# MORPHOLOGY, PHYSIOLOGY AND CYTOLOGY OF SYRINGOSPORA INEXORABILIS (MONILIA INEXORABILIS)

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While we were studying pathogenic species of Zymonema, Mazza and Palamedi ('32) described Monilia inexorabilis from a fatal case of blastomycosis of the skin and mucosa. His illustrations showed an imperfect stage which closely resembled some seen in lesions and in cultures of species of Zymonema. Dr. Mazza kindly sent us a culture, and although the organism has been grown for four years on a great variety of media, it has not shown any trace of sexuality or ascospore formation such as is common in Z. dermatitidis and Z. capsulatum. The study was resumed early in 1934, and sufficient morphological and cytological data have now accumulated to warrant their presentation.

The patient was a Spaniard, 53 years of age, who had lived for 21 years in the Chaco of Argentina. Ulcerating lesions on the buccal mucosae were diagnosed as blastomycotic, and a biopsy confirmed the diagnosis. The lesions were of a progressive nature and involved the whole respiratory tract, becoming generalized. A complete autopsy established this as "true" blastomycosis.

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### METHODS OF TECHNIQUE

For a morphological study of the organism, mounts of mycelium were made in Amann's lacto-phenol and 0.5 per cent cotton blue. Distilled-water mounts were also favorable because their uniform staining did not distort the material. Several dyes, such as 1 per cent aqueous crystal violet to the desired intensity in glycerine, methylene blue, neutral red, and iodine green, were used to bring out granulation. The life cycle of the fungus was observed from hanging-drop preparations made with lactose broth, nutrient broth (meat extract). and 2 per cent proteose peptone. Iodine potassium iodide (saturated solution) was used for studying the glycogen content of the cells and the possible chondriosomes, as advocated by Guilliermond; neutral-red and methylene blue, for studying volutin or metachromatic material. A pinch of benzidine sulphate added to a water mount of organisms showed the socalled "dancing bodies." Osmic acid 2 per cent, and platinic chloride 5 per cent, demonstrated fat and lipoidal substances.

For a study of the nuclear phenomena, agar slants of the culture were killed and fixed with Hermann's fluid and embedded in celloidin (Moore, '33). Sections were then cut to a thickness of 10  $\mu$ , and stained with Haidenhain's iron-alum haematoxylin with no counterstain. After being cleared in benzol they were mounted in Canada balsam. When embedded in celloidin the material has little or no shrinkage and is cleared very nicely.

### DESCRIPTION

The organism was obtained on a Sabouraud's glucose agar slant. In the host the parasite exists as a single or budding yeast-like cell which may very easily be confused with that found in blastomycosis. When cultivated on an artificial substrate, the cells send out germ-tubes or elongate (pl. 15, figs. 1-4) to produce hyphae of cells which are formed by the development of cross-walls (figs. 5-6). These vary from 2 to  $5 \mu$  in diameter according to the medium on which grown. On several media, however, the yeast-like budding cells may per-

sist, or at least predominate for a variable length of time (figs. 9, 25), and measure from 2 to 17  $\mu$  in diameter. Wort agar is especially adapted for the continuance of the yeast stage, and blood agar and serum media, to a certain degree (figs. 24–25).

The continued growth as a saprophyte results in morphological changes which help in the classification of these yeast-like fungi. The elongated forms developing from the germinating cell vary both in diameter and length. On media with beef extract near the neutral point or with a high pH, the filaments generally are long and have a small diameter (figs. 10–11, 23). On media with a low pH, as Raulin's malt extract, Czapek's, or wort agar, the cells are shorter and have a greater diameter (figs. 8–9, 12–13, 16–17). On wort agar (fig. 12) the organism becomes very large, spherical, and thick-walled. This peculiar condition is noticed to a lesser degree on malt extract agar (fig. 9), which is a modification of wort agar.

A verticillate or dendroid type of growth is rather common on most media for this fungus, but definitely characteristic on Raulin's solution (figs. 16–17). The cells of the main axes of the structure (fig. 17) are somewhat longer than those which bud off from them, the blastospores. The blastospores develop along with the hyphae as single or multiple cells, terminally (figs. 16–17, 20, 22), laterally (figs. 8, 10, 18–23), or at the point of division of two elongated cells of the filaments (fig. 17). They may either be spherical to ovoid or subpyriform to pyriform, and measure approximately 3–6  $\mu$  in diameter, usually 5  $\mu$  or more. These cells are uninucleate and are capable of developing new colonies if allowed to germinate under favorable conditions.

Besides the structures mentioned, there are also large terminal, clavate or obovate cells measuring 9–15  $\mu$  in diameter or long axis. They are double-contoured (thick-walled) structures and are found as modified cells on malt extract, glycerine, yeast dextrose, nutrient blood agar, and even in serum. Lanceolate cells 9–15  $\mu$  in long axis also occur, usually singly or budding, and are modifications of the spherical or ovoid cells found on various substrates. Another structure noted is the

thick-walled cell or cells sometimes found at the base of a verticillate growth (fig. 16). These are the germ cells which develop a thickened wall in much the same manner as would the root of a plant.

The nuclear phenomena of development will be considered under cytology.

# Cultural Characteristics

The organism when received was growing on a Sabouraud's agar slant. Subcultures from this growth were made on a number of different media, varying in hydrogen ion concentration, protein or protein decomposition products as peptones, and in the amounts of carbohydrate and nitrogen. All cultures were grown at room temperature, approximately 22° C., on the following media arranged in the order of increasing pH.

Raulin's solution (pH 4.1).—Sediment of growth on bottom of flask. Culture monilioid. Groups of budding yeast-like cells, double contoured,  $5-12 \mu$  in diameter. Filaments  $2-5 \mu$  in diameter; blastospores  $3-5 \mu$  in diameter; mycelium verticillate.

Czapek's Agar (pH 4.4).—Colony approximately 3.5 cm. in diameter after 40 days. Mycelium mostly submerged in agar, tending to form coremia of elongated cells approximately 3  $\mu$ in diameter. Spherical and ovoid, yeast-like cells 3–17  $\mu$  in diameter; racquet and verticillate mycelium; blastospores of varying proportions, spherical to ovoid.

Malt Extract Agar (Difco, pH 4.6).—Colony approximately 3.5 cm. in diameter after 40 days. Mycelium submerged in medium, with a general appearance similar to that on Czapek's agar. Color creamy-buff. Hyphae approximately 3  $\mu$  in diameter; blastospores approximately 6  $\mu$  in diameter. Budding cells 6–12  $\mu$  in diameter on surface of medium, with branching, coremioid mycelium. Terminal clavate, obovate to lanceolate cells approximately 12–15  $\mu$  in long axis.

Wort Agar (Difco, pH 4.8).—Colony approximately 3.5 cm. in diameter after 40 days. Color light coffee-brown. Culture

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shows excressences. Verticillate growth of cells 3–15  $\mu$  in diameter, the large spherical cells having a thick capsule. Budding cells present. Filamentous mycelium approximately 3  $\mu$ in diameter, in medium, with some elongated cells and blastospores approximately 4  $\mu$  in diameter. Terminal obovate to lanceolate cells present.

Sabouraud's Agar (pH 5.6).—Colony approximately 5 cm. in diameter after 40 days. Most of culture submerged, portion on surface approximately 2.5 cm. in diameter with a heaped-up growth in center. Growth excressences and radiating ridges to the exposed periphery evident. Color dark creamy-buff. Submerged mycelium filamentous, 2–5  $\mu$  in diameter, branching, coremioid and somewhat verticillate. Blastospores approximately 6  $\mu$  in diameter. Yeast-like, budding, also germinating, cells 3–12  $\mu$  in diameter. Obovate or clavate terminal cells on submerged filaments. Surface growth of yeast-like cells.

Corn-Meal Agar (Difco, pH 6.0).—Colony approximately 3.5 cm. in diameter after 40 days. Culture almost entirely submerged with a surface growth approximately 0.7 cm. in diameter. Color dull creamy-buff. Macroscopic appearance similar to that on Czapek's agar. Surface growth of yeast-like budding cells, same as on Sabouraud's agar. Filaments much thickened, branching, coremioid; blastospores thick-walled, approximately  $5 \mu$  in diameter.

Potato-Dextrose Agar (pH 6.2).—Colony approximately 3.5 cm. in diameter, with an irregular periphery. Growth on surface of yeast-like, budding cells approximately 2–10  $\mu$  in diameter. Submerged mycelium of filamentous forms with hyphae approximately 3  $\mu$  in diameter; blastospores approximately 6  $\mu$  in diameter. Mycelium verticillate as in other substrates.

Potato-Dextrose Broth  $(pH \ 6.2)$ .—Sediment at bottom of flask. Mycelium similar to that on the agar.

Nutrient Agar (Difco, pH 6.6).—Colony moist and irregular with growth partially submerged in agar, approximately 2.5

cm. in diameter. Cells on surface mostly spherical to ovoid, budding, up to  $12 \mu$  in diameter. Large cells  $15 \mu$  in diameter. Large spherical cells within medium and smaller ovoid to spherical forms on surface.

Nutrient Broth (Difco, pH 6.7).—Sediment at bottom of flask. Yeast-like cells as on the above medium, with filamentous forms monilioid in appearance, approximately 3  $\mu$  in diameter. Blastospores subpyriform, 5  $\mu$  in diameter.

Lactose Agar (Difco, pH 6.8).—Colony approximately 2 cm. in diameter. Submerged mycelium a verticillate, coremioid growth similar to that in Czapek's agar. Growth on surface moist and cream-colored, with many budding, yeast-like cells, spherical and ovoid,  $3-12 \mu$  in diameter. Filaments elongated,  $2-3 \mu$  in diameter, monilioid and blasto-dendroid, verticillate; blastospores pyriform, subclavate or ovoid, in varying proportions.

Glycerine Agar (nutrient agar plus 6 per cent glycerine, pH 7.0).—Colony approximately 5 cm. in diameter, both submerged in the agar and on the surface. Growth on surface approximately 3.5 cm. in diameter with an irregular periphery, dull cream in color. Central region of erupted, vesicular-like outgrowths. Yeast-like cells spherical and ovoid, singly or in chains, 3–9  $\mu$  in diameter. Elongated, branching filaments 2–4  $\mu$ in diameter. Subclavate cells terminally on filaments, 9–15  $\mu$  in long axis.

Yeast-Dextrose Agar (Difco, pH 7.0).—Macroscopic appearance similar to that on nutrient agar. Yeast-like budding cells  $3-12 \mu$  in diameter. Filaments monilioid, approximately  $3 \mu$  in diameter, branching, terminating in a double-contoured ovoid cell  $12 \mu$  in long axis; blastospores of varying proportions.

Nutrient Blood Agar (nutrient agar plus bacto-beef blood, pH 7.2).—Colony approximately 2 cm. in diameter with an irregular periphery. Yeast-like cells double-contoured, mostly spherical, in general  $9 \mu$  in diameter. Many smaller cells. Filaments 2–4  $\mu$  in diameter, cross-walled and branching.

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Serum (Bacto-beef blood serum, pH 7.3).—Thick sediment at bottom of flask. Mycelium of filaments approximately  $3 \mu$  in diameter, terminating in a thick-walled, spherical, ovoid or sublanceolate cell 9–15  $\mu$  in diameter; blastospores in varying proportions.

Endo's Agar (Difco, pH 7.5).—Colony approximately 3 cm. in diameter. Mycelium mostly submerged in agar. Many yeast-like, budding cells  $3-12 \mu$  in diameter. Hyphae  $2-4 \mu$  in diameter. Blastospores spherical, ovoid or pyriform, approximately  $6 \mu$  in diameter or long axis.

Carbohydrate reactions.—Acid and production of gas with dextrose, maltose, levulose, and d-mannose. Acid and no gas with galactose, lactose, saccharose. No acid or gas with larabinose, l-xylose, rhamnose, raffinose, mannitol, amygdalin or dextrin.

Gelatine.—Gelatine is liquefied.

Litmus milk .- Shows no acid or curdling.

# Cytology

As far as can be determined by the literature, there has been very little work on the cytology of the imperfect yeasts. Rajat ('06) described briefly a species of *Parendomyces* (?) under the name "champignon du muguet." The nuclear phenomena are often important in classification and in questions of phy-The single yeast-like cell is uninucleate (pl. 15, figs. logeny. 27-30). The granular nucleus is seen as a large body with a heavily staining nucleolus from which emanates a net-work along which are granules of volutin deeply stained with haematoxylin. As the cell grows older, it elongates (pl. 16, fig. 31) and the cell wall is constricted. The nuclear division which then occurs at the point of indentation is amitotic, of an advanced type. No chromosomes are seen in the division process; instead, as far as could be determined from fixed material. there is a clumping of the metachromatic material in two groups, within the nucleus and opposite to each other (figs.

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31-32, 35-36, 38, 42, 50). At this time, within the nucleus, fine reticulations with small granules extend between the two clumps. Directly after division these reticulations seem to spread out (figs. 33, 37, 50) and retain the type of structure seen in the large nuclei (figs. 27, 49).

When the young yeast-like cell or blastospores are allowed to germinate (pl. 15, figs. 1–4), the nucleus elongates, divides within the cell, and the daughter nucleus grows along with the tube as it grows out. In the formation of the cross-wall the daughter nucleus also elongates and divides amitotically as described above, accompanied by an abscission of the cell. One nucleus is thus carried into the newly formed cell. At a later stage, the mother cell divides in two, with one nucleus in each daughter cell, resulting in a uninucleate cell (pl. 16, figs. 39, 46, 48). In many of the yeast-like cells, the nucleus is at the apical portion growing into the newly forming bud (figs. 41–42). As the bud matures, there is an abscission from the mother cell and the nucleus is divided in the process (fig. 43).

The mycelium is uninucleate, with the extended nucleus centrally placed in the long cells (figs. 47–48). It is of interest that the division of a nucleus does not necessarily imply that a new cell is cut off at the same time. A nucleus may divide within a cell and then migrate into a newly formed bud (figs. 33, 36–37, 48–50). This is particularly true in the formation of the blastospores where the nucleus first divides amitotically and then migrates into the young blastospore which consequently is uninucleate.

The presence of a network and heavily granulated protoplasm is noted in all the cells. Except for the blastospores and the clavate, obovate or lanceolate cells which are modifications of the yeast-like cells, there are no distinctive structures in this organism.

Cellular contents.—Reserve materials are found normally in larger amounts in the older portions of the mycelium, as glycogen, lipoids, oil globules, metachromatic granules, or volutin, or as decomposition products, as nucleic acid substances, protein derivatives, or carbohydrates. These ma-

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terials have been discussed previously in greater detail (Rajat '06; Moore '33, '35).

Volutin.—Volutin or metachromatic material may be very easily demonstrated with methylene blue (fig. 54) or even with iron-alum haematoxylin, as substances within the cell, along the inner wall surface, or along the network, both within the nucleus and in the cytoplasm. With the latter dye they stain deeply, whereas with the former they appear as droplets. A pinch of benzidine sulphate added to a water mount of living organisms (fig. 52) reveals a vacuolar condition within which are a varying number of granules known as "dancing bodies." These granules are precipitated volutin in a state of Brownian movement. They take a blue coloration supposedly, because of the action of benzidine on the peroxidases.

*Glycogen.*—With saturated iodine potassium iodide glycogen can be demonstrated very easily (fig. 55), taking an orange-brown coloration. With neutral red it is not quite so clear but appears as a pink or red vacuole called the glycogen vacuole. Glycogen is more abundant in the older than in the younger cells.

Vacuoles.—Vacuoles are easily demonstrated with benzidine sulphate (fig. 52), methylene blue (fig. 54), and with iron-alum haematoxylin. In the last case they appear surrounded by the network. Vacuoles are further brought out with iodine, within which are found the crystalloid droplets comparable to those seen with neutral red. They can also be demonstrated with saturated iodine potassium iodide.

*Chondriosomes.*—Chondriosomes are shown with saturated iodine potassium iodide, as demonstrated by Guilliermond. In this fungus they appear as light yellow, refractile bodies or droplets of varying size, distributed within cytoplasmic substance. There are few in the young cells.

Fat, lipoidal substances.—In addition to the substances mentioned, there are also fats, lipoidal substances, other reserve materials, and secretion and even excretion products. What

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the nature of these substances may be is not entirely clear, but it is known that fatty acids, glycerides, phospholipides, glycerol and phospho-aminolipides (complex lipides) may be present in varying amounts. Several agents were tried to demonstrate them, each showing some advantage. With 2 per cent osmic acid, they are reduced and take a black coloration (fig. 51). In the young cells or filaments, the blackened portions are more abundant at points where the nuclei would be found. Many of the young, yeast-like cells show only a single small droplet, while older cells show numerous irregular globules throughout. With platinic chloride (5 per cent solution) much the same results are obtained (fig. 53). Iodine potassium iodide as applied for glycogen or chondriosomes shows lipoidal substances, as oil droplets, equally as well. These are very small and appear as refractile, hyaline bodies.

# Systematic Position

Since the literature dealing with the nomenclature of the imperfect yeasts is voluminous and has been thoroughly summarized in a recent work by the senior author ('35), it need not be reviewed here. Syringospora Quinquaud ('68) was based on S. Robinii, a renaming of Oidium albicans Robin ('53), which in turn was based on the work of Gruby (1842) on the common organism of thrush (muguet, Soor or sapinho). The morphology figured by Quinquaud is similar to that of our species or to that of Mycotorula Langeron & Talice ('32) non Will. In our organism, the verticils show some reduction, being less dense than in S. albicans and tending to produce one or two branched chains of blastospores at the septa as in the genus Mycotoruloides. The verticils of these chains of blastospores are also reduced, approaching a state shown in Mycocandida where the verticils normally contain only two blastospores per septum of the axial filament. The blastospores are generally ellipsoidal, rarely clavate or obovate, never pyriform or lacrimiform as those of Blastodendrion. Thick-walled cells (perhaps chlamydospores) appear to be basal either to a whole pseudo-mycelium or to a primary branch thereof. They are

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rarely terminal especially on serum (fig. 14 or 21), similar to those figured by Roger ('96) reproduced by Noisette ('98) for their strains of S. albicans.

In cultures our organism differs from S. albicans and S. Braulti in not forming a pellicle on any liquid media, although it is somewhat closer to the latter in morphology. Mazza & Palamedi report that sugars were not fermented nor gelatine liquefied, while Talice & Mackinnon ('34) and ourselves have found the organism to ferment glucose, fructose, mannose, and maltose, and to liquefy gelatine. It is possible that Mazza & Palamedi did not keep their cultures long enough to observe fermentation or liquefaction. After a study of Mazza & Palamedi's cultures. Talice & Mackinnon have reduced this species to synonymy with Syringospora albicans under the synonym Mucotorula albicans (Robin) Langeron & Talice. While this organism shows many characters in common with S. albicans, its invasive power is so much greater (being severely pathogenic to rabbit after two years in culture) that it hardly seems likely that it is the same as S. albicans which is a mild parasite of the mucosa of infants, or of extremely senile or moribund adults, having little or no power to invade the skin or lungs of adults. S. inexorabilis, starting in the lungs or buccal mucosa of a healthy farm laborer, produced a generalized infection involving the skin and invading the tissues.

While recognizing that only a thorough monograph along the lines of that undertaken by the Centraalbureau voor Schimmelcultures, but upon a variety of suitable media not used there, can settle the systematic position of these organisms, it seems better to transfer the species to *Syringospora*, where it may be characterized as follows:

SYRINGOSPORA INEXORABILIS (Mazza & Palamedi) Dodge, Med. Myc. 242. 1935.

Monilia inexorabilis Mazza & Palamedi, Reunión Soc. Argentina Patol. Reg. del Norte en Tucumán 7: 424–467, 1 pl. 50 fig. 1932.

Mycotorula albicans Talice & Mackinnon, Reunión Soc. Argentina Patol. Reg. del Norte, Santiago del Estero 8: 165–166. 1934.

Isolated from blastomycosis of the skin and mucosa of a patient in the Argentine Chaco by Mazza and Palamedi ('32); pathogenic to guinea pig and rabbit. Perhaps the case of Rockwood & Greenwood ('34) should be referred here, although the autopsy findings were less positive.

Yeast cells in tissue and pus, spherical. Yeast cells common in media of low hydrogen-ion concentration and higher oxygen tension, thin-walled except on serum. In higher hydrogenion concentrations pseudo-mycelium abundant in favorable media, true mycelium or racquet mycelium in unfavorable media. Blastospores single or in short, branched chains, usually at the septa, occasionally scattered in poor media.

# Related Species

Recently, Ciferri and Redaelli ('35) published notes on the morphology of several species of *Syringospora* and related genera, which had previously been very imperfectly known. As this information was received too late to incorporate in Dodge's 'Medical Mycology' ('35), it seems desirable to summarize it here and record the new combinations made necessary by the new information.

SYRINGOSPORA **dimorpha** (Redaelli & Ciferri) Dodge & Moore, n. comb.

Mycotorula dimorpha Redaelli & Ciferri, Arch. Mikrobiol. 6: 43-46, fig. 28, 29. 1935.

Mycotorula interdigitalis Redaelli, 1930. non Pollacci & Nannizzi.

Isolated from dysidrosiform lesions of the interdigital spaces of a patient in Cairo, Egypt. (Soliman, strain 3). Pathogenic for guinea pig.

Hyphae long, septate, little-branched, producing dense verticils of blastospores at the septa. Blastospores  $3 \times 3.5 \mu$ .

On glucose agar, colony rounded (3 cm. in 20 days), dense, creamy, dirty white tending toward yellowish, central portion somewhat elevated, broadly crateriform, smooth, with outer 6 mm. next margin grayish, deeply radially furrowed, margin round. On liquid media producing a ring on malt extract,

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otherwise with a few floating islets which settle as an abundant floccose sediment. Fermentation of glucose, fructose and mannose, acid with the other carbohydrates. No action on milk; gelatin liquefied within 5 days.

MYCOTORULOIDES **macedoniensis** (Castellani) Dodge & Moore, n. comb.

Monilia macedoniensis Castellani & Chalmers, Man. Trop. Med. ed. 3, 1087. 1919.

Myceloblastanon macedoniense Ota, Jap. Jour. Derm. Urol. 28: 127. 1928.

Castellania macedoniensis Dodge, Med. Myc. 259. 1935.

Mycotorula macedoniensis Redaelli & Ciferri in Ciferri & Redaelli, Arch. Mikrobiol. 6: 18. 1935.

Originally isolated from sputum of a patient in Macedonia by Castellani. The culture studied by Ciferri and Redaelli was received from Castellani under the name Monilia macedoniensis var. macedoniensoides Cast., isolated from sputum. Castellani & Taylor ('25) originally described Monilia macedoniensoides in a paper on vaginal monilias, and while they do not specifically state that this species came from the vagina one would assume that such is the case. Later the same year, Castellani, Douglas & Thompson ('25) list it in a paper on infections of the bronchi without giving data as to its origin. Ashford ('31) studied two strains of this species from Castellani, reporting glucose and fructose fermented by both strains; maltose, sucrose, galactose, and inulin fermented by one strain which fermented maltose only once in nine sowings. Acid was produced with glucose, maltose, galactose, xylose; about half the time with lactose and raffinose; and about one-fourth the time with mannitol and dextrin.

On liquid media only the yeast stage of ovoid cells  $3-4 \times 4.5-5 \mu$ , budding monopolar, rarely bipolar, rarely in short chains but no true filaments. On solid media (Difco nutrient agar with glucose) hyphae with verticils of blastospores at the septa. From Ciferri and Redaelli's illustrations, hyphae rarely branched, blastospores usually in compact verticils, occasionally in short, branched chains characteristic of Myco-

toruloides Lang. & Tal. Blastospores evidently variable, but no dimensions or magnifications of figures given.

Giant colony after 15 days in Difco nutrient agar with glucose at room temperatures, circular, 1 cm. in diameter, creamy white, smooth, shining, center slightly elevated, smooth, sloping gradually to the periphery with rare radial folds, margin smooth, thin, uniform or finally mammillate. Sanfilippo ('24), who studied Castellani's culture, reports sediment in broth; no turbidity nor pellicle.

Fermentation and acid production in glucose, fructose, galactose, sucrose, and inulin; milk coagulated; gelatin and serum not liquefied.

From both its morphology and biochemical characters this species seems to belong to *Mycotoruloides*, although the younger hyphae are suggestive of *Syringospora*. Var. macedoniensoides differs only in not coagulating milk.

Mycotoruloides trimorpha (Redaelli & Ciferri) Dodge & Moore, n. comb.

Mycotorula trimorpha Redaelli & Ciferri, Arch. Mikrobiol. 6: 35. 1935.

Candida insolita Redaelli in Graziano, Giorn. Batt. Immun. 5: 1070–1075. 1 fig. 1933. non (Cast.) Basgal, Contr. Estudo Blastomycoses Pulmonares, p. 49. 1931.

Isolated from feces in cases of enteritis of infants by Graziano ('33). Not pathogenic for guinea pig.

In solid media only yeast cells present. In liquid cultures, hyphae long, little-branched, of long cells bearing dense verticils at the septa, blastospores of the verticils in short-branched chains, 2.4  $\mu$  in diameter with a large hyaline cell (chlamydospore) at the tip of the hyphae up to 10  $\mu$  in diameter. Under some conditions these may proliferate to 8–10 cells in a chain, suggesting the condition found in *Monilia*.

On Sabouraud, carrot, and malt-extract agars, colony smooth, white, creamy, margin smooth, surface shining; on malt, carrot and potato, medium colored yellow. On gelatin colonies color similar but opaque, penetrating somewhat into the medium. In carrot decoction and malt extract, complete

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ring and incomplete thin pellicle, abundant sediment, and slight turbidity.

Obligate aerobe, optimum temperature 37° C., growth good but much slower at room temperature. Ferments glucose, fructose, maltose, galactose, and sucrose; slight acid production on most media; milk not coagulated nor digested; gelatin and serum not liquefied.

PARENDOMYCES Flareri (Redaelli & Ciferri) Dodge & Moore, n. comb.

Blastodendrion Flareri Redaelli & Ciferri, Arch. Mikrobiol. 6: 51-53, fig. 35, 36. 1935.

Strain 2 Cazzani was isolated by Flarer from scaling erythematous lesion of the human skin, and Strain 8 from an eczematous lesion, both in Messina, Italy. Not found pathogenic for guinea pig by intravenous injection.

Cells sprouting, forming small branched groups, rarely short, highly branched filaments, of blastospores variable in shape and size,  $1.5-2.3 \times 2-3 \mu$ ; chlamydospores  $5 \mu$  in diameter; cells filled with dense homogeneous protoplasm or uniguttulate except in old vacuolate cells.

On Sabouraud agar, colony round, dense, creamy, white, center elevated, slightly crateriform, sloping gently to the smooth margin, surface rather dull. In liquid media only an incomplete ring, with discrete sediment of conglutinate floccose colonies; in some media with a diffuse opalescence. No fermentation of carbohydrates; acid in dextrose, levulose, and mannose; no action on milk, nor on gelatine. Strain 8 also produced acid in galactose and raffinose.

Apparently differs from *P. Perryi* (Castellani) Dodge, in not producing acid on maltose.

PSEUDOMONILIA verticillata (Redaelli & Ciferri) Dodge & Moore, n. comb.

Mycotorula verticillata Redaelli & Ciferri, Arch. Mikrobiol. 6: 40. 1935.

Isolated at Milan, Italy, from a scaling erythematous lesion of the skin. Pathogenic for guinea pig.

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Hyphae branched with verticils at the septa, verticils often of only two cells. Occasionally tufts of mycelium rise above the colony when the verticils are denser, suggesting Syringospora; blastospores ovoid to spherical, about  $3.5 \mu$  in diameter.

On glucose agar, colony irregularly rounded, much elevated above the substrate (10–12 mm. in diameter in 20 days at room temperature), shining, whitish, opaque, cerebriform, irregular, marginal lobes smooth and thick. On liquid media, a ring with confluent islets forming a pellicle and abundant sediment. No fermentation of sugars; acid in glucose, mannose, fructose, galactose, and maltose; no action on milk or gelatin.

From its biochemical characters this species seems to belong rather to *Pseudomonilia* than to *Syringospora* (*Mycotorula* Lang. & Tal.). Morphologically the aerial mycelium sometimes suggests the latter, although in liquid media and within the colony it is typical of *Pseudomonilia*.

PSEUDOMONILIA **zeylanoides** (Shaw) Dodge & Moore, n. comb. *Monilia zeylanoides* Shaw, Centralbl. f. Bakt. I. **119**: 460–464. 1931. non Cast.

Mycotorula zeylanoides Redaelli & Ciferri, Arch. Mikrobiol. 6: 41-43, fig. 26, 27. 1935.

Isolated by Shaw from sputum and referred to *Monilia zeylanoides* Cast in 1931. Isolated by Carco from tonsillar lesions with the usual symptoms of a mycosis.

Hyphae long, hyaline, septate, with moderate branching, blastospores in reduced verticils at the septa.

On solid media colony dense, yellowish-white, becoming intense yellow in age, surface shining, margin smooth at first, becoming fringed. Giant colony showing three zones, center somewhat elevated and crateriform, second irregularly hilly suggesting lava flows, dense, milk-white, outer zone creamy color, thinner, opaque, finely fringed. On liquid media, complete ring, tough, dense, white, also thin pellicle of coalescing islets with abundant sediment. No fermentation of sugars; acid in glucose, fructose, mannose, galactose, maltose, inulin, and xylose; no action on milk; gelatin not liquefied.

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This species differs from *Parendomyces zeylanoides* (Cast.) Dodge, to which it was referred by Shaw, in not coagulating milk, and in its abundant hyphae. It seems more closely related to *Pseudomonilia matalensis* (Cast.) Dodge, from which it differs in the thinner white pellicle and abundant sediment in liquid media, and the production of acid in most sugars, acid production in *P. matalensis* being slight or none.

MYCODERMA **desidiosum** (Ciferri & Redaelli) Dodge & Moore, n. comb.

Candida desidiosa Ciferri & Redaelli, Arch. Mikrobiol. 6: 62–64. 1935.

Isolated from the intestinal contents of pigeons with experimental beriberi along with *Candida Krusei*. Pathogenic for guinea pigs.

Mycelium of short ellipsoidal blastospores, somewhat branched, cells filled with oil droplets.

On glucose agar, colony round, 1.5 cm. in diameter, cream to yellowish, dense, shining, central portion irregularly rounded, elevated but flat, smooth, with a few slight hillocks at the center; outer slope steep to margin which is smooth or slightly and indistinctly mammillate. In age, a slight grayish halo with a finely plumose margin so that three zones appear; a central plateau, an irregular mammillate, smooth, shining white zone, and a very thin grayish plumose margin. On liquid media, ring incomplete, better developed and yellowish on malt extract, slight turbidity, sediment in a single conglutinate mass. No fermentation.

CANDIDA **dendritica** (Ciferri & Redaelli) Dodge & Moore, n. comb.

Trichosporon (Geotrichoides) dendriticum Ciferri & Redaelli, Arch. Mikrobiol. 6: 53-58, fig. 37-38. 1935.

Isolated along with *Blastodendrion Pinoyi* (Cast.) Lang & Tal. from a patient with bronchomoniliasis in Milan, Italy. Pathogenicity not stated.

Mycelium 1.5–2.3  $\mu$  in diameter, somewhat verticillately branched. Arthrospores abundant, 2.3–2.5 × 7–9  $\mu$ ; blasto-

spores rare, ellipsoidal, tending to occur in verticils as in *Syringospora*.

On glucose agar, central portion whitish, creamy, mycelium mostly submerged in dendritic fascicles, with a thin, branched plumose margin. In liquid media a thin pellicle within 24 hours. Glucose and fructose fermented.

This species has been too imperfectly described for certain systematic position, but since *Trichosporon* is held to be untenable as a generic name, it seems best to place it here, pending further study.

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# EXPLANATION OF PLATE

#### PLATE 15

#### Syringospora inexorabilis (Monilia inexorabilis)

All figures drawn with the aid of a camera lucida at a magnification of  $\times$  1440 and reduced to  $\times$  765.

Figs. 1-6. Cells developing from blastospore or yeast-like cell.

Fig. 7. Collar-like cross-wall on Endo's agar.

Fig. 8. Type of mycelium on Czapek's agar.

Fig. 9. Group of budding yeast-like cells on malt extract agar.

Fig. 10. Type of mycelium with blastospores, on lactose agar.

Fig. 11. Type of mycelium on yeast-dextrose agar.

Fig. 12. Giant, spherical thick-walled cells on wort agar.

Fig. 13. Cells on wort agar.

Fig. 14. Type of cells in serum.

Fig. 15. Cells on yeast-dextrose agar.

Figs. 16-17. Type of mycelium on Raulin's solution.

Fig. 18. Blastospores on Czapek's agar.

Fig. 19. Mycelium with blastospores on Endo's agar.

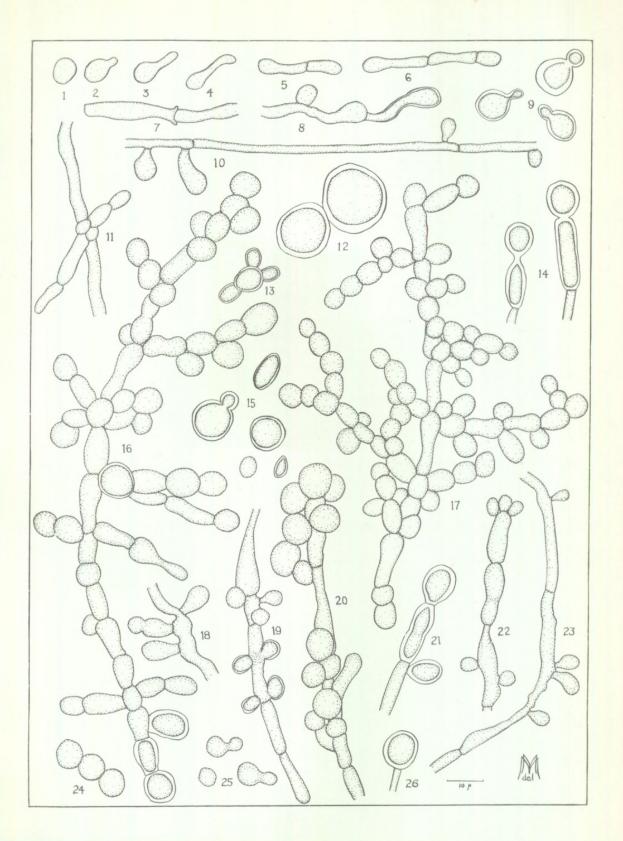
Fig. 20. Mycelium with blastospores on nutrient blood agar.

Fig. 21. Cells in serum.

Figs. 22-23. Mycelium on Endo's agar with peculiar oidioid cells at apex (fig. 22).

Figs. 24-25. Cells on nutrient blood agar.

Fig. 26. Cells in serum.





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