대한의진균학회
제10차 Workshop 초록

◐ 일 시 : 2014년 10월 25일(토)

◐ 장 소 : 서울 건국대병원 대강당

주최 : 대한의진균학회
대한피부과학회 피부진균연구회

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대한의진균학회 제10차 Workshop 진행계획표

일 시 : 2014년 10월 25일(토)
장 소 : 서울 건국대병원 대강당

13:30 ~ 14:00	등 록	
14:00 ~ 14:10	개회식	회장 : 안규중 교수 (건국의대)
14:10 ~ 15:40	강 의	
		좌장 : 유희준 (한양의대)
	Dermatophyte의 동정	
	.....	최종수 교수 (영남의대)
		좌장 : 서무규 (동국의대)
	*Candida*의 동정	
	.....	이미경 교수 (중앙의대)
		좌장 : 안규중 (건국의대)
	*Malassezia*의 동정	
	.....	이양원 교수 (건국의대)
15:40 ~ 16:00	Coffee Break	
16:00 ~ 17:00	조별 실습 (dermatophytes & Candida)	
17:00 ~ 17:20	질의 및 응답	
17:20 ~ 17:30	폐회식	

Dermatophytes 동정

최 종 수

영남대학교 의과대학 피부과학교실

진균 동정이 필요한 이유

- 진단
- 항진균제의 선택
- Epidemiology
  - 감염경로 : 동물 →사람, 사람 → 사람,
  - 지역 분포
  - 새로운 균종의 유입
  - 균종의 변화 : 증감, 소멸
  - 동일 개체 내 이동

Contents

Introduction of mycology
Identification of dermatophytes
Practice

Introduction of mycology

Kingdom of fungus
Eukaryotes
Excretion of enzyme- aspiration
Cell wall : glucan, chitin
10만 종
- 온열동물에 병원균 100종
- 기회감염 - 수백종

Terminology

- Mould (mold) : 사상균 filamentous fungi
  - Dermatophyte
  - Non dermatophytic mould
- Yeast : 효모균 unicellular growth form

- Dematiaceous : pigmented in olivaceous or brown colours
- Phaeo- : darkly pigmented
- Hyalin : colorless

- Dermatomycosis : 피부곰팡이증
- Dermatophytosis = tinea : 백선. 피부사상균에 의한 피부의 표재성 감염
- Onychomycosis : 손발톱곰팡이증
- Tinea unguium : 손발톱백선

- Anamorph : 불완전세대형, 무성생식형 asexual form of sporulation
- Teleomorph : 완전세대형, 유성생식형 sexual form of sporulation. nuclear recombination

- Spore: 포자, 홀씨 sexual propagule; general term
- Conidium(pl. conidia): 분생홀씨, 분생포자, 분생자 asexual propagule
- Microconidia, macroconidia
- Chlamidoconidium : 후막포자
- arthroconidium: 분절포자

- Hypha (pl. hyphae): 균사 septate, thread-like fungal element
- Pseudohypha (pl. Pseudohyphae) : 거짓균사 string of budding cells. No cytoplasmic connection

- Hypha (pl. hyphae) - mycelium (mycelia) 균사체– thallus 영상체– colony 집락

Taxonomy of fungus

Class	Hyphae	Sexual spore	Asexual spore
Zygomycetes	aseptated	zygospore	sporangium
*Ascomycetes**	septated	ascospore	conidium
Basidiomycetes	septated	basidiospore	conidium
Deuteromycetes	septated	not found	conidium

Reproduction

- Anamorph - asexual stage
- Teleomorph - sexual spore: ascospore: + or –

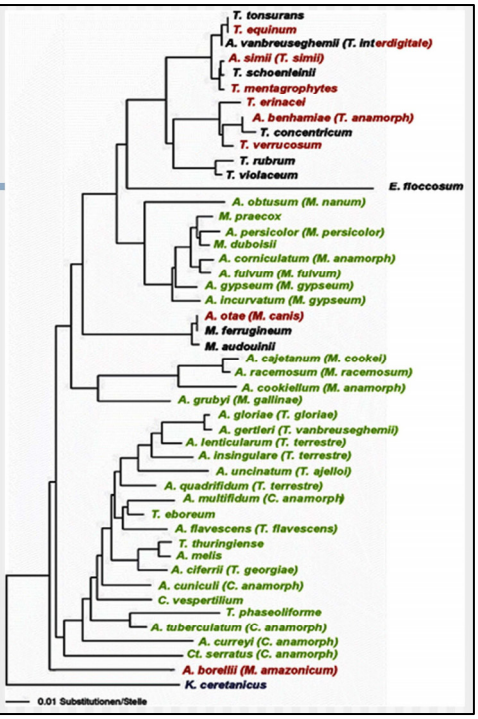
- *T. mentagrophytes*
  - var. *mentagrophytes* — *Arthroderma benhamiae*
  - var. *interdigitale* — *Arthroderma vanbreuseghemii*

Dermatophytes

- Taxonomy
  Ascomycota; Pezizomycotina;
  Eurotiomycetes; Eurotiomycetidae;
  Onygenales; Arthrodermataceae

- Classification
  - Anamorph state — Teleomorph state
    Trichophyton
    Microsporum — *Arthroderma*
    Epidermophyton

- Classification by host
  - Anthropophilic (black)
  - Zoophilic (red)
  - Geophilic (green)



by Graeser Y (2008)

우리나라에서 확인된 백선균

	서순봉 (1976-1995)	2000
T. rubrum	81.7 %	90.0%
T. mentagrophytes	5.8 %	7.9%
M. canis	5.7 %	1.4%
E. floccosum	1.0 %	0.1%
T. verrucosum	0.6 %	0.1%
M. gypseum	0.2 %	0.2%
M. ferrugineum	0.04%	
T. tonsurans	0.03%	0.3%
T. violaceum		

최근

Trichophyton(T.)
- *T. rubrum, T. mentagrophytes*
- *T. tonsurans, T. verrucosum*
- *T. erinacei*
- *T. violaceum*
- *T. schoenleinii*

Microsporum
- *M. canis,*
- *M. gypseum*
- *M. ferrugineum*

Epidermophyton
- *E. floccosum*

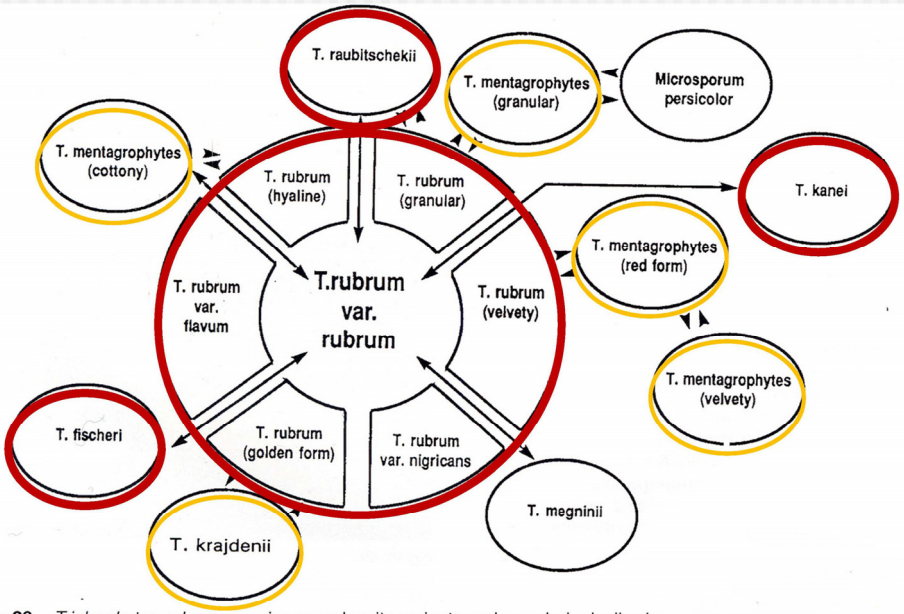
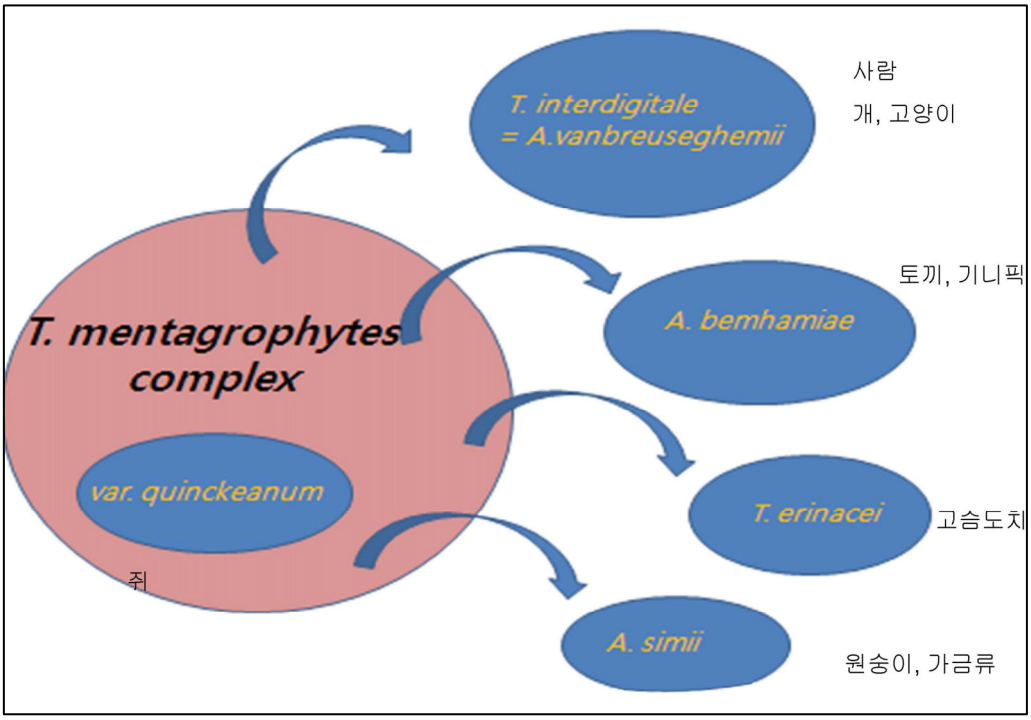


Figure 26. *Trichophyton rubrum* species complex; its variants and morphologically similar species. Note, the close apparent relationship among the common dermatophytes. The BCP Milk Solids Glucose agar was essential in establishing this revised taxonomy.



진균 동정

❖방법

- KOH 도말검사
- **진균배양검사***

- 생리검사
- 면역학적 검사
- 분자생리학적 방법

PDACT 배지

Potato Dextrose Agar-Corn meal-Tween 80

❖ 1986년 경북의대 서순봉 교수님이 개발

❖ 백선균 동정에 적합하다. (↔ SDA)

- *T.rubrum* –특유의 붉은 색, KOH/증류수 흡수
- 분색자를 잘 형성
- *T. mentagrophytes*의 아형 구분
- 융모변성 억제
- *C. albicans* 후막포자 촉진

❖ 조성

Potato dextrose agar (Oxoid)	20g
Corn mel agar (Difco)	20g
Peptone	4g
Tween 80	6ml
증류수	
	1 L

진균배양검사

❖ 육안적 관찰

- 성장속도
- 표면과 배면의 성상
- 대부분에서 동정 가능

❖ 현미경적 관찰

- Microconidia - *Trichophyton*
- Macroconidia- *Microsporum, Epidermophyton*
- Hyphae

❖ 생리적 검사

- Hair perforation test
- Urease test
- Growth factor requirement

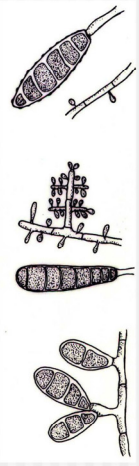
현미경 소견

❖ Hyphae
- Septation
- Pattern : spiral, raquet, chandelier, nodular body
- Pigmentation
- Vesicles or swollen cells

❖ Conidium (conidia)
- asexual reproduction ↔ spore
- Macroconidia
  Surface, number of septum, shape, wall thickness, size
- Microconidia
  Shape, group, 균사에 부착 양상

Dermatophytes

	Macroconidia	Microconidia	Involved
Microsporum spp.	numerous spindle-shaped thick walled spiny surface	numerous	skin, hair
Trichophyton spp.	rare pencil or fusiform thin walled smooth surface	Numerous 균에 따라 특징적	skin, hair, nail
Epidermophyton spp.	numerous boat-shaped thick or thin walled smooth surface	not produced	skin, nail

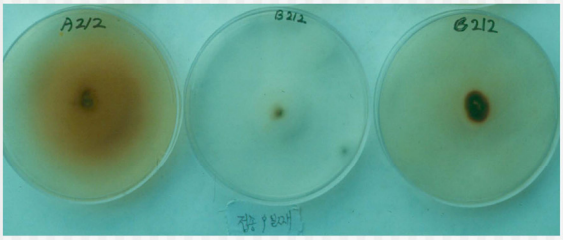


Contents

Classification of fungi
Identification of dermatophytes
Practice

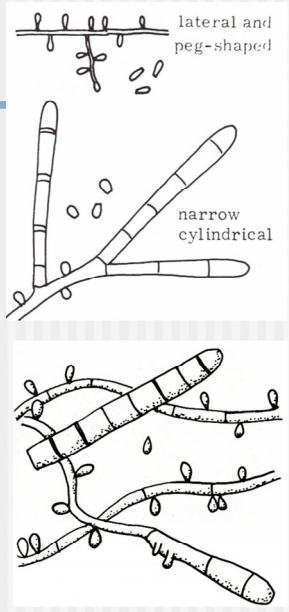
T. rubrum

- 성장속도 : slow 14일
- Colony 형태
  - 표면 : fluffy, white to buff
  - 배면 : **붉은 포도주색** >brown, yellow, no-color
  - 예외 :오염, 나쁜 배지

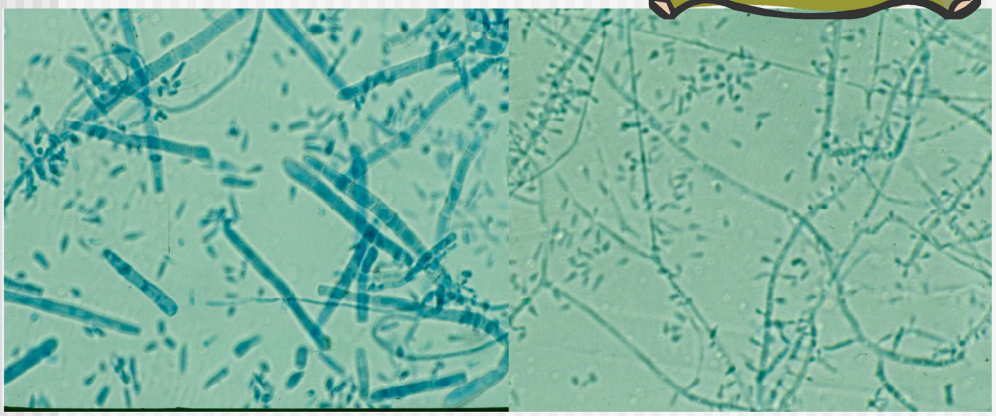


T. rubrum

❖ 현미경 소견
- Microconidia
  - Numerous to rare
  - Solitary along hyphae
  - tear-shaped
- Macroconidia
  - rare; pencil-shaped

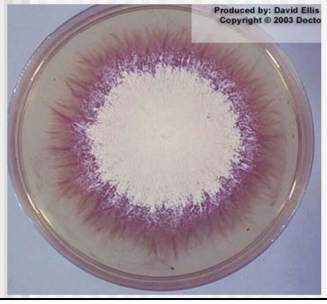


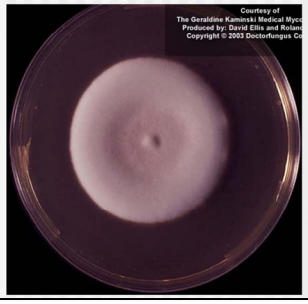
T. rubrum

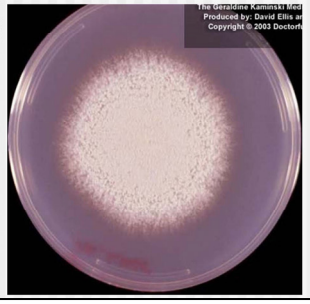


T. mentagrophytes (T. interdigitale)

- ❖ 성장속도 : moderate, 7-10일
- ❖ Colony 형태 : 매우 다양
  - zoophilic-과립형(granular)
  - anthropophilic –융모형(downy), 분말형(powdery), 복숭아색형(persicolor)
  - Powdery form : Radial or concentric fold
  - 배면 : brown > colorless, yellow, red







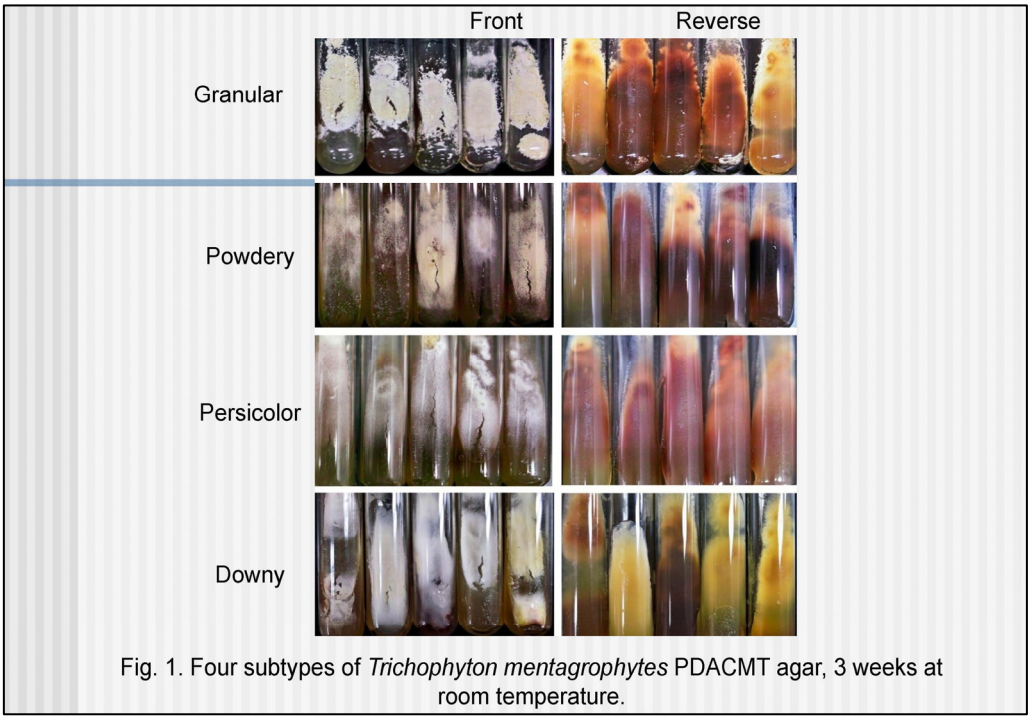
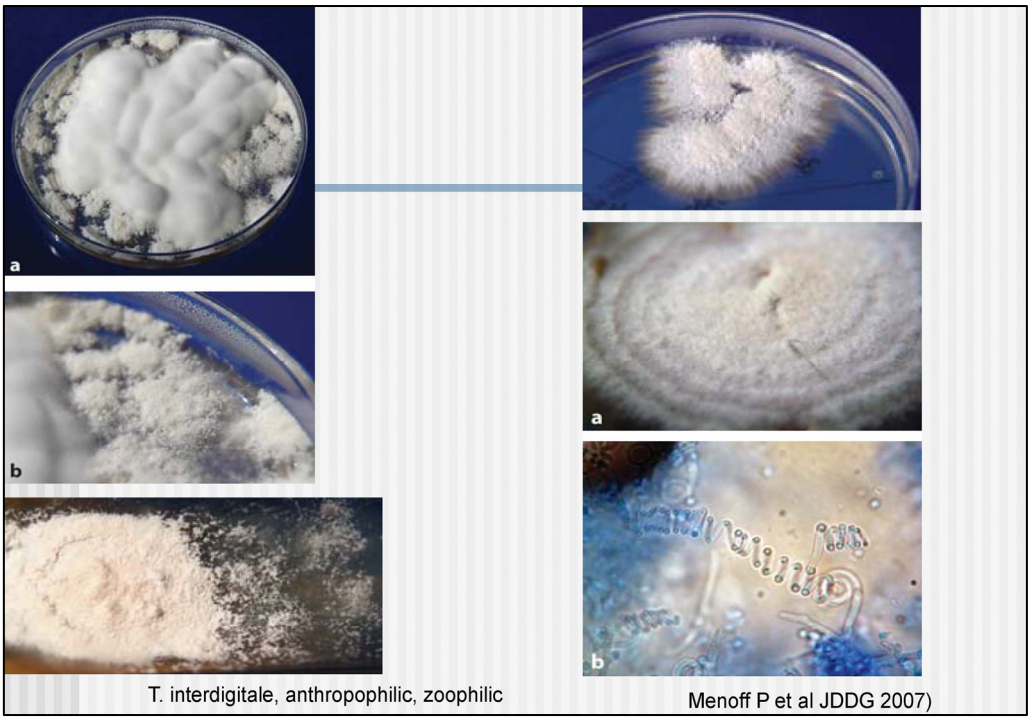


Fig. 1. Four subtypes of *Trichophyton mentagrophytes* PDACMT agar, 3 weeks at room temperature.



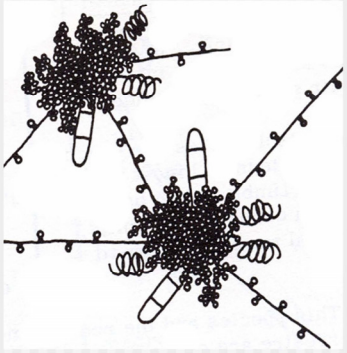
T. interdigitale, anthropophilic, zoophilic

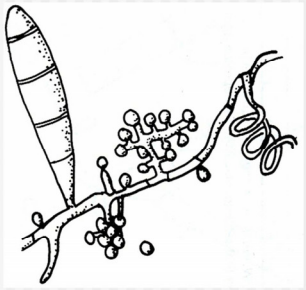
Menoff P et al JDDG 2007)

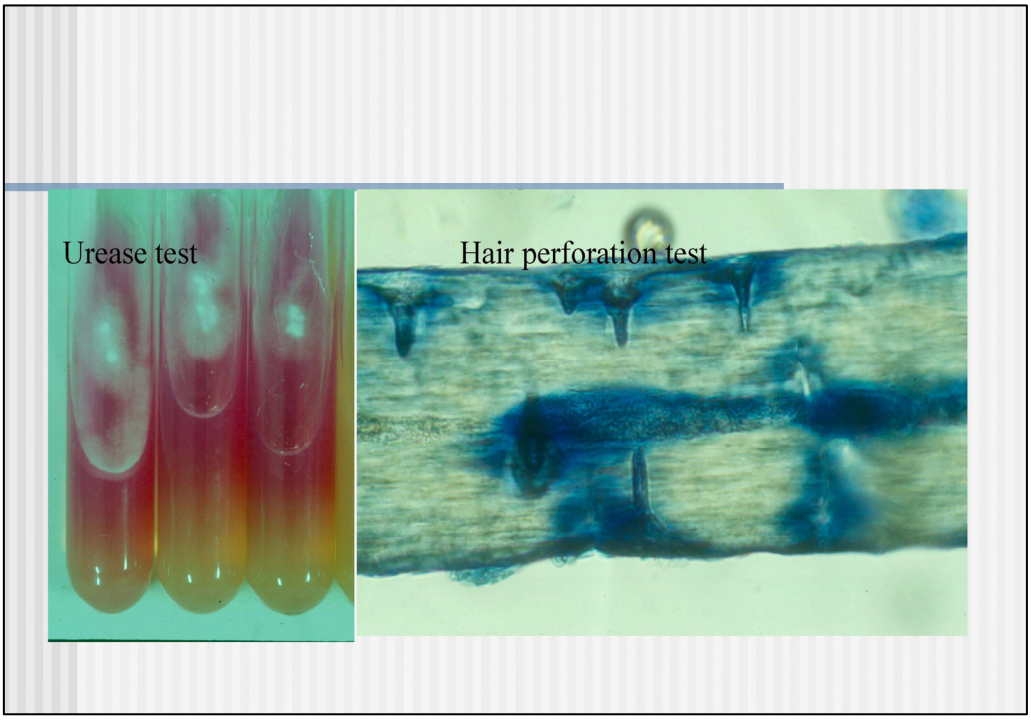
T. mentagrophytes (T. interdigitale)

❖ 현미경 소견

- Microconidia :very round, clustered
- Coiled spiral hyphae
- Macroconidia : 가끔, thin walled

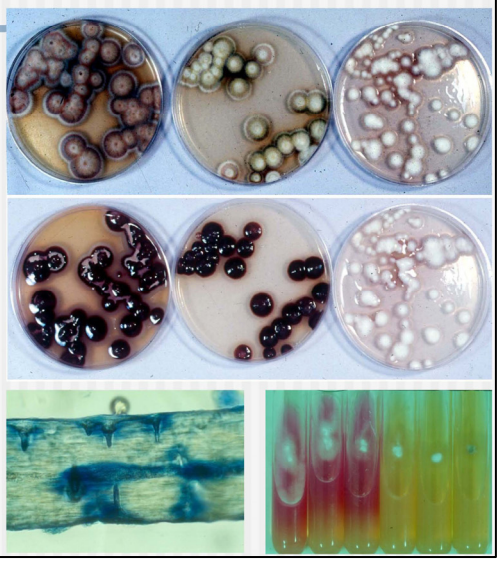




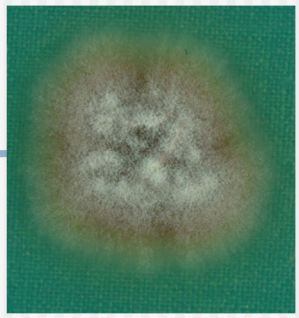


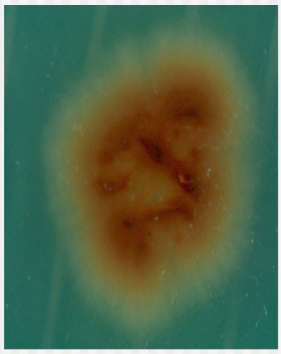
T. rubrum vs *T. mentagrophytes*

- PDACT
  - 붉은 포도주색
  - KOH 또는 증류수를 흡수 : *T. rubrum*
- 현미경 소견
  - coild hyphae,
  - clusted microconidia
- Hair perforation test :
  - *T. mentagrophytes* : 양성
- Urease test
  - *T. mentagrophytes* : 양성
  - Bacterially contaminated *T. rubrum*
  - *T. raubischeckii*



T. tonsurans





- ❖ 성장속도 : moderately slow, 12일
- ❖ Colony 형태
  - Sulfureum 형 : 황색조
  - Mahogany-red 형 : 처음에는 선홍색 반점,
    이후 회색 분말, 탁한 붉은 색으로 변한다.
  - 배면 : Mahogany-red

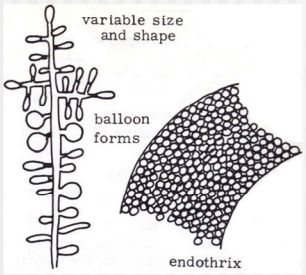
T. tonsurans

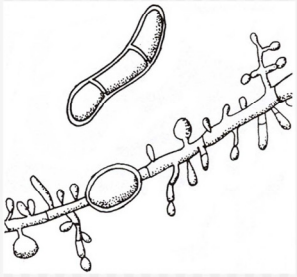
Mahogany red-brown var. sulfureum

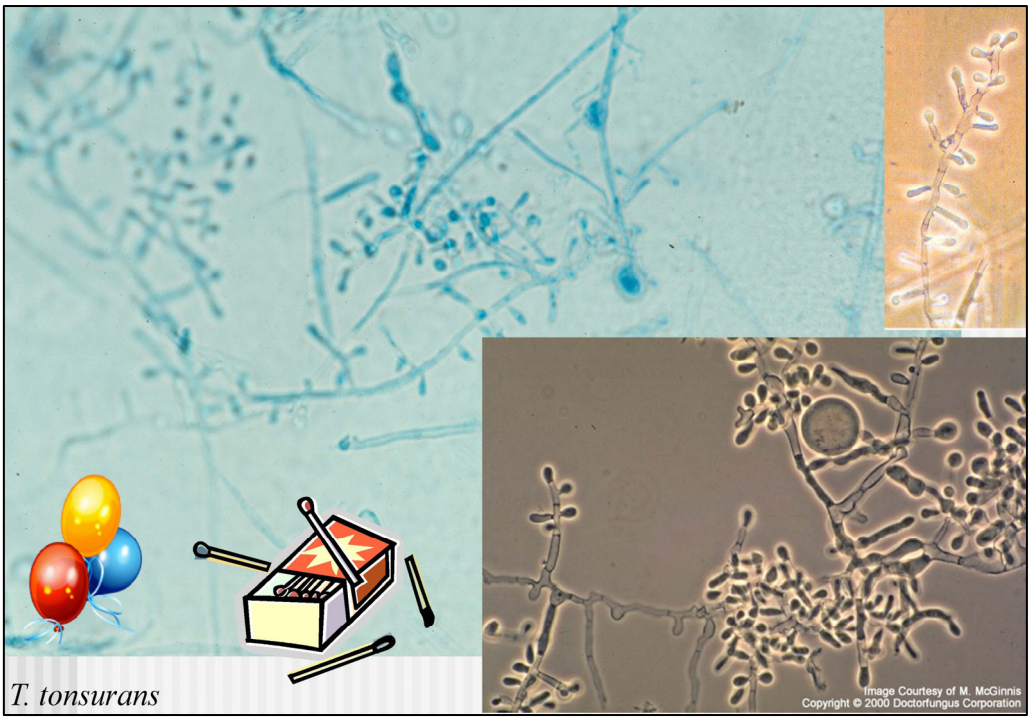
T. tonsurans

❖ 현미경 소견
- Microdonidia : diagnostic
  Variable: tear drop, 곤봉, **성냥알, balloon forms**
  Conidiophore : perpendicular to hyphae
- May spiral coils

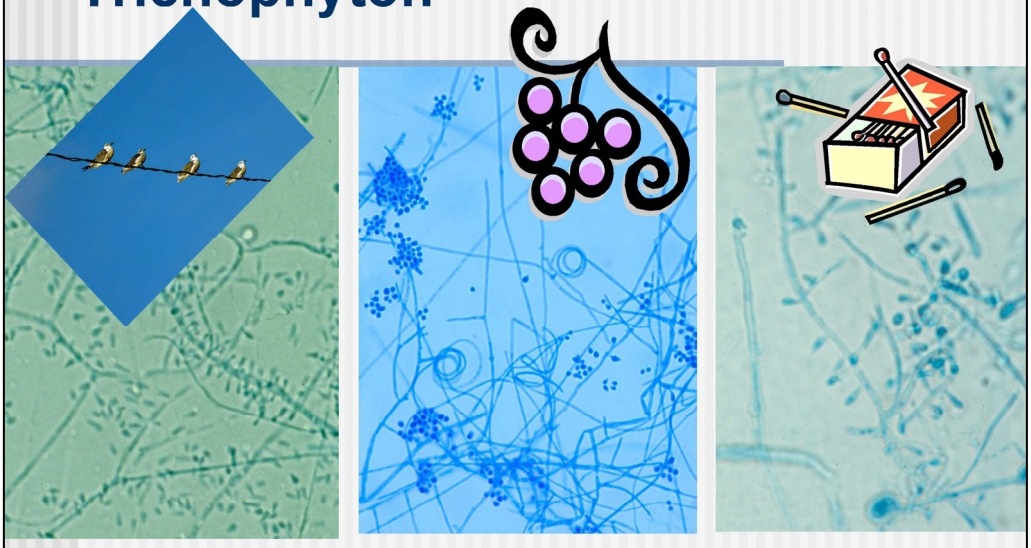
❖ Physiologic test : thiamine dependent







Trichophyton



T. rubrum *T. mentagrophytes* *T. tonsurans*

T. verrucosum

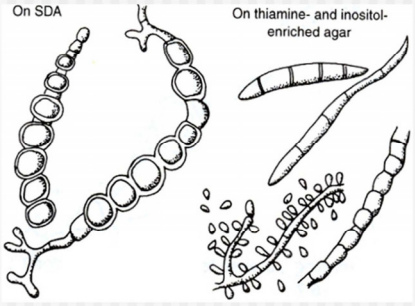
❖ 성장속도 : **very slow**; 21-30일
- 37도에서 더 잘 자란다

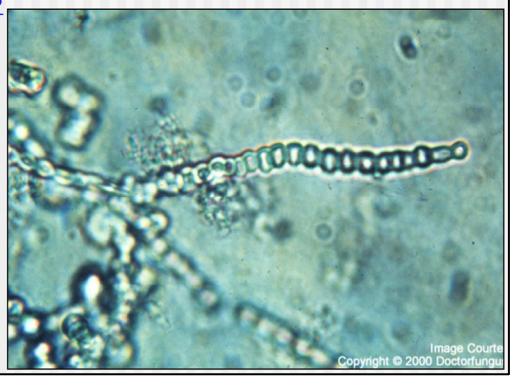
❖ Colony 형태
- Small, heaped, button-like> flat
- Texture : glabrous > downy
- White → gray or yellow
- 배면 : various

T. verrucosum

❖ 현미경 소견
- 염주양의 후막포자 (chlamidoconidia in chain)
- Macroconidia : 쥐꼬리 모양

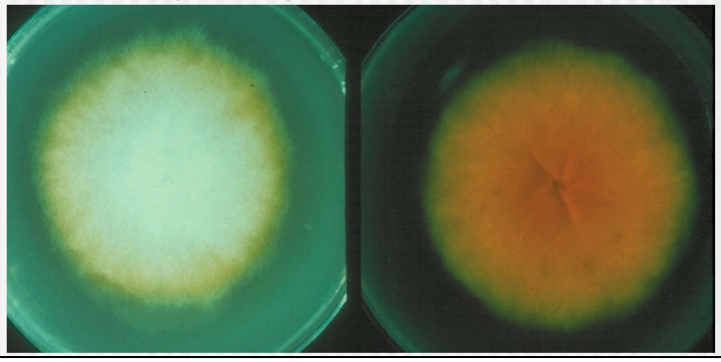
❖ Physiologic test : Thiamin, inositol 필요





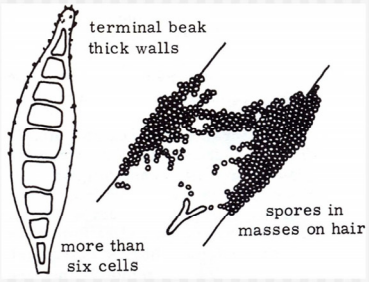
M. canis

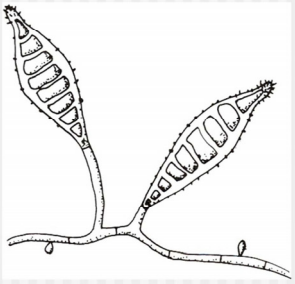
❖ 성장속도 : moderate, 6-10일 이내
❖ Colony 형태
- 표면: whitish , fluffy, 방사상 주름
- 가장자리 : 균사가 방사선상으로 퍼져나간다.
- 배면 : deep yellow → yellow brown

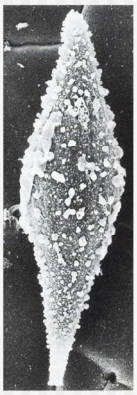


M. canis

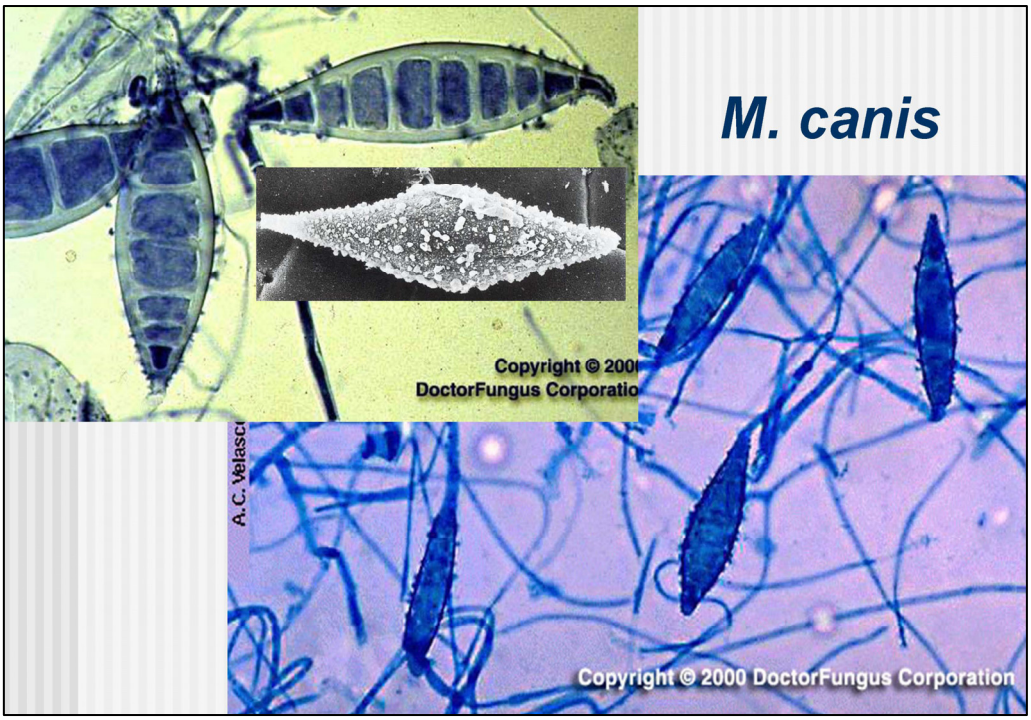
❖ 현미경 소견
- Macroconidia : 풍부하다. 특징적
  - Long, spindle shaped, **rough, thick walled**
  - 손잡이**(knob)** 같은 끝.
  - **6**개 이상의 세포
- Microconidia : a few







M. canis

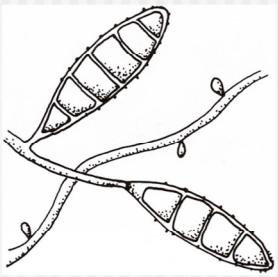


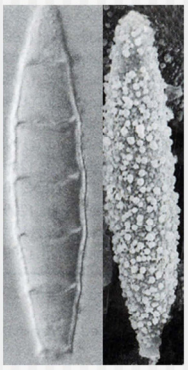
M. gypsesum

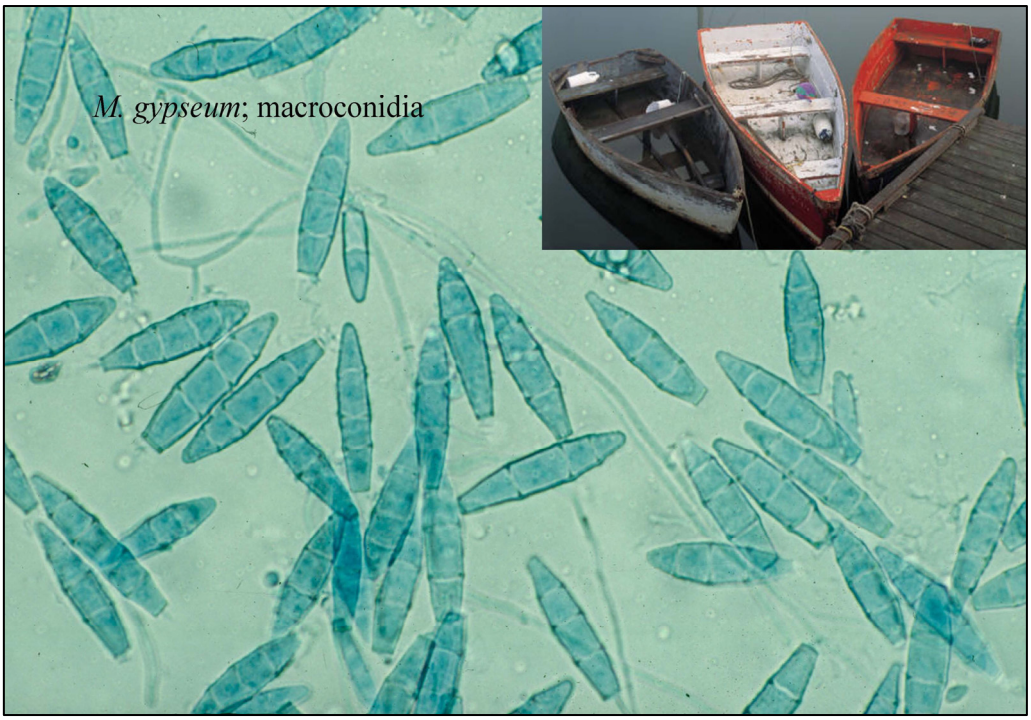
- 성장속도 : moderateley rapid, 6일 이내
- Colony 형태
  - 표면: flat, spreading, powdery to granular
    - Buff -> tan to cinnamon brown, 콩가루색깔
  - 배면 : variable

M. gypseum 현미경 소견

- Macroconidia: 매우 풍부, 특징적
  - Symmetric, rough, **thin walled**, **6개 이내의 세포**
  - **끝부분 : rounded** <-> pointed *M. canis*
- Microconidia : usually







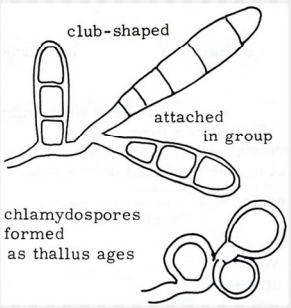
E. floccosum

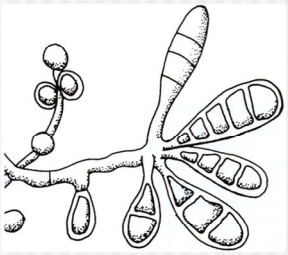
❖ 성장속도 : moderately slow,
15일 이내

❖ Colony 형태
- 표면: brownish yellow to olive gray or khaki
  - Lumpy and sparse
    -> folded, radial groove. velvety
  - 수주 후 fluffy
  - 배면 : orange to brownish,
    가끔 yellow border
- Pleomorphism이 잘 생긴다.

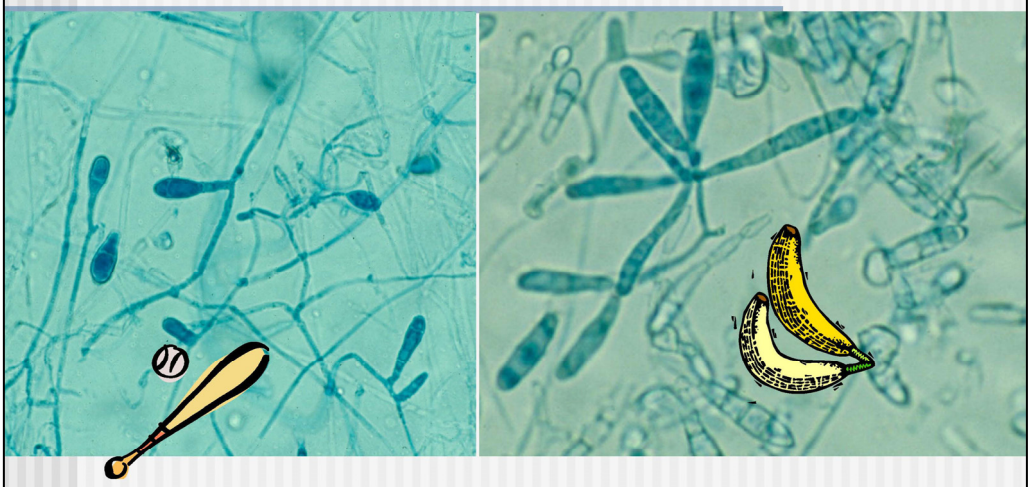
E. floccosum

❖ 현미경 소견
- Macroconidia:
  - 배양 초기에 발견된다.
  - Smooth, thin walled, club shaped, round ends
  - 2-6 세포; single or clusters
- **Microconidia : no**

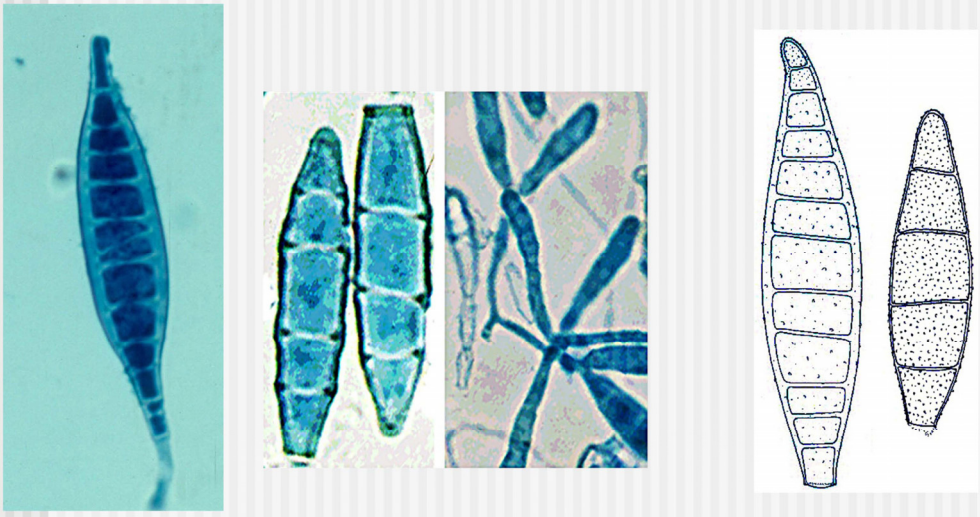




E. floccosum; 현미경 소견



Macroconidia

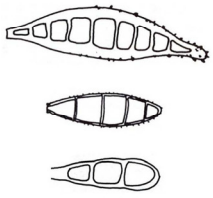


요약

Colony 색깔

포도주색: *T. rubrum*
황금색: *M. canis*
카키색 : *E. floccosum*
신나몬 : *M. gypseum*

Macroconidia

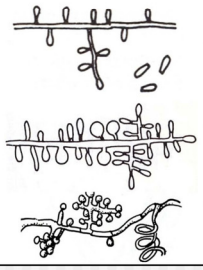


M. canis
M. gypseum
E. floccosum

성장 속도

빠르다 *M. gypseum, M. canis*
T. mentagrophytes
늦다 *E. floccosum, T. tonsurans*
T. rubrum
매우 늦다 *T. verrucosum, T. violaceum*
M. ferrugineum, T. schoenleinii

Microconidia



T. rubrum
T. tonsurans
T. mentagrophytes

Tip

- 배지 PDACT
  - *T. rubrum* 동정에 유리
- 따서 현미경으로 본다
- Primary culture가 중요

- Wine red & cottony
  - *T. rubrum* >>> *T. mentagrophytes*
- Granular
  - *T. mentagrophytes vs M. gypseum*

- Clinical information 참조

Clinical information

- ❖ Site
  - 발 : *T. rubrum, T. mentagrophytes*
  - 얼굴, 두피 : *M. canis, T. tonsurans*
- ❖ Animal 접촉
  - 고양이, 개 : *M. canis, T. mentagrophytes*
  - 소 : *T. verrucosum*
  - 고슴도치 : *T. erinacei*
- ❖ 운동선수 ; *T. tonsurans*
- ❖ 외국인, 외국여행

Next step

- ❖ 90%
- ❖ 10% :
  - Atypical strain
  - Rare species
  - First species in Korea
  - New species in the world
- ❖ 전문적인 동정
  - Special medias, physiologic test
  - Sequencing
  - Mating test
- ❖ 균 보관
  - 역사적 자료
  - 시간에 따른 변화
  - Epidemiology
  - Taxonomy
  - 자산
- ❖ 한국의진균자원센터
  - 무료보관
  - ykkim3245@konyang.ac.kr

Contents

Classification of fungi
Identification of dermatophytes
Practice

검체 채취

- 가장 중요
- Aseptic technique을 사용한다.
- 잘 씻는다.
- 살아 있는 균이 있는 곳을 선택한다.
  환상병소의 가장자리, 조갑 및 모발의 근위부
- 많은 양을 채취

배지

❖ 피부과
- PDACT

❖ 일반용도
- Sabouraud dextrose agar(SDA), Mycosel agar : 잘자란다, spore↓
- Potato dextrose agar (PDA) : conidiation 촉진
- Cornmeal with Tween 80 : conidiation 촉진, *T. rubrum*의 붉은 색
- Dermatophyte test medium (DTM) : phenol red : yellow → red

❖ 특수 용도
- Christensen urea agar
- Trichophyton agar #1 - #7 (Difco)
- Takashio medium (Diluted Sabouraud medium) : 교배용

배지 만들 때의 주의 사항 = 라면

❖ 신선한 재료를 사용한다.
❖ 너무 오래 끓이지 않는다.
❖ 사면 배지 -충분한 양을 넣고, 사면을 넓게 확보 (1:2)
❖ 김을 충분히 뺀다
지나친 물기는 contamination의 원인이 된다.
❖ 항생제와 cycloheximide
배지가 충분히 식은 후 (60℃) 첨가한다

보관 방법

❖ 1주 이내에 사용한다. 3주가 지나면 폐기한다.
❖ 마르지 않도록 밀봉하여 4℃에서 보관

검체 접종, 배양

❖ 접종
- tube를 2개 이상 사용한다. 또는 접종 면을 3등분하여
- 배지 표면에 넓게 펴 바른다.
- 배지 속으로 깊게 묻히거나 접촉이 안된 상태를 피한다.
- Aseptic - Alcohol lamp 위에서 모든 과정을 시행한다.
- Cycloheximide 넣은 것과 없는 2가지 배지를 사용한다.

❖ 배양
- 25℃와 37℃에서 배양한다.
- 충분한 기간

Contamination을 줄이는 방법

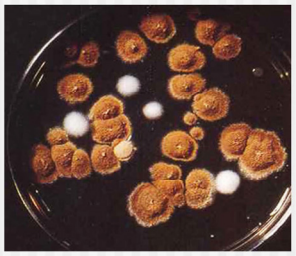
❖ 여러 병소에서 동시에 채취하여 배양
❖ 여러 개의 tube에 배양
❖ mite 제거 -나프탈렌
❖ 오염된 배지를 사용하지 않는다.
❖ 배지를 만들 때 물기를 충분히 말려서 보관
❖ 평판배지 보다는 tube를 사용한다.
❖ 검사실 환경을 깨끗하게
❖ Cycloheximide, 항생제 등을 첨가한 배지 사용
❖ 칼 소독
❖ 병변을 알코올로 소독한 후 가검물을 채취

Pathogen vs contaminant

- ❖ 여러 병소에서 동시에 채취하여 배양
- ❖ 여러 개의 tube에 동시에 배양
- ❖ 시간 간격을 두고 여러 번 배양
- ❖ 반복하여 검출이 되면 pathogen으로 판단한다.
- ❖ 검체를 채취한 부위를 고려한다.

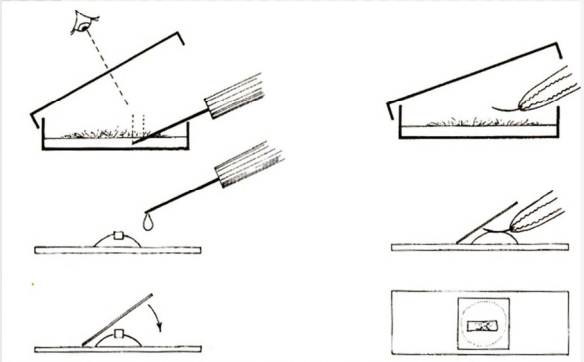
False negative (배양이 안되는 경우)

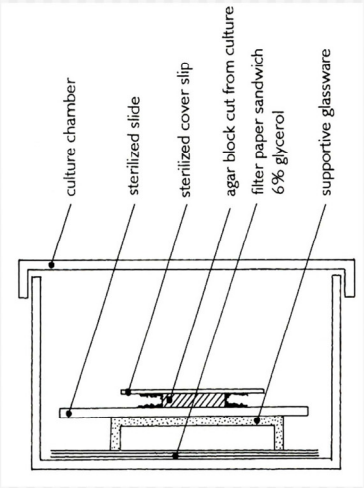
- ❖ 접종한 균이 적을 때
- ❖ 죽은 균, 항진균제 치료 중
- ❖ 접종을 잘못한 경우 배지 접촉 안됨, 뜨거운 칼
- ❖ 나쁜 배지
  - 오래된 배지, 조성이 잘못, 배지선택 잘못
- ❖ 마개를 꽉 막아 질식
- ❖ 온도가 맞지 않을 때 : 고온, 저온
- ❖ contamination
  - 백선균이 자라기 전에 배지 전체를 덮는다.
- ❖ 세균에 오염



Microscopic examination

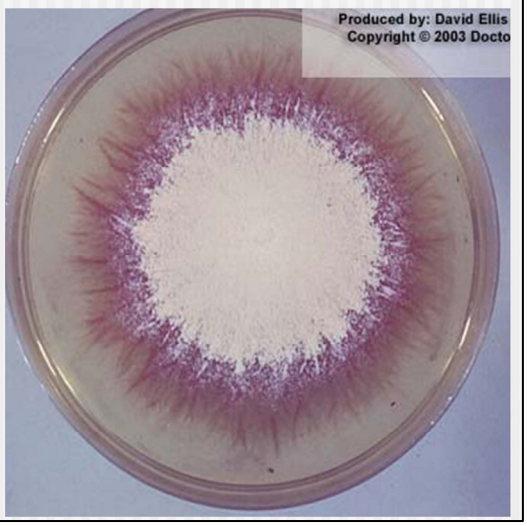
- Prepatation
  - Hook
  - Cellophan tape
  - Slide culture





Colony에서 채취부위

- 가장자리 : hyphae
- Center : Old conidia, degeneration
- 중간 : young conidia
- Cottony 부위 : conidia 거의 없다.



Mount 및 염색

- Water
- Lactic acid
- Cotton blue
- Lactophenol cotton blue
- 메니큐어로 가장자리 밀봉하면 장기간 유지할 수 있다.

Primary culture

Figure 9. The primary inoculated media were overgrown by molds. The scatter plate method yielded molds and small colonies of *T. rubrum*.

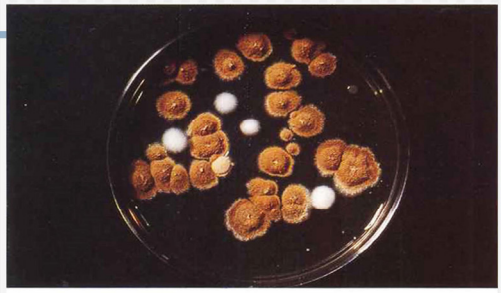


Figure 10. The primary isolation grew *Scopulariopsis brevicaulis*. Scraping scattered on SABCCG agar, also allowed the growth of a few colonies of *T. rubrum*.

- 여러 균종이 혼합되어 있거나 오염될 가능성- Pure culture
- 동정에 도움이 되는 특징적인 형태를 갖고 있다.
- 계대 배양을 할 수록 특징적인 소견을 잃는다-장기안전보존

진균의 장기 안전 보존

- 계대배양
  - 저영양 배지, 16-17 ℃
  - 손이 많이 간다, 오염 및 변이 가능성
  - *E. floccosum, T. rubrum*
- 물보존
  - 평판배지에 키운 후 잘라서 멸균증류수에 넣고 밀봉, 실온 보관
- 오일보존
  - 사면 배지에 배양 후 멸균한 mineral oil을 채워서 실온 보관
- 초저온 냉동고 보존
  - 글리세롤 또는 DMSO에 넣은 후 영하 80 ℃에 보관
- 액체질소 보존 : 가장 이상적
- 동결건조
  - 탈지유에 넣어 동결 건조 후 진공상태로 밀봉

연자 소개

Name Jong Soo Choi
Current Position Professor, Department of Dermatology, College of Medicine, Yeungnam University, Daegu, Korea

◖ Educational, research and professional experiences ◗

1973. 3 ~ 1979. 2	Yonsei University College of Medicine
1982. 9 ~ 1988. 8	M.D. and Ph.D. degree of dermatology at Yonsei University College of Medicine
1997. 3 ~ 1998. 2	Visiting researcher at Branch of Mycology, CDC, Atlanta, USA
2008. 10 ~ 2009. 8	Visiting researcher at laboratory of Professors Sybren de. Hoog, CBS fungal biodiversity center, Utrecht, Netherland
1983. 4 ~ present	Professor, Department of Dermatology, College of Medicine, Yeungnam University
2008	Academic secretary of the APSMM08, Seoul, Korea
2011. 6 ~ 2013. 6	Chief director of the Korean Society for Medical Mycology

◖ Membership of Societies ◗

1983 ~ present	The Korean Dermatological Association
1996 ~ present	The Korean Society for Medical Mycology
1997 ~ present	International Society for Human and Animal Mycology

MEMO

Candida Identification and Antifungal Drug Susceptibility

이 미 경

중앙대학교 의과대학 진단검사의학과

- **Fungal infections**
  (1) Healthcare-associated (HAI, nosocomial)
  : opportunistic mycoses
  (2) Community acquired
  : opportunistic + endemic mycoses

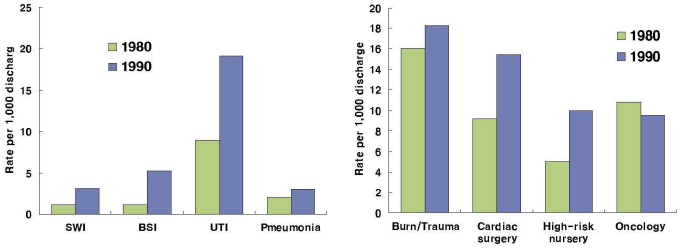
- **Incidence of fungal infections ⬆**
  · Immunocompromised pt. ↑
  (cancer, AIDS, large burn)
  · Aging population ↑
  · Use of new & aggressive therapeutic strategies (Antibiotics, ChemoTx, Transplantation)

Nosocomial fungal infections

CDC NNIS (National Nosocomial Infections Surveillance) System

: Rate of nosocomial fungal infections

2(1980) → 3.8(1990)/1000 discharges



- **Epidemiology of sepsis**
  - **· 1979-2000: 207% ⬆**
  - **· 24,170 nosocomial bloodstream infection (1995-2002, 49-centers)**
    : 4th leading cause

1. Coagulase-negative staphylococci	31.3%
2. *S. aureus*	20.2%
3. *Enterococcus* spp.	9.4%
4. *Candida* spp.	9.0%
5. *E. coli*	5.6%
6. *Klebsiella* spp.	4.8%
7. *P. aeruginosa*	4.3%
8. *Enterobacter* spp.	3.9%
9. *Serratia* spp.	1.7%
10. *A. baumannii*	1.3%

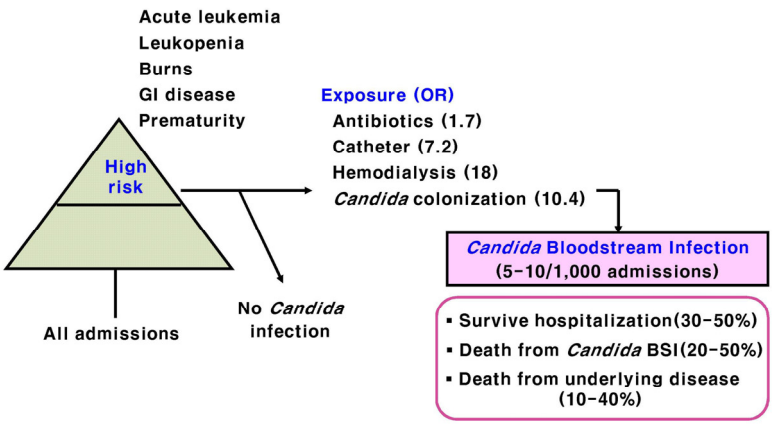
병원감염에서 분리된 균주의 분포(국내)

병원균	분리균주의 백분율(%)			
	1996년, 중환자실 (n=1,029)	1996년, 일반 병동 (n=2,743)	2004년, 중환자실 (n=534)	2010년 중환자실 (n=3865)
Staphylococcus aureus	20.80	18.00	23.2	12.2
(Methicillin-resistant *S. aureus*)	(19.14)	(12.32)	(21.5)	(10.9)
Pseudomonas aeruginosa	17.59	12.32	8.8	5.7
Candida spp.	9.52	3.72	14.8	22.0
Acinetobacter spp.	8.55	6.38	8.7	10.3
Klebsiella pneumoniae	7.58	7.77	6.6	7.5
Enterobacter spp.	6.51	5.21	2.6	2.6
Enterococcus spp.	5.83	8.35	12.8	14.1
Escherichia coli	5.25	14.62	5.5	6.0
Serratia marcescens	4.08	1.82	3.7	1.5
CNS	3.69	7.00	3.1	7.3
Others	10.68	15.86	NA	10.7
Total	100	100	100	100

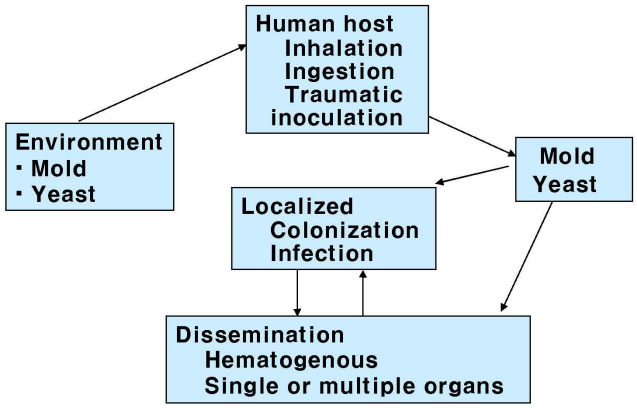
Risk factors for fungemia in hospitalized pts

Risk factor	Possible role in infection
Antimicrobial agent*	Promote fungal colonization
Corticosteroid	Immunosuppression
Chemotherapy*	〃
Malignancy	〃
Previous colonization*	Translocation across mucosa
Indwelling catheter*	Direct vascular access/Contamination
TPN	〃
Neutropenia*	Immunosuppression
Extensive surgery or burns	Route of infection, Direct vascular access
Assisted ventilation	Route of infection
Hospitalization, ICU	Exposure to pathogens/risk factors
Hemodialysis*	Immunosuppression
Malnutrition	〃

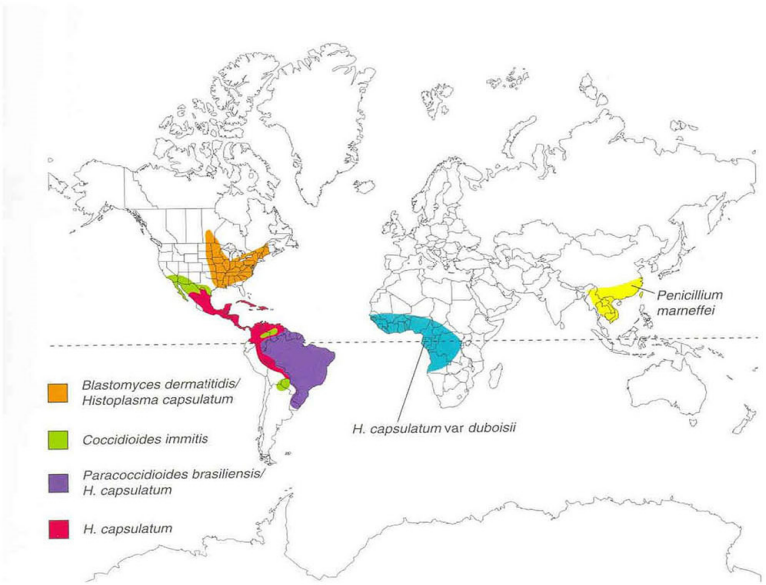
HA candidemia



Community-acquired fungal infections

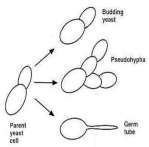


Endemic dimorphic pathogens



Description of Yeasts

- Unicellular, eukaryotic, budding cells
- Reproduction: budding (blastoconidia)
  ⇨ pseudohyphae
- Shape: generally round to oval
  (less often, elongate or irregular)
- Colonies: smooth & glabrous
  usually white to cream colored
- *C. albicans*
  - similar to *Saccharomyces cervisiae*
  - no sexual cycle / morphologic variation↑



Yeasts

Genus *Blastoschizomyces*
√Genus *Candida*
Genus *Clavispora*
√Genus *Cryptococcus*
Genus *Dabaryomyces*
Genus *Dipodascus*
√Genus *Malassezia*
Genus *Pichia*
Genus *Rhodotorula*
Genus *Saccharomyces*
Genus *Sporobolomyces*
√Genus *Trichosporon*

Selection of clinical specimens for recovery of yeasts

	Candida spp.	*Cryptococcus neoformans*	*Malassezia* spp.	*Trichosporon* spp.
Blood	○	○	○	○
Brain & CSF	○	○		
Bone marrow	○	○		
Catheter	○		○	○
Eye	○	○		
Respiratory sites	○	○		○
Skin, mucous memb.	○	○	○	○
Urine	○	○		○
Mul. systemic sites	○	○		○

Candida genus

- Approximately 200 species of 'yeast-like' fungi
  ⇨ 20 *Candida* spp. have been associated causing infection in humans
- Highly heterogenic group
  - Biochemistry
  - Morphology
  - Genetic composition
  - Ability to instigate human infection

Routes of transmission of *Candida* spp.

- Predominant source: patient him or herself
  · GI tract
  ↓ overgrowth of No. of yeast
  ↓ loss of the integrity of the GI mucosa
  Bloodstream
  · IV catheter, Respiratory tr, GU tr
- Exogenous transmission
  · contaminated materials (IV catheter, pressure-monitoring devices)
  · staff → pts, pts → pts
  ; burn, geriatric, hematology, intensive care, transplantation unit
- Neonates: from the maternal vagina
  hands of the hospital personnel

Candida infections of the skin & nails

- Invasive infections of the fingernails
  - *C. albicans*, *C. parapsilosis*
- Chronic swelling & inflammation of the nailfold
  - *C. albicans*
- A manifestation of hematogenous candidiasis
  : diagnostic value in neutropenic pts.
- Cutaneous infection: immunocompromised & premature neonates

Candida spp. can be found as commensals

- GI tract
- Vagina & urethra
- Skin
- Fingernails

Prevalence of *Candida* spp. in the mouth

Infants	5%
Middle aged	40%
AIDS	80%
Terminal Cancer	80%

Isolation of *Candida* spp.

- **Significant**
  : Blood, CSF, pleural, peritoneal & ascitic fluid
- **Often significant**
  : Urine
- **Seldom significant**
  : Sputum, stool

List of *Candida* species

- **Species commonly implicated in human infections**
  - *C. albicans*
  - *C. glabrata*
  - *C. parapsilosis*
  - *C. tropicalis*
  - *C. guilliermondii*
  - *C. krusei*
  - *C. lusitaniae*
- **Species uncommonly implicated in human infections**
  - *C. dubliniensis*
  - *C. famata*
  - *C. haemulonii*
  - *C. inconspicua*
  - *C. kefyr*
  - *C. lipolytica*
  - *C. pelliculosa*
  - *C. utilis*

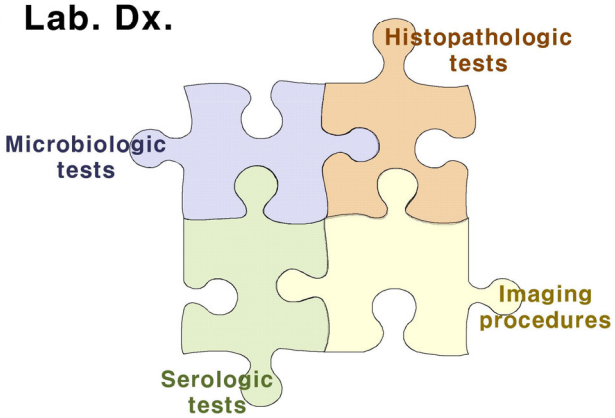
Species distribution of *Candida* and other yeast isolates by year in South Korea

Organism	2001		2002		2003		2004		2005		2006		2007	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Candida spp.														
C. albicans	426	93.42	588	89.36	914	86.64	687	81.40	473	70.28	766	68.09	671	78.60
C. glabrata	5	1.10	15	2.28	29	2.75	39	4.62	41	6.09	48	4.27	40	4.70
C. tropicalis	8	1.75	26	3.95	38	3.60	36	4.27	49	7.28	81	7.20	33	3.90
C. parapsilosis	8	1.75	22	3.34	57	5.40	58	6.87	41	6.09	74	6.58	40	4.70
C. guilliermondii	1	0.22			6	0.57	2	0.24	2	0.30	7	0.62	2	0.20
C. krusei			2	0.30	3	0.28	2	0.24	4	0.59	7	0.62	1	0.10
C. lusitaniae							3	0.36	2	0.30	3	0.27	3	0.40
C. pelliculosa			1	0.15	2	0.19	2	0.24	4	0.59				
C. famata	2	0.44											1	0.10
C. dubliniensis											2	0.18		
C. haemulonii											1	0.09		
Other *Candida* spp.			2	0.30							3	0.27		
C. neoformans					1	0.09	3	0.36	17	2.53	10	0.89	4	0.50
Trichosporon spp.														
T. beigelii/cutaneum	6	1.32	1	0.15					2	0.30	3	0.27		
T. asahii							1	0.12			1	0.09	1	0.10
T. inkin											1	0.09		
T. mucoides											1	0.09		
Other *Trichosporon* spp.							5	0.59			2	0.18	2	0.20
Rhodotorula spp.							1	0.12	1	0.15	1	0.09		
Saccharomyces spp.							1	0.12			3	0.27		
Pichia spp.									1	0.15	6	0.53		
Other yeasts			1	0.15	5	0.47	4	0.47	36	5.35	105	9.33	56	6.60
Total	456		658		1,055		844		673		1,125		854	

Korean J Lab Med 2010;30:364-372

Diagnosis of fungal infection

- Clinical Sx. & Sign
- Lab. Dx.



Laboratory Dx of invasive fungal infections

Conventional microbiologic
1. Direct microscopy (Gram,...)
2. Culture
3. Identification
4. Susceptibility testing

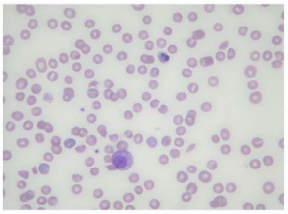
Histopathologic
1. Conventional microscopy (H&E PAS,...)
2. Direct immunofluorescence
3. In situ hybridization


Immunologic
1. Cryptococcal Ag test
2. Histoplasma Ag test
3. Galactomannan test
4. Mannan test

Molecular
1. Direct detection
2. Identification
3. Strain typing

Biochemical
1. Metabolites (D-arabinitol)
2. Cell wall components

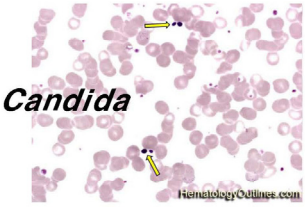

100 μm(0.1 mm)




Bacteria
0.1-5μm

RBC 6-8 μm


Virus
<0.01 μm



Incubation & Examination

- Incubated at 30℃ for mini. of 4 weeks
  Exam. : daily for first 7 days
  → at least twice/week
- Specimens from genital sites or mucosal surface (for *Candida* spp.)
  → discarded after 7 days
- *H. capsulatum* or *B. dermatitidis*
  → 6 to 8 weeks of incubation

Direct exam. of specimens

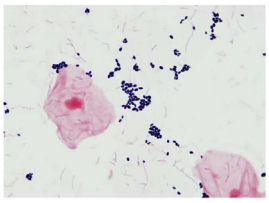
: Stain (Gram, calcofluor white,…)
KOH, India ink prep.

- size & shape
- morphology of the bud attachment site
- number of buds
- presence or absence of a capsule
- thickness of the cell wall
- presence of pseudohyphae
- presence of arthroconidia

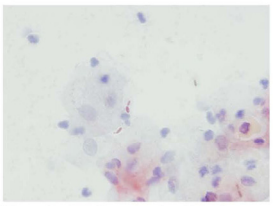
Potassium hydroxide (KOH)

- Wet mount prepared in a 10 to 30% KOH
- To distinguish fungi in mucoid secretions or in skin, hair, or nail
- Gentle warming ⇨ speed
- Dimethyl sulfoxide ⇨ to facilitate clearing
- Bubbles: confused with yeast cells
- Round & oval objects lacking buds
  : erroneously identified as yeasts
- Cotton swab: resemble hyphae ⇨ no use
- Calcofluor white stain

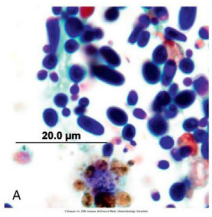
Stains (Gram, Pap, PAS)

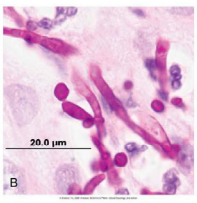


Dark blue, dense staining of yeasts (Gram stain, ×1000)



Yeasts and pseudophyphae (Papanicolaou stain, ×1000)





PAS stain

Identification of *Candida*

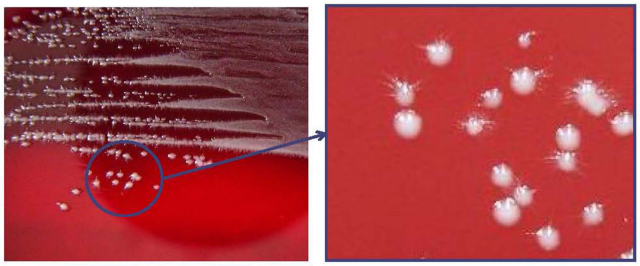
- Macroscopic characteristics
- Microscopic characteristics
- Germ tube test
- Chromogenic agars
- Commercially available kits
  : automated & semiautomated systems
- Molecular test

Macroscopic characteristics

- Most yeasts grow well on common media
  - exception: *Malassezia* spp.
- 30℃ (yeasts to grow at 37℃)
- Usually detected in 48-72 hr
- Colony
  - if mucoid ⇨ *C. neoformans*?
    (heavily encapsulated yeasts give a very moist, mucoid appearance)
  - if not mucoid ⇨ *Candida* spp.?
  - spiking on BAP ⇨ *C. albicans*

Spiking on BAP

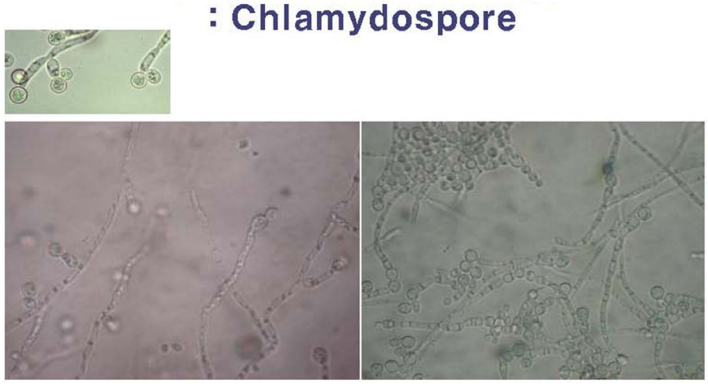
: Colonial morphology of *C. albicans* known as 'spiking' on a BAP in 5% CO_2 after 24 h incubation



Microscopic characteristics

Organism	Pseudohyphae	True hyphae	Blastoconidia	Arthroconidia	Annelloconidia	Chlamydospores	Ascospores
B. capitus	○	○	○		○		
C. albicans/C. dubliniensis	○	○	○			○	
Other *Candida* spp.	○	○	○				○
Cryptococcus spp.			○				
Geotrichum spp.		○		○			
Pichia spp.	○		○				○
Rhodotorula spp.			○				
Saccharomyces spp.	○		○				○
Trichosporon spp.	○	○	○	○			

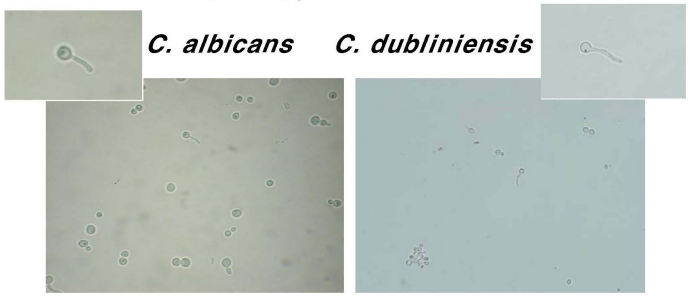
Cornmeal tween 80 agar
: Chlamydospore



C. albicans *C. dubliniensis*

Germ tube test

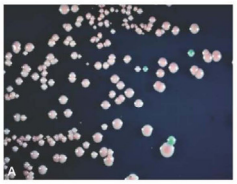
- Serum (human, rabbit, FBS) + yeast colony
  → incubate (37℃), 2-3 h

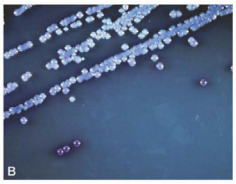


C. albicans *C. dubliniensis*

- False (+): *C. tropicalis*
- False (-): heavy inoculum, bacteria(+)

CHROMagar Candida

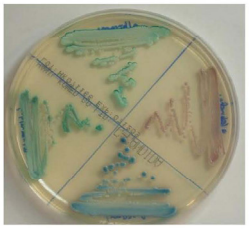


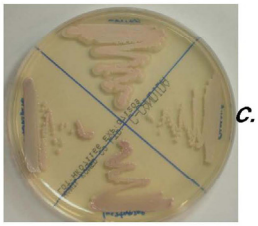


C. alb

C. dub C. gla

C. trop

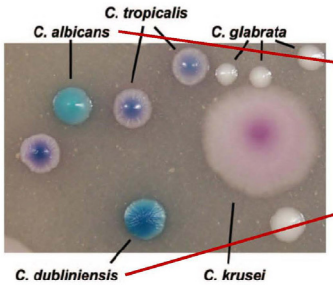


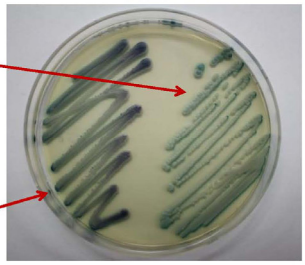


C. kru

C. guil C. lusi

C. para





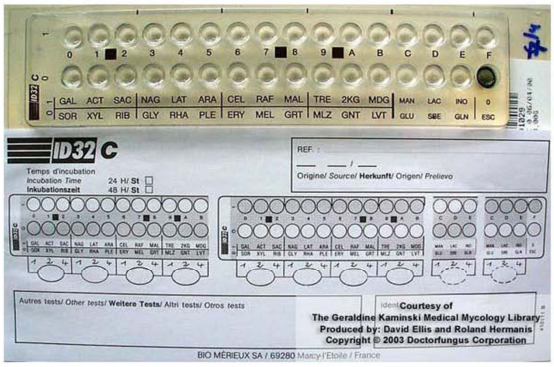
Colonies of different species of *Candida* after growing for 48 h at 37° C in CHROMagar Candida medium supplemented with Pal's agar (JCM 2006;43:5768-70)

Commercially available kits

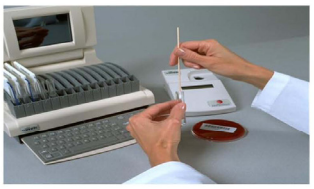
- For multiple genera
  - Vitek-Yeast Biochemical Card
  - Microbila Identification System (MIDI)
  - Microscan Rapid Yeast ID
  - API 20C AUX
  - API ID 32C
  - Vitek2 ID-YSD
  - Quantum II
  - Mass Spec and MALDI-TOF

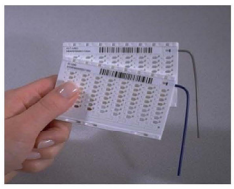
API 20C test(bioMerieux)

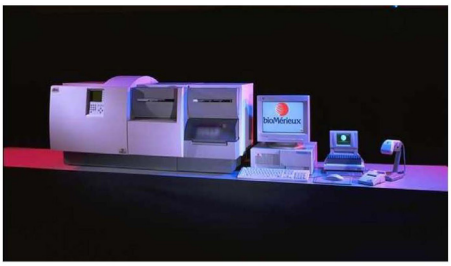
ID 32 C (bioMerieux)



Vitek YBC (bioMerieux)

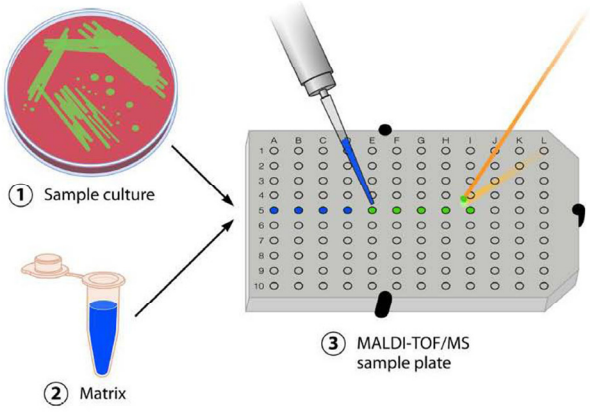




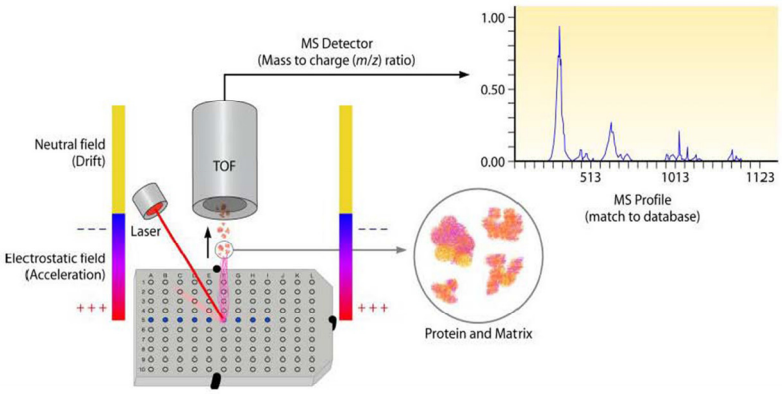


MALDI-TOF

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

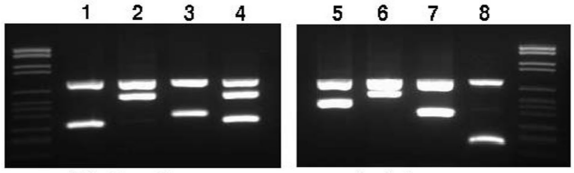


Clinical Microbiology Reviews, 2013



Clinical Microbiology Reviews, 2013

Molecular biology based identification



(A) Set A (B) Set B

Species-specific DNA amplifications by multiplex PCR in clinical isolates of *Candida* spp. Lane M, molecular size marker; Lane 1 (323 bp), *C. albicans*; Lane 2 (608 bp), *C. glabrata*; Lane 3 (379 bp), *C. krusei*; Lane 4 (323 bp and 608 bp), *C. albicans* and *C. glabrata*; Lane 5 (507 bp), *C. guilliermondii*; Lane 6 (603 bp), *C. tropicalis*; Lane 7 (419 bp), *C. parapsilosis*; Lane 8 (194 bp), *C. lusitaniae*. (KJCM 2006;9:119)

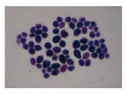
Performance of molecular assays

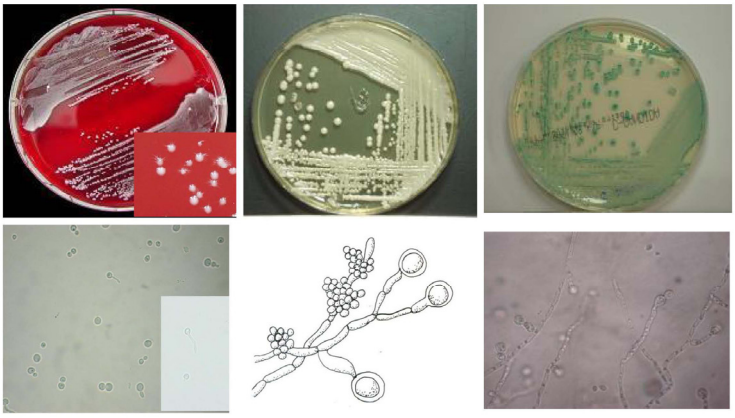
Molecular assay or format	Commercially available	DNA extraction procedure	*Candida* species detected	% Sensitivity*	% Specificity*
PCR with probe detection	No	Manual	Major *Candida* species	100	98-100
Seminested/nested PCR	No	Manual	Major *Candida* species	100	98-100
Multiplex PCR	No	Manual	Major *Candida* species	75	97
Real-time PCR	No	Manual	Major *Candida* species	91-100	97-100
SeptiFast	Yes	Automated	Five *Candida* species	90	97

*Cumulative values for sensitivity and specificity are based on several published studies

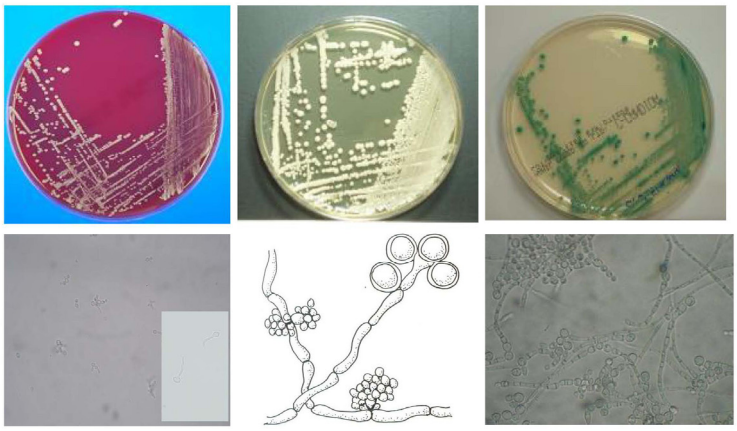
Indian J Med Microbiol 2013

C. albicans





C. dubliniensis



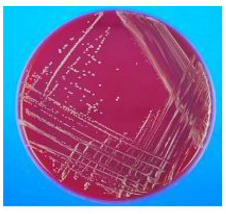
Case Report
Clinical Microbiology
Ann Lab Med 2012;32:225-228
http://dx.doi.org/10.3343/alm.2012.32.3.225
ISSN 2234-3806 · eISSN 2234-3814
ANNALS OF LABORATORY MEDICINE

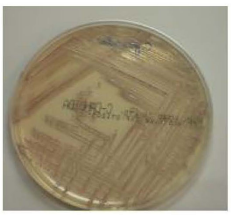
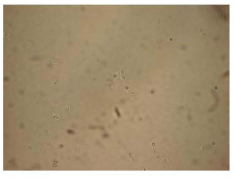
The First Korean Case of Candidemia due to *Candida dubliniensis*

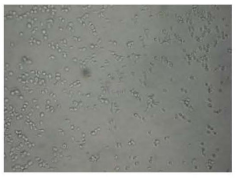
Nae Yu, M.D., Hye Ryoun Kim, M.D., and Mi-Kyung Lee, M.D.
Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Korea



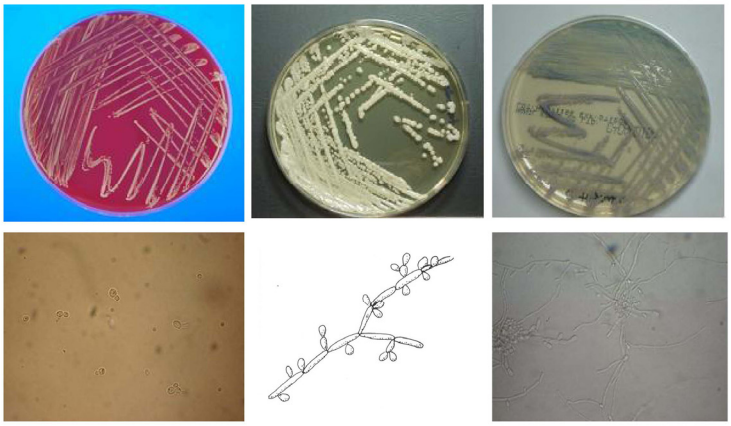
C. glabrata



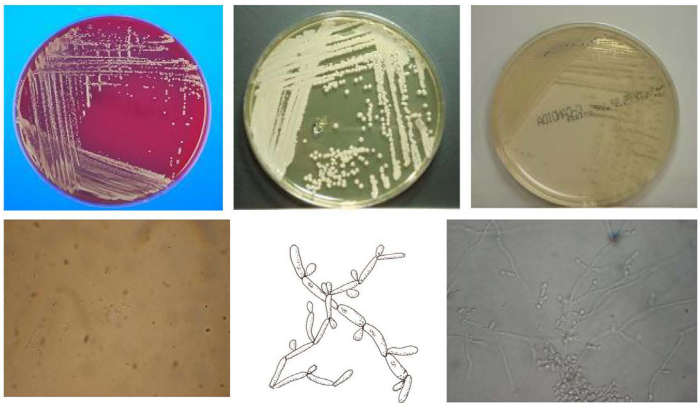





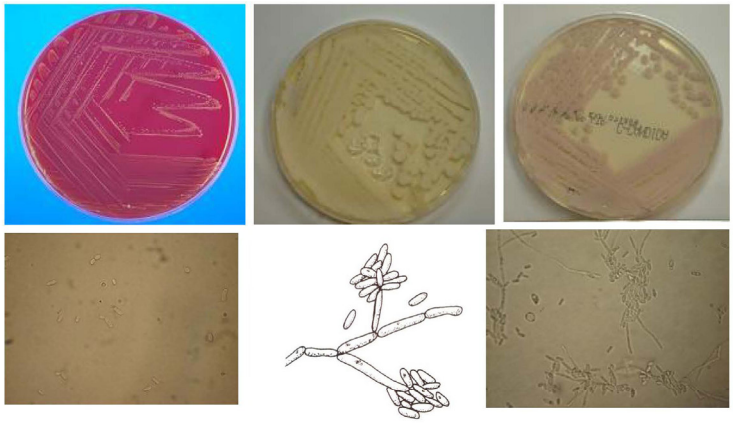
C. tropicalis



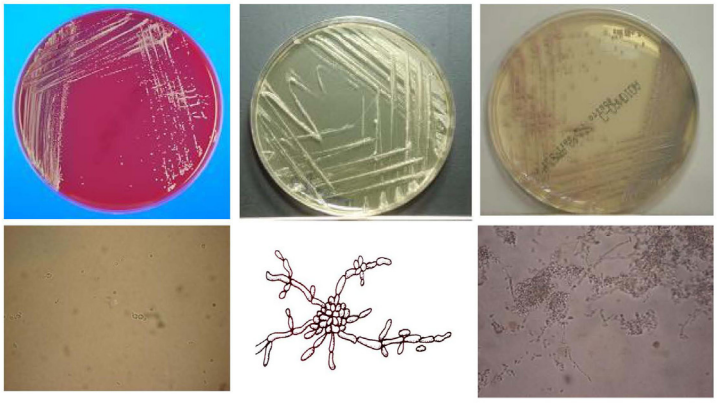
C. parapsilosis



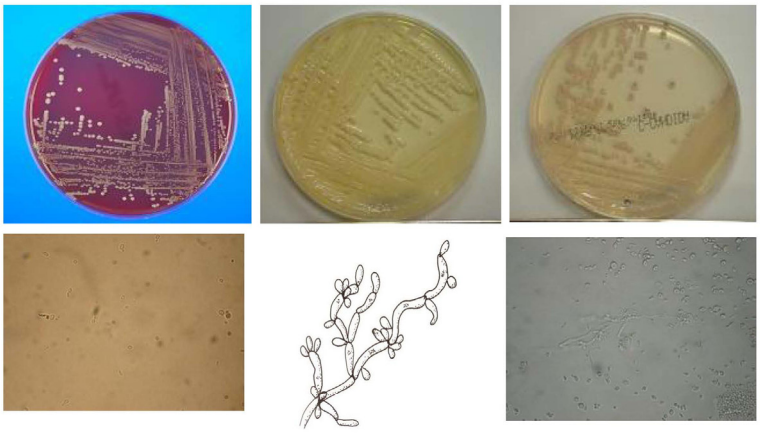
C. krusei



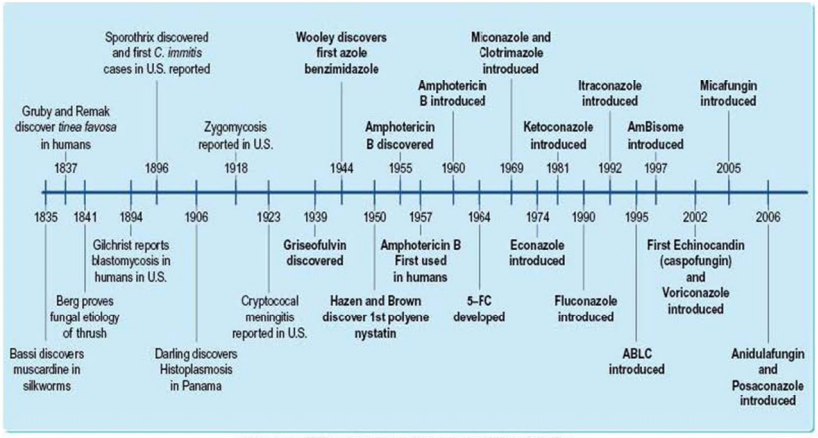
C. guiiliermondii



C. lusitaniae



History of antifungal development



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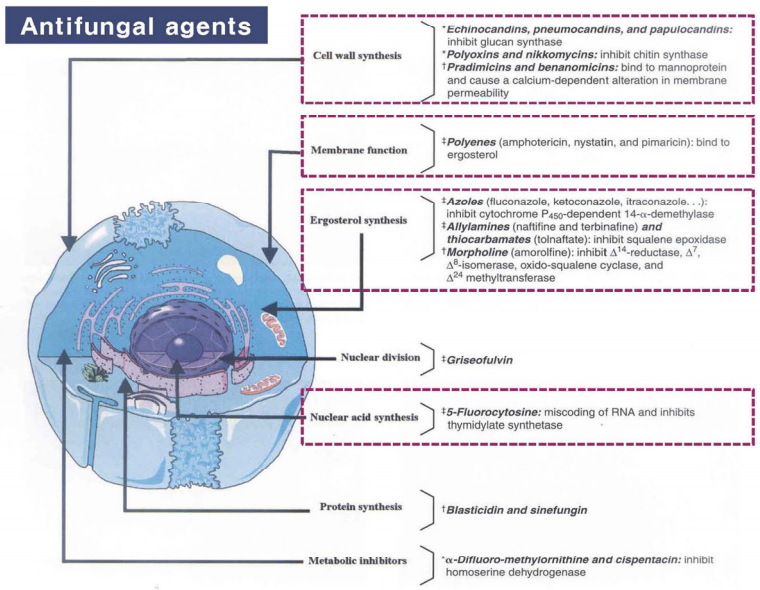


Mechanisms of action and resistance of major systemic antifungal agents

Antifungal agent	Mechanism(s) of action	Mechanism(s) of resistance
Antimetabolites		
Flucytosine	Cell death: inhibition of DNA and RNA synthesis	Enzymatic modifications: cytosine permease (*FCy2* gene); cytosine deaminase (*FCy1* gene); and phosphoribosyl transferase (*FUR1* gene)
Azoles		
Fluconazole, itraconazole, posaconazole and voriconazole	Perturbation of fungal membrane: inhibition of ergosterol synthesis by blocking P-450 14-α demethylase leads to accumulation of lanosterol	Decreased drug concentration by activation of efflux pumps (*CDR, MDR* and *atrF* genes); decreased affinity to the binding site (*ERG11* and *Cyp51A* genes); upregulation of target enzyme (*ERG11* and *Cyp51A* genes) Bypass pathways (*ERG3* gene)
Echinocandins		
Anidulafungin, caspofungin and micafungin	Formation of a defective cell wall leads to cell rupture (yeasts) or aberrant hyphal growth (molds): inhibition of β-1,3-D-glucan synthesis	Point mutations (*FKS1* and *FKS2* genes)
Polyenes		
Amphotericin B	Cell death: intercalation of pores across the membrane formed by eight polyene molecules linked to the ergosterol, through which the fungal cell leaks cytoplasmic components (protons and monovalent cations); oxidative damage of fungal membrane	Decreased access to the drug target by altered membrane ergosterol content, accumulation of other sterols and reduced intercalation (*ERG3* gene); decreased oxidative damage by increased catalase activity

Definition: Resistance

- Primary (intrinsic) resistance; resistant to a drug before it encounters the drugs
- Secondary (acquired) resistance; developed resistance in the presence of drugs
- Microbiologic resistance; inhibited by an antimicrobial agent concentration higher than the range seen for wild-type strains
- Clinical resistance; inhibited by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure

"90-60 rule"

: Arbitrary threshold for defining clinical response

- Susceptible isolates can be successfully treated 90% of the time
- Resistant isolates can be successfully treated 60% of the time

Azole MIC breakpoints: orophayngeal candidiasis

Antifungals	MIC (㎍/mL)	Interpretation	Cured
Fluconazole	≤ 8	S	97%
	16 – 32	SDD	82%
	≥ 64	R	60%
Itraconazole	≤ 0.125	S	90%
	0.25 - 0.5	SDD	63%
	≥ 1.0	R	53%

SDD: susceptible, dose dependent

NCCLS M27-A

Antifungal Drug Susceptibilities in *Candida*

- CDC, SENTRY, EIEIO, ARTEMIS,..

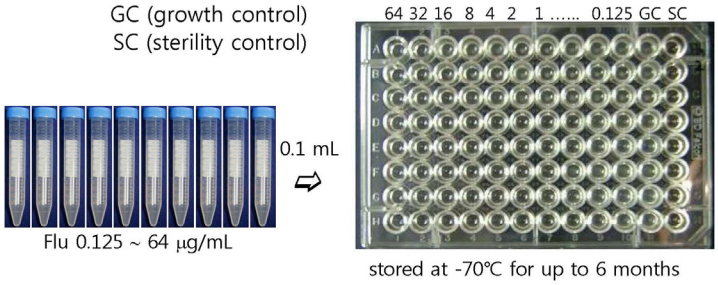
	Flu		Itra		Vori		Caspo	AmpB		5FC	
	MIC90	% R	MIC90	% R	MIC90	% R	MIC90	MIC90	% R	MIC90	% R
C. albicans	0.5-4	0-3.8	0.06-0.5	0-1	0.015-0.12	0.3	0.25	0.5-1	5	1-2	2.7
C. glabrata	16-64	4-50	2-4	24-53	0.5-1	0	0.25-0.5	0.5-1	3.4	0.12-0.25	
C. parapsilosis	1-2	0-15	0.25-0.5	0	0.03	0	2-4	0.5-2		0.25-0.5	
C. tropicalis	2-64	0-14.7	0.25-1	3.6-4.2	0.06-0.12	0	0.25-0.5	0.5-1.5		2-16	7.1
C. krusei	64	0-100	0-1	3-66	0.06		1	4-6		32	
C. guilliermondii	16		1				>8				
C. dubliniensis	16		0.25				0.5				
C. lusitaniae	2		0.25				1				

Antifungal susceptibility testing (AFST)

1. Broth-based method (M27-A3, M38-A2)
   - Macrobroth dilution
   - Microbroth dilution
   - Colorimetric
   - VITEK-2 system
2. Agar-based method
   - Agar dilution
   - E-test
   - Disk diffusion test (M44-A, M51-P)
3. Flow Cytometry (FCM)
4. Sterol Quantitation (SQM)

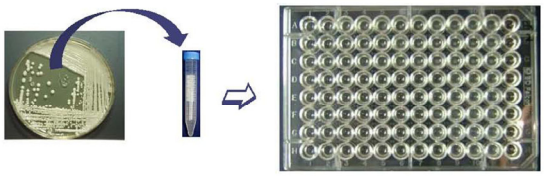
CLSI M27-A3: Test procedures

- **Broth medium**
  : RPMI 1640, 0.165 mol/L MOPS, pH 6.9-7.1
- **Preparing diluted antifungal agents (2x serial)**
  : microdilution plate (96 U-shaped wells)
  plastic test tubes



CLSI M27-A3: Test procedures

- **Inoculum preparation**
  1. subcultured onto SDA at 35℃ (purity & viability)
  2. *Candida* spp.(24h), *C. neoformans* (48h)
  3. 0.5 McF (1~5X10^6 cells/mL)
     1:1000 dilution (0.5~2.5X10^3 cells/mL)



- **Incubation at 35℃**
  Candida spp.(24~48h), *C. neoformans* (70~74h)

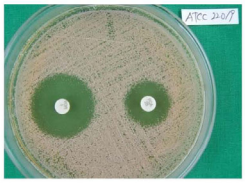
CLSI M27-A3: Reading results

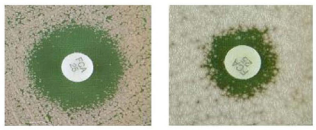
- **Amount of growth-compared visually with GC**
  0: optically clear
  1: slightly hazy
  2: prominent decrease (~50%) in turbidity
  3: slight reduction in turbidity
  4: no reduction in turbidity

- Score of 0: AMB
- Score of 2: 5-FC, Azole, Echinocandin

CLSI M44-A: Disk diffusion of Yeasts

- Mueller-Hinton agar
  + 2% Glucose (fungal growth)
  & 0.5 μg/mL Methylene Blue dye (zone edge definition)
- 0.5 McF standard (1~5X10^6 cells/mL)
- Reading: after 20-24 hrs of incubation





Vitek 2 Yeast AST card (bioMerieux, France)

- **The first automatic AFST**
- **More rapid results**
- **Eliminates the difficulties in reading of other methods (trailing zone)**
- **AMB, 5-FC, Fluconazole, Voriconazole, Caspofungin, Micafungin (Oct 2006: licence for Flu)**

Comparison of the Vitek 2 system with the CLSI broth microdilution, disk diffusion, and sterol quantitation methods for determining fluconazole susceptibility against *Candida* spp.

Isolate	Species	MIC, μ/mL					Zone diameter, mm
		BMD-24V	BMD-48V	BMD-48S	SQM	Vitek 2	Disk diffusion
1	*C. albicans*	0.125	64	0.125	<4	≤1	28
2	*C. albicans*	0.25	0.25	>64	<4	≤1	32
3	*C. albicans*	0.25	8	0.125	<4	≤1	26
4	*C. albicans*	0.5	64	0.25	<4	4	26
5	*C. albicans*	2	4	16	<4	4	17
6	*C. albicans*	0.25	4	0.25	<4	≤1	30
7	*C. albicans*	8	8	64	5.83	8	17
8	*C. albicans*	0.25	0.25	>64	<4	8	32
9	*C. glabrata*	16	8	64	16.69	4	16
10	*C. glabrata*	16	16	>64	14.04	4	18
11	*C. tropicalis*	0.25	0.25	>64	<4	≤1	27
12	*C. tropicalis*	0.5	64	1	<4	≤1	28
13	*C. tropicalis*	1	64	2	15.74	4	19
14	*C. tropicalis*	0.5	64	1	<4	≤1	25
15	*C. tropicalis*	>64	>64	0.25	<4	≤1	21
16	*C. albicans*	16	8	16	27.74	8	16
17	*C. albicans*	32	64	64	<4	8	31
18	*C. albicans*	32	32	16	52.59	32	8
19	*C. albicans*	64	64	64	<4	≤1	29
20	*C. glabrata*	16	16	16	26.71	8	15
21	*C. glabrata*	16	32	64	16.07	4	13
22	*C. glabrata*	16	32	32	19.33	8	13
23	*C. glabrata*	16	16	16	19.09	4	16
24	*C. glabrata*	16	32	32	14.03	4	17
25	*C. tropicalis*	64	64	64	<4	≤1	9
26	*C. tropicalis*	64	>64	>64	<4	≤1	24
27	*C. albicans*	0.25	64	64	<4	≤1	29
28	*C. albicans*	0.25	64	64	<4	≤1	29
29	*C. albicans*	0.25	64	>64	<4	≤1	32
30	*C. tropicalis*	0.25	64	>64	<4	≤1	32
31	*C. tropicalis*	1	64	64	<4	≤1	28
ATCC22019	*C. parapsilosis*	0.25	0.25	0.25	<4	2	27
ATCC6258	*C. krusei*	16	32	32	22.07	16	8

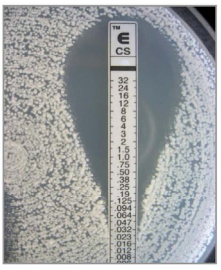
BMD, broth microdilution; BMD-24V, BMD-visual reading at 24 h of incubation; BMD-48V, BMD-visual reading after 48 h of incubation; BMD-48S, spectrophotometric reading after 48 h of incubation; SQM, sterol quantitation method; Vitek 2, Vitek 2 system.

Clin Chem Lab Med 2010;48(3)

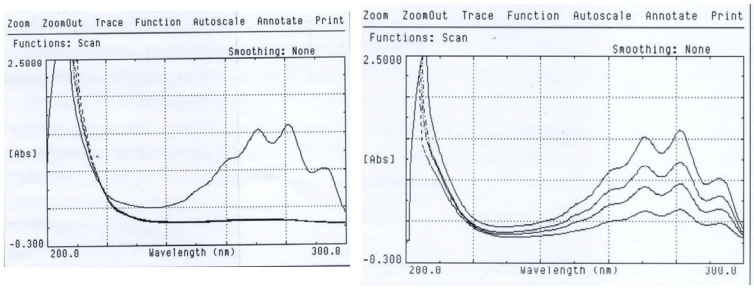
If the Vitek 2 system is used as a routine test for fluconazole MIC measurement, it is recommended that *Candida* spp. where the MIC is above 4 μg/mL be examined using a different method.

E-Test (AB Biodisk, Sweden)

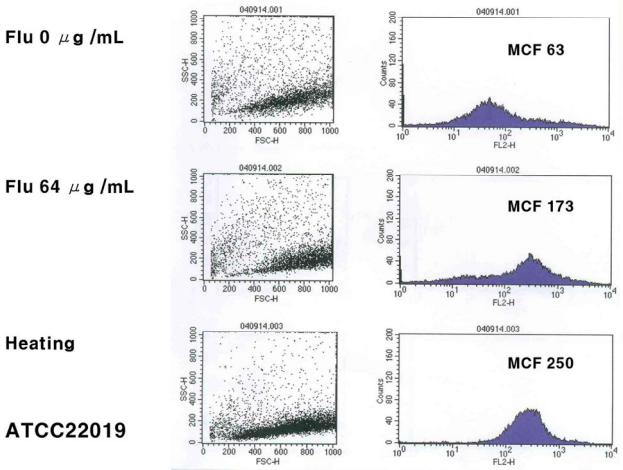
- **Quantitative agar diffusion method using antifungal gradient strips: direct reading of MICs.**
- **Available for all systemic AFST, excellent alternative to the CLSI standard.**



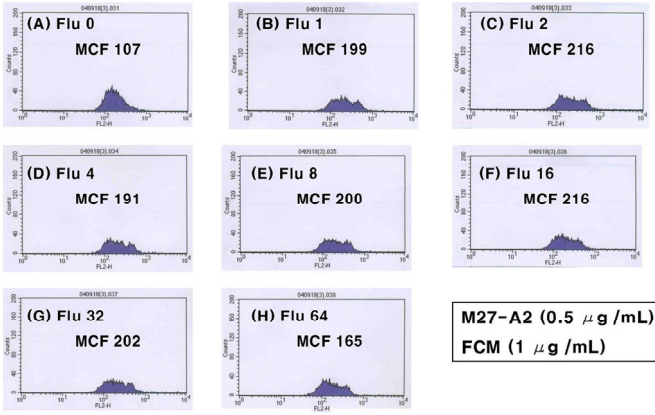
Sterol quantitation method (SQM)



Flowcytometry method: PI (Propidium iodide)

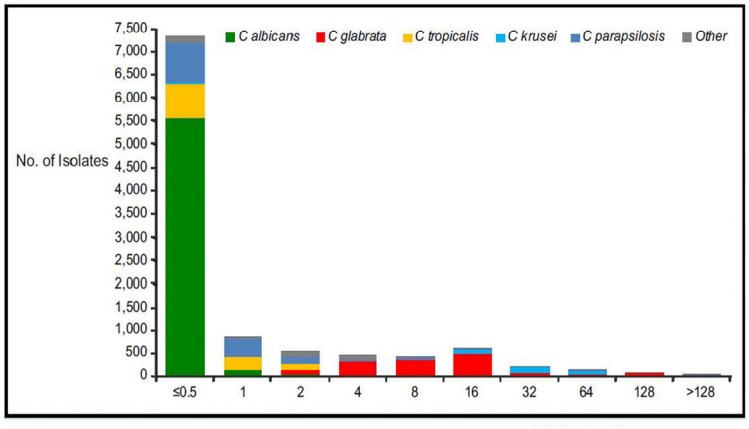


Ex: *C. tropicalis*



M27-A2 (0.5 μg /mL)
FCM (1 μg /mL)

Flu MIC distribution of 10,803 invasive *Candida* spp. using CLSI broth microdilution methods. Data compiled from Pfaller MA et al, 2010



Am J Med 2012;125:S3

Development of breakpoints

- Both CLSI and EUCAST have established clinical breakpoints (CBPs) for Flu and Vori versus *Candida by* taking into account the MIC distributions, pharmacokinetic (PK) and pharmacodynamic (PD) parameters, resistance mechanisms, and clinical outcomes as they relate to MIC values
- Epidemiologic cutoff values (ECVs)
- Species-specific CBPs
  ⇨ more sensitive & specific in detecting emerging resistance to antifungal agents

Interpretive guidelines for in vitro susceptibility testing of *Candida* spp. (CLSI)

항진균제	칸디다 종	판독 기준 (MIC, μg/mL)			
		S	SDD	I	R
Fluconazole	*C. albicans, C. parapsilosis, C. tropicalis*	≤ 2	4		≥ 8
	C. glabrata	–	≤ 32		≥ 64
Voriconazole	*C. albicans, C. parapsilosis, C. tropicalis*	≤ 0.12	0.25~0.5		≥ 1
	C. krusei	≤ 0.5	1		≥ 2
Anidulafungin Caspofungin	*C. albicans, C. tropicalis, C. krusei*	≤ 0.25		0.5	≥ 1
	C. glabrata	≤ 0.12		0.25	≥ 0.5
	C. parapsilosis. C. guilliermondii	≤ 2		4	≥ 8
Micafungin	*C. albicans, C. tropicalis, C. krusei*	≤ 0.25		0.5	≥ 1
	C. glabrata	≤ 0.06		0.12	≥ 0.25
	C. parapsilosis. C. guilliermondii	≤ 2		4	≥ 8

S, susceptible; SDD, susceptible-dose dependent; I, intermediate; R, resistant

Presumptive ID of *C. albicans*

- Germ tube test
- Spiking on blood agar plate (BAP)
- CHROMagar Candida
- Cornmeal tween 80 agar : Chlamydospore
- Preformed-enzyme test
  - Albistrip
  - Murex *C. albicans* CA50
- Monoclonal Ab
  - Bichro-latex albicans

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The Identification of *Malassezia* Yeasts

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Malassezia yeasts are lipophilic fungi that are recovered in 75~98% of healthy adults. The yeasts, since being first introduced in 1889, have been linked to various skin conditions such as pityriasis versicolor, seborrheic dermatitis, and *Malassezia* folliculitis, and most recently, atopic dermatitis. Its pathogenic ability is drawing attention more than ever as cases of confluent and reticulated papillomatosis and *Malassezia* onychomycosis, as well as systemic *Malassezia* infection in immunocompromised adults and neonates receiving intravenous fluid replacement have recently been reported.

Conventional studies and identification on *Malassezia* yeasts have traditionally been based on morphological and biochemical analyses. However, these methods often have dubious criteria, and environmental factors and genetic mutations are giving rise to new species. Therefore, new molecular biological methods, which would overcome these limitations, are now in demand.

The authors have already reported successful identification of *Malassezia* yeasts using 26S rDNA PCR-RFLP (polymerase chain reaction-restriction fragment polymorphism). RFLP methods enable us to analyze the pattern and size of fragmented amplified ribosomal DNA with the use of two restriction enzymes, *Hha*1, and *BstF*1. With these methods, genetic diversity can be examined, and it can be widely used in the rapid diagnosis and epidemiological study of fungal species because it is rapid, precise and cost-effective. In addition, the pyrosequencing method, which has recently been brought into the spotlight, enables us to identify the species with only a 30~40 bp sequence.

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MEMO

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2014년 10월 25일 발행
발행인 : 안 규 중
편집인 : 조 소 연
발행처 : 대한의진균학회

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Printing : October 21, 2014
Publishing : October 25, 2014
Publisher : Kyu Joong Ahn, M.D.
Editor : Soyun Cho, M.D.
Published by:
Korean Society for Medical Mycology

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