and sterilized in the autoclave.

Slide Cultures.

Two pieces of filter paper were put into the Petri dish. A clean slide was put on the filter paper; a cover glass, on the slide. Then it was sterilized in a dry oven for 15 minutes at 180 degrees C. Two pieces of paraffin were melted on the slide to make two parallel lines. The distance of these two lines was of about the

length of the cover glass. When the melted paraffin was about to harden the cover glass was mounted on it to make a chamber of about 1/10 mm. depth. A tube of sterile Modified Sabouraud's medium or Czapek's medium was melted and cooled to about 48 degrees C. It was then inoculated heavily with the conidia of the organism. A capillary pipette was used to transfer a minute drop of the suspension into one of the open sides between the cover glass and the slide. Due to the capillary attraction the suspension spread between the slide and cover glass. A few drops of sterile distilled water were added to the filter paper in the Petri dish to keep the moisture adequate.

MORPHOLOGICAL CHARACTERISTICS

Growth was studied in Sabouraud's broth, Modified Sabouraud's agar medium, Czapek's medium, plain nutrient broth, beef extract agar medium, carrot slant and slide culture.

In Sabouraud's broth this organism formed much submerged growth of loose texture and a superficial pale yellowish film of 2-5 mm. thickness after 48 hours' incubation at 37 degrees C. The color of the superficial films gradually turned to yellowish green, dirty green and ultimately chocolate brown after keeping at room temperature for two months. While in the plain nutrient broth, the superficial film was thinner and manifested avellaneous color instead of green, dirty green and chocolate brown. In both cases the films consisted mostly of densely interwoven vegetative mycelium, germinating spores and some aerial hyphae with very few fruiting structures and conidia. The germinating spores were round or pear-shaped with well-marked granulated contents. They varied in size from 6 by 7 to 13 by 15 microns. Occasionally the size may reach 17 microns in diameter. The superficial film in Sabouraud's broth was so densely interwoven with mycelium that it was very hard to break. The width of these vegetative hyphae grown in Sabouraud's broth were usually larger than those in

plain nutrient broth, the former varying from 3.5 to 6.5, the latter varying from 3.2 to 5.8 microns (figs. 9 & 10).

When it was transferred on Sabouraud's agar plate and incubated at 37 degrees C. for 24 hours the giant colonies were formed. The rapidly growing colonies measured in average about 15 mm. in diameter after 24 hours' incubation at 37 degrees C., thinly covered the medium, and gradually became greyish green and powdery due to the abundant production of conidia after keeping at room temperature for three weeks. When grown at room temperature for 48 hours the thin and minute avellaneous colonies were the result, but slowly enlarged and turned to yellowish green, greyish green, and powdery after four weeks.

When grown on Czapek's medium at 37 degrees C. for 24 hours a scanty growth of tiny avellaneous colonies were observed. After 72 hours' incubation they turned to green color also. If the cultures were kept at room temperature from the beginning the pale yellowish colonies gradually changed to an avellaneous, dirty green, and ultimately chocolate brown color after keeping for about a month.

When grown on beef extract nutrient agar medium the colonies were thinner and smaller. The color of the

colonies was at first pale yellowish, then turned to avellaneous, and ultimately pale brown after about a month. The color has never been green, greyish green or chocolate brown even after four months.

When grown on carrot slant for 24 hours at 37 degrees C. a green thick felt was usually formed. The color gradually changed to greyish green, brownish green, and ultimately chocolate brown after standing at room temperature for two weeks. Ascospores have never been found.

The vegetative mycelium was a complex network of septate branching, thin-walled hyphae. The entire vegetative mass was submerged in the substratum. The surface growth consisted of aerial hyphae arisen like the stems of a field of wheat. The submerged mycelium was usually massed within the first few milimeters below the surface. The width of the vegetative hyphae varied from 2.5 to 6 microns (fig. 3).

The superficial growth consisted of aerial mycelium and conidial fructifications (figs. 1, 2, 4, 5, 6, 7, and 8). The former consisted of colorless septate branched thin-walled hyphae varying from 3.5 to 8 microns in width. The aerial hyphae erected or partially erected or crept on the surface or looped partly below partly above the surface of the medium. Conidial fructifica-

tions were either terminal or on short branches on erect, partially erect or creeping hyphae, consisting of sterigmatic cells arisen from conidiophores, their branches, branchlets, or metulae as occasionally seen, or directly from fertile hyphae. Many conidiophores arose perpendicularly from the fertile hyphae. The sterigmata were irregularly distributed singly, in couple (conidiophores bearing dichotomous sterigmata) or in verticil. Their size varied from 3.5 to 6.8 by 14 to 24 microns, some with long tapering tubes bent from the axis of the sterigmatic cell and broadly divergent at the apices bearing long chains of conidia. The conidia were elliptical, varying in size from 3 to 4.5 by 4.5 to 7.4 microns. Under certain conditions, for instance, in an old culture the conidia were not rarely disposed in groups: the conidium at the end of a chain tilted over until it rested sideways on the one below, and this process continued until all the conidia were arranged in a group near or at the tip of the sterigmata (figs. 6 and 8, a).

The color exhibited in cultures of this species was due to the innumerable colored conidia produced on the fertile hyphae. The pigment of the conidia was soluble in ether, leaving the liquid yellowish green, avellaneous or pale brown in accordance with the original color of the conidia; it was insoluble in chloro-

form, alcohol, benzene, and distilled water. When suspended in alcohol, benzene, and distilled water the conidia sank slowly; while in chloroform, they rose slowly to the surface, leaving the liquid uncolored.

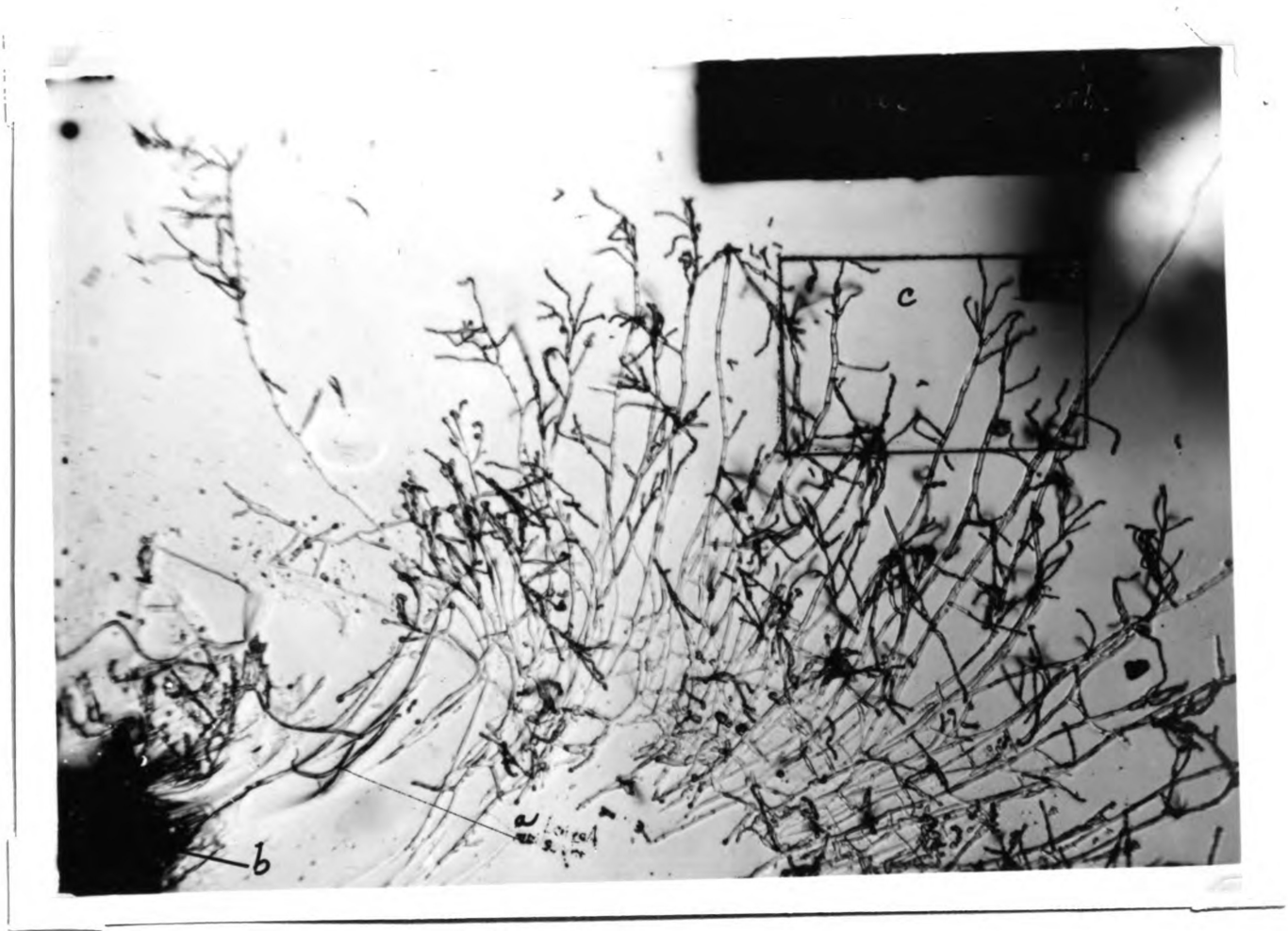

Fig. 1
Slide Culture
Incubated at 37 degree C. after 20 hours.
Magnification: 100 times.
a. Aerial hyphae. b. Czapek's medium.
c. Fig. 2

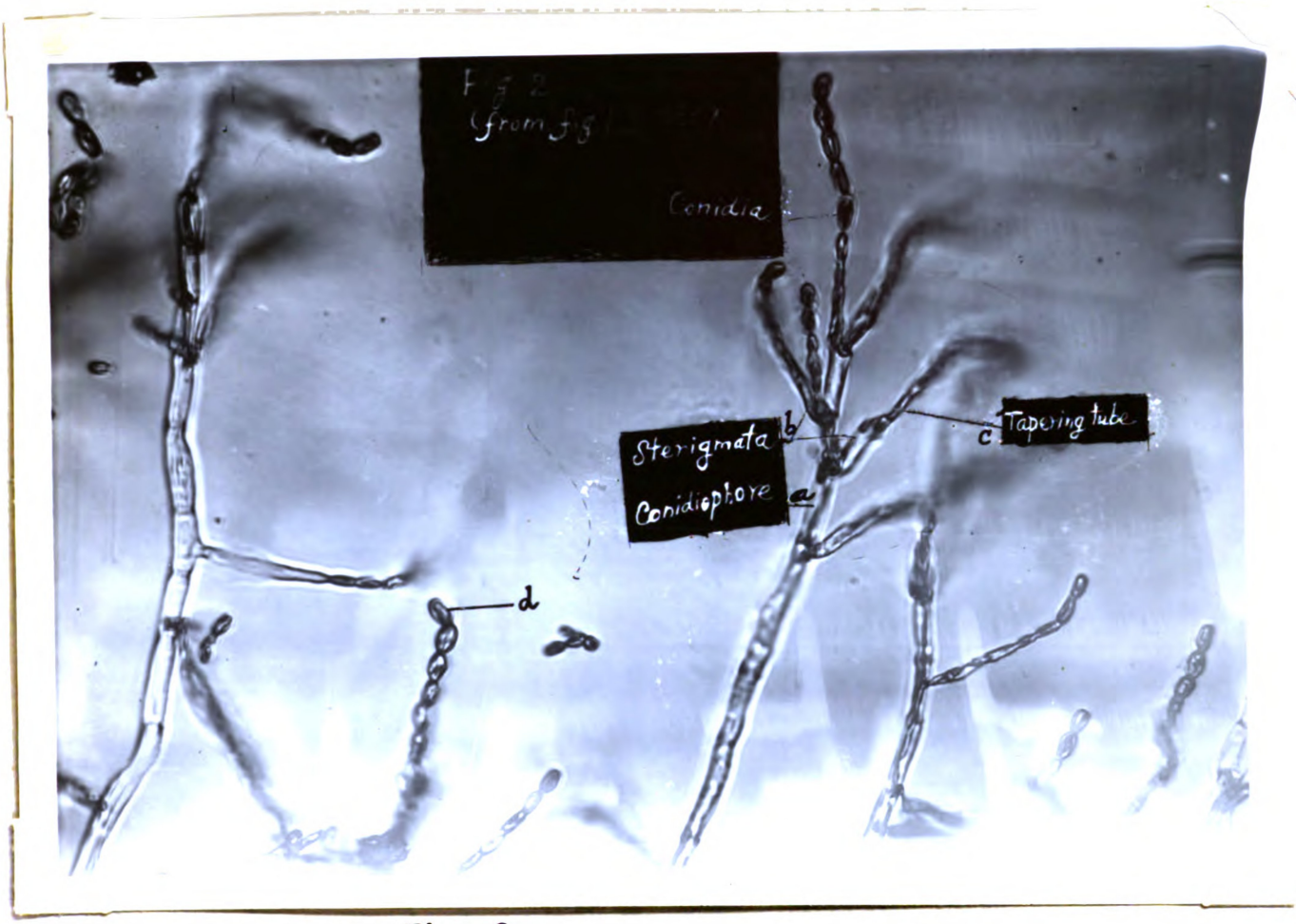

Fig. 2
Slide Culture
Incubated at 37 degree C. after 20 hours.
From Fig. 1 c.
Magnification: 450 times.
a. Conidiophore b. Sterigmata c. Tapering tube of the sterigma d. Conidium

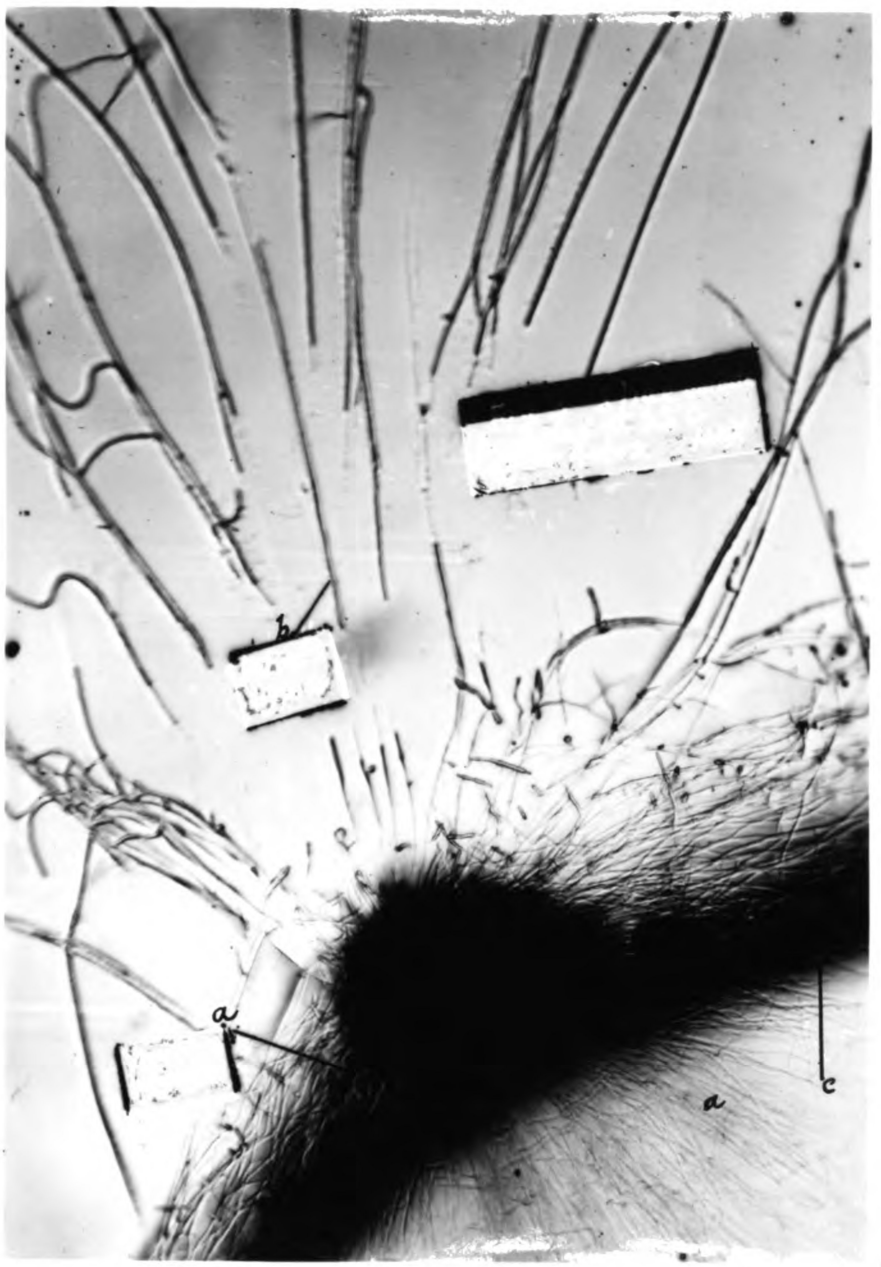

Fig. 3
Slide Culture
Incubated at 22 degree C. after 40 hours
Magnification: 100 times
a. Vegetative mycelium b. Aerial hypha
c. Czapek's medium

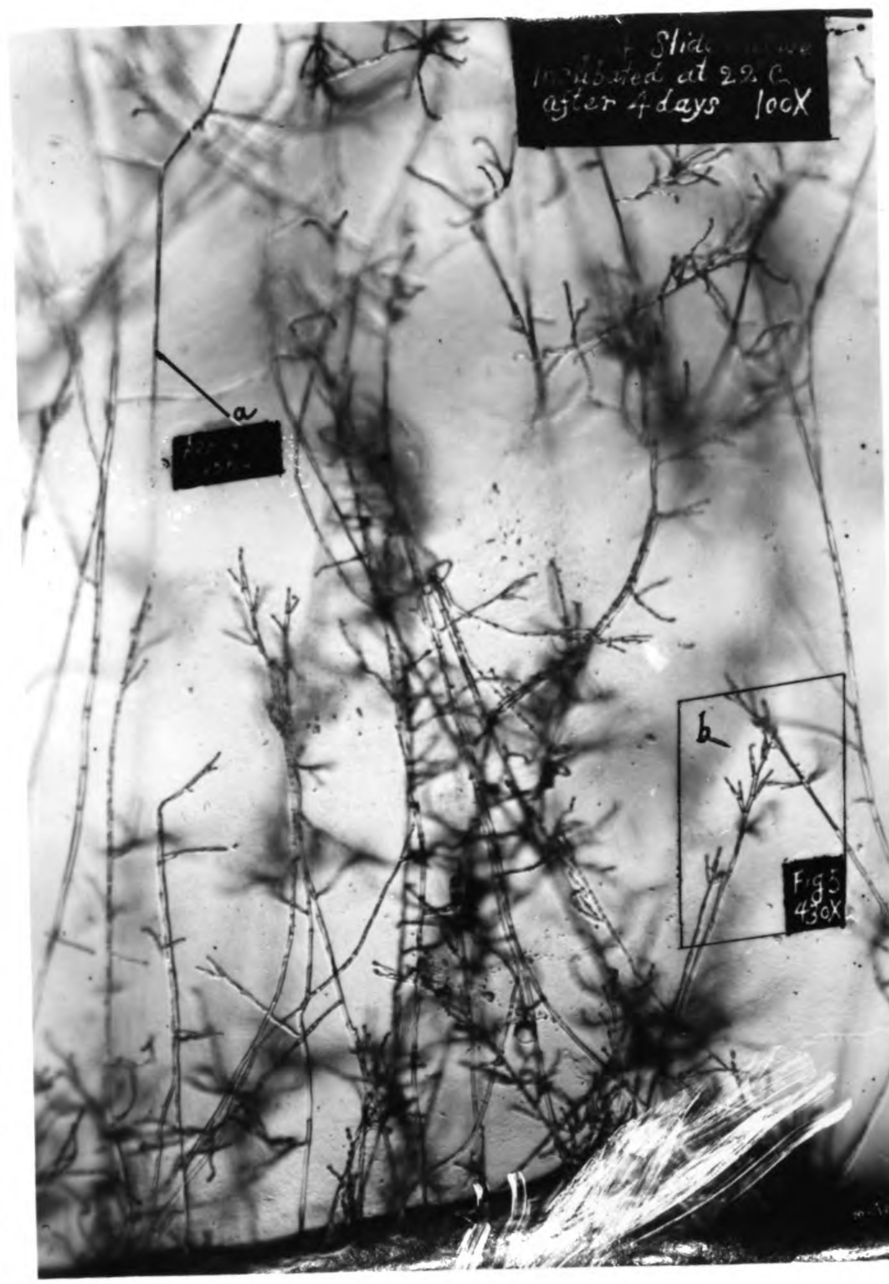

Fig. 4
Slide Culture
Incubated at 22 degree C. after 4 days.
a. Aerial hypha b. Fig. 5

Fig. 5
Slide Culture
From Fig. 4
Magnification: 430 times
a. Conidiophore
b. Sterigma
c. Conidium

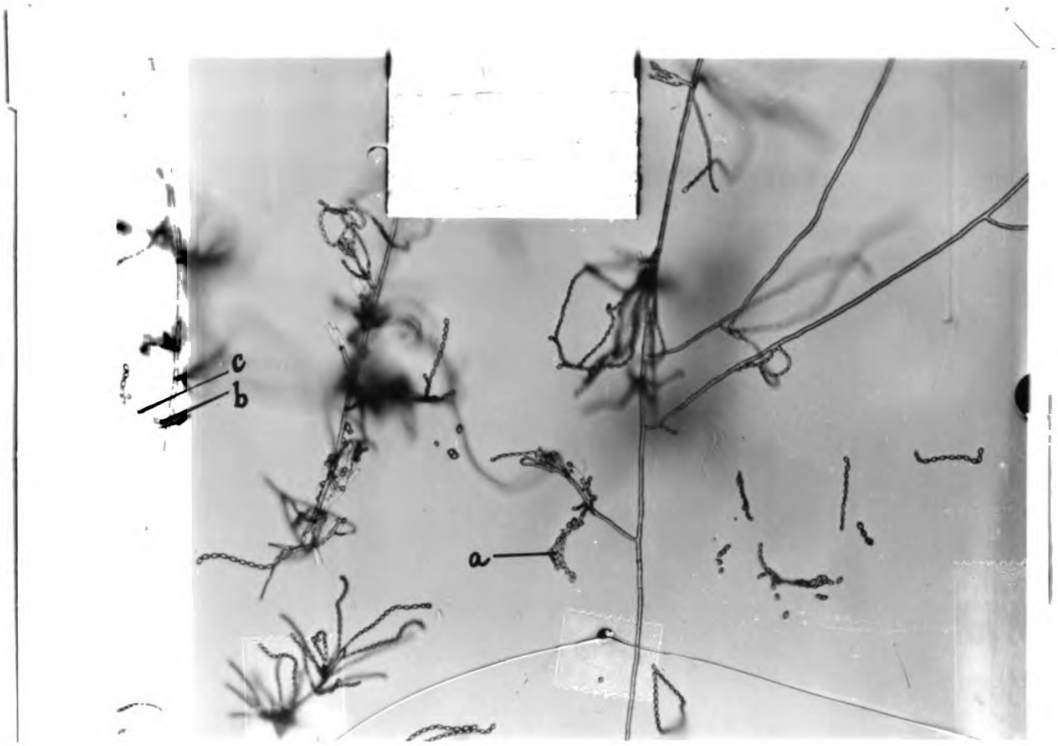

Fig. 6
Slide Culture
Incubated at 22 degree C. after 6 days.
Magnification: 100 times
a. A group of conidia b. Conidiophore
c. Dichotomous sterigmata

Fig. 7
Slide Culture
Incubated at 22 degree C. after 7 days.
Magnification: 100 times
a. Conidiophore b. Dichotomous sterigmata
c., d. Conidial chains.

Fig. 8
Slide Culture
Incubated at 22 degree C. after 8 days.
Magnification: 100 times
a. A group of conidia gathering at the tip of the sterigma
b. Sterigmata arranged in verticil

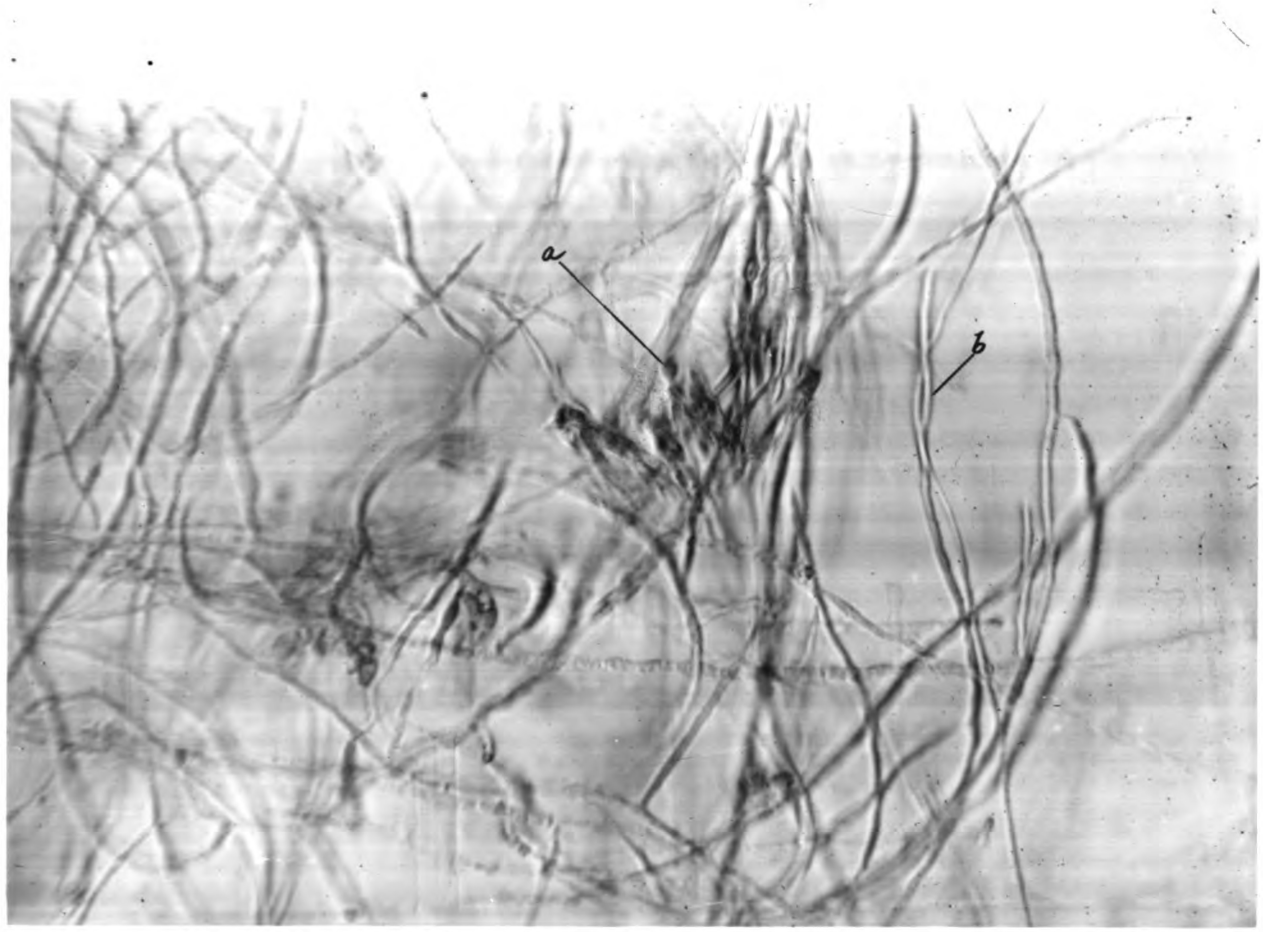

Fig. 9
Broth Culture
Grown in Sabouraud's broth at 37 degree C. after 48 hours. 100X
a. Germinating Conidium b. Vegetative hyphae

Fig. 10
Broth Culture
Grown in plain nutrient broth at 37 degree C. after 48 hours. 100 x
Showing the interwoven vegetative mycelium.

PHYSIOLOGICAL CHARACTERISTICS

Biochemical Reactions

For testing the biochemical reactions of this organism a standard loopful of 4 mm. diameter of the conidia was inoculated into each of the following fermentation tubes. The nutrient gelatin medium and starch medium were stabbed with the same organism. All of the tubes were incubated at 37 degrees C. for five days and the results recorded daily with the exception of the starch medium culture which was tested after 5 days' incubation as shown in the following table:

Table 1.

Biochemical Reactions of a New Species of Paecilomyces

Sugar Broth	Incubation Period in Days				
	1	2	3	4	5
Dextrose	A	A	A	A	A
Maltose	0	0	A	A	A
Sucrose	0	0	0	0	0
Lactose	0	0	0	0	0
Mannitol	0	0	0	0	0
Salicin	0	0	0	0	0
Xylose	0	0	0	0	0
Inositol	0	0	0	0	0

Sorbitol	0	0	0	0	0
Arabinose	0	0	0	0	0
Dulcitol	0	0	0	0	0
Rhamnose	0	0	0	0	0

Media

Nutrient Gelatin	-	-	-	-	-
Starch					+

Remark: A = Acid. 0 = No acid or gas.
- = No liquefaction of gelatin.
+ = Hydrolysis of starch.

As indicated in the above table no acid or gas was produced on any of the carbohydrate broths except in dextrose and maltose where a trace of acid was manifested. In the case of dextrose the Andrade's indicator turned to pale pink color after incubating for 24 hours and then became deeper pink after two days; while in maltose, the pink color did not appear until after the third day of incubation.

No proteolytic action was manifested by this organism as indicated by the fact that gelatin was not liquefied after five days' incubation.

After incubating at 37 degrees C. for five days a

few drops of Lugol's solution was added to the starch medium. No blue color was observed, while the non-inoculated control tubes developed distinct blue color. It is assumed that the starch had been hydrolyzed further than the stage of red erythrin.

Thermal Death Point in Moist Heat

Procedure: Sternberg's method of determining the thermal death point in moist heat was used throughout this test.

Results: Table 2

Thermal Death Point of a New Species of Paecilomyces in Moist Heat.

Exposure Time - 10 min.	Incubation Period in Hours					
	Set A			Set B		
Temperature ° C.	24	48	72	24	48	72
52 ----------	+	+	+	+	+	+
54 ----------	+	+	+	+	+	+
56 ----------	+	+	+	+	+	+
58 ----------	+	+	+	+	+	+
60 ----------	+	+	+	+	+	+
62 ----------	+	+	+	+	+	+
64 ----------	+	+	+	+	+	+
66 ----------	+	+	+	-	+	+
68 ----------	-	-	-	-	-	-
70 ----------	-	-	-	-	-	-

72 ----------	-	-	-	-	-	-
74 ----------	-	-	-	-	-	-
76 ----------	-	-	-	-	-	-

Remark: + = Growth - = No growth

The results show that the thermal death point of this organism in moist heat is 68 degrees C. for 10 minutes.

Thermal Death Point in Dry Heat

Procedure: The procedure used in this test was based upon Dr. W. L. Mallmann's Laboratory Manual of Antiseptics and Disinfectants. The 24-hour Czapek's medium slant culture of the organism incubated at 37 degrees C. was prepared. It was suspended with sterile physiological saline. Eight sterile cover glasses and 16 strips of sterile filter paper were dipped into the suspension of organisms. These were dried rapidly and aseptically in a sterile Petri dish. Into each of 8 sterile Petri dishes were placed two strips of the inoculated filter paper and one inoculated cover glass. The Petri dishes were heated at the different temperatures as shown in Table 3. The strips were then removed and dropped separately into the tubes of Sabouraud's broth and plain nutrient broth. Each one of the cover glasses was broken

into two peices and dropped separately into the tubes of Sabouraud's broth and plain nutrient broth. All of these tubes were incubated at 37 degrees C. for three days and results were recorded at the intervals of 24 hours.

Results: Table 3.

The Thermal Death Point of a New Species of Paecilomyces in Dry Heat

Exposure Time 10 min. Incubation Period in Hours

Temperature °C.	Sabouraud's Broth						Plain Nutrient Broth					
	Set A			Set B			Set A			Set B		
	24	48	72	24	48	72	24	48	72	24	48	72
70	+	+	+	+	+	+	+	+	+	+	+	+
80	+	+	+	+	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+	+	+	+
120	+	+	+	+	+	+	+	+	+	+	+	+
140	-	+	+	-	-	+	-	-	+	-	-	+
160	-	-	-	-	-	-	-	-	-	-	-	-
180	-	-	-	-	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-	-	-	-

Remark: Set A. Inoculated filter paper.

Set B. Inoculated cover glass.

+ = Growth. - = No growth.

The filter paper strips dipped in the organism suspension and heated at 140 degrees C. for 10 minutes showed a trace of growth after 48 hours' incubation in Sabouraud's broth or after 72 hours' incubation in plain nutrient broth;

while the cover glasses, after 72 hours' incubation in both kinds of broth. All of the filter paper strips and cover glasses heated above 160 degrees C. for 10 minutes showed no trace of growth, therefore the thermal death point of the organism in dry heat was estimated to be approximately 160 degrees C. for 10 minutes.

One of the most important factors which affects the thermal death point of an organism is the coagulation of proteins. Proteins coagulate at lower temperature in the presence of moisture; for instance, albumin coagulates at 56 degrees C. in the presence of 50 % of moisture, and at 160-170 degrees C. in the absence of moisture. This accounts for the wide difference in the thermal death points in dry and in moist heat.

Resistance to Ultraviolet Light

Procedure: Nine sterile Czapek's medium plates were prepared. With a wax pencil, a 2-inch square on the bottom of each plate was marked. A sterile cotton swab was dipped in the physiological saline suspension of the organism. With the inoculated swab the 2-inch squares on all nine plates were uniformly smeared, and then exposed directly to the light of a quartz mercury lamp of 110-Watt at a distance of four inches with the cover of the Petri dish removed. The time of exposure was 10, 30, 60, 120, 180, 240, 300 and 600 seconds respectively. All these cultures were incubated at 37 degrees C. for 48 hours.

Results: Table 4.

Ultraviolet Light Radiation Test.

Time of Radiation in Seconds.	No. of Colonies after 48 Hours' Incubation.
10	numerous
30	numerous
60	numerous
120	3,500
180	2,400
240	800
300	146
600	32
Control	
No radiation	numerous

The results show that this organism has marked resistance to ultraviolet light radiation. When it was exposed directly to a quartz mercury lamp of 110-Watt at a distance of four inches even for 60 seconds no perceptible decrease in number of colonies could be observed.

Resistance to Desiccation

Procedure: A physiological saline suspension of a 48-hour Czapek's medium culture of the organism was prepared. About 200 strips of filter paper and cover glasses were dipped in the suspension and dried in sterile Petri dishes. At 24-hour intervals, two pieces of the inoculated

filter paper and the cover glass were planted in Sabouraud's broth and incubated at 37 degrees C. for three days to determine its viability.

Results: The organism remained alive after desiccation for 40 days.

Antibiotic Test

Procedure: A sterile wire loop was dipped into the bacterial suspension of the test organism and rubbed back and forth on the surface of an agar plate, making successive streaks as close togeter as possible. A central straight streak of the fungus suspension was made, crossing the bacterial streaks. It was then incubated at 37 degrees C. for three days and the results were observed at intervals of 24 hours.

Results: Some strains of Staphylococcus aureus, Salmonella enteritidis, Salmonella paratyphi, Salmonella pullorum, Salmonella typhimurium, Salmonella abortivo-equina, Salmonella choleraesuis and Salmonella Schott-muelleri were used for antibiotic tests with this new species of Paecilomyces. The results were all negative.

All of these test organisms were obtained from the Department of Bacteriology and Public Health, Michigan State College.

Determination of Optimum Temperature

Procedure: Eight plates and eight slants of Modified Sabouraud's agar medium and beef extract medium were prepared. A standard loopful of 4 mm. diameter of this organism was inoculated on the surface of each tube and plate. The duplicate slants and plates were incubated at the different temperatures as shown in Table 5. The growth was observed and recorded at intervals of 24 hours for three days.

Results: Table 5.

Growth Rate at Different Temperatures

Incubation Period in Hours.		Temperature °C.			
24		4	22	30	37
Modified Sabouraud's Slant	A	-	+	++	+++
" " "	B	-	+	++	+++
" " Plate	A	-	+	++	+++
" " "	B	-	+	++	+++
Beef Extract Agar Slant	A	-	-	+	++
" " " "	B	-	-	+	++
" " " Plate	A	-	-	+	++
" " " "	B	-	-	+	++
48					
Modified Sabouraud's Slant	A	-	+	+++	+++
" " "	B	-	+	+++	+++
" " Plate	A	-	+	+++	+++
" " "	B	-	+	+++	+++
Beef Extract Agar Slant	A	-	+	+++	+++
" " " "	B	-	+	++	+++
" " " Plate	A	-	+	++	+++
" " " "	B	-	+	++	+++
72					
Modified Sabouraud's Slant	A	-	++	+++	+++
" " "	B	-	++	+++	+++
" " Plate	A	-	++	+++	+++
" " "	B	-	++	+++	+++
Beef Extract Agar Slant	A	-	++	+++	+++
" " " "	B	-	++	+++	+++
" " " Plate	A	-	+	++	+++
" " " "	B	-	+	++	+++

Remark: - = No growth + = Slight growth

++ = Fair growth +++ = Heavy growth

Abundant growth was observed on the modified Sabouraud's medium after incubating at 37 degrees C. for 24 hours; moderate growth at 30 degrees C.; and slight growth at 22 degrees C.

When transferred on beef extract medium this organism showed more scanty growth, and no trace of growth could be observed when incubated at 22 degrees C. until the second day. At 4 degrees C. no trace of growth was detected in any of the media after three days' incubation.

The results show that the optimum temperature for the growth of this organism is about 37 degrees C. or higher. No attempt has been made to determine its precise optimum temperature.

DISCUSSION

In view of its morphological and physiological characteristics this organism belongs to the class Deuteromycetes, order Hyphomycetales (or Moniliales) family Mucedinaceae (or Moniliaceae), genus Paecilomyces.

According to Vuillemin's classification which is in general use by French mycologists, this organism belongs to the class Fungi Imperfecti because of non-sexual reproductive structure, order Conidiosporales because of reproducing conidia, suborder Phialidineae because of reproducing conidia borne upon phialides or sterigmata. But according to Saccardo's classification which is followed by most mycologists today, this organism belongs to the order Hyphomycetales, frequently called Moniliales, because of conidiophores are produced neither in pycnidia (Sphaeropsidales) nor upon acervuli (Melanconiales) but are formed from superficial hyphae over the entire surface of the colony; family Mucedinaceae, because of mycelium is colorless in contrast to the family Dematiaceae whose mycelium is dark or smoky such as Hormondendrum, Cladosporum, Alternaria etc. It should be classified under the genus Paecilomyces, because it is distinguishable from Penicillium by its longer tubular sterigmata, many tubular processes being

bent away from the axis of the sterigmata, and by greater irregularity of the branching which is only in part verticillate.

Up to the present time only 14 species, possessing the characteristics of the genus Paecilomyces, have been reported. Generally speaking, very few studies on the physiology of Paecilomyces have been carried out. For convenient review the following table of morphological and physiological comparisons of the various species of Paecilomyces is compiled. This organism is distinguishable from the other known 14 species of Paecilomyces by the size of sterigmata and conidia, optimum temperature, and some biochemical reactions.

Since this organism showed no morphological and physiological characteristics in common with either one of the known 14 species, it is therefore considered to be a new species of Paecilomyces and the name Paecilomyces michiganesis is proposed. Because of lack of evidence as to real natural relationship among these species the names of these species in the following table are arranged alphabetically.

Table 6
Morphological and Physiological Comparisons of
Various Species of Paecilomyces.

Strains	Size of Sterigmata (Microns)	Forms of Conidia	Size of Conidia (Microns)	Color of Conidia	Optimum Temperature (°C.)	Biochemical Reactions
Penicillium aurenarium Shaposhnikov & Manteifel (1923)	25 by 5-6	lemon-shaped	10-11		35-40	
Paecilomyces aureo-cinnamomeum (Biourge)Thom (1923)	13-30 by 1.5-2.5	elliptical	2.4-4.5 by 2.4 to 6.4 by 3.6			
Paecilomyces austriacus Szilviny(1941)	12-13 by 2-3	oval	3 by 2			
Paecilomyces burci Pollacci(1921)	10-12 in length	globose, ellipsoi-dal	4-5(Thom: 6-8 by 2.5-4)	almost colorless		
Eidamia catenulata Horne & Williamson (1923)	1-2.5 by 8-16	elliptical	4-7 by 2-3.5	pale cream, yellow, olive, avellaneous, cinnamon brown, etc.	30	hydrolyses starch inverts sucrose decomposes protein and asparagin
Corollium dermatophagum Sopp (1912)	50-60 by 10-12	oval	9-10 by 3-4	yellowish brown	38-40	liquefies gelatin
Penicillium elegans Corda (1838)		elliptical				
Spicaria fimetaria Moesz(1921)		ellipsoid	6.5-10 by 5-6			
Penicillium flavum El. & Em. Marchal (1921)	13-19 by 2.3-3	ovoid	4.2-6 by 3-4			
Paecilomyces hibernicum Kennelley & Grimes (1930)		elliptical	4 by 2.6	pink		partially liquefies gelatin
Paecilomyces mandshuricum Saito (1921)		elliptical to ovate	6.5 by 4.5		36	liquefies gelatin & starch media
Paecilomyces michiganensis (1947 n.sp.)	3.5-6.8 by 14-24	elliptical	3-4.5 by 4.5-7.4	yellow, yellowish green, avellaneous pale brown	37	ferments dextrose & maltose, hydrolyzes starch
Paecilomyces repandum Bainier & Sartory (1913)	15-20 by 7-9		3.5-4 by 6-8	yellowish		
Paecilomyces subflavus Szilvinyi (1941)	11.3-12.5 by 1-1.5	oval	1.1 by 2.5-3.5			
Paecilomyces varioti Bainier (1907) (Synonym:Penicillium divarication Thom)	15-20 by 3	elliptical or fusiform	5-7 by 2.5-3	yellowish to brownish		gelatin not liquefied

The so-called "macrospores" observed by Horne and Williamson have never been found in the writer's culture even those incubated at 22 degrees C. The above-mentioned authors indicated that when kept at 20 or 25 degrees C. these hyaline macrospores occurred on all the culture media employed. However, the term "macrospores" can not be used as a definite determinative criterion, because they are of transformed units, homologous to sterigmatic cells and conidia under certain conditions as demonstrated by Kita and Wai and other investigators.

That this organism can utilize inorganic instead of organic nitrogen as the only source from which to build protoplasm, as most of the molds do, is clearly indicated by the abundant growth on Czapek's medium which is an organic nitrogen free medium, i.e., sodium nitrate is the only source of nitrogen. Carbohydrate compounds greatly enrich the growth of this organism, and probably contribute directly or indirectly to the production of green pigment of the conidia as was the case when grown on modified Sabouraud's and Czapek's media in contrast to the avellaneous color developed when grown on beef extract medium.

Ordinarily this organism may be saprophytic, but under certain conditions, i.e., when it enters the animal body in sufficient numbers either through experimental injection or natural infection, it may exist for

certain periods of time, without causing pathological manifestations.

SUMMARY

(1) Up to the present time 14 species possessing the characteristics of the genus Paecilomyces have been reported in literature.

(2) The salient morphological features and some physiological characteristics of a new species of Paecilomyces are described, and the name Paecilomyces michiganesis is proposed.

(3) The organism was found to produce acid but no gas in dextrose and maltose broth and to hydrolyze starch. It does not ferment sucrose, lactose, mannitol, salicin, xylose, inositol, arabinose, dulcitol and rhamnose nor does it liquefy gelatin.

(4) The thermal death point of this organism was determined to be 68 degrees C. for 10 minutes in moist heat and 160 degrees C. for 10 minutes in dry heat.

(5) Ultraviolet light radiation of 110-Watt quartz mercury lamp at a distance of 4 inches for 60 seconds failed to reduce the number of organisms; a small number of organisms survived an exposure of 600 seconds.

(6) This organism was viable after desiccation for 40 days.

(7) It manifested no antibiotic properties toward Staphylococcus aureus and several salmonellae.

(8) The optimum temperature of this organism seems to be about 37 degrees C. No attempt has been made to determine its precise optimum temperature.

(9) The pigment of the conidia is soluble in ether, insoluble in chloroform, benzene, alcohol and distilled water.

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