A NEW MONILIA-LIKE FUNGUS

CHARACTERISTICS OF AN ORGANISM ASSOCIATED WITH A DERMATOsis PECULIAR TO WORKERS IN CANNERIES

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In a previous report 1 was presented evidence of the mycotic origin of an occupational dermatosis occurring among workers in certain fruit canneries of the Pacific Northwest. The disease is known locally as "fruit poisoning." At the time the first paper was written, only three strains of the monilia-like yeast had been obtained. Since then, eight additional strains have been added to the collection. In a careful search of the medical literature a description of a similar organism could not be found. The following account of the characteristics of the new fungus will therefore be of interest.

SOURCE AND MORPHOLOGY

The three original cultures were taken in January, 1924, from two patients with paronychia and from one with interdigital erosion, all of whom were infected the previous pear season at the same cannery. Six of the eight new strains were obtained from typical cases of paronychia or of interdigital erosion; a seventh was isolated from a box of pears just delivered to the cannery, and the eighth from a tray of pear parings at the workers' table. The organism was found in all typical lesions from which cultures were taken, but nontypical lesions yielded only bacteria or "wild yeasts." Cultures from conveying belts and vats were negative for the organism. The fact that one of the strains was isolated from a box of pears opens the possibility of the fruit being a source of the infection.

Cells from a twenty-four hour growth on solid mediums were round or oval budding forms, measuring from 5 to 8 microns in diameter. 3 An occasional elongated cell, 5 by 10 microns in diameter, was found. Rarely were true typhae found in young cultures on solid mediums. A definite cell membrane enclosed clear protoplasm con-

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2. All the mediums used in these experiments were prepared in the Department of Bacteriology.
3. Thiennes (footnote 1, figure 7).
taining an eccentric, highly refractile spherical body which was usually larger in cells grown on acid mediums. This refractile body did not stain with Giemsa’s fluid but rather with sudan III and was considered to be a fat globule (figs. 1 and 2). Treatment with a diluted compound solution of iodine demonstrated glycogen granules throughout the cell. Within from forty-eight to seventy-two hours vacuoles filled with a glycogen-free fluid containing one or two chromophilic, coccoid granules in rapid brownian motion appeared in many of the cells. Often such vacuoles nearly filled the cells of old cultures. Cells from old colonies were of many sizes and bizarre shapes. Some seemed to be shells containing only a fat globule, while others were elongated, containing one or more vacuoles and several fat globules; still others were cystlike, either clear or granular like chlamydospores, and measuring as much as 15 microns in diameter. Small greenish bodies, difficult to distinguish from fat globules, were found floating free in hanging drop preparations from old colonies; these were at first thought to be ascospores, but they never were seen to germinate. Tortuous hyphal forms of from one to six segments, some of which were branched, were abundant in the gnarled masses of giant colonies.

In liquid mediums, twenty-four hour cultures consisted of septate hyphae, some of which were branched, together with the round or oval budding forms similar to those on solid mediums. At one end of a segmented hyphal thread the enlarged, round mother cell was frequently found, sometimes budding and usually containing a vacuole and fat globule, as shown in figure 1. Segments of the hyphae varied from 10 to 60 microns in length and from 2 to 3 microns in diameter, except for the tapering terminal segments which measured as much as 100 microns in length. Side-buds or conidia without sterigmas occurred, occasionally pleurogenous, but usually at the ends of segments; the conidia themselves often bore buds, and these, in turn, others. The end of the conidium-bearing segment was enlarged, like the joint of an insect’s leg. Terminal hyphal segments, placed on a hanging drop of solid medium, were seen to become septate; the new terminal segment

Fig. 1.—Camera lucida drawing of various forms from galactose broth, stained to show glycogen granules. The solid black bodies are fat globules. The vacuoles should be noted; × 1,000.

Fig. 2.—Photomicrograph of old hanging drop. The beaded appearance of hyphae due to fat globules should be noted; × 800.
elongating and again septating or giving rise to a bud, which either
elongated or budded further. Such a preparation rapidly became over­
grown by the budding conidia, obscuring the threads. Ascitic fluid was
an especially good medium for the development of conidia-bearing
hyphae. Hyphae of old hanging drop cultures in sugar broth were
given a beaded appearance by numerous fat droplets, shown in figure 2.
In superficial skin lesions, the organism developed both budding
forms and hyphae, the latter predominating. In the deeper tissues,
such as the lung, liver and kidney of experimental animals, the round
cells predominated, and hyphae present were composed of only from
two to four short segments. The "double contour" was not a
constant feature in stained tissue sections. Figures showing the forms
obtained from tissues are to be found in a previous report.

Reproduction was always by gemmation, attempts to develop asci
on gypsum blocks, on Gorodkowa's medium and in old cultures having
been unsuccessful.

The fungus was gram-positive, but not acid-fast. Young cells took
the ordinary stains, but older cells stained poorly, except with aniline
gentian violet or carbol fuchsin. A nucleus was not demonstrable by
the iron-hematoxylin method. In thin tissue sections, the Gram-Weigert
method was fairly efficient in demonstrating the organism.

Dilute suspensions of a twenty-four hour broth culture in extract
broth were killed in ten minutes at 54°C.

CULTURAL CHARACTERISTICS

Twenty-four hours at 37°C, or three days at room temperature were
usually sufficient for an abundant growth. The appearance on solid
mediums varied with the medium, being most abundant on alkaline
dextrose agar (pH 7.2), and decreasing in the order named: acid
dextrose agar (pH 6.4), autoclaved carrot, autoclaved pear, autoclaved
potato, plain agar, plain gelatin, Löffler's serum medium, blood agar,
raw carrot, raw liver and clotted blood. On solid mediums, the sur­
faco of the growth was slightly moist, dull white and raised; during the
first week, colonies coalesced, becoming light cream colored, later show­
ing a faint tinge of green. The consistency was that of thick top
cream. On fruit, the growth was spreading and more moist; on
potato it was dry, with the development of minute, chalklike pillars. In
liquid mediums, the organism settled to the bottom or adhered to the
lower side of leaning tubes. A ring usually developed at the surface,
amounting to a collar in fermenting sugar mediums.

In gelatin stabs, hyphae grew out from the line of inoculation.
There was no characteristic appearance, such as the pine-tree growth

of Monilia pilosis of Ashford, but different tubes of the same strain
in the same batch of gelatin exhibited different arrangements of out­
growth. Gelatin was not liquefied.

A loopful of a twenty-four hour broth culture of each strain was
inoculated on 75 cc. each of alkaline dextrose agar (4 per cent dextrose,
and acid dextrose agar (4 per cent dextrose, pH 6.4) in 250 cc.

Fig. 3.—Giant colonies from three months' growth at room temperature; left,
acid dextrose agar; right, alkaline dextrose agar.

Fig. 4.—Giant colonies from five months' growth at room temperature, during
warm weather; left, alkaline dextrose agar; right, acid dextrose agar.

tinged with green. Later, bubbles formed and ruptured, causing a
gnarled, crumpled-paper-like mass, as in figure 4, especially on those
colonies grown during the warm months. The piled-up cells were of
both round and thread forms.

Dry aerial hyphae were not developed under any conditions tried,
either aerobic or anaerobic, although cultures were kept for as long as
one year.

Whether protected from drying or not, most of the cultures were
alive at the end of six months, but not at the end of one year. Cells
placed on gypsum blocks and kept moist with sterile distilled water
either aerobic or anaerobic, although cultures were kept for as long as
six months.

Fermentation Tests

The tube-within-a-tube method, with 0.5 per cent solutions of
various sugars in extract broth, was found to be satisfactory. Tests
were run at both room and incubator temperature. Dextrose, levulose
and maltose were fermented, with the production of alcohol, acetic acid
and carbon dioxide; galactose was fermented with the production of
alcohol and acetic acid but not with carbon dioxide. Fermentation was
practically complete in forty-eight hours in the incubator or in one
week at room temperature, except in the case of maltose, which required
a day longer in the incubator and from two to four days at room
temperature. Saccharose, lactose, lev-o-arabinose, raffinose, mannite,
inulin, salicin, xylite, glycerol and dextrin were not fermented. All
sugar broths not fermented were made more alkaline than the control.

There was no change in the pH of dextrin broth. After two or three
weeks, the sugar broths which had been fermented became alkaline, with
the absorption of carbon dioxide.

Litmus milk was rendered alkaline and clotted within seventy-two
hours at 37 C. or within ten days at room temperature.

Neither nitrite nor indole was formed. Calcium oxalate crystals
were found in old liquid cultures.

Comment

Morphologically and culturally, the organism described in this paper
had many characteristics in common with Monilia psilosis of Ashford,
although the fact that it failed to develop the “inverted fir tree” growth
in gelatin definitely differentiates it from the sprue fungus. There are
also other reported fungi which it resembles; yet it differs from each

5. One of the strains on gelatin was sent to Dr. R. K. Ashford in Porto Rico.
This strain had been shown previously to ferment maltose, but Dr. Ashford
reported that it had not fermented maltose in his laboratory.

in some important feature. The morphologic characteristics of the
organism and its failure to produce ascospores suggest a close relation-
ship of the fruit-canners’ fungus to the “parasaccharomyces” of Ander-
sor and to Monilia as listed by Clements, but the present confusion
in the taxonomy of “blastomyces” does not permit of a definite
classification.

Summary

1. A new fungus is described. It is considered to be the etiologic
agent in an occupational dermatosis among workers in canneries.

2. Morphologically, the fungus occurred both as a round yeastlike
form, and as branched, septate hyphae with conidia. Dry, aerial hyphae
were not observed.

3. Reproduction was by budding. Ascii were not observed.

4. Gelatin was not liquefied. Litmus milk was clotted and rendered
alkaline, and dextrose, levulose and maltose were fermented, with the
production of alcohol and acetic acid only.

5. Although closely resembling Monilia or parasaccharomyces, the
organism cannot yet be definitely classified.

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