

The background of the entire page is a light blue, semi-transparent microscopic image. It shows numerous small, round yeast cells, some in chains and some in clusters. A prominent feature is a long, thin, curved hypha or filament extending from the right side towards the center. The overall appearance is that of a yeast culture under a microscope.

# **MYCOLOGY PROFICIENCY TESTING PROGRAM**

**Critique**  
**January 2001**

**Wadsworth Center**  
**New York State Department of Health**



# TABLE OF CONTENTS

STAFF AND CONTACT .....	2
METHODS AND GRADING .....	3
ANSWER KEY .....	4
RESULTS .....	5
TEST STATISTICS .....	6
MOLD DESCRIPTIONS .....	8 - 19
<i>Aspergillus niger</i> .....	8 - 9
<i>Aspergillus nidulans</i> .....	10 - 11
<i>Rhizopus</i> species .....	12 - 13
<i>Aspergillus fumigatus</i> .....	14 - 15
<i>Aspergillus flavus</i> .....	16 - 17
Scheme for differentiation of <i>Aspergilli</i> .....	19
YEAST DESCRIPTIONS .....	20 - 29
<i>Saccharomyces cerevisiae</i> .....	20 - 21
<i>Candida parapsilosis</i> .....	22 - 23
<i>Cryptococcus terreus</i> .....	24 - 25
<i>Candida kefyr</i> .....	26 - 27
<i>Candida zeylanoides</i> .....	28 - 29
BIBLIOGRAPHY .....	30

# MYCOLOGY LABORATORY

## STAFF

**Dr. Vishnu Chaturvedi, Director**

## PROFICIENCY TESTING LAB

**Dr. Rama Ramani, PT Coordinator**

## REFERENCE LAB

**Ms. Susan Larsen, Supervisor**  
**Ms. Sally Gromadzki, Lab technician**  
**Ms. Andrea Doney, Lab technician**

## RESEARCH LAB

**Dr. Sudha Chaturvedi, Research Scientist**  
**Ms. Birgit Rodeghier, Research technician**

## CONTACT

**Mycology Laboratory**  
**Wadsworth Center**  
**New York State Department of Health**  
**120 New Scotland Avenue**  
**Albany, NY 12208.**

**Phone: (518) 474-4177**

**FAX: (518) 486-7811**

**E-mail: [mycology@wadsworth.org](mailto:mycology@wadsworth.org)**

## METHODS

Minimums of two strains of each of the proposed mold specimens were examined for inclusion in the proficiency test. These strains were clinical isolates available in the Fungus Culture Collection at NYS Dept. of Health. The colony morphology of all strains was studied on Sabouraud dextrose agar. Microscopic morphologic features were examined on potato dextrose agar slide cultures. Biochemical characteristics such as cycloheximide sensitivity, growth at higher temperatures, conversion to yeast phase etc., were investigated with the appropriate test media. The single strain, which best demonstrated the morphologic and physiologic characteristics of each of the proposed test specimens, was used in the test.

Similarly, a minimum of two strains of each of the proposed yeast specimens was examined for inclusion in the proficiency test. The morphology of all yeast strains was studied on cornmeal + Tween 80 agar plates inoculated by the Dalmau or streak-cut method. On occasion, the morphologic features were examined on corn meal + Tween 80 slide cultures. 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 investigated through the use of Durham tubes in 3 ml of Wickerham fermentation broth in 16 x 125 mm screw-cap tubes. Supplemental physiologic characteristics, such as nitrate assimilation, urease activity, cycloheximide sensitivity, etc., were investigated with the appropriate test media. The strain demonstrating best morphologic and physiologic characteristics of each of the proposed test specimens was used in the test.

## GRADING

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. Referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of the New York State and have a record of excellent performance during the last three years.

The grading for each specimen is based on the following formula:

$$\frac{\text{Number of correct responses}}{\text{\# of fungi present + \# incorrect responses}} \quad \text{X0}$$

Participating laboratories must achieve a score of 80% or greater 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 <i>Aspergillus niger</i>	<i>Aspergillus niger</i>	
M-2 <i>Aspergillus nidulans</i>	<i>Aspergillus nidulans</i>	<i>Emericella nidulans</i>
M-3 <i>Rhizopus species</i>	<i>Rhizopus species</i>	<i>Rhizopus microsporus</i> <i>Rhizopus arrhizus</i>
M-4 <i>Aspergillus fumigatus</i>	<i>Aspergillus fumigatus</i>	
M-5 <i>Aspergillus flavus</i>	<i>Aspergillus flavus</i>	

## MYCOLOGY – YEAST ONLY

Specimen Key	Validated Specimen	Acceptable Answers
Y-1 <i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	
Y-2 <i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>	
Y-3 <i>Cryptococcus terreus</i>	<i>Cryptococcus terreus</i>	
Y-4 <i>Candida kefyr</i>	<i>Candida kefyr</i>	<i>Candidapseudotropicalis</i>
Y-5 <i>Candida zeylanoides</i>	<i>Candida zeylanoides</i>	

# RESULTS

## MYCOLOGY - GENERAL

		<b>CORRECT RESPONSES/ TOTAL # LABS (%)</b>	<b>REFEREE LABS (%)</b>
<b>M- 1</b>	<i>Aspergillus niger</i>	<b>82/82 (100%)</b>	<b>10/10 (100%)</b>
<b>M- 2</b>	<i>Aspergillus nidulans</i>	<b>76/82 (92.6%)</b>	<b>9/10 (90%)</b>
<b>M- 3</b>	<i>Rhizopus sp.</i>	<b>81/82 (98.8%)</b>	<b>10/10 (100%)</b>
<b>M- 4</b>	<i>Aspergillus fumigatus</i>	<b>82/82 (100%)</b>	<b>10/10 (100%)</b>
<b>M- 5</b>	<i>Aspergillus flavus</i>	<b>80/82 (97.5%)</b>	<b>9/10 (90%)</b>

## MYCOLOGY – YEAST ONLY

		<b>CORRECT RESPONSES/ TOTAL # LABS (%)</b>	<b>REFEREE LABS (%)</b>
<b>Y - 1</b>	<i>Saccharomyces cerevisiae</i>	<b>70/70 (100%)</b>	<b>10/10 (100%)</b>
<b>Y - 2</b>	<i>Candida parapsilosis</i>	<b>70/70 (100%)</b>	<b>10/10 (100%)</b>
<b>Y - 3</b>	<i>Cryptococcus terreus</i>	<b>69/70 (98.6%)</b>	<b>10/10 (100%)</b>
<b>Y - 4</b>	<i>Candida kefyr</i>	<b>69/70 (98.6%)</b>	<b>10/10 (100%)</b>
<b>Y - 5</b>	<i>Candida zeylanoides</i>	<b>70/70 (100%)</b>	<b>10/10 (100%)</b>

# TEST STATISTICS

## MYCOLOGY - GENERAL

Number of participating laboratories	82	
Number of REFEREE laboratories	10	
Number of laboratories responding by deadline	82	
Number of laboratories responding after deadline [TEST NOT GRADED]		0
Number of laboratories not responding	0	
Number of laboratories successfully completing this test	82	
Number of laboratories unsuccessfully completing this test	0	

## 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 [TEST NOT GRADED]		0
Number of laboratories not responding	0	
Number of laboratories successfully completing this test	70	
Number of laboratories unsuccessfully completing this test	0	

### Commercial Yeast Identification Systems Used\*

AMS Vitek system -----	23
API 20C -----	17
API 20C AUX -----	09
Microscan -----	03
Remel Uni-Yeast-Tek -----	05
Other -----	15

\*Includes multiple systems used by some labs.



## M – 1 *Aspergillus niger*

Source: Ear

Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 82

### Clinical Significance:

It commonly causes ear infection, also implicated in pulmonary aspergilloma and rarely in primary cutaneous infection.

### Ecology:

Cosmopolitan in soil and on plants.

### Laboratory Diagnosis:

- a. Culture – It is a fast growing fungus, covering entire plate in 2 – 3 days. At 25<sup>o</sup> C, initial growth is white, becoming black giving “salt and pepper appearance “ and reverse turning pale yellow in 1 – 2 days (**Fig. 1**). Good growth is seen at 37<sup>o</sup> C.
- b. Microscopic morphology –Lactophenol cotton blue or Calcofluor mount shows septate hyphae with smooth-walled, simple conidiophores measuring upto 1mm in length. Conidiophore bears conidial heads, which split with age. Conidiophores end in vesicle, which is globose and entirely covered (radiating) with two series of sterigmata (biseriate). Conidia produced from these sterigmata are brown to black, round, rough walled, and in chains measuring 4 –5  $\mu$ m in diameter (**Fig. 2**).
- c. Differentiation from other *Aspergillus* species - Rapid growing, black colonies, microscopically revealing biseriate, radiating heads with black, round, rough conidia.

- d. Molecular tests – PCR method has been described by Accensi et al (1) to differentiate various species in the *Aspergillus niger* aggregate.
- e. In vitro susceptibility testing – Susceptibility testing results indicate that most clinical isolates are susceptible to amphotericin B and variably susceptible to itraconazole and resistant to fluconazole.

### Comments:

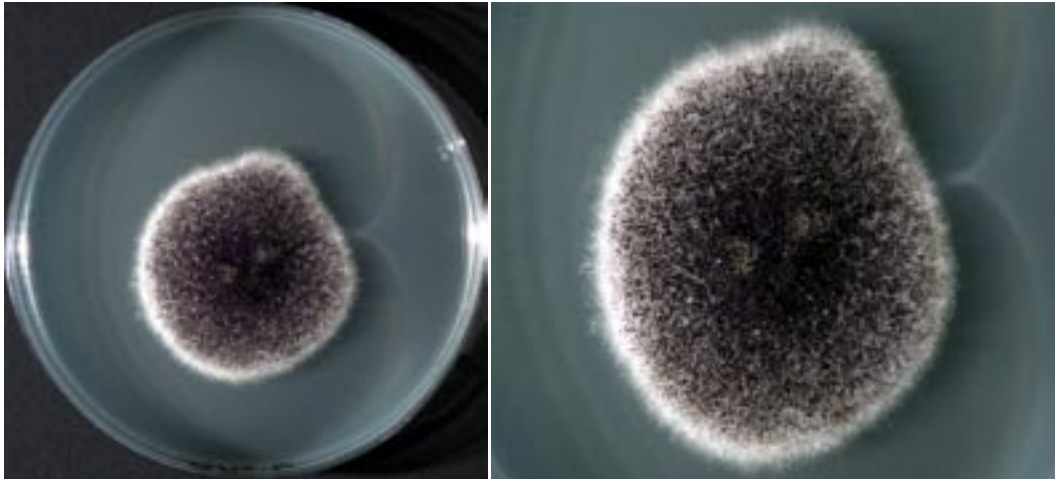
All labs were able to make correct specific identification.

### Further reading:

1. Accensi F, Cano J, Figuera L, Abarca ML, Cabanes FJ. 1999. New PCR method to differentiate species in the *Aspergillus niger* aggregate. FEMS Microbiology Letters. 180 (2): 191 – 196.
2. Amod FC, Coovadia YM, Pillay T, Ducasse G. 2000. Primary cutaneous aspergillosis in ventilated neonates. Pediatric Infect Dis J. 19 (5): 482 – 483.
3. Graham RM, Burrell MG, Bainton R, Carson L, Needham G. 1999. Aspergillosis with calcification of the maxillary sinus. British J Oral & Maxillofacial Surgery. 37 (5): 425 – 426.
4. Gupta A, Gupta V, Dogra MR, Chakrabarti A, Ray P, Ram J, Patnaik B. 2000. Fungal endophthalmitis after a single intravenous administration of presumably contaminated dextrose infusion fluid. Retina. 20 (3): 262 – 263.
5. Hoshino H, Tagaki S, Kon H, Shibusu T, Takabatake H, Fujita A, Sekine K, Abe S. 1999. Allergic bronchopulmonary aspergillosis due to *Aspergillus niger* without bronchial asthma. Respiration. 66 (4): 369 – 372.
6. Kermani NK and Aggarwal SP. 2000. Isolated post – operative *Aspergillus niger* endophthalmitis. Eye. 14: 114 – 116.
7. Kreiss Y, Vered Z, Keller N, Kochva I, Sidi Y, Gur H. 2000. *Aspergillus niger* endocarditis in an immunocompetent patient: an unusual course. Postgraduate Med J. 76 (892) 105 – 106.
8. Laurel VL, Meier PA, Astorga A, Dolan D, Brockett R, Rinaldi MG. 1999. Pseudoepidemic of *Aspergillus niger* infections traced to specimen contamination in the microbiology laboratory. J Clin Microbiol. 37 (5): 1612 – 1616.

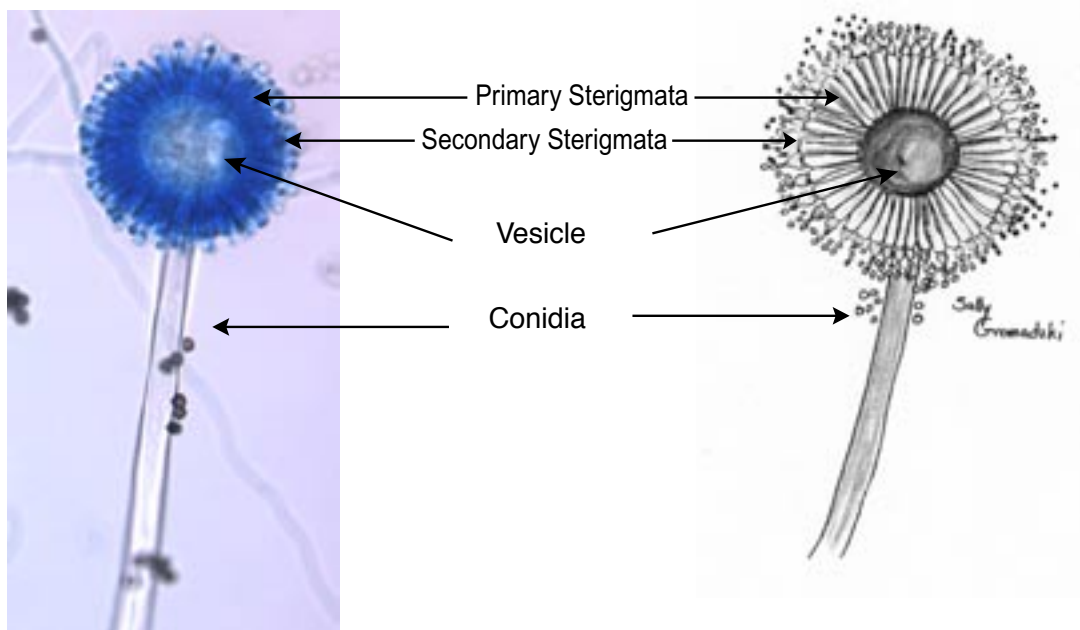
**Figure 1.**

Left, a three-day-old colony of *Aspergillus niger* on Sabouraud's dextrose agar. Right, a close up of the colony showing typical 'salt and pepper appearance', which results from darkly pigmented conidia borne in large numbers on conidiophores.



**Figure 2.**

Microscopic morphology of *Aspergillus niger* showing globose vesicle with biserial, radiating head and dark, round conidia (left, 400X magnification; right, line drawing not to scale).



## M – 2 *Aspergillus nidulans*

Source: Lung

Correct answers:

Referee Labs: 9/10  
Labs with Correct ID 76

Labs with incorrect answers:

*Aspergillus versicolor*: 3  
*Aspergillus terreus*: 1  
*Aspergillus sp.*: 2

### Clinical Significance:

Human infections of *Aspergillus nidulans* have been rarely reported. Most of these reports were from patients with chronic granulomatous disease involving skin, sinus, lungs etc.

### Ecology:

Cosmopolitan in soil.

### Laboratory Diagnosis:

- a. Culture – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar are dark green with purplish peripheral pigment and purple reverse, powdery and rapid growing (**Fig.3**).
- b. Microscopic morphology – Lactophenol cotton blue or Calcofluor mount – septate hyphae with brown, wavy conidiophores. Conidiophore ends in vesicle, which is subglobose with its upper half covered by two series of sterigmata (biseriate). Conidia, measuring 5 –7  $\mu\text{m}$  in diameter, are round, smooth- rough walled. Round hulle cells and reddish color cleistothecia are also seen. Hulle cells are specialized structures made up of loose net-work of hyphae, having globose, vesiculose cells with thick walls that occur in certain groups of *Aspergilli*. Their characteristic shape provides a valuable diagnostic tool. Cleistothecia are sexual structures i. e network of hyphae where mating between a and  $\alpha$  strains occur. Ascospores (sexual spores) produced within

these cleistothecia, are purple in color, lens shaped with equatorial crests, (**Fig. 4**).

- c. Differentiation from other *Aspergilli* – *Aspergillus nidulans* can be distinguished by its dark green colony with purple reverse; microscopically, brown conidiophores, biseriate phialides, round hulle cells, cleistothecia with lens shaped ascospores with equatorial crests are characteristics.
- d. Molecular tests – *Aspergillus nidulans* has a well-defined genetic system, which allows it to be used as model organism in basic and applied research.
- e. In vitro susceptibility testing – Susceptibility testing results indicate that most of the isolates are susceptible to amphotericin B and variably susceptible to itraconazole.

### Comments:

Three labs reported this organism as *Aspergillus versicolor* while one lab reported *Aspergillus terreus*. Microscopy easily differentiates *A. nidulans* from *A. versicolor* by presence of cleistotheca and ridged ascospores. Similarly, colony morphology differentiates it from *A. terreus*.

### Further reading:

1. de Souza CC, Pellizzon CH, Hiraishi M, Goldman MH, Goldman GH. 1998. Isolation and characterisation of cycloheximide – sensitive mutants of *Aspergillus nidulans*. *Current Genetics*. 33 (1): 60 – 69.
2. Kim M, Shin JH, Suh SP, Ryang DW, Park CS, Kim C, Kook H, Kim J. 1997. *Aspergillus nidulans* infection in a patient with chronic granulomatous disease. *J Korean Medical Sci*. 12 (3): 244 – 248.
3. Lucas GM, Tucker P, Merz WG. 1999. Primary cutaneous *Aspergillus nidulans* infection associated with a Hickman catheter in a patient with neutropenia. *Clin Infect Dis*. 29(6): 1594 –1546.
4. Mitchell RG, Chaplin AJ, Mackenzie DW. 1987. *Emericella nidulans* in a maxillary sinus fungal mass. *J Medical & Veterinary Mycology*. 25 (5): 339 – 341.

5. Mizuki M, Chikuba K, Tanaka K. 1994. A case of chronic necrotizing pulmonary aspergillosis due to *Aspergillus nidulans*. *Mycopathologia*. 128 (2): 75 – 79.
6. Ng KP, Saw TL, Madasamy M, Soo Hoo T. 1999. Onychomycosis in Malaysia. *Mycopathologia*. 147 (1): 29 – 32.
7. Tong QJ, Chai WX, Wang ZF, Kou JF, Qi ZT, Wang DL. 1990. A case of cerebral aspergillosis caused by *Aspergillus nidulans*. *Clinical, pathologic and mycologic identifications*. *Chinese Medical J.* 103 (6): 518 – 522.
8. Yano S, Kobayashi K, Shishido S, Nakano H. 1999. Intrabronchial *Aspergillus nidulans* infection in an immunocompetent man. *Internal Medicine*. 38 (4): 372 – 375.

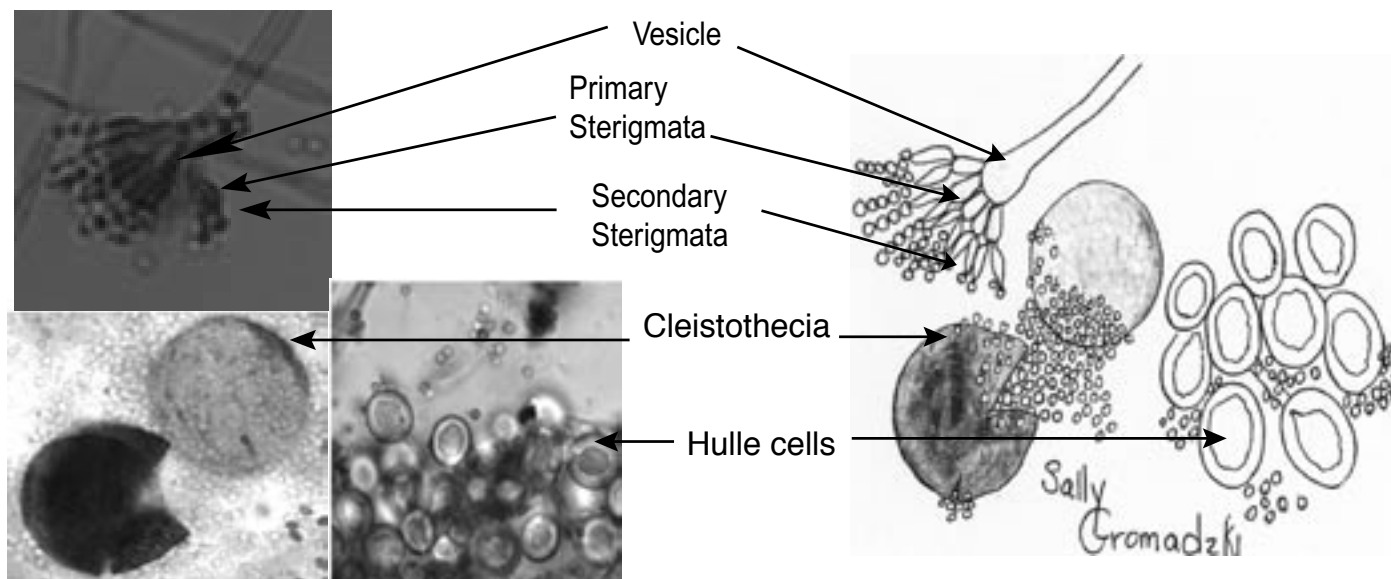
**Figure 3.**

Left, one day old colony of *Aspergillus nidulans* on Sabouraud’s dextrose agar. Right, a close up of the colony showing powdery texture. with dark green center and purplish peripheral pigment.



**Figure 4.**

Microscopic morphology of *Aspergillus nidulans* showing subglobose vesicle with biserial, columnar head, cleistothecia with ascus and ascospores, and hulle cells (left, 400 X magnification; right, line drawing not to scale).



## M – 3 *Rhizopus* species

Source: Sinus

### Correct answers

Referee Labs: 10/10

Labs with correct ID: 81

### Incorrect answers

*Rhizomucor pusillus*: 1

### **Clinical Significance:**

This organism is the most common agent of zygomycosis. The predisposing factors are diabetic ketoacidosis, malnutrition, burns, immunocompromising conditions like hematologic malignancy, corticosteroid therapy, etc.

**Ecology:** It is cosmopolitan in distribution, mainly isolated from soil, decaying vegetables and bread.

### **Pathogenicity:**

It rapidly causes necrosis of tissue and produces infarcts in various organs.

### **Laboratory Diagnosis:**

- a. **Culture** – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar, are wooly in texture, greyish brown, growing very rapidly, filling the culture plate in 24 –48 h (**Fig.5**).
- b. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mount show broad, aseptate hyphae, either single or tufts of brown sporangiophores (conidiophores) arising from hyphae (stolons) opposite well-developed rhizoids (root like structures). Sporangiophores end in sporangia with a round columella (vesicle, enlarged at the apex), producing round to oval sporangiospores or sexual spores (**Fig. 6**).
- c. **Differentiation from other zygomycetes** – *Rhizopus* species is distinguished from other members by the presence of well-developed rhizoids situated opposite sporangiophores.

Sporangiophores are often unbranched and in tufts unlike in *Mucor*, *Rhizomucor*, *Absidia*.

- d. **Molecular tests** – PCR assay for the rapid and accurate identification of the agents of mucormycosis has been reported by Voigt et al (5).
- e. **In vitro susceptibility testing** – Most of the clinical isolates are susceptible to amphotericin B. *Rhizopus sp.* shows variable susceptibility to azoles.

### **Comments:**

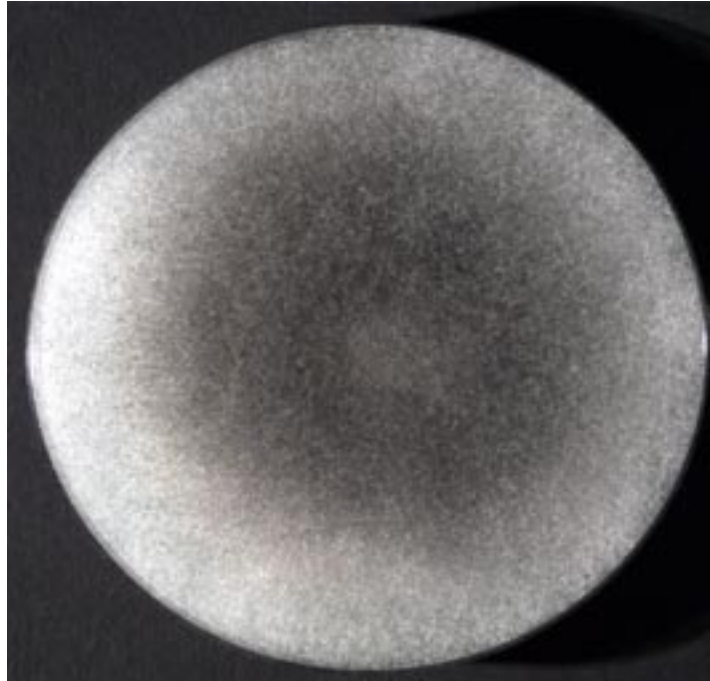
This isolate is *Rhizopus microsporus* NRRL 28630. All the labs except one reported correct identification. Among the participating labs, 5 labs reported additional organism. Of the five, three labs reported *Aspergillus flavus*, another lab reported *Aspergillus glaucus* group, and a third lab reported *Aspergillus* species. *Aspergilli* ought to be handled with good aseptic precautions to eliminate chances of specimen cross-contamination.

### **Further reading:**

1. Ciesla MC, Kammeyer PL, Yeldandi V, Petruzzelli GJ, Yong SL. 2000. Identification of the asexual state of *Rhizopus* species on histologic tissue sections in a patient with rhinocerebral mucormycosis. Archives Pathology & Lab Medicine. 124 (6): 883 – 887.
2. Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV. 2000. Zygomycosis in the 1990s in a tertiary care cancer center. Clin Infect. Dis. 30 (6): 851 –856.
3. Locher DH, Adesina A, Wolf TC, Imes CB, Chodosh J. 1998. Postoperative *Rhizopus* scleritis in a diabetic man. J Cataract & Refractive Surgery. 24 (4): 562 –565.
4. Ribes JA, Vanover – Sams CL, Baker DJ. 2000. Zygomycetes in human disease. Clin Microbiol Reviews. 13 (2): 236 – 301.
5. Voigt K, Cigelnik E, O'donnell K. 1999. Phylogeny and PCR identification of clinically important zygomycetes based on nuclear ribosomal – DNA sequence data. J Clin Microbiol. 37 (12): 3957 – 3964.

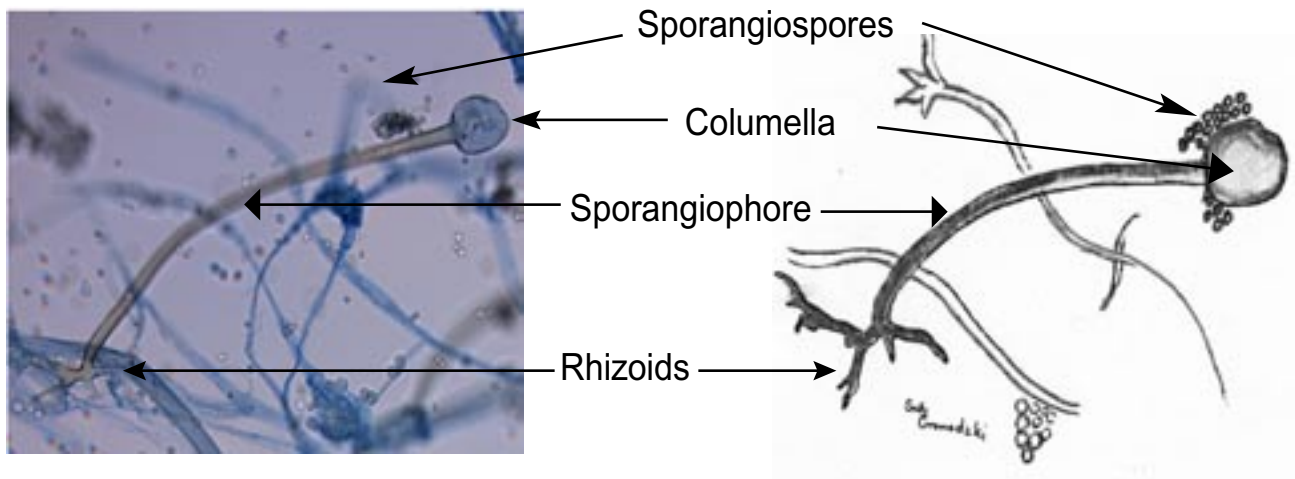
**Figure 5.**

Two-day-old grayish brown colony of *Rhizopus* sp. filling Sabouraud's dextrose agar plate.



**Figure 6.**

Microscopic morphology of *Rhizopus* sp. showing broad, aseptate hyphae, sporangiophores arising opposite rhizoids, sporangia with round columella, and oval sporangiospores (left; 100X magnification, right; line drawing not to drawn to scale).



## M – 4 *Aspergillus fumigatus*

Source: Sputum

### Correct answers

Referee Labs: 10/10  
*Aspergillus fumigatus*: 82

### **Clinical Significance:**

*Aspergillus fumigatus* is the most frequent etiologic agent of aspergillosis in humans. It causes pulmonary, sinus, cerebral, bone, ocular, cardiovascular and other organ diseases especially in immunocompromised host.

**Pathogenicity:** *Aspergillus fumigatus* causes allergy (allergic broncho-pulmonary aspergillosis), local colonization (aspergilloma) and systemic infection (invasive aspergillosis). The fungus has a pronounced tendency to invade blood vessels (angioinvasion), which often causes fatal outcome.

### **Ecology:**

It is cosmopolitan in distribution; isolated from compost, soil and plant materials. Among various *Aspergilli*, *A. fumigatus* is the most common in nature with a wide range of temperature tolerance.

### **Laboratory Diagnosis:**

- a. **Culture** – At 25° C, colonies on Sabouraud's dextrose agar are blue – green, powdery and rapid growing, covering the entire surface of the plate in 48 h (**Fig.7**). Generally, all strains of *Aspergillus fumigatus* grow well at 45° C, which is used as a diagnostic feature for identification.
- b. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mount show septate hyphae with smooth – walled, green shaded conidiophores. Conidiophore ends in vesicle, which is subglobose in shape and its upper half portion is covered (columnar) with one series of sterigmata (uniseriate). Conidia produced from these sterigmata are round, smooth – delicately rough

walled and in chains (**Fig. 8**)

- c. **Differentiation from other *Aspergilli*** – *Aspergillus fumigatus* is differentiated from other *Aspergillus* species by blue – green colonies, columnar conidial heads with uniseriate sterigmata and good growth at 45° C.
- d. **Molecular tests** – For the molecular epidemiology of *Aspergillus fumigatus* three typing methods have been used: multi – locus enzyme electrophoresis, random amplified polymorphic DNA, and sequence – specific DNA primers (8).
- e. ***In vitro* susceptibility testing** – Susceptibility testing results indicate that isolates are variably susceptible to amphotericin B and to itraconazole, but more susceptible to newer azoles like voriconazole and posaconazole.

### **Comments:**

All labs correctly identified this important pathogen

### **Further reading:**

1. Asanza CG, Menchen PL, Senent C, Jimenez P, Perez I, Cos E. 2000. Esophageal aspergillosis: an unusual endoscopic finding. *Endoscopy*. 32 (1): S7.
2. Gabal MA, el – Sherif AM, Enany MS, Soliman SS. 1999. A polymerase chain reaction 'PCR' for a quick diagnosis of aspergillosis. *Mycoses*. 42 (9 – 10): 515 – 520.
3. Kaur R, Mittal N, Kakkar M, Aggarwal AK, Mathur MD. 2000. Ootomycosis: a clinicomycologic study. *Ear, Nose, & Throat J*. 79 (8): 606 – 609.
4. Kawamura S, Maesaki S, Omagari K, Hashiguchi K, Tomono K, Tashiro T, Kohno S. 1999. Invasive pulmonary aspergillosis diagnosed early by polymerase chain reaction assay. *Internal Medicine*. 38 (9): 744 – 746.
5. Rabkin JM, Orolloff SL, Corless CL, Benner KG, Flora KD, Rosen HR, Olyaei AJ. 2000. Association of fungal infection and increased mortality in liver transplant recipients. *Am J Surgery*. 179 (5): 426 – 430.
6. Latge JP. 1999. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Reviews*. 12(2): 310 – 350.
7. Rajesh GD, Sony N, Biswal N, Nalini P, Kanungo R, Kumar A. 2000. Perinephric abscess due to *Aspergillus fumigatus*. *Indian J of Pediatrics*. 67 (7): 545 – 546.

8. Rodriguez E, Symoens F, Mondon P, Mallie M, Piens MA, Lebeau B, Tortorano AM, Chaib F, Carlotti A, Villard J, Viviani MA, Chapuis F, Nolard N, Grillot R, Bastide JM. 1999. Combination of three typing methods for the molecular epidemiology of *Aspergillus fumigatus* infections. *J Medical Microbiol.* 48 (2): 181 –194.
9. Seki M, Maesaki S, Hashiguchi K, Tomiyama Y, Tomono K, Tashiro T, Kohno S. 2000. *Aspergillus fumigatus* isolated from blood samples of a patient with pulmonary aspergilloma after embolization. *Internal medicine.* 39 (2): 188 – 190.
10. Shaw CL, McCleave M, Wormald PJ. 2000. Unusual presentations of isolated sphenoid fungal sinusitis. *J of Laryngology & Otology.* 114 (5): 385 – 388.
11. Verweij PE, Brinkman K, Kremer HPH, Kullberg BJ, Meis JF. 1999. *Aspergillus meningitis: diagnosis by non – culture – based microbiological methods and management.* *J Clin Microbiol.* 37 (4): 1186 – 1189.

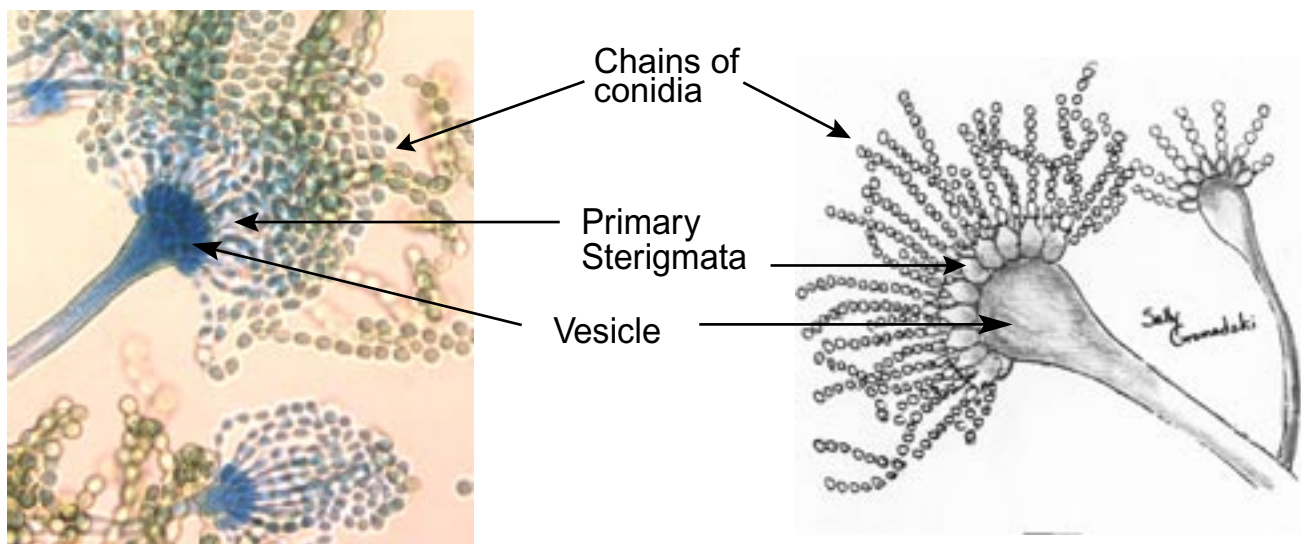
**Figure 7.**

Two-day-old, blue-green, powdery colony of *Aspergillus fumigatus* filling Sabouraud’s dextrose agar plate.



**Figure 8.**

Microscopic morphology of *Aspergillus fumigatus* showing typical, columnar conidiophores consisting of subglobose vesicle with uniseriate sterigmata and large chains of round conidia (left;400X magnification, right; line drawing not to scale).



## M – 5 *Aspergillus flavus*

Source: Bronchial wash

Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 80

Labs with Incorrect answers:

*Aspergillus glaucus* group: 1  
*Aspergillus* sp.: 1

### Clinical Significance:

*Aspergillus flavus* causes pulmonary and disseminated infection in immunocompromised patients. It is the second most common species causing aspergillosis. Occasionally, this fungus can cause infection of sinus, eye, ear and nails. *Aspergillus flavus* produces aflatoxins in certain foodstuff like peanuts that can cause mycotoxicosis.

### Pathogenicity:

*Aspergillus flavus* is angioinvasive, producing extensive damage to blood vessels, leading to infarction and necrosis.

### Ecology:

It is found worldwide in plants and organic materials.

### Laboratory Diagnosis:

- a. Culture – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar are yellow – green with pale yellow reverse, powdery and rapidly growing in 2 –3 days (**Fig.9**).
- b. Microscopic morphology – Lactophenol cotton blue or Calcofluor mount shows septate hyphae with roughened, colorless conidiophores. Conidiophores end in vesicle, which is globose and the entire surface is covered (radiating) with one series or two series of sterigmata (uni or biseriata). Conidia measuring 3 – 6 μm, produced from these sterigmata are round, rough walled and in chains (**Fig. 10**).

- c. Differentiation from other *Aspergilli* – *Aspergillus flavus* is differentiated from other *Aspergilli* by yellow – green colonies, rough walled conidiophores, radiating conidial heads with uniseriate or biseriata sterigmata.
- d. Molecular tests – A PCR based amplification of gene fragments – encoding alkaline proteases from *Aspergillus fumigatus* and *Aspergillus flavus* was used to detect these organisms in respiratory specimens (7). For molecular epidemiology, RAPD fingerprinting method has been used (1). Farber et al used a PCR based reaction to detect aflatoxigenic *Aspergillus flavus* strains in figs (2).
- e. In vitro susceptibility testing – Susceptibility testing results of clinical isolates of *Aspergillus flavus* show variable susceptibility to amphotericin B and to itraconazole.

### Comments:

All participating labs except two correctly identified *A. flavus*.

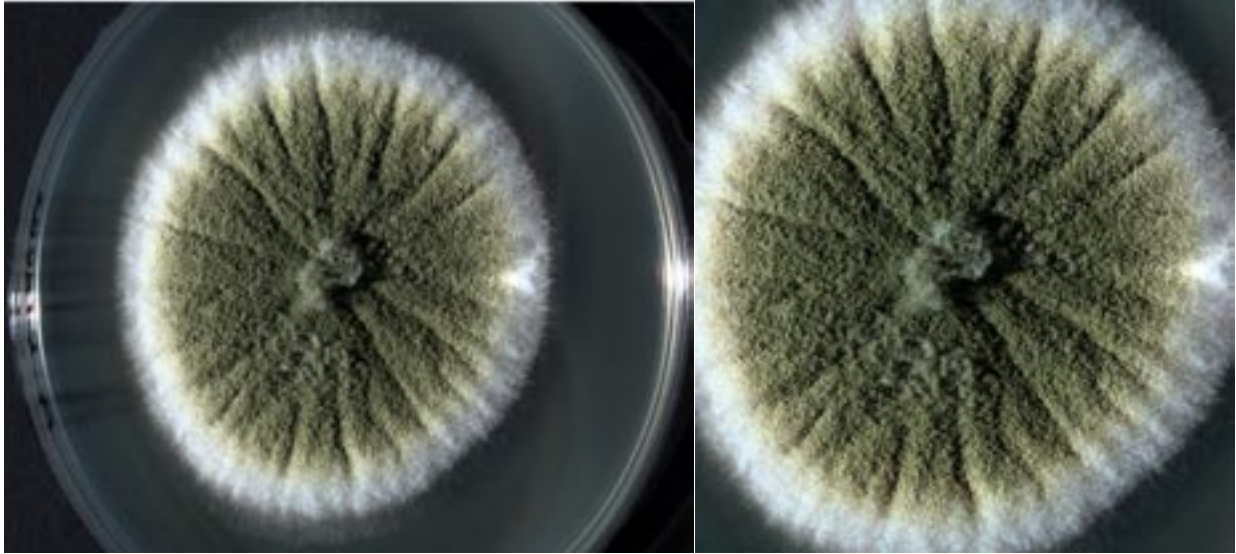
### Further reading:

1. Diaz – Guerra TM, Mellado E, Cuenca – Estrella M, Gaztelurrutia L, Navarro JI, Tudela JL. 2000. Genetic similarity among one *Aspergillus flavus* strain isolated from a patient who underwent heart surgery and two environmental strains obtained from the operating room. J Clin Microbiol. 38 (6): 2419 – 2422.
2. Farber P, Geisen R, Holzappel WH. 1997. Detection of aflatoxinogenic fungi in figs by a PCR reaction. International J of Food Microbiol. 36 (2-3): 215 – 220.
3. Gokahmetoglu S, Koc AN, Patiroglu T. 2000. Fatal *Aspergillus flavus* pericarditis in a patient with acute myeloblastic leukaemia. Mycoses. 43 (1-2): 65 –66.
4. Leenders A, van Belkum A, Janssen S, de Marie S, Kluytmans J, Wielenga J, Lowenberg B, Verbrugh H. 1996. Molecular epidemiology of apparent outbreak of invasive aspergillosis in a hematology ward. J Clin Microbiol. 34(2):345 –351.
5. Mori T, matsumura M, Yamada K, Irie S, Oshimi K, Suda K, Oguri T, Ichinoe M. 1998. Systemic aspergillosis caused by an aflatoxin – producing strain of *Aspergillus flavus*. Medical Mycology. 36 (2): 107 –112.
6. Sridhar MS, Garg P, Bansal AK, Gopinathan U. 2000. *Aspergillus flavus* keratitis after laser in situ keratomileusis. Am J of Ophthalmology. 129 (6): 802 – 804.

7. Tang CM, Holden DW, Aufauvre – Brown A, Cohen J. 1993. The detection of *Aspergillus* sp. By the polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. *Am Review of Respiratory Dis.* 148 (5): 1313 –1317.

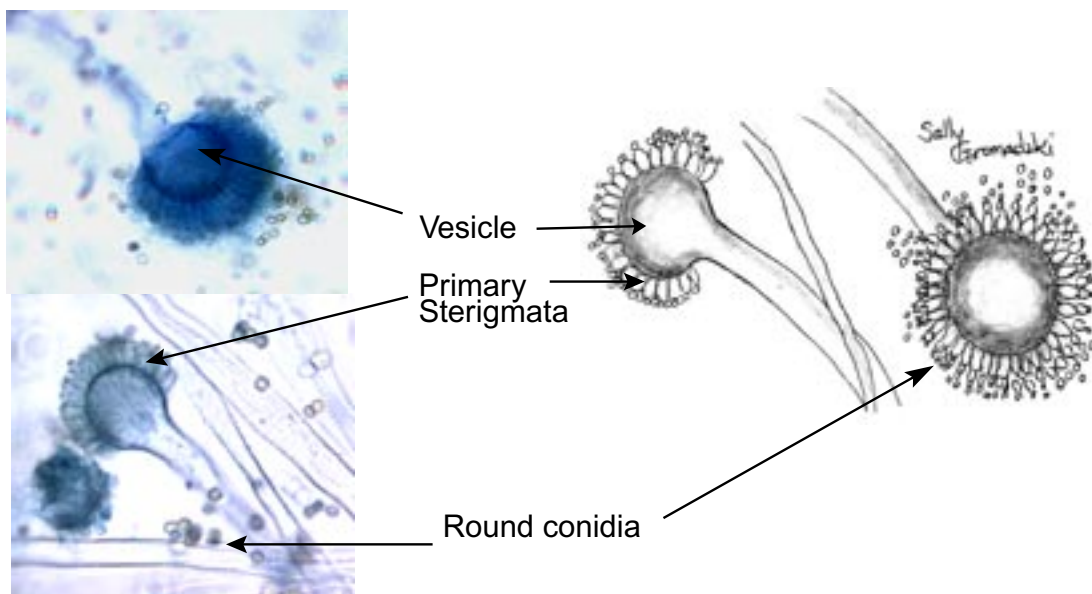
**Figure 9.**

Left, two-day-old, yellow-green colony of *Aspergillus flavus* on Sabouraud’s dextrose agar plate. Right, close up of the colony showing powdery texture.















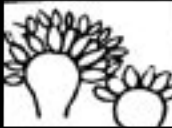


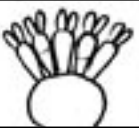











**Figure 10.**

Microscopic morphology of *Aspergillus flavus* depicting typical radiate heads with globose vesicle, uniseriate sterigmata, and round conidia (left; 400X magnification, right; line drawing not to scale).





**Scheme for differentiation of *Aspergilli*  
most commonly involved in human diseases.**

	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>A. nidulans</i>	<i>A. niger</i>	<i>A. terreus</i>	<i>A. versicolor</i>
Colony	Yellow - green	Blue - green	Dark- green	Black	Tan - buff	Pale - green
Conidiophores						
Vesicle						
Sterigmata						
Conidia						
Other Structures						

## Y-1 *Saccharomyces cerevisiae*

Source: Skin

Correct answers:

Referee Labs: 10/10  
Labs with correct ID: 70

### Clinical Significance:

*Saccharomyces cerevisiae* causes disseminated infection in immunocompromised hosts.

### Epidemiology:

It is cosmopolitan in distribution.

### Laboratory Diagnosis:

- a. Culture – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar are cream, smooth, dull butyrous in 3 –5 days (**Fig. 11**).
- b. Microscopic morphology– On corn meal agar with Tween – 80, round to oval yeast cells with no pseudohyphae or rudimentary pseudohyphae (**Fig. 12**). On special media like V – 8 agar or malt agar, characteristic ascospores encased in asci are seen.
- c. Differentiation from other yeasts - *Saccharomyces cerevisiae* ferments glucose, maltose and sucrose, does not grow on the media containing cycloheximide, and grows at 37<sup>o</sup> C. On the API 20C AUX, a specific assimilation biocode identifies this organism.
- d. Molecular tests - *Saccharomyces cerevisiae* is the most intensely studied model organism also being the first eukaryote to have its entire genome sequenced and mapped.
- e. In vitro susceptibility testing –Most isolates are susceptible to amphotericin B, 5 FC, and to azoles like fluconazole, miconazole, etc.

### Comments:

All labs identified this organism.

### Further Reading:

1. Barchiesi F, Arzeni D, Compagnucci P, Di Francesco LF, Giacometti A, Scalise G. 1998. In vitro activity of five antifungal agents against clinical isolates of *Saccharomyces cerevisiae*. *Medical Mycology*. 36 (6): 437 – 440.
2. Eng RH, Drehmel R, Smith SM, Goldstein EJ. 1984. *Saccharomyces cerevisiae* infections in man. *Sabouraudia*. 22 (5): 403 –407.
3. Fiore NF, Conway JH, West KW, Kleiman MB. 1998. *Saccharomyces cerevisiae* infections in children. *Pediatric Infectious Disease J*. 17 (12): 1177 –1179.
4. Konecny P, Drummond FM, Tish KN, Tapsall JW. 1999. *Saccharomyces cerevisiae* oesophagitis in an HIV – Infected patient. *International J STD & AIDS*. 10 (12): 821 –822.
5. McCullough MJ, Clemons KV, Farina C, McCusker JH, Stevens DA. 1998. Epidemiological investigation of vaginal *Saccharomyces cerevisiae* isolates by a genotypic method. *J Clin Microbiol*. 36 (2): 557 –562.
6. Posteraro B, Sanguinetti M, Masucci L, Romano L, Morace G, Fadda G. 2000. Reverse cross blot hybridization assay for rapid detection of PCR – amplified DNA from *Candida* species, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* in clinical samples. *J Clin Microbiol*. 38 (4): 1609 – 1614.
7. Ramani R, Gromadzki S, Pincus DH, Salkin IF, Chaturvedi V. 1998. Efficacy of API 20C and ID 32C systems for identification of common and rare clinical yeast isolates. *J Clin Microbiol*. 36 (11): 3396 – 3398.
8. Xu J, Boyd CM, Livingston E, Meyer W, Madden JF, Mitchell TG. 1999. Species and genotypic diversities and similarities of pathogenic yeasts colonizing women. *J Clin Microbiol*. 37 (12): 3835 – 3843.

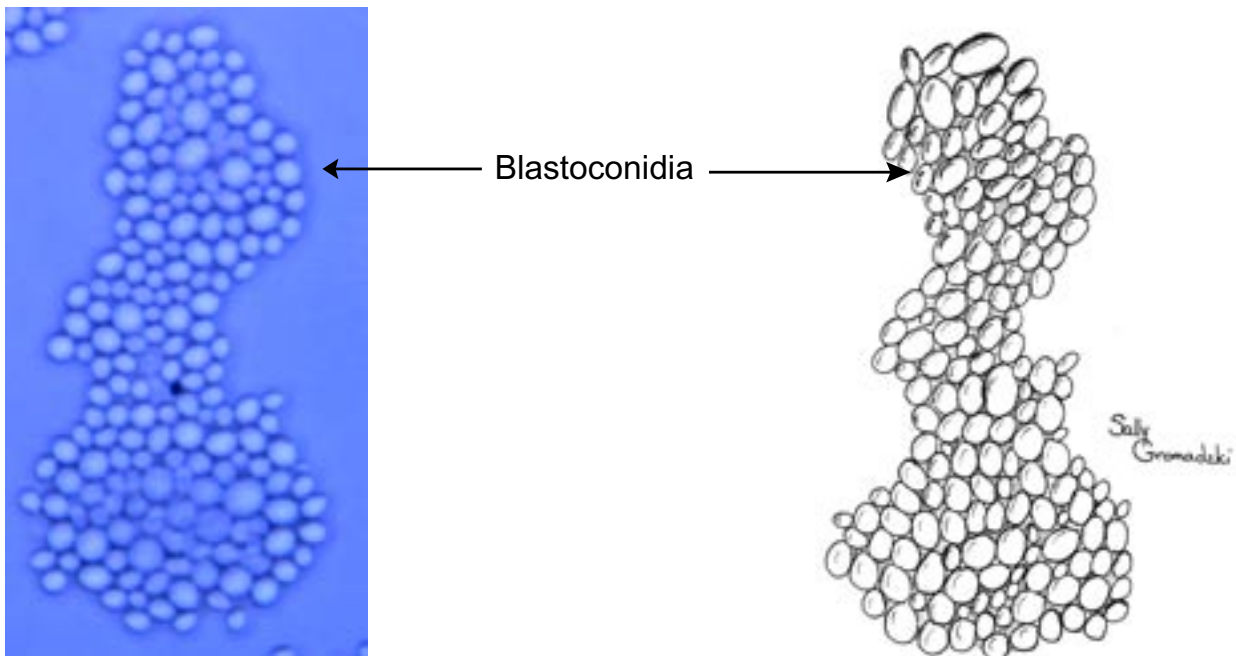
**Figure 11.**

Four-day-old creamish white, butyrous, raised colony of *Saccharomyces cerevisiae* on Sabouraud's dextrose agar.



**Figure 12.**

Microscopic morphology of *Saccharomyces cerevisiae* on cornmeal agar showing round, oval blastoconidia (left; 200X magnification, right; line drawing not to scale).



## Y-2 *Candida parapsilosis*

Source: Catheter tip

Correct answers:

Referee Labs: 10/10  
Labs with correct ID: 70/70

### Clinical Significance:

*Candida parapsilosis* is frequently isolated from blood and catheter tips in clinical labs. It is implicated in candidal endocarditis, endophthalmitis, fungemia, and infection in burn patients. It is an important nosocomial pathogen in various hospital outbreaks like neonatal fungemia, endophthalmitis after cataract surgery, etc.

### Ecology:

It is found on the skin of humans and other mammals, fruit juices, water.

### Laboratory Diagnosis:

- a. Culture – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar are white to cream in color, smooth to wrinkle in 2 – 5 days (**Fig. 13**).
- b. Microscopic morphology – On corn meal agar with Tween 80, long, multibranched, pseudohyphae with clusters of blastoconidia are seen (**Fig. 14**).
- c. Differentiation from other yeasts - Biochemically, *Candida parapsilosis* ferments only glucose, does not grow on media containing cycloheximide, grows at 37<sup>o</sup> C, and is urea and nitrate negative. Microscopically – it forms long pseudohyphae that differentiates it from *Candida lusitanae*.
- d. Molecular tests – PCR assay of the ITS1 and ITS2 region of ribosomal DNA was used to identify *Candida parapsilosis* in clinical specimens (7). Chromosome length polymorphism and random amplified polymorphic DNA procedures were used to characterize the genetic

diversity of this organism (3, 4, and 8).

- e. In vitro susceptibility testing – *Candida parapsilosis* is susceptible to amphotericin B, 5FC, and various azoles like itraconazole, ketoconazole and fluconazole. Few clinical isolates are resistant to fluconazole.

### Comments:

This clinical isolate has been identified to genus and species level by all participating labs.

### Further Reading:

1. Costa SF, Marinho I, Araujo EA, Manrique AE, Medeiros EA, Levin AS. 2000. Nosocomial fungemia: a 2 – year prospective study. *J Hospital Infect.* 45 (1): 69 –72.
2. Darwazah A, Berg G, Faris B. 1999. *Candida parapsilosis*: an unusual organism causing prosthetic heart valve infective endocarditis. *J Infect.* 38 (2): 130 –131.
3. Dassanayake RS, Samaranyake LP. 2000. Characterization of the genetic diversity in superficial and systemic human isolates of *Candida parapsilosis* by randomly amplified polymorphic DNA (RAPD). *APMIS.* 108 (2): 153 – 160.
4. Fernando PH, Samaranyake LP. 1998. Chromosome length polymorphism in clinical isolates of *Candida parapsilosis*. *APMIS* 106 (10): 941 –946.
5. Fujita S, Hashimoto T. 2000. DNA fingerprinting patterns of *Candida* species using *HinfI* endonuclease. *International J Systematic & Evolutionary Microbiology.* 50 (3): 1381 – 1389.
6. Kappstein I, Krause G, Hauer T, Jonas D. 1998. Pseudo – outbreak of candidaemia with *Candida parapsilosis*. *J Hospital Infect.* 40 (2): 164 –165.
7. Krcmery V Jr, Spanik S, Grausova S, Trupl J, Krupova I, Salek T, Sufliarsky J, Mardiak J. 1998. *Candida parapsilosis* fungemia in cancer patients – incidence, risk factors and outcome. *Neoplasma.* 45 (5): 336 – 342.
8. Riederer K, Fozo P, Khatib R. 1998. Typing of *Candida albicans* and *Candida parapsilosis*: species – related limitations of electrophoretic karyo typing and restriction endonuclease analysis of genomic DNA. *Mycoses.* 41 (9 – 10): 397 – 402.
9. Trizna Z, Chen SH, Lackhart S, Lundquist KF, Smith EB, Wagner RF. 2000. *Candida parapsilosis* chondritis successfully treated with oral fluconazole. *Archives Dermatology.* 136 (6): 804.
10. Wong PN, Mak SK, Lo KY, Tong GM, Wong AK. 2000. A retrospective study of seven cases of *Candida parapsilosis* peritonitis in CAPD patients: the therapeutic implications. *Peritoneal Dialysis International.* 20 (1): 76 – 79.

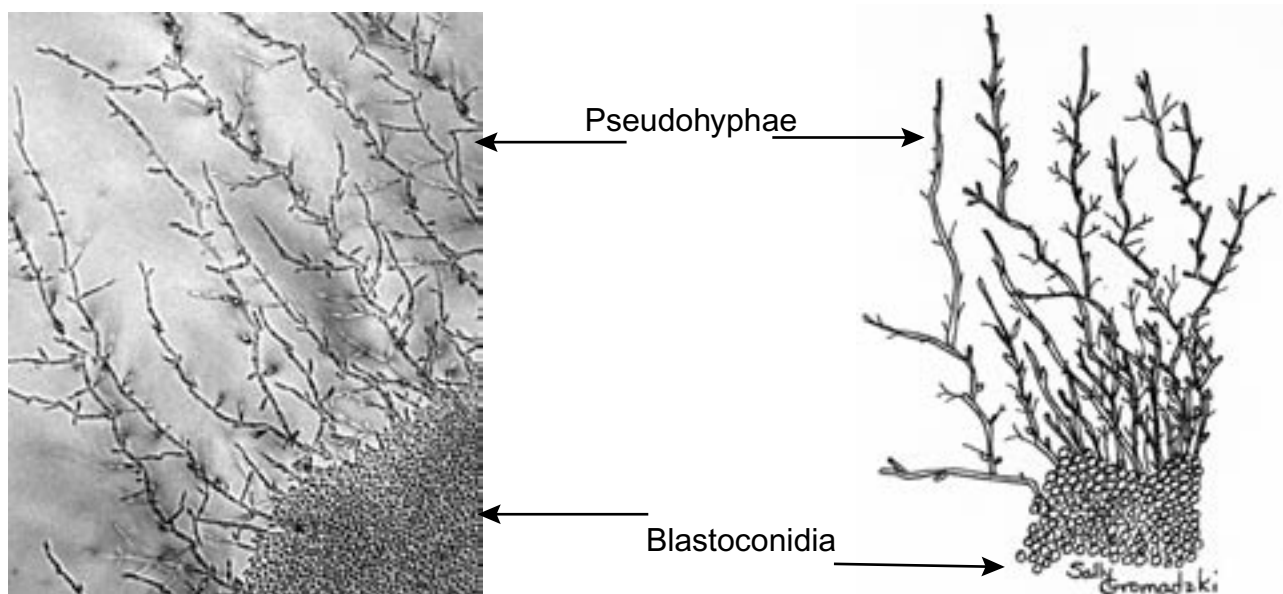
**Figure 13.**

Four-day-old, creamish white, butyrous, smooth colony of *Candida parapsilosis* on Sabouraud's dextrose agar.



**Figure 14.**

Microscopic morphology of *Candida parapsilosis* on cornmeal agar showing pseudohyphae and blastoconidia (left; 200X magnification, right; line drawing not to scale).



## Y-3 *Cryptococcus terreus*

Source: Eye

### Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 69

### Labs with Incorrect answer:

*Candida parapsilosis*: 1

### **Clinical Significance:**

*Cryptococcus terreus* is not a known pathogen; it is occasionally isolated as a contaminant in clinical labs.

### **Ecology:**

*Cr. terreus* has worldwide distribution in soil.

### **Laboratory Diagnosis:**

- a. Culture – At 25<sup>o</sup> C colonies, on Sabouraud's dextrose agar are smooth, soft but not mucoid, white turning tan with age (**Fig. 15**).
- b. Microscopic morphology – Corn meal agar with Tween 80 shows round to ovoid cells either singly or in groups and no pseudohyphae (**Fig. 16**).
- c. Differentiation from other yeasts- Biochemically, *Cryptococcus terreus* closely resembles *Cryptococcus albidus*. Both organisms are urea positive, assimilate nitrate, and do not grow on media containing cycloheximide, with maximum growth temperature of 35<sup>o</sup> C. However, *Cr. terreus* does not assimilate sucrose and melezitose while *Cr. albidus* assimilates both.
- d. Molecular tests – No information available
- e. In vitro susceptibility testing – No information available.

### **Comments:**

Eight labs reported two organisms from this specimen. The mixed organisms were either *Candida parapsilosis* or *Candida tropicalis*. All the referee labs reported only single organism i.e. *Cr. terreus*. Use of good laboratory practices can eliminate cross-contamination of specimens.

### **Further Reading:**

1. de Minna, ME. 1954. *Cryptococcus terreus* n. sp. from soil in New Zealand. J Gen. Microbiol. 11: 195 –197.
2. Fell JW and StatzellTallman A. 1992. Systematic placement of the basidiomycetous yeast *Cystofilobasidium lari* – Marini comb. Nov. as predicted by rRNA nucleotide sequence analysis. Antonie van Leeuwenhoek. 62: 209 – 213.
3. Spencer JFT, Gorin PAJ, Tulloch AP. 1970. *Torulopsis bombicola* sp. n. Antonie van Leeuwenhoek. 36: 129 –133.

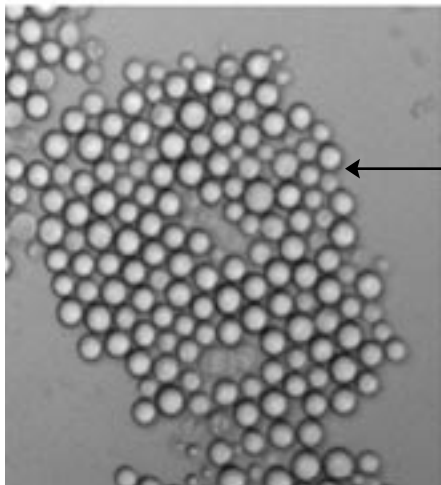
**Figure 15.**

Four-day-old, non-mucoid, tan colony of *Cryptococcus terreus* on Sabouraud's dextrose agar.

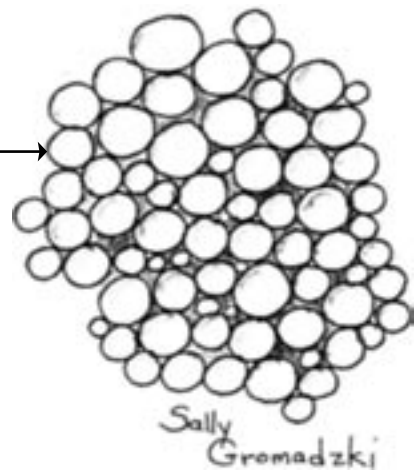


**Figure 16.**

Microscopic morphology of *Cryptococcus terreus* on corn meal agar showing round blastoconidia (left; 200X magnification, right; line drawing not to scale).



Blastoconidia



## Y-4 *Candida kefyr*

Source: Blood

### Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 69

### Labs with Incorrect ID:

*Malassezia pachydermatis*: 1

### **Clinical Significance:**

*Candida kefyr* rarely causes infection in humans. It has been isolated from blood, vagina, urine, etc.

### **Ecology:**

It is found in dairy products, grains and on other mammals.

### **Laboratory Diagnosis:**

- a. Culture – At 25° C, colonies on Sabouraud's dextrose agar are smooth, dull, soft, butyrous turning yellow with age (**Fig.17**).
- b. Microscopic morphology – On cornmeal agar with Tween 80, long, abundant pseudohyphae bearing oval to elongate blastoconidia are seen (**Fig. 18**). On special media like V - 8 agar or malt agar, *C. kefyr* produces asci containing ascospores.
- c. Differentiation from other yeasts - Biochemically, *Candida kefyr* ferments glucose, sucrose, and lactose. It grows on media containing cycloheximide, with maximum growth temperature of 45° C.
- d. Molecular tests –No information available.
- e. In vitro susceptibility testing – Almost all isolates are susceptible to amphotericin B but some strains are less susceptible against fluconazole.

### **Comments:**

All the labs except one identified this organism as *Candida kefyr*. The older, obsolete name *Candida pseudotropicalis* was used by one lab.

### **Further Reading:**

1. Neoff P, Oswald U, Hausteil UF. 1999. In vitro susceptibility of yeasts for fluconazole and itraconazole. Evaluation of a microdilution test. *Mycoses*. 42 (11 – 12): 629 –639.
2. Farina C, Vailati F, Manisco A, Goglio A. 1999. Fungaemia survey: a 10 year experience in Bergamo, Italy. *Mycoses*. 42 (9 –10): 543 –548.
3. Carrillo – Munoz AJ, Quindos G, Tur C, Ruesga MT, Miranda Y, del Valle O, Cossum PA, Wallace TL. 1999. In vitro antifungal activity of liposomal nystatin in comparison with nystatin, amphotericin B cholesteryl sulphate, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B desoxycholate, fluconazole and itraconazole. *J of Antimicrobial Chemotherapy*. 44 (3): 397 –401.
4. Abu –Elteen KH, Abdul Malek AM, Abdul Wahid NA. 1997. Prevalence and susceptibility of vaginal yeast isolates in Jordan. *Mycoses*. 40 (5 – 6): 179 –185.
5. Tumbarello M, Caldarola G, Tacconelli E, Morace G, Posteraro B, Cauda R, Ortona L. 1996. Analysis of the risk factors associated with the emergence of azole resistant oral candidosis in the course of HIV infection. *J Antimicrobial Chemotherapy*. 38 (4): 691 – 699.
6. Listermann H, Schulz KD, Wasmuth R, Begemann F, Meigel W. 1998. Oesophagitis caused by *Candida kefyr*. *Mycoses*. 41 (7 –8): 343 – 344.
7. Ramani R, Gromadzki S, Pincus DH, Salkin IF, Chaturvedi V. 1998. Efficacy of API 20C and ID 32C systems for identification of common and rare clinical isolates. *J Clin Microbiol*. 36 (11): 3396 –3398.

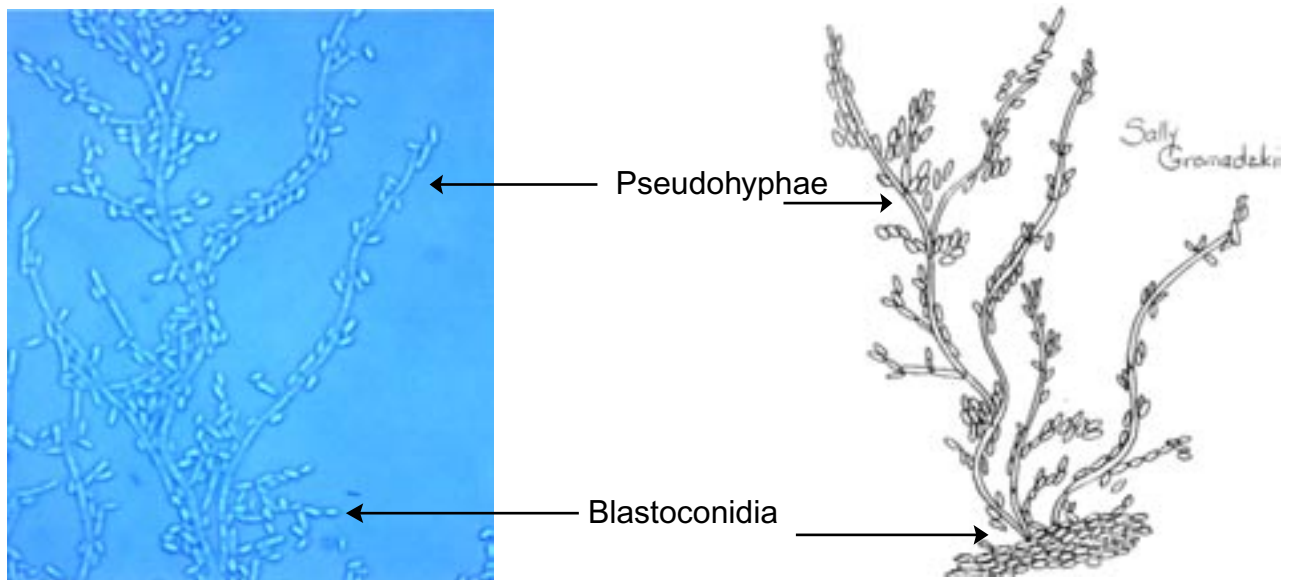
**Figure 17.**

Four-day-old, creamish yellow, butyrous colony of *Candida kefyr* on Sabouraud's dextrose agar.



**Figure 18.**

Microscopic morphology of *Candida kefyr* on cornmeal agar showing abundant pseudohyphae with oval blastoconidia (left; 200X magnification, right; line drawing not to scale).



## Y-5 *Candida zeylanoides*

Source: Urine

Correct answers:

Referee Labs:	10/10
Labs with correct ID	70

### Clinical Significance:

It is a relatively rare pathogen in humans. In immunocompromised patients, *C. zeylanoides* causes fungemia, endocarditis, and arthritis. In immunocompetent patients, it causes skin and nail infections.

### Ecology:

It is cosmopolitan found in water, meat, and on human body.

### Laboratory Diagnosis:

- a. Culture – At 25<sup>o</sup> C, *Candida zeylanoides* produces smooth, cream – colored, butyrous raised colonies (**Fig. 19**).
- b. Microscopic morphology – On corn meal agar with Tween 80, *Candida zeylanoides* forms long pseudohyphae with verticillate, ovoid blastoconidia (**Fig. 20**). Blastoconidia are produced in whorls around the pseudohyphae.
- c. Differentiation from other yeasts - *C. zeylanoides* does not ferment any carbohydrates, grows at 37<sup>o</sup> C, grows on media containing cycloheximide, and assimilates limited carbohydrates.
- d. Molecular tests – No information available
- e. In vitro susceptibility testing – *C. zeylanoides* is susceptible to amphotericin B and to the commonly used azoles.

### Comments:

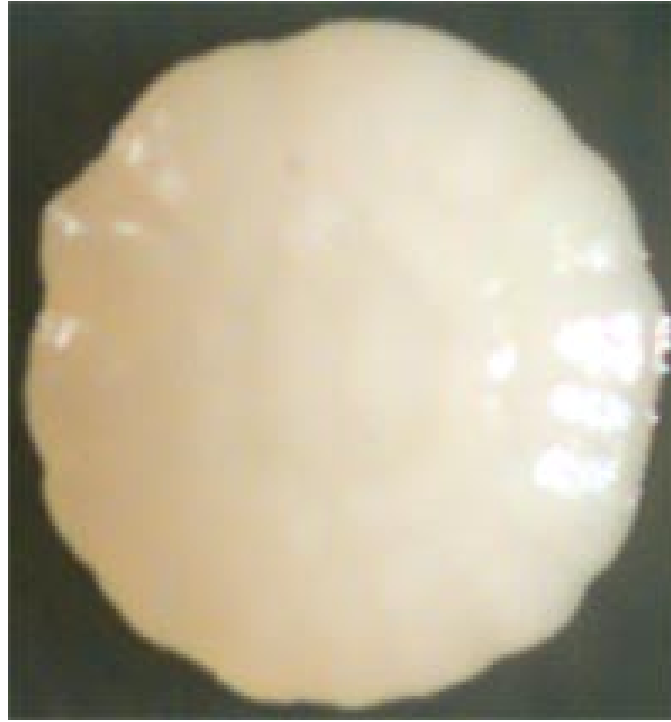
All the participating labs identified this organism to the species level.

### Further Reading:

1. Bisbe J, Vilardell J, Valls M, Moreno A, Brancos M, Andreu J. 1987. Transient fungemia and Candida arthritis due to *Candida zeylanoides*. European J Clin Microbiol. 6 (6): 668 –669.
2. Crozier WJ. 1993. Two cases of onychomycosis due to *Candida zeylanoides*. Australasian J Dermatology. 34 (1): 23 –25.
3. Levenson D, Pfaller MA, Smith MA, Hollis R, Gerarden T, Tucci CB, Isenberg HD. 1991. *Candida zeylanoides*: another opportunistic yeast. J Clin Microbiol. 29 (8): 1689 –1692.
4. Liao WQ, Li ZG, Guo M, Zhang JZ. 1993. *Candida zeylanoides* causing candidiasis as tinea cruris. 1993. Chinese Medical J. 106 (7): 542 – 545.
5. Ramani R, Gromadzki S, Pincus DH, Salkin IF, Chaturvedi V. 1998. Efficacy of API 20C and ID 32C systems for identification of common and rare clinical isolates. J Clin Microbiol. 36 (11): 3396 –3398.
6. Whitby S, Madu EC, Bronze MS. 1996. *Candida zeylanoides* infective endocarditis complicating infection with the human immunodeficiency virus. Am J the Medical Sciences. 312 (3): 138 –139.

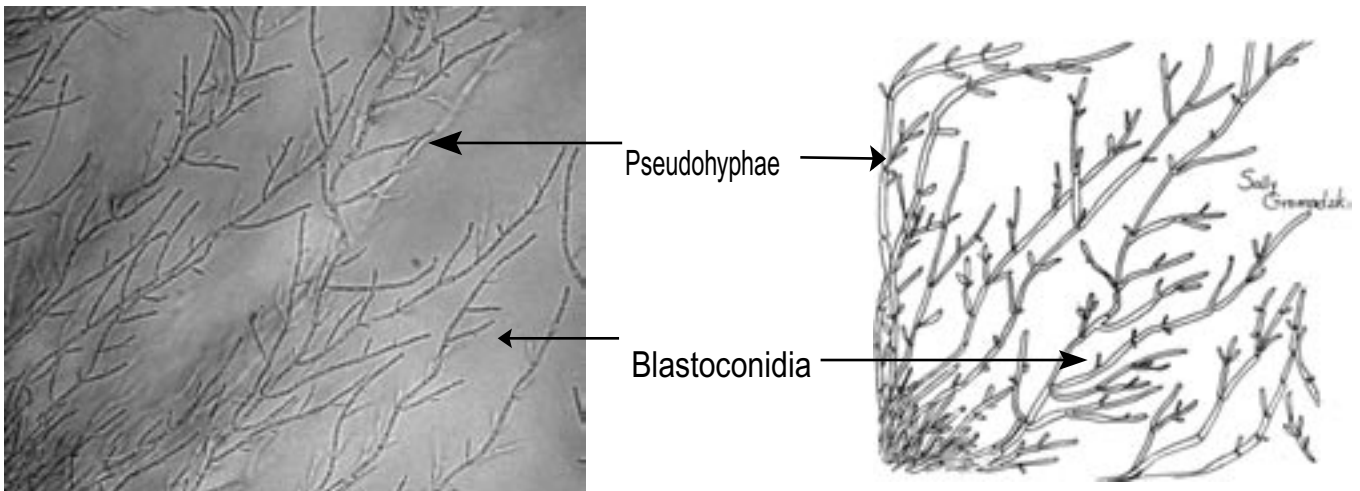
**Figure 19.**

Four-day-old, creamish white, butyrous, raised colony of *Candida zeylanoides* on Sabouraud's dextrose agar.



**Figure 19.**

Microscopic morphology of *Candida zeylanoides* on cornmeal agar showing long pseudohyphae with verticillate, ovoid blastoconidia (left; 100X magnification, right; line drawing not to scale).



# BIBLIOGRAPHY

1. CP Kurtzman, and JW Fell. 1998. *The Yeasts, a taxonomic study*. 4th ed. Elsevier, New York, NY.
2. DA Sutton, AW Fothergill, and MG Rinaldi. 1998. *Guide to Clinically Significant Fungi*. Williams and Wilkins, Baltimore.
3. DH Larone. 1995. *Medically Important Fungi – A Guide to Identification*. 3rd Edition, ASM Press, Washington, D.C.
4. E Kiffer, and M Morelet. 1999. *The Deuteromycetes – Mitosporic Fungi, Classification and Generic Keys*. Science Publishers Inc.
5. ES Beneke, and AL Rogers. 1996. *Medical Mycology and Human Mycoses*. Star Publishing Company.
6. F Fisher and NB Cook. 1998. *Fundamentals of Diagnostic Mycology*. W.B. Saunders Company, Philadelphia.
7. G St-Germain and R Summerbell. 1996. *Identifying Filamentous Fungi – A Clinical laboratory handbook*. Star Publishing Company.
8. GL Barron. 1968. *The Genera of Hyphomycetes from Soil*. Williams and Wilkins Co., Baltimore.
9. HL Barnett, and BB Hunter. 1987. *Illustrated genera of Imperfect Fungi*. 4th ed. Macmillan Publishing Co. New York.
10. JA Arx von. 1981. *The Genera of Fungi Sporulating in Pure Culture*. 3rd ed. J. Cramer, Vaduz, Germany.
11. JA Barnett, RW Payne, D Yarrow. 2000. *Yeasts: Characteristics and Identification*. 3rd ed. Cambridge University Press, UK.
12. JC Gilman. 1957. *A Manual of Soil Fungi*. 2nd ed. Iowa State University Press, Ames, Iowa.
13. JW Carmichael, WB Kendrick, IL Conners, L Sigler. 1980. *Genera of Hyphomycetes*. University of Alberta Press, Edmonton, Alberta, Canada
14. JW Rippon. 1988. *Medical Mycology – The Pathogenic Fungi and the Pathogenic Actinomycetes*. W.B. Saunders Company, Philadelphia.
15. KB Raper, and DI Fennell. 1973. *The Genus Aspergillus*. Robert E. Krieger Publishing Company, Huntington, New York.
16. KH Domsch, W Gams, TH Anderson. 1980. *Compendium of Soil Fungi*, Vols. 1 and 2. Academic Press. New York.
17. KJ Kwon-Chung, JE Bennett. 1992. *Medical Mycology*. Lea & Febiger, Philadelphia.
18. MB Ellis. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.
19. ME Kern and KS Blevins. 1997. *Medical Mycology – A Self-Instructional Text*. 2nd Edition. F.A. Davis Company, Philadelphia
20. MR McGinnis. 1980. *Laboratory Handbook of Medical Mycology*. Academic Press, New York.
21. PR Murray, EJ Baron, MA Pfaller, FC Tenover, RH Tenover. 1998. *Manual of clinical Microbiology*. Seventh Edition. ASM Press, Washington, D.C.
22. R Hamlin. 1990. *Illustrated Genera of Ascomycetes*. APS press. The American Phytopathological Society. St. Paul, Minnesota
23. WB Kendrick, JW Carmichael. 1973. *Hyphomycetes*. In Ainsworth GCM Sparrow FK, Sussman AS. (eds). *The Fungi*. Vol. IVA. Academic Press, New York, 323-509.

**Cover and interior design: Stuart Lehrman of Wadsworth Center Photo and Illustration Department**