FURTHER STUDIES ON THE PRACTICAL CLASSIFICATION OF THE MONILIAS[1]

DONALD S. MARTIN AND CLAUDIUS P. JONES

Department of Bacteriology and the Department of Obstetrics and Gynecology, Duke University School of Medicine and Duke Hospital, Durham, North Carolina

Received for publication October 20, 1939

INTRODUCTION

One of the greatest handicaps to the clinical study of fungus disease is the lack of uniform methods of identification and classification of the fungi isolated from the human body. Most of the medically important fungi belong to the *Fungi imperfecti* which do not develop diagnostic sexual forms. These imperfect fungi, although relatively simple in comparison with the highly developed forms found in nature, possess many structures of differential importance but disagreements in regard to their interpretation have led only to confusion in classification.

In no other group of medically important fungi is there any greater confusion than that concerned with the classification of the non-ascosporogenous mycelia-producing yeastlike organisms known to the medical men as the "*Monilias.*" For example, Langeron and Guerra (1938) in France listed 102 synonyms for the thrush fungus *Candida albicans*, and the same year Ciferri, Redaelli, and Cavallero (1938) in Italy listed 121 synonyms for the same species under the name *Mycotorula albicans*. However, these two groups agree with each other in only 51 synonyms, the French group listing 51 names not recorded by the Italians, who list 70 synonyms not mentioned by Langeron and Guerra. These 121 names upon which there is no agreement actually represent only some 40 or more different species of fungi, the other synonyms

[1] Aided by a grant from the John and Mary R. Markle Foundation.

having been suggested previously by workers in other laboratories. Since many strains were unavailable, both synonymies were based chiefly on descriptions found in the literature. The confusion caused by the use of such methods is well illustrated by Dodge (1935), who splits into 5 different genera (*Syringospora*, *Blastodendrion*, *Zymonema*, *Castellania*, and *Candida*), those 51 fungi in regard to which there is complete agreement between Langeron and Guerra (1938) and Ciferri *et al.* (1938) that they all belong to a single species *albicans*.

In 1937 we published a method of classification based on the study of 172 strains of anascosporogenous mycelia-producing yeastlike fungi, 169 of which could be classified into one of 6 species, including a new species, *Monilia stellatoidea*. The methods were essentially bacteriologic in nature beginning with the streaking of a blood agar plate from a 48-hour growth in glucose broth. This blood agar plate was an essential part of the procedure because it not only allowed the detection of bacterial contaminants, but also enabled the observer to pick a pure smooth colony for subculture. By following a rigid technique it was found that the carbohydrate fermentations and microscopic morphology on corn meal agar were remarkably constant for each species. By thus combining many of the essential points in the identification of pathogenic bacteria, i.e., microscopic morphology, colony morphology, carbohydrate fermentations, growth in broth, agglutination and animal pathogenicity, it was possible to establish diagnostic criteria for these mycelia-producing non-ascosporogenous yeasts.

Since publication of this paper, we have received, from various mycologists, numerous criticisms attacking our retention of the name "*Monilia*," the use of such an unusual medium as "blood agar," and the synonymy as presented in that paper. The name "*Monilia*" was retained by us because it is a generic name which is recognized by the clinician and the bacteriologist. We have been unable to convince ourselves that it would be worthwhile to enter into this controversy over taxonomy. Since the acceptable generic name is determined by priority of usage and the cultures studied by these early investigators are no longer

available, it is very doubtful that this problem will be settled within the near future. We have no preference and will accept any generic name which has the sanction of a majority of mycologists.[2]

Langeron and Guerra (1938) published a method for classifying these fungi in which they criticized our use of the species names *candida*, *parapsilosis*, and *mortifera* rather than *tropicalis*, *parakrusei*, and *pseudotropicalis* respectively. They were correct in that the latter species names are preferable because of prior usage but until the appearance of Langeron's paper with Guerra in 1938, no one had published a comparative study proving their identity. In fact our use of the species name *mortifera* was based on a culture received from Langeron in 1934 under the name *Mycocandida mortifera*, but which was changed by him in 1938 to *Candida pseudotropicalis*. This author also criticized our technique as being too complicated, but his recent paper with Guerra (1938) included many of the features we recommended, i.e., plating on glucose agar (instead of blood agar) and testing for fermentation of carbohydrates under a paraffin seal[3] (instead of vaseline). They also recommend testing for the assimilation of 5 carbohydrates (glucose, sucrose, maltose, lactose and raffinose) and determining the utilization of nitrogen from peptone, asparagine, histidine, urea and ammonium sulphate.

The synonymy as given in our 1937 paper is subject to the criticism that it included names of cultures which were not studied directly. Thus *Monilia pseudotropicalis* was given as a synonym of *Monilia albicans* because Stovall and Bubolz, studying a culture of this fungus from the American Type Culture Collection, found it to be identical with their *Monilia* type II

[2] In accord with a decision reached at an informal meeting of medical mycologists attending the Third International Congress for Microbiology, September 2–9, 1939 in New York City, we have substituted the generic term *Candida* for *Monilia*. Although Berkhout's original description of the genus *Candida* will require amending, it was the consensus of opinion of those present that the existing confusion would be greatly clarified if workers in this field would use only one generic term while awaiting official action by the Rules Committee of the International Botanical Congress.

[3] The introduction of this anaerobic method of testing for fermentations of carbohydrates should be credited to Guerra (1935).

(*Monilia albicans*). We agree now with Langeron and Guerra, since our study of a culture obtained from the Centraalbureau voor Schimmelcultures shows that this fungus should have been included as a synonym of *M. mortifera*. The criticism of this method of preparing synonymies may be applied equally well to the extensive synonymies published by Langeron and Guerra (1938), Ciferri, Redaelli and Cavallero (1938), Dodge (1935), and many others.

This report is concerned with results obtained by the study of 535 strains of non-ascosporogenous mycelia producing yeastlike fungi. It was felt that the multiplicity of names for yeastlike fungi to be found in the literature could be effectively reduced and confusion lessened by careful comparative studies using the rigid technique previously described (1937).

EXPERIMENTAL

In our previous studies only "typical smooth" colonies on blood agar were selected for subsequent planting on corn meal agar and inoculation of carbohydrate fermentation tubes because we had noted that rough or bizarre colonies frequently resulted from bacterial contamination. Atypical colonies were ignored in the same manner that a bacteriologist selects only smooth typical colonies for pure culture study. The microscopic and macroscopic morphologic changes brought about by enforced dissociation studied recently in this laboratory by Mickle and Jones (1940), may explain some of the difficulties encountered in other laboratories in the morphologic study of old cultures. In all but 1 of our 461 freshly isolated strains, all or the great majority of the colonies appearing on the first blood agar plating were smooth. In contrast, many of the previously named strains had been kept for many years (*Monilia tumefaciens* (*alba*) was isolated in 1900) on different media through numerous transplants. Many of the blood agar colonies on first plating of these strains bore no resemblance at all to *Candida* colonies, but by means of colony selection, maintenance by rapid transfer on glucose agar and numerous replatings, some of these old strains could be satisfactorily classified.

STUDY OF UNNAMED STRAINS

Up to the present time, in this laboratory, we have studied 461 previously unnamed cultures of *Candida*, most strains having been isolated in this hospital. These cultures include the 153 strains previously reported, 108 fecal strains reported by Schnoor (1939), 169 vaginal strains to be reported by Carter and Jones, and a number of miscellaneous cultures isolated here or sent to this laboratory for identification. Of these 461 strains, 457 (99.13 per cent) were readily identified, and did not differ in any essential detail from the criteria previously described. Since the 4 unclassified strains differed from each other, we hesitated to

TABLE 1

Candida albicans (*Monilia albicans*)	233 strains	(50.5 per cent)
Candida stellatoidea (*Monilia stellatoidea*)	110 strains	(23.9 per cent)
Candida parakrusei (*Monilia parapsilosis*)	47 strains	(10.2 per cent)
Candida krusei (*Monilia krusei*)	36 strains	(7.8 per cent)
Candida tropicalis (*Monilia candida*)	28 strains	(6.7 per cent)
Candida pseudotropicalis (*Monilia mortifera*)	3 strains	(0.7 per cent)
Candida sp. (*unclassified*)	4 strains	(0.9 per cent)
Total	461	

give them species rank and thus add more names to the literature. The frequency of isolation of these species is listed in table 1.

STUDY OF PREVIOUSLY NAMED STRAINS

Seventy-four previously named cultures were obtained from the Centraalbureau voor Schimmelcultures,[4] Langeron, Benham, Castellani, Stovall, and others, and include those previously reported (Martin *et al.*, 1937). These cultures were sent under 62 different names or combinations of names, representing 49 differently named species (table 2).

All cultures were examined by the procedures used for identification of freshly isolated strains as reported in our previous paper (1937) with the exception that the carbohydrate fermentations were limited to 4 carbohydrates: glucose, sucrose, lactose and maltose.

[4] Hereinafter referred to as the C. B. S.

TABLE 2

NUMBER	CULTURE	DONOR	YEAR RECEIVED
M-49	*Candida tropicalis* (Cast.) Berkhout	Langeron # 255	1938
75	*Candida tropicalis* (Cast.) Berkhout	Langeron # 677	1934
406	*Candida tropicalis* (Cast.) Berkhout	C. B. S.	1938
636	*Monilia tropicalis* Cast.	Castellani	1939
C-70	*Geotrichoides krusei* (Cast.) Lang. and Tal.	Langeron # 683	1933
424	*Geotrichoides krusei* (Cast.) Lang. and Tal.	C. B. S.	1938
465	*Candida krusei* (Cast.) Berkhout	Langeron # 632	1938
635	*Candida krusei* (Cast.) Berkhout	Castellani	1939
M-69	*Monilia krusei* Cast.	Benham	1932
443	*Mycotorula albicans* (Robin) Lang. and Tal.	C. B. S.	1938
459	*Candida albicans* (Robin) Berkhout	Langeron # 308	1938
M-87	*Monilia albicans* (Robin) Zopf	Benham # 1755	1932
M-103	*Monilia albicans* (Robin) Zopf	Benham # 1773	1932
M-47	*Mycocandida mortifera* (Red.) Lang. and Tal.	Langeron # 516	1933
427	*Candida mortifera* Red.	C. B. S.	1938
407	*Candida mortifera* var. *alpha* Red.	C. B. S.	1938
408	*Monilia pseudotropicalis* Cast.	C. B. S.	1938
637	*Monilia pseudotropicalis* Cast.	Castellani	1939
469	*Candida pseudotropicalis* Cast.	Langeron # 681	1938
C-76	*Candida parapsilosis* Ashford	Langeron # 341	1934
421	*Candida parapsilosis* Ashford	C. B. S.	1938
M-88	*Monilia parapsilosis* Ashford	Benham	1932
405	*Candida macedoniensis* (Cast.) Berkhout	C. B. S.	1938
413	*Candida macedoniensis* var. *macedoniensoides*	C. B. S.	1938
638	*Monilia macedoniensis* (Cast.)	Castellani	1939
M-46	*Mycotorula psilosis* (Ashford) Lang. and Tal.	Langeron # 340	1933
412	*Candida psilosis* Ashford	C. B. S.	1938
418	*Mycotoruloides aldoi* (Pereira) Lang. and Tal.	C. B. S.	1938
464	*Candida aldoi* Pereira	C. B. S.	1938
431	*Mycotoruloides triadis* Lang. and Tal.	C. B. S.	1938
466	*Candida triadis* Lang. and Tal.	Langeron # 587	1938
470	*Candida parakrusei* Cast.	Langeron # 517	1938
410	*Monilia guilliermondii* Cast.	C. B. S.	1938
633	*Monilia guilliermondii* Cast.	Castellani	1939
462	*Candida guilliermondii* Cast.	Langeron # 648	1938
M-96	*Monilia type I* Stovall and Bubolz	Stovall	1932
M-92	*Monilia type II* Stovall and Bubolz	Stovall	1932
M-238	*Monilia type II* Stovall and Bubolz	Reed and Johns.	1935
M-93	*Monilia type III* Stovall and Bubolz	Stovall	1932

TABLE 2—*Concluded*

NUMBER	CULTURE	DONOR	YEAR RECEIVED
M-239	*Monilia type III* Stovall and Bubolz	Reed and Johns.	1935
M-240	*Monilia type IV* Reed and Johns.	Reed and Johns.	1935
M-242	*Monilia type VI* Reed and Johns.	Reed and Johns.	1935
425	*Mycotoruloides ovalis* Lang. and Tal.	C. B. S.	1938
M-44	*Mycotoruloides ovalis* Lang. and Tal.	Langeron #296	1933
426	*Candida mycotoruloidea* Red. and Cif.	C. B. S.	1938
428	*Monilia alba* Cast.	C. B. S.	1938
429	*Mycotorula sinensis*	C. B. S.	1938
430	*Monilia ashfordi* Anderson	C. B. S.	1938
634	*Monilia pinoyi* Cast.	Castellani	1939
433	*Mycotorula verticillata* Red. and Cif.	C. B. S.	1938
436	*Mycotorula dimorpha* Red. and Cif.	C. B. S.	1938
437	*Monilia fioccoi* Poll. and Nann.	C. B. S.	1938
M-171	*Blastodendrion intermedium* Cif. and Ashford	Langeron #493	1933
439	*Candida vulgaris* Berkhout	C. B. S.	1938
440	*Monilia onychophila* Poll. and Nann.	C. B. S.	1938
442	*Mycotorula zeylanoides* (Cast.) Cif. and Red.	C. B. S.	1938
444	*Mycotorula aegyptiaca* Cif. and Red.	C. B. S.	1938
419	*Monilia richmondi* Shaw	C. B. S.	1938
445	*Monilia pseudolondinensis* Cast.	C. B. S.	1938
446	*Blastodendrion flareri* Red. and Cif.	C. B. S.	1938
472	*Blastodendrion flareri* Red. and Cif.	Langeron #583	1938
447	*Mycocandida paratropicalis* (Cast.) Lang. and Guerra	C. B. S.	1938
450	*Candida metalondinensis* (Cast.) Berkhout	C. B. S.	1938
451	*Mycocandida pinoyisimilis* (Cast.) Red. and Cif.	C. B. S.	1938
460	*Candida lodderi*	C. B. S.	1938
471	*Candida brumpti* (Lang. and Guerra) Guerra	Langeron #415	1938
409	*Candida chevalieri* (Guilliermond) Westerdijk	C. B. S.	1938
415	*Candida kefyr* (Beijerinck) Westerdijk	C. B. S.	1938
417	*Candida desidiosa* Cif. and Red.	C. B. S.	1938
420	*Monilia tumefaciens alba* (Foulerton) Poll. and Nann.	C. B. S.	1938
422	*Candida ravauti* Lang. and Guerra	C. B. S.	1938
423	*Blastodendrion erectum* Lang. and Tal.	C. B. S.	1938
M-114	*Monilia candida* (from ATCC #2113)	Benham #1956	1932
411	*Mycotorula trimorpha* Red. and Cif.	C. B. S.	1938

Candida tropicalis

(Previously described by us (1937) as *Monilia candida*)

The criteria previously described for the identification of this species are as follows: (1) bubbles with narrow film on the surface of Sabouraud's glucose acid broth[5] incubated at 37°C. for 48 hours; (2) large gray colonies surrounded by a mycelial fringe on blood agar plates streaked from Sabouraud's broth and incubated for 10 days at 37°C.; (3) acid and gas formation in glucose, sucrose and maltose, and no fermentation of lactose in 1 per cent carbohydrate broths inoculated after several transplants on sugar-free media, and incubated under a vaseline seal for 10 days at 37°C.; (4) typical well-developed branched mycelium containing numerous conidia and no chlamydospores on corn meal agar streaked from sugar-free media and incubated for several days at room temperature.

The following cultures conformed in all details to the description cited above: *Candida tropicalis* (M-49, 75, 406); *Monilia tropicalis* (636); *Monilia candida* (M-114); *Mycotorula dimorpha* (436); *Mycotorula trimorpha* (411); *Monilia* type III (M-93, M-239); *Blastodendrion intermedium* (M-171); *Candida vulgaris* (439); *Mycotorula aegyptiaca* (444); *Candida kefyr* (415).

QUESTIONABLY MISLABELED STRAINS

Candida psilosis. The species *psilosis* has been studied by other observers and found to be indistinguishable from *albicans.* The culture (412) received from the C. B. S. had all the characteristics of *tropicalis* (*candida*), but should not be included in the synonymy of *tropicalis.* The culture received from Langeron, *Mycotorula psilosis* (M-46) proved to be identical with *C. albicans* and is described later.

Mycotorula zeylanoides (442). This strain, which was identified by Ciferri and Redaelli as *Mycotorula zeylanoides,* is obviously not the noncarbohydrate-fermenting species *zeylanoides* described by Castellani. The strain we studied, which had all the

[5] This is unadjusted broth containing 4 per cent glucose and 1 per cent Fairchild's peptone.

characteristics of *tropicalis* (*candida*), was obtained from the C. B. S. where it had been sent by Redaelli and Ciferri. Langeron and Guerra obtained the same reactions in their study of *Mycotorula zeylanoides* sent to them by Redaelli. These authors then obtained directly from Castellani a culture which agreed with Castellani's original description in that it fermented none of the carbohydrates and is therefore given a separate species rank, *Candida zeylanoides.*

ATYPICAL STRAINS

Monilia onychophila (440) was similar in all respects to *tropicalis* (*candida*) except for the absence of the typical mycelial fringe on blood agar plates. Repeated subcultures were tried but we were never able to obtain this feature. The growth in broth, carbohydrate fermentations and corn meal agar cultures were characteristic of *C. tropicalis.*

Monilia tumefaciens (*alba*) (420) was atypical in that mycelial formation on blood agar plates was somewhat irregular. Some of the pure colonies, when picked and transplanted for corn meal agar and sugar fermentations gave irregular results, either failing to form mycelia regularly, or producing no change or only acid in maltose. After a series of replatings and several colony selections, a subculture was obtained which corresponded in every detail to the criteria described for *Candida tropicalis.*

Comment

There is complete agreement with Langeron and Guerra (1938) in respect to the cultures *tropicalis, dimorpha, trimorpha, vulgaris, kefyr, tumefaciens alba,* and *zeylanoides* (Ciferri's strain). They did not study *Mycotorula aegyptiaca,* but included it in the synonomy of *Candida tropicalis.* These authors consider *Monilia onychophila* to be identical with *Candida parakrusei* but our culture was more nearly related to *tropicalis.*

Blastodendrion intermedium is given species rank as *Candida intermedia* by Langeron and Guerra (1938) although it differs from other strains only in the assimilation of lactose. Since only one strain was observed by those authors and this strain

is otherwise indistinguishable from *tropicalis* (*candida*), we prefer to classify it as a member of this species.

The species name *tropicalis* is preferred over *candida* since Castellani (1910) introduced the name *Oidium tropicale* for a yeastlike fungus isolated from the sputum of a patient in Ceylon. For the origin of the word *candida* as a species name, reference must be made to the older literature.

Candida albicans

The criteria previously described for the identification of this species are: (1) no surface growth on Sabouraud's glucose acid broth; (2) typical, fairly uniform, large, round, grayish colonies on blood agar streaked from Sabouraud's broth and incubated for 10 days at 37°C.; (3) fermentation of glucose and maltose with acid and gas, formation of acid in sucrose and no fermentation of lactose in 1 per cent carbohydrate broths inoculated from sugar-free media and incubated under a vaseline seal for 10 days at 37°C.; (4) production of typical branching tree-like mycelium with chlamydospores on corn meal agar inoculated from sugar-free media.

The following cultures conformed in all details to the description cited above: *Mycotorula albicans* (443); *Candida albicans* (459); *Monilia albicans* (M-87, M-103); *Mycotorula psilosis* (M-46); *Mycotoruloides triadis* (431); *Monilia* type II (M-92, M-238); *Mycotoruloides ovalis* (425, M-44); *Monilia pinoyi* (634); *Candida metalondinensis* (450).

Excluding the strains reported in our previous paper (Martin *et al.* 1937), only 4 of these typical strains (443, 431, 425, and 450) were tested for pathogenicity in rabbits. Two rabbits inoculated with *Mycotoruloides triadis* and *Mycotoruloides ovalis* died within a week and the cultures of *Mycotorula albicans* and *Candida metalondinensis* were non-virulent.

ATYPICAL (QUESTIONABLY DISSOCIATED) STRAINS

Monilia alba (428). The first colonies of this organism on blood agar were rough, dry, and granular in appearance. Repeated subcultures on Sabouraud's medium and streaking of plates from

broth resulted in obtaining pure colonies typical in every way of *C. albicans.*

Candida triadis (466) had the same sugar fermentations as *albicans* but the colonies on blood agar were noticeably smaller than the typical *albicans* colonies. The mycelial development on corn meal agar was atypical in that long narrow hyphae were arranged in wavy parallel rows suggesting the mycelial arrangement brought about by lithium chloride dissociation (Mickle and Jones, 1940). The organism was not pathogenic for rabbits.

Candida mycotoruloidea (426) was atypical in that the colonies which developed on the first blood agar plating were very rough. The coarsely radiated surface was bound down so tightly to the medium by subsurface mycelia that the colonies had to be picked up whole for transplanting. Repeated plating and rapid passage on Sabouraud's agar produced only one smooth colony which appeared on a single plate and immediately reverted to the rough stage on subculture. The carbohydrate fermentations were typical of *albicans* and chlamydospores were found on corn meal agar although the mycelial development on this medium was not typical. The strain was non-virulent for rabbits.

Mycotorula sinensis (429) did not grow readily and the first colonies on blood agar were small, dry, yellow, and wrinkled. Smooth colonies having the dull gray color of *albicans* were obtained by transfer on Sabouraud's agar and replating, but the colonies were always smaller than those obtained with freshly isolated *M. albicans.* The sugar fermentations were those of *albicans,* but we were unable to obtain a satisfactory development of mycelia on corn meal agar. There were no chlamydospores. A rabbit inoculated with this culture died a month after inoculation.

Monilia ashfordi (430) differed from typical *Candida albicans* in that the first transplant showed a bubbly appearance on the surface of Sabouraud's broth and irregular colonies on blood agar superficially resembling those of *C. stellatoidea.* Typical *albicans* colonies were obtained by picking smooth colonies and replating. The sugar fermentations were those of *C. albicans* and chlamydo-

spores were found on corn meal agar although the mycelia were not as typical as those produced by freshly isolated cultures. The culture killed a rabbit 2 weeks after inoculation.

Monilia richmondi (419). This organism was shown by Stovall and Bubolz (1932) to be *Monilia* type II (*albicans*) and is listed in the synonymy of *Candida albicans* by Langeron and Guerra (1938), although they did not study a culture. Our culture from the C. B. S. differed from the typical *albicans* in that no chlamydospores were formed in corn meal agar and the arrangement of mycelia in this medium closely resembled that of dissociated *Candida albicans*, (Mickle and Jones 1940). The first cultures on blood agar gave rhizoid colonies and smears from Sabouraud's medium showed long narrow yeastlike cells superficially resembling *C. krusei*. The sugar fermentations were those of *C. albicans*.

Monilia pseudolondinensis (445) differed from typical *C. albicans* in colony formation on blood agar. The colonies were tough, dry, and had a course mycelial appearance, several of them showing a distinct central crater. The fermentations were those of *C. albicans* and chlamydospores were found in corn meal agar. An unusual feature was the occurrence of numerous short chains of very small budding yeast cells in the mycelial matrix.

Candida desidiosa (417) differed from *C. albicans* only in the appearance of the blood agar colonies which were rough, irregular, and appeared corrugated. Chlamydospores were present and the sugar fermentations were those of *C. albicans*.

Blastodendrion erectum (423) produced only small colonies on blood agar about the usual size for *C. parakrusei* although the color was typical of *C. albicans*. The sugar fermentations were those of *C. albicans*. No chlamydospores were found on corn meal agar.

Mycocandida pinoyisimilis (451) had the sugar fermentations of *C. albicans* but the blood agar colonies were darker than usual and the corn meal agar slide culture showed mycelial development similar to that of *C. albicans* dissociated by lithium chloride (Mickle and Jones, 1940). There were no chlamydospores. Diddens and Lodder (1939) have reported asci in a culture

of *Mycocandida pinoyisimilis* var. *citelliana* obtained from Ciferri and Redaelli. They consider it identical with *Saccharomyces fragilis*.

Comment

There is complete agreement with Langeron and Guerra (1938) in regards to the cultures *Mycotorula psilosis*, *Mycotoruloides ovalis*, *Monilia pinoyi*, *Candida metalondinensis*, *Blastodendrion erectum*, *Candida mycotoruloidea*, and *Mycocandida pinoyisimilis*. They did not study the species *alba*, *richmondi*, and *pseudolondinensis* but include them among the synonyms of *Candida albicans*.

These authors, however, prefer to place *ashfordi* in a different species *Candida guilliermondi*. This was based on a culture of *Parasaccharomyces ashfordi* from an unknown source. These authors also separate the species *Candida triadis* which differs from *Candida albicans* by only one feature—formation of a "veil" in broth after 13 days' incubation. However, our strain of *Mycotoruloides triadis* (431) from the C. B. S. was pathogenic for rabbits and was typical of *C. albicans* in every detail. We have observed repeatedly that a film or veil occurs irregularly in broth cultures incubated longer than 48 hours, and, therefore, have selected the 48-hour period in order to preserve this criterion for the diagnosis of the species *krusei* and *tropicalis* (*candida*). We do not feel that these authors are justified in separating this species from *albicans* especially since the organism is pathogenic for rabbits.

The latest contribution to the confusion concerning the proper name of this species is in a recent paper by Redaelli, Ciferri and Cavallero (1939). These authors studied a strain of *Monilia albicans* obtained from the Department of Bacteriology, University of Illinois and found numerous chlamydospores. This culture is considered identical with the classical *Endomyces albicans* of Vuillemin, the authors believing that Vuillemin mistook the chlamydospores for asci. They propose that the culture be labeled *Mycotorula albicans* var. *vuillemini*.

The occurrence of large numbers of chlamydospores in this culture is evidently a surprise to these workers although we have

found them in all 233 of our freshly isolated cultures of *Candida albicans* when inoculated into corn meal agar from sugar-free media.

Candida krusei

The criteria previously described for the identification of this species are: (1) wide film on surface of Sabouraud's broth tubes after 48 hours' incubation at 37°C.; (2) small, irregularly shaped, flat or heaped-up colonies on blood agar incubated at 37°C. for 10 days after streaking from Sabouraud's broth; (3) fermentation of glucose and no fermentation of lactose, sucrose, or maltose in 1 per cent broths incubated under a vaseline seal for 10 days after inoculation from a sugar-free medium; (4) typical "crossed sticks" mycelial appearance on corn meal slide cultures inoculated from a sugar-free medium.

The following cultures conformed in all details to the description cited above: *Geotrichoides krusei* (C-70, 424); *Candida krusei* (465, 635); *Monilia krusei* (M-69); *Candida chevalieri* (409).

Comment

The formation of a wide film on the surface of Sabouraud's broth, the small irregular blood agar colonies, and the weak fermentative activity of this species are so distinctive that little difficulty is encountered in diagnosis. Stovall and Bubolz (1932) placed this species in the genus *Mycoderma* because of the formation of a pellicle in broth. However, since it is a budding, yeastlike, non-ascospore producing, mycelia-forming fungus, we prefer to retain it as a member of the genus *Candida*, agreeing with Langeron and Guerra (1938) who have discarded the name *Geotrichoides* of Langeron and Talice (1932) and placed it in their genus *Candida*.

Candida pseudotropicalis

(Previously described by us (1937) as *Monilia mortifera*)

The criteria previously described for the identification of this species are: (1) no surface growth in Sabouraud's broth after 48 hours' incubation at 37°C.; (2) very small, non-characteristic, irregular colonies on blood agar streaked from Sabouraud's

broth and incubated at 37°C. for 10 days; (3) formation of acid and gas in glucose, sucrose, and lactose and no fermentation of maltose in 1 per cent carbohydrate broths inoculated from sugar-free media and incubated for 10 days at 37°C. under a vaseline seal; (4) formation, with difficulty, of a branched mycelium without chlamydospores.

The following cultures conformed in all details to the description cited above: *Mycocandida mortifera* (M-47); *Candida mortifera* (427); *Candida mortifera* var. *alpha* (407); *Monilia pseudotropicalis* (408, 637); *Candida pseudotropicalis* (469).

QUESTIONABLY MISLABELED STRAINS

The culture labeled *Mycocandida paratropicalis* (447) obtained from the C. B. S. was typical of *pseudotropicalis* (*mortifera*). This organism was described by Castellani as being very similar to *M. tropicalis* and Langeron and Guerra, who did not study a culture, included it in their synonomy of *Candida tropicalis*. Since the organism was originally described by Castellani as a non-lactose fermenter, it is possible that our strain obtained from the C. B. S. was mislabeled.

Comment

This vigorous lactose-fermenting organism is comparatively rare in our experience since we have isolated it only 3 times (twice from sputum and once from feces). The species name *pseudotropicalis* is preferred over *mortifera* since Castellani first used the name *Endomyces pseudotropicalis* in 1910, the name *mortifera* not appearing until introduced by Redaelli in 1925.

Diddens and Lodder (1939) describe *Candida pseudotropicalis* as the imperfect stage of *Saccharomyces fragilis* because it resembles a culture of *Mycocandida pinoyisimilis* var. *citelliana* in which they found asci and renamed *Saccharomyces fragilis*.

Candida parakrusei

(Previously described by us (1937) as *Monilia parapsilosis*)

The criteria previously described for the identification of this species are: (1) no surface growth in Sabouraud's broth incubated for 48 hours at 37°C.; (2) typical small, round, brilliantly

white colonies on blood agar streaked from Sabouraud's broth and incubated at 37°C. for 10 days; (3) fermentation of glucose with acid and gas (occasionally acid only) after inoculation of 1 per cent carbohydrate broths from sugar-free media and incubation at 37°C. for 10 days under a vaseline seal; (4) mycelia formed with difficulty, but well developed after growth has occurred.

The following cultures conformed in all details to the description cited above: *Candida parakrusei* (470); *Candida parapsilosis* (C- 76, 421); *Monilia parapsilosis* (M-88); *Monilia* type I (M-96); *Monilia* type IV (M-240); *Monilia* type VI (M-242); *Candida lodderi* (460); *Candida brumpti* (471).

QUESTIONABLY MISLABELED STRAINS

Monilia fioccoi (437) as received by us from the C. B. S. had all the characteristics of *M. parakrusei* (*parapsilosis*) but according to the original description should probably be considered as *albicans* because of chlamydospore formation.

ATYPICAL STRAINS

Mycotorula verticillata (433) on the first blood agar platings produced only raised, yellowish-tinged colonies which had a peculiar "fuzzy" appearance. After several transplantings the colonies became smooth and finally attained the brilliant white color of *C. parakrusei* although the colonies were larger than typical freshly isolated strains. Acid only was produced in glucose and the mycelial development on corn meal agar was typical of *parakrusei*.

Blastodendrion fareri from the C. B. S. (446) on the first blood agar plating produced 3 types of colonies; some were bright yellow and heaped-up, some were flat, dry and gray, and there was 1 yellow rough colony which was hard to pick up. After several platings, smooth colonies typical of *parakrusei* were obtained and the sugar fermentations and corn meal slide cultures were typical. The strain of this fungus obtained from Langeron (472) gave smooth colonies on the first transplant but the mycelial development on corn meal agar was atypical and no fermentation of any sugar occurred in 10 days.

Candida ravauti (422) produced colonies which resembled *albicans* more than *parakrusei* but glucose only was fermented and the corn meal agar slide culture, though not typical, was more characteristic of *C. parakrusei* than *C. albicans*.

Comment

This species probably offers the most diagnostic difficulties because of its relatively slow growth, feeble fermentative powers, and reluctance to form mycelia. Reed and Johnstone (1935), for example, did not recognize *Monilia* type I (*C. parakrusei*) of Stovall and Bubolz (1932) and described 2 new types, IV and VI, for fungi that were indistinguishable from *C. parakrusei* by the procedure we outlined.

The species name *parakrusei* antedated *parapsilosis*, and is, therefore, the preferred name.

The cultures labeled *flareri* and *brumpti* are given species rank by Langeron and Guerra. Their table for sugar fermentations gives identical sugar reactions but separates them on their different reactions in utilization of various carbohydrates. *Candida lodderi* is included in their species *Candida chalmersi*. They also include *Monilia onychophila* as *Candida parakrusei*. Their culture was obtained from the same source as ours (C. B. S.) but our culture had all the characteristics of *tropicalis* (*candida*) except for the failure to form a mycelial fringe around the colonies on blood agar.

OTHER STRAINS

Three strains of the species *macedoniensis* (405, 413, 638) produced a bubbly surface growth on Sabouraud's broth and produced acid and gas in glucose and sucrose. No fermentative action took place with lactose and maltose. The blood agar colonies of 2 strains (405, 638) were about the size of typical colonies of *pseudotropicalis*, but the colonies of one strain (413) resembled those of *C. albicans* in size and color. We were unable to obtain definite mycelia on corn meal agar slide cultures. Stovall and Bubolz (1932) reported asci in their strain obtained from the American Type Culture Collection and consider the fungus as a member of the genus *Endomyces*. Langeron and

Guerra (1938), however, classify it as a *Saccharomyces* because of the lack of mycelial formation. We could not find asci in carrot plug cultures in these 3 strains and were unable to obtain satisfactory mycelial development. According to our findings, the organism more nearly resembles a species of *Cryptococcus*. However, Diddens and Lodder (1939) reported the finding of asci in 2 strains of *Blastodendrion macedoniensis* obtained from Langeron and in 1 strain of *Candida macedoniensis* var. *macedoniensoides* sent to them by Castellani. These asci developed on carrot at 25°C. and these authors therefore changed the name to *Saccharomyces macedoniensis*. On the other hand, no asci were found in a culture of *Monilia macedoniensis* sent to the culture bureau by Joekes or in a culture of *Saccharomyces fragrans* isolated at Delft by Mayer. Since these cultures were identical, Diddens and Lodder (1939) renamed them *Candida macedoniensis*, considering them as imperfect stages of *Saccharomyces macedoniensis*.

Mycotoruloides aldoi (418) and *Candida aldoi* (464) from the C. B. S. gave rough colonies on the first plating on blood agar. It was only after several replatings that colonies resembling the smooth *C. albicans* type could be found. The sugar fermentations of these rough strains were irregular but the last 2 checks on *C. aldoi* (464) gave the reactions of *C. albicans*. *M. aldoi* (418) fermented only glucose but developed chlamydospores on corn meal agar. We were unable to obtain any mycelial growth on *C. aldoi* (464). These findings suggest that this fungus may have been *Candida albicans* before it had undergone dissociative changes.

Three strains of the species *guilliermondi* (410, 462, and 633) were all similar in that the colonies on blood agar superficially resembled those of *C. albicans* but none fermented any of the 4 carbohydrates and no chlamydospores were formed on corn meal agar. The organism then deserves species rank as advised by Langeron and Guerra, who reported acid and gas production in glucose and sucrose when incubated at 25 instead of 37°C. We have never encountered this species in our 461 freshly isolated strains.

DISCUSSION

There are still many differences of opinion concerning generic and species relationships among the bacteria in spite of their very simple mode of reproduction. It is not surprising, therefore, that attempts to classify the "*Candidas*" should cause differences of opinion since these fungi can present variations in many features, such as the size and shape of the cell, the site of budding on the cell, the amount and arrangement of the mycelia, the arrangement of conidia around the branches and the formation of special structures like chlamydospores. Thus, if we discard everything but microscopic morphology, the number of combinations is infinite and greatly handicaps attempts to classify these fungi, since each group of workers tends to disregard some features while emphasizing others. Thus Langeron and Talice (1932) classified these organisms into 6 genera. At that time these authors considered the arrangement of the conidial whorls around the mycelium to be sufficiently important to separate the genus *Mycotoruloides* from *Mycotorula*. Later, Langeron and Guerra (1938) not only reduced all their 6 genera to a single genus *Candida* but evidently discarded the conidial whorl arrangement as unimportant even in species differentiation. The reduction of these fungi to a single genus is very commendable and most workers (Diddens and Lodder, 1939; Ciferri and Redaelli, 1939) are now approaching this view in contrast to that of Dodge (1935) who subdivides them into 13 genera containing 122 different species.

Langeron and Guerra (1938) should be commended also for reducing the number of species to 16. In our opinion, this number could be reduced still further as we consider the species *albicans* and *triadis* synonymous and also believe *intermedia* to be indistinguishable from *tropicalis*. The species *brumpti* and *flareri* are closely related to *parakrusei*, although the cultures were not typical. We did not study *zeylanoides*, *deformans*, *pelliculosa*, *chalmersi*, or *suaveolens*. The species *guilliermondi* is distinct but we have never isolated a fungus of this description among our 461 freshly isolated strains. It is very interesting

that their list of species most commonly found in man corresponds, with one exception, to ours published in 1937 and extended in this present paper. These species are *albicans*, *tropicalis* (*candida*), *pseudotropicalis* (*mortifera*), *krusei*, and *parakrusei* (*parapsilosis*). They report *guilliermondi* as being of frequent occurrence and do not report the isolation of the species *stellatoidea* reported by us. Our high incidence of *C. stellatoidea* is due to the large number of vaginal cultures made by Carter and Jones (to be published).

We have received some correspondence criticizing our complication of the system of classification by separating the species *stellatoidea* from *albicans*. We have injected more than 50 strains of *C. albicans* into rabbits and all but 1 died with typical lesions in 2 to 7 days. The non-pathogenic strain had all the characteristics of dissociated *Candida albicans* described by Mickle and Jones (1940). On the other hand, more than 40 strains of *C. stellatoidea* have been injected and none was pathogenic. Since the pathogenicity, blood agar colonies and sugar fermentations in this species are constantly different from *C. albicans*, we prefer to retain this species as a separate fungus.

Blood agar is apparently unfavorable for growth, since many of the old strains develop on this medium as rough and irregular colonies. The growth on glucose agar, however, is uniformly moist and creamy, even in those strains which grow as very dry and wrinkled colonies on the blood medium. Except for strains showing complete dissociation (Mickle and Jones, 1940), this medium has a distinct differential value in the detection of dissociative changes which are not revealed by the glucose agar method recommended by Langeron and Guerra (1938).

The merits of any system of classification or identification can be determined only by the results obtained in other laboratories by other workers. New species and sub-groups will undoubtedly be found but we believe that the number of new species and the resulting addition of new names to the literature will be greatly reduced if the strains are examined carefully by the technique outlined in our previous paper.

SUMMARY

Five hundred and thirty-five strains of non-ascus-forming, mycelia-producing, yeastlike fungi were studied by the previously described technique.

Of 461 recently isolated strains, 457 (99.13 per cent) agreed in all details with the criteria established for the previously described 6 species.

Forty-six of the 73 previously named strains were likewise identified as members of the previously described 6 species.

Sixteen of the remaining previously named strains were tentatively classified as belonging to the previously described species but differed from typical strains in some detail (blood agar colony or mycelium production).

Four strains were apparently mislabeled as they did not correspond to the original description or to the descriptions of other workers.

The remaining 8 strains did not fit into the classification previously proposed and are described under the heading "other strains."

CONCLUSIONS

The great majority of freshly isolated non-ascosporogenous mycelia-producing, yeastlike fungi (the *Candidas*) can be identified by the procedure formerly described from this laboratory.

Cultures which have been kept on artificial media for many years become dissociated or changed in such a way that they are no longer typical and, therefore, cannot be used for comparison in the identification of recently isolated strains.

Slight variations in technique of study produce such great changes in growth and biological characteristics that many of the older descriptions must be interpreted with great care before arriving at definite opinions concerning their position in more modern systems of classification.

REFERENCES

CASTELLANI, A. 1910 Tropical bronchomycosis. Observations on a new species of *Epidermophyton* found in tinea cruris. A new intestinal spirillum. Philippine J. Sci. (B) **5**, 197–207.

CIFERRI, R., AND REDAELLI, P. 1939 *Mycotorula* vs. *Candida:* A plea. Mycopathologia, **2**, 73–74.

CIFERRI, R., REDAELLI, P., AND CAVALLERO, C. 1938 *L'Oidium albicans* Robin. Mycopathologia, **1**, 115–161.

DIDDENS, H. A., AND LODDER, J. 1938 An appeal for unification of the generic taxonomy in the *Mycotoruloidea.* Mycopathologia, **2**, 1–6.

DIDDENS, H. A., AND LODDER, J. 1938 On some sporogenous yeasts and their imperfect stages. Mycopathologia, **2**, 28–36.

DODGE, C. W. 1935 Medical Mycology. C. V. Mosby Co., St. Louis.

GUERRA, P. 1935 Rôle des levures en dermatologie. Étude clinique et mycologique. These Fac. Med. Paris (quoted from Langeron and Guerra, 1938).

LANGERON, M., AND GUERRA, P. 1938 Nouvelles recherches de zymologie medicale, Ann. parasitol. humaine compar., **16**, 36–84, 162–179, 429–476, 481–525.

LANGERON, M., AND TALICE, R.-V. 1932 Nouvelles méthodes d'étude et essai de classification des champignons levuriformes. Ann. parasitol. humaine compar., **10**, 1–80.

MARTIN, D. S., JONES, C. P., YAO, K. F., AND LEE, L. E. 1937 A practical classification of the *Monilias.* J. Bact., **34**, 99–128.

MICKLE, W. A., AND JONES, C. P. 1940 Dissociation of *Candida albicans* by lithium chloride and immune serum. J. Bact. (in press).

REDAELLI, P., CIFERRI, R., AND CAVALLERO, C. 1939 Sul presunto *Endomyces albicans* Vuillemin. Mycopathologia **2**, 116–121.

REED, A. C., AND JOHNSTONE, H. G. 1935 A clinical study of intestinal fungi. Am. J. Trop. Med., **15**, 155–174.

SCHNOOR, T. G. 1939 The occurrence of *Monilia* in normal stools. Am. J. Trop. Med., **19**, 163–169.