Handout #8
Bio 329
2007

Fungi Imperfecti: "yeast" form-genera.

Form-genera

TABLE 20–1. ~~Classification~~ of Medically Important Yeasts of the Form-Phylum Deuteromycota

Form-Class: Blastomycetes *

Form-Family: Cryptococcaceae *

Genus 1: *Cryptococcus.* Unicellular budding cells only; reproduce by blastoconidia pinched off the mother cell. Most are urease-positive. Cell surrounded by a heteropolysaccharide capsule and produces starchlike compounds; carotenoid pigments are usually lacking. Inositol is assimilated; sugars are not fermented.
Example: *Cryptococcus neoformans* (cryptococcal meningitis).

Genus 2: *Malassezia.* Mostly unicellular budding cells that reproduce by blastoconidia that develop from a reduced phialide. Cells may adhere, forming short hyphal strands. Growth stimulated by lipids. There is no fermentative ability. **
Example: *Malassezia furfur* (pityriasis versicolor)

Genus 3: *Rhodotorula.* Unicellular budding forms that rarely produce pseudomycelium, are generally encapsulated, but do not produce starchlike substance. They do not assimilate inositol or ferment sugars. Carotenoid pigments are produced.
Example: *Rhodotorula rubra* (rare pulmonary and systemic infections)

Genus 4: *Candida.* Reproduction is by pinched blastoconidia. They may form pseudomycelium or true mycelium; generally urease-negative; capsules are not formed; starch or carotenoid pigments are not produced; inositol is not assimilated.
Example: *Candida albicans* (candidiasis)

Genus 5: *Trichosporon.* Reproduction is by blastoconidia and arthroconidia. Mycelium and pseudomycelium are formed. ***
Example: *Trichosporon beigelii* (white piedra and systemic infections)

~~Genus 6: Torulopsis. Reproduction by pinched blastoconidia. Capsules, starchlike substances, and carotenoid pigments, and mycelium not formed. No assimilation of inositol.~~
~~Example: *Torulopsis glabrata* (rare opportunistic infections)~~

* no so-called black yeasts
** form-genus that includes agent of pityriasis versicolor
*** form-genus that includes agent of white piedra & trichosporosis

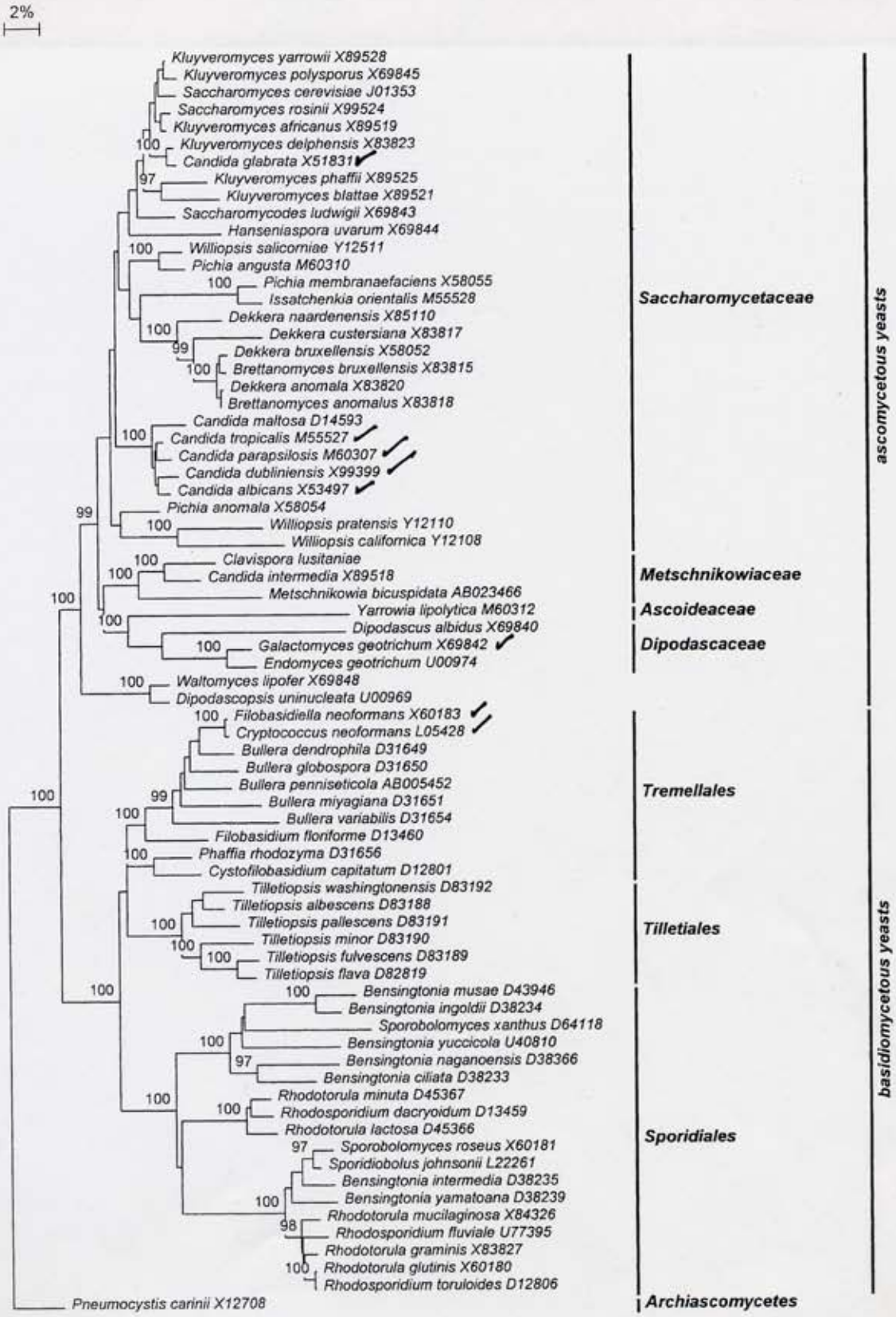


Fig. 17. Phylogenetic tree of the *Archiascomycetes* and the asco- and basidiomycetous yeasts, based on confidently aligned, near-complete SSU rDNA sequences using Neighbor joining algorithm with Kimura correction. Bootstrap values >90 from 100 resampled datasets are shown. *Pneumocystis carinii* was chosen as outgroup. All ascomycetous yeasts (*Hemiascomycetes*) are classified in a single order, the *Saccharomycetales*. In the basidiomycetous yeasts the diversity is larger, which has led to the distinction of several classes (see Table 1 on p. 19). All basidiomycetous orders contain numerous hyphal representatives.

Atlas of Clinical Fungi
de Hoog et al., 2000
Centraalbureau voor Schimmel-
cultures, The Netherlands

Basidiomycetous yeasts

Atlas of Clinical Fungi'
de Hoog et al., 2000
Centraalbureau voor Schim-
melcultures, The Netherlands.
(on Reserve)

General remarks. Basidiomycetous yeasts are anamorphs of members of jelly fungi (*Hymenomycetes; Tremellales*) or of smuts (*Ustilaginomycetes, Ustilaginales*). They are recognized by presence of urease and extracellular DNAse, and by the less widely used Diazonium Blue B (DBB) staining reaction, which is also positive. In addition, mostly extracellular starch-like compounds are produced, inositol is mostly assimilated, and sugars are not fermented or only in amounts that are not detected by standard methods. Bud formation mostly percurrent. Generative reproduction is mostly produced after mating of suitable partners. A clamped mycelium with thick-walled, brown teliospores is formed, which eventually germinate with a non-septate basidium (holobasidium) or a septate basidium (phragmobasidium), bearing sessile basidiospores. Ultrastructure: cell walls are multilamellar; septa have dolipores or simple pores.

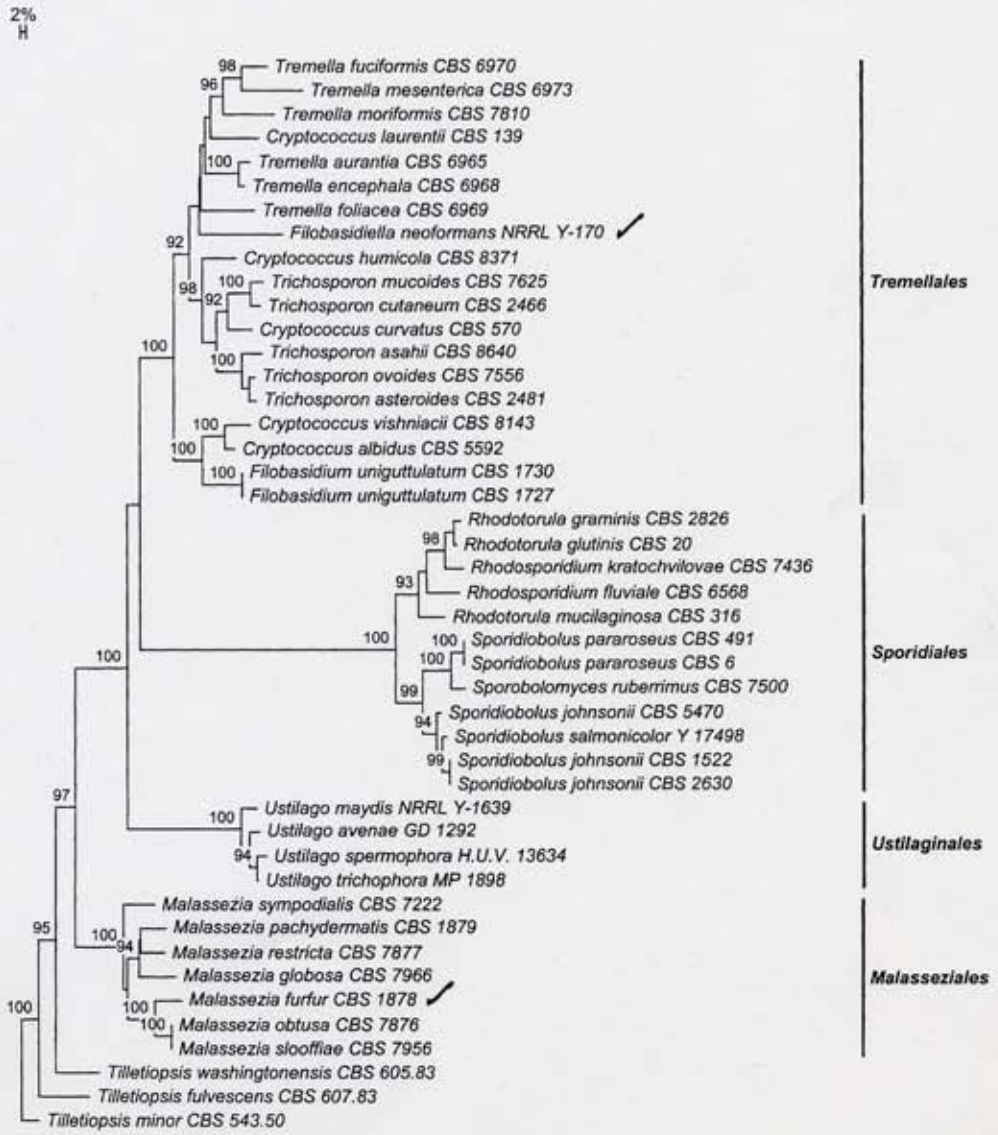


Fig. 18. Phylogenetic tree of basidiomycetous yeasts based on confidently aligned, D1/D2 domains of LSU rDNA, using Neighbor joining algorithm with Kimura correction. Bootstrap values >90 from 100 resampled datasets are shown. *Tilletiopsis minor* was chosen as outgroup. Note that numerous orders of basidiomycetous yeasts are known, which do not contain any clinically significant representatives. Medical fungi are mainly found in two orders, the *Tremellales* and the *Sporidiales*, which are clearly apart from each other. *Malassezia* takes an isolated position and therefore Begerow *et al.* (2000) maintained the order *Malasseziales* for this group.

Table 1.1. An overview of yeast genera[1].

Teleomorphic[2] Ascomycetous Genera (Ascomycotina)	Anamorphic[2] Ascomycetous Genera (Deuteromycotina)	Teleomorphic Heterobasidiomycetous Genera (Basidiomycotina)	Anamorphic Heterobasidiomycetous Genera (Basidiomycotina)
Ambrosiozyma (2 species);	*Aciculoconidium* (1)	*Bulleromyces* (1)	*Bensingtonia* (10);
Arthroascus (4);	*Arxula* (2);	*Chinosphaera* (1);	*Bullera* (14);
Arxiozyma (1);	*Brettanomyces* (3);	*Cystofilobasidium* (4);	*Cryptococcus* (40);
Ascoidea (6);	*Candida* (152);	*Erythrobasidium* (1);	*Fellomyces* (4);
Ashbya (1);	*Kloeckera* (1);	*Filobasidiella* (1);	*Itersonilia* (1);
Botryoascus (1);	*Myxozyma* (9);	*Filobasidium* (5);	*Kockovaella* (2);
Cephaloascus (2);	*Oosporidium* (1);	*Leucosporidium* (3);	*Kurtzmanomyces* (2);
Citeromyces (1);	*Saitoella* (1);	*Kondoa* (1);	*Malassezia* (7);
Clavispora (2);	*Schizoblastosporion* (2);	*Mrakia* (4);	*Phaffia* (1);
Coccidiascus (1);	*Sympodiomyces* (1);	*Rhodosporidium* (9);	*Rhodotorula* (37);
Cyniclomyces (1);	*Trigonopsis* (1)	*Sporidiobolus* (3);	*Sporobolomyces* (27);
Debaryomyces (10);		*Sterigmatosporidium* (1);	*Sterigmatomyces* (2);
Dekkera (2);		*Tilletiaria* (1);	*Sympodiomycopsis* (1);
Eremothecium (2);		*Tremella* and	*Tilletiopsis* (6);
Galactomyces (2)		*Sirobasidium* (12);	*Trichosporon* (20);
Guilliermondella (1);		*Udeniomyces* (3);	*Tsuchiyaea* (1)
Hanseniaspora (6);		*Xanthophyllomyces* (1)	
Hansenula[3] (1);			
Hormoascus (3);			
Hyptopichia (1);			
Issatchenkia (4);			
Kluyveromyces (17);			
Lipomyces (5);			
Lodderomyces (1);			
Metschnikowia (10);			
Nadsonia (3);			
Nematospora (1);			
Pachysolen (1);			
Pichia (87);			
Saccharomyces[4] (16);			
Saccharomycodes (2);			
Saccharomycopsis (6);			
Saturnispora (4);			
Schizosaccharomyces (3);			
Schwanniomyces (1);			
Sporopachydermia (3);			
Stephanoascus (2);			
Torulaspora (3);			
Wickerhamia (1);			
Wickerhamiella (1);			
Williopsis (5);			
Yarrowia (1);			
Zygoascus (1);			
Zygosaccharomyces (9);			
Zygozyma (4)			

[1] Information from Boekhout and Kurtzman (1996) and A. Vaughan-Martini and A. Martini (University of Perugia, personal communication, 1997). The latest (4th) edition of *The Yeasts. A Taxonomic Study* (edited by C.P. Kurtzman and J.W. Fell and published by Elsevier, 1997) was in press at the time of writing.

[2] Teleomorphic and anamorphic refers to, respectively, meiosporic and mitosporic expression of yeast species.

[3] The sole *Hansenula* species is *H. misumaiensis*. Other *Hansenula* species, including *H. polymorpha* which is referred to elsewhere in this book, are not listed above due to the fact that Kurtzman (1984) transferred *Hansenula* species with hat-shaped spores to *Pichia* and those with saturn-shaped spores to *Williopsis*. (Thus, *H. polymorpha* is now *Pichia angusta*.)

[4] *Saccharomyces sensu stricto* have been separated into four species: *S. bayanus*, *S. cerevisiae*, *S. paradoxus* and *S. pastorianus* (see Vaughan-Martini and Martini, 1993).

See Landcover

Table 1 General Morphological and Physiological Differences Between Anamorphs of Ascomycetous and Basidiomycetous Yeasts

	Ascomycetous	Basidiomycetous
Diazonium B blue reaction	No color change	Red color reaction
Cell walls[a]	Two or three layers, lacking xylose, chitin content relatively low, mannan high	Laminar, xylose, galactose, or fucose present,[b] chitin content relatively high, mannan low
G + C	30-60%	49-70+%
Urease reaction	Generally lacking	Usually strong
Coenzyme Q	Q_6-Q_9	Q_8-Q_{10}
Mitosis[a]	Intranuclear usually in neck of bud	Nuclear membrane partially breaks down, bud mitosis
Fermentative metabolism	Often present	Infrequent

[a]Limited number of observations.
[b]Xylose found in Filobasidiaceae; galactose and fucose found in Sporobolomycetaceae.

* many exceptions.

166

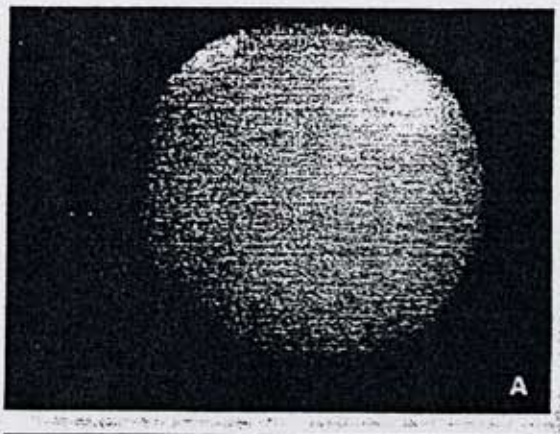
Hemiascomycetes

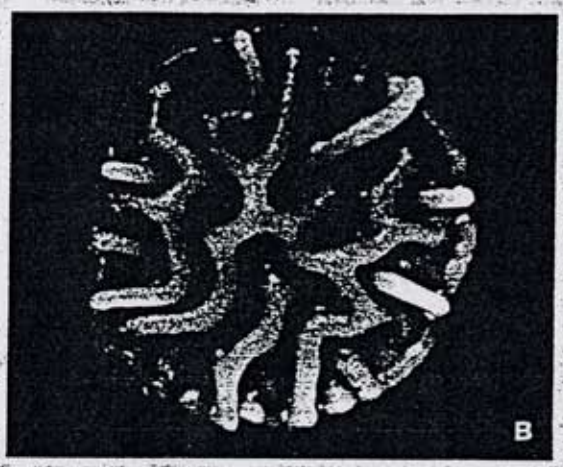
Table 13.3. Teleomorphs of *Candida* Species Isolated from Clinical Specimens

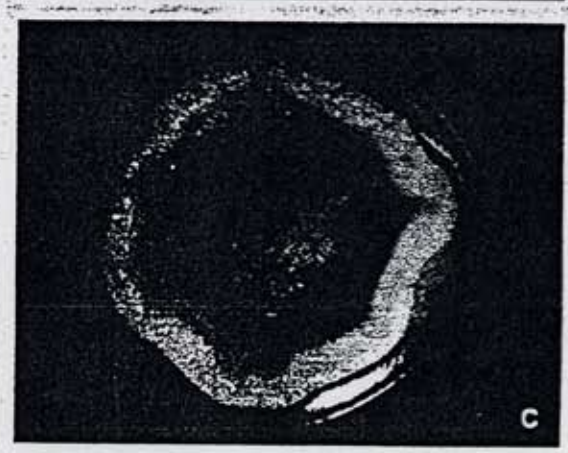
Form-Species	Teleomorph	Sexuality
• *C. albicans*	none	none*
C. famata	*Debaryomyces hansenii*	Homothallic
• *C. glabrata*	none	none
C. guilliermondii var. *guilliermondii*	*Pichia guilliermondii*	Heterothallic
C. guilliermondii var. *membranaefaciens*	*Pichia ohmeri*	Heterothallic
C. haemulonii	none	none
C. kefyr	*Kluyveromyces marxianus*	Heterothallic
• *C. krusei*	*Issatchenkia orientalis*	Heterothallic(?)
C. lusitaniae	*Clavispora lusitaniae*	Heterothallic
C. norvegensis	*Pichia norvegensis*	Homothallic
• *C. parapsilosis*	none	none
• *C. tropicalis*	none	none
C. viswanathii	none	none

• main pathogenes
* depends on definition (see RR Sz 25 about this)

Candida's ability to 'transform' may cause therapeutic difficulties







Cellular changes noted. Shown in (A) is an example of an original smooth colony phenotype, (B) a switch "irregular wrinkle" phenotype, and (C) a "ring" phenotype.

ATLANTA—"It may be that you can't diagnose *Candida* as *Candida* anymore—there are too many different types which emanate from a single cell." At the IX International Congress of the International Society for Human and Animal Mycology, Dr. David Soll reported that *Candida albicans* is capable of "switching" heritability at high frequency between at least seven general phenotypes that can be identified by colony morphology. He stated, "This unusual phenomenon may play a role in the organism's capacity to invade different types of body tissues, evade the immune system, or evade antibody treatment."

The ability to transform into an entirely different "being" prompted Dr. Soll to describe *Candida* as having a "Dr. Jekyll and Mr. Hyde syndrome," since switching is also reversible at high frequency. It should be noted, he added, that switching between the many colony phenotypes is superimposed on the basic capacity of *Candida* dimorphism; for example, even when cells are in different switch phenotypes, they are still capable of growing in the alternative forms of bud and hypha.

According to Dr. Soll, follow-up of the clinical implications is already under way. "We are collaborating with four clinical laboratories, and we're picking up different switch phenotypes in different disease processes." He added, "Many of the switch phenotypes have increased antibiotic resis-

(continued on page 8)

See for example SZ 2 for importance of white-opaque switch

Table I: Human Yeast Pathogens in the Genus *Candida*

Species	Clinical Manifestations
Predominant	
• *C albicans*	Most commonly isolated and considered by some to be most pathogenic. Clinical syndromes variable and include cutaneous, mucocutaneous, CNS, UTI, vaginitis, pulmonary, and generalized or systemic infections.
• *C tropicalis*	Arthritis, bronchopulmonary, endophthalmitis, onychomycosis, peritonitis, systemic, UTI, vaginitis.
Occasional	
• *C parapsilosis*	Endocarditis, otitis externa, paronychia, blood.
• *C krusei*	Rarely endocarditis, fungemia, UTI, vaginitis.
C pseudotropicalis	UTI, vaginitis.
Uncommon and Rare	
C guilliermondii	Dermatologic, endocarditis, onychomycosis, UTI.
C lusitaniae	Mucocutaneous, blood.
C pulcherrima	Systemic
C ravautii	Onychomycosis
C stellatoidea	Endocarditis, vaginitis.
C viswanathii	CNS
C dubliniensis	See RR Sz 19 6

Candida species implicated but not proven as human pathogens include: *brumptii, claussenii, humicola, ingens, intermedia, lipolytica, melinii, mogii, paratropicalis, reukaufii, scottii, solani, utilis, zeylanoides*.

TABLE 20–3. Clinical Manifestations of Chronic Mucocutaneous Candidiasis

Dysgenesis of Thymus
Dysplasia of thymus with agammaglobulinemia (Swiss type)
Dysplasia of thymus without agammaglobulinemia (Nezelof-Allibone syndrome)
Absence of thymus and parathyroid (DiGeorge syndrome)
Polyendocrine Dysfunction
Familial juvenile hypoparathyroidism and hypoadrenocorticism
Thymoma
Defective Immune Responsiveness
No MIF*, no DH (macular lesions)
MIF, no DH, defective phagocytosis (granulomas), ? chronic granulomatous disease of children (myeloperoxidase lacking)
No MIF, no Ca DH, + PPD, DNCB; sp. inhib. *Candida* reaction
Defect, as yet undelineated
Others
Specific antibody against normal serum candida clumping factor

*MIF = migration inhibiting factor; DH = dermal hypersensitivity; Ca = *Candida;* DNCB = dinitrochlorobenzene; PPD = purified protein derivative (tuberculosis).

Rippon page 549

Table 3.1 Summary of clinical groups and/or predisposing factors for invasive candidiasis.

Neutropenia (especially >7 days).
Hematological malignancy.
Solid tumor malignancy.
Postsurgical intensive care patients.
Prolonged intravenous catheterization.
> Broad-spectrum or multiple antibiotic therapy.
Diabetes mellitis.
Parental nutrition.
Severe burns.
Neonates.
Corticosteroid therapy.
Intravenous drug abuse.

AIDS

85 → 95% of all HIV+ patients will develop oral/esophageal candidiasis at some time.

HIV-POSITIVE INDIVIDUALS

CD4+ T-cells		Clinical categories		
		A		
		Asymptomatic, acute (primary) HIV	B	C
No.	%	or PGL	Symptomatic, not (A) or (C)	AIDS
1. ≥500/μl	>28	A1	B1	C1
2. 200-499/μl	14-28	A2	B2	C2
3. <200/μl	<14	A3	B3	C3

PGL, persistent generalized lymphadenopathy.

A. Asymptomatic HIV infection
Persistent generalized lymphadenopathy
Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

B. Candidiasis, oropharyngeal (thrush)
Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
Constitutional symptoms, such as fever (38.5°C) or diarrhea lasting >1 month
Herpes zoster (shingles), involving at least two distinct episodes
Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess
Peripheral neuropathy

C. Candidiasis of bronchi, trachea, or lungs, esophageal
Cervical cancer, invasive
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex: chronic ulcer(s) (>1 month's duration); or bronchitis, pneumonitis, or esophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi's sarcoma
Lymphoma
Mycobacterium avium complex or other species; disseminated or extrapulmonary
Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)
Pneumocystis carinii pneumonia
Pneumonia, recurrent, any cause
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to HIV

See earlier handout #1

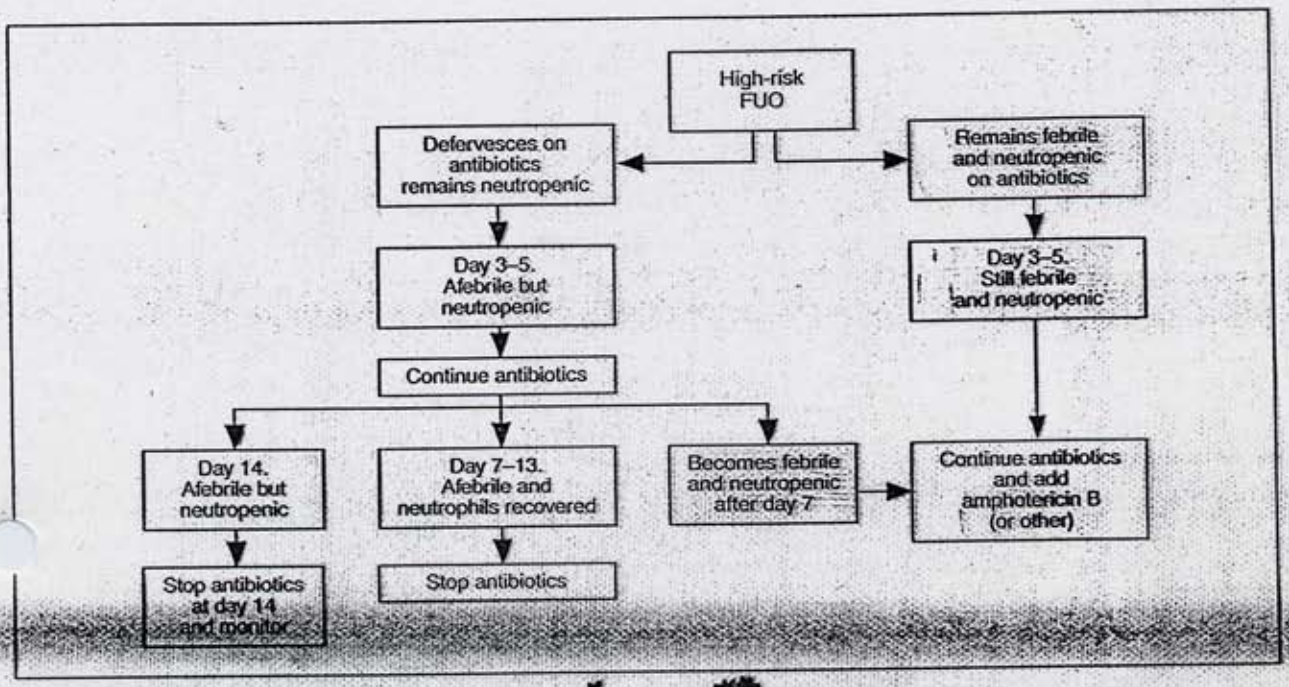


Figure 3. Algorithm for the initial management of febrile* neutropenic** patients. Adapted from [15].

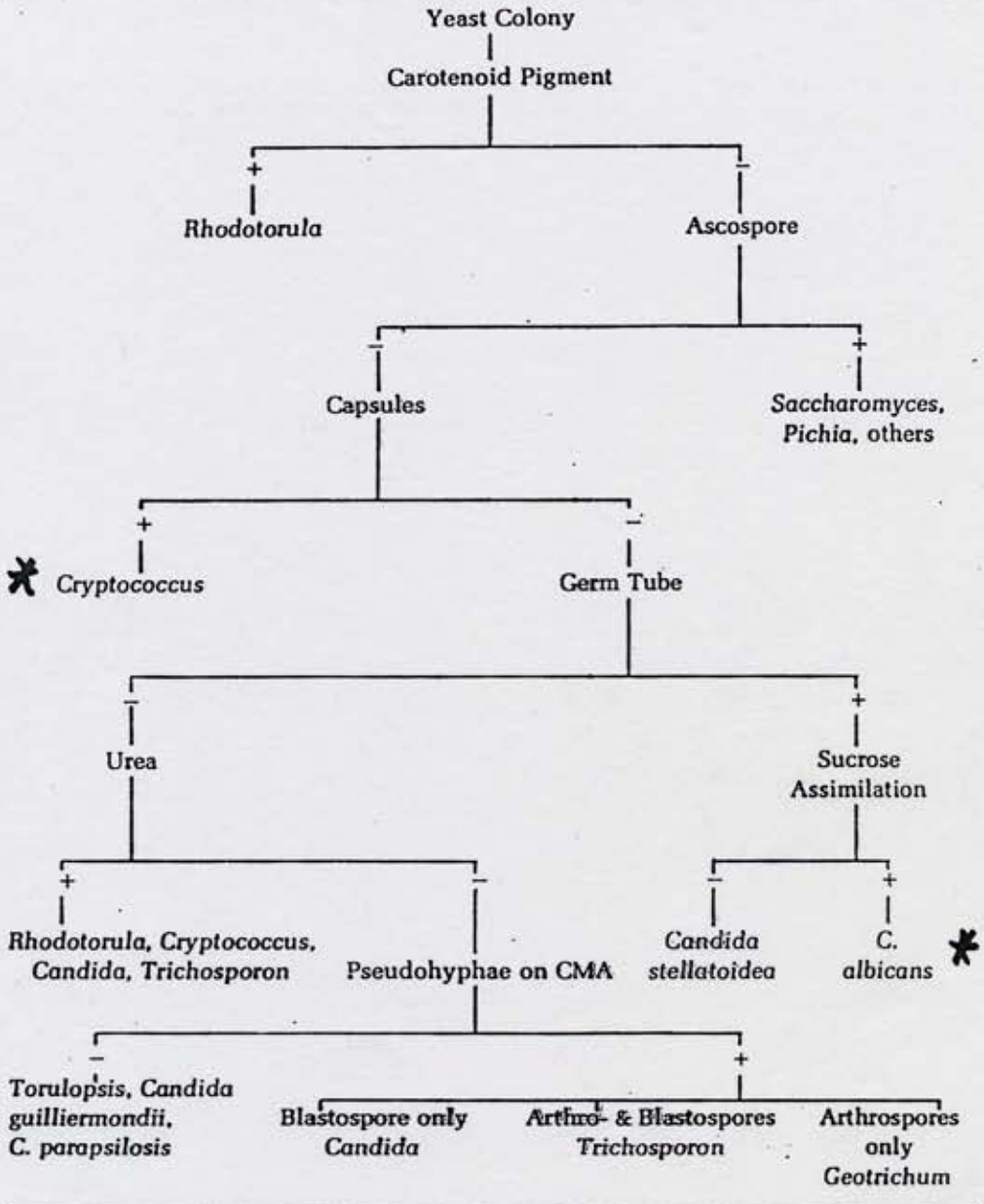
* feverish (often accompanied by increase in pulse and respiration rates)

** low number of neutrophils (neutral dye-staining leukocytes in blood), which should be 40-60% of the white cell count.

See handout

Simplified

Table 12. Yeast Identification Schema



Microbiol. Mol. Biol. Rev 64 (2000) 746-785.

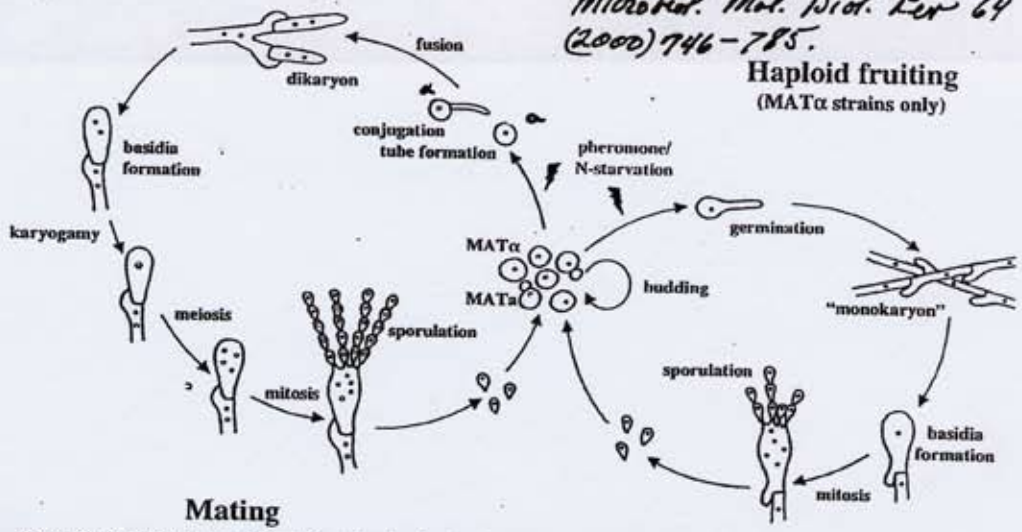


FIG. 9. Life cycle of *C. neoformans*. Mating begins with conjugation tube formation, leading to cell fusion, followed by formation of dikaryotic filaments with fused clamp connections. Terminal basidia form from the tips of mating filaments, where nuclear fusion (karyogamy) occurs. Meiosis and sporulation follow, and long chains of basidiospores are produced, which subsequently germinate into yeast cells. Filamentation independent of a mating partner (haploid fruiting) is observed in MATα strains in response to nitrogen limitation and desiccation.

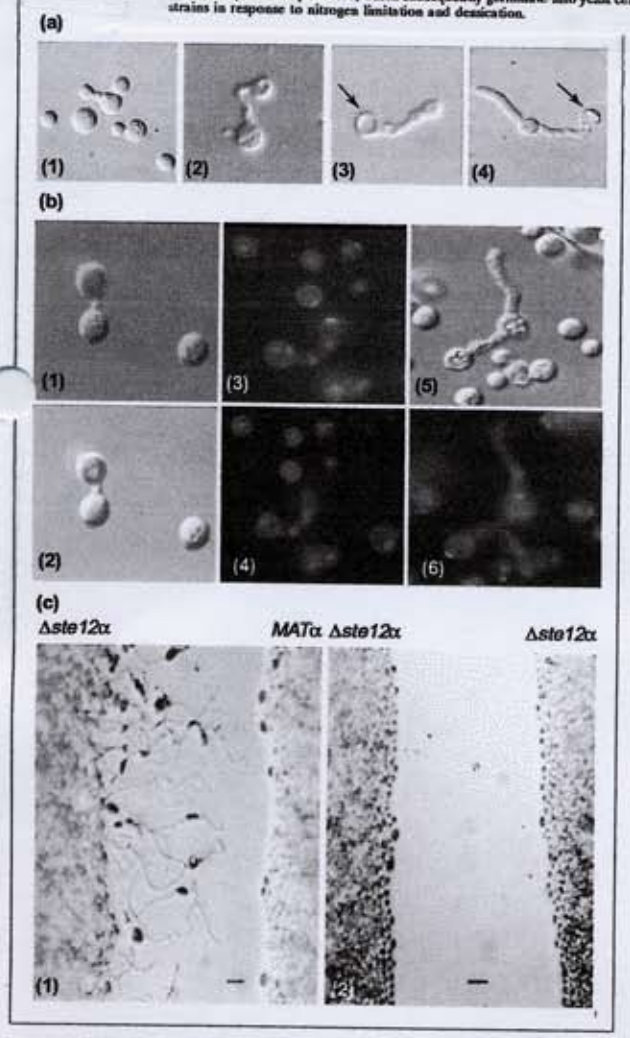


Figure 3. Patterns of interaction between *MATα* and *MATa* cells in *Cryptococcus neoformans* are unique. **(a)** Four different pairs of conjugated cells. (1) Two cells with a short conjugation tube appear unaltered. (2) Cells with a longer conjugation tube. The smaller cell shows signs of vacuolar disposition. (3 and 4) One conjugated cell from each pair appears void of cellular contents (arrows), whereas the presumed recipient forms a hypha. Scale bar represents 2 μm. **(b)** Identification of *MAT* type in conjugated cells. (1 and 2) Overlay of differential interference contrast (DIC) and fluorescence microscopy shows a conjugated pair to be from one of h mating type (*MATa*, red; *MATα*, green). (3 and 4) DAPI staining (blue) reveals t the nucleus from the *MATα* cell (green) migrates into the conjugation tube. (5) DIC and (6) fluorescence microscopy of hyphal production from the *MATa* cell (red) of a conjugated pair. Scale bar represents 2 μm. **(c)** Conjugation tube formation on nitrogen starvation medium (SLAD agar). (1) *MATα* cells lacking the *STE12α* gene produce abundant conjugation tubes (not hyphae) towards *MATa* cells. (2) *STE12α* deletant cells do not produce conjugation tubes in response to cells of the same mating type. Scale bar represents 15 μm. (c) Reproduced, with permission, from Ref. [7].

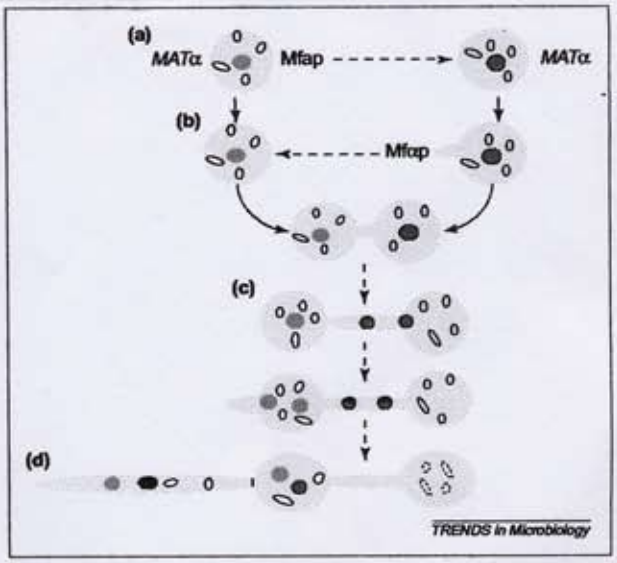


Figure 4. The sequence of events leading to conjugation and hyphal formation in *Cryptococcus neoformans*. **(a)** *MATa* cells secrete *MFa* pheromone (MFap) when exposed to nitrogen starvation conditions, such as V-8 medium. **(b)** The *MATα* cells respond by producing *MFα* pheromone (MFα p) and forming a conjugation tube. The length of the conjugation tube is determined by the proximity of mating-competent *MATα* and *MATa* cells. The cells then fuse. **(c)** The nucleus of the conjugated *MATα* cell divides and the sister nucleus migrates into the conjugation tube. At the same time, the *MATa* nucleus divides and the cell initiates hyphal formation. The *MATα* mother nucleus also migrates into the conjugation tube. **(d)** *MATα*- and *MATa*-type nuclei migrate into the hyphae produced by the *MATa* cell. The *MATa* cell contributes almost all mitochondria present in the hyphae. *MATα*-specific mitochondria are diluted out or destroyed. A septum is formed separating the hyphae from the *MATa* cell.

See Sz 27 for details

Original article

© 2005 Blackwell Publishing Ltd • *Mycoses*, **48**, 122–125

Cryptococcosis: the 1981–2000 epidemic

Gary D. Friedman,[1] W. Jeffrey Fessel,[2] Natalia V. Udaltsova[1] and Leo B. Hurley[1]

[1]Division of Research and [2]San Francisco Medical Center, Kaiser Permanente Medical Care Program, Oakland, CA, USA

Summary

1981 ~ start of AIDS Epidemic: 1995 ~ start of HAART.

The annual incidence of cryptococcosis during 1981–2000 was determined in subscribers of a large integrated health care program in Northern California and in those among them who were HIV positive. The incidence of cryptococcosis had been measured in this setting in the previous decade. The 20-year incidence per million person-years was 19.0 in males and 2.6 in females. In males, annual incidence rose sharply but irregularly from 1981 to 1992, then decreased irregularly. In females, trends were less marked, with maximum incidence in 1997. In HIV-positive patients cryptococcosis incidence was highest in 1981–85 and decreased thereafter in men. In women, maximum incidence occurred in 1986–90 and was followed by a decrease. Cryptococcosis was rare in the non-predisposed. Thus, cryptococcosis incidence increased markedly in men early in the AIDS epidemic, and began to decrease in both male and female HIV-positive patients well before highly active antiretroviral therapy became available.

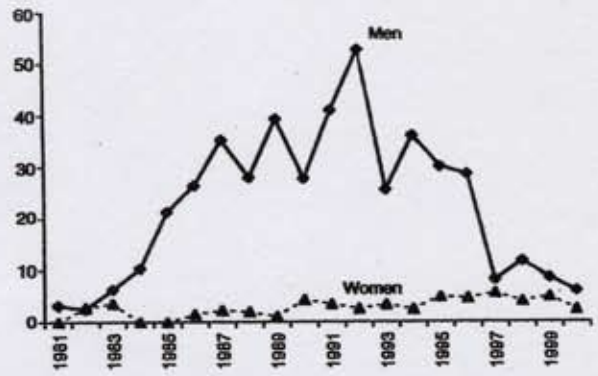


Figure 1 Annual incidence of cryptococcosis per million person-years in males and females by year. Kaiser Permanente Medical Care Program subscribers in Northern California, 1981–2000. Age-adjusted by the direct method to the 1990 subscriber population.

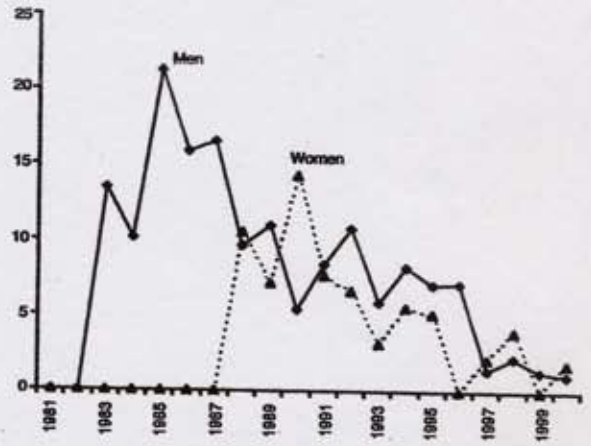


Figure 2 Annual incidence of cryptococcosis per thousand person-years in men and women with HIV infection, by year. Kaiser Permanente Medical Care Program subscribers in Northern California, 1981–2000.

Table 1 Incidence of cryptococcosis per million person-years, by age. Kaiser Permanente Medical Care Program subscribers in Northern California, 1981–2000.

Age group (years)	<20	20–29	30–39	40–49	50–59	60–69	70+
Males	0.5	11.4	41.0	38.9	21.9	17.6	11.9
Females	0.1	1.3	2.5	2.6	3.3	6.5	10.2

Table 4. Variables associated with death in patients with central nervous system *Cryptococcus neoformans* infection

Variable (no. of patients for whom data available)	Death (%)	Survival (%)	p value
Mean age in yrs	40.6	42.4	NS[a]
Fever (29)	34 (10/29)	66 (10/29)	NS
No fever (7)	43 (3/7)	57 (4/7)	
Headache (20)	25 (5/20)	75 (15/20)	NS
No headache (21)	52 (11/21)	48 (10/21)	(0.09)
Abnormal mental status (20)	55 (11/20)	45 (9/20)	NS
Normal mental status (26)	31 (8/26)	69 (18/26)	
White blood cell >20/mm^3 (20)	40 (8/20)	60 (12/20)	NS
White blood cell <20/mm^3 (13)	62 (8/13)	38 (5/13)	
Cryptococcal antigen titer ≥1,024 (10)	20 (2/10)	80 (8/10)	NS
Cryptococcal antigen titer <1,024 (17)	35 (6/17)	65 (11/17)	
Positive blood culture (8)	13 (1/8)	87 (7/8)	NS
Negative blood culture (16)	50 (8/16)	50 (8/16)	
Renal failure (22)	54 (12/22)	46 (10/22)	0.011
No renal failure (12)	8 (1/12)	92 (11/12)	
Therapy			NS
AmB[b] alone (55)	47 (26/55)	53 (29/55)	
AmB + 5 FC[c] (32)	50 (16/32)	50 (16/32)	
Fluconazole (5)	40 (2/5)	60 (3/5)	

[a]NS = not significant, p >0.05.
[b]AmB = Amphotericin B deoxycholate.
[c]FC = flucytosine.

From EID 7(2001)375-381