Mycology Proficiency Testing Program

Critique

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Test Specimens

A minimum of two strains of each of the proposed mold specimens were examined for inclusion in the proficiency test event of October 2001. The colony morphology of these strains was studied on Sabouraud dextrose agar. The microscopic morphologic features were examined by potato dextrose agar slide cultures. The physiological characteristics, such as cycloheximide sensitivity, and growth at higher temperatures, were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of the proposed fungal pathogen was used in the test. Similarly, two or more strains of each of the proposed yeast pathogens were examined for inclusion in the proficiency test. The morphology of all yeast isolates was studied on Cornmeal - Tween 80 agar plates inoculated by the Dalmau or streak-cut method. Carbohydrate assimilation was studied with the API 20C AUX identification kit. The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, were also investigated. Additionally, physiologic characteristics, such as nitrate assimilation, urease activity, and cycloheximide sensitivity, were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of each of the proposed yeast pathogens was used in the test.

Grading Policy

A laboratory's response for each sample is compared with the response that reflects 90 percent agreement of 10 referee laboratories or 90 percent of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The grading formula used for each specimen is:

$$\frac{\text{\# of correct responses}}{\text{\# of fungi present} + \text{\# incorrect responses}} \times 100$$

Participating laboratories must achieve a score of 80% or better on two (2) of three (3) consecutive test events to maintain acceptable proficiency levels.

Answer Key

Mycology - General

	Specimen Key	Validated Specimen	Acceptable Answers
M-1	*Epidermophyton floccosum*	*Epidermophyton floccosum*	
M-2	*Microsporum canis*	*Microsporum canis*	
M-3	*Fusarium* species	*Fusarium* species	
M-4	*Trichophyton mentagrophytes*	*Trichophyton mentagrophytes*	
M-5	*Trichophyton rubrum*	*Trichophyton rubrum*	
Ed.Sp.	*Microsporum audouinii*		

Mycology - Yeast Only

	Specimen Key	Validated Specimen	Acceptable Answers
Y-1	*Candida guilliermondii*	*Candida guilliermondii*	*Pichia guilliermondii*
Y-2	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa*	*Rhodotorula rubra*
Y-3	*Candida tropicalis*	*Candida tropicalis*	
Y-4	*Cryptococcus albidus*	*Cryptococcus albidus*	
Y-5	*Sporobolomyces salmonicolor*	*Sporobolomyces salmonicolor*	
Ed.Sp.	*Pichia anomala*		*Hansenula anomala* *Candida pelliculosa*

Laboratory Results

Mycology - General

		No. Labs responding/ Total Labs (%)	referee labs/total (%)
M- 1	*Epidermophyton floccosum*	77/78 (99%)	10/10 (100%)
M- 2	*Microsporum canis*	66/78 (85%)	10/10 (100%)
M- 3	*Fusarium* species	77/78 (99%)	10/10 (100%)
M- 4	*Trichophyton mentagrophytes*	73/78 (94%)	10/10 (100%)
M- 5	*Trichophyton rubrum*	76/78 (97%)	10/10 (100%)

Mycology - Yeast Only

		No. Labs responding/ Total Labs (%)	referee labs/total (%)
Y - 1	*Candida guilliermondii*	66/70 (94%)	10/10 (100%)
Y - 2	*Rhodotorula mucilaginosa*	66/70 (94%)	10/10 (100%)
Y - 3	*Candida tropicalis*	70/70 (100%)	10/10 (100%)
Y - 4	*Cryptococcus albidus*	70/70 (100%)	10/10 (100%)
Y - 5	*Sporobolomyces salmonicolor*	69/70 (99%)	10/10 (100%)

Test Statistics

Mycology - General

Number of participating laboratories	78
Number of referee laboratories	10
Number of laboratories responding by deadline	78
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	75
Number of laboratories unsuccessfully completing this test	3

Mycology - Yeast Only

Number of participating laboratories	70
Number of referee laboratories	10
Number of laboratories responding by deadline	70
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	70
Number of laboratories unsuccessfully completing this test	0

Commercial Identification Systems Used*

AMS Vitek system	29
API 20C	26
API 20C AUX	12
Microscan	03
Remel Uni-Yeast-Tek	04
Other	N/A

(* Includes multiple systems used by some labs)

Source: Skin Scrapings

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	77
Labs with incorrect ID:	1
(Trichophyton tonsurans)	(1)

Clinical Significance: A frequent casual agent of nail and skin infections in feet and groin. Unlike many other dermatophytes, *E. floccosum* does not infect hair. There is one case report on invasive disease in immunocompromised patient with Behcet's syndrome.

Ecology: Anthropophilic (associated with humans), found world wide.

Laboratory Diagnosis:

1. Culture – *E. floccosum* is a slow-growing fungus. Colonies measure up to 3 cm in 2 weeks. At 25^0 C, on Sabouraud's dextrose agar, colonies are initially white to yellow, later becoming greenish-yellow, folded in center and radially grooved, reverse turning tan (Fig. 1).
2. Microscopic morphology – Lactophenol Cotton Blue or Calcofluor mounts show septate hyphae; macroconidia single or in clusters, smooth, thin-walled, club-shaped, with 2-6 septations (Fig. 2). No microconidia are seen. Chlamydoconidia are present in old cultures.
3. Differentiation from other dermatophytes – *E. floccosum* is differentiated from other fungi by slow growth, absence of microconidia, and club-shaped macroconidia. The second species in this genus, *E. stockdaleae,* has been isolated from soil. It is differentiated from *E. floccosum* by its production of longer conidia with nine septations.
4. Molecular tests – ITS1 sequences of clinical isolates are species-specific (5). Specific DNA bands in arbitrarily primed polymerase chain reaction (AP – PCR) also provide rapid identification of dermatophytes including *E. floccosum* (4, 6).
5. *In vitro* susceptibility testing – Most clinical isolates are susceptible to terbinafine and variably to griseofulvin, itraconazole, ketoconazole, and clotrimazole (1).

Comments: One lab reported this organism as *T. tonsurans* based upon teardrop-shaped microconidia and intercalary chlamydoconidia. However, *E. floccosum* does not produce any microconidia.

Further reading:

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3. **Korting, H. C., and S. Rosenkranz.** 1990. In vitro susceptibility of dermatophytes from Munich to griseofulvin, miconazole and ketoconazole. Mycoses. **33**(3): 136-9.
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6. **Mochizuki, T., N. Sugie, and M. Uehara.** 1997. Random amplification of polymorphic DNA is useful for the differentiation of several anthropophilic dermatophytes. Mycoses. **40**(11-12): 405-9.
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8. **Weitzman, I., N. X. Chin, N. Kunjukunju, and P. Della-Latta.** 1998. A survey of dermatophytes isolated from human patients in the United States from 1993 to 1995. J Am Acad Dermatol. **39**(2 Pt 1): 255-61.

M-2 Microsporum canis

Source: Skin

Scoring:	No. Labs		
Referee labs with correct ID:	10		
Labs with correct ID:	66		
Labs with incorrect ID:	12		
(Chrysosporium species)	(1)	*(Trichophyton mentagrophytes)*	(1)
(Microsporum audouinii)	(2)	*(Trichophyton rubrum)*	(1)
(Microsporum ferrugineum)	(2)	*(Trichophyton terrestre)*	(2)
(Microsporum gypseum)	(1)	*(Trichophyton tonsurans)*	(1)
(Trichophyton ajelloi)	(1)		

Clinical Significance: A frequent casual agent of scalp and skin infections, most commonly in children. It rarely causes infection of the nail (6). Humans acquire infection through dogs or cats harboring this organism (zoophilic).

Ecology: Cosmopolitan, isolated from dogs and cats.

Laboratory Diagnosis:

1. Culture – *M. canis* is a moderately fast-growing fungus. Colonies measure up to 3 cm in 5-7 days. At 25^0 C, on Sabouraud's dextrose agar, colonies are yellowish-white, wooly, with yellow-orange reverse (Fig.3).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae with macroconidia and microconidia. The macroconidia are fusoid, thick-walled, with curved apex and up to 15 septations (Fig. 4). The microconidia are club–shaped.
3. Differentiation from other dermatophytes – *M. canis* can be differentiated from other dermatophytes on the basis of macroscopic and microscopic features, notably yellowish colonies with yellowish-orange reverse and thick-walled macroconidia containing up to 15 cells.
4. Molecular tests – Identification of *M. canis* by the random amplification of polymorphic DNA (RAPD) method and Southern hybridzation was reported by Kano and coworkers (3). Liu and coworkers developed specific PCR for identification of *M. canis* (5). Kano and coworkers reported detection of *M.canis* in skin scrappings of dogs based on sequences of chitin synthase 1 gene (2).
5. *In vitro* susceptibility testing – Susceptibility testing results indicate that most *M. canis* isolates are susceptible to terbinafine and itraconazole (1).

Comments: This isolate produced abundant microconidia, but few spindle–shaped macroconidia. Many labs incorrectly identified this isolate in the absence of characteristic macroconidia. Sporulation is usually lost when the cultures are maintained on agar media for long time, but it is regained by subcultures on media that induce sporulation, e.g., potato dextrose agar or cereal agar.

Further reading:

1. **Jessup, C. J., N. S. Ryder, and M. A. Ghannoum.** 2000. An evaluation of the in vitro activity of terbinafine. Med Mycol. **38**(2):155-9.
2. **Kano, R., Y. Nakamura, S. Watanabe, and A. Hasegawa.** 2000. Detection of *Microsporum canis* in the skin scrapings and hairs of dogs with dermatophytosis based on sequences of the chitin synthase 1 gene. Microbiol Immunol. **44**(7):605-7.
3. **Kano, R., Y. Nakamura, T. Watari, S. Watanabe, H. Takahashi, H. Tsujimoto, and A. Hasegawa.** 1998. Identification of clinical isolates of *Microsporum canis* and *M. gypseum* by random amplification of polymorphic DNA (RAPD) and Southern hybridization analyses. Mycoses. **41**(3-4):139-43.
4. **King, D., L. W. Cheever, A. Hood, T. D. Horn, M. G. Rinaldi, and W. G. Merz.** 1996. Primary invasive cutaneous *Microsporum canis* infections in immunocompromised patients. J Clin Microbiol. **34**(2):460-2.
5. **Liu, D., L. Pearce, G. Lilley, S. Coloe, R. Baird, and J. Pedersen.** 2001. A specific PCR assay for the dermatophyte fungus *Microsporum canis*. Med Mycol. **39**(2):215-9.
6. **Romano, C., E. Paccagnini, and L. Pelliccia.** 2001. Case report. Onychomycosis due to *Microsporum canis*. Mycoses. **44**(3-4):119-20.

M-3 *Fusarium* species

Source: Nails

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	77
Labs with incorrect ID:	1
(Microsporum nanum)	(1)

Clinical Significance: A frequent casual agent of keratitis, endophthalmitis, and onychomycosis in healthy individuals. It has been reported from peritonitis and disseminated infection in immunocompromised patients.

Ecology: Cosmopolitan in soil and plants. Some species of *Fusarium* are major plant pathogens.

Laboratory Diagnosis:

1. Culture – *Fusarium* is a fast-growing fungus. Colonies measure up to 5 cm in 5 days. At 25^0 C, on Sabouraud's dextrose agar, colonies are rapid-growing, pink–purple in color, wooly, with red–violet reverse (Fig.5).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts show septate hyphae, with short or long phialides. Microconidia are ovoid, and macroconidia are septated and curved-boat/banana-shaped (Fig. 6). Chlamydospores may be present.
3. Differentiation from other molds – *Fusarium* species produces curved, septate macroconidia along with single-cell microconidia, which distinguish them from other hyphomycetes, especially *Acremonium* species.
4. Molecular tests – Hue and coworkers (3) described a PCR method for rapid detection and identification of *Fusarium* species from culture and clinical samples. Jaeger and coworkers (4) used pan-fungal PCR, followed by nested PCR with species–specific primers, for rapid detection of *Fusarium* DNA in ocular samples.
5. *In vitro* susceptibility testing – Most clinical isolates are susceptible to amphotericin B. Some isolates are variably susceptible to azoles (7).

Comments: One lab reported this organism as *Microsporum nanum* based upon rough,thin-walled, egg-shaped macroconidia with 1-3 septations, and smooth-walled, club-shaped microconidia. However, these features are not typical of *Fusarium* species sent in this testing event.

Further reading:

1. **Guarro, J., M. Nucci, T. Akiti, J. Gene, M. D. Barreiro, and R. T. Goncalves.** 2000. Fungemia due to *Fusarium sacchari* in an immunosuppressed patient. J Clin Microbiol. **38**(1):419-21.
2. **Guarro, J., M. Nucci, T. Akiti, and J. Gene.** 2000. Mixed infection caused by two species of *Fusarium* in a human immunodeficiency virus-positive patient. J Clin Microbiol. **38**(9): 3460-2.
3. **Guarro, J., and J. Gene.** 1995. Opportunistic fusarial infections in humans. Eur J Clin Microbiol Infect Dis. **14**(9): 741-54.
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7. **Petrikkou, E., J. L. Rodriguez-Tudela, M. Cuenca-Estrella, A. Gomez, A. Molleja, and E. Mellado.** 2001. Inoculum standardization for antifungal susceptibility testing of filamentous fungi pathogenic for humans. J Clin Microbiol. **39**(4): 1345-7.

M-4 *Trichophyton mentagrophytes*

Source: Hair

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	73
Labs with incorrect ID:	5
(*Trichophyton terrestre*)	(2)
(*Trichophyton tonsurans*)	(3)

Clinical Significance: A frequent casual agent of skin, nail, and hair infections. Anthropophilic isolates, i.e., *T. mentagrophytes* var. *interdigitale,* cause chronic infection of nails, feet, and groin, while zoophilic isolates, i.e. *T. mentagrophytes* var. *mentagrophytes,* cause infection of skin, scalp, and beard.

Ecology: Cosmopolitan; zoophilic isolates are present in rodents, rabbits, and small animals.

Laboratory Diagnosis:

1. Culture – *T. mentagrophytes* is a fast-growing fungus. Colonies measure up to 2 cm in 5 days. At 25^0 C, on Sabouraud's dextrose agar, colonies grow rapidly in 3–5 days, buff, cream-colored, powdery, with tan reverse (Fig.7).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae with macro- and micro-conidia. Macroconidia are thin–walled, slender, with 5–6 septations. Microconidia are round, clustered on conidiophores (Fig. 8). Spiral hyphae are often seen.
3. Differentiation from other dermatophytes – Microscopically, *T. mentagrophytes* is differentiated from *T. rubrum* by its round conidia produced in clusters, and the presence of spiral hyphae. *T. mentagrophytes* is urease-positive, hair perforation-positive, and has no specific growth requirements. It is differentiated from *M. persicolor* by alkaline reaction on BCP–milk–glucose agar. It is differentiated from *T. terrestre* by good growth at 37^0 C.
4. Molecular tests – ITS1 sequences of clinical isolates are species-specific (7). Species-specific primers of chitin synthase 1 gene have been used to differentiate the *Trichophyton mentagrophytes* complex in specimens from humans and animals (6). The random amplified polymorphic DNA (RAPD) method has been used to study the genetic diversity of clinical isolates (5).
5. *In vitro* susceptibility testing – Susceptibility testing using the NCCLS protocol (M38 – P) indicated that common clinical isolates are susceptible to terbinafine and itraconazole (1).

Comments: Some labs reported this organism as *T. tonsurans* on the basis of tear-drop or club-shaped microconidia, balloon forms, smooth-walled, and sinuous macroconidia, and the requirement of thiamine for growth. Other labs misidentified this isolate as *T. terrestre* on the basis of club-shaped microconidia and no growth at 37^0 C. However, these features are not typical of *T. mentagrophytes* sent in this testing.

Further reading:

1. **Fernandez-Torres, B., A. J. Carrillo, E. Martin, A. Del Palacio, M. K. Moore, A. Valverde, M. Serrano, and J. Guarro.** 2001. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrob Agents Chemother. **45**(9): 2524-8.
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4. **Kano, R., Y. Nakamura, T. Watari, S. Watanabe, H. Takahashi, H. Tsujimoto, and A. Hasegawa.** 1998. Molecular analysis of chitin synthase 1 (CHS1) gene sequences of *Trichophyton mentagrophytes* complex and *T. rubrum*. Curr Microbiol. **37**(4):236-9.
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6. **Makimura, K., Y. Tamura, T. Mochizuki, A. Hasegawa, Y. Tajiri, R. Hanazawa, K. Uchida, H. Saito, and H. Yamaguchi.** 1999. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Clin Microbiol. **37**(4):920-4.
7. **Sommer, S., R. C. Barton, S. M. Wilkinson, W. J. Merchant, E. G. Evans, and M. K. Moore.** 1999. Microbiological and molecular diagnosis of deep localized cutaneous infection with *Trichophyton mentagrophytes*. Br J Dermatol. **141**(2):323-5.

M-5 Trichophyton rubrum

Source: Toes

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	76
Labs with incorrect ID:	2
(Trichophyton mentagrophytes)	(1)
(Trichophyton tonsurans)	(1)

Clinical Significance: A frequent causal agent of infections of the feet, toes, groin, finger nails, and skin. It rarely causes infection of scalp or hair. Most common dermatophytic pathogen in humans.

Ecology: Cosmopolitan, anthropophilic.

Laboratory Diagnosis:

1. Culture – *T. rubrum* is a fast-growing fungus. Colonies measure up to 5 cm in 5 days. At 25^0 C, on Sabouraud's dextrose agar, colonies are fluffy to powdery, white to buff, with wine–red to brown in color (Fig.9).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae, microconidia: teardrop-shaped, solitary along the hyphae; macroconidia are pencil-shaped, multiseptate, and rarely seen (Fig. 10). Some strains form arthroconidia.
3. Differentiation from other dermatophytes – *T. rubrum* can be differentiated from *T. mentagrophytes* by teardrop-shaped solitary microconidia, no urease negativity, no hair perforation, and no specific growth requirements. It is differentiated from *T. terrestre* by good growth at 37^0 C, and on potato-glucose agar or cornmeal glucose agar, *T. rubrum* produces red pigment on reverse. Three new species, *T. fischeri, T. raubitschekii, and T. kanei* were described to be closely related to *T. rubrum*. Two of these species, *T. raubitschekii* and *T. kanei,* have been isolated from skin lesions. *T. raubitschekii* and *T. kanei* form urease enzyme in broth medium; *T. kanei* lacks microconidia, while *T. raubitschekii* produces variably shaped microconidia; these characters serve to differentiate these two species from *T. rubrum*. *T. fischeri* resembles *T. rubrum* closely, but is non-pathogenic for humans.
4. Molecular tests – A species–specific DNA probe using highly variable internal transcribed spacer 2 region of the ribosomal DNA (ITS2) was developed to detect *T. rubrum* in culture and from clinical samples (1). Species identification of dermatophytes was done based on DNA sequences of nuclear ribosomal internal transcribed spacer regions (ITS), and of the 5.8S ribosomal DNA region, and comparison with DNA sequence database (4).
5. *In vitro* susceptibility testing – *T. rubrum* are highly susceptible to terbinafine and variably to azoles (2).

Comments: One lab reported this fungus as *T. tonsurans* on the basis of varying shapes and sizes of microconidia, balloon forms, and requirement of thiamine for growth. Another lab reported this organism as *T. mentagrophytes* on the basis of teardrop microconidia in clusters. However, these features are not typical of *T. rubrum* sent in this testing.

Further reading:

1. **El Fari, M., H. J. Tietz, W. Presber, W. Sterry, and Y. Graser.** 1999. Development of an oligonucleotide probe specific for *Trichophyton rubrum*. Br J Dermatol. **141**(2): 240-5.
2. **Fernandez-Torres, B., H. Vazquez-Veiga, X. Llovo, M. Pereiro, Jr., and J. Guarro.** 2000. In vitro susceptibility to itraconazole, clotrimazole, ketoconazole and terbinafine of 100 isolates of *Trichophyton rubrum*. Chemotherapy. **46**(6): 390-4.
3. **Kornbleuth, S., and S. Hsu.** 1999. White superficial onychomycosis of the fingernail caused by *Trichophyton rubrum* in an immunocompetent patient. Cutis. **64**(2): 99-100.
4. **Makimura, K., Y. Tamura, T. Mochizuki, A. Hasegawa, Y. Tajiri, R. Hanazawa, K. Uchida, H. Saito, and H. Yamaguchi.** 1999. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Clin Microbiol. **37**(4):920-4.
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6. **Summerbell, R. C., S. A. Rosenthal, and J. Kane.** 1988. Rapid method for differentiation of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and related dermatophyte species. J Clin Microbiol. **26**(11): 2279-82.

Source: Scalp

Clinical Significance: A causal agent of scalp and skin infections in young children. It rarely afflicts adults.

Ecology: Cosmopolitan, anthropophilic. A decade ago, this fungus was the commonest cause of tinea capitis in North America but it is now seen mostly in Africa and South America.

Laboratory Diagnosis:

1. Culture – At 25^0 C, on Sabouraud's dextrose agar, colonies grow rapidly in 3 –7 days; downy, white to gray in color on the surface, with pale pink to salmon reverse (Fig.11).
2. Microscopic morphology – Lactophenol cotton blue or Calcofluor mounts show hyaline septate hyphae. Macroconidia and microconidia are rarely produced. Generally, sterile and pectinate hyphae are seen, with terminal or intercalary chlamydospores (Fig. 12).
3. Differentiation from other dermatophytes – It is differentiated from other *Microsporum* species by its production of brownish pigment on autoclaved rice grain. It does not perforate hair, and has no specific growth requirements.
4. Molecular tests – Species identification of dermatophytes was done based on DNA sequences of nuclear ribosomal internal transcribed spacer regions and of the 5.8S ribosomal DNA region, and comparison with DNA sequence database (2).
5. *In vitro* susceptibility testing – Clinical isolates of *M. audouinii* were susceptible to various antifungal agents, but they have high MIC to fluconazole (1).

Further reading:

1. **Fernandez-Torres, B., A. J. Carrillo, E. Martin, A. Del Palacio, M. K. Moore, A. Valverde, M. Serrano, and J. Guarro.** 2001. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrob Agents Chemother. **45**(9):2524-8.
2. **Graser, Y., A. F. Kuijpers, M. El Fari, W. Presber, and G. S. de Hoog.** 2000. Molecular and conventional taxonomy of the *Microsporum canis* complex. Med Mycol. **38**(2):143-53.
3. **Gupta, A. K., and R. C. Summerbell.** 1998. Increased incidence of *Trichophyton tonsurans* tinea capitis in Ontario, Canada between 1985 and 1996. Med Mycol. **36**(2):55-60.
4. **Liu, D., S. Coloe, R. Baird, and J. Pedersen.** 2000. Application of PCR to the identification of dermatophyte fungi. J Med Microbiol. **49**(6):493-7.

Y-1 *Candida guilliermondii*

Source: Blood

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	66
Labs with incorrect ID:	4
(Candida famata)	(4)

Clinical Significance: A frequent causal agent of nosocomial fungemia in immunosuppressed patient. Also, infrequent casual agent of urinary tract infections, brain abscess, and ocular infections.

Epidemiology: Cosmopolitan in distribution.

Laboratory Diagnosis:

1. Culture – At 25⁰ C, on Sabouraud's dextrose agar, colonies are flat, smooth, cream-yellow in 3 days (Fig. 13).
2. Microscopic morphology – On cornmeal agar with Tween 80, few short pseudohyphae with clusters of blastoconidia (Fig. 14).
3. Differentiation from other yeasts – *Candida guilliermondii* ferments glucose, sucrose, and trehalose, grows at 37⁰ C, and grows on media containing cycloheximide. It does not form pink pigment thereby differentiating from *Rhodotorula* species. It does not produce true hyphae, which differentiates it from *Candida ciferrii* and *Trichosporon beigelii*. Unlike *C. lusitaniae,* it is unable to grow at 45⁰ C.
4. Molecular tests – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate *C. guilliermondii* from *C. famata/Debaryomyces hansenii* complex (4). Isolates of *C. guilliermondii* were identified using PCR to amplify ribosomal DNA, followed by restriction digestion of the PCR product (3).
5. *In vitro* susceptibility testing – Most clinical isolates are susceptible to amphotericin B, 5 FC, and azoles such as fluconazole, itraconazole, and ketoconazole; a few isolates are reported to have high MIC to azoles (1, 6).

Comments: Four labs reported this isolate as *C. famata*, probably beacuse in the API 20C AUX yeast identification system, *C. guilliermondii* and *C. famata* are assigned the same biocode. However, *C. guilliermondii* assimilates melezitose and raffinose frequently (90%), while *Candida famata* assimilates the two carbohydrates infrequently (60%).

Further Reading:

1. **Barchiesi, F., A. M. Tortorano, L. F. Di Francesco, M. Cogliati, G. Scalise, and M. A. Viviani.** 1999. In-vitro activity of five antifungal agents against uncommon clinical isolates of *Candida* spp. J Antimicrob Chemother. **43**(2): 295-9.
2. **Mardani, M., H. A. Hanna, E. Girgawy, and I. Raad.** 2000. Nosocomial *Candida guilliermondii* fungemia in cancer patients. Infect Control Hosp Epidemiol. **21**(5): 336-7.
3. **Mason, M. M., B. A. Lasker, and W. S. Riggsby.** 1987. Molecular probe for identification of medically important *Candida* species and *Torulopsis glabrata*. J Clin Microbiol. **25**(3): 563-6.
4. **Nishikawa, A., T. Sugita, and T. Shinoda.** 1997. Differentiation between *Debaryomyces hansenii/Candida famata* complex and *Candida guilliermondii* by polymerase chain reaction. FEMS Immunol Med Microbiol. **19**(2): 125-9.
5. **Tietz, H. J., V. Czaika, and W. Sterry.** 1999. Case report. Osteomyelitis caused by high resistant *Candida guilliermondii*. Mycoses. **42**(9-10):577-80.
6. **Williams, D. W., M. J. Wilson, M. A. Lewis, and A. J. Potts.** 1995. Identification of *Candida* species by PCR and restriction fragment length polymorphism analysis of intergenic spacer regions of ribosomal DNA. J Clin Microbiol. **33**(9): 2476-9.

Y-2 Rhodotorula mucilaginosa

Source: Skin

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	66
Labs with incorrect ID:	4
(Rhodotorula glutinis)	(4)

Clinical Significance: A not very common casual agent of catheter-associated fungemia, dialysis-related peritonitis, and post-surgery ventriculitis, endocarditis and meningitis.

Ecology: Cosmopolitan

Laboratory Diagnosis:

1. Culture – At 25^0 C, colonies on Sabouraud's dextrose agar are smooth, moist, soft, pink to coral red, in 2 days (Fig. 15).
2. Microscopic morphology – On cornmeal agar with Tween 80, oval to round yeast cells, sometimes in short chains (Fig. 16). Rarely a faint capsule and rudimentary pseudohyphae are seen.
3. Differentiation from other yeasts – *R. mucilaginosa* does not ferment any carbohydrate, grows at 37^0 C, and does not grow on media containing cycloheximide. It forms pink pigment, thereby differentiating it from other yeast species. It does not produce ballistoconidia, thus differing from *Sporobolomyces* species. *R. mucilaginosa* does not assimilate nitrate or nitrite, which distinguishes it from *R. glutinis*.
4. Molecular tests – Using species-specific oligonucleotide primers for PCR, identification of the basidiomycetous yeasts *Cr. neoformans, T. cutaneum,* and *R. mucilaginosa* were done from single and mixed yeast populations. The cytochrome b sequences were used to identify various genera and species, and phylogenetic relationships among basidiomycetous yearts (1).
5. *In vitro* susceptibility testing – *Rhodotorula mucilaginosa* is susceptible to amphotericin B and 5FC, variably susceptible to itraconazole, and resistant to fluconazole (6).

Comments: Four labs reported this isolate as *R. glutinis* because of positive nitrate assimilation. All referee labs and a majority of participating labs reported negative nitrate assimilation. This is a critical biochemical test used to differentiate between *R. mucilaginosa* and *R. glutinis.*

Further Reading:

1. **Biswas, S. K., K. Yokoyama, K. Nishimura, and M. Miyaji.** 2001. Molecular phylogenetics of the genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial cytochrome b gene. Int J Syst Evol Microbiol. **51**(Pt 3):1191-9.
2. **Gyaurgieva, O. H., T. S. Bogomolova, and G. I. Gorshkova.** 1996. Meningitis caused by *Rhodotorula rubra* in an HIV-infected patient. J Med Vet Mycol. **34**(5): 357-9.
3. **Huttova, M., K. Kralinsky, J. Horn, I. Marinova, K. Iligova, J. Fric, S. Spanik, J. Filka, J. Uher, J. Kurak, and V. Krcmery, Jr.** 1998. Prospective study of nosocomial fungal meningitis in children—report of 10 cases. Scand J Infect Dis. **30**(5): 485-7.
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6. **Posteraro, B., L. Romano, M. Sanguinetti, L. Masucci, G. Morace, and G. Fadda.** 2000. Commercial systems for fluconazole susceptibility testing of yeasts: comparison with the broth microdilution method. Diagn Microbiol Infect Dis. **38**(1):29-36.

Y-3 *Candida tropicalis*

Source: Sputum

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	70
Labs with incorrect ID:	0

Clinical Significance: A frequent casual agent of sepsis, wound infections, and disseminated infections in immunocompromised patients.

Ecology: Cosmopolitan, found in water and in alimentary tract of lower mammals, and in humans.

Laboratory Diagnosis:

1. Culture – At 25^0 C, on Sabouraud's dextrose agar colonies are smooth-to-wrinkled, cream-colored, rapid-growing (Fig. 17).
2. Microscopic morphology – On cornmeal agar with Tween 80, shows long true hyphae and pseudohyphae, with either single or small clusters of blastoconidia (Fig. 18).
3. Differentiation from other yeasts – *C. tropicalis* is differentiated from *C. albicans* and *C. dubliniensis* by variable growth on media containing cycloheximide, and by its fermentation of glucose, maltose, sucrose, and trehalose. Occasionally, *C. tropicalis* can produce chlamydospores on cornmeal agar.
4. Molecular tests – Martin and coworkers (6) used reverse-hybridzation line probe assay combined with PCR amplification of internal transcribed-spacer (ITS) regions for rapid identification of clinically significant fungal pathogens including *C. tropicalis.* The combination of pan-fungal PCR and multiplex liquid hybridzation of ITS regions was developed for detection and identification of fungi in tissue specimens (1).
5. *In vitro* susceptibility testing – Few strains of *C. tropicalis* has been reported with high amphotericin B MIC (3). *C. tropicalis* is generally susceptable to azoles, but variably susceptible to flucytosine (2).

Further Reading:

1. **Hendolin, P. H., L. Paulin, P. Koukila-Kahkola, V. J. Anttila, H. Malmberg, M. Richardson, and J. Ylikoski.** 2000. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. J Clin Microbiol. **38**(11): 4186-92.
2. **Hoban, D. J., G. G. Zhanel, and J. A. Karlowsky.** 1999. In vitro susceptibilities of *Candida* and *Cryptococcus neoformans* isolates from blood cultures of neutropenic patients. Antimicrob Agents Chemother. **43**(6): 1463-4.
3. **Jandourek, A., P. Brown, and J. A. Vazquez.** 1999. Community-acquired fungemia due to a multiple-azole-resistant strain of *Candida tropicalis*. Clin Infect Dis. **29**(6): 1583-4.
4. **Kurup, A., M. N. Janardhan, and T. Y. Seng.** 2000. *Candida tropicalis* pacemaker endocarditis. J Infect. **41**(3): 275-6.
5. **Makhoul, I. R., I. Kassis, T. Smolkin, A. Tamir, and P. Sujov.** 2001. Review of 49 neonates with acquired fungal sepsis: further characterization. Pediatrics. **107**(1): 61-6.
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Cryptococcus albidus

Source: Eye

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	70
Labs with incorrect ID:	0

Clinical Significance: A rare casual agent of sepsis, wound infection, and pneumonia in immunocompromised patients.

Ecology: Cosmopolitan, found in plants and water. It is also found on skin of lower animals and on humans.

Laboratory Diagnosis:

1. Culture – At 25^0 C, on Sabouraud's dextrose agar colonies are soft, mucoid, cream to pink, in 4 days (Fig.19).
2. Microscopic morphology – On cornmeal agar with Tween 80, large, round budding yeast cells, no true hyphae or pseudohyphae (Fig. 20).
3. Differentiation from other yeasts – *C. albidus* does not grow on media containing cycloheximide, grows poorly at 37^0 C, produces urease enzyme, and assimilates nitrate. It is differentiated from *C. neoformans* by its inability to form brown colonies on niger seed agar. Although *C. terreus* is also nitrate-positive, it differs from *C. albidus* in assimilation of sorbitol and N-acetylglucosamine.
4. Molecular tests – Ribosomal DNA sequence analysis revealed diversity in *C. albidus* (1).
5. *In vitro* susceptibility testing – Almost all isolates are susceptible to amphotericin B, flucytosine, and azoles.

Further Reading:

1. **Fonseca, A., G. Scorzetti, and J. W. Fell.** 2000. Diversity in the yeast *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. Can J Microbiol. **46**(1):7-27.
2. **Gluck, J. L., J. P. Myers, and L. M. Pass.** 1987. Cryptococcemia due to *Cryptococcus albidus*. South Med J. **80**(4): 511-3.
3. **Kordossis, T., A. Avlami, A. Velegraki, I. Stefanou, G. Georgakopoulos, C. Papalambrou, and N. J. Legakis.** 1998. First report of *Cryptococcus laurentii* meningitis and a fatal case of *Cryptococcus albidus* cryptococcaemia in AIDS patients. Med Mycol. **36**(5):335-9.
4. **Loison, J., J. P. Bouchara, E. Gueho, L. de Gentile, B. Cimon, J. M. Chennebault, and D. Chabasse.** 1996. First report of *Cryptococcus albidus* septicaemia in an HIV patient. J Infect. **33**(2):139-40.
5. **Narayan, S., K. Batta, P. Colloby, and C. Y. Tan.** 2000. Cutaneous *Cryptococcus* infection due to *C. albidus* associated with Sezary syndrome. Br J Dermatol. **143**(3):632-4.
6. **Wells, G. M., A. Gajjar, T. A. Pearson, K. L. Hale, and J. L. Shenep.** 1998. Brief report. Pulmonary cryptosporidiosis and *Cryptococcus albidus* fungemia in a child with acute lymphocytic leukemia. Med Pediatr Oncol. **31**(6):544-6.

Sporobolomyces salmonicolor

Source: Wound

Scoring:	No. Labs
Referee labs with correct ID:	10
Labs with correct ID:	69
Labs with incorrect ID:	1
(Hensenula polymorpha)	(1)

Clinical Significance: An infrequent casual agent of dermatitis. It has also been isolated from blood, sputum, and urine as a contaminant.

Ecology: Cosmopolitan, isolated from air, plants. It has also been isolated from skin of humans.

Laboratory Diagnosis:

1. Culture – At 25^0 C, colonies on Sabouraud's dextrose agar are coral to salmon pink color, smooth to wrinkled, with satellite colonies, in 3 –5 days (Fig. 21).
2. Microscopic morphology – On cornmeal agar with Tween 80, oval to elongate yeastlike cells, kidney-shaped ballistoconidia are seen (Fig. 22). True hyphae and pseudohyphae rarely produced.
3. Differentiations from other yeasts – Abundant satellite colonies are formed from the ballistoconidia, differentiating *S. salmonicolor* from pink–red *Rhodotorula* species.
4. Molecular tests – Cytochrome b sequences were used for both species identification and the study of phylogenetic relationships among basidiomycetous yeast (1). The random amplified polymorphic DNA (RAPD) method was used to differentiate the members of *Sporobolomyces* from *Sterigmatomyces* and *Tilletiopsis* (4).
5. *In vitro* susceptibility testing – Most isolates of *S. salmonicolor* are susceptible to amphotericin B and to commonly used azoles like fluconazole and itraconazole (3).

Comments: One lab reported this isolate as *H. polymorpha* on the basis of a specific biocode generated using API 20C AUX. *S. salmonicolor* produces ballistoconidia, while *H. polymorpha* forms ascospores.

Further Reading:

1. **Biswas, S. K., K. Yokoyama, K. Nishimura, and M. Miyaji.** 2001. Molecular phylogenetics of the genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial cytochrome b gene. Int J Syst Evol Microbiol. **51**(Pt 3): 1191-9.
2. **Bross, J. E., P. Manning, D. Kacian, and G. H. Talbot.** 1986. Pseudomeningitis caused by *Sporobolomyces salmonicolor*. Am J Infect Control. **14**(5): 220-3.
3. **Espinel-Ingroff, A., M. Pfaller, S. A. Messer, C. C. Knapp, S. Killian, H. A. Norris, and M. A. Ghannoum.** 1999. Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeasts and yeast-like organisms. J Clin Microbiol. **37**(3): 591-5.
4. **Messner, R., H. Prillinger, F. Altmann, K. Lopandic, K. Wimmer, O. Molnar, and F. Weigang.** 1994. Molecular characterization and application of random amplified polymorphic DNA analysis of *Mrakia* and *Sterigmatomyces* species. Int J Syst Bacteriol. **44**(4): 694-703.
5. **Plazas, J., J. Portilla, V. Boix, and M. Perez-Mateo.** 1994. *Sporobolomyces salmonicolor* lymphadenitis in an AIDS patient. Pathogen or passenger? Aids. **8**(3):387-8.

Source: Urine

Clinical Significance: An infrequent causal agent of nosocomial infections. It also causes fungemia in neonates, and endocarditis in immunosuppressed patients.
Ecology: It is cosmopolitan, found in plants. It is also found on skin of humans and lower animals.
Laboratory Diagnosis:

1. Culture – At 25^0 C, colonies on Sabouraud's dextrose agar are cream-colored, soft, and butyrous, in 2 – 3 days (Fig. 23).
2. Microscopic morphology – On cornmeal agar with Tween 80, blastoconidia with ascospores, but no pseudohyphae, are seen. (Fig. 24). Some strains may produce elaborate pseudohyphae.
3. Differentiation from other yeasts – The anamorph (asexual form) of this yeast is *Candida pelliculosa*. It does not grow on media containing cycloheximide, shows no growth at 42^0 C, assimilates nitrate, and does not produce urease enzyme.
4. Molecular tests – Polymerase chain reaction assays were performed with B2F/B4R primers for amplifying a 700-bp fragment of 17S rDNA, and heteroduplex mobility assays were performed for identification of clinically important yeasts (4). Phylogenetic analysis of domain D1/D2 sequences placed four new species in the *P. anomala* clade (2).
5. *In vitro* susceptibility testing – *P. anomala* is susceptible to amphotericin B, 5-flucytosine, and azoles like fluconazole, clotrimazole, and itraconazole (1).

Further Reading:

1. **Chakrabarti, A., K. Singh, A. Narang, S. Singhi, R. Batra, K. L. Rao, P. Ray, S. Gopalan, S. Das, V. Gupta, A. K. Gupta, S. M. Bose, and M. M. McNeil.** 2001. Outbreak of *Pichia anomala* infection in the pediatric service of a tertiary-care center in Northern India. J Clin Microbiol. **39**(5):1702-6.
2. **Kurtzman, C. P.** 2000. Four new yeasts in the *Pichia anomala* clade. Int J Syst Evol Microbiol. **50 Pt 1**:395-404.
3. **Ma, J. S., P. Y. Chen, C. H. Chen, and C. S. Chi.** 2000. Neonatal fungemia caused by *Hansenula anomala*: a case report. J Microbiol Immunol Infect. **33**(4):267-70.
4. **Olicio, R., C. A. Almeida, and H. N. Seuanez.** 1999. A rapid method for detecting and distinguishing clinically important yeasts by heteroduplex mobility assays (HMAs). Mol Cell Probes. **13**(4):251-5.
5. **Wong, A. R., H. Ibrahim, H. Van Rostenberghe, Z. Ishak, and M. J. Radzi.** 2000. *Hansenula anomala* infection in a neonate. J Paediatr Child Health. **36**(6):609-10.
6. **Yamada, Y., K. Maeda, and K. Mikata.** 1994. The phylogenetic relationships of the hat-shaped ascospore-forming, nitrate-assimilating *Pichia* species, formerly classified in the genus *Hansenula* Sydow et Sydow, based on the partial sequences of 18S and 26S ribosomal RNAs (Saccharomycetaceae): the proposals of three new genera, *Ogataea*, *Kuraishia*, and *Nakazawaea*. Biosci Biotechnol Biochem. **58**(7):1245-57.

Figure 1. Four-day-old, yellowish-white colony of *E. floccosum* on Sabouraud's dextrose agar.

Macroconidia

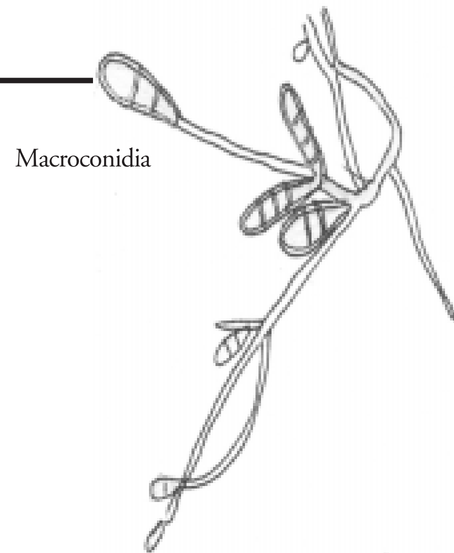


Figure 2. Microscopic morphology of *Epidermophyton floccosum*. Hyphae and smooth, thin-walled, club-shaped macroconidia are seen (left; 400X magnification, right; line drawing not to scale).

Figure 3. Four-day-old, yellowish-white, wooly colony of *Microsporum canis* on Sabouraud's dextrose agar.

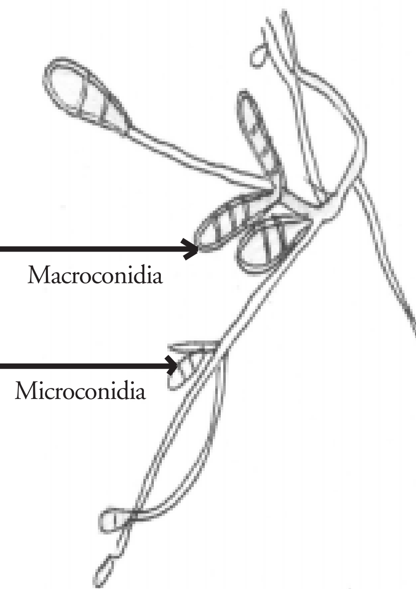
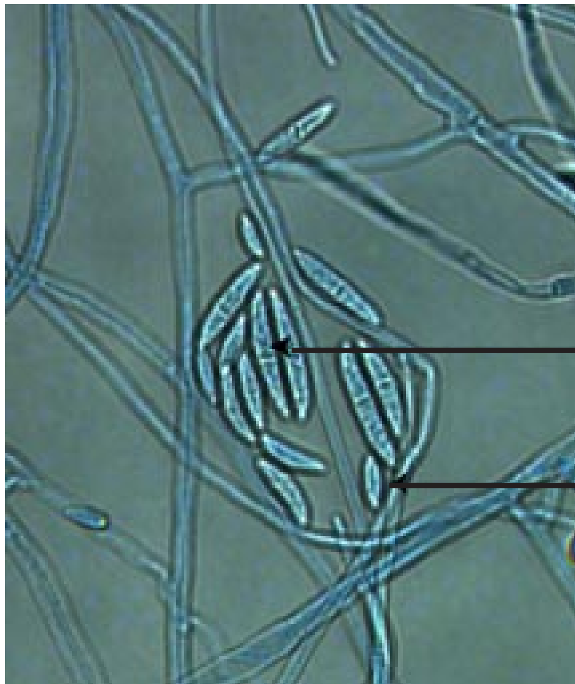


Figure 4. Microscopic morphology of *Microsporum canis*. The fusoid, thick-walled macroconidia with curved apex and a few microconidia are seen (left; 200X magnification, right; line diagram not to scale).

Figure 5. Four-day-old, wooly, purplish-pink colony of *Fusarium* species on Sabouraud's dextrose agar.



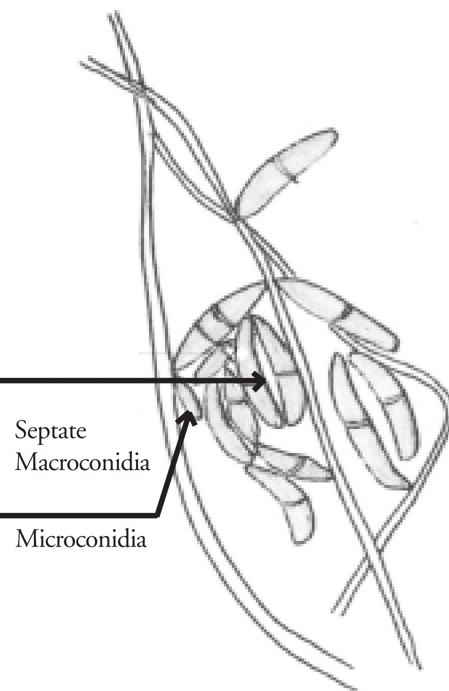
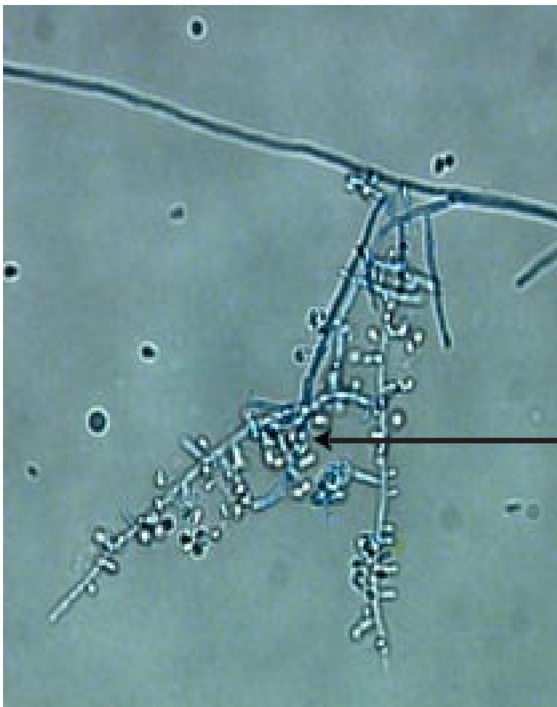


Figure 6. Microscopic morphology of *Fusarium* species. Curved, septate macroconidia and elongate microconidia are seen (left; 400X magnification, right; line diagram, not to scale).

Figure 7. Four-day-old, cream-colored, powdery colony of *Trichophyton mentagrophytes* on Sabouraud's dextrose agar.



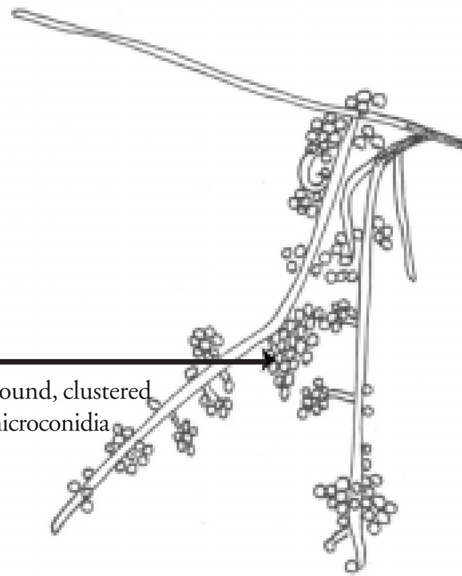
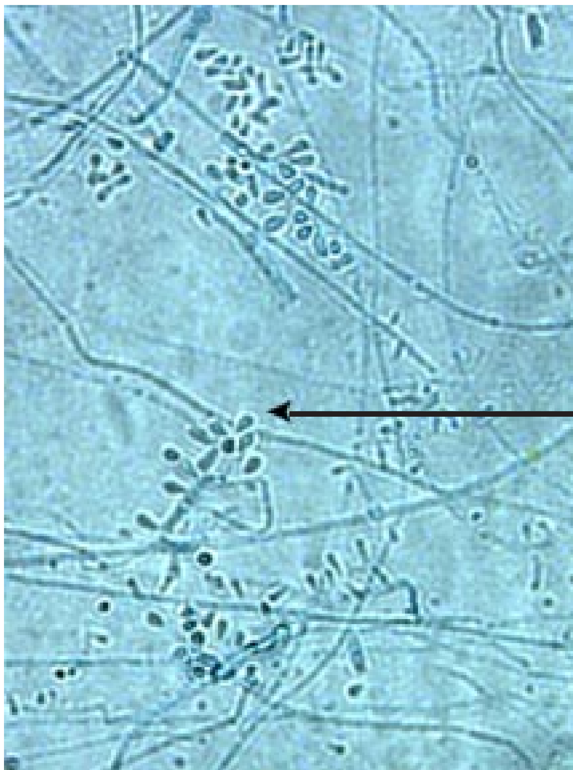


Figure 8. Microscopic morphology of *Trichophyton mentagrophytes*. Round microconidia clustered on conidiophores are seen (left; 200X magnification, right; line diagram not to scale).

Figure 9. Four-day-old, buff, fluffy colony of *Trichophyton rubrum* on Sabouraud's dextrose agar.



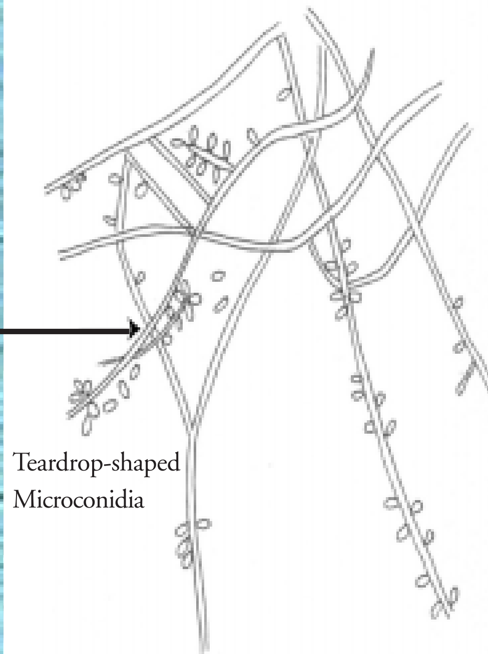
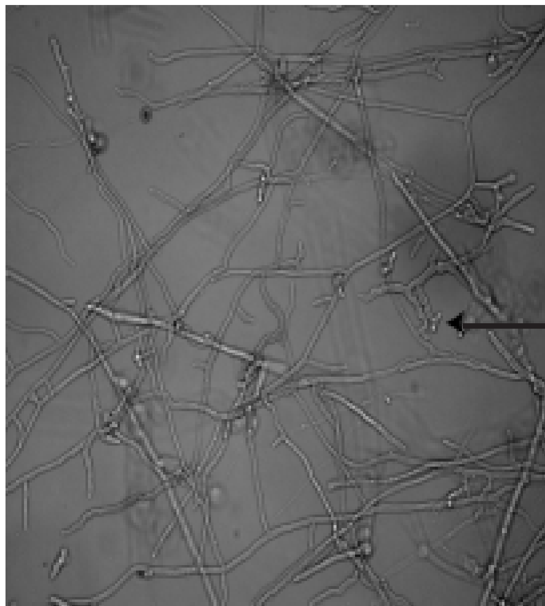


Figure 10. Microscopic morphology of *Trichophyton rubrum*. Teardrop-shaped microconidia, solitary along the hyphae are seen (left; 200X magnification, right; line diagram not to scale).

Figure 11. Four-day-old, downy, white colony of *Microsporum audouinii* on Sabouraud's dextrose agar.



Pectinate hyphae

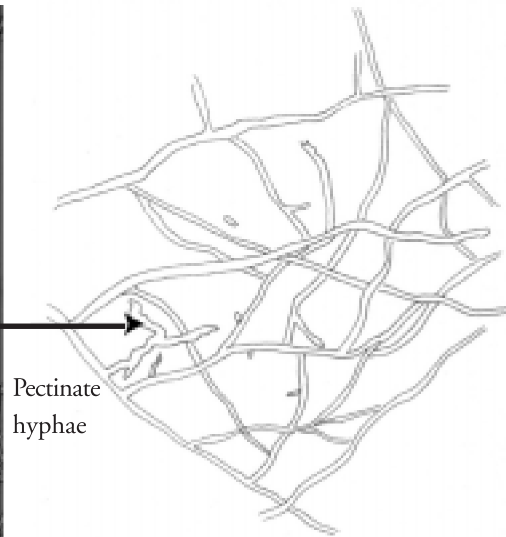


Figure 12. Microscopic morphology of *Microsporum audouinii*. Pectinate and sterile hyphae are seen (left; 200X magnification, right; line diagram not to scale).

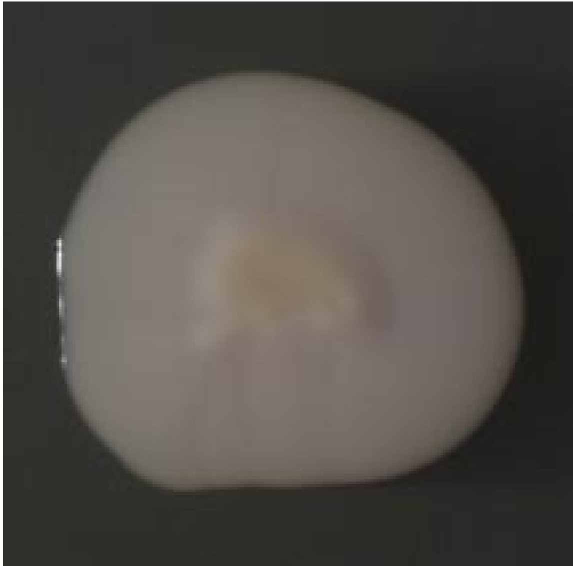


Figure 13. Two-day-old, smooth colony of *Candida guilliermondii* on Sabouraud's dextrose agar.

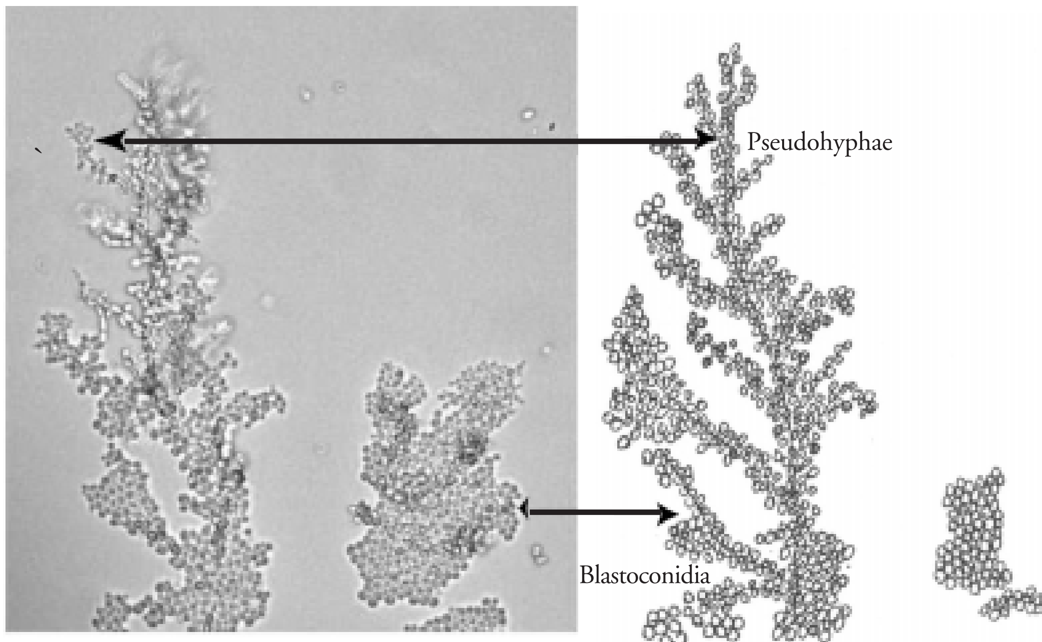
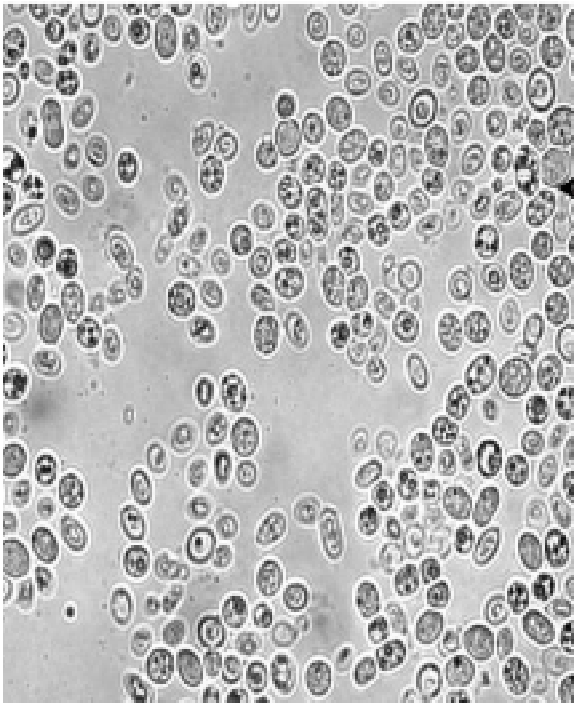


Figure 14. Microscopic morphology of *Candida guilliermondii*. On cornmeal agar culture, short pseudohyphae with clusters of blastoconidia are seen (left; 200X magnification, right; line diagram not to scale).

Figure 15. Two-day-old, coral-red colony of *Rhodotorula mucilaginosa* on Sabouraud's dextrose agar.



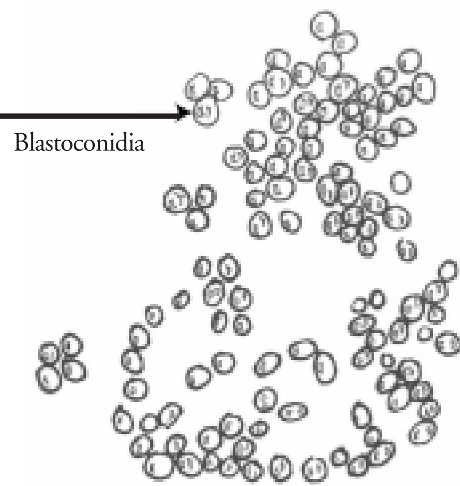


Figure 16. Microscopic morphology of *Rhodotorula mucilaginosa*. On cornmeal agar culture with oval to round blastoconidia are seen (left; 400X magnification, right; line diagram not to scale).

Figure 17. Two-day-old, wrinkled, cream-colored colony of *Candida tropicalis* on Sabouraud's dextrose agar.

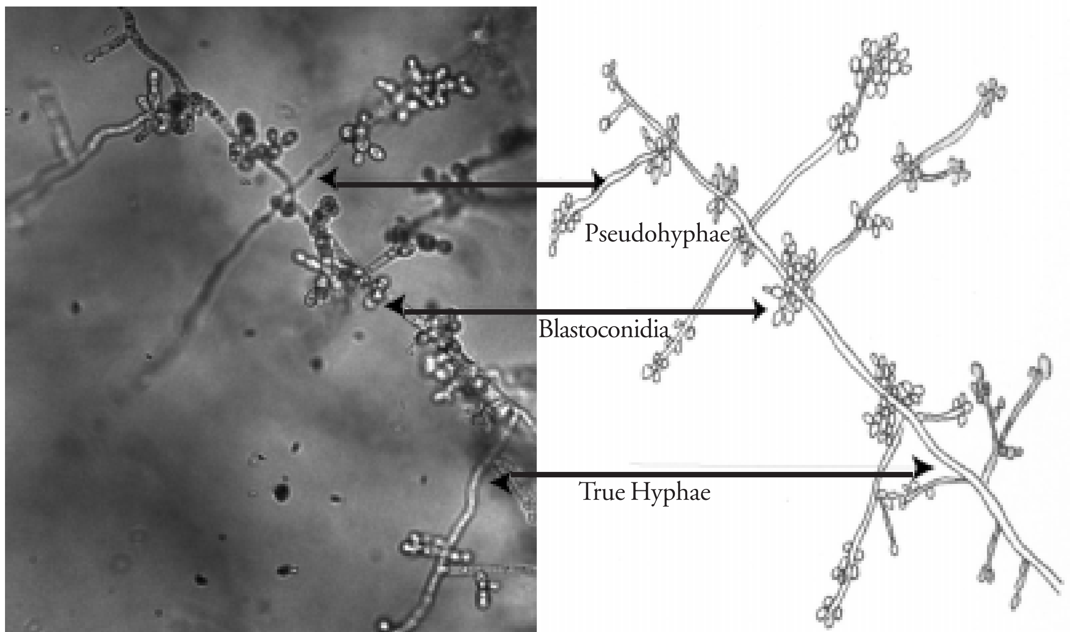


Figure 18. Microscopic morphology of *Candida tropicalis*. On cornmeal agar culture, long true hyphae and pseudohyphae with clusters of blastoconidia are seen (left; 200X magnification, right; line diagram not to scale).

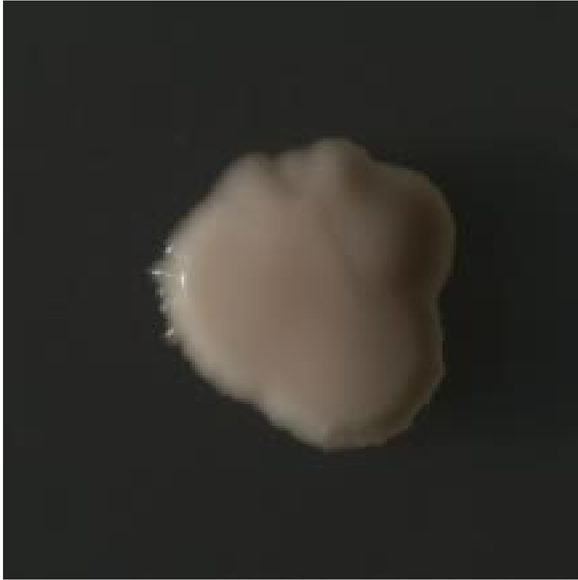


Figure 19. Two-day-old, mucoid, soft colony of *C. albidus* on Sabouraud's dextrose agar.

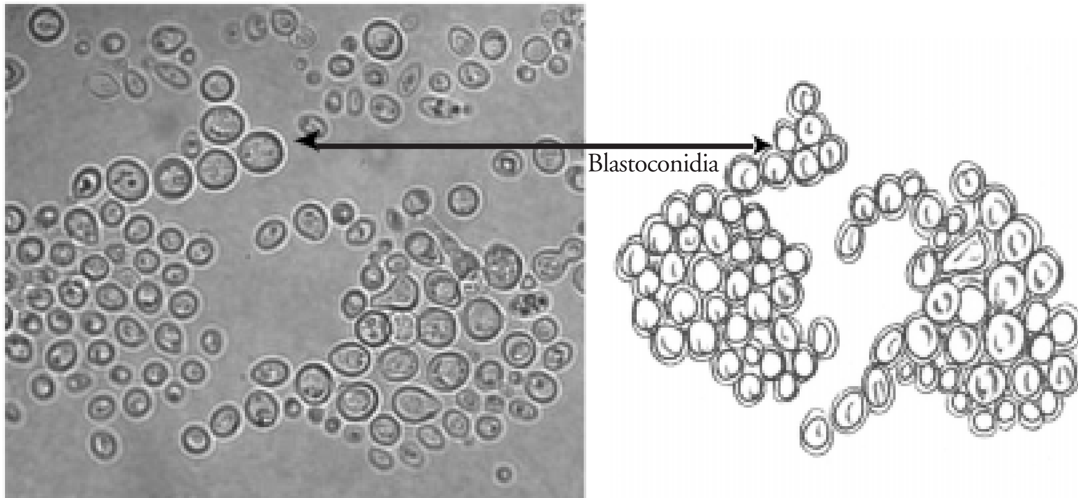


Figure 20. Microscopic morphology of *Cryptococcus albidus*. On cornmeal agar culture, large, round blastoconidia are seen (left; 400X magnification, right; line diagram not to scale).

Figure 21. Five-day-old, salmon-pink colony with satellite colonies, of *Sporobolomyces salmonicolor* on Sabouraud's dextrose agar.

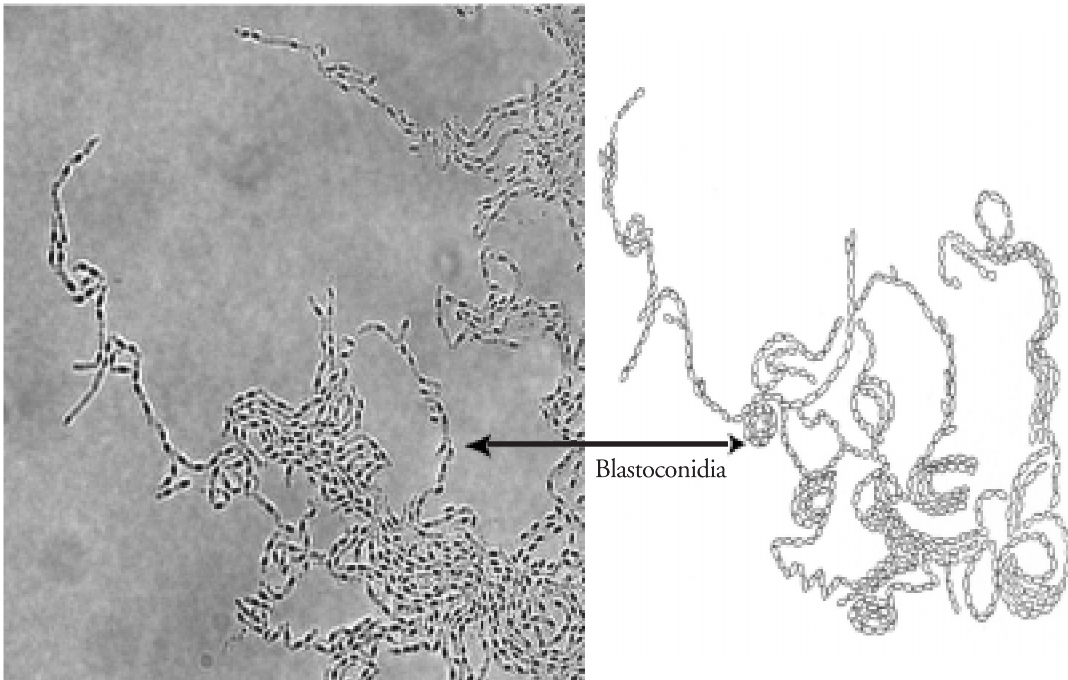


Figure 20. Microscopic morphology of *Sporobolomyces salmonicolor*. On cornmeal agar culture, oval to elongate yeastlike cells are seen (left; 200X magnification, right; line diagram not to scale).

Figure 23. Two-day-old, cream-colored, soft colony of *Pichia anomala* on Sabouraud's dextrose agar.

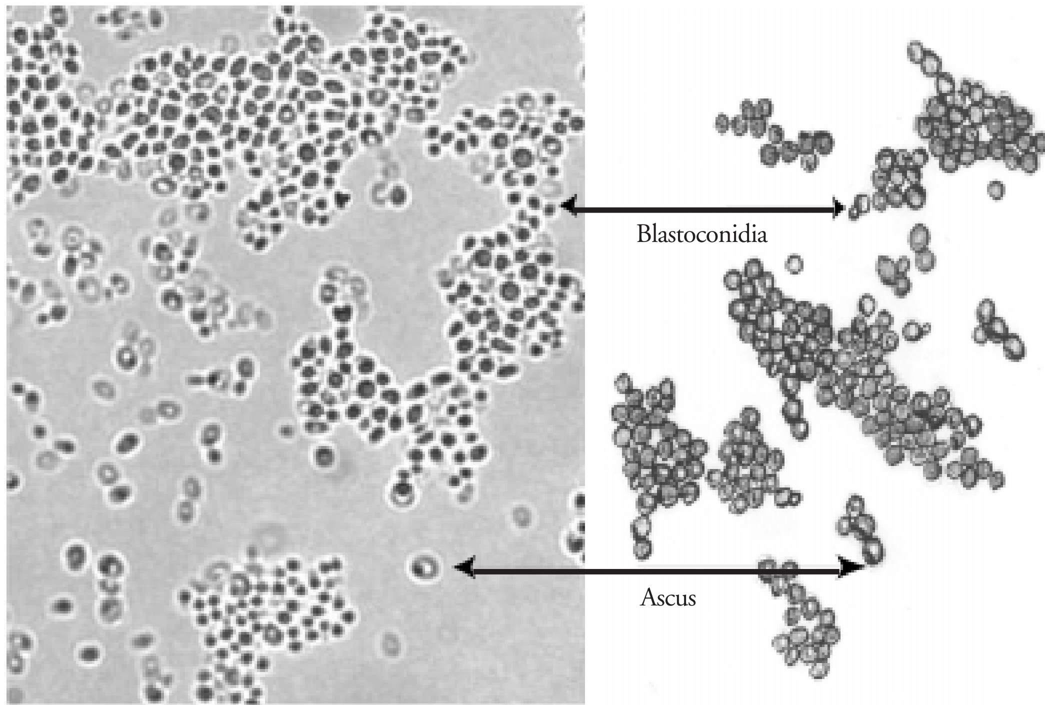


Figure 24. Microscopic morphology of *Pichia anomala*. On cornmeal agar culture, blastoconidia and ascus are seen (left; 200X magnification, right; line diagram not to scale).

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