

# DESCRIPTIONS OF MEDICAL FUNGI

SECOND EDITION

DAVID ELLIS  
STEPHEN DAVIS  
HELEN ALEXIOU  
ROSEMARY HANDKE  
ROBYN BARTLEY

MYCOLOGY UNIT  
WOMEN'S AND CHILDREN'S HOSPITAL  
SCHOOL OF MOLECULAR & BIOMEDICAL SCIENCE  
UNIVERSITY OF ADELAIDE

ADELAIDE

AUSTRALIA

2007

Cover: *Cryptococcus neoformans*, and montages including *Microsporum*, *Candida*, *Schizophyllum*, *Sordaria*, *Conidiobolus*, *Fusarium*, *Bipolaris*, *Aspergillus*, *Curvularia*, *Saksenaea*, *Gliocladium*, *Trichophyton* and *Phialophora*.

Published by the Authors

Mycology Unit  
Women's and Children's Hospital  
North Adelaide 5006  
AUSTRALIA

Direct Phone: (08) 8161 7365  
International + 618 8161 7365

Direct Fax: (08) 8161 7589  
International + 618 8161 7589

Email: [dellis@adelaide.edu.au](mailto:dellis@adelaide.edu.au)

[www.mycology.adelaide.edu.au](http://www.mycology.adelaide.edu.au)

© Copyright 2007

The National Library of Australia Cataloguing-in-Publication entry:

Descriptions of medical fungi.

2nd ed.  
Bibliography.  
Includes index.  
ISBN 9780959851267 (pbk.).

1. Fungi - Indexes. 2. Mycology - Indexes. I. Ellis, David (David H.).

579.5

Printed in Adelaide by  
Nexus Print Solutions  
153 Holbrooks Road  
Underdale, South Australia 2032

---

## PREFACE

The Mycology Unit at the Adelaide Women's and Children's Hospital has played a key role in the provision of the Mycology component of the Microbiology Quality Assurance Program (QAP) organised by the Royal College of Pathologists of Australasia since its inception in 1979. The idea to provide all laboratories with a set of description sheets covering medical fungi evolved in the late 1980s and the first edition of this book was published in 1992. We now provide an updated edition which includes new and revised descriptions. We have endeavoured to reconcile current morphological descriptions with more recent genetic data, however in some cases, especially for the anthropophilic dermatophytes this is currently not possible.

These descriptions have by necessity been kept brief and many have been based on previous descriptions by other authors. For further information regarding any of the mycoses or pathogenic fungi mentioned, the reader is referred to the references cited. For the precise definitions of the mycological terminology used, the reader is referred to Ainsworth and Bisby's Dictionary of the Fungi (Kirk *et al.* 2001).

For many species, antifungal susceptibility data has also been provided. This has been derived from both the literature and in-house data from Australian clinical isolates generated by using the CLSI M27-A2 protocol for yeasts and the CLSI M38-A protocol for moulds. This composite data is provided as a guide only. MIC<sub>90</sub>s for *Aspergillus*, *Candida*, *Cryptococcus* and *Scedosporium* species are provided from large Australian studies based predominantly on primary isolates. In many cases the clinical relevance of *in vitro* antifungal susceptibility results remains difficult to interpret, and expert advice from a consulting microbiologist or infectious disease specialist may be required.

Risk group (RG) recommendations are based on published data and on current laboratory safety procedures in accordance with the Australian/New Zealand Standard AS/NZS 2243.3:2002. Safety in laboratories Part 3: Microbiological aspects and containment facilities.

David Ellis BSc (Hons), MSc, PhD, FASM, FRCPA (Hon).  
Associate Professor  
School of Molecular & Biomedical Sciences  
University of Adelaide  
Head, Mycology Unit  
Women's and Children's Hospital  
North Adelaide, Australia 5006

September, 2007



---

## CONTENTS

<i>Absidia corymbifera</i>	1	<i>Cunninghamella bertholletiae</i>	55
<i>Acremonium</i>	2	<i>Curvularia</i>	57
<i>Acrophialophora fuispora</i>	3	<i>Cylindrocarpon</i>	58
<i>Alternaria</i>	4	<i>Drechslera</i>	59
<i>Aphanoascus fulvescens</i>	5	<i>Epicoccum purpurascens</i>	60
<i>Apophysomyces elegans</i>	6	<i>Epidermophyton floccosum</i>	61
<i>Aspergillus</i>	8	<i>Exophiala dermatitidis</i>	62
<i>Aspergillus flavus</i>	9	<i>Exophiala jeanselmei</i> complex	63
<i>Aspergillus fumigatus</i>	10	<i>Exophiala spinifera</i> complex	65
<i>Aspergillus nidulans</i>	11	<i>Exserohilum</i>	66
<i>Aspergillus niger</i>	12	<i>Fonsecaea</i>	67
<i>Aspergillus terreus</i>	13	<i>Fusarium</i>	68
<i>Aureobasidium pullulans</i>	14	<i>Fusarium oxysporum</i>	69
<i>Basidiobolus ranarum</i>	15	<i>Fusarium solani</i>	70
<i>Beauveria</i>	16	<i>Geotrichum candidum</i>	71
<i>Bipolaris</i>	17	<i>Geotrichum capitatum</i>	72
<i>Blastomyces dermatitidis</i>	19	<i>Gliocladium</i>	73
<i>Candida</i>	20	<i>Graphium</i>	74
<i>Candida albicans</i>	23	<i>Histoplasma capsulatum</i>	75
<i>Candida colliculosa</i>	24	<i>Hortaea werneckii</i>	77
<i>Candida dubliniensis</i>	25	<i>Lasiodiplodia theobromae</i>	78
<i>Candida fabianii</i>	26	<i>Lecythophora hoffmannii</i>	79
<i>Candida famata</i>	27	<i>Madurella grisea</i>	80
<i>Candida glabrata</i>	28	<i>Madurella mycetomatis</i>	81
<i>Candida guilliermondii</i>	29	<i>Malassezia</i>	82
<i>Candida haemulonii</i>	30	<i>Malbranchea</i>	83
<i>Candida inconspicua</i>	31	<i>Microsporum</i>	84
<i>Candida kefyr</i>	32	<i>Microsporum audouinii</i>	85
<i>Candida krusei</i>	33	<i>Microsporum canis</i>	86
<i>Candida lipolytica</i>	34	<i>Microsporum canis</i> var. <i>distortum</i>	88
<i>Candida lusitaniae</i>	35	<i>Microsporum canis</i> var. <i>equinum</i>	89
<i>Candida norvegensis</i>	36	<i>Microsporum cookei</i>	90
<i>Candida parapsilosis</i>	37	<i>Microsporum ferrugineum</i>	91
<i>Candida pelliculosa</i>	38	<i>Microsporum fulvum</i>	92
<i>Candida rugosa</i>	39	<i>Microsporum gallinae</i>	93
<i>Candida tropicalis</i>	40	<i>Microsporum gypseum</i>	94
<i>Chaetomium</i>	41	<i>Microsporum nanum</i>	95
<i>Chrysosporium tropicum</i>	42	<i>Microsporum persicolor</i>	96
<i>Cladophialophora bantiana</i>	43	<i>Mortierella wolfii</i>	97
<i>Cladophialophora carrionii</i>	44	<i>Mucor</i>	98
<i>Cladosporium</i>	45	<i>Mucor amphibiorum</i>	99
<i>Coccidioides immitis</i>	46	<i>Mucor circinelloides</i>	100
<i>Colletotrichum</i>	48	<i>Mucor indicus</i>	100
<i>Conidiobolus coronatus</i>	49	<i>Mucor ramosissimus</i>	100
<i>Cryptococcus</i>	50	<i>Nattrassia mangiferae</i>	101
<i>Cryptococcus albidus</i>	51	<i>Ochroconis gallopava</i>	102
<i>Cryptococcus laurentii</i>	51	<i>Onychocola canadensis</i>	103
<i>Cryptococcus gattii</i>	52	<i>Paecilomyces</i>	104
<i>Cryptococcus neoformans</i>	53	<i>Paecilomyces lilacinus</i>	105

---

## CONTENTS

<i>Paecilomyces variotii</i>	106	<i>Trichophyton rubrum</i> granular type	160
<i>Paracoccidioides brasiliensis</i>	107	<i>Trichophyton schoenleinii</i>	162
<i>Penicillium</i>	108	<i>Trichophyton soudanense</i>	163
<i>Penicillium marneffeii</i>	110	<i>Trichophyton terrestre</i>	164
<i>Phaeoacremonium parasiticum</i>	111	<i>Trichophyton tonsurans</i>	165
<i>Phialophora</i>	112	<i>Trichophyton verrucosum</i>	167
<i>Phialophora richardsiae</i>	112	<i>Trichophyton violaceum</i>	169
<i>Phialophora verrucosa</i>	113	<i>Trichosporon</i>	170
<i>Phoma</i>	114	<i>Trichosporon asahii</i>	171
<i>Pithomyces</i>	115	<i>Trichosporon asteroides</i>	172
<i>Prototheca</i>	116	<i>Trichosporon cutaneum</i>	172
<i>Ramichloridium</i>	117	<i>Trichosporon inkin</i>	172
<i>Ramichloridium schulzeri</i>	117	<i>Trichosporon mucoides</i>	173
<i>Rhinocladiella</i>	118	<i>Trichosporon ovoides</i>	173
<i>Rhinocladiella atrovirens</i>	118	<i>Trichothecium roseum</i>	174
<i>Rhizomucor</i>	119	<i>Ulocladium</i>	175
<i>Rhizomucor miehei</i>	119	<i>Veronaea botryosa</i>	176
<i>Rhizomucor pusillus</i>	120	<i>Verticillium</i>	177
<i>Rhizopus</i>	121		
<i>Rhizopus azygosporus</i>	122	<b>Microscopy Stains &amp; Techniques</b>	178
<i>R. microsporus</i> var. <i>microsporus</i>	122	Calcofluor White with 10% KOH	178
<i>R. microsporus</i> var. <i>oligosporus</i>	123	KOH with Chlorazol Black	178
<i>R. microsporus</i> var. <i>rhizopodiformis</i>	123	India Ink Mounts	178
<i>Rhizopus oryzae</i>	124	Lactophenol Cotton Blue (LPCB)	179
<i>Rhodotorula</i>	125	Direct Microscopic Preparations	179
<i>Rhodotorula glutinis</i>	126	Cellotape Flag Preparations	179
<i>Rhodotorula mucilaginosa</i>	127	Slide Culture Preparations	180
<i>Saccharomyces cerevisiae</i>	128	<b>Specialised Culture Media</b>	181
<i>Saksenaea vasiformis</i>	129	Bird seed agar	181
<i>Scedosporium apiospermum</i>	131	Bromcresol Purple Milk Agar	181
<i>Scedosporium aurantiacum</i>	131	CDBT media	182
<i>Scedosporium prolificans</i>	134	CGB media	182
<i>Schizophyllum commune</i>	135	Cornmeal agar	183
<i>Scopulariopsis</i>	136	Cornmeal glucose sucrose agar	183
<i>Sepedonium</i>	137	Czapek Dox agar	183
<i>Sporothrix schenckii</i>	138	Dixon's agar	183
<i>Stemphylium</i>	140	Hair perforation test	184
<i>Syncephalastrum</i>	141	Lactritmel agar	184
<i>Trichoderma</i>	142	Littman oxgall agar	184
<i>Trichophyton</i>	143	Malt extract agar	185
<i>Trichophyton ajelloi</i>	144	1% Peptone agar	185
<i>Trichophyton concentricum</i>	145	Potato dextrose agar	185
<i>Trichophyton equinum</i>	147	Rice grain slopes	185
<i>Trichophyton erinacei</i>	149	Sabouraud dextrose agar	186
<i>Trichophyton interdigitale</i>	151	Sabouraud dextrose agar 5% NaCl	186
<i>T. interdigitale</i> var. <i>nodulare</i>	153	Tap water agar	187
<i>Trichophyton mentagrophytes</i>	154	Urease agar with 0.5% glucose	187
<i>T. mentag.</i> var. <i>quinckeanum</i>	156	Vitamin free agar	187
<i>Trichophyton rubrum</i>	158	<b>References</b>	188

## ***Absidia corymbifera* (Cohn) Saccardo & Trotter**

The genus *Absidia* is characterised by a differentiation of the hyphae into arched stolons bearing more or less verticillate sporangiophores at the raised part of the stolon (internode), and rhizoids formed at the point of contact with the substrate (at the node). This feature separates species of *Absidia* from the genus *Rhizopus*, where the sporangia arise from the nodes and are therefore found opposite the rhizoids. The sporangia are relatively small, globose, pyriform or pear-shaped and are supported by a characteristic funnel-shaped apophysis. This distinguishes *Absidia* from the genera *Mucor* and *Rhizomucor*, which have large, globose sporangia without an apophysis. *Absidia* currently contains 21 mostly soil-borne species. *A. corymbifera* is a known human pathogen causing pulmonary, rhinocerebral, disseminated, CNS or cutaneous zygomycosis.

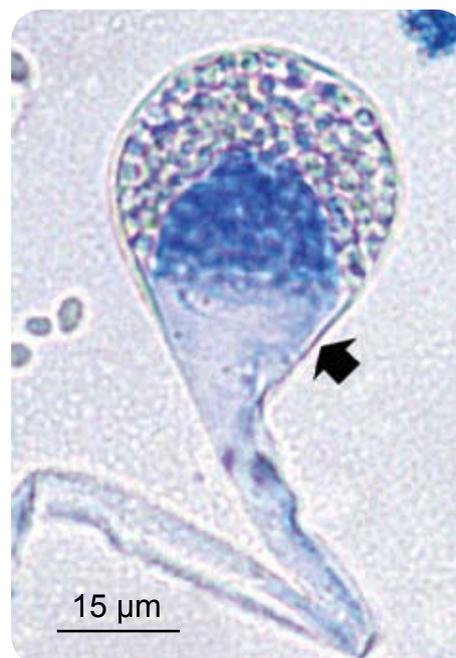
Colonies are fast growing, floccose, white at first becoming pale grey with age, and up to 1.5 cm high. Sporangiophores are hyaline to faintly pigmented, simple or sometimes branched, arising solitary from the stolons, in groups of three, or in whorls of up to seven. Rhizoids are very sparingly produced and may be difficult to find without the aid of a dissecting microscope to examine the colony on the agar surface. Sporangia are small (10-40 µm in diameter) and are typically pyriform in shape with a characteristic conical-shaped columella and pronounced apophysis, often with a short projection at the top. Sporangiospores vary from subglobose to oblong-ellipsoidal (3-7 x 2.5-4.5 µm), hyaline to light grey and smooth-walled. Temperature: optimum 35-37°C; maximum 45°C. RG-2 organism.

For descriptions of species, keys to taxa and additional information see Ellis and Hesse (1965 and 1966), Hesse and Ellis (1964 and 1966), Nottebrock *et al.* (1974), O'Donnell (1979), Samson *et al.* (1995), Domsch *et al.* (1980), McGinnis (1980), de Hoog *et al.* (2000) and Ellis (2005b).

**Key Features:** zygomycete, small pyriform-shaped sporangia with a characteristic conical-shaped columellae and pronounced apophysis, rapid growth at 40°C.

Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>
Amphotericin B	0.03-2	1
Flucytosine	>256	>256
Fluconazole	>16	>16
Itraconazole	0.03-2	0.5
Posaconazole	0.03 - 1	0.25
Voriconazole	2->64	>16

Very limited data, antifungal susceptibility testing of individual strains is recommended. Sun *et al.* (2002), Dannaoui *et al.* (2003), Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003, 2006), Singh *et al.* (2005), Sabatelli *et al.* (2006) and WCH in-house data.



*A. corymbifera* showing a typical pyriform-shaped sporangium with a conical-shaped columella and pronounced apophysis (arrow).

## ***Acremonium* Link ex Fries**

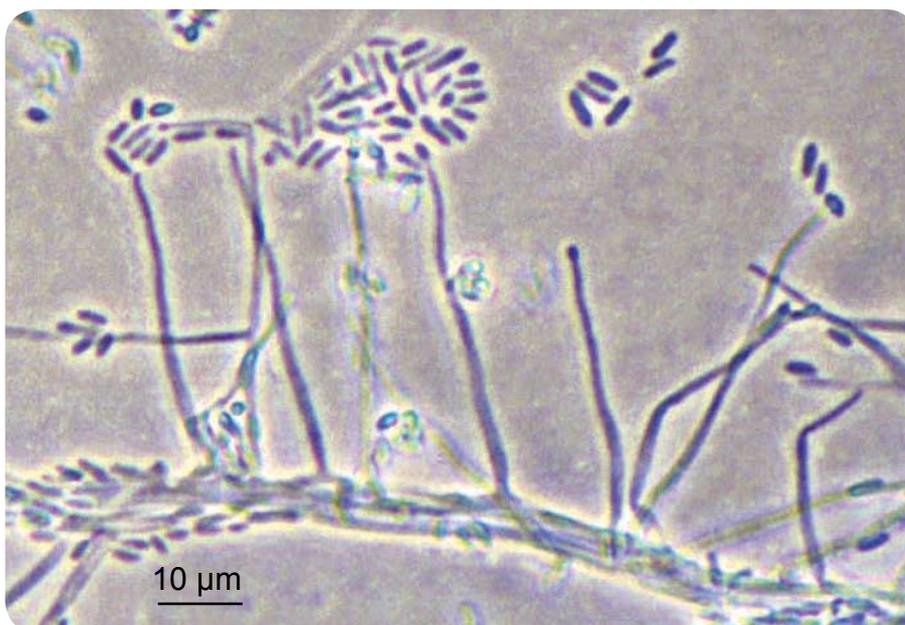
Colonies are usually slow growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in colour. Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides. Conidia are usually one-celled (ameroconidia), hyaline or pigmented, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide.

The genus *Acremonium* currently contains 100 species, most are saprophytic being isolated from dead plant material and soil. However a number of species including *A. falciforme*, *A. kiliense*, *A. recifei*, *A. alabamensis*, *A. roseogriseum* and *A. strictum* are recognised as opportunistic pathogens of man and animals, causing mycetoma, mycotic keratitis and onychomycosis. RG-2 for species isolated from humans.

Microconidial *Fusarium* isolates may be confused with *Acremonium*, but they usually grow faster and have colonies with a characteristic fluffy appearance.

**Key Features:** hyphomycete with solitary, erect, hyaline, awl-shaped phialides producing single-celled, globose to cylindrical conidia, mostly in slimy heads.

For descriptions of species, keys to taxa and additional information see Gams (1971), Domsch *et al.* (1980), Samson *et al.* (1995) and de Hoog *et al.* (2000).



*Acremonium* showing long awl-shaped phialides producing cylindrical, one-celled conidia mostly aggregated in slimy heads at the apex of each phialide.

Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range
Itraconazole	0.5->8	Amphotericin B	0.5-16
Posaconazole	0.06-4	Caspofungin	0.03->8
Voriconazole	0.06-4	Anidulafungin	0.5->8

Very limited data, antifungal susceptibility testing of individual strains is recommended. Guarro *et al.* (1997), Pfaller *et al.* (1998, 2002a), Espinel-Ingroff (2003), Cuenca-Estrella *et al.* (2006) and WCH in-house data.

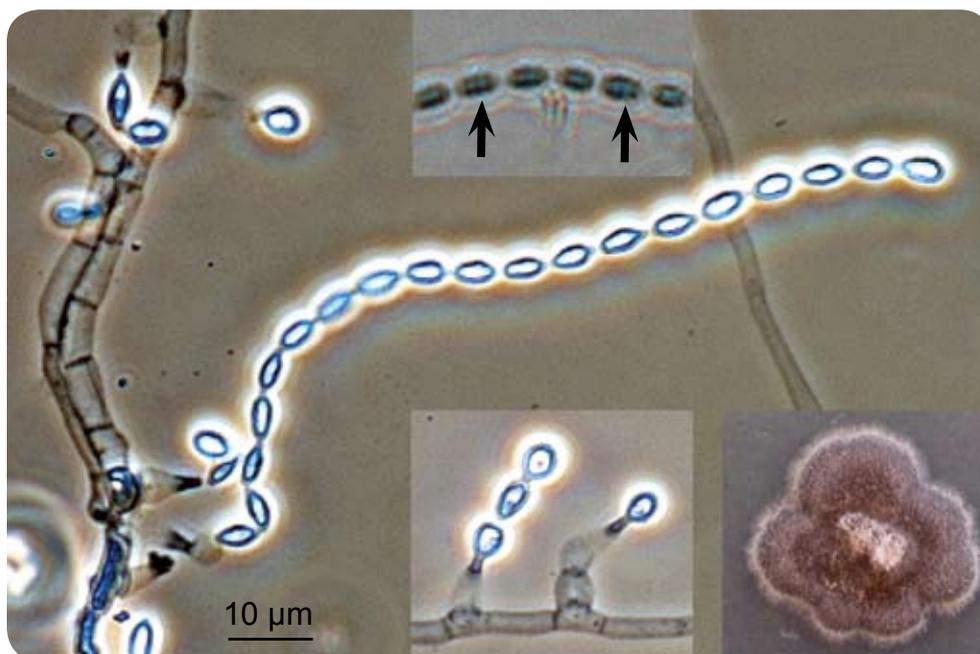
### ***Acrophialophora fusispora* (S.B. Saksena) Samson**

Colonies fast growing, greyish-brown with a black reverse. Conidiophores arising singly, terminally and laterally from the hyphae, erect, straight or slightly flexuose, tapering towards the apex, pale brown, rough-walled, up to 15 µm long, 2-5 µm wide, with whorls of phialides on the upper part. Phialides flask-shaped with a swollen base and a long, narrow neck, hyaline, smooth-walled or echinulate, 9-15 × 3-4.5 µm in the broadest part. Conidia in long chains, limoniform, one-celled, pale brown 5-12 × 3-6 µm, smooth to finely echinulate with indistinct spiral bands. Temperature: optimum 40°C; maximum 50°C.

The genus *Acrophialophora* contains 3 species and is most commonly associated with soil, especially from India. *A. fusispora* is a rare human pathogen. RG-1 organism.

**Key Features:** hyphomycete with flask-shaped phialides producing long chains of one-celled, limoniform, pale brown conidia, with indistinct spiral bands.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), de Hoog *et al.* (2000) and Al-Mohsen *et al.* (2000).



Culture, phialides and conidia with striations (arrows) of *A. fusispora*.

Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range
Fluconazole	8-32	Amphotericin B	0.25-2
Itraconazole	0.06-0.125	Flucytosine	>64
Voriconazole	0.06	Posaconazole	0.03

Very limited data, antifungal susceptibility testing of individual strains is recommended. Al-Mohsen *et al.* (2000) and WCH in-house data.

## ***Alternaria* Nees ex Fries**

Colonies are fast growing, black to olivaceous-black or greyish, and are suede-like to floccose. Microscopically, branched acropetal chains (blastocatenate) of multicellular conidia (dictyoconidia) are produced sympodially from simple, sometimes branched, short or elongate conidiophores. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak, pale brown, smooth-walled or verrucose.

The genus contains 44 species, most are plant parasites, but a few species are ubiquitous and are also frequently soil-borne. *A. alternata* is the most common of these. Although usually seen as saprophytic contaminants, *Alternaria* species are recognised causative agents of mycotic keratitis.

*Alternaria* species soon lose their ability to sporulate in culture. Potato dextrose agar and cornmeal agar are the most suitable media to use, and incubation under near ultra-violet light is recommended to maintain sporulation. Temperature: optimum 25-28°C; maximum 31-32°C. RG-1 organism.

**Key Features:** dematiaceous hyphomycete producing chains of darkly pigmented, ovoid to obclavate dictyoconidia, often with short conical or cylindrical beaks.

For descriptions of species, keys to taxa and additional information see Simmons (1967), Ellis (1971), Domsch *et al.* (1980), Samson *et al.* (1995), de Hoog *et al.* (2000).



*Alternaria alternata* showing branched acropetal chains and multi-celled, obclavate to obpyriform conidia with short conical beaks.

Antifungal	MIC $\mu\text{g/mL}$		Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	16->64	>64	Amphotericin B	0.125->16	2 (4)
Itraconazole	0.125-2	1	Flucytosine	>128	>128
Voriconazole	0.5-2	1	Posaconazole	0.06-0.25	0.25

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Pujol *et al.* (2000), Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Sabatelli *et al.* (2006) and WCH in-house data.

## ***Aphanoascus fulvescens* (Cooke) Apinis**

Colonies are moderately fast growing, white to tan with the production of numerous spherical, pseudoparenchymatous, buff to light brown cleistothecia (non-ostiolate ascocarps). Asci are subspherical to ellipsoidal and eight-spored. Ascospores light brown, yellowish to pale brown in mass, irregularly reticulate, lens-shaped, 3.5-4.7 x 2.5-3.5  $\mu\text{m}$ . *Aphanoascus fulvescens* has a *Chrysosporium* anamorph showing typical pyriform to clavate-shaped conidia with truncated bases, 15.0-17.5 x 3.7-6.0  $\mu\text{m}$ , which are formed either intercalary, laterally or terminally.

*Aphanoascus fulvescens* is a soil keratinolytic ascomycete which occasionally causes dermatomycosis in man and animals. RG-2 organism.

**Key Features:** keratinolytic ascomycete with a *Chrysosporium* anamorph.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980) and de Hoog *et al.* (2000).



10  $\mu\text{m}$

Culture, cleistothecium and conidia of *Aphanoascus fulvescens*.

## ***Apophysomyces elegans* Misra, Srivastava & Lata**

Colonies are fast growing, white, becoming creamy white to buff with age, downy with no reverse pigment, and are composed of broad, sparsely septate (coenocytic) hyphae typical of a zygomycetous fungus. Sporangiohores are unbranched, straight or curved, slightly tapering towards the apex, up to 200 µm long, 3- 5 µm in width near the apophysis, and hyaline when young but developing a light to dark brown pigmentation and a conspicuous sub-apical thickening 10-16 µm below the apophysis with age. Sporangiohores arise at right angles from the aerial hyphae and often have a septate basal segment resembling the “foot cell” commonly seen in *Aspergillus*. Rhizoids are thin-walled, subhyaline and predominantly unbranched. Sporangia are multispored, small (20-50 µm diameter), typically pyriform in shape, hyaline at first, sepia-coloured when mature, columellate and strongly apophysate. Columellae are hemispherical in shape and the apophyses are distinctively funnel or bell-shaped. Sporangiospores are smooth-walled, mostly oblong, occasionally subglobose, (3-4 x 5-6 µm), subhyaline to sepia in mass. Good growth at 26°C, 37°C and 42°C. RG-2 organism.

*Apophysomyces elegans* is readily distinguishable from other zygomycetes of medical importance, especially the morphologically similar, strongly apophysate pathogen *Absidia corymbifera*, by having sporangiohores with distinctive funnel or bell-shaped apophyses and hemispherical-shaped columellae. In addition, there is a conspicuous pigmented sub-apical thickening which constricts the lumen of the sporangiohore below the apophysis, and there are also distinctive foot cells.

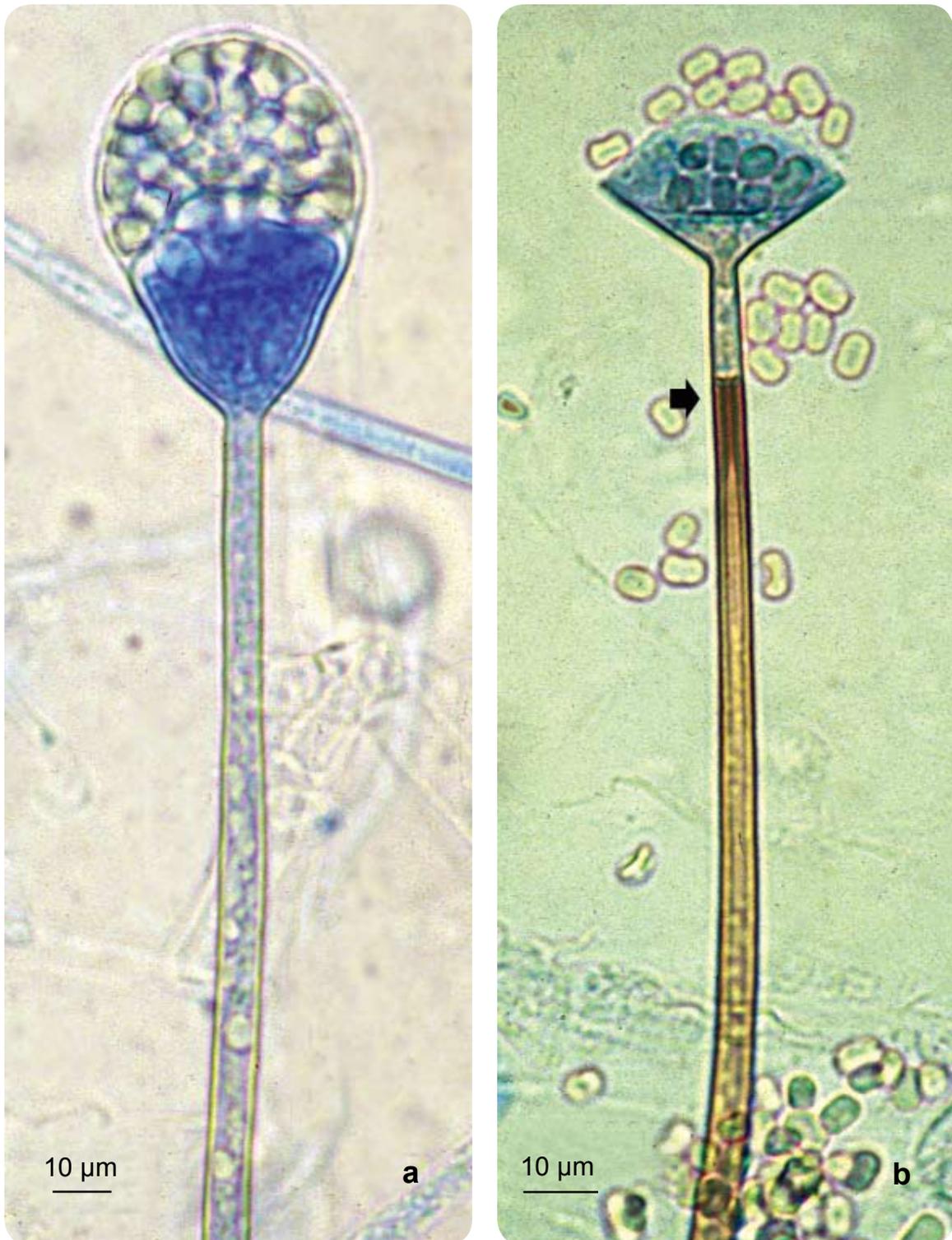
Laboratory identification of this fungus may be difficult or delayed because of the mould's failure to sporulate on the primary isolation media or on subsequent subculture onto potato dextrose agar. Sporulation may be stimulated by the use of nutrient deficient media, like cornmeal-glucose-sucrose-yeast extract agar, Czapek Dox agar, or by using the agar block method described by Ellis and Ajello (1982) and Ellis and Kaminski (1985).

**Key Features:** zygomycete, rare human pathogen usually associated with invasive lesions following the traumatic implantation of the fungus through the skin. Soil fungus with a tropical to sub-tropical distribution. Characteristic “cocktail glass” apophysate sporangial morphology with conspicuous sub-apical thickening of the sporangiohore, rapid growth at 42°C.

For descriptions of species, keys to taxa and additional information see Cooter *et al.* (1990), Ellis and Ajello, (1982), Misra *et al.* (1979), Padhye and Ajello (1988), Lawrence *et al.* (1986), Wieden *et al.* (1985), de Hoog *et al.* (2000) and Ellis (2005b).

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	>64	Amphotericin B	0.03-2
Itraconazole	0.03-8	Flucytosine	>256
Posaconazole	0.03-4	Voriconazole	8->64

Very limited data, antifungal susceptibility testing of individual strains is recommended. Sun *et al.* (2002), Dannaoui *et al.* (2003), Sabatelli *et al.* (2006) and WCH in-house data.

***Apophysomyces elegans* Misra, Srivastava & Lata**

(a) Young, multisporous, pyriform sporangium of *A. elegans* showing a typical funnel-shaped apophysis but without the sub-apical thickening of a more mature sporangiophore. (b) Mature sporangium of *A. elegans* showing distinctive funnel-shaped apophyses, columellae, and a conspicuous pigmented sub-apical thickening which constricts the lumen of the sporangiophore below the apophysis (arrow). Sporangiospores are smooth-walled, oblong and subhyaline.

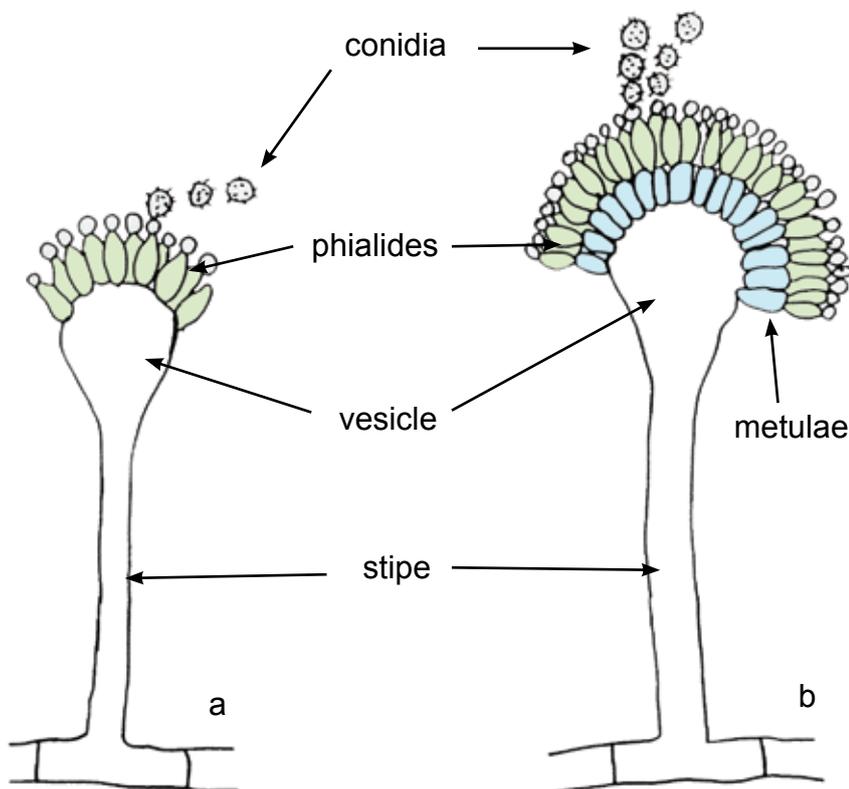
## ***Aspergillus Micheli ex Link***

Colonies are usually fast growing, white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores. Conidiophores terminate in a vesicle covered with either a single palisade-like layer of phialides (uniseriate) or a layer of subtending cells (metulae) which bear small whorls of phialides (the so called biseriata structure). The vesicle, phialides, metulae (if present) and conidia form the conidial head. Conidia are one-celled, smooth or rough walled, hyaline or pigmented are produced in long dry chains which may be divergent (radiate) or aggregated in compact columns (columnar). Some species may produce Hülle cells or sclerotia.

For identification, isolates are usually inoculated at three points on Czapek Dox agar and 2% malt extract agar and incubated at 25°C. Most species sporulate within 7 days. Descriptions are primarily based on colony pigmentation and morphology of the conidial head. Microscopic mounts are best made using cellotape flag or slide culture preparations mounted in lactophenol cotton blue. A drop of alcohol is usually needed to remove bubbles and excess conidia.

**Key Features:** hyaline hyphomycete showing distinctive conidial heads with flask-shaped phialides arranged in whorls on a vesicle.

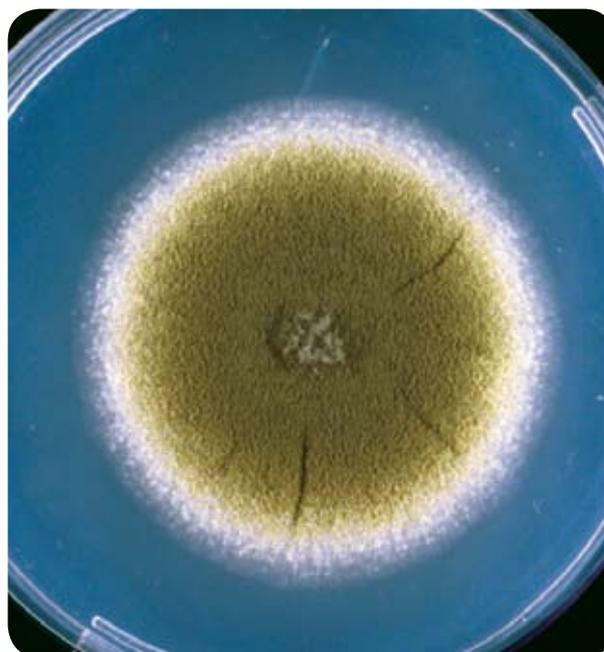
For descriptions of species, keys to taxa and additional information see Raper and Fennell (1965), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Samson and Pitt (1990), Samson *et al.* (1995), de Hoog *et al.* (2000) and Klich (2002).



Conidial head morphology in *Aspergillus* (a) uniseriate, (b) biserial.

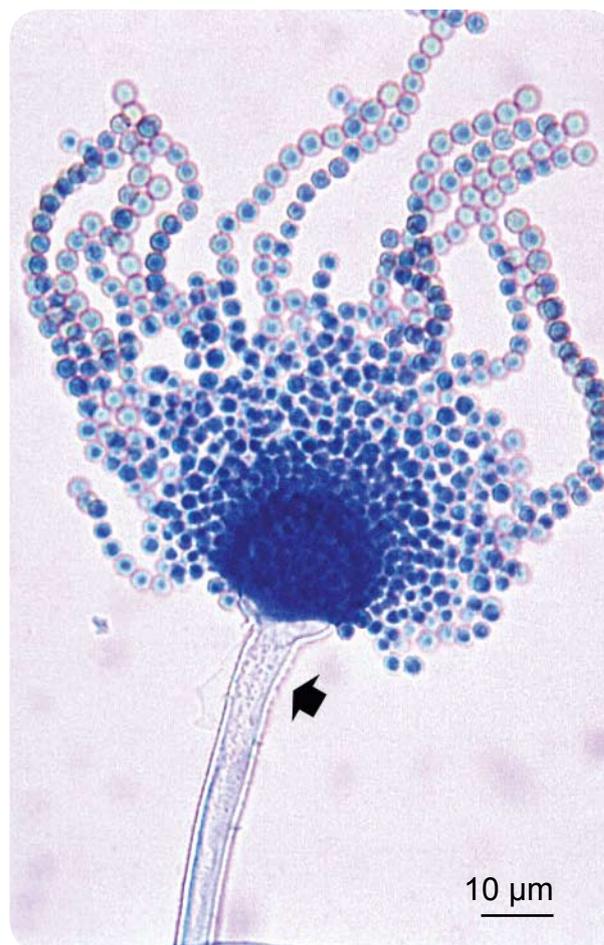
### ***Aspergillus flavus* Link ex Grey**

On Czapek Dox agar, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.



*A. flavus* has a world-wide distribution and normally occurs as a saprophyte in soil and on many kinds of decaying organic matter, however, it is also a recognised pathogen of humans and animals. RG-2 organism.

**Key Features:** spreading yellow-green colonies, rough-walled stipes, mature vesicles bearing phialides over their entire surface and conspicuously echinulate conidia.



Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>
Amphotericin B	0.06->8	4
Itraconazole	0.03-8	0.5
Voriconazole	0.03-2	0.5
Posaconazole	0.03-1	0.5
Anidulafungin	<0.03-0.125	nd
Caspofungin	<0.03->8	nd

Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002a), Diekema *et al.* (2003), Espinel-Ingroff (2001, 2003), Serrano *et al.* (2003), Cuenca-Estrella *et al.* (2006), Sabatelli *et al.* (2006). MIC<sub>90</sub>s from Australian clinical isolates (nd = not done).

Culture and conidial head of *A. flavus*. Note: rough-walled stipe near vesicle (arrow) and that both uniseriate and biseriate conidial heads may be present.

## ***Aspergillus fumigatus* Fresenius**

On Czapek Dox agar, colonies show typical blue-green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores. Conidial heads are typically columnar (up to 400 x 50 µm but often much shorter and smaller) and uniseriate. Conidiophore stipes are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to subglobose (2.5-3.0 µm in diameter), green and rough-walled to echinulate. Note: This species is thermotolerant and grows at temperatures up to 55°C.

*A. fumigatus* is truly a cosmopolitan mould and has been found almost everywhere on every conceivable type of substrate. It is an important pathogen of humans and animals. RG-2 organism.

**Key Features:** uniseriate and columnar conidial heads with the phialides limited to the upper two thirds of the vesicle and curving to be roughly parallel to each other.



Culture and conidial head morphology of *A. fumigatus*. Note: uniseriate row of phialides on the upper two thirds of the vesicle.

Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>
Amphotericin B	0.03->8	2
Itraconazole	<0.03->16	0.5
Voriconazole	<0.03-8	0.25
Posaconazole	<0.03-2	0.125
Anidulafungin	<0.03-0.125	nd
Caspofungin	0.015->8	nd

Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002a), Diekema *et al.* (2003), Espinel-Ingroff (2001, 2003), Serrano *et al.* (2003), Cuenca-Estrella *et al.* (2006), Sabatelli *et al.* (2006). MIC<sub>90</sub>s from Australian clinical isolates (nd = not done).

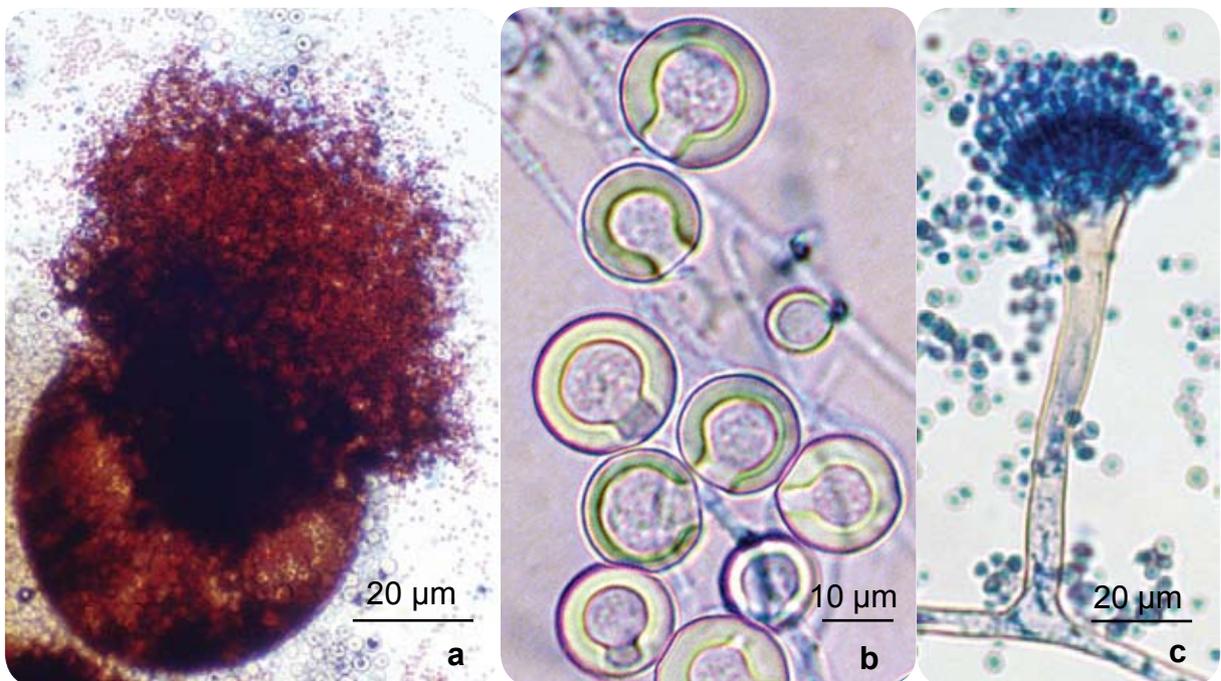
## ***Aspergillus nidulans* (Eidam) Wint.**

**Teleomorph:** *Emericella nidulans* (Eidam) Vuill.

On Czapek Dox agar, colonies are typically plain green in colour with dark red-brown cleistothecia developing within and upon the conidial layer. Reverse may be olive to drab-grey or purple-brown. Conidial heads are short columnar (up to 70 x 30 µm in diameter) and biseriate. Conidiophore stipes are usually short, brownish and smooth-walled. Conidia are globose (3-3.5 µm in diameter) and rough-walled.

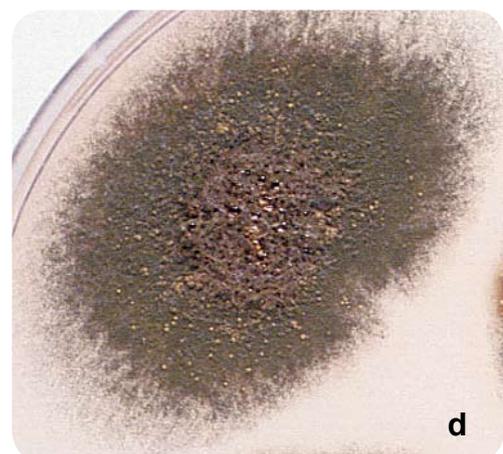
*A. nidulans* is a typical soil fungus with a world-wide distribution, it has also been reported causing disease in human and animals. RG-1 organism.

**Key Features:** conidial heads are short columnar and biseriate. Stipes are usually short, brownish and smooth-walled. Conidia are globose and rough-walled.



(a) Cleistothecium of *Emericella nidulans* (anamorph *Aspergillus nidulans*) showing numerous reddish-brown ascospores and thick-walled hülle cells; (b) cleistothecia are often surrounded by a mass of hülle cells which are up to 25 µm in diameter; (c) conidial head and stipe and (d) culture of *A. nidulans*.

Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>
Amphotericin B	0.125-4	2
Itraconazole	0.03-8	0.25
Voriconazole	0.125-4	0.25
Posaconazole	0.03-1	0.25
Caspofungin	0.125-8	nd
Espinel-Ingroff <i>et al.</i> (2001), Espinel-Ingroff (2003), Cuenca-Estrella <i>et al.</i> (2006). MIC <sub>90</sub> s from Australian clinical isolates (nd = not done).		



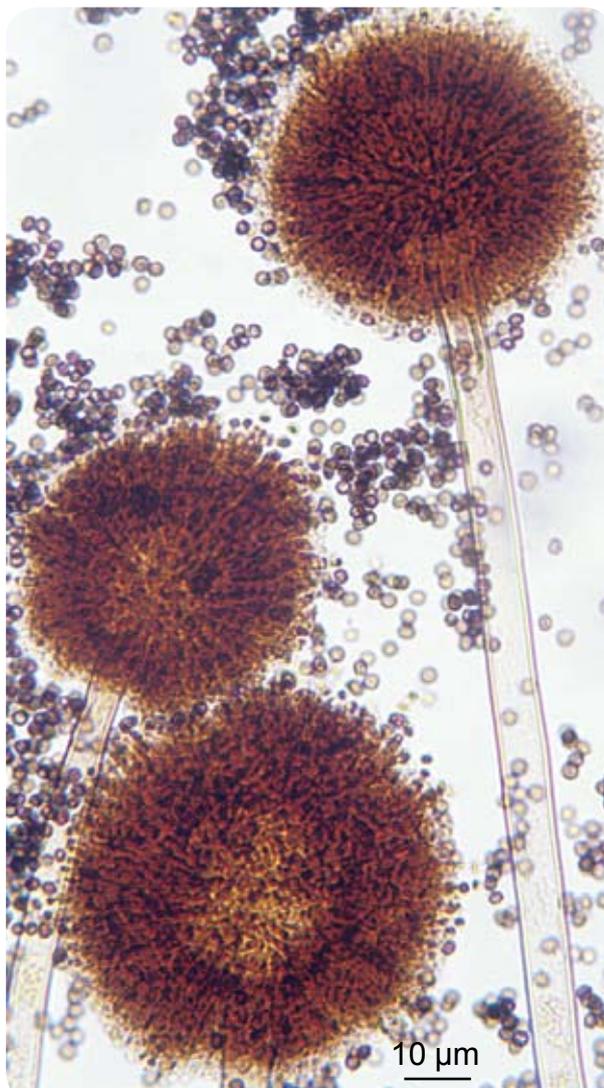
## ***Aspergillus niger* van Tieghem**

On Czapek Dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm by 15 to 20  $\mu\text{m}$  in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5  $\mu\text{m}$  in diameter), dark brown to black and rough-walled.



*A. niger* is one of the most common and easily identifiable species of the genus *Aspergillus*, with its white to yellow mat later bearing black conidia. This species is very commonly found in aspergillomas and is the most frequently encountered agent of otomycosis. It is also a common laboratory contaminant. RG-1 organism.

**Key Features:** conidial heads are dark brown to black, radiate and biseriate with metulae twice as long as the phialides. Conidia brown and rough-walled.



Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>
Amphotericin B	0.125-2	2
Itraconazole	0.03->8	0.5
Voriconazole	<0.03-4	0.5
Posaconazole	0.03-1	0.25
Anidulafungin	0.03	nd
Caspofungin	0.015-0.25	nd

Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002), Diekema *et al.* (2003), Espinel-Ingroff (2003), Serrano *et al.* (2003), Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from Australian clinical isolates (nd = not done).

Culture and conidial head morphology of *A. niger*. Note: conidial heads are biseriate, large, globose, dark brown, becoming radiate with the phialides borne on metulae.

### ***Aspergillus terreus* Thom**

On Czapek Dox agar, colonies are typically suede-like and cinnamon-buff to sand brown in colour with a yellow to deep dirty brown reverse. Conidial heads are compact, columnar (up to 500 x 30-50 µm in diameter) and biseriate. Conidiophore stipes are hyaline and smooth-walled. Conidia are globose to ellipsoidal (1.5-2.5 µm in diameter), hyaline to slightly yellow and smooth-walled.

*A. terreus* occurs commonly in soil and is occasionally reported as a pathogen of humans and animals. RG-2 organism.

**Key Features:** cinnamon-brown cultures, conidial heads biseriate with metulae as long as the phialides.

For descriptions of species, keys to taxa and additional information see Raper and Fennell (1965), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Samson and Pitt (1990), Samson *et al.* (1995), de Hoog *et al.* (2000) and Klich (2002).



Culture and conidial head and conidiophore of *A. terreus*. Note: conidial heads are biseriate.

Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>
Amphotericin B	0.06-16	4
Itraconazole	0.03-1	0.25
Voriconazole	0.06-2	0.25
Posaconazole	0.03-2	0.125
Anidulafungin	0.03	nd
Caspofungin	0.015-0.5	nd

Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002), Diekema *et al.* (2003), Espinel-Ingroff (2003), Serrano *et al.* (2003), Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from Australian clinical isolates (nd = not done).

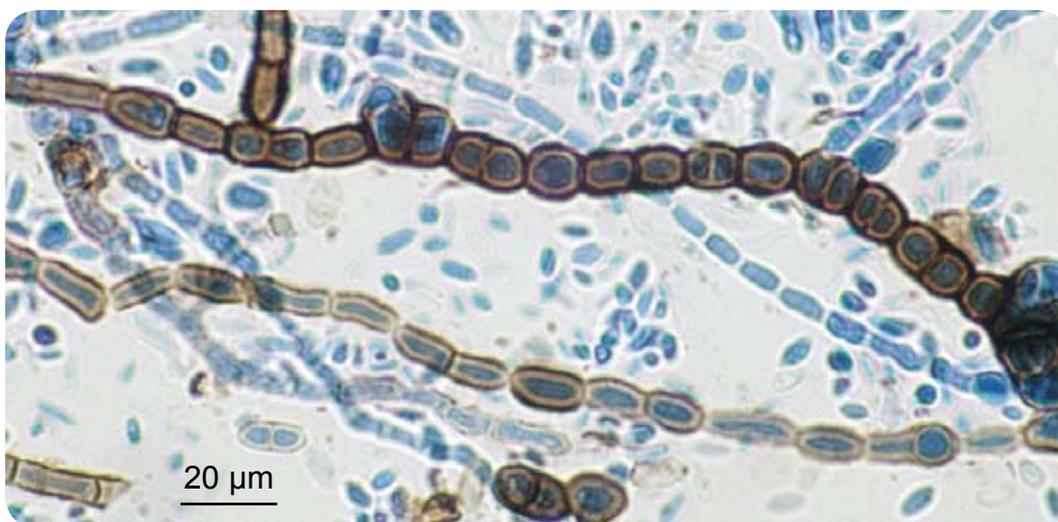
### ***Aureobasidium pullulans* (de Bary) Arnaud**

Colonies are fast growing, smooth, soon covered with slimy masses of conidia, cream or pink to brown or black. Hyphae hyaline and septate, frequently becoming dark-brown with age and forming chains of one- to two-celled, thick-walled, darkly pigmented arthroconidia. These arthroconidia actually represent the *Scytalidium* anamorph of *Aureobasidium* and are only of secondary importance in recognising members of this genus. Conidia are produced synchronously in dense groups from indistinct scars or from short denticles on undifferentiated, hyaline to sub-hyaline hyphae. Conidia are hyaline, smooth-walled, single-celled, ellipsoidal but of very variable shape and size (8-12 × 4-6 µm), often with an indistinct hilum (= a mark or scar at the point of attachment). Temperature: optimum 25°C; maximum 35-37°C.

This species has two varieties: *A. pullulans* var. *pullulans*, with a colony which remains pink, light brown, or yellow for at least three weeks, and *A. pullulans* var. *melanogenum* which soon becomes black or greenish-black due to dark hyphae which often fall apart into separate cells. *A. pullulans* has a world-wide distribution and is usually isolated as a saprophyte, occasionally from skin and nails. However, it has also been reported as a rare causative agent of phaeohyphomycosis, mycotic keratitis and peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). RG-1 organism.

**Key Features:** hyphomycete (so called black yeast) producing hyaline blastoconidia simultaneously from the vegetative hyphae, which may also form chains of darkly pigmented, thick-walled arthroconidia.

For descriptions of species, keys to taxa and additional information see Hermanides-Nijhof (1977), Domsch *et al.* (1980), McGinnis (1980) and de Hoog *et al.* 2000.



*A. pullulans* showing chains of one- to two-celled, darkly pigmented arthroconidia of the *Scytalidium* anamorph of *Aureobasidium* and the presence of numerous hyaline, single-celled, ovoid-shaped conidia which are produced on short denticles.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.125-2	Itraconazole	0.03-0.25	Voriconazole	0.03-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001) and WCH in-house data.

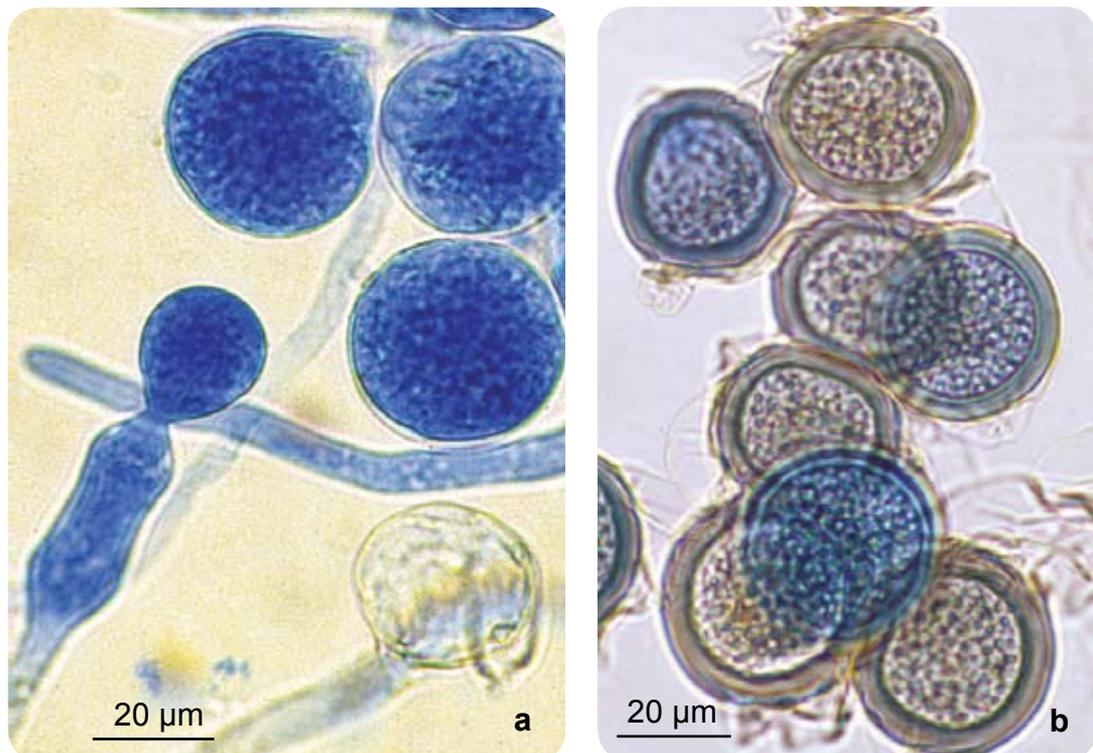
## ***Basidiobolus ranarum* Eidem**

**Synonyms:** *Basidiobolus meristosporus* Drechsler; *Basidiobolus heterosporus* Srinivasan & Thirumalachar; *Basidiobolus haptosporus* Drechsler.

Colonies are moderately fast growing at 30°C, flat, yellowish-grey to creamy-grey, glabrous, becoming radially folded and covered by a fine, powdery, white surface mycelium. Note: satellite colonies are often formed by germinating conidia ejected from the primary colony. Microscopic examination usually shows the presence of large vegetative hyphae (8-20 µm in diameter) forming numerous round (20-50 µm in diameter), smooth, thick-walled zygosporangia that have two closely appressed beak-like appendages. The production of “beaked” zygosporangia is diagnostic for the genus. Two types of asexual conidia are formed, although isolates often lose their sporulating ability with subculture and special media incorporating glucosamine hydrochloride and casein hydrosolate may be needed to stimulate sporulation (Shipton and Zahari, 1987). Primary conidia are globose, one-celled, solitary and are forcibly discharged from a sporophore. The sporophore has a distinct swollen area just below the conidium that actively participates in the discharge of the conidium. Secondary (replicative) conidia are clavate, one-celled and are passively released from a sporophore. These sporophores are not swollen at their bases. The apex of the passively released spore has a knob-like adhesive tip. These spores may function as sporangia, producing several sporangiospores. RG-2 organism.

*Basidiobolus ranarum* is commonly present in decaying fruit and vegetable matter, and as a commensal in the intestinal tract of frogs, toads and lizards. It has been reported from tropical Africa, India, Indonesia and South East Asia including Australia.

For descriptions of species, keys to taxa and additional information see Strinivasan and Thirumalachar (1965), Greer and Friedman (1966), Dworzack *et al.* (1978), McGinnis (1980), King (1983), Rippon (1988), Davis *et al.* (1994), Jong and Dugan (2003), de Hoog *et al.* (2000) and Ellis (2005a).



(a) Sporophore and conidia and (b) zygosporangia of *Basidiobolus ranarum*.

## ***Beauveria* Vuillemin**

Colonies are usually slow growing, mostly not exceeding 2 cm in ten days at 20°C, downy, at first white but later often becoming yellow to pinkish. The genus *Beauveria* is characterised by the sympodial development of single-celled conidia (ameroconidia) on a geniculate or zig-zag rachis. Conidiogenous cells are flask-shaped, rachiform, proliferating sympodially and are often aggregated into sporodochia or synnemata. Conidia are hyaline and globose or ovoid in shape. RG-1 organism.

Three species are recognised, two of which are well known parasites of insects. *B. bassiana* is the most common species and is best known as the causal agent of the disastrous muscardine in silkworms. *Beauveria* species are occasionally isolated in the clinical laboratory as saprophytic contaminants.

**Key Features:** hyphomycete showing sympodial development of ameroconidia on a geniculate or zig-zag rachis emanating from a flask-shaped conidiophore.

For descriptions of species, keys to taxa and additional information see de Hoog (1972), Domsch *et al.* (1980), McGinnis (1980) and de Hoog *et al.* (2000).



*Beauveria bassiana* showing sympodial development of conidia on a geniculate or zig-zag rachis. Conidiogenous cells are flask-shaped, rachiform, proliferating sympodially and are often aggregated into sporodochia or synnemata. Conidia are hyaline and globose or ovoid in shape, 2-3  $\mu\text{m}$  diameter (phase contrast image).

## ***Bipolaris* Shoemaker**

**Teleomorph:** *Cochliobolus* Drechsler

Colonies are moderately fast growing, effuse, grey to blackish brown, suede-like to floccose with a black reverse. Microscopic morphology shows sympodial development of pale brown pigmented, pseudoseptate conidia on a geniculate or zig-zag rachis. Conidia are produced through pores in the conidiophore wall (poroconidia) and are straight, fusiform to ellipsoidal, rounded at both ends, smooth to finely roughened, germinating only from the ends (bipolar).

The genus *Bipolaris* contains about 45 species which are mostly subtropical and tropical plant parasites; however several species, notably *B. australiensis*, *B. hawaiiensis* and *B. spicifera* are well documented human pathogens. RG-1 organisms.

**Key Features:** dematiaceous hyphomycete producing sympodial, pseudoseptate, pale brown, straight, fusiform to ellipsoidal poroconidia, which are rounded at both ends.

The genera *Drechslera*, *Bipolaris*, *Curvularia* and *Exserohilum* are all closely related and differentiation of the genera relies upon a combination of characters including conidial shape, the presence or absence of a protruding hilum, the contour of the basal portion of the conidium and its hilum, the point at which the germ tube originates from the basal cell and, to a lesser degree, the sequence and location of the first three conidial septa. The table below is modified from Domsch *et al.* (1980).

<b>Anamorph</b>	<b>Main characters</b>	<b>Teleomorph</b>
<i>Drechslera</i>	Conidia cylindrical, germinating from any cell, hilum not protuberant	<i>Pyrenophora</i>
<i>Bipolaris</i>	Conidia fusiform-ellipsoidal, central cells not much darker and broader than the distal ones, hilum not protuberant, germination bipolar.	<i>Cochliobolus</i>
<i>Curvularia</i>	Conidia with 2-3 broader and darker central cells, often curved, with or without a prominent hilum, germination bipolar.	<i>Cochliobolus</i>
<i>Exserohilum</i>	Conidia fusiform-cylindrical to obclavate, with a protuberant hilum germination bipolar.	<i>Setosphaeria</i>

Species of *Bipolaris*, *Curvularia* and *Exserohilum* are causative agents of phaeohyphomycosis which is an emerging mycotic infection of humans and lower animals caused by a number of dematiaceous (brown-pigmented) fungi where the tissue morphology of the causative organism is mycelial. This separates it from other clinical types of disease involving brown-pigmented fungi where the tissue morphology of the organism is a grain (mycotic mycetoma) or sclerotic body (chromoblastomycosis).

For descriptions of species, keys to taxa and additional information see Ellis (1971 and 1976), Luttrell (1978), Domsch *et al.* (1980), Alcorn (1983), Padhye *et al.* (1986), McGinnis *et al.* (1986b), Sivanesan (1987), Rippon (1988) and de Hoog *et al.* (2000). Also see Descriptions for *Curvularia*, *Drechslera* and *Exserohilum*.

***Bipolaris* Shoemaker**

*Bipolaris australiensis* showing sympodial development of pale brown, fusiform to ellipsoidal, pseudoseptate, poroconidia on a geniculate or zig-zag rachis.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range
Itraconazole	0.03-1	Amphotericin B	0.06-2
Posaconazole	0.06-0.05	Anidulafungin	1-4
Voriconazole	0.06-0.05	Caspofungin	1-4

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002a), Espinel-Ingroff (2003), McGinnis and Pasarell (1998) and WCH in-house data.

## ***Blastomyces dermatitidis* Gilchrist & Stokes**

Colonies (SDA) at 25°C are variable in morphology and rate of growth. They may grow rapidly, producing a fluffy white mycelium or slowly as glabrous, tan, nonsporulating colonies (Fig. a). Growth and sporulation may be enhanced by yeast extract. Most strains become pleomorphic with age. Microscopically, hyaline, ovoid to pyriform, one-celled, smooth-walled conidia (2-10 µm in diameter) of the *Chrysosporium* type, are borne on short lateral or terminal hyphal branches.

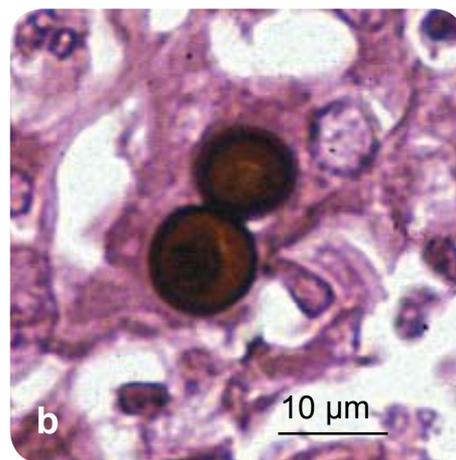
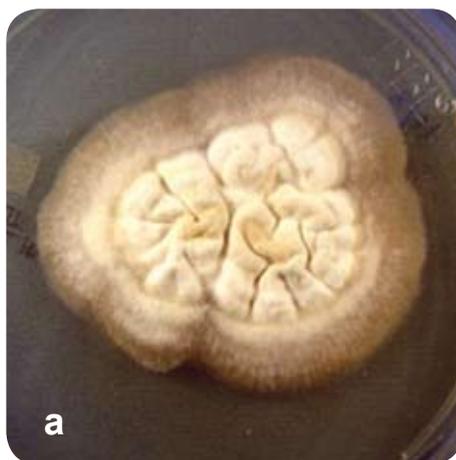
Colonies on blood agar at 37°C are wrinkled and folded, glabrous and yeast-like. Microscopically, the organism produces the characteristic yeast phase as seen in tissue pathology; ie. *B. dermatitidis* is a dimorphic fungus.

**WARNING:** RG-3 organism. Cultures of *Blastomyces dermatitidis* may represent a biohazard to laboratory personnel and should be handled in an appropriate pathogen handling cabinet. In the past, conversion from the mould form to the yeast form was necessary to positively identify this dimorphic pathogen from species of *Chrysosporium* or *Sepedonium*; however, culture identification by exoantigen test is now the method of choice.

**Histopathology:** Tissue sections show large, broad-based, unipolar budding yeast-like cells, which may vary in size from 8-15 µm (Fig. b), with some larger forms up to 30 µm in diameter. Note: tissue sections need to be stained by Grocott's methenamine silver method to clearly see the yeast-like cells, which are often difficult to observe in H&E preparations.

**Key Features:** clinical history, tissue pathology, culture identification by positive exoantigen test.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Chandler *et al.* (1980), Kaufman and Standard (1987) and Rippon (1988).



Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125-64	4-16	Amphotericin B	0.03-1	0.5
Itraconazole	0.03->16	0.125-2	Voriconazole	0.03-16	0.25
Posaconazole	0.03-2	0.125	Caspofungin	0.5-8	2

Limited data available. Sugar and Liu (1996), Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Gonzales *et al.* (2005) and Sabatelli *et al.* (2006).

## Candida Berkhout

The genus *Candida* is characterised by globose to elongate yeast-like cells or blastoconidia that reproduce by multilateral budding, polar budding if present on a narrow base, pseudohyphae and occasionally true hyphae may also be present. Arthroconidia, ballistoconidia and colony pigmentation are always absent. Fermentation or not: Nitrate assimilation or not: Inositol assimilation or not, however all inositol positive strains form pseudohyphae. In the past, the genus *Torulopsis* was separated from the genus *Candida* by the absence of pseudomycelium. However, in 1978 Yarrow & Meyer amended the description of *Candida* to include all species previously included in *Torulopsis*.

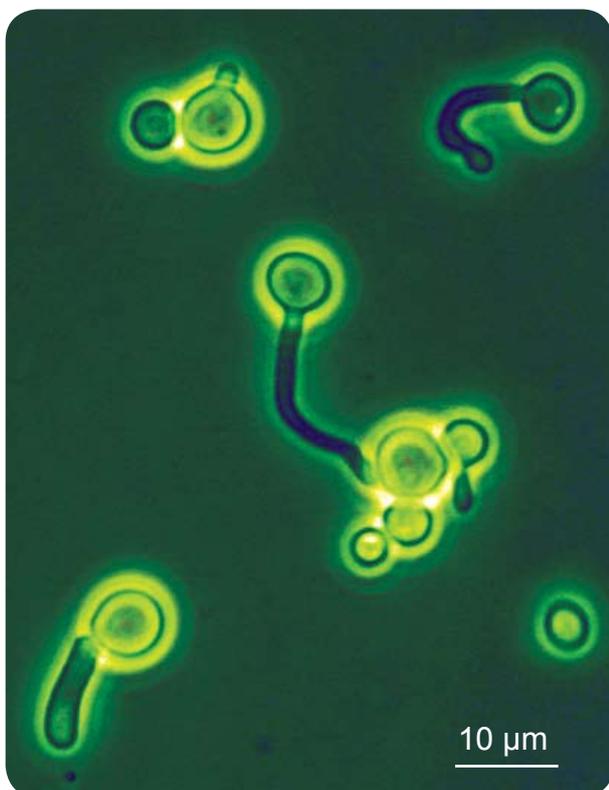
Several species of *Candida* may be aetiological agents, most commonly *C. albicans*, followed by *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. tropicalis*. However a number of other species may also be isolated (see table below). All are ubiquitous and occur naturally on humans.

### Identification:

Ensure that you start with a fresh growing pure culture; streak for single colony isolation if necessary.

**Chromogenic agars** are now being used for primary isolation for both the detection of mixed flora and rapid species identification, especially from non-sterile sites.

**Germ Tube Test.** A rapid screening test for *Candida albicans* and *Candida dubliniensis*. 0.5 mL of serum, containing 0.5% glucose, is lightly inoculated with the test organism and incubated at 35°C for 2-3 hours. On microscopy, the production of germ tubes by the cells is diagnostic for *Candida albicans*.



Production of germ tubes by *C. albicans*.

Species distribution from 944 patients with candidemia (Australian Candidemia Study 2002-2004).

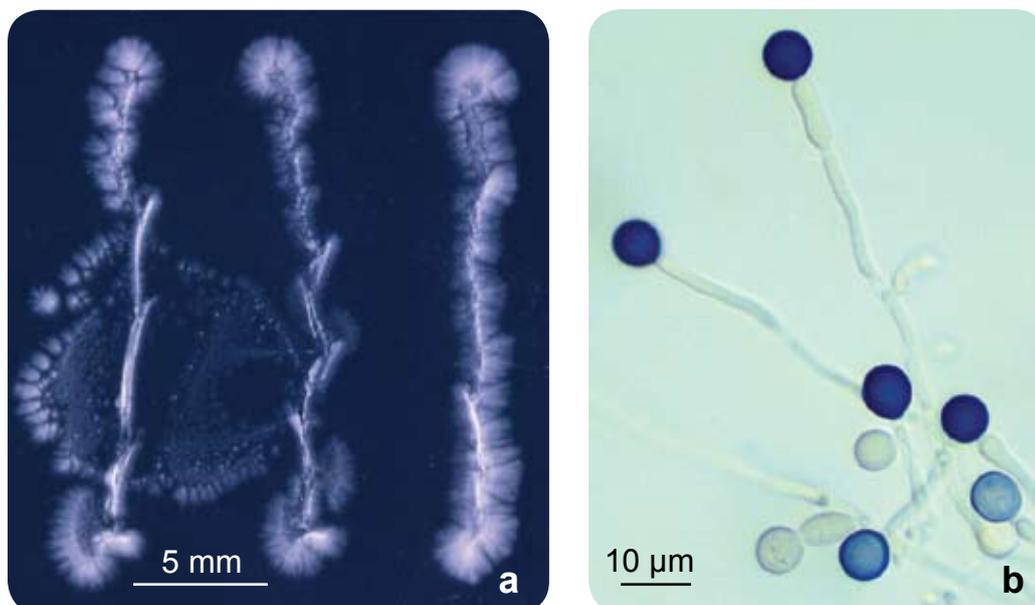
Species	No	%
<i>C. albicans</i>	447	47.3
<i>C. parapsilosis</i>	182	19.3
<i>C. glabrata</i>	167	17.8
<i>C. krusei</i>	46	4.9
<i>C. tropicalis</i>	46	4.9
<i>C. dubliniensis</i>	22	2.3
<i>C. guilliermondii</i>	11	1.2
<i>C. lusitaniae</i>	8	0.8
<i>C. kefyr</i>	5	0.5
<i>C. pelliculosa</i>	3	0.3
<i>C. rugosa</i>	2	0.2
<i>C. colliculosa</i>	1	0.1
<i>C. famata</i>	1	0.1
<i>C. inconspicua</i>	1	0.1
<i>C. lipolytica</i>	1	0.1
<i>C. fabianii</i>	1	0.1

## ***Candida* Berkhout**

**For the full identification of germ tube negative yeasts, morphological (Dalmau plate culture), physiological and biochemical tests are essential.**

(a) Dalmau Plate Culture: To set up a yeast morphology plate, dip a flamed sterilised straight wire into a culture to make a light inoculum and then lightly scratch the wire into the surface of a cornmeal/tween 80, rice/tween 80 or yeast morphology agar plate, then place a flamed coverslip onto the agar surface covering the scratches. Dalmau morphology plates are examined *in-situ* directly under the lower power of a microscope for the presence of pseudohyphae which may take up to 4-5 days at 26°C to develop. *Candida albicans* also produces characteristic large, round, terminal, thick-walled vesicles (often called chlamydo spores). The key features to remember are to use a light inoculum and to scratch the surface of the agar with the wire when inoculating.

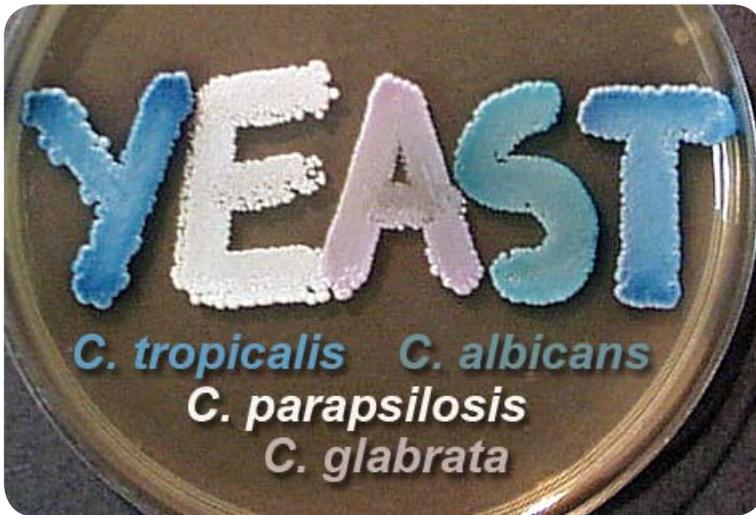
(b) Physiological and biochemical tests including fermentation and assimilation studies should be performed based on those used at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. Reference “The Yeasts: a taxonomic study”, edited by Kurtzman and Fell (1998), Elsevier Science Publishers B.V. Amsterdam. Reliable commercially available yeast identification kits are the API 20C, ID32C, MicroScan and Vitek systems. For specific identification of species see appropriate text book.



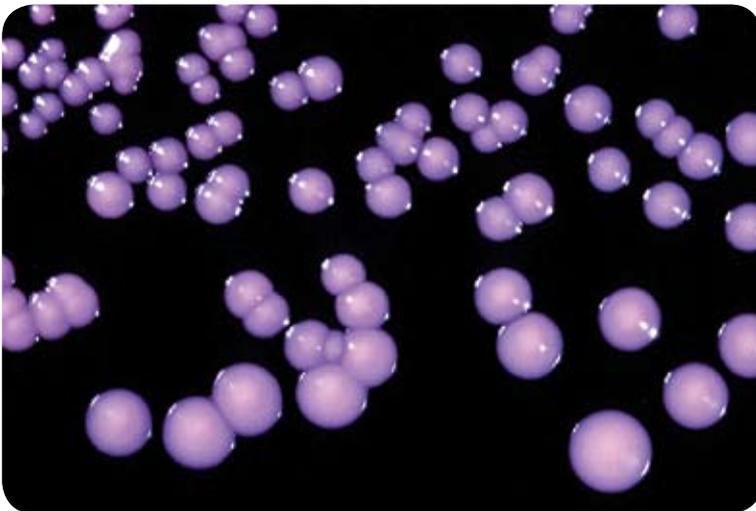
(a) Dalmau plate culture showing colonies of *C. albicans* growing out from scratches on the surface of a cornmeal/tween 80 agar plate. Note: a coverslip has been placed onto the agar surface covering the scratches. (b) Confirmatory test for *C. albicans*. Production of large round, thick-walled vesicles (often called chlamydo spores) in Dalmau plate cultures.

For descriptions of species, keys to taxa and additional information see Barnett *et al.* (1983), Kurtzman and Fell (1988) and de Hoog *et al.* (2000).

## *Candida albicans* (Robin) Berkhout



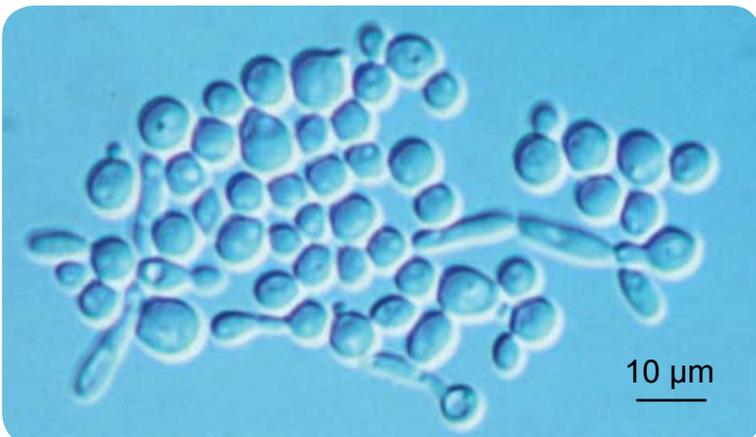
CHROMagar *Candida* plate showing chromogenic colour change for *C. albicans* (green), *C. tropicalis* (blue), *C. parapsilosis* (white) and *C. glabrata* (pink).



*Candida albicans* on Sabouraud's dextrose agar showing typical cream coloured, smooth surfaced, waxy colonies.



Direct smear of urine from a patient with candidiasis of the kidney showing *C. albicans* in mycelial or tissue phase with blastoconidia budding from the pseudohyphae.



Microscopic morphology of *C. albicans* showing budding spherical to ovoid blastoconidia.

## ***Candida albicans* (Robin) Berkhout**

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spherical to subspherical budding blastoconidia, 2-7 x 3-8 µm in size.

**India Ink Preparation:** Negative - No capsules present.

**Dalmau Plate Culture on Cornmeal and Tween 80 Agar:** Branched pseudohyphae with dense verticils of blastoconidia. Spherical chlamydoconidia, mostly terminal, often on a slightly swollen subtending cell are formed near the edge of the cover slip.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	+	L-Sorbose	v	L-Arabinose	v	D-Glucitol	-(s)
<b>Fermentation</b>		Sucrose	v	D-Arabinose	v	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	-(s)	D-Gluconate	-(s)
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-(s)	Trehalose	+(s)	D-Glucosamine	v	myo-Inositol	+
Maltose	+	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	+

**Key Features:** germ tube positive, production of chlamydoconidia on Dalmau plate culture, fermentation of glucose, sugar assimilation profile and a distinctive green colour on CHROMagar. Note: germ tube negative variants, known as *C. clausenii*, and sucrose-negative variants described as *C. stellatoidea* have proven to be synonymous with *C. albicans*. *C. albicans* is a commensal of mucous membranes and the gastrointestinal tract. Environmental isolations have been made from sources contaminated by human or animal excreta, such as polluted water, soil, air and plants. RG-2 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.03->64	2	Amphotericin B	0.03-4	0.25
Itraconazole	0.008->8	0.125	Flucytosine	0.03->64	0.5
Posaconazole	0.008->8	0.016	Caspofungin	0.008->4	0.125
Voriconazole	0.008->8	0.03	Anidulafungin	0.008->8	nd

Good data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002b, 2006, 2007), Espinel-Ingroff (2003), Hajjeh *et al.* (2004), Richter *et al.* (2005) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida colliculosa* (Hartmann) S.A. Meyer & Yarrow**

**Teleomorph:** *Torulasporea delbrueckii* (Lindner) Lindner.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spherical to ellipsoidal budding blastoconidia, 2-6 x 3-7 µm in size. Ascospores may be produced on 5% malt extract or cornmeal agar after 5-30 days at 25°C.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Budding yeast cells only. No pseudohyphae or true hyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	v
<b>Fermentation</b>		Sucrose	v	D-Arabinose	-	α-M-D-glucoside	v
Glucose	+	Maltose	v	D-Ribose	-	D-Gluconate	v
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	v	Trehalose	-,s	D-Glucosamine	-	myo-Inositol	-
Maltose	v	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	v
Trehalose	v	Raffinose	v	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	v	D-Mannitol	+	Growth at 37°C	v

**Key Features:** asci containing 1-4 spheroidal ascospores, variable growth at 37°C and a variable sugar assimilation profile. *C. colliculosa* is a rare cause of candidemia. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	8	Amphotericin B	0.25
Itraconazole	0.25	Flucytosine	0.03
Posaconazole	0.25	Caspofungin	0.06
Voriconazole	0.06	Anidulafungin	nd

Very limited data, antifungal susceptibility testing of individual stains is recommended. Data from the Australian Candidemia Study (nd = not done).

### ***Candida dubliniensis* Sullivan et al.**

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spherical to subspherical budding blastoconidia, 3-8 x 2-7 µm in size.

**India Ink Preparation:** Negative - No capsules present.

**Dalmau Plate Culture on Cornmeal and Tween 80 Agar:** Branched pseudohyphae with dense verticils of blastoconidia and spherical, mostly terminal chlamydoconidia.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	+	L-Sorbose	-	L-Arabinose	-	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+,s
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	-,s
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	+	D-Glucosamine	-,s	myo-Inositol	-
Maltose	+	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	+,s	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	+,s	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	v	D-Mannitol	+	Growth at 40°C	+

**Key Features:** germ tube positive, similar to *C. albicans*, except for absence of growth at 45°C; glycerol (mostly +), methyl-α-D-glucoside (-), trehalose (-), and D-xylose (-). Initial colonies dark green colour on CHROMagar and producing rough colonies on bird seed agar. *C. dubliniensis* is an uncommon cause of candidemia and mucosal infection, especially in HIV patients. RG-2 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.05->64	1	Amphotericin B	0.03-2	0.125
Itraconazole	0.008->8	0.125	Flucytosine	0.03-64	0.125
Posaconazole	0.03-1	0.125	Caspofungin	0.008-1	0.25
Voriconazole	0.008-2	0.016	Anidulafungin	<0.125-8	nd

Good data available. Espinel-Ingroff et al. (2001), Pfaller et al. (2002b, 2007), Espinel-Ingroff (2003) and Hajjeh et al. (2004). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida fabianii* (Hartmann) S.A. Meyer & Yarrow**

**Teleomorph:** *Pichia fabianii* (Wickerham) Kurtzman

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spheroidal to ellipsoidal budding blastoconidia, 3.0-6.5 x 2-5.5 µm in size. No pseudohyphae produced. Asci when present spherical, containing 1-4 spherical, faintly roughened ascospores.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Spherical to ovoid budding yeast cells and occasional pseudohyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	+
Galactose	-	Cellobiose	+	L-Rhamnose	-	DL-Lactate	+
Sucrose	+	Trehalose	+	D-Glucosamine	-	myo-Inositol	-
Maltose	+,s	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	+	Erythritol	-	Nitrate	+
<b>Assimilation</b>		Melezitose	+	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. Molecular identification may be required. *Candida fabianii* is a rare cause of candidemia. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	8	Amphotericin B	0.125
Itraconazole	0.5	Flucytosine	0.03
Posaconazole	0.5	Caspofungin	0.5
Voriconazole	0.125	Anidulafungin	nd

Very limited data, antifungal susceptibility testing of individual strains is recommended. Data from the Australian Candidemia Study (nd = not done).

## ***Candida famata* (Harrison) S.A. Meyer & Yarrow**

**Teleomorph:** *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ovoid to broadly ellipsoidal budding blastoconidia, 3.5-5 × 2-3.5 µm in size. No pseudohyphae produced. Asci when present spherical, persistent, containing 1-2 spherical ascospores with rough walls.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Spherical to ovoid budding yeast cells only. No pseudohyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+,w	D-Glucitol	+,w
<b>Fermentation</b>		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	+
Glucose	-,w	Maltose	+	D-Ribose	v	D-Gluconate	+,w
Galactose	-,w	Cellobiose	+	L-Rhamnose	v	DL-Lactate	v
Sucrose	-,w	Trehalose	+	D-Glucosamine	v	myo-Inositol	-
Maltose	-	Lactose	v	N-A-D-glucosamine	v	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	+	D-Glucuronate	v
Trehalose	-,w	Raffinose	+	Erythritol	v	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	v	0.1% Cycloheximide	v
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	+,w

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida famata* is a common environmental isolate, however it is only rarely recovered from clinical specimens, usually associated with skin. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	0.125->64	Amphotericin B	0.06-2
Itraconazole	0.03->8	Flucytosine	0.06-128
Posaconazole	0.06-1	Caspofungin	0.06->16
Voriconazole	0.03-1	Anidulafungin	0.008->16

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2003, 2007), Espinel-Ingroff (2003), Cuenca-Estrella *et al.* (2006) and the Australian Candidemia Study.

## ***Candida glabrata* (Anderson) S.A. Meyer & Yarrow**

**Synonym:** *Torulopsis glabrata* (Anderson) Lodder & de Vries

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ovoid to ellipsoidal budding blastoconidia, 3.4 × 2.0 µm in size. No pseudohyphae or chlamydospores produced.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Ovoid budding yeast cells only. No pseudohyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-	Trehalose	-	D-Glucosamine	-	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	v
Lactose	-	Melibiose	-	Glycerol	+,s	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida glabrata* is one of the most common yeast species to be found on the body surface and is often isolated as an incidental finding from skin and urine. It has been implicated as an “opportunistic” cause of both superficial and systemic infections, especially in immunocompromised patients, and it has been isolated from patients with septicemia, pyelonephritis, pulmonary infections, endocarditis and hyperalimentation. Approximately 10% of clinical isolates show azole cross resistance. RG-2 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.03->128	128	Amphotericin B	0.008-2	0.5
Itraconazole	0.008->16	16	Flucytosine	0.008-16	0.03
Posaconazole	0.008-8	8	Caspofungin	0.008->8	0.25
Voriconazole	0.008-16	2	Anidulafungin	0.008-8	nd

Good data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002b, 2006, 2007), Espinel-Ingroff (2003), Hajjeh *et al.* (2004), Richter *et al.* (2005) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (note: in this study 10% of primary blood isolates were azole cross-resistant, nd = not done).

## ***Candida guilliermondii* (Castellani) Langeron & Guerra**

**Teleomorph:** *Pichia guilliermondii* Wickerham.

**Culture:** White to cream-coloured smooth, glabrous yeast-like colonies.

**Microscopy:** Spherical to subspherical budding yeast-like cells or blastoconidia, 2.0-4.0 × 3.0-6.5 μm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Branched pseudohyphae with dense verticils of blastoconidia.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
<b>Fermentation</b>		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	+	D-Gluconate	v
Galactose	v	Cellobiose	v	L-Rhamnose	v	DL-Lactate	v
Sucrose	+	Trehalose	+	D-Glucosamine	+	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	+	D-Glucuronate	-
Trehalose	+	Raffinose	+	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	+	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	v	0.1% Cycloheximide	v
Galactose	+	D-Xylose	+	D-Mannitol	v	Growth at 37°C	v

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida guilliermondii* has been isolated from numerous human infections, mostly of cutaneous origin. It is also found from normal skin and in sea water, faeces of animals, fig wasps, buttermilk, leather, fish, and beer. RG-1 organism.

Antifungal	MIC μg/mL		Antifungal	MIC μg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125->128	16	Amphotericin B	0.03-1	0.5
Itraconazole	0.03-8	1.0	Flucytosine	0.03-8	0.125
Posaconazole	0.03-8	0.5	Caspofungin	0.125->8	0.5
Voriconazole	0.03-8	0.25	Anidulafungin	0.06-4	nd

Good data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2003, 2006, 2007), Espinel-Ingroff (2003) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida haemulonii* (van Uden & Kolipinski) Meyer & Yarrow**

**Synonym:** *Torulopsis haemulonii* van Uden & Kolipinski

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ovoid to globose, budding yeast-like cells or blastoconidia, 3.0-5.0 x 3.0-6.5 µm. No pseudohyphae produced.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Spherical to ovoid, budding yeast-like cells only. No pseudohyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-,s	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-,s	α-M-D-glucoside	-
Glucose	+	Maltose	+	D-Ribose	-,s	D-Gluconate	+
Galactose	-	Cellobiose	-	L-Rhamnose	+,s	DL-Lactate	-
Sucrose	+	Trehalose	+	D-Glucosamine	+,s	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+,s	D-Glucuronate	-
Trehalose	+,s	Raffinose	+,s	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+,s	Ribitol	s	Urease	-
Glucose	+	Soluble Starch	v	Galactitol	-,s	0.1% Cycloheximide	+
Galactose	-,s	D-Xylose	-,s	D-Mannitol	+	Growth at 37°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. Molecular identification may be required. *Candida haemulonii* has been reported from a few cases of fungemia but clinical isolations remain rare. It has also been isolated from fish and a dolphin. *C. haemulonii* may be difficult to distinguish from *C. famata* using some commercial yeast identification systems due to data base limitations. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	32->256	Amphotericin B	2-8
Itraconazole	0.125-4	Flucytosine	0.008-0.125
Voriconazole	0.06-0.5	Caspofungin	0.03-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. Rodero *et al.* (2002) and Khan *et al.* (2007).

***Candida inconspicua* (Lodder & Kreger-van Rij) S.A.Meyer & Yarrow**

**Synonym:** *Torulopsis inconspicua* Lodder & Kreger-van Rij.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ovoidal budding blastoconidia, 2.0-5 x 5.0-11.0 µm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmou Plate Culture on Cornmeal and Tween 80 Agar:** Spherical to ovoid budding yeast cells only. Primitive pseudohyphae may be produced after 14 days.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	+	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida inconspicua* is a rare cause of candidemia. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	4-128	Amphotericin B	0.125->8
Itraconazole	0.25-8	Flucytosine	1-64
Posaconazole	0.5-8	Caspofungin	0.008-0.25
Voriconazole	0.125-4	Anidulafungin	nd

Very limited data, antifungal susceptibility testing of individual strains is recommended. Pfaller *et al.* (2003), Espinel-Ingroff (2003) and the Australian Candidemia Study (nd = not done).

## ***Candida kefyr* (Beijerinck) van Uden & Buckley**

**Synonym:** *Candida pseudotropicalis* (Castellani) Basgal.

**Teleomorph:** *Kluyveromyces marxianus* (Hansen) van der Walt.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Short-ovoid to long-ovoid, budding blastoconidia, 3.0-6.5 × 5.5-11.0 μm, sometimes becoming elongate (up to 16.0 μm).

**India Ink Preparation:** Negative - No capsules present.

**Dalmiau Plate Culture on Cornmeal and Tween 80 Agar:** Abundant, long, wavy, branched pseudohyphae usually formed, with ovoid blastoconidia, budding off singly, in pairs or chains, often in a verticillated position. Note: in some strains pseudohyphae may be scarce or almost absent.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	v	D-Gluconate	-
Galactose	+,s	Cellobiose	v	L-Rhamnose	-	DL-Lactate	+
Sucrose	+	Trehalose	-,w	D-Glucosamine	-	myo-Inositol	-
Maltose	-	Lactose	v	N-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	v	Melibiose	-	Glycerol	s	D-Glucuronate	-
Trehalose	-	Raffinose	+	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	+
Galactose	s	D-Xylose	s	D-Mannitol	v	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida kefyr* is a rare cause of candidiasis and is usually associated with superficial cutaneous manifestations rather than systemic disease. Environmental isolations have been made from cheese and dairy products. RG-1 organism.

Antifungal	MIC μg/mL		Antifungal	MIC μg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125-4	0.5	Amphotericin B	0.125->8	1
Itraconazole	0.03-0.5	0.06	Flucytosine	0.03-64	16
Posaconazole	0.03-0.25	0.25	Caspofungin	0.03-1	0.125
Voriconazole	0.008-0.125	0.016	Anidulafungin	0.03-0.5	nd

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2003, 2006), Espinel-Ingroff (2003) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida krusei* (Castellani) Berkhout**

**Teleomorph:** *Issatchenkia orientalis* Kudryavtsev.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Predominantly small, elongated to ovoid budding blastoconidia, 2.0-5.5 x 4.0-15.0 µm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Abundant long, wavy, branched pseudohyphae with elongated to ovoid blastoconidia, budding off in verticillate branches.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	+	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	+	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	v
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida krusei* is regularly associated with some forms of infant diarrhoea and occasionally with systemic disease. It has also been reported to colonise the gastrointestinal, respiratory and urinary tracts of patients with granulocytopenia. Environmental isolations have been made from beer, milk products, skin, faeces of animals and birds and pickle brine. RG-2 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.25->128	64	Amphotericin B	0.06->8	1
Itraconazole	0.03->8	0.5	Flucytosine	0.5-64	16
Posaconazole	0.03-1	1	Caspofungin	0.125->4	1
Voriconazole	0.03-4	0.5	Anidulafungin	0.008-8	nd

Good data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002b, 2003, 2007), Espinel-Ingroff (2003), Hajjeh *et al.* (2004), Richter *et al.* (2005) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida lipolytica* (F.C. Harrison) Diddens & Lodder**

**Teleomorph:** *Yarrowia lipolytica* (Wickerham *et al.*) van der Walt & von Arx.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spherical, ellipsoidal to elongate budding blastoconidia, 3.0-5 x 3.3-15.0  $\mu\text{m}$ .

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Pseudohyphae and true hyphae are produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	+
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	$\alpha$ -M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	v	D-Gluconate	v
Galactose	-	Cellobiose	w,-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	-	<i>myo</i> -Inositol	-
Maltose	-	Lactose	-	<i>N</i> -A-D-glucosamine	+	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	+	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	-	D-Mannitol	+	Growth at 37°C	v

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida lipolytica* is a rare cause of candidemia. RG-1 organism.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range
Fluconazole	1->64	Amphotericin B	0.06-1
Itraconazole	0.06-8	Flucytosine	0.125-64
Posaconazole	0.03-4	Caspofungin	0.25-2
Voriconazole	0.03-1	Anidulafungin	0.125-0.5

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2003), Espinel-Ingroff (2003) and Australian Candidemia Study.

## ***Candida lusitaniae* van Uden & do Carmo-Sousa**

**Teleomorph:** *Clavispora lusitaniae* Rodrigues de Miranda.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ovoid to ellipsoidal budding blastoconidia, 1.5-6.0 x 2.5-10.0 µm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Abundant pseudohyphae with short chains of blastoconidia.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	+	L-Arabinose	v	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	s
Galactose	v	Cellobiose	+	L-Rhamnose	v	DL-Lactate	+,w
Sucrose	v	Trehalose	+	D-Glucosamine	-	myo-Inositol	-
Maltose	v	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	v	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida lusitaniae* is a known cause of disseminated candidiasis, including septicemia and pyelonephritis. *C. lusitaniae* was first isolated from the alimentary tract of warm blooded animals and environmental isolations have been made from cornmeal, citrus peel, fruit juices, and milk from cows with mastitis. *C. lusitaniae* may also be difficult to distinguish from *C. tropicalis* using some yeast identification systems. RG-2 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125-64	8	Amphotericin B	0.03-8	0.125
Itraconazole	0.06-2	0.125	Flucytosine	0.03-128	0.03
Posaconazole	0.008-0.5	0.25	Caspofungin	0.125-4	0.5
Voriconazole	0.008-2	0.125	Anidulafungin	0.03->8	nd

Some data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002b, 2003), Espinel-Ingroff (2003) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida norvegensis* Dietrichson ex van Uden & Buckley**

**Teleomorph:** *Pichia norvegensis* Leask & Yarrow.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ovoid, budding blastoconidia, 2.0-3.5 x 3.5-5.0 µm. Pseudohyphae rarely produced.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Spherical to ovoid budding yeast cells only. No pseudohyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	s	Maltose	-	D-Ribose	-	D-Gluconate	-
Galactose	-	Cellobiose	+	L-Rhamnose	-	DL-Lactate	w
Sucrose	-	Trehalose	-	D-Glucosamine	+	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	-	D-Xylose	-	D-Mannitol	-	Growth at 37°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida norvegensis* is a very rare clinical isolate that has been reported as a causative agent of peritonitis and disseminated candidiasis in a patient on CAPD. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	16	Amphotericin B	1
Itraconazole	0.25	Flucytosine	8
Voriconazole	0.125	Posaconazole	0.125

Very limited data, antifungal susceptibility testing of individual strains is recommended. Pfaller *et al.* (2002b).

## ***Candida parapsilosis* Complex**

Recently *Candida parapsilosis* has been recognised as 3 species: *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis* (Tavanti *et al.* 2005). These three species are phenotypically indistinguishable and are best distinguished by genetic analysis. Antifungal susceptibility data from the Australian Candidemia Study also shows no significant differences between the species.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Predominantly small, globose to ovoid budding blastoconidia, 2.0-3.5 x 3.0-4.5 µm, with some larger elongated forms present.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Abundant, much-branched pseudohyphae in a delicate tree-like pattern with 2-3 blastoconidia in small clusters at intervals along the pseudohyphae.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	+s	L-Arabinose	+	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	v	D-Gluconate	+,s
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	-
Sucrose	-,s	Trehalose	+	D-Glucosamine	v	myo-Inositol	-
Maltose	-,s	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-,s	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	+,s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida parapsilosis* is an opportunistic human pathogen which may cause cutaneous infections, especially of the nail and systemic disease, especially endocarditis. Other clinical manifestations include endophthalmitis and fungemia. Environmental isolations have been made from intertidal and oceanic waters, pickle brine, cured meats, olives and normal skin, and faeces. RG-1 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125->64	8	Amphotericin B	0.016-2	0.5
Itraconazole	0.015-2	0.25	Flucytosine	0.03->64	0.25
Posaconazole	0.008-0.5	0.03	Caspofungin	0.03->8	1
Voriconazole	0.008-2	0.25	Anidulafungin	0.008->8	nd

Good data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002b, 2006, 2007), Espinel-Ingroff (2003), Hajjeh *et al.* (2004), Richter *et al.* (2005) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

## ***Candida pelliculosa* Redaelli**

**Teleomorph:** *Pichia anomala* (Hansen) Kurtzman.

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spherical to ellipsoidal budding blastoconidia, 2-4 × 2-6 µm. Pseudohyphae may be present. Asci when present, containing 1-4 hat-shaped ascospores.

**India Ink Preparation:** Negative - No capsules present.

**Dalmou Plate Culture on Cornmeal and Tween 80 Agar:** Spherical to ellipsoidal budding yeast cells and abundant pseudohyphae in most strains.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	v	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	+
Glucose	+	Maltose	+	D-Ribose	v	D-Gluconate	v
Galactose	v	Cellobiose	+	L-Rhamnose	-	DL-Lactate	+
Sucrose	+	Trehalose	+	D-Glucosamine	-	myo-Inositol	-
Maltose	v	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	n
Trehalose	-	Raffinose	+	Erythritol	+	Nitrate	+
<b>Assimilation</b>		Melezitose	+	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	v	D-Mannitol	+	Growth at 37°C	v

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida pelliculosa* has been reported from cases of candidemia and catheter related infections in humans and has been isolated from soil, grains, fruit and warm blooded animals. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	2-16	Amphotericin B	0.125-2
Itraconazole	0.25-2	Flucytosine	0.03-64
Posaconazole	0.125-1	Caspofungin	0.06-0.5
Voriconazole	0.06-0.25	Anidulafungin	nd

Limited data available, antifungal susceptibility testing of individual strains is recommended. Pfaller *et al.* (2003), Espinel-Ingroff (2003), Cuenca-Estrella *et al.* (2006) and the Australian Candidemia Study (nd = not done).

## ***Candida rugosa* (Anderson) Diddens & Lodder**

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Ellipsoidal to elongate budding blastoconidia, 6-10 × 2-3.5 µm. Sometimes short pseudohyphae may be produced.

**India Ink Preparation:** Negative - No capsules present.

**Dalmou Plate Culture on Cornmeal and Tween 80 Agar:** Ellipsoidal budding yeast cells only and densely branched pseudohyphae produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	+,s
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	-	D-Gluconate	v
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+,s
Sucrose	-	Trehalose	-	D-Glucosamine	-	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	s	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	-,s	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	+	D-Xylose	v	D-Mannitol	+,s	Growth at 37°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida rugosa* has been associated with catheter related fungemia and has been isolated from human and bovine faeces, sea water and soil. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	1-16	Amphotericin B	0.25-4
Itraconazole	0.03-1	Flucytosine	0.06-16
Posaconazole	0.06-0.25	Caspofungin	0.25-2
Voriconazole	0.008-0.25	Anidulafungin	0.03-4

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2003), Espinel-Ingroff (2003) and the Australian Candidemia Study.

## ***Candida tropicalis* (Castellani) Berkhout**

**Culture:** Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.

**Microscopy:** Spherical to subspherical budding yeast-like cells or blastoconidia, 3-5.5 x 4-9 µm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmat Plate Culture on Cornmeal and Tween 80 Agar:** Abundant, long, wavy, branched pseudohyphae with numerous ovoid blastoconidia, budding off. Terminal vesicles (chlamydospores) are not produced.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	+
<b>Fermentation</b>		Sucrose	v	D-Arabinose	-	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	-,s	D-Gluconate	v
Galactose	+	Cellobiose	+,s	L-Rhamnose	-	DL-Lactate	v
Sucrose	v	Trehalose	+	D-Glucosamine	v	myo-Inositol	-
Maltose	+	Lactose	-	N-A-D-glucosamine	+	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	+,s	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	+,s	Urease	-
Glucose	+	Soluble Starch	+	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. *Candida tropicalis* is a major cause of septicemia and disseminated candidiasis. It is also found as part of the normal human mucocutaneous flora and environmental isolations have been made from faeces, shrimp, kefir, and soil. RG-2 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125->128	2	Amphotericin B	0.03-8	0.5
Itraconazole	0.03->8	0.5	Flucytosine	0.03->64	0.125
Posaconazole	0.008->8	0.06	Caspofungin	0.03->8	0.25
Voriconazole	0.008->8	0.25	Anidulafungin	0.03->8	nd

Good data available. Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002b, 2006, 2007), Espinel-Ingroff (2003), Hajjeh *et al.* (2004), Richter *et al.* (2005) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian Candidemia Study (nd = not done).

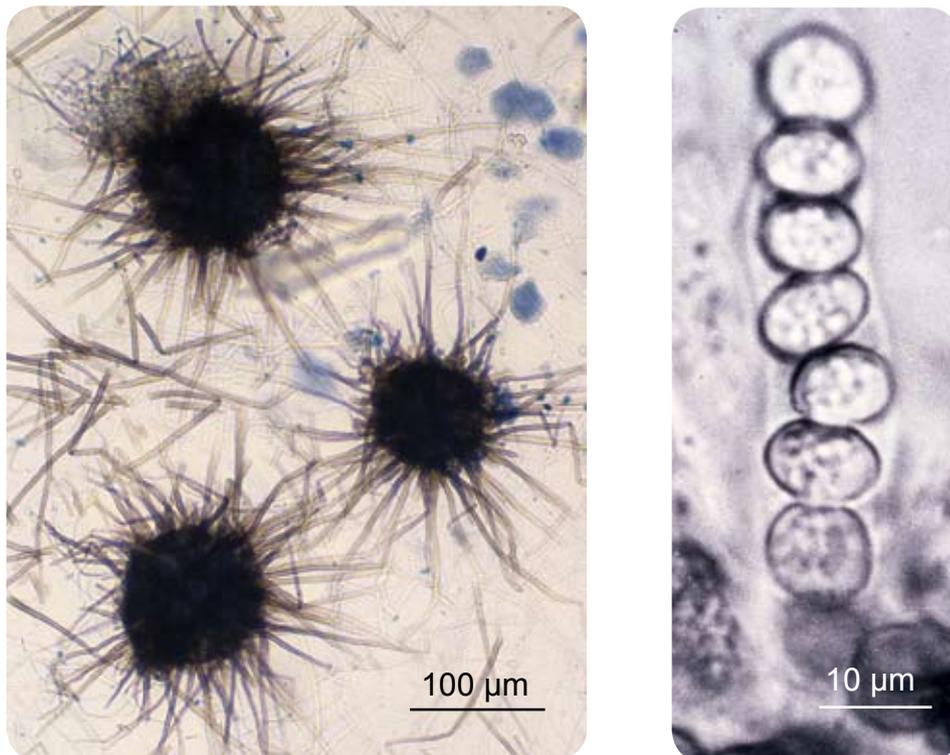
### ***Chaetomium* Kunze ex Fries**

This genus is a common ascomycete (pyrenomycete) characterised by the formation of darkly-pigmented, globose, ovoid, barrel to flask-shaped, ostiolate ascocarps (perithecia) beset with dark-coloured terminal hairs (setae) which are straight, branched or curved. Asci are clavate to cylindrical, typically eight-spored and evanescent. Ascospores are one-celled, darkly-pigmented, smooth-walled, of varying shape, mostly ovoid, ellipsoidal or lemon-shaped. Chlamydospores and solitary conidia may also be produced. RG-1 organism.

The genus contains between 160 and 180 species, and all are saprophytic being isolated from soil, straw, dung and plant debris. However, quite a few species are thermophilic and can grow at temperatures above 37°C. *Chaetomium* species are important agents for the decomposition of cellulose waste and plant materials, and are only rarely isolated in medical mycology laboratories.

**Key Features:** ascomycete producing darkly-pigmented ostiolate perithecia beset with long dark terminal setae.

For descriptions of species, keys to taxa and additional information see Ames (1963), Seth (1970), Millner (1975), Domsch *et al.* (1980), Ellis and Keane (1981), Ellis (1981) and de Hoog *et al.* (2000).



Ascocarp (perithecia), terminal hairs, asci and ascospores of *Chaetomium*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.125-16	Itraconazole	0.03-0.125	Voriconazole	0.125-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

## ***Chrysosporium* Corda**

Colonies are moderately fast growing, flat, white to tan to beige in colour, often with a powdery or granular surface texture. Reverse pigment absent or pale brownish-yellow with age. Hyaline, one-celled conidia are produced directly on vegetative hyphae by non-specialised conidiogenous cells. Conidia are typically pyriform to clavate with truncate bases and are formed either intercalary (arthroconidia), laterally (often on pedicels) or terminally.

Species of *Chrysosporium* are occasionally isolated from skin and nail scrapings, especially from feet, but because they are common soil saprophytes they are usually considered as contaminants. There are about 22 species of *Chrysosporium*, several are keratinolytic with some also being thermotolerant, and cultures may closely resemble some dermatophytes, especially *Trichophyton mentagrophytes*, and some strains may also resemble cultures of *Histoplasma* and *Blastomyces*.

## ***Chrysosporium tropicum* Carmichael**

Colonies are flat, white to cream-coloured with a very granular surface. Reverse pigment absent or pale brownish-yellow with age. Microscopically, conidia are numerous, hyaline, single-celled, clavate to pyriform, smooth, slightly thick-walled (6-7 x 3.5-4 µm), and have broad truncate bases and pronounced basal scars. The conidia are formed at the tips of the hyphae, on short or long lateral branches, or sessile along the hyphae (intercalary). No macroconidia or hyphal spirals are seen. RG-2 organism.

For descriptions of species, keys to taxa and additional information see Carmichael (1962), Rebell and Taplin (1970), Sigler and Carmichael (1976), Van Oorschot (1980), Domsch *et al.* (1980) and de Hoog *et al.* (2000).



*Chrysosporium tropicum* showing typical pyriform to clavate-shaped conidia with truncated bases which may be formed either intercalary, laterally or terminally.

### ***Cladophialophora bantiana* (Saccardo) de Hoog et al.**

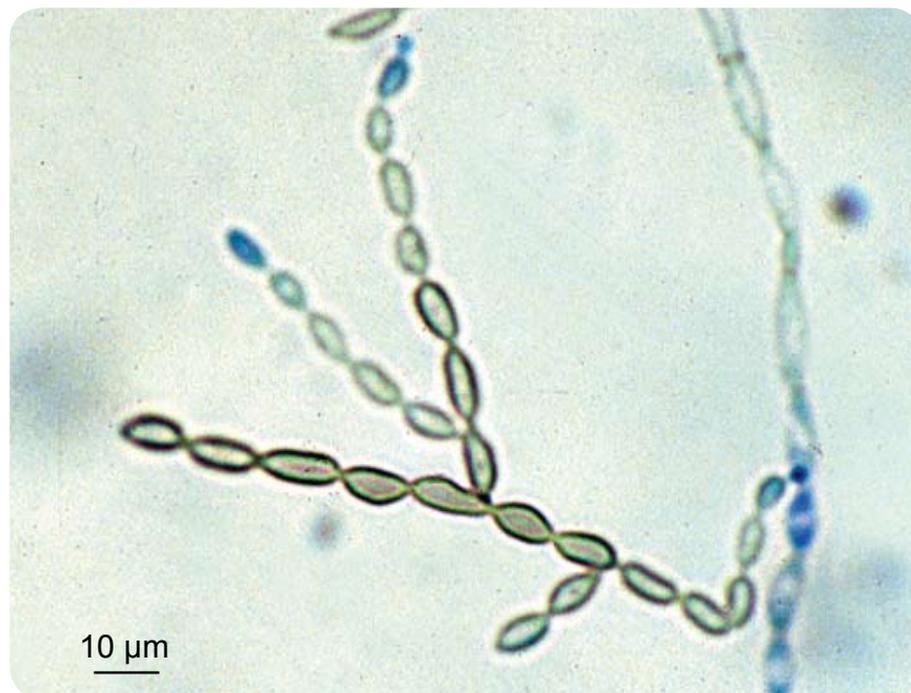
**Synonym:** *Xylohypha bantiana* (Saccardo) McGinnis, Borelli and Ajello

Colonies are moderately fast growing, olivaceous-grey, suede-like to floccose and grow at temperatures up to 42-43°C. Conidia are formed in long, sparsely branched, flexuose, acropetal chains from undifferentiated conidiophores. Conidia are one-celled (very occasionally two-celled), pale brown, smooth-walled, ellipsoid to oblong-ellipsoid and are 2-3 x 4-7 µm in size.

*Cladophialophora bantiana* has been isolated from soil and is a recognised agent of cerebral phaeohyphomycosis. The fungus is neurotropic and may cause brain abscesses in both normal and immunosuppressed patients. RG-2 organism.

*Cladophialophora bantiana* may be distinguished from *Cladosporium* species by the absence of conidia with distinctly pigmented hila, the absence of characteristic shield cells and by growth at 42°C (compared with *Cladophialophora carrionii* which has a maximum growth temperature of 35-36°C, and *Cladosporium* species which have a maximum of less than 35°C).

For descriptions of species, keys to taxa and additional information see McGinnis (1980), McGinnis and Borelli (1981), McGinnis et al. (1986a), Rippon (1988), Kwon-Chung and Bennett (1992) and de Hoog et al. (2000).



Conidiophore and conidia of *Cladophialophora bantiana*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.03-2	0.5	Posaconazole	0.008-0.06	0.06
Itraconazole	0.03-0.5	0.5	Voriconazole	0.03-1	0.125

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff et al. (2001), Espinel-Ingroff (2001) and WCH in-house data.

## *Cladophialophora carrionii* (Trejos) de Hoog *et al.*

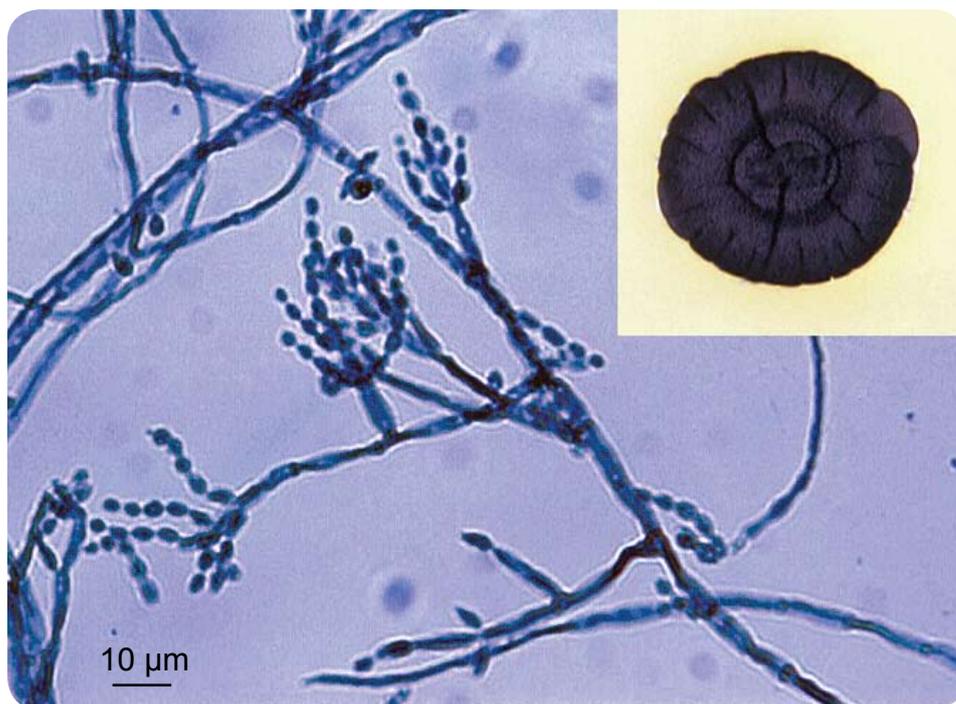
**Synonym:** *Cladosporium carrionii* Trejos

Colonies are slow growing, reaching 3-4 cm in diameter after one month, with a compact suede-like to downy surface and are olivaceous-black in colour. Microscopy shows ascending to erect, olivaceous-green, apically branched, elongate conidiophores producing branched acropetal chains of smooth-walled conidia. Conidia are pale olivaceous, smooth-walled or slightly verrucose, limoniform to fusiform, 1.5-3.0 × 2.0-7.0 µm in size. Bulbous phialides with large collarettes and minute, hyaline conidia are occasionally formed on nutritionally poor media. Maximum growth temperature 35-37°C. RG-2 organism.

*Cladophialophora carrionii* is a recognised agent of chromoblastomycosis and it has been isolated from soil and fence posts made from *Eucalyptus* sp. Cases of chromoblastomycosis caused by *C. carrionii* are commonly found in Australia, Venezuela, Madagascar and South America. Isolates from phaeomycotic cysts and opportunistic infections have also been reported.

**Key Features:** conidia are smaller and comprise heavily branched systems which fall apart much more easily than in the other *Cladophialophora* species.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Rippon (1988), de Hoog *et al.* (1995) and de Hoog *et al.* (2000).



Conidiophores and conidia of *Cladophialophora carrionii*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.06-4	1	Posaconazole	0.06-0.5	0.25
Itraconazole	0.03-0.5	0.5	Voriconazole	0.03-0.5	0.25

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001), Gonzales *et al.* (2005) and WCH in-house data.

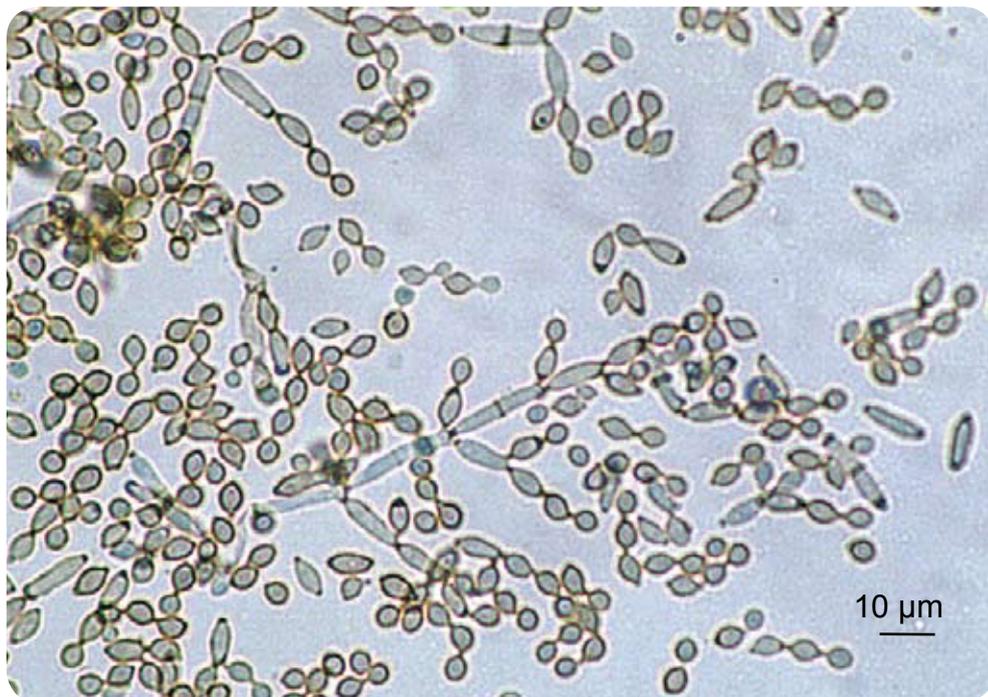
### **Cladosporium Link ex Fries**

Colonies are rather slow growing, mostly olivaceous-brown to blackish brown but also sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia. Vegetative hyphae, conidiophores and conidia are equally pigmented. Conidiophores are more or less distinct from the vegetative hyphae, being erect, straight or flexuose, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia are produced in branched acropetal chains, being smooth, verrucose or echinulate, one- to four-celled, and have a distinct dark hilum. The term blastocatenate is often used to describe chains of conidia where the youngest conidium is at the apical or distal end of the chain. Note: the conidia closest to the conidiophore, and where the chains branch, are usually “shield-shaped”. The presence of shield-shaped conidia, a distinct hilum, and chains of conidia that readily disarticulate, are diagnostic for the genus *Cladosporium*.

*Cladosporium* species have a world-wide distribution and are amongst the most common of air-borne fungi. Some 500 species have been described. Isolates of *Cladosporium* are frequently isolated as contaminants. RG-1 organisms. The pathogenic species have now been transferred to the genus *Cladophialophora*.

**Key Features:** dematiaceous hyphomycete forming branched acropetal chains of conidia, each with a distinct hilum.

For descriptions of species, keys to taxa and additional information see Ellis (1971 and 1976), Domsch *et al.* (1980), McGinnis (1980) and de Hoog *et al.* (2000).



Conidiophores and conidia of *Cladosporium cladosporioides*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.03-8	Itraconazole	0.03-32	Voriconazole	0.06-1

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

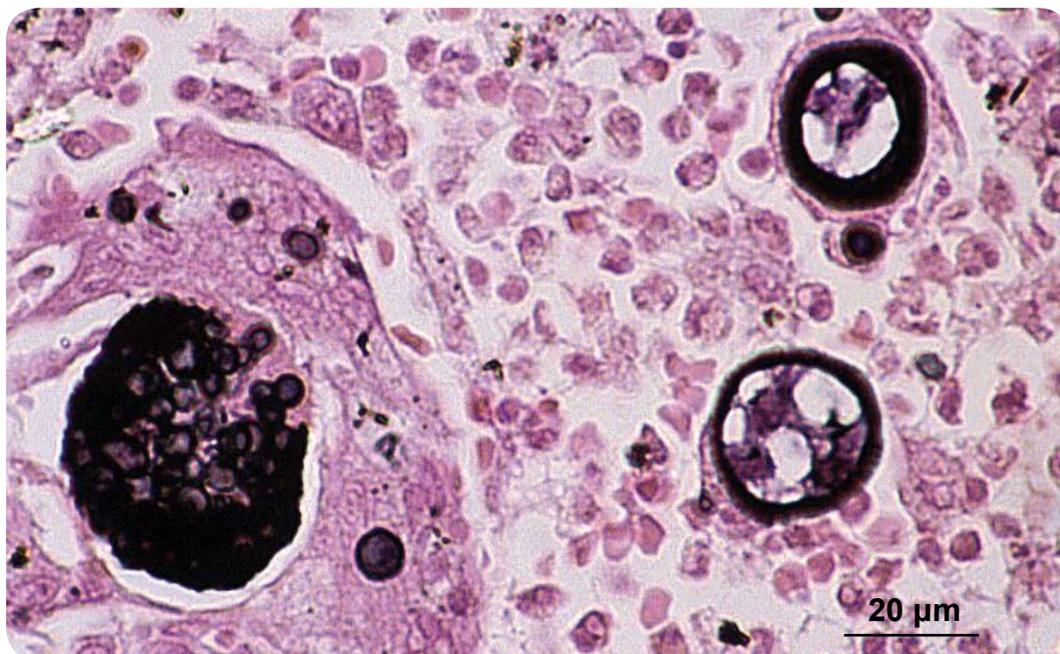
## ***Coccidioides immitis/posadasii* complex**

Recently *Coccidioides immitis* has been recognised as 2 species: *C. immitis* and *C. posadasii* (Fisher *et al.* 2002). The two species are morphologically identical and can be distinguished only by genetic analysis and different rates of growth in the presence of high salt concentrations (*C. posadasii* grows more slowly). *C. immitis* is geographically limited to California's San Joaquin Valley region, whereas *C. posadasii* is found in the desert regions of the USA southwest, Mexico and South America. The two species appear to coexist in the desert regions of the USA southwest and Mexico.

Colonies of *C. immitis/posadasii* on Sabouraud's dextrose agar at 25°C are initially moist and glabrous, but rapidly become suede-like to downy, greyish white with a tan to brown reverse, however considerable variation in growth rate and culture morphology has been noted. Microscopy shows typical single-celled, hyaline, rectangular to barrel-shaped, alternate arthroconidia, 2.5-4 × 3-6 µm in size, separated from each other by a disjunctive cell. This arthroconidial state has been classified in the genus *Malbranchea* and is similar to that produced by many non-pathogenic soil fungi, e.g. *Gymnoascus* species.

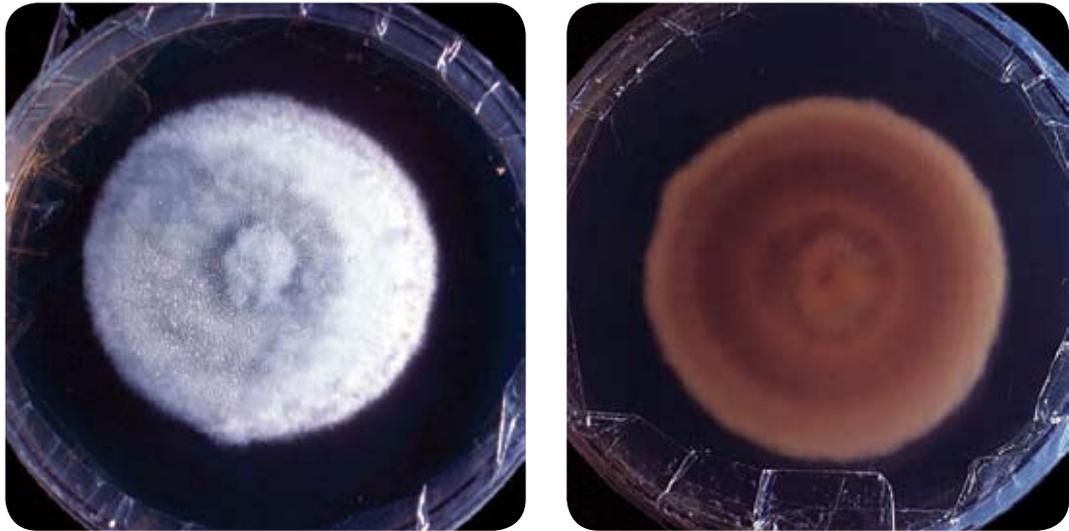
**WARNING:** RG-3 organism. Cultures of *Coccidioides immitis/posadasii* represent a severe biohazard to laboratory personnel and must be handled with extreme caution in an appropriate pathogen handling cabinet. *C. immitis/posadasii* is a dimorphic fungus, existing in living tissue as spherules and endospores, and in soil or culture in a mycelial form. Culture identification by exoantigen test is now the method of choice.

**Key Features:** clinical history, tissue pathology, culture identification by positive exoantigen test.



Tissue morphology showing typical endosporulating spherules of *C. immitis*. Young spherules have a clear centre with peripheral cytoplasm and a prominent thick-wall. Endospores (sporangiospores) are later formed within the spherule by repeated cytoplasmic cleavage. Rupture of the spherule releases endospores into the surrounding tissue where they re-initiate the cycle of spherule development.

***Coccidioides immitis/posadasii* complex**



Culture and arthroconidia separated from each other by disjunctive cells of *Coccidioides immitis/posadasii*.

For descriptions of species, keys to taxa and additional information see Ajello (1957), Steele *et al.* (1977), McGinnis (1980), Chandler *et al.* (1980), Catanzaro (1986), Rippon (1988), de Hoog *et al.* (2000) and Fisher *et al.* (2002).

Antifungal	MIC $\mu\text{g/mL}$		Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.06-2	1	Posaconazole	0.03-1	0.5
Fluconazole	2-64	32	Voriconazole	0.03-1	0.5
Itraconazole	0.03-2	0.5			

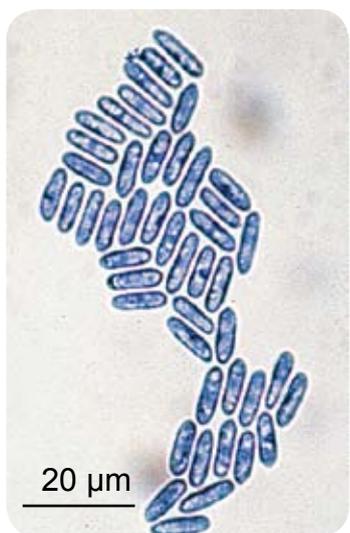
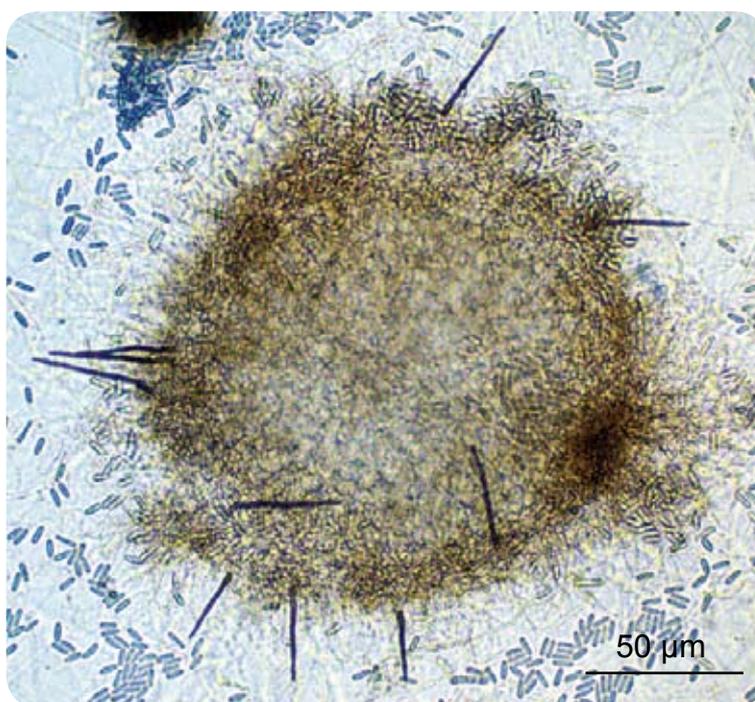
Limited data available. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Gonzalez *et al.* (2005) and Sabatelli *et al.* (2006).

### ***Colletotrichum coccodes* (Wallroth) S. Hughes**

Colonies usually darkly pigmented with white aerial mycelium, consisting of numerous black sclerotia and light brown-coloured conidial masses, reverse is dark brown. Sclerotia are usually abundant, setose, spherical and are often confluent. Conidia are straight, fusiform, attenuated at the ends, 16-22 x 3-4  $\mu\text{m}$ . Appressoria are common, clavate, brown, 11-16.5 x 6-9.5  $\mu\text{m}$ , variable in shape. RG-1 organism.

Over 500 *Colletotrichum* species have been reported. *C. coccodes* is a common soil and plant pathogen widely distributed in Africa, Asia, Australasia, Europe, and the Americas. It has been reported from a case of human mycotic keratitis.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980) and de Hoog *et al.* (2000).



Sclerotia with setae, conidia and appressoria of *C. coccodes*.

## ***Conidiobolus coronatus* (Costantin) Batko**

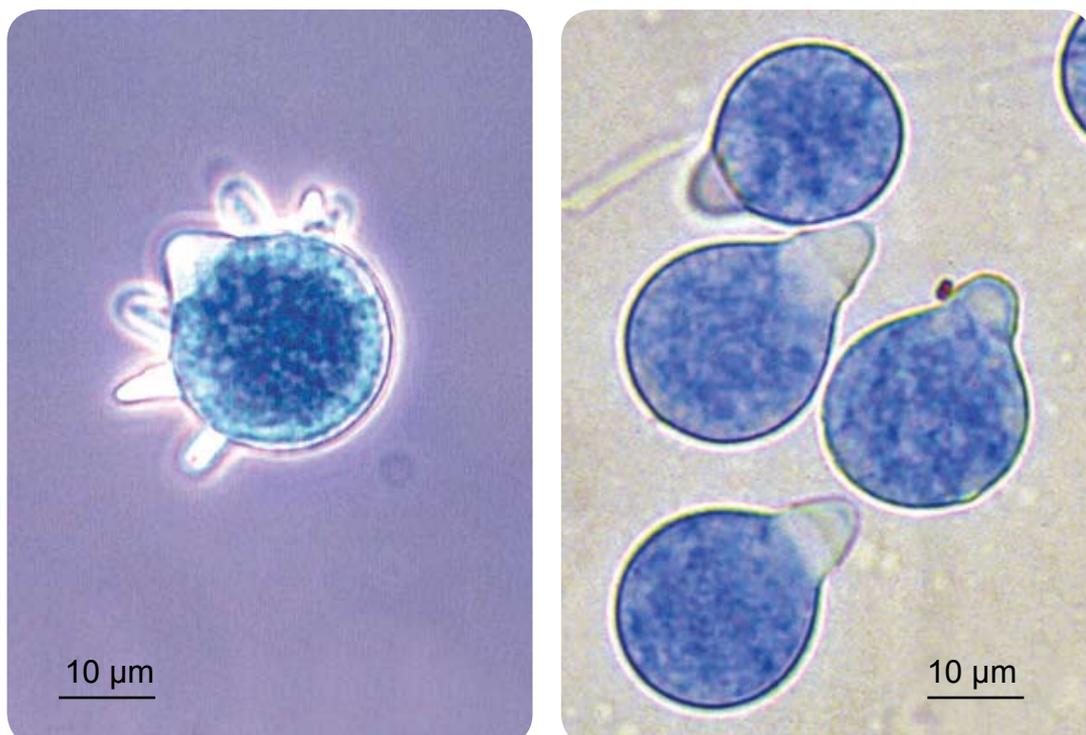
**Synonym:** *Entomophthora coronata* (Costantin) Kevorkian

The species of the genus *Conidiobolus* produce characteristic multinucleate primary and secondary (replicative) conidia on top of unbranched conidiophores. Each subspherical conidium is discharged as a result of the pressure developed within the conidium, and each bears a more or less prominent papilla after discharge (King 1983). The genus contains 27 species, however *C. coronatus* and *C. incongruus* are the only species that are known to cause human disease, although *C. lamprauges* has also been reported once from a horse (Humber, Brown and Kornegay, 1989).

Colonies of *C. coronatus* grow rapidly and are flat, cream-coloured, glabrous becoming radially folded and covered by a fine, powdery, white surface mycelium and conidiophores. The lid of the petri dish soon becomes covered with conidia, which are forcibly discharged by the conidiophores. The colour of the colony may become tan to brown with age. Conidiophores are simple forming solitary, terminal conidia which are spherical, 10 to 25 µm in diameter, single-celled and have a prominent papilla. Conidia may also produce hair-like appendages, called villae. Conidia germinate to produce either, (1) single or multiple hyphal tubes that may also become conidiophores which bear secondary conidia, or (2) replicate by producing multiple short conidiophores, each bearing a small secondary conidium. RG-2 organism.

*Conidiobolus coronatus* is commonly present in soil and decaying leaves. It has a world-wide distribution especially tropical rain forests of Africa. Human infections are usually restricted to the rhinofacial area. However, there are occasional reports of dissemination to other sites. All human infections have been confined to the tropics.

For descriptions of species, keys to taxa and additional information see Emmons and Bridges (1961), King (1976a, 1976b, 1983), McGinnis (1980), Rippon (1988), Kwon-Chung and Bennett (1992), de Hoog *et al.* (2000) and Ellis (2005a).



Spherical conidia with hair-like appendages (villae) and prominent papillae characteristic of *Conidiobolus coronatus*.

## ***Cryptococcus* Kützing emend. Phaff & Spencer**

The genus *Cryptococcus* is characterised by globose to elongate yeast-like cells or blastoconidia that reproduce by multilateral budding, by polar budding on a narrow base (may or may not be present), and pseudohyphae being absent or rudimentary. Most strains have capsulated cells; extent of capsule formation depends on the medium. Under certain conditions of growth the capsule may contain starch-like compounds, which are released into the medium by many strains. On solid media the cultures are generally mucoid or slimy in appearance; red, orange or yellow carotenoid pigments may be produced, but young colonies of most species are usually non-pigmented, being cream in colour. No fermentation: Nitrate assimilated or not: Inositol assimilated. The genus *Cryptococcus* is similar to the genus *Rhodotorula*. The distinctive difference between the two is the assimilation of inositol, which is positive in *Cryptococcus*.

Cryptococcosis is a chronic, subacute to acute pulmonary, systemic or meningitic disease, initiated by the inhalation of basidiospores and/or desiccated yeast cells of *Cryptococcus* species. Primary pulmonary infections have no diagnostic symptoms and are usually subclinical. On dissemination, the fungus usually shows a predilection for the central nervous system, however skin, bones and other visceral organs may also become involved. Although *C. neoformans* and *C. gattii* are regarded as the principle pathogenic species, *Cryptococcus albidus* and *Cryptococcus laurentii* have on occasion also been implicated in human infection.



Culture appearances on Bird Seed Agar of *Cryptococcus neoformans* (brown colonies) and *Candida albicans* (white colonies) and India ink preparation of *C. neoformans* surrounded by a characteristic wide gelatinous capsule.

For descriptions of species, keys to taxa and additional information see Rippon (1982), Barnett *et al.* (1983), McGinnis (1980), Kurtzman and Fell (1988), Casadevall and Perfect (1988) and de Hoog *et al.* (2000).

### ***Cryptococcus albidus* (Saito) Skinner**

**Culture:** Colonies (SDA) are cream-coloured smooth, mucoid glabrous yeast-like. Budding yeast cells only. No pseudohyphae present.

**Microscopy:** Globose to ovoid budding yeast-like cells, 3.5-8.8 × 5.5-10.2 µm.

**India Ink Preparation:** Positive - Distinct capsules are present.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	v
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	+	L-Rhamnose	v	DL-Lactate	v
Sucrose	-	Trehalose	+,w	D-Glucosamine	-	myo-Inositol	+
Maltose	-	Lactose	v	N-A-D-glucosamine	-	2-K-D-gluconate	+
Lactose	-	Melibiose	v	Glycerol	v	D-Glucuronate	+
Trehalose	-	Raffinose	+	Erythritol	v	Nitrate	+
<b>Assimilation</b>		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	v	Galactitol	v	0.1% Cycloheximide	-
Galactose	v	D-Xylose	+	D-Mannitol	+	Growth at 37°C	v

*C. albidus* has variable growth at 37°C, and rare human infections have been reported however its pathogenicity is questionable. RG-1 organism.

### ***Cryptococcus laurentii* (Kufferath) Skinner**

**Culture:** Colonies (SDA) are cream-coloured, often becoming a deeper orange-yellow with age, with a smooth mucoid texture. Budding yeast cells only. No pseudohyphae present.

**Microscopy:** Spherical and elongated budding yeast-like cells or blastoconidia, 2.0-5.5 × 3.0-7.0 µm. No pseudohyphae present.

**India Ink Preparation:** Positive - Narrow but distinct capsules are present.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	+	α-M-D-glucoside	+
Glucose	-	Maltose	+	D-Ribose	+	D-Gluconate	+
Galactose	-	Cellobiose	+	L-Rhamnose	+	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	-	myo-Inositol	+
Maltose	-	Lactose	+	N-A-D-glucosamine	-	2-K-D-gluconate	+
Lactose	-	Melibiose	+	Glycerol	v	D-Glucuronate	+
Trehalose	-	Raffinose	+	Erythritol	v	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	+	Urease	+
Glucose	+	Soluble Starch	v	Galactitol	+	0.1% Cycloheximide	-
Galactose	v	D-Xylose	+	D-Mannitol	+	Growth at 37°C	-,w

Note: some strains of *C. laurentii* may develop a brown pigment on Bird Seed agar and turn CGB media blue, similar to *C. gattii*, however *C. laurentii* assimilates both lactose and melibiose while *C. gattii* does not. Rare human infections have been reported however its pathogenicity is questionable. RG-1 organism.

## ***Cryptococcus gattii* (Vanbreus. & Takashio) Kwon-Chung & Boekhout**

**Teleomorph:** *Filobasidiella bacillispora* Kwon-Chung,

**Synonym:** *Cryptococcus neoformans* var. *gattii* Vanbreus & Takashio

*Cryptococcus gattii* has two serotypes (B and C) and has recently been reclassified as a separate species (Kwon-Chung *et al.* 2002). *C. gattii* generally has a more restricted geographical distribution than *C. neoformans*, causing human disease in climates ranging from temperate to tropical Australia, Papua New Guinea, parts of Africa, India, southeast Asia, Mexico, Brazil, Paraguay and Southern California, although recent infections have also been reported from Vancouver Island, Canada. Non-immunocompromised hosts are usually affected and large mass lesions in the lung and/or brain (cryptococcomas) are often present (Sorrell, 2001).

**Canavanine glycine bromothymol blue (CGB) agar** (Kwon-Chung *et al.* 1982) is the media of choice to differentiate *C. gattii* from *C. neoformans*. This simple biotype test is based on the ability of *C. gattii* isolates to grow in the presence of L-canavanine and to assimilate glycine as a sole carbon source.



*C. gattii* turns CGB agar blue within 2-5 days;  
*C. neoformans* does not grow on this medium

**Culture:** Colonies (SDA) cream-coloured smooth, mucoid yeast-like colonies.

**Microscopy:** Globose to ovoid budding yeast-like cells 3.0-7.0 x 3.3- 7.9 µm.

**India Ink Preparation:** Positive - Distinct, wide gelatinous capsules are present.

**Dalmat Plate Culture on Cornmeal and Tween 80 Agar:** Budding yeast cells only. No pseudohyphae present.

**Bird Seed Agar:** Colonies turn dark brown in colour as they selectively absorb a brown pigment from this media. Colonies are often more mucoid when compared to *C. neoformans* (Staib, 1987).

**Canavanine-Glycine-Bromothymol Blue (CGB) Agar:** turns blue within 2-5 days.

**Key Features:** encapsulated yeast; absence of pseudohyphae; growth at 37°C; positive hydrolysis of urea; negative fermentation of sugars and positive assimilation of glucose, maltose, sucrose, galactose, trehalose, raffinose, inositol, cellobiose, rhamnose, arabinose, melezitose and xylose, and negative assimilation of nitrate, lactose, melibiose, erythritol and soluble starch; growth on bird seed (*Guizotia abyssinica* seed) or caffeic acid agar - colonies turn a dark brown colour; growth on CGB agar turning it blue within 2-5 days. RG-2 organism, however mating experiments for the production of basidiospores should be done in an appropriate pathogen handling cabinet.

## ***Cryptococcus gattii* (Vanbreus. & Takashio) Kwon-Chung & Boekhout**

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+ ,w	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	+	$\alpha$ -M-D-glucoside	+
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	+ ,w	L-Rhamnose	+	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	v	<i>myo</i> -Inositol	+
Maltose	-	Lactose	-	<i>N</i> -A-D-glucosamine	v	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	-	D-Glucuronate	+
Trehalose	-	Raffinose	+ ,w	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	+	Galactitol	+	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

Antifungal	MIC $\mu$ g/mL		Antifungal	MIC $\mu$ g/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	1->64	16	Amphotericin B	0.03-2	0.25
Itraconazole	<0.03-1	0.25	Flucytosine	0.03-64	4
Voriconazole	<0.03-2	0.25	Posaconazole	0.3-0.5	0.25

Good data available. Trilles *et al.* (2004) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian *Cryptococcus* Study.

## ***Cryptococcus neoformans* (Sanfelice) Vuillemin**

**Teleomorph:** *Filobasidiella neoformans* Kwon-Chung.

**Synonym:** *Cryptococcus neoformans* var. *neoformans*.

This species has two varieties: *C. neoformans* var. *grubii* [serotype A] and *C. neoformans* var. *neoformans* [serotype D].

*C. neoformans* var. *grubii* [serotype A] has a world-wide distribution. It has been isolated from various sources in nature and is noted for its association with accumulations of avian guano, especially with pigeon excreta. The fungus has also been isolated from the dung of caged birds including canaries, parrots and budgerigars. Other environmental isolations of *C. neoformans* var. *grubii* have been from rotting vegetables, fruits and fruit juices, wood, dairy products and soil.

*C. neoformans* var. *neoformans* [serotype D] has a more restricted distribution with infections being more prevalent in France, Italy and Denmark. Moreover, infections are more strongly correlated to older patients, the skin, and the use of corticosteroids (Franzot *et al.* 1999).

**Creatinine dextrose bromothymol blue thymine (CDBT) agar** is the medium of choice for the differentiation of *Cryptococcus neoformans* var. *neoformans* and *Cryptococcus neoformans* var. *grubii*. *Cryptococcus neoformans* var. *neoformans* grows as bright red colonies, turning the medium a bright orange after 5 days (Irokanulo *et al.* 1994).

## ***Cryptococcus neoformans* (Sanfelice) Vuillemin**

**Culture:** Colonies (SDA) cream-coloured smooth, mucoid yeast-like colonies.

**Microscopy:** Globose to ovoid budding yeast-like cells 3.0-7.0 x 3.3- 7.9 µm.

**India Ink Preparation:** Positive - Distinct, wide gelatinous capsules are present.

**Dalmau Plate Culture on Cornmeal and Tween 80 Agar:** Budding yeast cells only. No pseudohyphae present.

**Bird Seed Agar:** Colonies turn dark brown in colour as colonies selectively absorb a brown pigment from this media (Staib, 1987).

**Canavanine-Glycine-Bromothymol Blue (CGB) Agar:** leaves this medium unchanged.

**Creatinine dextrose bromothymol blue thymine (CDBT) agar:** *Cryptococcus neoformans* var. *neoformans* grows as bright red colonies, turning the medium a bright orange after 5 days. No colour change is observed for *C. neoformans* var. *grubii*.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	+,w	D-Glucitol	+
<b>Fermentation</b>		Sucrose	+	D-Arabinose	+	α-M-D-glucoside	+
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	+,w	L-Rhamnose	+	DL-Lactate	-
Sucrose	-	Trehalose	+	D-Glucosamine	v	myo-Inositol	+
Maltose	-	Lactose	-	N-A-D-glucosamine	v	2-K-D-gluconate	+
Lactose	-	Melibiose	-	Glycerol	-	D-Glucuronate	+
Trehalose	-	Raffinose	+,w	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	+	Galactitol	+	0.1% Cycloheximide	-
Galactose	+	D-Xylose	+	D-Mannitol	+	Growth at 37°C	+

**Key Features:** encapsulated yeast; absence of pseudohyphae; growth at 37°C; positive hydrolysis of urea; negative fermentation of sugars and positive assimilation of glucose, maltose, sucrose, galactose, trehalose, raffinose, inositol, cellobiose, rhamnose, arabinose, melezitose and xylose, and negative assimilation of nitrate, lactose, melibiose, erythritol and soluble starch; growth on bird seed (*Guizotia abyssinica* seed) or caffeic acid agar - colonies turn a dark brown colour; does not growth on CGB agar (no colour change). RG-2 organism, however mating experiments for the production of basidiospores should be done in an appropriate pathogen handling cabinet.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	1->64	8	Amphotericin B	0.03-2	0.5
Itraconazole	<0.03-1	0.25	Flucytosine	0.03-64	4
Voriconazole	<0.03-2	0.125	Posaconazole	0.03-0.5	0.25

Good data available. Espinel-Ingroff et al. (2001), Espinel-Ingroff (2003), Trilles et al. (2004), Cuenca-Estrella et al. (2006). MIC<sub>90</sub>s from the Australian *Cryptococcus* Study.

## ***Cunninghamella bertholletiae* Stadel**

**Synonyms:** *Cunninghamella elegans* Lendner  
*Cunninghamella echinulata* var. *elegans* (Lendner) Lunn & Shipton

The genus *Cunninghamella* is characterised by white to grey, rapidly growing colonies, producing erect, straight, branching sporangiophores. These sporangiospores end in globose or pyriform-shaped vesicles from which several one-celled, globose to ovoid, echinulate or smooth-walled sporangia develop on swollen denticles. Chlamydospores and zygospores may also be present.

Colonies are very fast growing, white at first, but becoming rather dark grey and powdery with sporangia development. Sporangiophores to 20 µm wide, straight, with verticillate or solitary branches. Vesicles subglobose to pyriform, the terminal ones up to 40 µm and the lateral ones 10-30 µm in diameter. Sporangia are globose (7-11 µm diameter), or ellipsoidal (9-13 x 6-10 µm), verrucose or short-echinulate, hyaline singly but brownish in mass. Temperature: optimum 25 to 30°C: maximum up to 50°C. RG-2 organism.

*Cunninghamella* species are mainly soil fungi of the Mediterranean and subtropical zones; they are only rarely isolated in temperate regions. The genus now contains seven species with *C. bertholletiae* the only known species to cause disease in man and animals which is often associated with trauma and immunosuppression.

**Key Features:** zygomycete, clinical isolates grow at 40°C, one-celled, globose to ovoid, echinulate sporangia borne on swollen terminal or lateral globose to clavate fertile vesicles.

Once again, there has been some confusion as to the correct name of this zygomycete. Many medical mycologists (McGinnis, 1980, Weitzman, 1984, and Rippon, 1988) preferred the name *Cunninghamella bertholletiae* because of the thermophilic nature of human isolates that grow at temperatures as high as 45°C. However, Samson (1969) and Domsch *et al.* (1980) preferred the name *Cunninghamella elegans* and Lunn and Shipton (1983) went further and reduced *C. elegans* (= *C. bertholletiae*) to a variety of *Cunninghamella echinulata*; i.e. *C. echinulata* var. *elegans*. However, *C. bertholletiae* is currently the most acceptable name; *C. elegans* differs by having purely grey colonies and by not growing at temperatures above 40°C (Weitzman and Crist, 1979 and de Hoog *et al.* 2000).

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	>64	>64	Amphotericin B	0.125-8	2
Itraconazole	0.125-4	2	Flucytosine	>256	>256
Posaconazole	0.003-1	1	Voriconazole	8->64	>64

Very limited data, antifungal susceptibility testing of individual strains is recommended. Sun *et al.* (2002), Dannaoui *et al.* (2003), Espinel-Ingroff (2001, 2003), Singh *et al.* (2005), Sabatelli *et al.* (2006) and WCH in-house data.

### *Cunninghamella bertholletiae* Stadel



Microscopic morphology of *Cunninghamella bertholletiae* showing simple sporangiophores forming a swollen, terminal vesicle around which single-celled, globose to ovoid sporangia develop on swollen denticles.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Weitzman (1984), Lunn and Shipton (1983), Domsch *et al.* (1980), Samson (1969), de Hoog *et al* (2000) and Ellis (2005b).

## Curvularia Boedijn

**Teleomorph:** *Cochliobolus* Drechsler

Colonies are fast growing, suede-like to downy, brown to blackish brown with a black reverse. Conidia are pale brown, with three or more transverse septa (phragmoconidia) and are formed apically through a pore (poroconidia) in a sympodially elongating geniculate conidiophore similar to *Drechslera*. Conidia are cylindrical or slightly curved, with one of the central cells being larger and darker, germination is bipolar and some species may have a prominent hilum.

The genus *Curvularia* contains some 35 species which are mostly subtropical and tropical plant parasites; however three ubiquitous species, *C. lunata*, *C. pallescens* and *C. geniculata* have been recovered from human infections, principally from cases of mycotic keratitis. However, cases of subcutaneous, sinusitis, endocarditis, peritonitis and disseminated infection have also been reported in immunosuppressed patients. RG-1 organisms.

**Key Features:** dematiaceous hyphomycete producing sympodial, pale brown, cylindrical or slightly curved phragmoconidia, with one of the central cells being larger and darker.

For descriptions of species, keys to taxa and additional information see Ellis (1971), Domsch *et al.* (1980), McGinnis (1980), Rippon (1988) and de Hoog *et al.* (2000).



Conidia of *Curvularia lunata*.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range		Range
Amphotericin B	0.03-16	Itraconazole	0.03-32	Voriconazole	0.06-1

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001) and WCH in-house data.

## ***Cylindrocarpon* Wollenw.**

**Teleomorph:** *Nectria* (Fries) Fr.

Colonies are fast growing, hyaline or bright-coloured, suede-like or woolly. Sporodochia may occasionally be present. Conidiophores consist of simple or repeatedly verticillate phialides, arranged in brush-like structures. Phialides are cylindrical to subulate, with small collarettes producing hyaline, smooth-walled conidia, which are arranged in slimy masses. Two types of conidia may be produced; macroconidia which are one to several septate, hyaline, straight or curved, cylindrical to fusiform, with a rounded apex and flat base; and microconidia which are one-celled, which are usually clearly distinct from the macroconidia. Chlamydospores may be present or absent, hyaline to brown, spherical, formed singly, in chains or in clumps, intercalary or terminal. RG-2 organism if isolated from humans.

The genus contains 35 species, is widespread, isolated mostly from soil and is recorded as an occasional human and animal pathogen. *Cylindrocarpon* differs from *Fusarium* by lacking an asymmetrical foot-cell on the macroconidia.

For descriptions of species, keys to taxa and additional information see Booth (1966), Domsch *et al.* (1980) and de Hoog *et al.* (2000).



Culture, chlamydospores and macroconidia of *Cylindrocarpon lichenicola*.

## ***Drechslera* Ito**

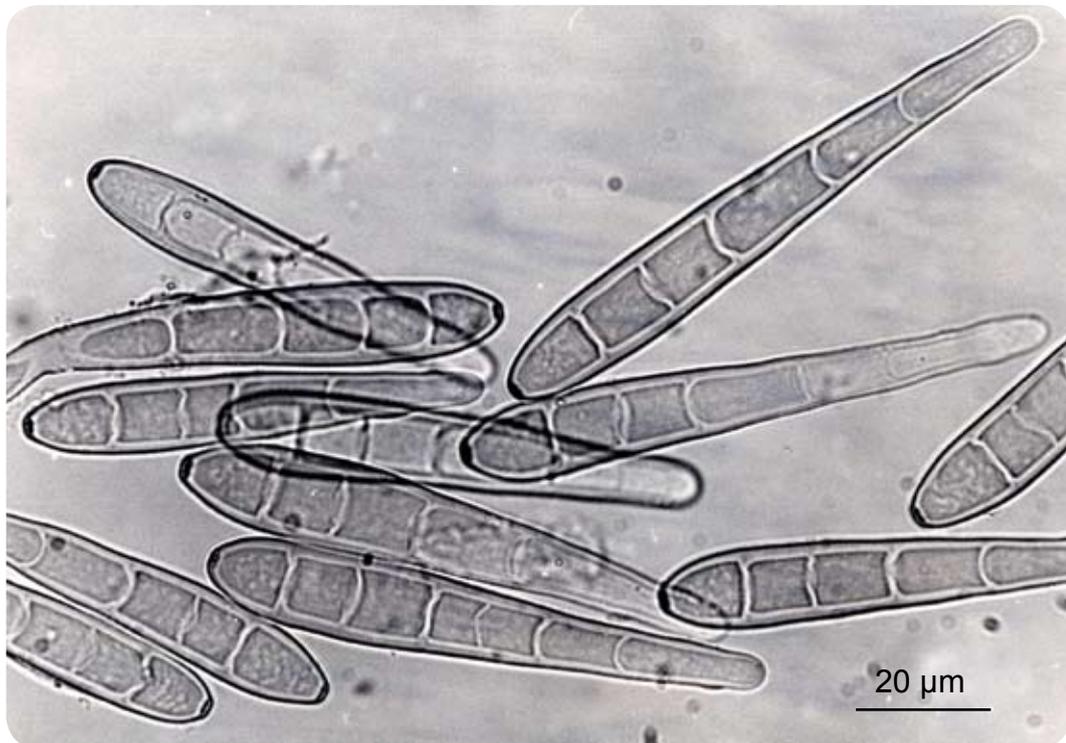
**Teleomorph:** *Pyrenophora* Fries

Colonies are fast growing, suede-like to downy, brown to blackish brown with a black reverse. Conidia are pale to dark brown, usually cylindrical or subcylindrical, straight, smooth-walled, and are formed apically through a pore (poroconidia) on a sympodially elongating, geniculate conidiophore. Conidia are transversely septate (phragmoconidia), with the septum delimiting the basal cell formed first during conidium maturation. Germinating is from any or all cells and the hilum is not protuberant. RG-1 organism.

McGinnis *et al.* (1986b) have reviewed the isolates from human and animal disease purported to be *Drechslera* or *Helminthosporium* and concluded that all pathogenic isolates examined actually belong to the genera *Bipolaris* or *Exserohilum*.

**Key Features:** dematiaceous hyphomycete producing sympodial, pale brown, cylindrical or subcylindrical, transversely septate poroconidia.

For descriptions of species, keys to taxa and additional information see Luttrell (1978), Ellis (1971 and 1976), Domsch *et al.* (1980), McGinnis (1980), McGinnis *et al.* (1986b), Sivanesan (1987), Rippon (1988) and de Hoog *et al.* (2000). Also see Descriptions for *Bipolaris*, *Curvularia* and *Exserohilum*.



Conidia of *Drechslera*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.25	Itraconazole	0.25	Voriconazole	0.06

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998).

***Epicoccum purpurascens* Ehrenb. ex Schlecht.**

**Synonym:** *Epicoccum nigrum* Link

Colonies are fast growing, suede-like to downy, with a strong yellow to orange-brown diffusible pigment. When sporulating numerous black sporodochia (aggregates of conidiophores) are visible. Conidia are formed singly on densely compacted, non-specialised, determinant, slightly pigmented conidiophores. Conidia are globose to pyriform, mostly 15-25 µm diameter with a funnel-shaped base and broad attachment scar, often seceding with a protuberant basal cell; i.e. aleuric or rhexolytic dehiscence of conidia. Conidia become multicellular (dictyoconidia), darkly pigmented and have a verrucose external surface. RG-1 organism.

*Epicoccum purpurascens* is a cosmopolitan saprophyte of world-wide distribution which is occasionally isolated as a contaminant from clinical specimens like skin.

**Key Features:** dematiaceous hyphomycete producing darkly pigmented, large globose to pyriform, verrucose dictyoconidia on a sporodochium.

For descriptions of species, keys to taxa and additional information see Ellis (1971), Domsch *et al.* (1980), McGinnis (1980) and Samson *et al.* (1995).



Conidia of *Epicoccum purpurascens*.

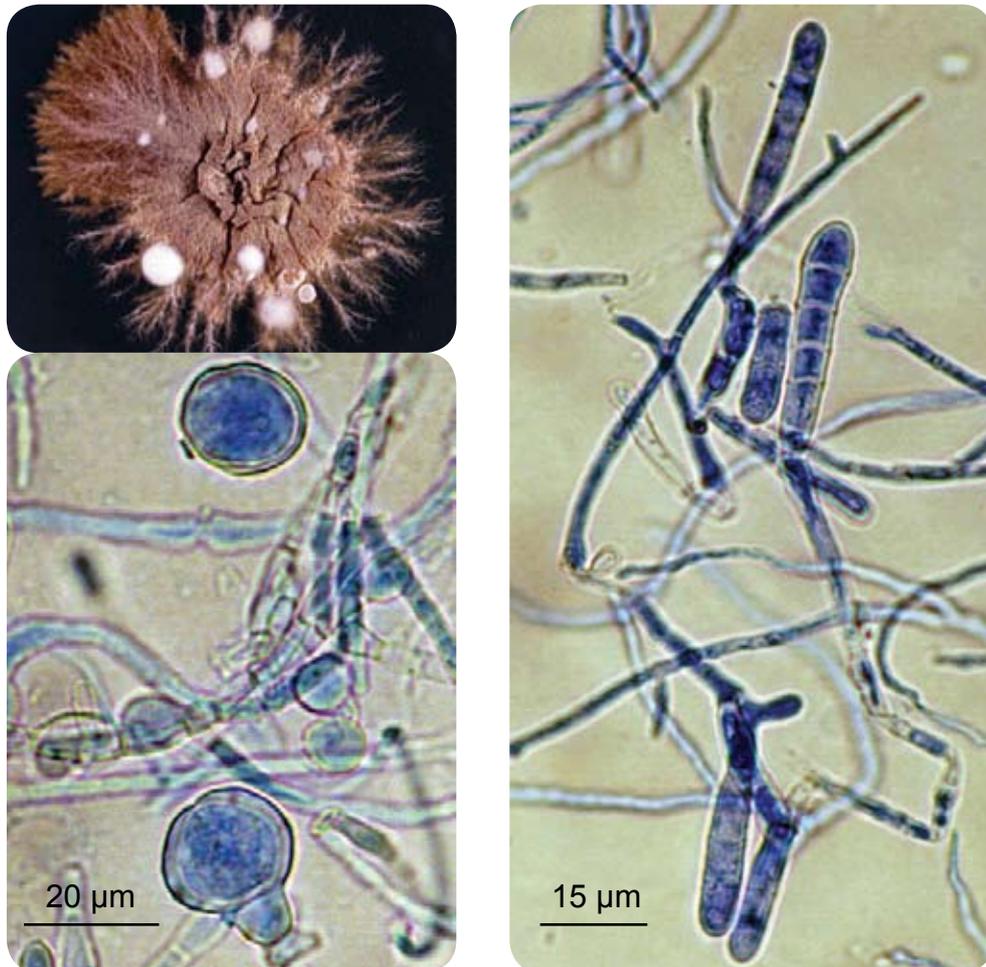
### ***Epidermophyton floccosum* (Harz) Langeron et Milochevitch**

Colonies (SDA) are usually slow growing, greenish-brown or khaki-coloured with a suede-like surface, raised and folded in the centre, with a flat periphery and submerged fringe of growth. Older cultures may develop white pleomorphic tufts of mycelium. A deep yellowish-brown reverse pigment is usually present. Microscopic morphology shows characteristic smooth, thin-walled macroconidia which are often produced in clusters growing directly from the hyphae. Numerous chlamydospores are formed in older cultures. Microconidia are not formed.

*Epidermophyton floccosum* is an anthropophilic dermatophyte with a world-wide distribution which often causes tinea pedis, tinea cruris, tinea corporis and onychomycosis. It is not known to invade hair *in vivo* and no specific growth requirements have been reported. RG-2 organism.

**Key Features:** culture characteristics, microscopic morphology and clinical disease.

For descriptions of species, keys to taxa and additional information see Rebell and Taplin (1970), Mackenzie *et al.* (1987), Rippon (1988) and de Hoog *et al.* (2000).



Culture, macroconidia and chlamydospores of *E. floccosum*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Griseofulvin	0.06-2	1	Amphotericin B	0.03-0.5	0.25
Itraconazole	0.01-8	0.125	Fluconazole	0.5->64	>64
Terbinafine	0.01-1	0.06	Voriconazole	0.01-8	0.125

Fernandez-Torres *et al.* (2001) and Sabatelli *et al.* (2006) and WCH in-house data.

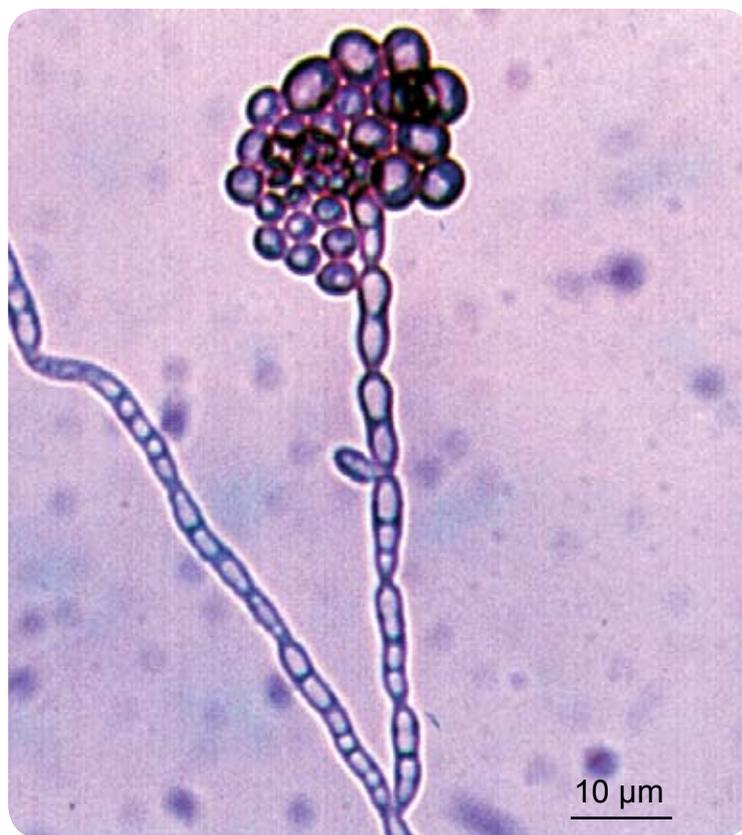
## *Exophiala dermatitidis* (Kano) de Hoog

**Synonym:** *Wangiella dermatitidis* (Kano) McGinnis

Colonies are slow growing, initially yeast-like and black, becoming suede-like, olivaceous grey with the development of aerial mycelium with age. The initial yeast-like phase is referred to as the *Phaeococcomyces exophialae* synanamorph, which is characterised by unicellular, ovoid to elliptical, budding yeast-like cells. The yeast-like cells are hyaline and thin-walled when young becoming darkly pigmented (dematiaceous) and thick-walled when mature. With the development of mycelium, flask-shaped to cylindrical annellides are produced. Conidia are hyaline to pale brown, one-celled, round to obovoid, 2.0-4.0 × 2.5-6.0 µm, smooth-walled and accumulate in slimy balls at the apices of the annellides or down their sides. Cultures grow at 42°C.

*E. dermatitidis* has been isolated from plant debris and soil and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans. RG-2 organism.

For descriptions of species, keys to taxa and additional information see de Hoog and Hermanides-Nijhof (1977), McGinnis (1980), Hohl *et al.* (1983), Nishimura and Miyaji (1983), Matsumoto *et al.* (1984), Dixon and Polak-Wyss (1991), de Hoog *et al.* (2000).



Annellides and conidia of *Exophiala dermatitidis*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.03-2	0.5	Amphotericin B	0.03-2	0.5
Voriconazole	0.06-0.5	0.25	Posaconazole	0.03-1	nd

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff (2001), Espinel-Ingroff *et al.* (2001), Pfaller *et al.* (2002a) and WCH in-house data (nd = not done).

## ***Exophiala jeanselmei* Complex**

**Synonyms:** *Phialophora jeanselmei* (Langeron) Emmons

*E. jeanselmei* has long been recognised as heterogeneous (de Hoog 1977). Recent molecular studies have redefined *Exophiala jeanselmei* and three additional species have been identified: *E. oligosperma*, *E. nishimurae*, and *E. xenobiotica* (Vitale and de Hoog, 2002, de Hoog *et al.* 2003, 2006). These species are morphologically very similar and can best be distinguished by genetic analysis.

Conidiogenous cells are predominantly annellidic and erect, multicellular conidiophores are absent. No growth at 40°C.

<i>E. jeanselmei</i>	Mature conidiogenous cells rocket-shaped, slightly darker than the supporting hyphae, with regular tapering annellated zones.
<i>E. oligosperma</i>	Mature conidiogenous cells remain concolorous with supporting hyphae and may be intercalary and lateral, the latter being flask or rocket-shaped. Annellated zones have the appearance of inconspicuous flat scars. Chlamydospores are absent.
<i>E. nishimurae</i>	Similar morphology to <i>E. oligosperma</i> , however large chlamydospore-like cells are present.
<i>E. xenobiotica</i>	A segregant genotype of the <i>E. jeanselmei</i> complex with less melanised conidiogenous cells.

Colonies are initially smooth, greenish-grey to black, mucoid and yeast-like, becoming raised and developing tufts of aerial mycelium with age, often becoming dome-shaped and suede-like in texture. Reverse is olivaceous-black. Numerous ellipsoidal, yeast-like, budding cells are usually present, especially in young cultures. Scattered amongst these yeast-like cells are larger, inflated, subglobose to broadly ellipsoidal cells (germinating cells) which give rise to short torulose hyphae that gradually change into unswollen hyphae. Conidia are formed on lateral pegs either arising apically or laterally at right or acute angles from essentially undifferentiated hyphae or from strongly inflated detached conidia. Conidiogenous pegs are 1-3 µm long, slightly tapering and imperceptibly annellate. Conidia are hyaline, smooth, thin-walled, broadly ellipsoidal, 3.2-4.4 × 1.2-2.2 µm, and with inconspicuous basal scars. Cultures grow at 37°C but not at 40°C. RG-2 organism.

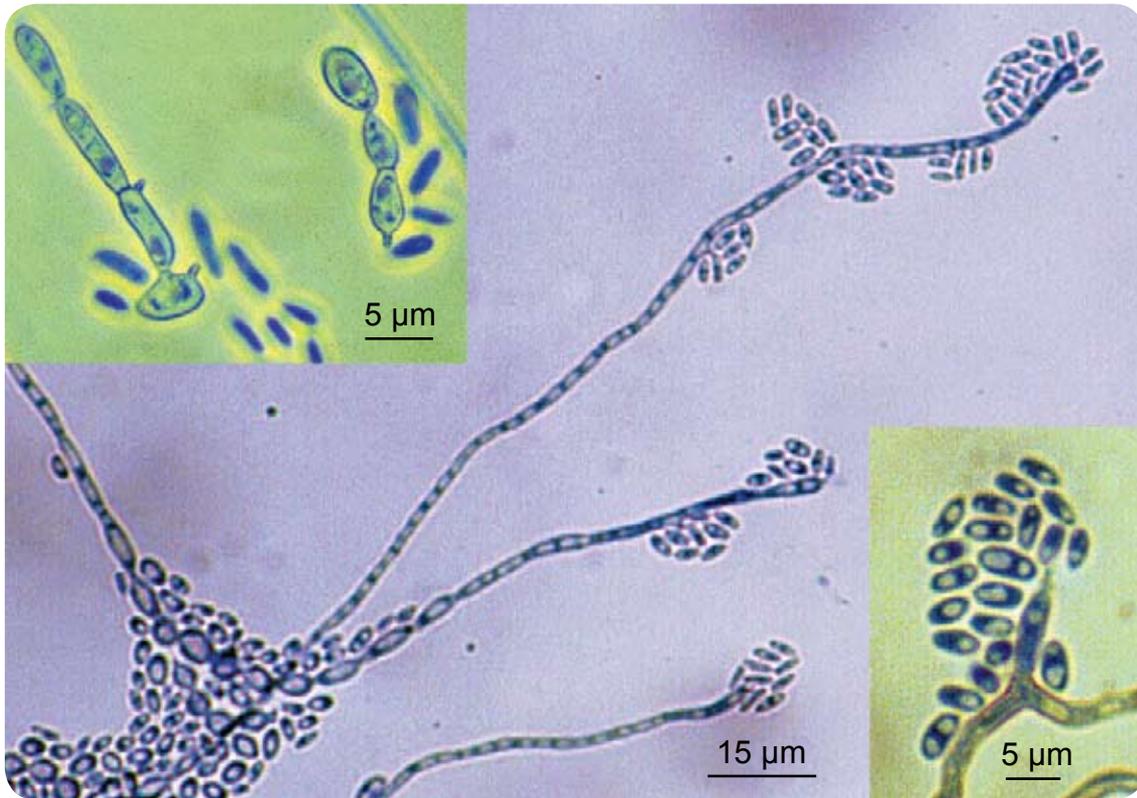
*E. jeanselmei* has a world-wide distribution and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans.

For descriptions of species, keys to taxa and additional information see de Hoog and Hermanides-Nijhof (1977), de Hoog (1977, 1985), McGinnis and Padhye (1977), McGinnis (1978, 1980), Domsch *et al.* (1980), Nishimura and Miyaji (1983), Matsumoto *et al.* (1987), Dixon and Polak-Wyss (1991) and de Hoog *et al.* (2000, 2003, 2006).

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.03-2	0.5	Amphotericin B	0.03-4	0.5
Voriconazole	0.06-2	0.5	Posaconazole	0.25-0.5	0.5

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001), Nucci *et al.* (2001) and WCH in-house data.

### *Exophiala jeanselmei/spinifera* Complex



Annellides, conidia and conidiogenous pegs (annellides) on yeast-like cells and torulose hyphae of *Exophiala jeanselmei*.



Erect, multiseptate conidiophores that are darker than the supporting hyphae, with long annellated zones and conidia of *Exophiala spinifera*.

## ***Exophiala spinifera* Complex**

**Synonyms:** *Phialophora spinifera* Nielsen & Conant  
*Rhinoctadiella spinifera* (Nielsen & Conant) de Hoog

Recent molecular studies have re-examined *Exophiala spinifera* and have recognised two species: *E. spinifera* and *E. attenuata* (Vitale and de Hoog, 2002). These two species are morphologically very similar and can best be distinguished by genetic analysis.

Conidiogenous cells are predominately annellidic and erect, multicellular conidiophores that are darker than the supporting hyphae are present. No growth at 40°C.

<i>E. spinifera</i>	Annellated zones are long with clearly visible, frilled annellations.
<i>E. attenuata</i>	Annellated zones are inconspicuous and degenerate.

Colonies are initially mucoid and yeast-like, black, becoming raised and developing tufts of aerial mycelium with age, finally becoming suede-like to downy in texture. Reverse is olivaceous-black. Conidiophores are simple or branched, erect or sub-erect, spine-like with rather thick brown pigmented walls. Conidia are formed in basipetal succession on lateral pegs either arising apically or laterally at right or acute angles from the spine-like conidiophores or from undifferentiated hyphae. Conidiogenous pegs are 1-3 µm long, slightly tapering and imperceptibly annellate. Conidia are one-celled, subhyaline, smooth, thin-walled, subglobose to ellipsoidal, 1.0-2.9 × 1.8-2.5 µm, and aggregate in clusters at the tip of each annellide. Toruloid hyphae and yeast-like cells with secondary conidia are typically present. No growth at 40°C. RG-2 organism.

*E. spinifera* has a world-wide distribution and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans.

For descriptions of species, keys to taxa and additional information see de Hoog and Hermanides-Nijhof (1977), McGinnis and Padhye (1977), Domsch *et al.* (1980), McGinnis (1980), Nishimura and Miyaji (1983), de Hoog (1985), Matsumoto *et al.* (1987), Dixon and Polak-Wyss (1991) and de Hoog *et al.* (2000, 2003, 2006).

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.125-1	Itraconazole	0.03-1	Voriconazole	0.125-1

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001) and WCH in-house data.

## Exserohilum Leonard and Suggs

Colonies are grey to blackish-brown, suede-like to floccose in texture and have an olivaceous black reverse. Conidia are straight, curved or slightly bent, ellipsoidal to fusiform and are formed apically through a pore (poroconidia) on a sympodially elongating geniculate conidiophore. Conidia have a strongly protruding, truncate hilum and the septum above the hilum is usually thickened and dark, with the end cells often paler than other cells, walls often finely roughed. Conidial germination is bipolar.

The genus *Exserohilum* may be differentiated from the closely related genera *Bipolaris* and *Drechslera* by forming conidia with a strongly protruding truncate hilum (i.e. **exserted hilum**). The hilum is defined as “a scar on a conidium at the point of attachment to the conidiophore”. In *Drechslera* species, the hilum does not protrude; in *Bipolaris* species the hilum protrudes only slightly. Several species of *Exserohilum* have been reported as agents of phaeohyphomycosis, notably *E. rostratum* (= *E. halodes*), *E. mcginnisii* and *E. longirostratum*. RG-1 organisms.

**Key Features:** dematiaceous hyphomycete producing sympodial, transverse septate, ellipsoidal to fusiform conidia with a strongly protruding, truncate hilum.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), Alcorn (1983), Adam *et al.* (1986), McGinnis *et al.* (1986b), Rippon (1988), Burges *et al.* (1987), Dixon and Polak-Wyss (1991) and de Hoog *et al.* (2000).



Conidiophores and conidia with distinctive hilum (arrow) of *E. rostratum*.

Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range
Amphotericin B	0.125-2	Itraconazole	0.03-0.5	Voriconazole	0.03-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

### ***Fonsecaea pedrosoi/monophora* Complex**

Morphologically the genus *Fonsecaea* is defined by the presence of indistinct melanised conidiophores with blunt, scattered denticles bearing conidia singly or in short chains that eventually become branched. de Hoog *et al.* (2004) revised the genus on the basis of ribosomal DNA internal transcribed spacer (ITS) sequence data recognising two species; *F. pedrosoi* and *F. monophora*. The previously described species *F. compacta* was found to be a morphological variant of *F. pedrosoi*. Morphological *F. pedrosoi* and *F. monophora* are very similar and can best be distinguished by genetic analysis. *F. monophora* on average has slightly longer conidial chains and slightly shorter denticles than *F. pedrosoi*. All strains grow at 37°C but not at 40°C. Both species are recognised etiologic agents of chromoblastomycosis. RG-2 organism.

Colonies are slow growing, flat to heaped and folded, suede-like to downy, olivaceous to black with black reverse. Conidiogenous cells pale olivaceous, arranged in loosely branched systems, with prominent denticles. Conidia pale olivaceous, clavate to ellipsoidal, in short chains, subhyaline, smooth and thin-walled, 3.5-5 × 1.5-2 µm.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Dixon and Polak-Wyss (1991), de Hoog *et al.* (2000), de Hoog *et al.* (2004).



Conidiophores and conidia of *Fonsecaea*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.03-1	0.25	Amphotericin B	0.03-2	1
Voriconazole	0.06-1	0.06	Posaconazole	0.06-1	nd

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003) and WCH in-house data (nd = not done).

## ***Fusarium* Link ex Fries**

Colonies are usually fast growing, pale or bright coloured (depending on the species) with or without a cottony aerial mycelium. The colour of the thallus varies from whitish to yellow, pink, red or purple shades. Species of *Fusarium* typically produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia are one- or two-celled, hyaline, smaller than macroconidia, pyriform, fusiform to ovoid, straight or curved. Chlamydo spores may be present or absent.



Cultures of *F. oxysporum* showing purple pigmentation and *F. subglutinans* showing pink pigmentation.

Identification of *Fusarium* species is often difficult due to the variability between isolates (e.g. in shape and size of conidia and colony colour) and because not all features required are always well developed (e.g. the absence of macroconidia in some isolates after subculture). The important characters used in the identification of *Fusarium* species are as follows. Note: sporulation may need to be induced in some isolates and a good slide culture is essential.

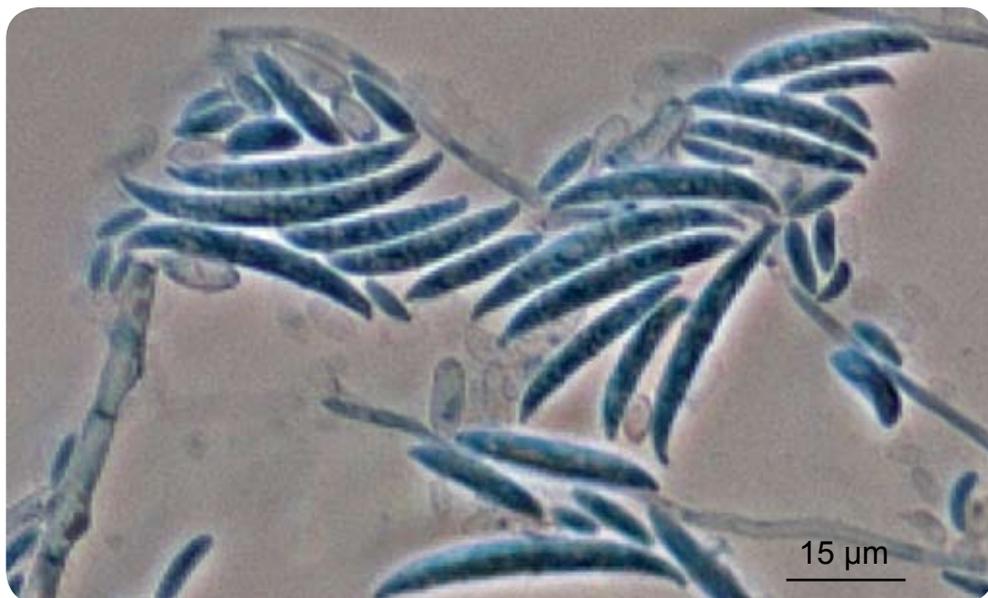
1. Colony growth diameters on potato dextrose agar and/or potato sucrose agar after incubation in the dark for 4 days at 25°C.
2. Culture pigmentation on potato dextrose agar and/or potato sucrose agar after incubation for 10-14 days with daily exposure to light.
3. Microscopic morphology including shape of the macroconidia; presence or absence of microconidia; shape and mode of formation of microconidia; nature of the conidigenous cell bearing microconidia; and presence or absence of chlamydo spores.

Most *Fusarium* species are soil fungi and have a world-wide distribution. Some are plant pathogens, causing root and stem rot, vascular wilt or fruit rot. Several species, notably *F. oxysporum*, *F. solani* and *F. moniliforme* are recognised as being pathogenic to man and animals causing hyalohyphomycosis (especially in burn victims and bone marrow transplant patients), mycotic keratitis and onychomycosis. Other species cause storage rot and are important mycotoxin producers.

For descriptions of species, keys to taxa and additional information see Booth (1971 and 1977), Domsch *et al.* (1980), McGinnis (1980), Burgess and Liddell (1983), Rippon (1988), Samson *et al.* (1995) and de Hoog *et al.* (2000).

### ***Fusarium oxysporum* Schlecht**

Colonies growing rapidly, 4.5 cm in 4 days, aerial mycelium white, becoming purple, with discrete orange sporodochia present in some strains; reverse hyaline to dark blue or dark purple. Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Macroconidia are fusiform, slightly curved, pointed at the tip, mostly three septate, basal cells pedicellate, 23-54 x 3-4.5 µm. Microconidia are abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved, 5-12 x 2.3 - 3.5 µm. Chlamyospores are terminal or intercalary, hyaline, smooth or rough-walled, 5-13 µm. In contrast to *F. solani* the phialides are short and mostly non-septate. RG-2 organism.



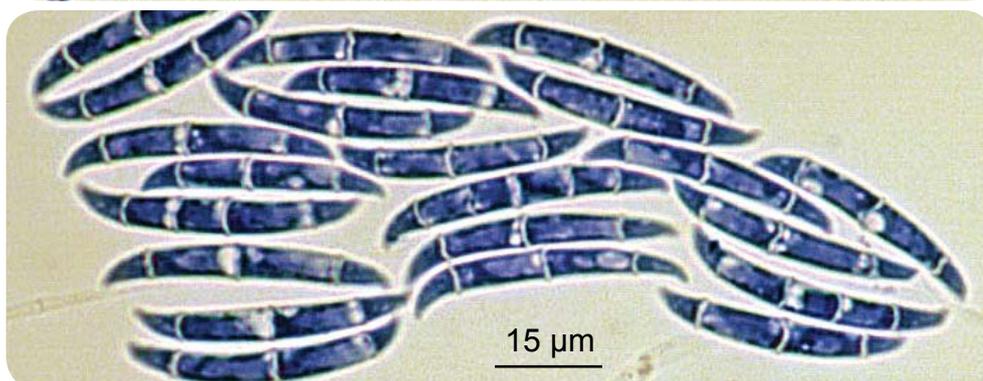
Microconidia on short phialides and macroconidia of *F. oxysporum*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.5->16	>8	Amphotericin B	0.25->16	1-2
Voriconazole	0.25->8	1-2	Posaconazole	1->8	4

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2001, 2003), Diekema *et al.* (2003), Cuenca-Estrella *et al.* (2006), Sabatelli *et al.* (2006) and WCH in-house data.

### *Fusarium solani* (Mart.) Sacc.

Colonies growing rapidly, 4.5 cm in 4 days, aerial mycelium white to cream, becoming bluish-brown when sporodochia are present. Macroconidia are formed after 4-7 days from short multi-branched conidiophores which may form sporodochia. They are 3- to 5- septate (usually 3- septate), fusiform, cylindrical, often moderately curved, with an indistinctly pedicellate foot cell and a short blunt apical cell, 28-42 x 4-6  $\mu\text{m}$ . Microconidia are usually abundant, cylindrical to oval, one- to two-celled and formed from long lateral phialides, 8-16 x 2-4.5  $\mu\text{m}$ . Chlamydo-spores are hyaline, globose, smooth to rough-walled, borne singly or in pairs on short lateral hyphal branches or intercalary, 6-10  $\mu\text{m}$ . RG-2 organism.



Microconidia on long phialides, macroconidia and chlamydo-spores of *F. solani*.

Antifungal	MIC $\mu\text{g/mL}$		Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.25->16	>8	Amphotericin B	0.25->16	4
Voriconazole	0.125->8	4 (>8)	Posaconazole	>8	>8

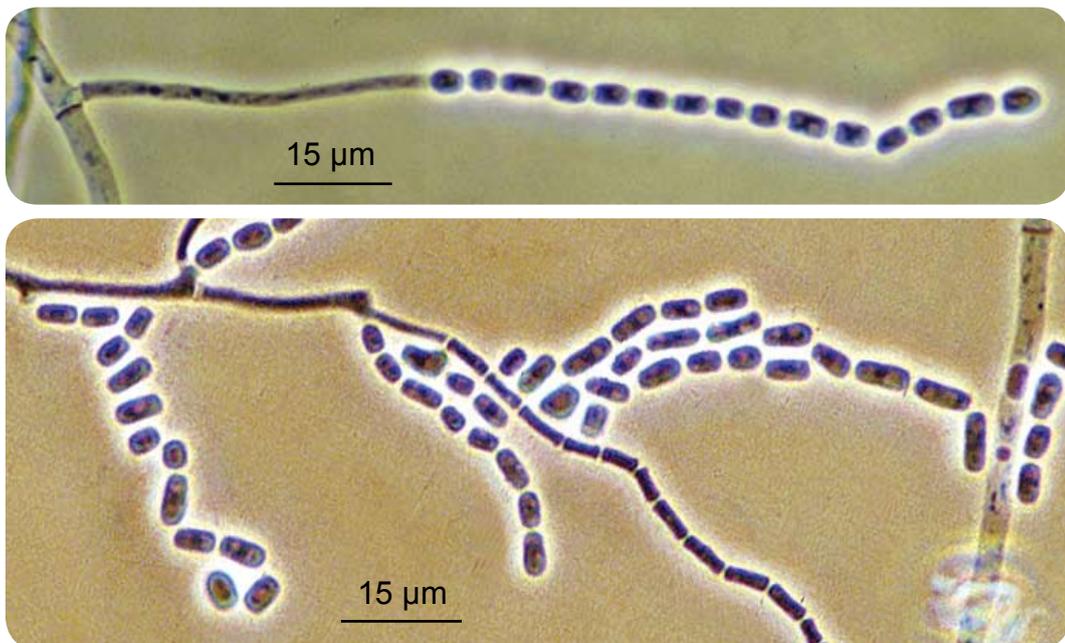
Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2001, 2003), Diekema *et al.* (2003), Cuenca-Estrella *et al.* (2006), Sabatelli *et al.* (2006) and WCH in-house data.

### Geotrichum Link

Species of the genus *Geotrichum* typically produce chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia by the holoarthric fragmentation of undifferentiated hyphae. Conidia may also develop sympodially and chlamydoconidia and endoconidia may also be present. The arthroconidia, which are quite variable in size may germinate at one end giving the appearance of a bud. However, the latter develops into a septate mycelium. True blastoconidia production is not found in the genus. This characteristic distinguishes the genus *Geotrichum* from *Trichosporon* which usually does produce blastoconidia.

The need to exercise care when identifying species of *Geotrichum* must be stressed, as this name has often been used erroneously to describe any hyaline hyphomycete producing arthroconidia (McGinnis, 1980). *Geotrichum* species may be differentiated from each other using physiological and biochemical tests similar to those used for the identification of yeasts (Gueho, 1979 and Buchta and Otcenasek, 1988).

For descriptions of species, keys to taxa and additional information see Gueho (1979), Domsch *et al.* (1980), McGinnis (1980), Barnett *et al.* (1983), Buchta and Otcenasek (1988), Samson *et al.* (1995), Kurtzman and Fell (1998) and de Hoog *et al.* (2000).



Arthroconidium formation in *G. candidum*. Hyphal elements are progressively compartmentalised by fragmentation of septa. Conidial secession is by the centripetal separation (schizolysis) of a so called double septum and concomitant rupture of the original outer hyphal wall layer.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.25-32	8-32	Amphotericin B	0.06-1.0	0.125
Itraconazole	0.03->32	>32	Flucytosine	0.125-16	4
Voriconazole	0.03-0.5	0.25			

Limited data, antifungal susceptibility testing of individual strains is recommended. Girmenia *et al.* (2003), Kucukates *et al.* (2005) and WCH in-house data.

## ***Geotrichum candidum* Link**

**Teleomorph:** *Galactomyces geotrichum* (Butler & Petersen) Redhead & Malloch

Colonies are fast growing, flat, white to cream, dry and finely suede-like with no reverse pigment. Hyphae are hyaline, septate, branched and break up into chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia. They are 6-12 x 3-6 µm in size and are released by the separation of a double septum. RG-1 organism.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow, n No data							
Germ Tube	-	L-Sorbose	+	L-Arabinose	-	D-Glucitol	+
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	v	Maltose	-	D-Ribose	v	D-Gluconate	-
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	-	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	n	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	v	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	+	D-Mannitol	v	Growth at 37°C	v

*Geotrichum candidum* is an extremely common fungus with a world-wide distribution. Pulmonary involvement is the most frequently reported form of the disease, but bronchial, oral, vaginal, cutaneous and alimentary infections have also been noted.

## ***Geotrichum capitatum* (Diddens & Lodder) v. Arx**

**Teleomorph:** *Dipodascus capitatis* de Hoog *et al.*

**Synonyms:** *Trichosporon capitatum*, *Blastoschizomyces capitatis*

Colonies are moderately fast growing, flat, whitish, and finely suede-like with no reverse pigment. Hyphae are profusely branched at acute angles, with terminal and intercalary conidiogenous cells which form long, cicatrized rachids on which conidia are borne. Conidia are hyaline, smooth, one-celled, cylindrical to clavate, with a rounded apex and flat base, 7-10 x 2.5-3.5 µm. Rectangular arthroconidia are also often present. RG-2 organism.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow, n No data							
Germ Tube	-	L-Sorbose	v	L-Arabinose	-	D-Glucitol	-
<b>Fermentation</b>		Sucrose	-	D-Arabinose	-	α-M-D-glucoside	-
Glucose	-	Maltose	-	D-Ribose	v	D-Gluconate	-
Galactose	-	Cellobiose	-	L-Rhamnose	-	DL-Lactate	+
Sucrose	-	Trehalose	-	D-Glucosamine	-	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	n	2-K-D-gluconate	-
Lactose	-	Melibiose	-	Glycerol	+	D-Glucuronate	-
Trehalose	-	Raffinose	-	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	-	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	+
Galactose	+	D-Xylose	-	D-Mannitol	-	Growth at 40°C	+

*Geotrichum capitatum* occurs quite commonly in humans, usually as a transient component of normal skin flora and sputum. Systemic infections including pulmonary, fungemia and endocarditis have been reported in immunosuppressed patients.

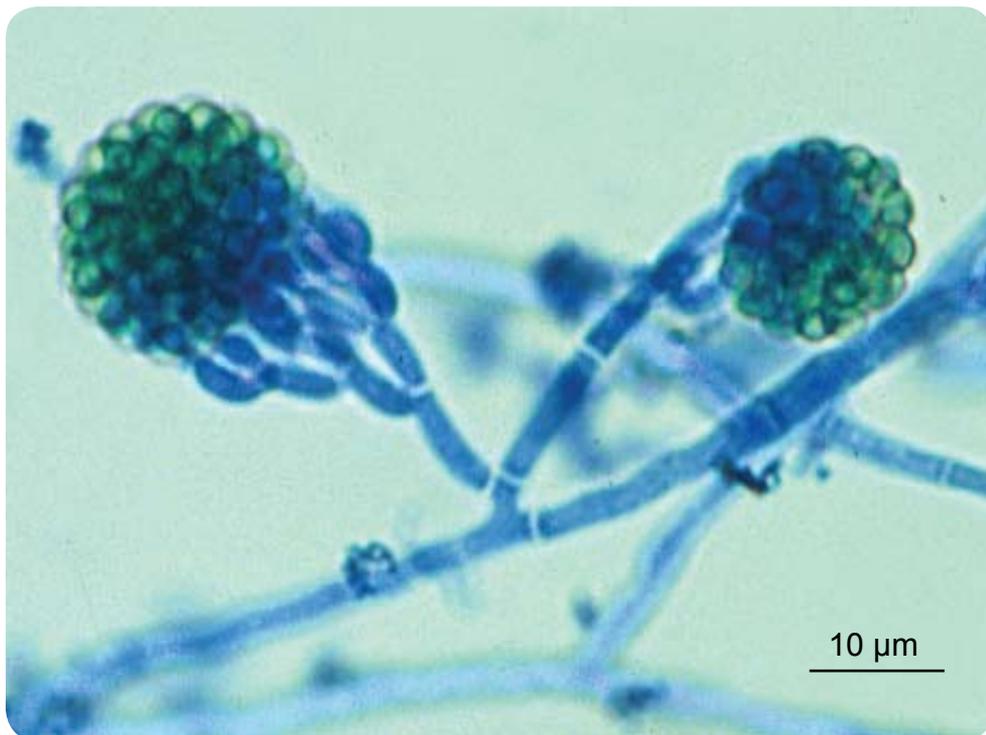
## ***Gliocladium* Corda**

The genus *Gliocladium* is often described as a counterpart of *Penicillium* with slimy conidia. Colonies are fast growing, suede-like to downy in texture, white at first, sometimes pink to salmon, becoming pale to dark green with sporulation. The most characteristic feature of the genus is the distinctive erect, often densely penicillate conidiophores with phialides which bear slimy, one-celled hyaline to green, smooth-walled conidia in heads or columns. Although, some penicillate conidiophores are always present, *Gliocladium* species may also produce verticillate branching conidiophores which can be confused with *Verticillium* or *Trichoderma*.

*Gliocladium* species have a world-wide distribution and are commonly isolated from a wide range of plant debris and soil. RG-1 organism.

**Key Features:** hyphomycete producing distinctive erect penicillate conidiophores with phialides bearing clusters of single-celled conidia.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Rippon (1988), de Hoog *et al.* (2000).



Conidiophore and conidia of *Gliocladium*.

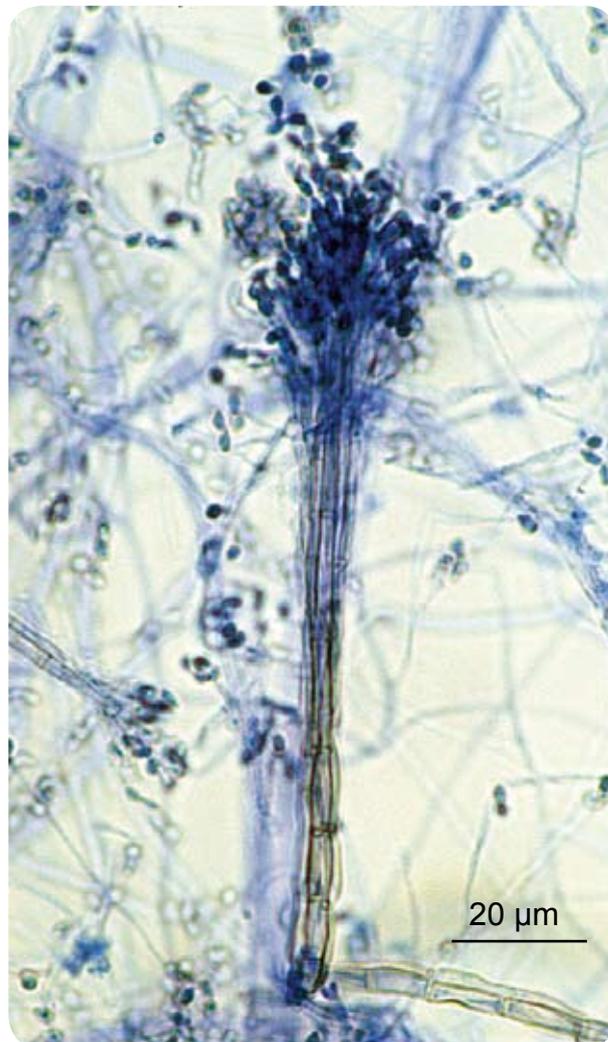
## ***Graphium Corda***

The genus *Graphium* is characterised by the formation of synnemata which consist of a more or less compact group of erect conidiophores that are cemented together, usually splaying out and bearing conidia at the apex. Synnemata are darkly pigmented, erect and occur solitary or in clusters. Conidia are hyaline, one-celled, smooth, subglobose to ovoid and are usually aggregated in slimy heads at the apex of the synnemata. Colonies are effuse, grey, olivaceous brown or black.

*Graphium eumorphum* is one of the synanamorphs of *Pseudallescheria boydii* and is commonly found on woody plant material. RG-1 organism.

**Key Features:** dematiaceous hyphomycete producing erect synnemata with apical aggregates of single-celled conidia in slimy heads.

For descriptions of species, keys to taxa and additional information see Barron (1968), Ellis (1971), McGinnis (1980) and de Hoog *et al.* (2000).



Synnemata and conidia of *Graphium*.

## ***Histoplasma capsulatum* Darling**

*Histoplasma capsulatum* exhibits thermal dimorphism growing in living tissue or in culture at 37°C as a budding yeast-like fungus and in soil or culture at temperatures below 30°C as a mould.

Colonies (SDA) at 25°C are slow growing, white or buff-brown, suede-like to cottony with a pale yellow-brown reverse. Other colony types are glabrous or verrucose, and a red pigmented strain has been noted (Rippon, 1988). Microscopic morphology shows the presence of characteristic large, rounded, single-celled, 8-14 µm in diameter, tuberculate macroconidia formed on short, hyaline, undifferentiated conidiophores. Small, round to pyriform, 2-4 µm in diameter, microconidia borne on short branches or directly on the sides of the hyphae may also be present.

On brain heart infusion agar containing blood incubated at 37°C colonies are smooth, moist, white and yeast-like. Microscopically, numerous small round to oval budding yeast-like cells, 3-4 x 2-3 µm in size are observed.

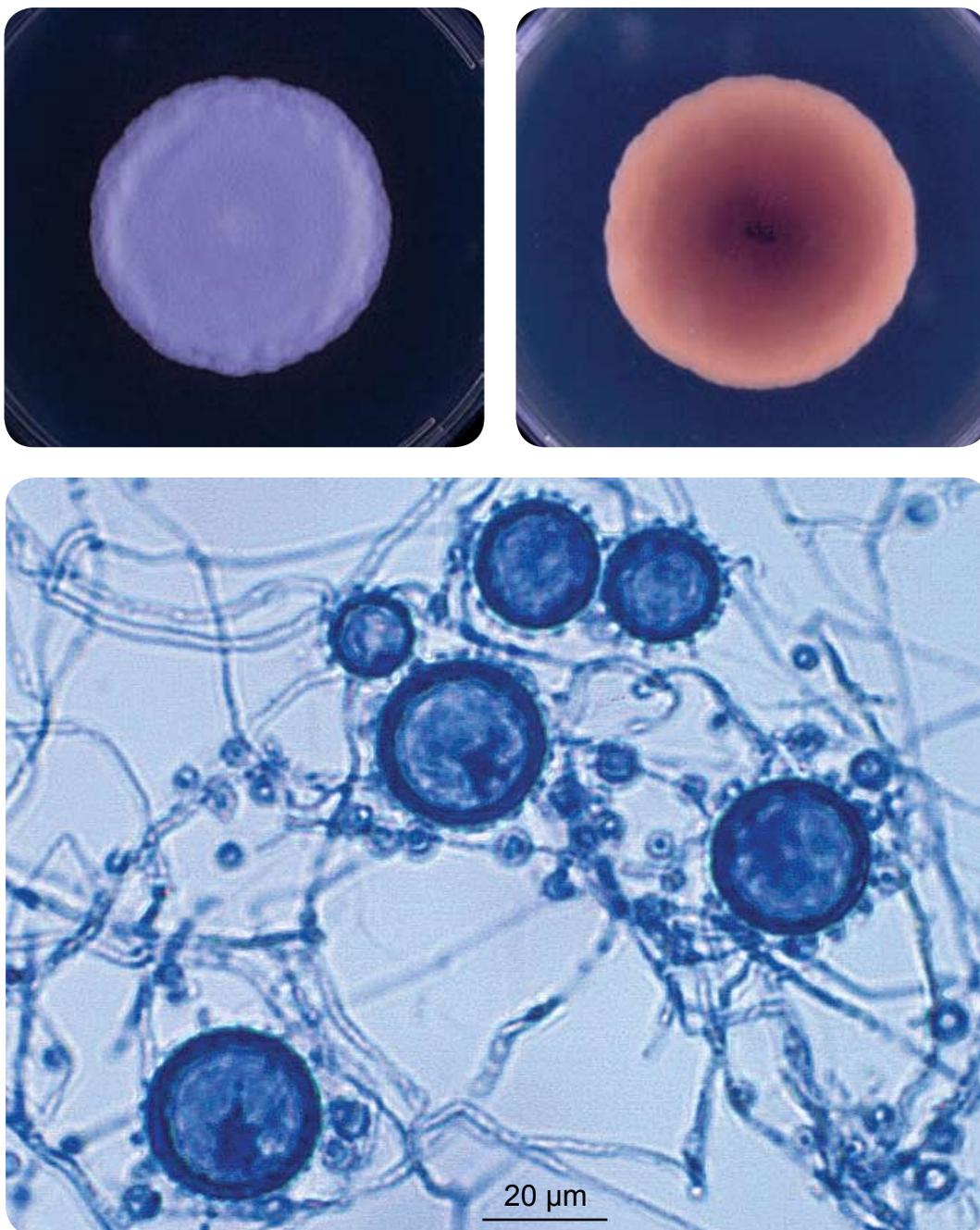
Three varieties of *Histoplasma capsulatum* are recognised, depending on the clinical disease: var. *capsulatum* is the common cause of histoplasmosis; var. *duboisii* is the African type and var. *farciminosum* causes lymphangitis in horses. *Histoplasma* isolates may also resemble species of *Sepedonium* and *Chrysosporium*. Traditionally, positive identification required conversion of the mould form to the yeast phase by growth at 37°C on enriched media, however culture identification by exoantigen test is now the method of choice.

**WARNING:** RG-3 organism. Cultures of *Histoplasma capsulatum* represent a severe biohazard to laboratory personnel and must be handled with extreme caution in an appropriate pathogen handling cabinet.

**Key Features:** clinical history, tissue morphology, culture morphology and positive exoantigen test.

*Histoplasma capsulatum* has a world wide distribution, however the Mississippi-Ohio River Valley in the USA is recognised as a major endemic region. Environmental isolations of the fungus have been made from soil enriched with excreta from chicken, starlings and bats. Histoplasmosis is an intracellular mycotic infection of the reticuloendothelial system caused by the inhalation of the fungus. Approximately 95% of cases of histoplasmosis are inapparent, subclinical or benign. Five percent of the cases have chronic progressive lung disease, chronic cutaneous or systemic disease or an acute fulminating fatal systemic disease. All stages of this disease may mimic tuberculosis. Sporadic cases do occur in Australia.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Chandler *et al.* (1980), George and Penn (1986), Rippon (1988) and de Hoog *et al.* (2000).

***Histoplasma capsulatum* Darling**

Culture and microscopic morphology of the saprophytic or mycelial form of *H. capsulatum* showing characteristic large, rounded, single-celled, tuberculate macroconidia formed on short, hyaline, undifferentiated conidiophores.

Antifungal	MIC $\mu\text{g/mL}$		Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.03-2	0.5-1	Posaconazole	0.25-1	0.25 (2)
Fluconazole	0.125-64	16	Voriconazole	0.03-2	0.25 (1)
Itraconazole	0.03-8	0.06 (1)			

Limited data available. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Gonzales *et al.* (2005) and Sabatelli *et al.* (2006).

## ***Hortaea werneckii* (Horta) Nishimura & Miyaji**

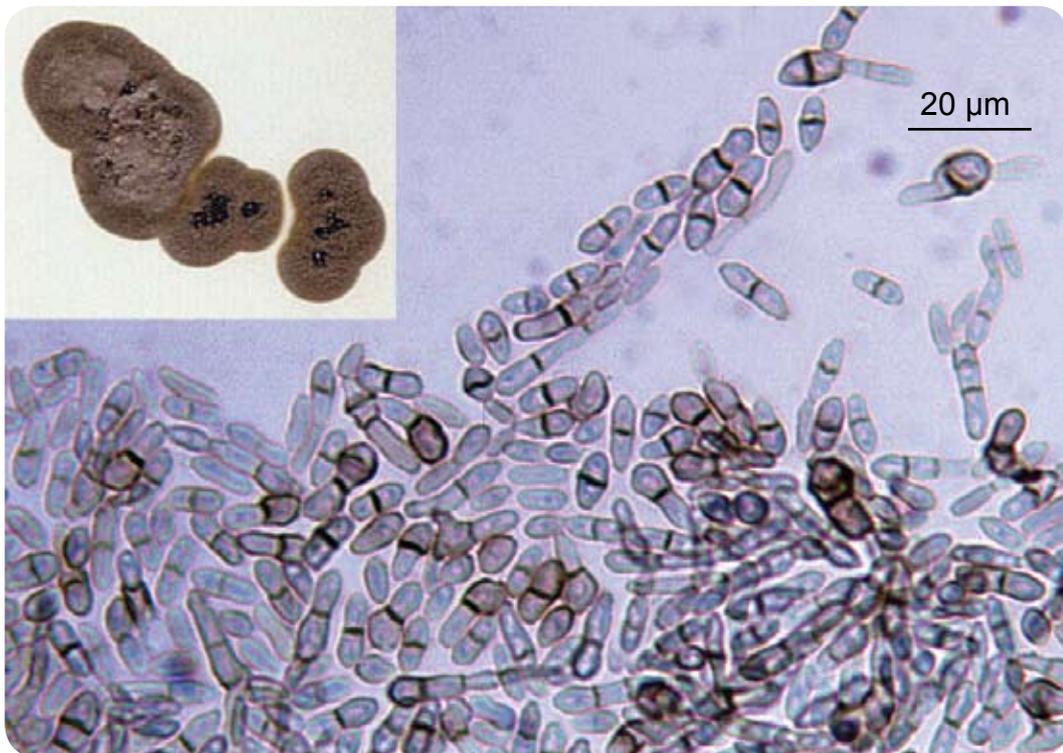
**Synonyms:** *Phaeoannellomyces werneckii* (Horta) McGinnis & Schell  
*Exophiala werneckii* (Horta) v. Arx

Colonies are slow growing, initially mucoid, yeast-like and shiny black. However with age they develop abundant aerial mycelia and become dark olivaceous in colour. Microscopically, colonies consist of brown to dark olivaceous, septate hyphal elements and numerous two-celled, pale brown, cylindrical to spindle-shaped yeast-like cells that taper towards the ends to form an annellide. Most yeast-like cells also have prominent darkly-pigmented septa. Annellides may also arise from the hyphae. Conidia are one to two-celled, cylindrical to spindle-shaped, hyaline to pale brown and usually occur in aggregated masses. RG-1 organism.

*Hortaea werneckii* is a common saprophytic fungus believed to occur in soil, compost, humus and on wood in humid tropical and sub-tropical regions and is the causative agent of tinea nigra in humans.

**Key Features:** dematiaceous hyphomycete, two-celled yeast-like cells producing annelloconidia.

For description of species, keys to taxa and additional information see Mok (1982), McGinnis (1980), McGinnis *et al.* (1985), Rippon (1988) and de Hoog *et al.* (2000).



Culture and conidia of *Hortaea werneckii*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.03-1	Itraconazole	0.03-0.25	Voriconazole	0.03-0.125

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

## *Lasiodiplodia theobromae* (Pat.) Griffon & Maublanc

**Teleomorph:** *Botryosphaeria rhodina* (Berk. & Curt. v. Arx

**Synonym:** *Botryodiplodia theobromae* Patouillard

Colonies are greyish sepia to mouse grey to black, fluffy with abundant aerial mycelium; reverse fuscous black to black. Pycnidia are simple or compound, often aggregated, stromatic, ostiolate, frequently setose, up to 5 mm wide. Conidiophores are hyaline, simple, sometimes septate, rarely branched cylindrical, arising from the inner layers of cells lining the pycnidial cavity. Conidiogenous cells are hyaline, simple, cylindrical to subobpyriform, holoblastic, annellidic. Conidia are initially unicellular, hyaline, granulate, subovoid to ellipsoide-oblong, thick-walled, base truncate; mature conidia one-septate, cinnamon to fawn, often longitudinally striate, 20-30 × 10-15 µm. Paraphyses when present are hyaline, cylindrical, sometimes septate, up to 50 µm long.

*Lasiodiplodia theobromae* is a well known plant pathogen and it has been reported from about 500 host plants, mainly confined to an area 40° north to 40° south of the equator. It has also been associated with mycotic keratitis, lesions on nail and subcutaneous tissue. RG-1 organism.

**Key Features:** coelomycete, with pycnidia producing characteristic two-celled, dark brown, striated conidia.

For description of species, keys to taxa and additional information see de Hoog *et al.* (2000).



Mature two-celled dark brown conidia with typical striations of *L. theobromae*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.03	Itraconazole	16	Voriconazole	0.25

Very limited data, antifungal susceptibility testing of individual strains is recommended. WCH in-house data only.

### ***Lecythophora* Nannfeldt**

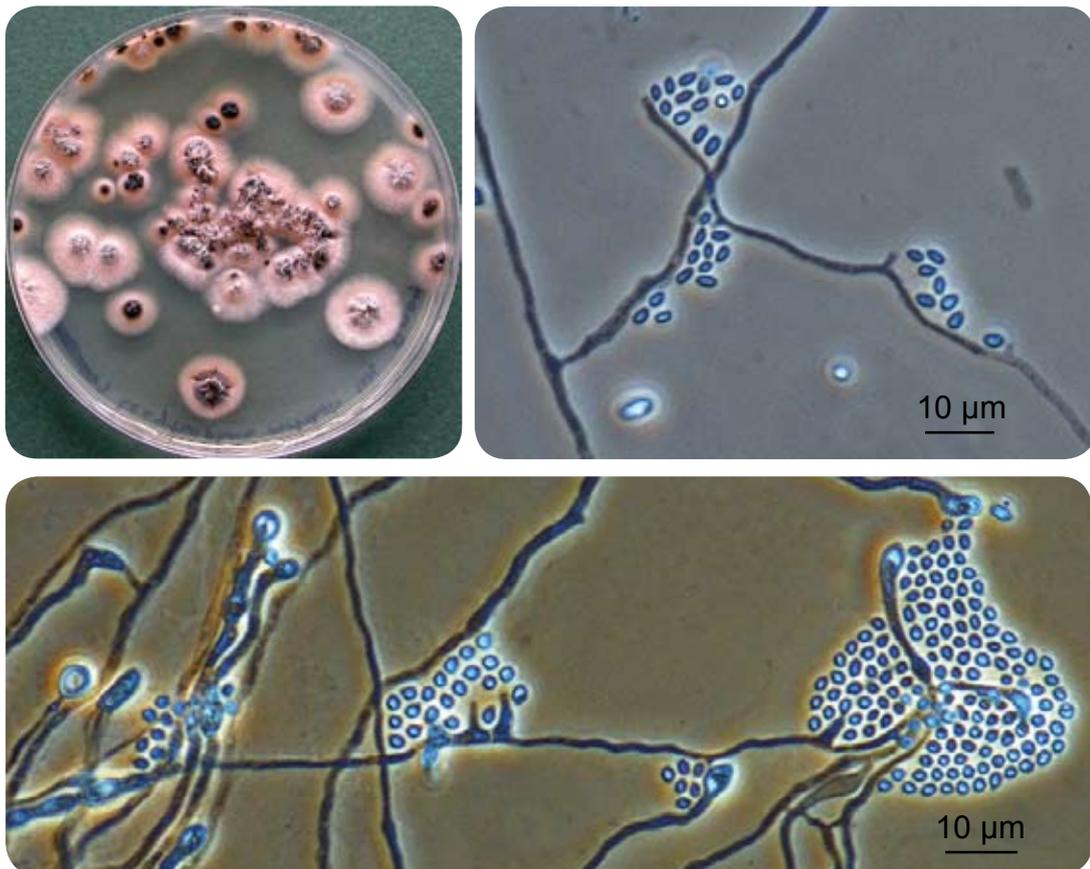
Colonies are pink to salmon, later becoming blackish, smooth, often mucoid or yeast-like. Conidiogenous cells are poorly differentiated, usually lateral or intercalary, hyphae bearing one or several scattered inconspicuous collarettes, either sessile or on small outgrowths. Conidia are hyaline, smooth and thin walled, broadly ellipsoidal, cylindrical, reniform or allantoid. Chlamydo-spores may be present. *Lecythophora* contains 6 species, with two species of medical interest; *L. hoffmannii* and *L. mutabilis*.

#### ***Lecythophora hoffmannii* (J.F.H. Beyma) W. Gams & McGinnis**

**Teleomorph:** *Coniochaeta ligniaria* (Grev.) Cooke

Colonies are flat, smooth, moist, pink to orange, with regular and sharp margin; reverse pink. Hyphae are narrow, hyaline, producing conidia laterally from small collarettes directly on the hyphae, or from lateral cells which are sometimes arranged in dense groups; lateral cells flask-shaped or nearly cylindrical. Collarettes are unpigmented, about 1.5 µm wide. Conidia are hyaline, smooth and thin walled, broadly ellipsoidal to cylindrical or allantoid, 3.0-3.5 × 1.5-2.5 µm, produced in slimy heads. RG-1 organism.

For description of species, keys to taxa and additional information see de Hoog (1983) and de Hoog *et al.* (2000).



Culture, hyphae with small collarettes and conidia of *Lecythophora hoffmannii*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.06-0.5	Itraconazole	0.06-32	Voriconazole	0.125-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998).

## ***Madurella grisea* Mackinnon, Ferrada and Montemayer**

The genus *Madurella* is based on tissue morphology (mycetoma with black grains) and the formation of sterile cultures on mycological media. Both *M. mycetomatis* and *M. grisea* have been isolated from soil and are one of the major causative agents of mycetoma.

Colonies are slow growing, dark, leathery, folded with radial grooves and with a light brown to greyish surface mycelium. With age, colonies become dark brown to red-dish-brown and have a brownish-black reverse. Microscopically, cultures are sterile, although hyphae of two widths have been described, thin at 1-3  $\mu\text{m}$  in width or broad at 3-5  $\mu\text{m}$  in width. The optimum temperature for growth of *M. grisea* is 30°C; this fungus does not grow at 37°C. RG-2 organism.

*M. grisea* can be distinguished from *Madurella mycetomatis* by the inability to grow at 37°C and to assimilate lactose.

**Key Features:** black grain mycetoma, no growth at 37°C, no diffusible brown pigment produced on culture and absence of conidia.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Chandler *et al.* (1980), Rippon (1988) and de Hoog *et al.* (2000).



Grains of *Madurella grisea* (tissue microcolonies) are black, round to lobed, soft to firm, up to 1.0 mm, with two distinctive zones, a hyaline to weakly pigmented central zone and a deeply pigmented periphery.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range		Range
Amphotericin B	0.25	Itraconazole	0.5	Voriconazole	0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001).

### ***Madurella mycetomatis* (Laveran) Brumpt**

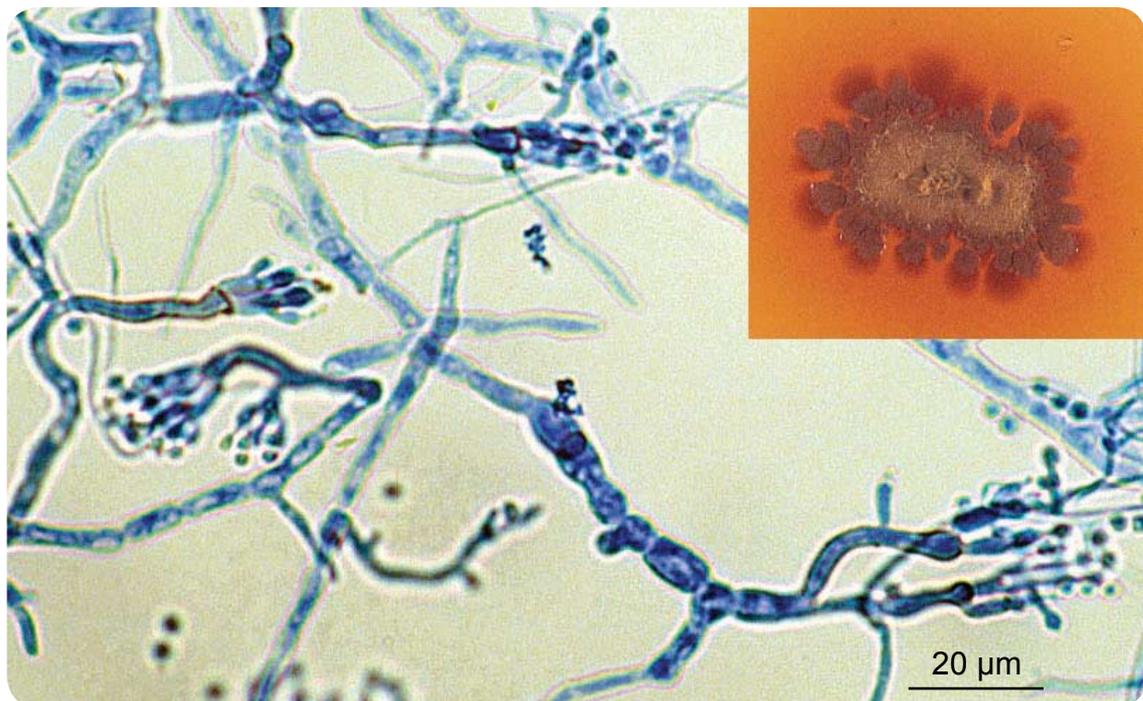
Colonies are slow growing, flat and leathery at first, white to yellow to yellowish-brown, becoming brownish, folded and heaped with age and with the formation of aerial mycelia. A brown diffusible pigment is characteristically produced in primary cultures. Although most cultures are sterile, two types of conidiation have been observed, the first being flask-shaped phialides that bear rounded conidia, the second being simple or branched conidiophores bearing pyriform conidia (3-5 µm) with truncated bases. The optimum temperature for growth of this mould is 37°C. RG-2 organism

Grains of *Madurella mycetomatis* (tissue microcolonies) are brown or black, 0.5-1.0 mm in size, round or lobed, hard and brittle, composed of hyphae which are 2-5 µm in diameter, with terminal cells expanded to 12-15 (30) µm in diameter.

*M. mycetomatis* can be distinguished from *Madurella grisea* by growth at 37°C and its inability to assimilate sucrose.

**Key Features:** black grain mycetoma, growth at 37°C, diffusible brown pigment produced on culture and the occasional presence of phialides.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Chandler *et al.* (1980), Rippon (1988) and de Hoog *et al.* (2000).



Culture showing brown diffusible pigment and phialides of *M. mycetomatis*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.03	Itraconazole	0.03-0.125	Voriconazole	0.03-0.6

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and Espinel-Ingroff *et al.* (2001).

## **Malassezia Baillon**

*Malassezia* is characterised by globose, oblong-ellipsoidal to cylindrical, yeast cells. Reproduction is by budding on a broad base and from the same site at one pole (unipolar). With the exception of *M. pachydermatis*, *Malassezia* are lipophilic yeasts, therefore *in vitro* growth must be stimulated by natural oils or other fatty substances. The most common method used is to overlay Sabouraud's dextrose agar containing cycloheximide (actidione) with olive oil or alternatively to use a more specialised media like Dixon's agar which contains glycerol mono-oleate. On such media, colonies are cream to yellowish, smooth or lightly wrinkled, glistening or dull, and with the margin being either entire or lobate (see photo). Seven species have now been recognised (Gueho *et al.* 1996). RG-2 organisms.

Species	Source
<i>M. furfur</i>	humans, normal flora, pityriasis
<i>M. globosa</i>	humans, normal flora, pityriasis
<i>M. pachydermatis</i>	animals, especially dogs
<i>M. obtusa</i>	humans, normal flora, atopic dermatitis
<i>M. restricta</i>	humans, normal flora
<i>M. slooffiae</i>	human and pig normal flora
<i>M. sympodialis</i>	humans, normal flora



**Identification criteria for the differentiation of *Malassezia* species (de Hoog *et al.* 2000).**

	Buds	SDA	40°C	Cremophor EL	Tween 80	Tween 40	Tween 20	Esculine	Catalase
<i>M. furfur</i>	wide	-	+	+	+	+	+	W	+
<i>M. globosa</i>	narrow	-	-	-	-	-	-	-	+
<i>M. pachydermatis</i>	wide	+	+	-,W	+	+	-,W	V	V
<i>M. obtusa</i>	wide	-	-	-	-	-	-	+	+
<i>M. restricta</i>	narrow	-	-	-	-	-	-	-	-
<i>M. slooffiae</i>	wide	-	+	-	+,W	+	+	-	+
<i>M. sympodialis</i>	narrow	-	+	-,W	+	+	+	+	+

For descriptions of species, keys to taxa and additional information see Guillot and Gueho (1995), Gueho *et al.* (1996), Guillot *et al.* (1996, 2000), de Hoog *et al.* (2000).

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.125->64	4 (8)	Amphotericin B	0.03-16	1 (8)
Itraconazole	0.03-16	0.125	Voriconazole	0.03-16	0.125 (1)
Ketoconazole	0.03-4	0.25	Posaconazole	0.03-32	0.125 (2)

Very limited data available. Special growth conditions are needed for antifungal susceptibility testing. Nakamura *et al.* (2000), Velegraki *et al.* (2004) and Miranda *et al.* (2007).

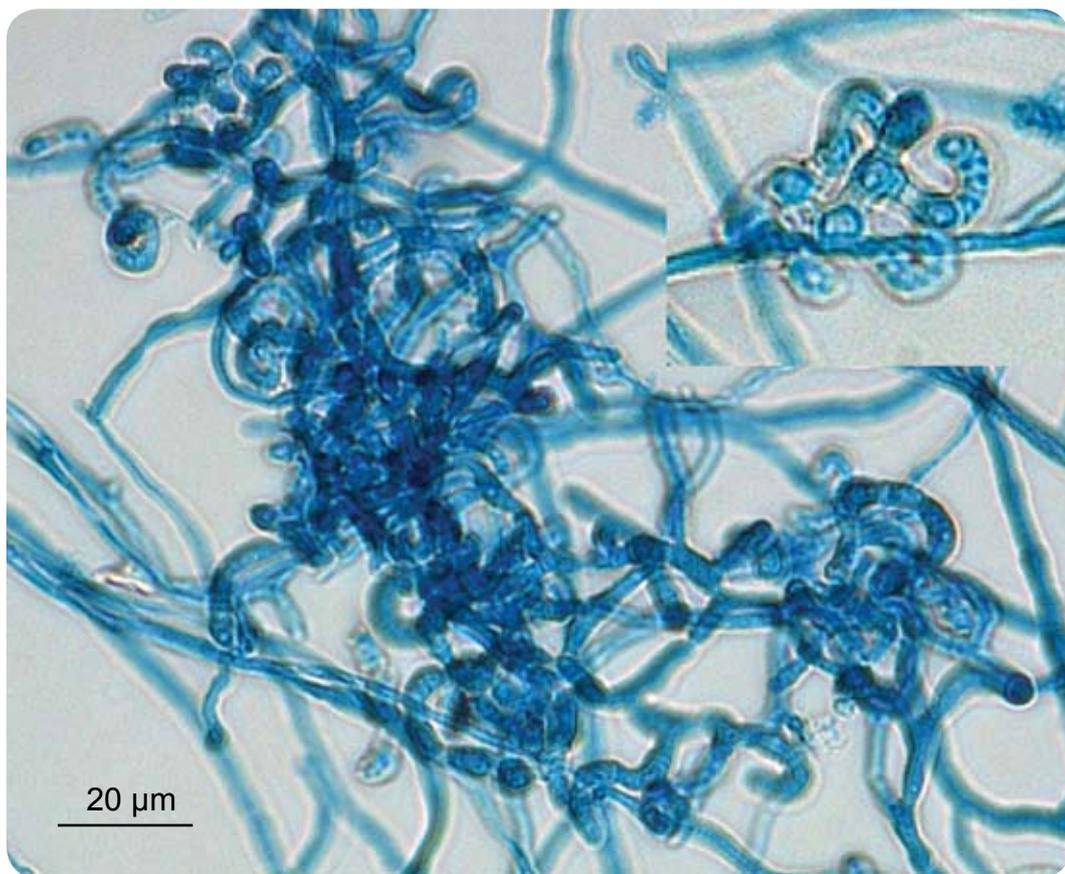
## ***Malbranchea* Saccardo**

Colonies are white to sulphur-yellow to ochre-brown in colour, suede-like in texture, with a reddish-brown reverse, and often a reddish diffusible pigment. Microscopic morphology shows typical hyaline, one-celled, cylindrical, truncate, alternate arthroconidia produced in terminal fertile portions of the hyphae. Arthroconidia are released by lysis of the disjuncter cells. These arthroconidia may be perceived as a yellow dust when released at maturity. RG-1 organisms.

**Key Features:** hyphomycete producing alternate arthroconidia with disjuncter cells.

*Malbranchea* species are soil fungi of world-wide distribution which microscopically may resemble *Coccidioides immitis/posadasii*. Exoantigen tests are now the method of choice for culture identification of *C. immitis/posadasii*.

For description of the species, keys to taxa and additional information see Cooney and Emerson (1964), McGinnis (1980), Rippon (1988) and de Hoog *et al.* (2000).



Arthroconidia of *Malbranchea*.

## ***Microsporium* Gruby**

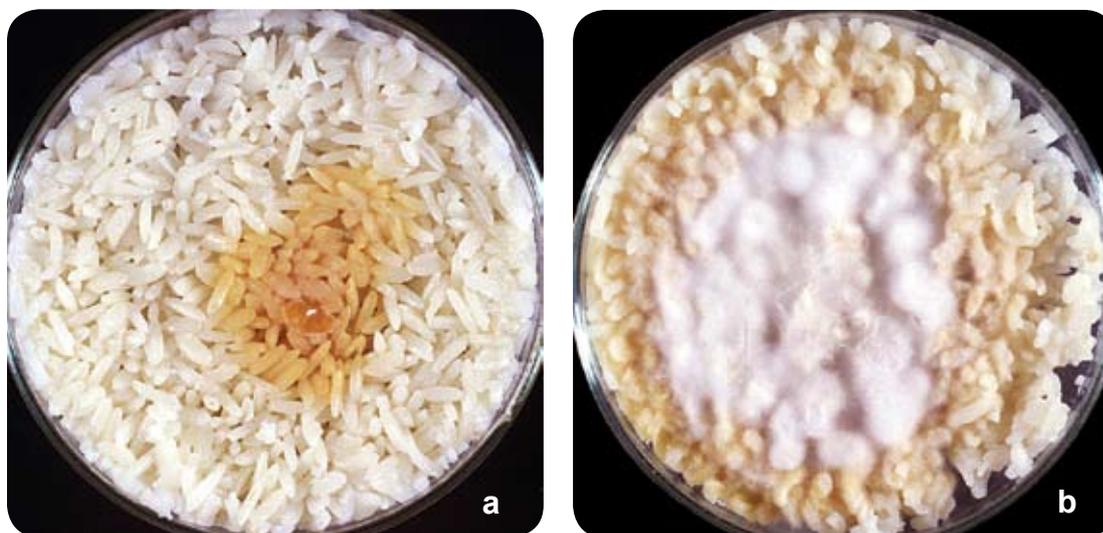
**Teleomorph:** *Arthroderma* Currey and Berkeley emend Weitzman *et al.*

*Microsporium* species form both macro- and microconidia on short conidiophores. Macroconidia are hyaline, multiseptate, variable in form, fusiform, spindle-shaped to obovate, ranging from 7-20 x 30-60 µm in size, with thin- or thick- echinulate to verrucose cell walls. Their shape, size and cell wall features are important characteristics for species identification. Microconidia are hyaline, single-celled, pyriform to clavate, smooth-walled, 2.5-3.5 x 4-7 µm in size and are not diagnostic for any one species.

The separation of this genus from *Trichophyton* is essentially based on the roughness of the macroconidial cell wall, although in practice this may sometimes be difficult to observe. Seventeen species of *Microsporium* have been described (Rippon, 1988) however only the more common species are included in these descriptions.

It is essential to observe macroconidia when identifying species of *Microsporium*. Strains of *M. canis* often do not produce macroconidia and/or microconidia on primary isolation media and it is recommended that sub-cultures be made onto polished rice grains to stimulate sporulation. These non-sporulating strains of *M. canis* are often erroneously identified as *M. audouinii* and it is surprising just how many laboratories have difficulty in differentiating between *M. canis* and *M. audouinii*.

For descriptions of species, keys to taxa and additional information see Rebell and Taplin (1970), Vanbreusegham *et al.* (1978), Rippon (1988), McGinnis (1980), Domsch *et al.* (1980), Ajello (1977), Weitzman *et al.* (1986), Mackenzie *et al.* (1986), Kane *et al.* (1997) and de Hoog *et al.* (2000).



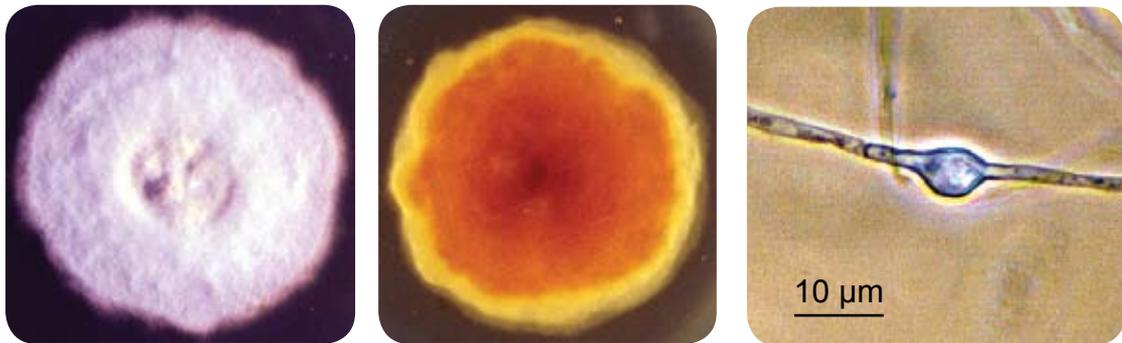
(a) *M. audouinii* showing poor growth on rice grains, usually being visible only as a brown discoloration. (b) *M. canis* on rice grains showing good growth, yellow pigmentation and sporulation.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Griseofulvin	0.125-2	1	Amphotericin B	0.03-8	1-2
Itraconazole	0.01-4	0.25-0.5	Fluconazole	0.06->64	64
Terbinafine	0.01-16	0.06	Voriconazole	0.007-1	0.5

Fernandez-Torres *et al.* (2001), Sabatelli *et al.* (2006) and WCH in-house data.

### ***Microsporum audouinii* Gruby**

Colonies (SDA) are flat, spreading, greyish-white to light tan-white in colour, and have a dense suede-like to downy surface, suggestive of mouse fur in texture. Reverse can be yellow-brown to reddish-brown in colour. Some strains may show no reverse pigment. Macroconidia and microconidia are only rarely produced, most cultures are sterile or produce only occasional thick-walled terminal or intercalary chlamydospores. When present macroconidia may resemble those of *M. canis* but are usually longer, smoother and more irregularly fusiform in shape; microconidia when present are pyriform to clavate in shape and are similar to those seen in other species of *Microsporum*. So called pectinate (comb-like) hyphae and racquet hyphae (a series of hyphal segments swollen at one end) may also be present. RG-2 organism.



Culture and a thick-walled intercalary chlamydospore of *M. audouinii*. Note: macroconidia and microconidia are only rarely produced.

**Growth on Rice Grains:** Very poor or absent, usually being visible only as a brown discolouration. This is one of the features which distinguish *M. audouinii* from *M. canis*.

**Reverse Pigment on Potato Dextrose Agar:** Salmon to pinkish-brown (*M. canis* is bright yellow).

**Lactritmel Agar:** Colonies are usually flat, spreading, with a fine, whitish suede-like surface and a very pale yellow-brown reverse. Microscopic morphology as described above.

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements. Cultures are flat, white, suede-like to downy, with a yellow-brown reverse. Note: growth of some strains of *M. audouinii* is enhanced by the presence of thiamine (Trichophyton agar No.4).

**Hair Perforation Test:** Negative after 28 days.

**Key Features:** absence of conidia, poor or no growth on polished rice grains, inability to perforate hair *in vitro*, and culture characteristics.

*M. audouinii* is an anthropophilic fungus causing non-inflammatory infections of scalp and skin especially in children. Once the cause of epidemics of tinea capitis in Europe and North America, it is now becoming less frequent. Invaded hairs show an ectothrix infection and usually fluoresce a bright greenish-yellow under Wood's ultra-violet light. Only rarely found in Australasia, most reports are in fact non-sporulating strains of *M. canis*.

## ***Microsporium canis* Bodin**

**Teleomorph:** *Arthroderma otae* (Hasegawa and Usui) McGinnis, *et al.*

Colonies (SDA) are flat, spreading, white to cream-coloured, with a dense cottony surface which may show some radial grooves. Colonies usually have a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur. Macroconidia are typically spindle-shaped with 5-15 cells, verrucose, thick-walled and often have a terminal knob, 35-110 x 12-25 µm. A few pyriform to clavate microconidia are also present. Macroconidia and/or microconidia are often not produced on primary isolation media and it is recommended that sub-cultures be made onto Lactritmel Agar and/or boiled polished rice grains to stimulate sporulation. RG-2 organism.

**Growth on Rice Grains:** good growth of white aerial mycelium with production of yellow pigment. Microscopy reveals numerous macroconidia and microconidia similar to those described above.

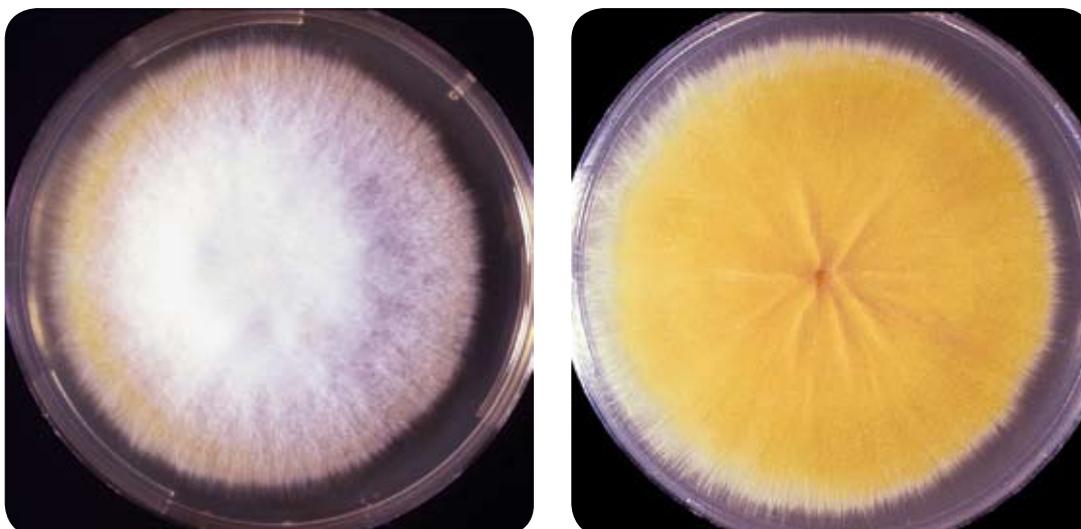
**Lactritmel Agar:** Flat, white suede-like to cottony colony with a bright yellow reverse. Microscopy reveals moderate numbers of thick-walled, multiseptate, long, spindle-shaped macroconidia, some of which show a terminal knob. Walls of macroconidia are slightly rough or echinulate especially at terminal knobs. Numerous clavate to pyriform microconidia are also present in this strain. Lactritmel agar with sterile soil added is also an excellent medium for the stimulation of macroconidial development in *M. canis*.

**Reverse Pigment on Potato Dextrose Agar:** Bright yellow (both *M. audouinii* and *M. canis* var. *equinum* are salmon to pinkish-brown).

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements. Cultures are flat, white, suede-like to downy, with a yellow to pale yellow-brown reverse.

**Hair Perforation Test:** Positive at 14 days.

**Key Features:** distinctive macroconidia and culture characteristics. Abundant growth and sporulation on polished rice grains and *in vitro* perforation of hair.



Culture of *Microsporium canis*.

### *Microsporium canis* Bodin



Typical spindle-shaped macroconidia of *Microsporium canis*.



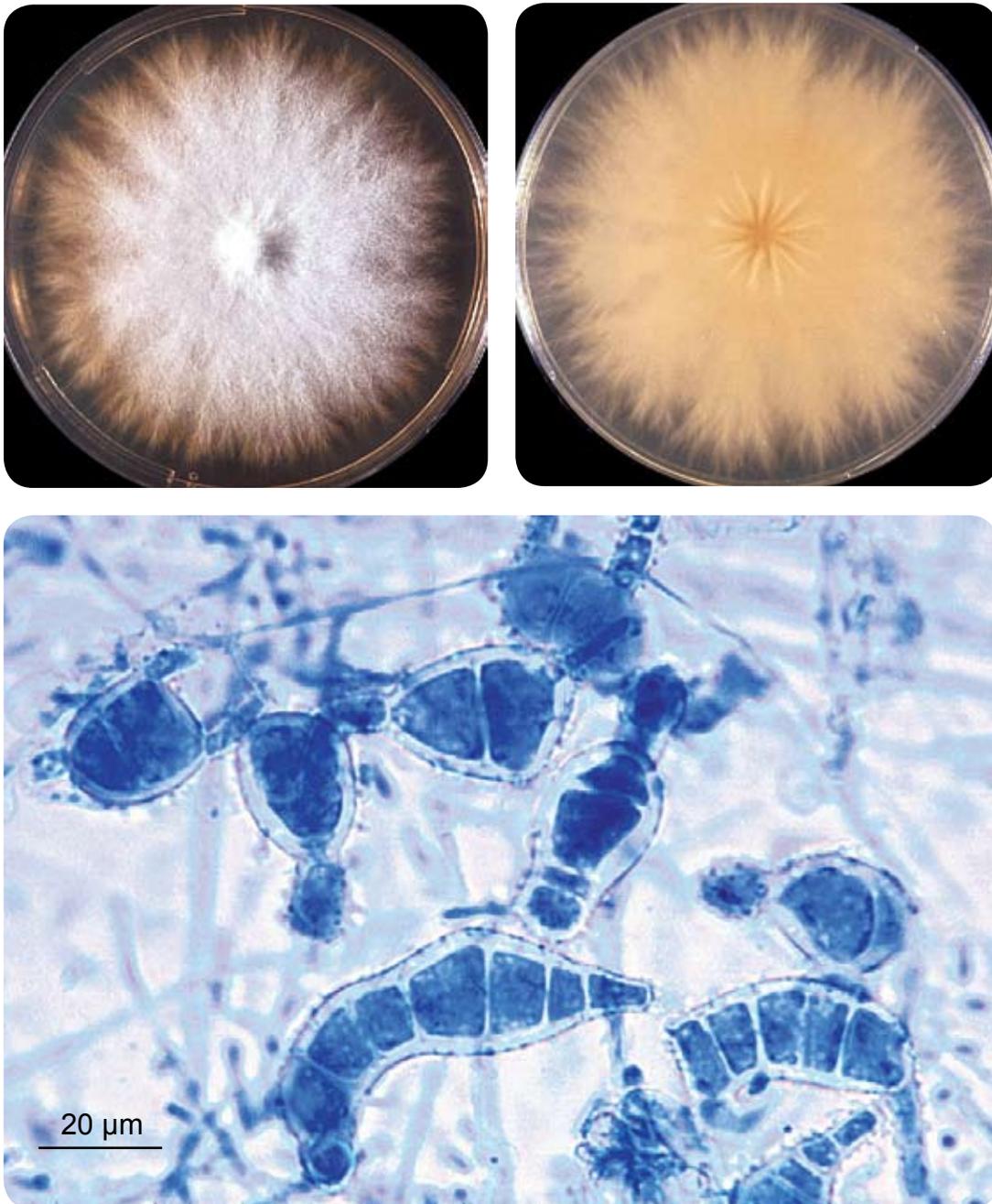
Dysgonic strains of *M. canis* are rare but may also occur. These dysgonic strains have a typically heaped and folded, yellow-brown thallus and macroconidia are usually absent. However, typical colonies and macroconidia of *M. canis* are usually produced by this variant when subcultured onto polished rice grains. Note: the dysgonic type colony of *M. canis* is similar to that of *Microsporium ferrugineum*.

*M. canis* is a zoophilic dermatophyte of world-wide distribution and is a frequent cause of ringworm in humans, especially children. Invades hair, skin and rarely nails. Cats and dogs are the main sources of infection. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light.

***Microsporium canis* var. *distortum* di Menna & Marples**

Supplementary description for *Microsporium canis* var. *distortum*, a dysgonic variant of *M. canis* with distinctive distorted macroconidia. Abundant growth and sporulation on rice grains. RG-2 organism.

*Microsporium canis* var. *distortum* is a zoophilic fungus known to cause infections in cats, dogs and other animals. It is a rare cause of tinea capitis in New Zealand, Australia and North America. Clinical disease is similar to *M. canis*. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light.



Culture and distorted macroconidia of *M. canis* var. *distortum*.

### ***Microsporium canis* var. *equinum* (Delacroix & Bodin) comb. nov.**

**Basionym:** *Microsporium equinum* (Delacroix & Bodin) Gueguen

*Microsporium equinum* is now considered to be a genotypic synonym of *Microsporium canis* (de Hoog *et al.* 2000), however we propose it be maintained as a variety of *M. canis* due to phenotypic and epidemiologic differences.

Colonies are flat, spreading, suede-like, pale buff to pale salmon, usually with some radial furrows. A buff to pinkish-buff to yellow-brown reverse pigment is produced. Note: only a few strains produce conidia on primary isolation, however growth on urea agar usually stimulates the production of macroconidia. Macroconidia are small (especially when compared to those produced by *M. canis*), broad, irregular, spindle-shaped, 18-60 × 5-15 μm with rough thick walls and few septa. Microconidia are pyriform to clavate in shape, 3-9 × 1.5-3.5 μm, but are rarely produced. RG-2 organism.

**Growth on Rice Grains:** Poor with minimal surface growth, usually being visible only as a brown discolouration. This is one of the features which distinguish *M. canis* var. *equinum* from *M. canis*.

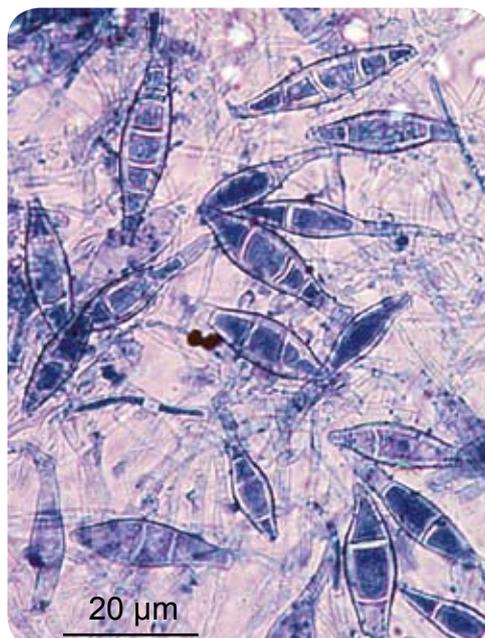
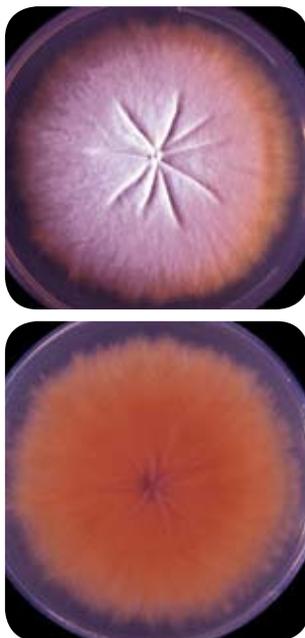
**Reverse Pigment on Potato Dextrose Agar:** Salmon to pinkish-brown (*M. canis* is bright yellow).

**Lactrimel Agar:** Colonies are usually flat, spreading, with a fine, whitish suede-like surface and a very pale yellow-brown reverse. Microscopic morphology as described above.

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements. Cultures are flat, white, suede-like to downy, with a yellow-brown reverse.

**Hair Perforation Test:** Negative after 28 days.

**Key Features:** macroconidial shape and size, inability to perforate hair *in vitro*, and poor growth on polished rice grains.



*Microsporium equinum* is a rare cause of ringworm of horses. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light. Rarely infects man or other animal species. Reported from Australia, Europe and North America.

Culture and macroconidia of *M. canis* var. *equinum*.

## *Microsporium cookei* Ajello

**Teleomorph:** *Arthroderma cajetani* Ajello, Weitzman, McGinnis & Padhye

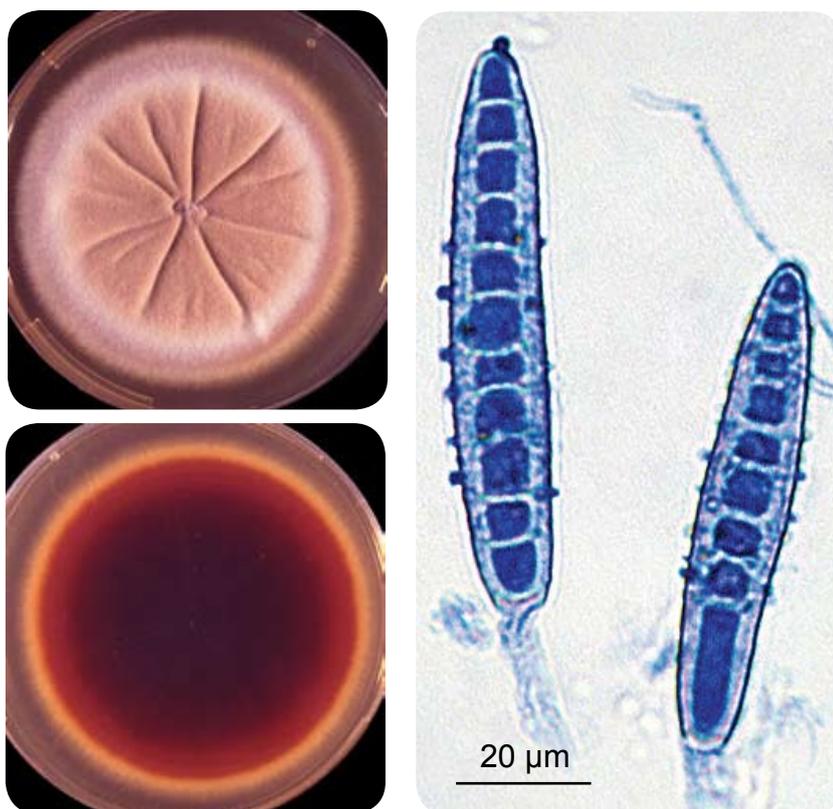
Colonies (SDA) are flat, spreading, buff to pale brown, powdery to suede-like, with a slightly raised and folded centre and some radial grooves. Reverse pigment dark reddish brown. Numerous large, very thick-walled, echinulate (rough) elliptical macroconidia with predominantly 5-6 septa but may be 2-8 septate. Occasional spirals may be seen. Moderate numbers of mainly slender clavate with some pyriform microconidia are present. The macroconidia are quite characteristic and diagnostic of *M. cookei* and further tests are not necessary. The thick walls and usually larger size of the macroconidia distinguish *M. cookei* from *M. gypseum*. RG-1 organism.

**Lactritmel Agar:** Flat, buff-coloured, suede-like to powdery colony with a deep red-brown reverse. Microscopic morphology as described above for the primary culture.

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements, pinkish-buff-coloured, suede-like colony with a deep magenta red reverse.

**Hair Perforation Test:** Positive.

**Key Features:** distinctive macroconidial morphology and culture characteristics.



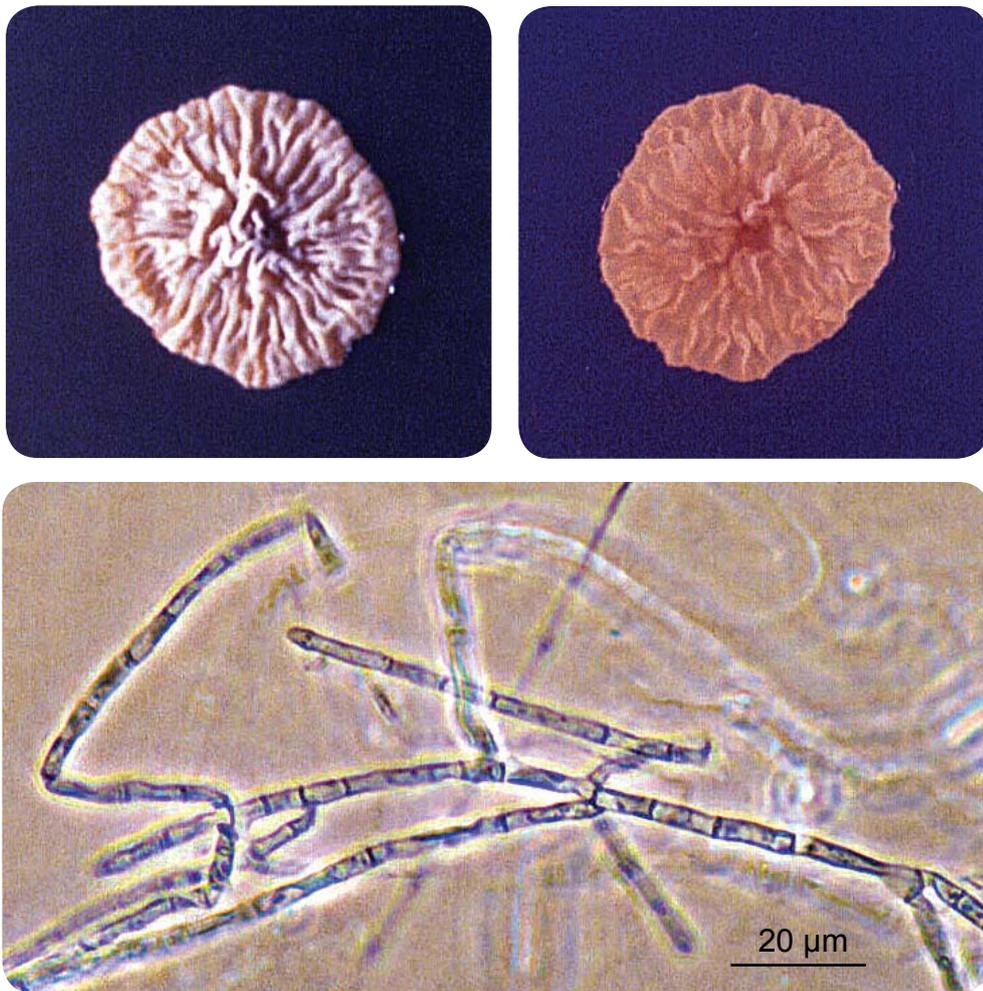
Culture and macroconidia of *Microsporium cookei*.

*Microsporium cookei* is a geophilic fungus which has been isolated from hair of small mammals showing no clinical lesions. Infection has been reported in rodents, dogs and rarely in humans. It is not known to invade hair *in vivo*, but produces hair perforations *in vitro*. *M. cookei* has a world-wide distribution.

### ***Microsporium ferrugineum* Ota**

Colonies (SDA) are slow growing, forming a waxy, glabrous, convoluted thallus with a cream to buff-coloured surface and no reverse pigment. Note: surface pigmentation may vary from cream to yellow to deep red and a flatter white form sometimes occurs. Cultures rapidly become downy and pleomorphic. Microconidia or macroconidia are not produced. However, irregular branching hyphae with prominent cross walls (“bamboo hyphae”) and chlamydo spores are seen. The so-called “bamboo hyphae” is a characteristic of this species. RG-2 organism.

**Key Features:** clinical history, culture characteristics and distinctive “bamboo” hyphae.



Culture and “bamboo hyphae” of *Microsporium ferrugineum*.

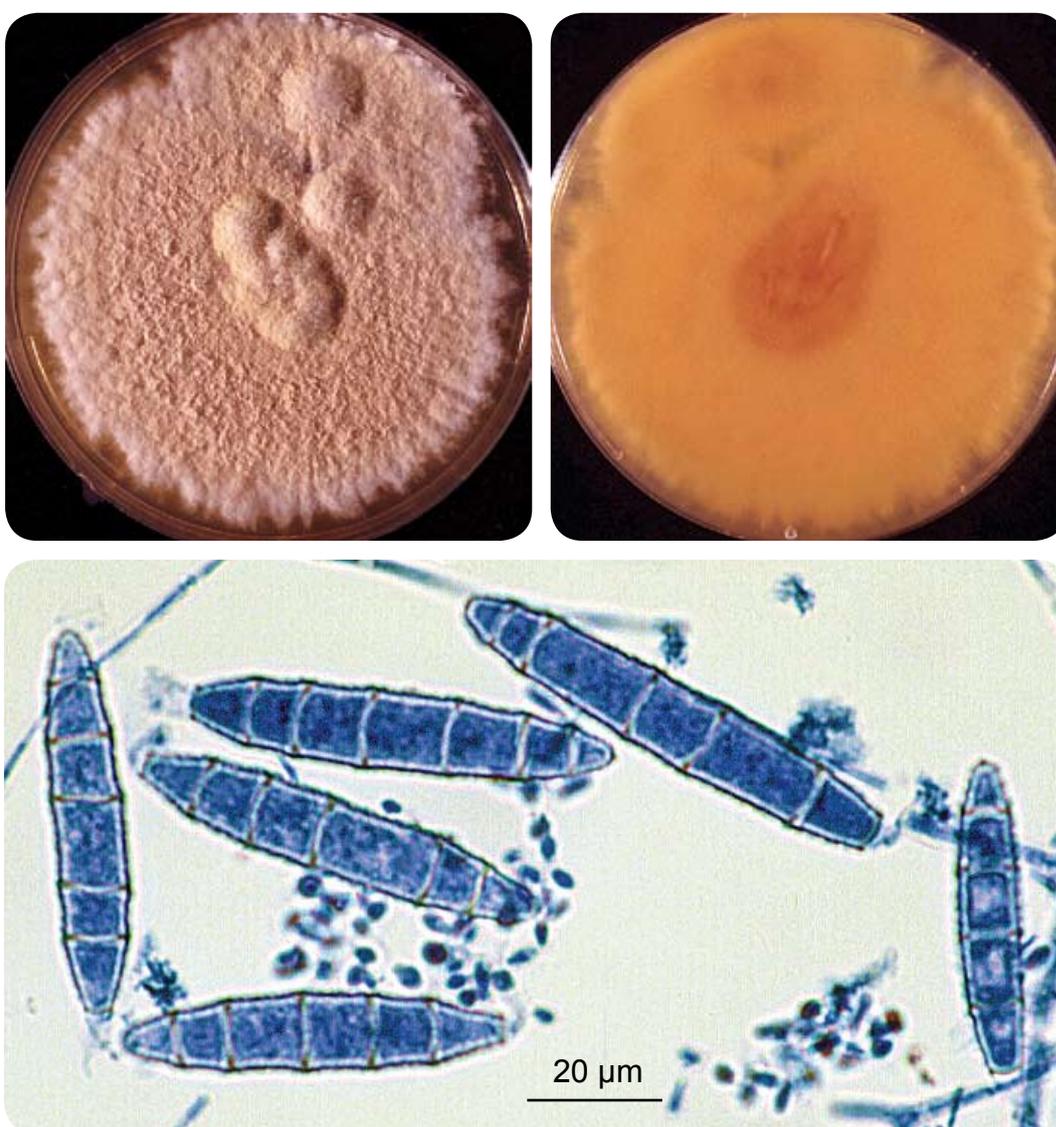
*Microsporium ferrugineum* is an anthropophilic fungus causing epidemic juvenile tinea capitis in humans. The clinical features are similar to those of infections caused by *M. audouinii*. Invaded hairs show an ectothrix infection and fluoresce a greenish-yellow under Wood’s ultra-violet light. Reported from Asia (including China and Japan), USSR, Eastern Europe and Africa.

## *Microsporium fulvum* Uriburu

**Teleomorph:** *Arthroderma fulvum* (Stockdale) Weitzman *et al.*

Colonies (SDA) are fast growing, flat, suede-like, tawny-buff to pinkish-buff in colour and frequently have a fluffy white advancing edge. A dark red under surface is occasionally seen, otherwise it is colourless to yellow brown. Abundant thin-walled, elongate, ellipsoidal macroconidia are formed which closely resemble those of *M. gypseum*, except they are longer and more bullet-shaped (clavate) with 3 to 6 septa. Numerous spiral hyphae, which are often branched are seen. Numerous pyriform to clavate microconidia are also produced but these are not diagnostic. RG-1 organism.

**Key Features:** macroconidial morphology and culture characteristics.



Culture and macroconidia of *Microsporium fulvum*.

*Microsporium fulvum* is a geophilic fungus of world-wide distribution which may cause occasional infections in humans and animals. Clinical disease is similar to *M. gypseum* but less common. Invaded hairs show a sparse ectothrix infection but do not fluoresce under Wood's ultra-violet light.

### ***Microsporium gallinae* (Megnin) Grigorakis**

Colonies (SDA) are flat with a suede-like texture and are white in colour with a pinkish tinge. Some cultures show radial folding. An orange-pink “strawberry-coloured” reverse pigment is usually present. Macroconidia when present are usually five- to six-celled, thin to thick-walled, slightly echinulate, cylindrical to clavate with narrow base and blunt tip, 15-60 x 6-10 µm. Microconidia are ovoidal to pyriform in shape. RG-2 organism.

**Key Features:** macroconidial morphology, culture characteristics and clinical lesions in chickens.



Culture and macroconidia of *Microsporium gallinae*.

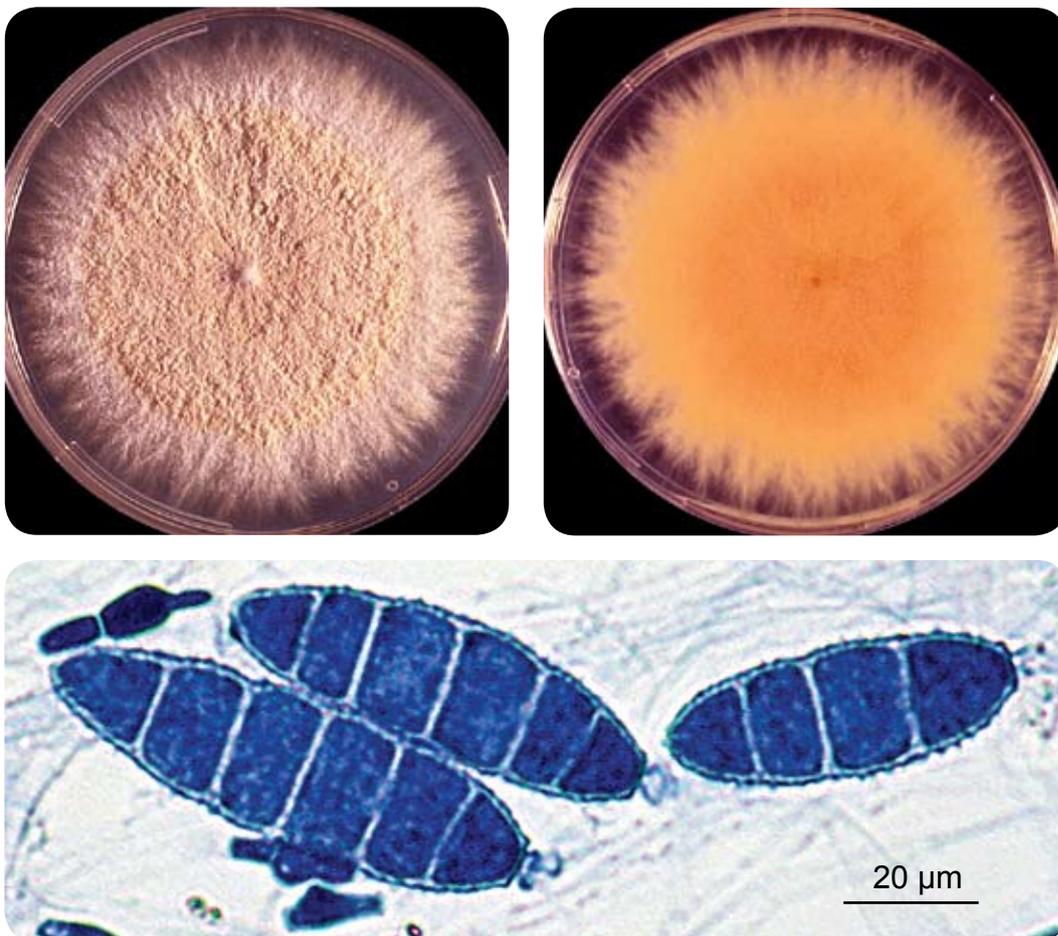
*Microsporium gallinae* is a zoophilic fungus causing fowl favus in chickens and other fowl, affecting the comb and wattles producing “white comb” lesions. A rare cause of tinea in humans. Invaded hairs show a sparse ectothrix infection but do not fluoresce under Wood’s ultra-violet light.

## ***Microsporium gypseum* (Bodin) Guiart & Grigorakis**

**Teleomorphs:** *Arthroderma gypsea* (Nannizzi) Weitzman *et al.*  
*Arthroderma incurvatum* (Stockdale) Weitzman *et al.*

Colonies (SDA) are usually flat, spreading, suede-like to granular, with a deep cream to tawny-buff to pale cinnamon-coloured surface. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral border. A yellow-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains. Cultures produce abundant, symmetrical, ellipsoidal, thin-walled, verrucose, four- to six-celled macroconidia. The terminal or distal ends of most macroconidia are slightly rounded, while the proximal ends (point of attachment to hyphae) are truncate. Numerous clavate-shaped microconidia are also present, but these are not diagnostic. RG-1 organism.

**Key Features:** distinctive macroconidia and culture characteristics.



Culture and macroconidia of *Microsporium gypseum*.

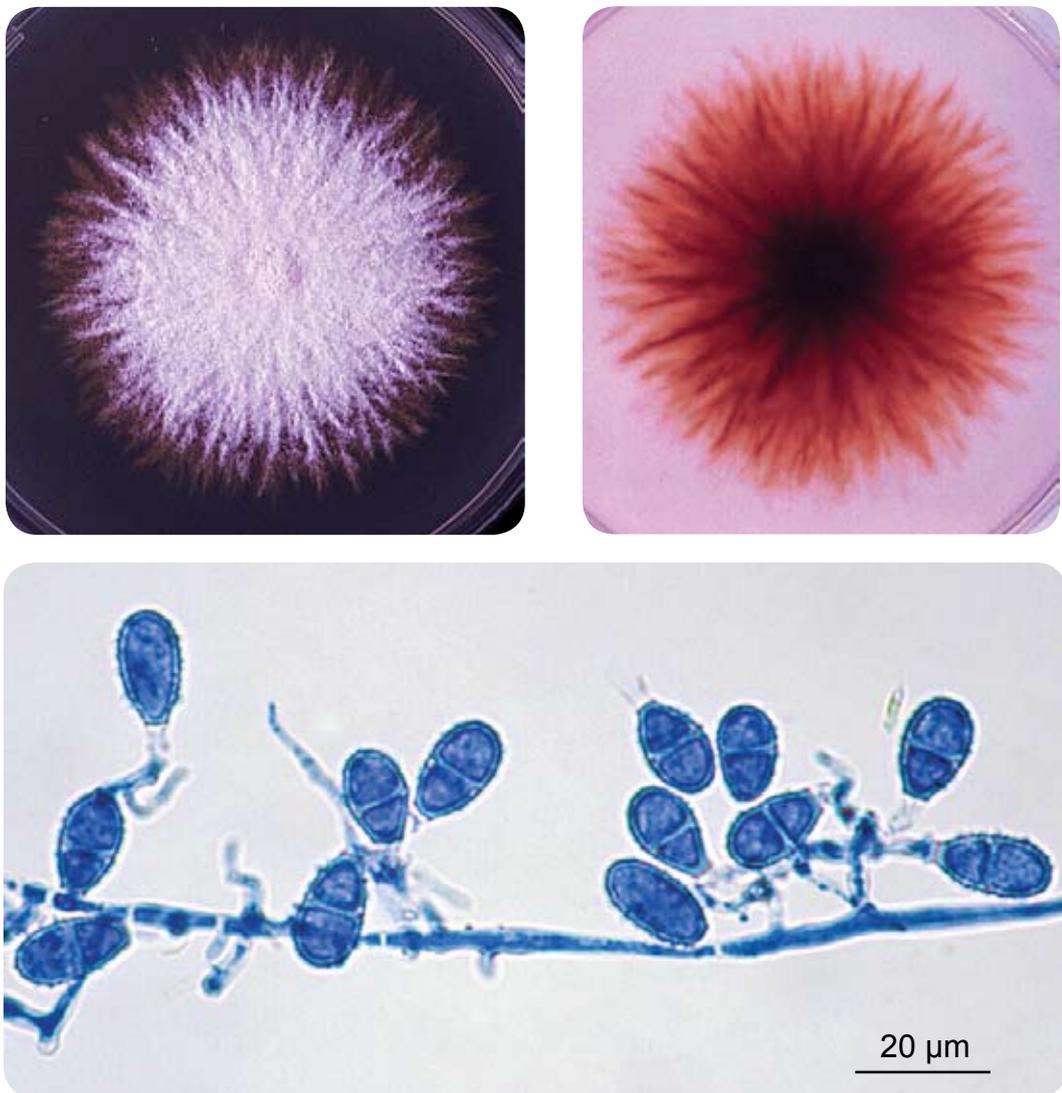
*Microsporium gypseum* is a geophilic fungus with a world-wide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a single inflammatory skin or scalp lesion. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light.

## ***Microsporium nanum* Fuentes**

**Teleomorph:** *Arthroderma obtusum* (Dawson and Gentles) Weitzman *et al.*

Colonies (SDA) are flat, cream to buff in colour with a suede-like to powdery surface texture. Young colonies have a brownish-orange pigment which deepens into a dark reddish-brown with age. Cultures produce numerous small ovoid to pyriform macroconidia with one to three (mostly 2) cells, with relatively thin, finely echinulate (rough) walls, and broad truncate bases. Many macroconidia are borne on conidiophores (stalks) which do not stain readily. Occasional clavate microconidia are present, which distinguishes *M. nanum* from some species of *Chrysosporium*. RG-2 organism.

**Key Features:** distinctive macroconidia and culture characteristics.



Culture and macroconidia of *Microsporium nanum*.

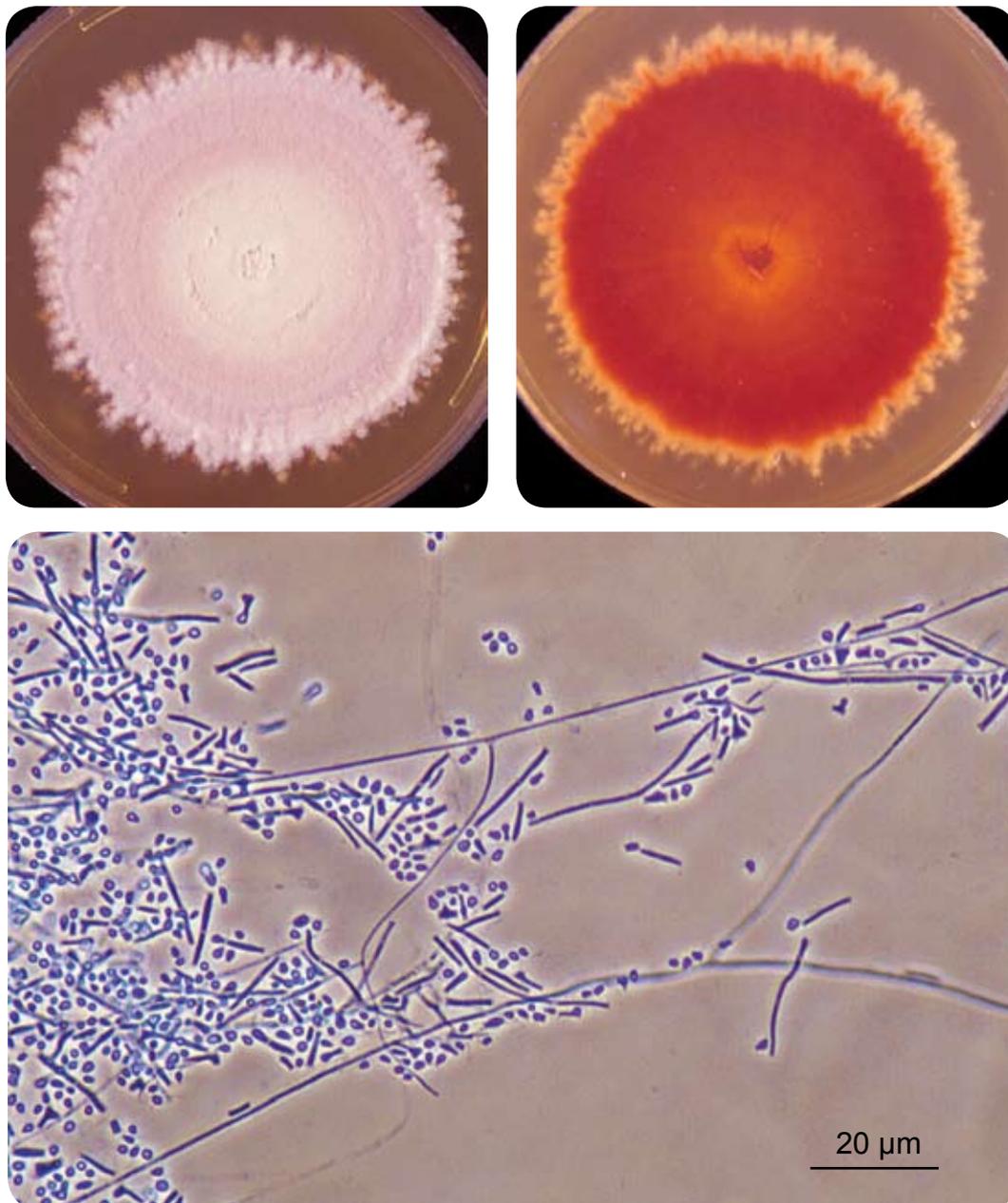
*Microsporium nanum* is a geophilic and zoophilic fungus frequently causing chronic non-inflammatory lesions in pigs and a rare cause of tinea in humans. Also present in soil of pig-yards. Infections in man are usually contacted directly from pig or fomites. Invaded hairs may show a sparse ectothrix or endothrix infection but do not fluoresce under Wood's ultra-violet light. The geographical distribution is world-wide.

## ***Microsporium persicolor* (Sabouraud) Guiart & Grigorakis**

**Teleomorph:** *Arthroderma persicolor* (Stockdale) Weitzman *et al.*

Colonies (SDA) are generally flat, white to pinkish in colour, with a suede-like to granular texture and peripheral fringe. Reverse pigmentation is orange to red. Macroconidia are thin-walled, cigar-shaped, four- to seven-celled, 40-60 x 6-8 µm but are only rarely produced. Microconidia are abundant, spherical to pyriform. RG-2 organism.

**Key Features:** microscopic morphology and culture characteristics.



Culture and microconidia of *Microsporium persicolor*.

*Microsporium persicolor* is a zoophilic fungus often occurring as a saprophyte on voles and bats. A rare cause of tinea corporis in humans. Not known to invade hair *in vivo*, but produces hair perforations *in vitro*. Distribution: Africa, Australia, Europe and North America.

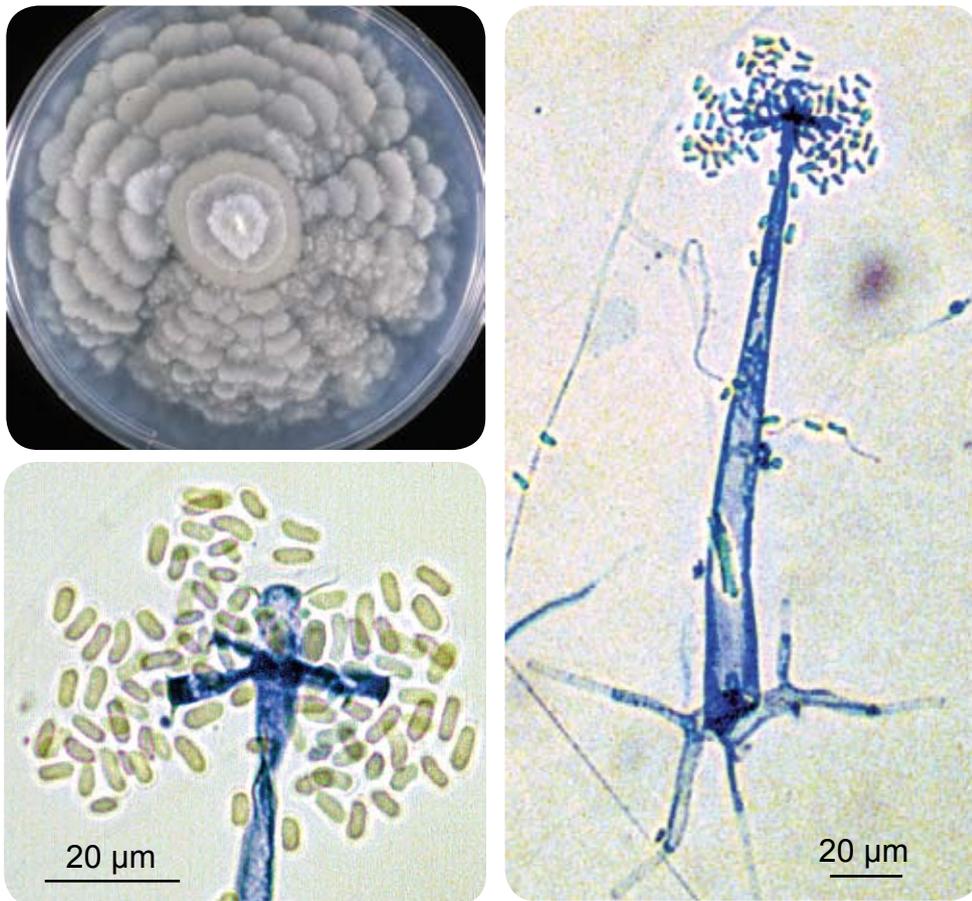
## *Mortierella wolfii* Mehrotra & Baijal

The genus *Mortierella* has now been placed in a separate order, the Mortierellales (Cavalier-Smith 1998), and the genus contains about 90 recognised species, however *Mortierella wolfii* is probably the only pathogenic species being an important causal agent of bovine mycotic abortion, pneumonia and systemic mycosis in New Zealand, Australia, Europe and USA.

Cultures are fast growing, white to greyish white, downy, often with a broadly zonate or lobed (rosette-like) surface appearance and no reverse pigment. Sporangiphores are typically erect, delicate, 80-250  $\mu\text{m}$  in height, 6-20  $\mu\text{m}$  wide at the base, arising from rhizoids or bulbous swellings on the substrate hyphae and terminating with a compact cluster of short acrotonous (terminal) branches. Sporangia are usually 15-48  $\mu\text{m}$  in diameter, with transparent walls and a conspicuous collarette is usually present following dehiscence of the sporangiospores. Columella are generally lacking and sporangiospores are single-celled, short-cylindrical, 6-10 x 3-5  $\mu\text{m}$ , with a double membrane. Chlamydospores with or without blunt appendages (amoeba-like) may be present, zygospores have not been observed. Temperature: grows well at 40-42°C; maximum 48°C. RG-2 organism.

**Key Features:** zygomycete, rapid growth at 40°C (thermotolerant), and characteristic delicate acrotonous branching sporangia without columellae.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), Smith (1989) and de Hoog *et al.* (2000).



Culture of *M. wolfii* showing a broadly zonate or lobed rosette-like surface appearance and sporangium, showing a sporangiophore, wide at the base, arising from rhizoids, and acrotonous (terminal) branches, collarettes and sporangiospores.

## *Mucor Micheli ex Staint-Amans*

The genus *Mucor* can be differentiated from *Absidia*, *Rhizomucor* and *Rhizopus* by the absence of stolons and rhizoids. Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia. Sporangiohores are erect, simple or branched, forming large (60-300  $\mu\text{m}$  in diameter), terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae. A conspicuous collarette (remnants of the sporangial wall) is usually visible at the base of the columella after sporangiospore dispersal. Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled or finely ornamented. Chlamydo spores and zygo spores may also be present.

**Key Features:** zygomycete, large, spherical, non-apophysate sporangia with pronounced columellae and conspicuous collarette at the base of the columella following sporangiospore dispersal.



Sporangia, columella with a conspicuous collarette (arrow) and sporangiospores of *Mucor*.

Antifungal	MIC $\mu\text{g/mL}$		Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	>64	>64	Amphotericin B	0.03-4	1
Itraconazole	0.125-8	2	Flucytosine	>256	>256
Posaconazole	0.06-8	1	Voriconazole	8->64	>64

Very limited data, antifungal susceptibility testing of individual strains is recommended. Sun *et al.* (2002), Dannaoui *et al.* (2003), Espinel-Ingroff (2001, 2003), Singh *et al.* (2005), Sabatelli *et al.* (2006) and WCH in-house data.

## ***Mucor Micheli* ex Staint-Amans**

The genus *Mucor* contains about 50 recognised taxa, many of which have widespread occurrence and are of considerable economic importance (Zycha *et al.* 1969, Schipper 1978, Domsch *et al.* 1980). However, only a few thermotolerant species are of medical importance and human infections are only rarely reported. Most infections reported list *M. circinelloides* and similar species such as *M. indicus* (*M. rouxii*), *M. ramosissimus* and *M. amphibiorum* as the causative agents. However, *M. hiemalis* and *M. racemosus* have also been reported as infectious agents, although their inability to grow at temperatures above 32°C raises doubt as to their validity as human pathogens and their pathogenic role may be limited to cutaneous infections (Scholer *et al.* 1983, Goodman and Rinaldi 1991, Kwon-Chung and Bennett 1992, de Hoog *et al.* 2000).

Maximum temperature for growth of the reported pathogenic species of *Mucor*.

Species	Max temp. (°C)	Pathogenicity
<i>M. amphibiorum</i>	36	Animals, principally amphibians
<i>M. circinelloides</i>	36-40	Animals, occasionally humans
<i>M. hiemalis</i>	30	Questionable cutaneous infections only
<i>M. indicus</i>	42	Humans and animals
<i>M. racemosus</i>	32	Questionable
<i>M. ramosissimus</i>	36	Humans and animals

For descriptions of species, keys to taxa and additional information see Schipper (1978), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Scholer *et al.* (1983), Rippon (1988), Goodman and Rinaldi (1991), Samson *et al.* (1995), de Hoog *et al.* (2000), Schipper and Staplers (2003) and Ellis (2005b).

### ***Mucor amphibiorum* Schipper**

Colonies are greyish-brown, slightly aromatic and do not grow at 37°C (maximum temperature for growth is 36°C). Sporangiophores are hyaline, erect and mostly unbranched, rarely sympodially branched. Sporangia are dark-brown, up to 75 µm in diameter, and are slightly flattened with a diffluent membrane. Columellae are subglobose to ellipsoidal or pyriform, up to 60 × 50 µm, with small collarettes. Sporangiospores are smooth-walled, spherical, and 3.5-5.5 µm in diameter. Zygosporangia, when formed by compatible mating types, are spherical to slightly compressed, up to 70 × 60 µm in diameter, with stellate projections. *Mucor amphibiorum* is distinguished by poor ramification of the sporangiophores and by globose sporangiospores. Ethanol and nitrate are not assimilated (Schipper 1978, Scholer *et al.* 1983, Hoog *et al.* 2000). RG-2 organism.

### ***Mucor circinelloides* v. Tiegh**

*M. circinelloides* is a common and variable species that includes 4 forms: *circinelloides*, *lusitanicus*, *griseo-cyanus* and *janssenii* (Schipper 1978, Scholer *et al.* 1983). Colonies are floccose, pale greyish-brown and grow poorly at 37°C (maximum growth temperature 36-40°C). Sporangiohores are hyaline and mostly sympodially branched with long branches erect and shorter branches becoming circinate (recurved). Sporangia are spherical, varying from 20-80 µm in diameter, with small sporangia often having a persistent sporangial wall. Columellae are spherical to ellipsoidal and are up to 50 µm in diameter. Sporangiospores are hyaline, smooth-walled, ellipsoidal, and 4.5-7 x 3.5-5 µm in size. Chlamydospores are generally absent. Zygosporangia are only produced in crosses of compatible mating types and are reddish-brown to dark-brown, spherical with stellate spines, up to 100 µm in diameter and have equal to slightly unequal suspensor cells. *M. circinelloides* differs from other species of *Mucor* in its formation of short circinated (coiled), branched sporangiohores bearing brown sporangia and its ability to assimilate ethanol and nitrate (Schipper 1976, Scholer *et al.* 1983, Samson *et al.* 1995, de Hoog *et al.* 2000, Schipper and Staplers 2003). RG-1 organism.

### ***Mucor indicus* Lendner**

Colonies are characteristically deep-yellow, aromatic and have a maximum growth temperature of 42°C. Sporangiohores are hyaline to yellowish, erect or rarely circinate and repeatedly sympodially branched, with long branches. Sporangia are yellow to brown, up to 75 µm in diameter, with diffluent membranes. Columellae are subglobose to pyriform, often with truncate bases, up to 40 µm high. Sporangiospores are smooth-walled, subglobose to ellipsoidal, and 4-5 µm in diameter. Chlamydospores are produced in abundance, especially in the light. Zygosporangia when formed by crosses of compatible mating strains are black, spherical up to 100 µm in diameter, with stellate spines and unequal suspensor cells. *Mucor indicus* differs from other species of *Mucor* by its characteristic deep-yellow colony colour, growth at over 40°C, assimilating ethanol, but not nitrate, and being thiamine dependent (Schipper 1978, de Hoog *et al.* 2000, Schipper and Staplers 2003). RG-1 organism.

### ***Mucor ramosissimus* Samutsevich**

Colonial growth is restricted, greyish and does not grow at 37°C (maximum temperature for growth is 36°C). Sporangiohores are hyaline, slightly roughened, tapering towards the apex and are erect with repeated sympodial branching. Sporangia are grey to black, globose or somewhat flattened, up to 80 µm in diameter and have very persistent sporangial walls. Columellae are applanate (flattened), up to 40-50 µm in size and are often absent in smaller sporangia. Sporangiospores are faintly brown, smooth-walled, subglobose to broadly ellipsoidal, 5-8 x 4.5-6 µm in size. Oidia may be present in the substrate hyphae, chlamydospores and zygosporangia are absent. Assimilation of ethanol is negative and that of nitrate is positive. *Mucor ramosissimus* differs from other species of *Mucor* by its low, restricted growth on any medium, extremely persistent sporangial walls, columellae that are applanate or absent in smaller sporangia (often resembling *Mortierella* species), short sporangiohores that repeatedly branch sympodially as many as 12 times, and the occurrence of racket-shaped enlargements in the sporangiohores (Hesseltine and Ellis 1964, Schipper 1976, Scholer *et al.* 1983, de Hoog *et al.* 2000, Schipper and Staplers 2003). RG-1 organism.

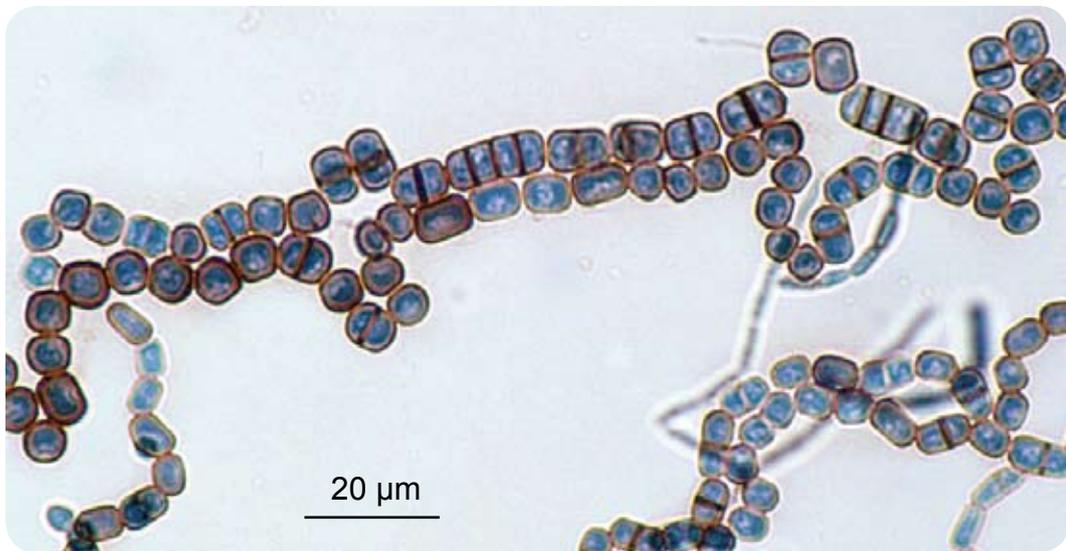
### ***Nattrassia mangiferae* (H. Syd. & Syd) Sutton & Dyko**

**Pycnidial synanamorph:** *Hendersonula toruloidea* Nattras  
**Arthroconidial synanamorph:** *Scytalidium dimidiatum* (Penzig) Sutton & Dyko  
*Scytalidium hyalinum* Campbell & Mulder

This coelomycete is characterised by the presence of black, ostiolate pycnidia containing numerous hyaline, flask-shaped phialides. Phialoconidia are at first one-celled and hyaline, later becoming three-celled and brown, with the centre cell darker than the end cells. Conidia are ovoid to ellipsoidal in shape. Pycnidia are only occasionally formed in older cultures. In culture the *Scytalidium dimidiatum* anamorph is typically present showing chains of one- to two-celled, darkly pigmented arthroconidia, 3.5-5 x 6.5-12 µm, produced by the holothallic fragmentation of undifferentiated hyphae. Cultures are effuse, hairy, dark grey to blackish-brown, or white to greyish, with a cream-coloured to deep ochraceous-yellow colony reverse. Colourless (hyaline) mutants (= *Scytalidium hyalinum*) often occur.

*Nattrassia mangiferae* (arthric synanamorph = *Scytalidium dimidiatum*) is a recognised agent of onychomycosis and superficial skin infections, especially in tropical regions. However, isolates are sensitive to cycloheximide (actidione) which is commonly added to primary isolation media used for culturing skin scrapings. RG-2 organism.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Moore (1986), Rippon (1988), Frankel and Rippon (1989), Sutton and Dyko (1989) and de Hoog *et al.* (2000).



Microscopic morphology of the *Scytalidium dimidiatum* synanamorph of *Nattrassia mangiferae* showing chains of one- to two-celled, darkly pigmented arthroconidia.

Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range
Amphotericin B	0.125-2	Itraconazole	0.03-32	Voriconazole	0.03-0.5

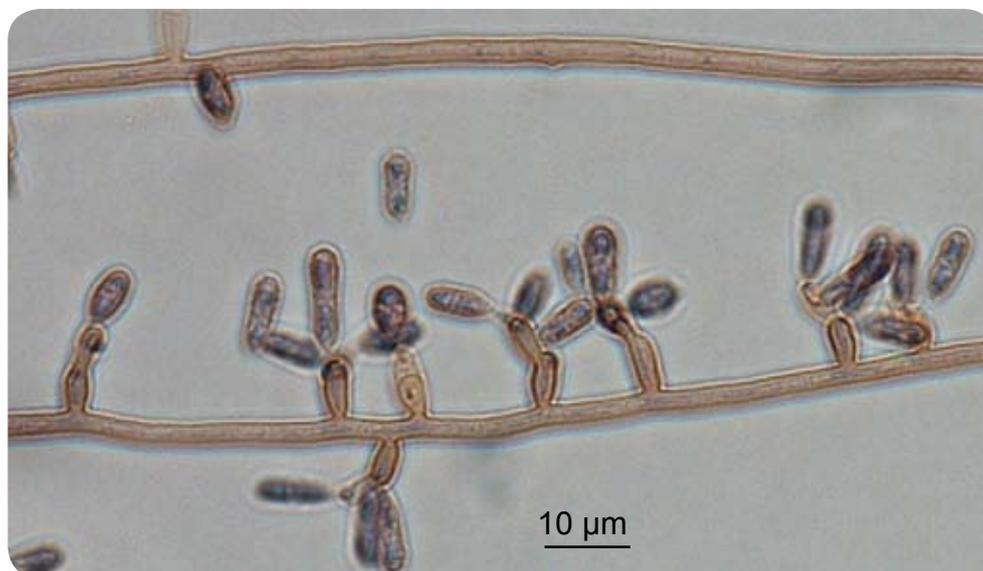
Very limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001) and WCH in-house data.

### *Ochroconis gallopava* (W.B. Cooke) de Hoog

Colonies are smooth to suede-like, dry, flat, tobacco-brown to brownish-black with a dark brown diffusible pigment. Hyphae are brown with relatively thick walls. Conidiophores are mostly cylindrical to acicular, sometimes poorly differentiated, bearing a few conidia at the tip. Conidia are two-celled, subhyaline to pale brown, smooth-walled to verrucose, cylindrical to clavate, constricted at the septum, 11-18 x 2.5-4.5 µm in size, with the apical cell wider than the basal cell. A remnant of a denticle may also be seen at the conidial base. Optimum growth at 35°C, tolerant to 40°C. RG-2 organism.

*Ochroconis gallopava* is a well recognised species and has been reported as an avian pathogen, especially in poultry. Occasional human infections have also been reported.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* 1980, McGinnis 1980 and de Hoog *et al.* 2000.



Hyphae, conidiophores and conidia of *Ochroconis gallopava*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.03-2	Itraconazole	0.03-0.5	Voriconazole	0.03-1

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff (2001) and WCH in-house data.

## ***Onychocola canadensis* Sigler**

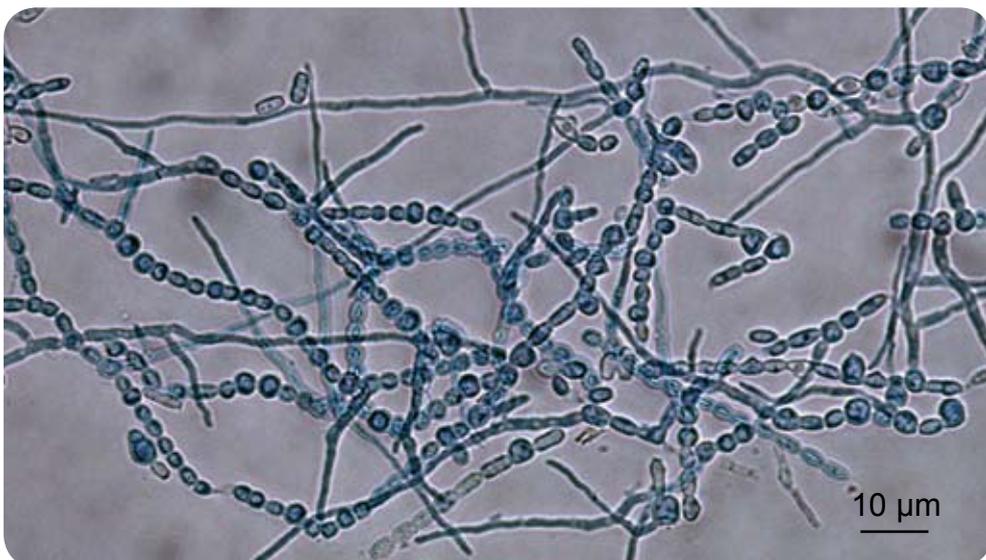
**Teleomorph:** *Arachnomyces nodososetosus* Sigler & Abbott

Colonies grow slowly and are velvety to lanose, white to yellowish, with a brownish reverse. Arthroconidia are cylindrical to broadly ellipsoidal, one- or two-celled, hyaline to subhyaline, 4-16 × 2-5 µm in size, forming long chains. Older cultures may show broad, brown, rough-walled hyphae. RG-2 organism.

*Onychocola canadensis* is an uncommon cause of distal and lateral subungual or white superficial onychomycosis. However, it may sometimes be present in an abnormal-appearing nail as an insignificant finding, not acting as a pathogen.

**Key Features:** slow growing, white, arthroconidial mould isolated from nails.

For descriptions of species, keys to taxa and additional information see Sigler and Congly (1990), Sigler *et al.* (1994), Gupta *et al.* (1998) and de Hoog *et al.* (2000).



Culture and arthroconidia of *Onychocola canadensis*.

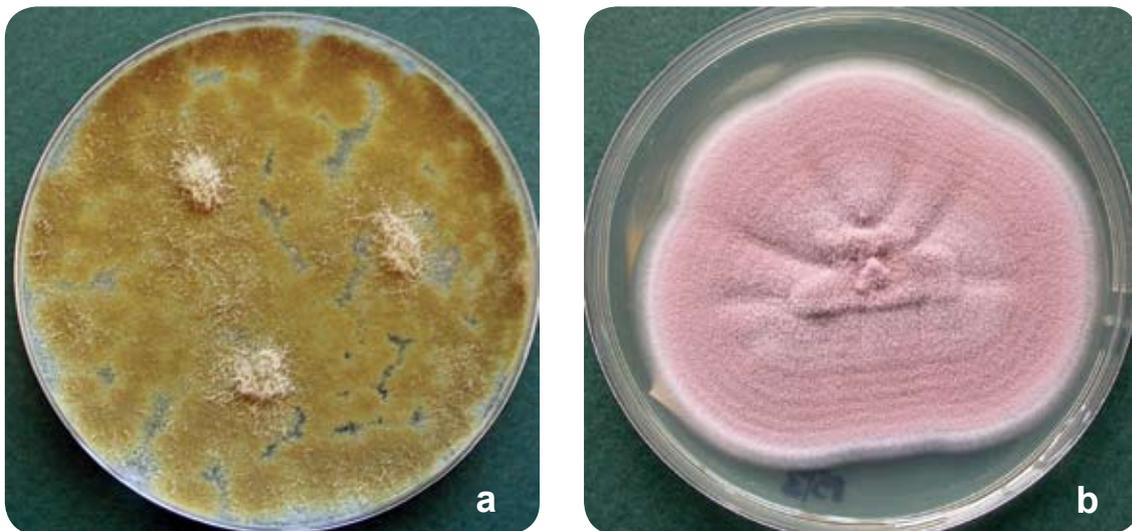
## ***Paecilomyces* Bain**

Colonies are fast growing, powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan, but never green or blue-green as in *Penicillium*. Phialides are swollen at their bases, gradually tapering into a rather long and slender neck, and occur solitarily, in pairs, as verticils, and in penicillate heads. Long, dry chains of single-celled, hyaline to dark, smooth or rough, ovoid to fusoid conidia are produced in basipetal succession from the phialides.

The genus *Paecilomyces* may be distinguished from the closely related genus *Penicillium* by having long slender divergent phialides and colonies that are never typically green. *Paecilomyces* species are common environmental moulds and are seldom associated with human infection. However, some species, *P. variotii*, *P. marquandii* and *P. lilacinus* are emerging as causative agents of mycotic keratitis and of hyalohyphomycosis in the immunocompromised patient.

**Key Features:** long slender divergent phialides and culture pigmentation.

For descriptions of species, keys to taxa and additional information see Samson (1974), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Rippon (1988) and de Hoog *et al.* (2000).



Cultures of *P. variotii* (a) and *P. lilacinus* (b) showing colony pigmentation.

### *Paecilomyces lilacinus* (Thom) Samson

Colonies are fast growing, suede-like to floccose, vinaceous to violet-coloured. Conidiophores are erect 400-600 µm in length, bearing branches with densely clustered phialides. Conidiophore stipes are 3-4 µm wide, yellow to purple and rough-walled. Phialides are swollen at their bases, gradually tapering into a slender neck. Conidia are ellipsoidal to fusiform, smooth-walled to slightly roughened, hyaline to purple in mass, 2.5-3.0 × 2-2.2 µm, and are produced in divergent chains. Chlamyospores are absent. Growth at 38°C. RG-1 organism.

**Key Features:** colony pigmentation, phialides with swollen bases, and pigmented and rough-walled conidiophore stipes, absence of chlamyospores.



Conidiophores, phialides and conidia of *Paecilomyces lilacinus*. Note: rough-walled conidiophore (arrow).

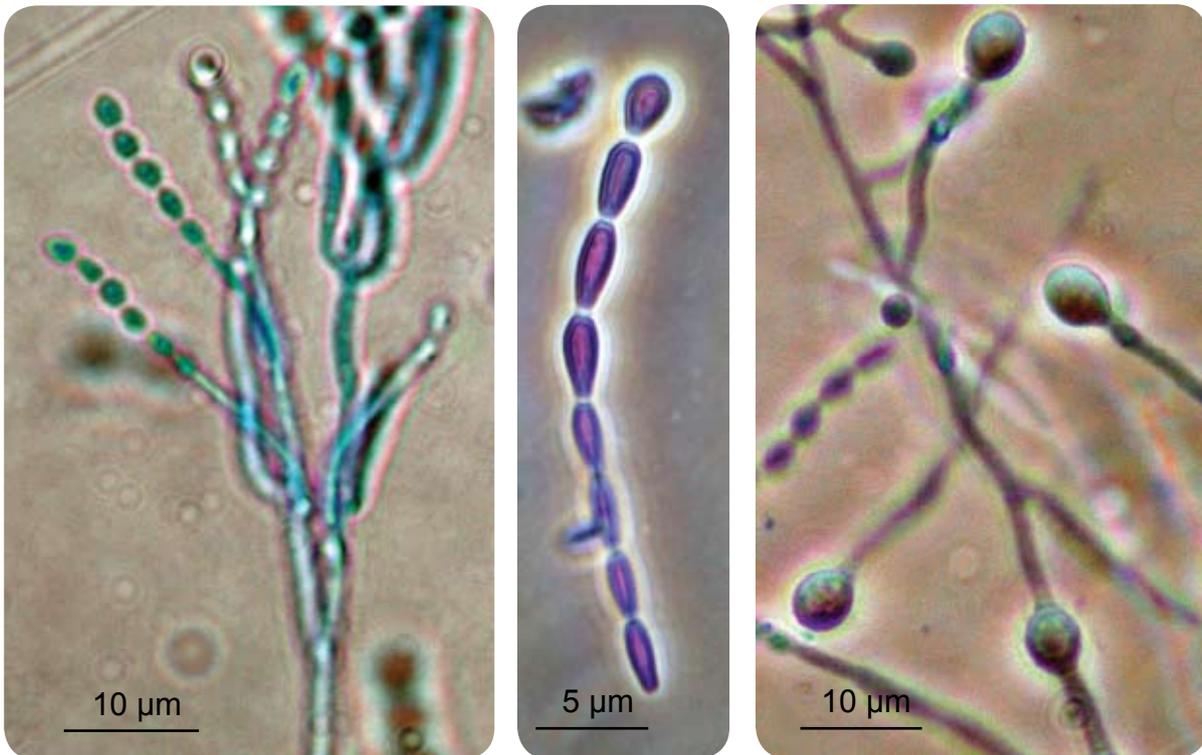
Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	2-16	8	Posaconazole	0.06-2	0.5 (2)
Itraconazole	0.5-16	16	Voriconazole	0.06-4	0.25 (2)

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2001, 2003), Gonzales *et al.* (2005), and WCH in-house data.

### *Paecilomyces variotii* Bain

Colonies are fast growing, powdery to suede-like, funiculose or tufted, and yellow-brown or sand-coloured. Conidiophores bearing dense, verticillately arranged branches bearing phialides. Phialides are cylindrical or ellipsoidal, tapering abruptly into a rather long and cylindrical neck. Conidia are subspherical, ellipsoidal to fusiform, hyaline to yellow, smooth-walled, 3-5 × 2-4 µm and are produced in long divergent chains. Chlamydospores are usually present, singly or in short chains, brown, subspherical to pyriform, 4-8 µm in diameter, thick-walled to slightly verrucose. RG-2 organism.

**Key Features:** yellow-brown colony pigmentation, cylindrical phialides, and presence of chlamydospores.



Conidiophores, phialides, conidia and terminal chlamydospores of *P. variotii*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.06-1	1	Posaconazole	0.03-0.5	0.5
Itraconazole	0.03-8	0.5	Voriconazole	0.03-2	0.5

Limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003) and WCH in-house data.

### ***Paracoccidioides brasiliensis* (Splendore) Almeida**

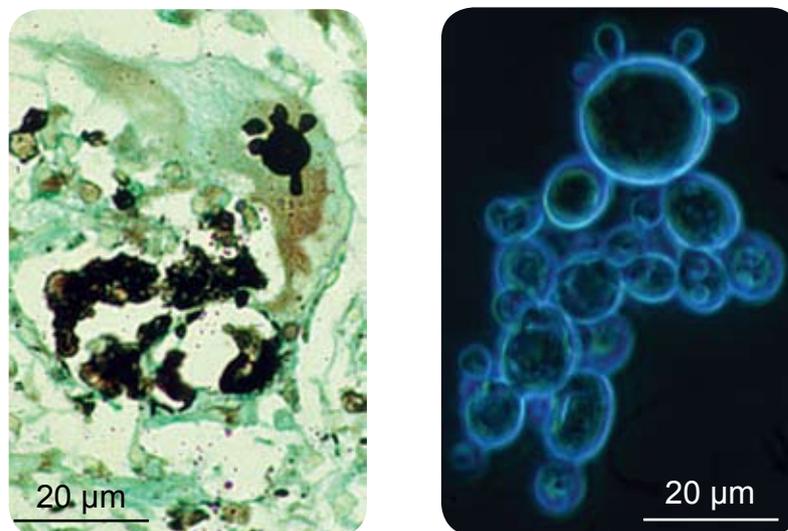
Colonies (SDA) at 25°C are slow growing and variable in morphology. Colonies may be flat, wrinkled and folded, glabrous, suede-like or downy in texture, white to brownish with a tan or brown reverse. Microscopically, a variety of conidia may be seen, including pyriform microconidia, chlamydospores and arthroconidia. However, none of these are characteristic of the species, and most strains may grow for long periods of time without the production of conidia.

On BHI blood agar at 37°C, the mycelium converts to the yeast phase and colonies are white to tan, moist and glabrous and become wrinkled, folded and heaped. Microscopically, numerous large, 20-60 µm, round, narrow base budding yeast cells are present. Single and multiple budding occurs, the latter are thick-walled cells that form the classical “steering wheel” or “mickey mouse” structures that are diagnostic for this fungus, especially in methenamine silver stained tissue sections.

**WARNING:** RG-3 Organism. Cultures of *Paracoccidioides brasiliensis* may represent a biohazard to laboratory personnel and should be handled with extreme caution in an appropriate pathogen handling cabinet. *P. brasiliensis* is geographically restricted to areas of South and Central America (Rippon, 1988).

**Key Features:** clinical history, tissue pathology, culture identification with conversion to yeast phase at 37°C.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Chandler *et al.* (1980), Rippon (1988) and de Hoog *et al.* (2000).



Multiple, narrow base budding yeast cells “steering wheels” of *P. brasiliensis*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.03-4	0.25	Itraconazole	0.03-1	0.06
Fluconazole	0.125-64	na	Voriconazole	0.03-2	na

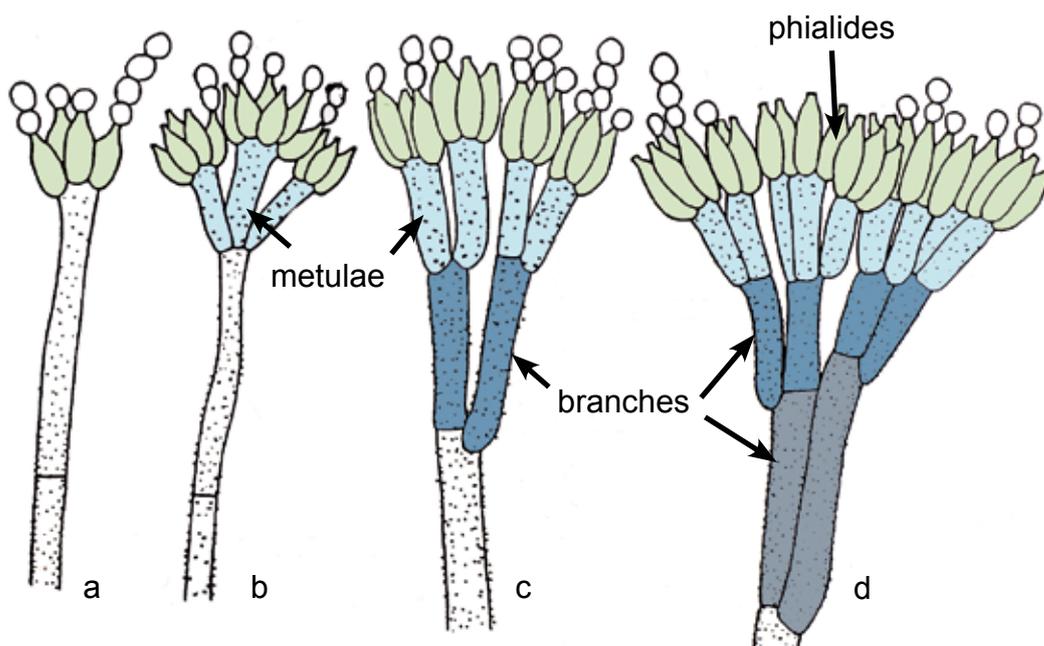
Limited data available. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2001) and Sabatelli *et al.* (2006) (na = not available).

## ***Penicillium* Link: Fries**

Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores. Microscopically, chains of single-celled conidia are produced in basipetal succession from a specialised conidiogenous cell called a phialide. The term basocatenate is often used to describe such chains of conidia where the youngest conidium is at the basal or proximal end of the chain. In *Penicillium*, phialides may be produced singly in groups or from branched metulae, giving a brush-like appearance (a penicillus). The penicillus may contain both branches and metulae (penultimate branches which bear a whorl of phialides). All cells between the metulae and the stipes of the conidiophores are referred to as branches. The branching pattern may be either simple (non-branched or monoverticillate), one-stage branched (biverticillate-symmetrical), two-stage branched (biverticillate-asymmetrical) or three-to more-staged branched. Conidiophores are hyaline, smooth or rough-walled. Phialides are usually flask-shaped, consisting of a cylindrical basal part and a distinct neck, or lanceolate (more or less with a narrow basal part tapering to a somewhat pointed apex). Conidia are in long dry chains, divergent or in columns, are globose, ellipsoidal, cylindrical or fusiform, hyaline or greenish, smooth or rough-walled. Sclerotia are produced by some species. RG-1 organisms, with the exception of *P. marneffe* which is an RG-3 organism.

For identification, isolates are usually inoculated at three points on Czapek Dox agar and 2% Malt extract agar and incubated at 25°C. Most species sporulate within 7 days. Microscopic mounts are best made using a cellotape flag or a slide culture preparation mounted in lactophenol cotton blue. A drop of alcohol is usually needed to remove bubbles and excess conidia (see Samson *et al.*, 1995).

**Key Features:** hyphomycete, flask-shaped phialides arranged in groups from branched metulae forming a penicillus.



Morphological structures and types of conidiophore branching in *Penicillium*. (a) simple; (b) one-stage branched; (c) two-stage branched; (d) three-stage branched (see Samson *et al.* 1995).

### ***Penicillium* Link:Fries**

Many species of *Penicillium* are common contaminants on various substrates and are known as potential mycotoxin producers. Correct identification is therefore important when studying possible *Penicillium* contamination of food. In some species odour and exudate production will help to recognise the taxa, but it should be pointed out that inhalation of conidia and volatiles may affect health. Human pathogenic species are rare, however opportunistic infections leading to mycotic keratitis, otomycosis and endocarditis (following insertion of valve prosthesis) have been reported (see Samson *et al.*, 1995 and Rippon, 1988).

For descriptions of species, keys to taxa and additional information see Raper and Thom (1949), Pitt (1979), Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Ramirez (1982), Samson *et al.* (1995) and de Hoog *et al.* (2000).



Conidiophores of *P. verrucosum* var. *cyclopium* showing two-stage branching. Simple conidiophore of *P. cheresanum* showing long chains of single-celled conidia.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Amphotericin B	0.125-2	1-2	Posaconazole	0.03-2	0.25-1
Itraconazole	0.03-2	0.5-2	Voriconazole	0.03->8	0.5-2

Limited data, antifungal susceptibility testing of individual strains is recommended. Pfaller *et al.* (2002), Diekema *et al.* (2003), Espinel-Ingroff (2003) and WCH in-house data.

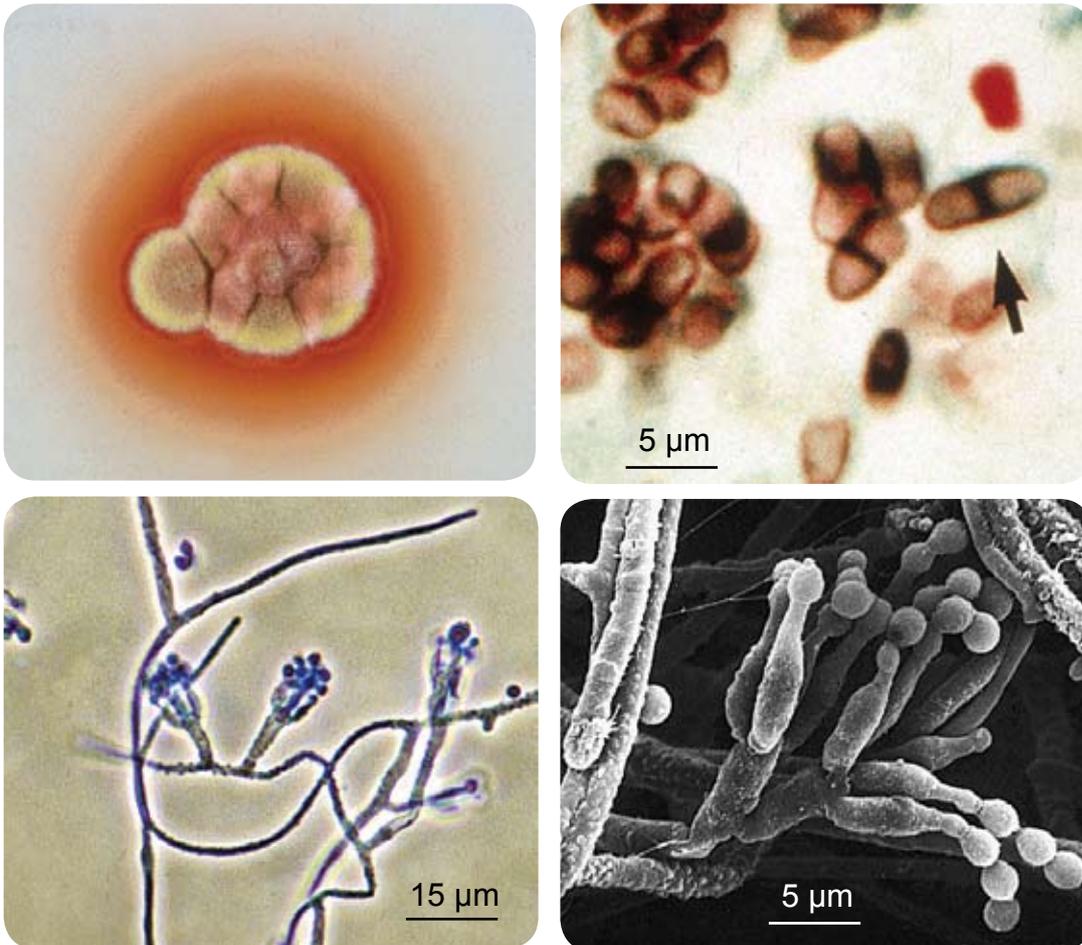
### ***Penicillium marneffe* Segretain**

Colonies (SDA) at 25°C are fast growing, suede-like to downy, white with yellowish-green conidial heads. Colonies become greyish-pink to brown with age and produce a diffusible brownish-red to wine-red pigment. Conidiophores are hyaline, smooth-walled and bear terminal verticils of 3-5 metulae, each bearing 3-7 phialides. Conidia are globose to subglobose, 2 to 3 µm in diameter, smooth-walled and are produced in basipetal succession from the phialides.

On brain heart infusion (BHI) blood agar incubated at 37°C, colonies are rough, glabrous, tan-coloured and yeast-like. Microscopically, yeast cells are spherical to ellipsoidal, 2-6 µm in diameter, and divide by fission rather than budding. Numerous short hyphal elements are also present.

**WARNING:** RG-3 organism. Cultures of *Penicillium marneffe* may represent a biohazard to laboratory personnel and should be handled with caution in an appropriate pathogen handling cabinet. *P. marneffe* exhibits thermal dimorphism and is endemic in Southeast Asia and the southern region of China.

Tissue sections show small, oval to elliptical yeast-like cells, 3 µm in diameter, either packed within histiocytes or scattered through the tissue. Occasional, large, elongated sausage-shaped cells, up to 8 µm long, with distinctive septa may be present.



Colony, a giemsa stained touch smear showing typical septate yeast-like cells (arrow), phialides and conidia of *Penicillium marneffe*.

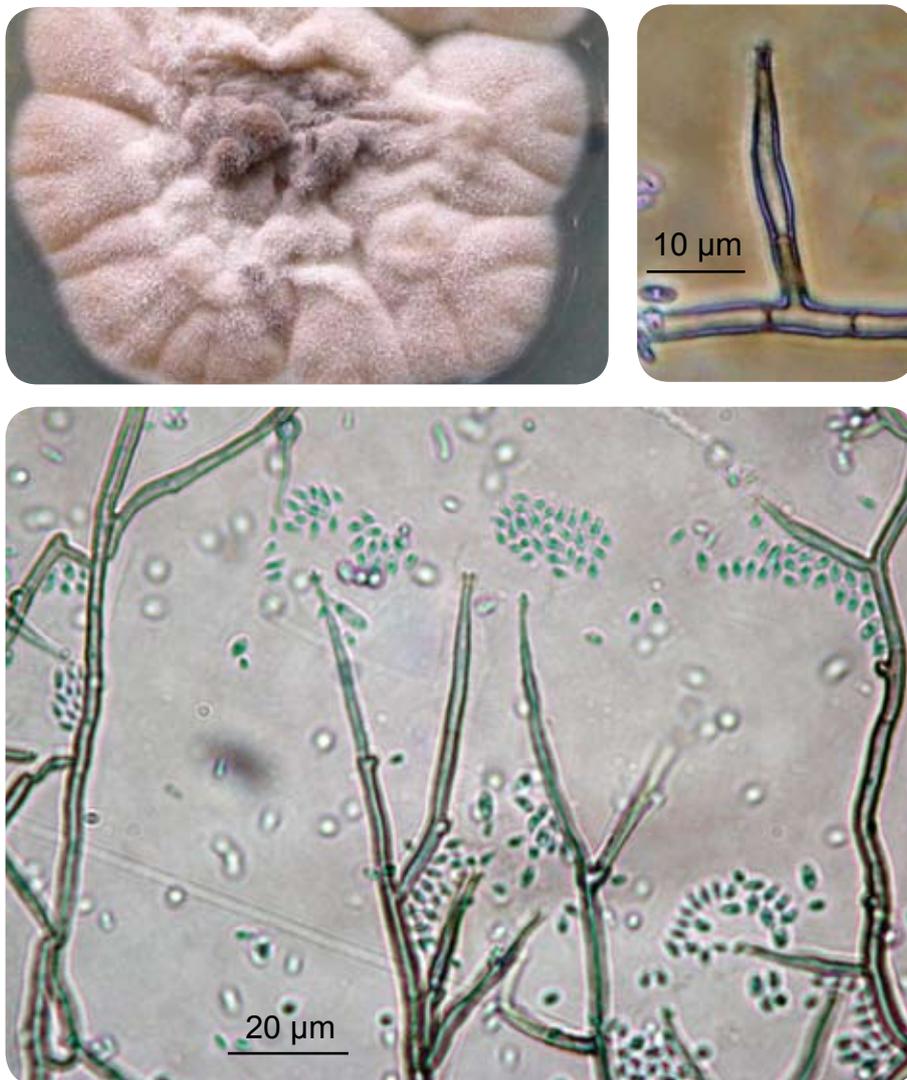
***Phaeoacremonium parasiticum* (Ajello et al.) W. Gams et al.**

**Synonym:** *Phialophora parasiticum* Ajello, Gerog & Wang

Cultures are usually slow growing, suede-like with radial furrows, initially whitish-grey becoming olivaceous-grey with age. Hyphae hyaline, later becoming brown and some becoming rough-walled. Phialides are brown, thick-walled, slender, acular to cylindrical slightly tapering towards the tip, 15-50 µm long, often proliferating, with small, funnel-shaped collarettes. Conidia, often in balls, are hyaline, thin-walled, cylindrical to sausage-shaped, 3-6 x 1-2 µm, later inflating (de Hoog et al. 2000). RG-2 organism.

*P. parasiticum* is a plant pathogen but it has also been reported from cases of subcutaneous infection, arthritis, mycetoma, endocarditis and mycotic keratitis.

For descriptions of species and additional information see de Hoog et al. (2000).



Colony, phialides and conidia of *Phaeoacremonium parasiticum*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.06-16	Itraconazole	0.06-32	Voriconazole	0.06-2

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

## *Phialophora* Medlar

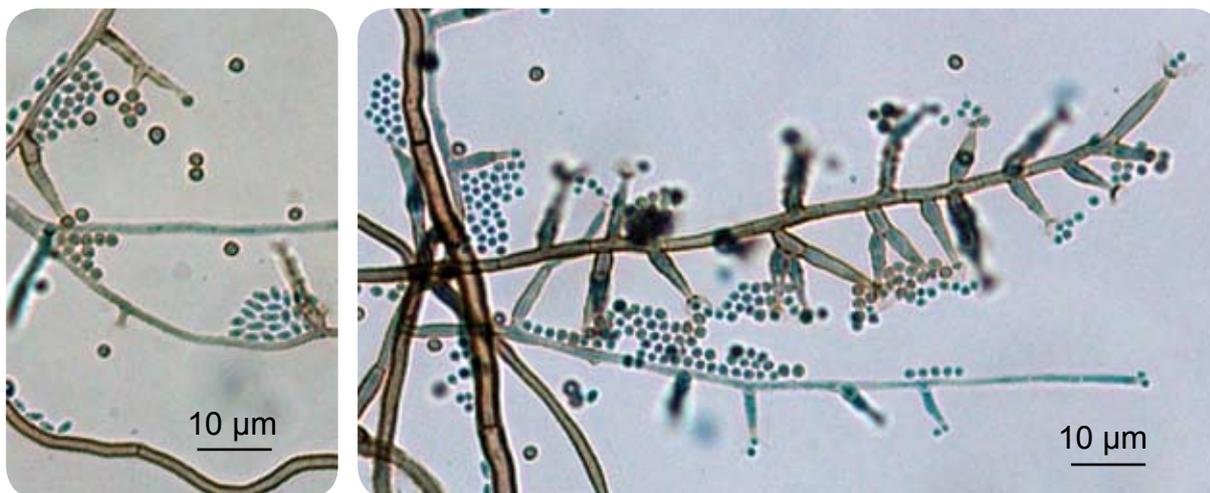
Colonies are usually slow growing, grey to olivaceous-black, often becoming brown with age. Microscopically, members of the genus *Phialophora* produce clusters of single-celled conidia in basipetal succession from characteristic flask-shaped or cylindrical phialides which have distinctive collarettes. Conidia are hyaline to olivaceous brown, smooth-walled, ovoid to cylindrical or allantoid, and usually aggregate in slimy heads at the apices of the phialides, which may be solitary, or in a brush-like arrangement.

The genus *Phialophora* contains more than 40 species, most are saprophytes commonly found in soil or on decaying wood. However, several species have been documented as causing either chromoblastomycosis (*P. verrucosa*) or phaeohyphomycosis (*P. verrucosa* and *P. richardsiae*).

### *Phialophora richardsiae* (Nannf.) Conant

Colonies grow rapidly, and are powdery to woolly or tufted, greyish-brown with a grey-brown to olivaceous-black reverse. Two conidial types are produced; (1) hyaline conidia which are allantoid or cylindrical, 3-6 × 1.5-2.5 μm in size, formed on inconspicuous, peg-like phialides on thin-walled hyphae; and (2) brown, thick-walled conidia which are spherical to sub-spherical, 2.5-3.5 × 2-3 μm, formed on dark brown, slender, tapering phialides with flaring collarettes. RG-2 organism.

*Phialophora richardsiae* is a soft rot fungus of wood and is an uncommon cause of human infection. However, cases of subcutaneous phaeohyphomycosis have been reported.



Phialides of *P. richardsiae* producing 2 types of conidia. (1) hyaline conidia, formed on inconspicuous, peg-like phialides on thin-walled hyphae; and (2) brown, thick-walled conidia formed on dark brown, slender, tapering phialides with flaring collarettes.

Antifungal	MIC μg/mL	Antifungal	MIC μg/mL	Antifungal	MIC μg/mL
	Range		Range		Range
Amphotericin B	0.125-1	Itraconazole	0.03-2	Voriconazole	0.125-2

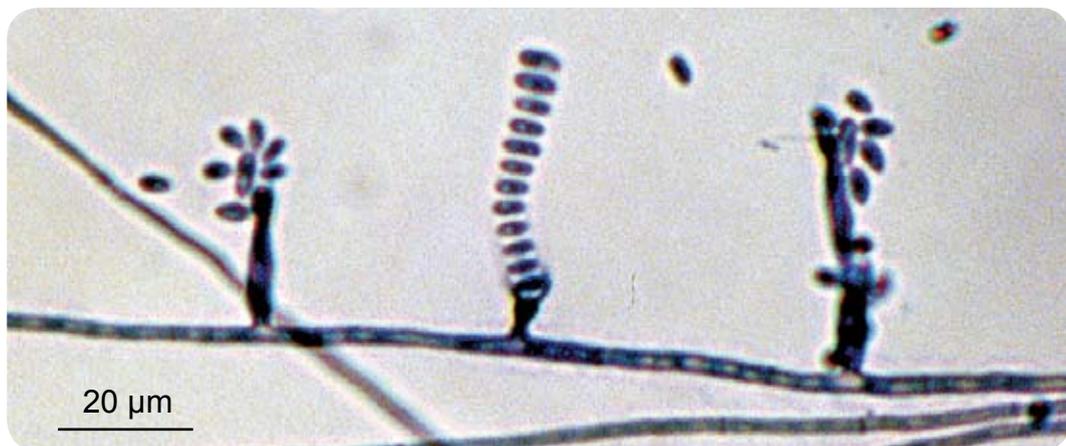
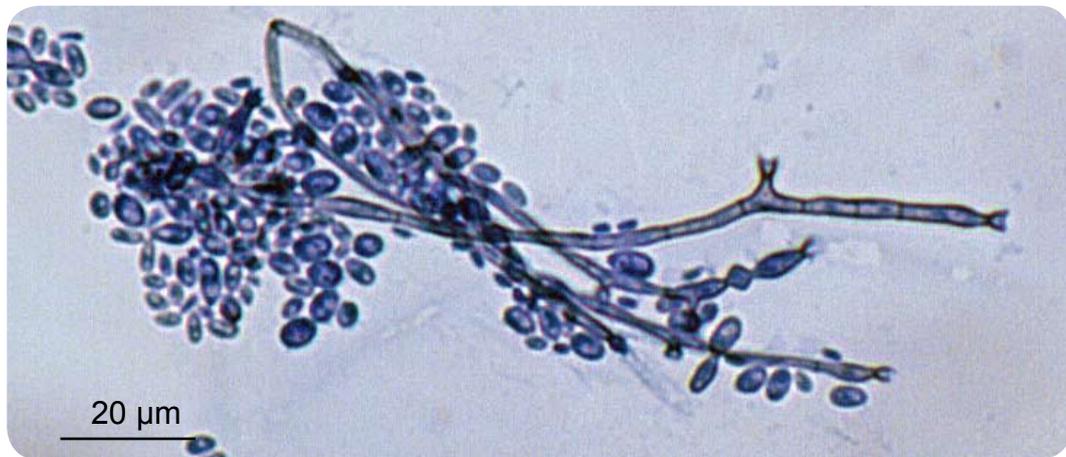
Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

### ***Phialophora verrucosa* Medlar**

Colonies (SDA) are slow growing, initially dome-shaped, later becoming flat, suede-like and olivaceous to black in colour. Phialides are flask-shaped or elliptical with distinctive funnel-shaped, darkly pigmented collarettes. Conidia are ellipsoidal, smooth-walled, hyaline, mostly 3.0-5.0 × 1.5-3.0 µm, and aggregate in slimy heads at the apices of the phialide. RG-2 organism.

*Phialophora verrucosa* is a well documented causative agent of chromoblastomycosis, and mycetoma. It produces characteristic flask-shaped phialides with distinctive funnel-shaped, darkly pigmented collarettes.

For descriptions of species, keys to taxa and additional information see Ellis (1971), McGinnis (1978), Domsch *et al.* (1980), McGinnis (1980) and de Hoog *et al.* (2000).



Phialides and conidia of *Phialophora verrucosa*.

Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range	Antifungal	MIC µg/mL Range
Amphotericin B	0.03-4	Itraconazole	0.03-0.06	Voriconazole	0.03-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

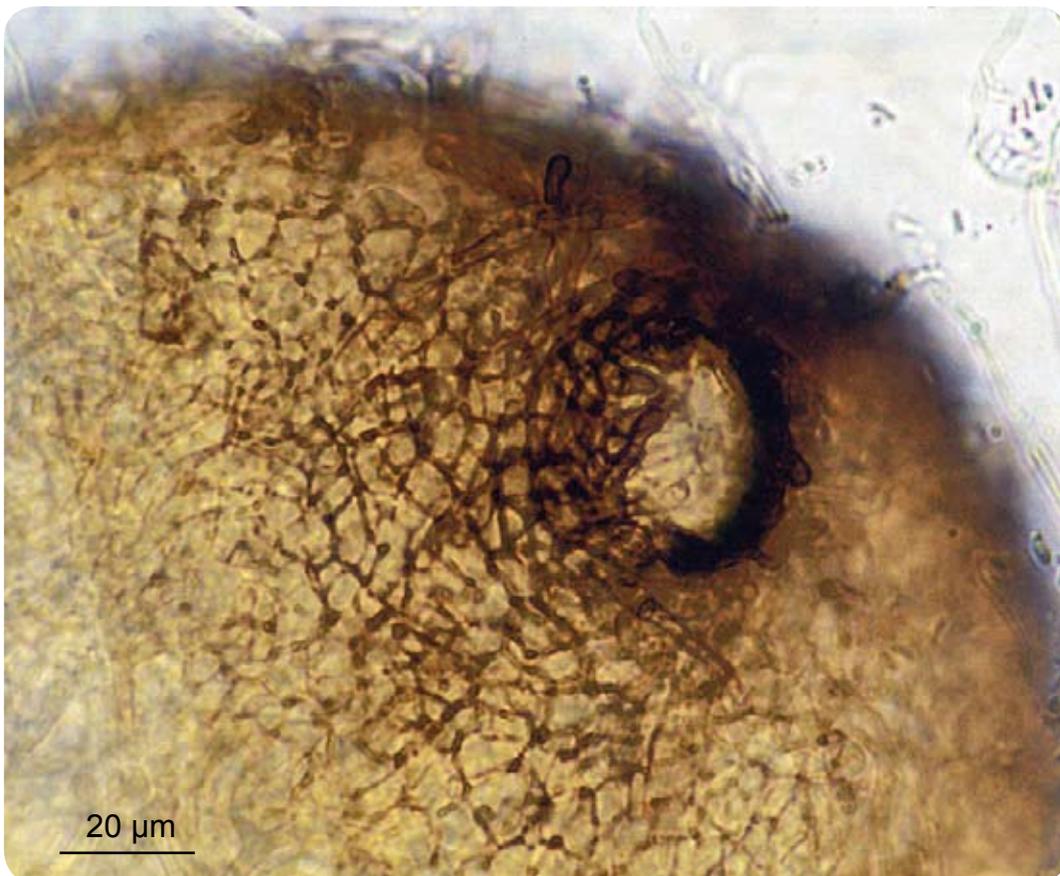
## ***Phoma* Saccardo**

Colonies are spreading, greyish-brown, powdery or suede-like and produce large, globose, membranous to leathery, darkly pigmented, ostiolate pycnidia. Conidia are produced in abundance within the pycnidia on narrow thread-like phialides, which are hardly differentiated from the inner pycnidial wall cells. Conidia are globose to cylindrical, one-celled, hyaline, and are usually extruded in slimy masses from the apical ostiole. RG-1 organism.

Members of the genus *Phoma* have a world-wide distribution and are ubiquitous in nature, with over 2000 species having been described from soil, as saprophytes on various plants, and as pathogens to plants and humans.

**Key Features:** coelomycete, ostiolate pycnidia producing masses of slimy, hyaline, single-celled conidia.

For descriptions of species, keys to taxa and additional information see Punithalingam (1979), McGinnis (1980), Sutton (1980), Rippon (1988), Montel *et al.* (1991), Samson *et al.* (1995) and de Hoog *et al.* (2000).



Pycnidia of *Phoma*.

## ***Pithomyces* Berkeley and Broome**

Colonies are fast growing, dark grey to black, suede-like to downy and produce darkly pigmented, multicellular conidia formed on small peg-like branches of the vegetative hyphae. Conidia are broadly elliptical, pyriform, oblong, and are commonly echinulate or verrucose.

The genus *Pithomyces* contains about 15 species commonly found from a very wide range of plant material, also from air, soil, hay, sawn timber and ceiling plaster. However, one species, *Pithomyces chartarum* is often involved with facial eczema of sheep.

### ***Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis**

Colonies are fast growing, suede-like to downy and black. Conidiophores are pale olive, smooth or verrucose, 2.5-10 x 2-3.5  $\mu\text{m}$ . Conidiogenous cells integrated, intercalary or terminal, indeterminate, with 1-2 loci of similar width in the conidiogenous cells. Conidia muriform, medium to dark brown, echinulate to verrucose, 3 (-4)-euseptate, slightly constricted at the septa, with one or both median cells divided by longitudinal septa, thick-walled, broadly ellipsoidal, apex obtuse, base truncate and characteristically with part of the conidiogenous cell remaining attached as a small pedicel, 18-29 x 10-17  $\mu\text{m}$ . RG-1 organism.

**Key Features:** dematiaceous hyphomycete with multi-celled conidia produced on small peg-like branches of the vegetative hyphae.

For descriptions of species, keys to taxa and additional information see Ellis (1971 and 1976), Domsch *et al.* (1980) and Rippon (1988).



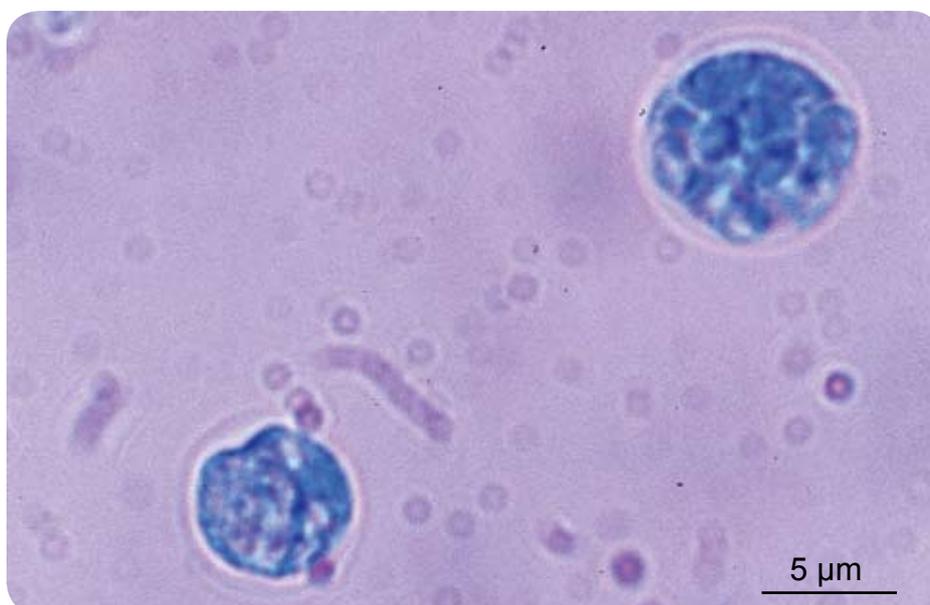
Conidiophores and conidia of *Pithomyces chartarum*.

## ***Prototheca* Kruger**

*Prototheca* species are achlorophyllous algae with phylogenetic affinities to the genus *Chlorella*. Colonies are smooth, moist, white to cream and yeast-like. Cultures are sensitive to cycloheximide (actidione) and optimal growth occurs at 25°C to 30°C. Mycelium and conidia are absent. Vegetative cells are globose to ovoid, hyaline, varying in size from approximately 8-20 µm, and have a relatively thick and highly refractile wall. No budding cells are present; reproduction is by the development of large sporangia (theca) which contain from 2-20 or more small sporangiospores (endospores or autospores) which are asexually produced by nuclear division and cleavage of the cytoplasm (Kaplan, 1977, McGinnis, 1980, Rippon, 1988, Pore, 1985). RG-1 organism.

**Key Features:** achlorophyllous algae reproducing by sporangia (theca) and sporangiospores (autospores). The genus *Prototheca* contains four species which can be differentiated by assimilation tests and morphological criteria as outlined below. The API 20C yeast identification strip may be used for species identification. So far only *P. wickerhamii* and *P. zopfii* have been involved in human or animal infections.

	<i>P. wickerhamii</i>	<i>P. zopfii</i>	<i>P. stagnora</i>	<i>P. moriformis</i>
Growth at 37°C	+	+	-	-
Glucose	+	+	+	+
Trehalose	+	-	-	-
L-propanol	-	+	+/-	+
Acetate (pH5)	-	+	+/-	+
Galactose	+	-	+	-
Capsule	-	-	+	+



Thecae and autospores of *Prototheca wickerhamii*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.25-0.5	Voriconazole	0.25	Posaconazole	0.25
WCH in-house data only.					

## ***Ramichloridium* Stahel ex de Hoog**

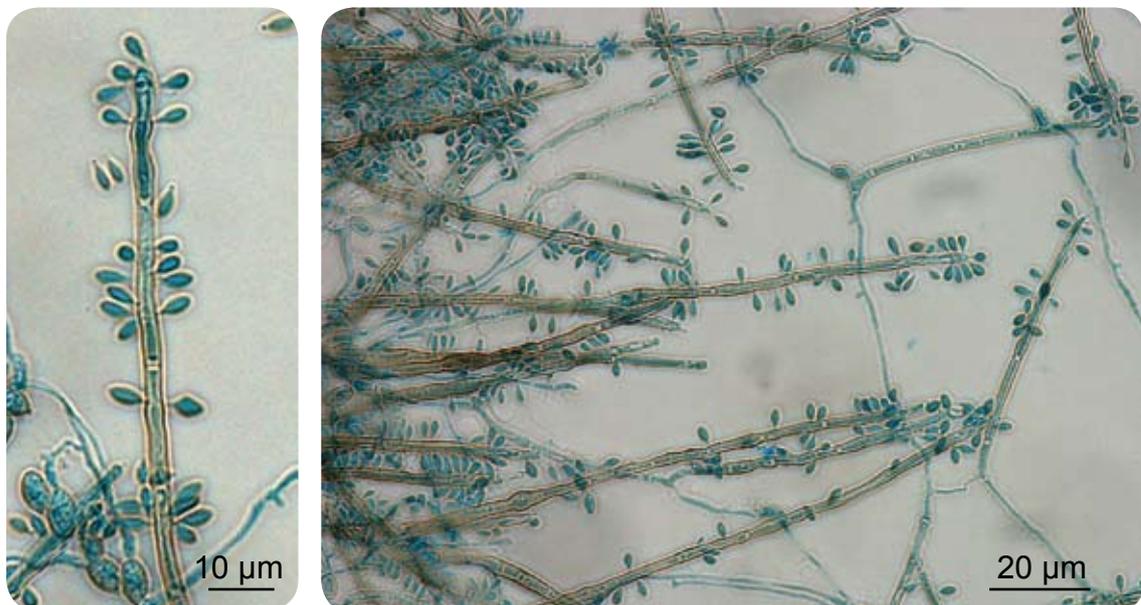
Colonies are rapid growing, smooth, farinose or velvety, brown or olivaceous-green, often with orange or yellow soluble pigments. Conidiophores are erect, brown, apically with small denticles on which conidia are produced in sympodial succession. Conidia are one-celled, hyaline to pale brown.

*Ramichloridium* contains about 25 species that are usually associated with forest litter and rotting wood, however the genus contains two species of medical interest; *R. mackenziei* and *R. schulzeri*.

## ***Ramichloridium schulzeri* (Sacc.) de Hoog**

Colonies growing moderately rapidly, consisting of a rather compact, flat, submerged mycelium, pale orange, locally with some powdery, brownish aerial mycelium; reverse pink to orange. Conidiophores are erect, straight, unbranched, thick-walled, reddish-brown, up to 250  $\mu\text{m}$  high, gradually becoming paler towards the apex, of variable length, elongating sympodially during conidiogenesis, with scattered, pimple-shaped conidium bearing denticles which have unpigmented scars. Conidia are subhyaline, smooth-walled or slightly rough-walled, ellipsoidal, obovoidal or fusiform, 6.5-10.0  $\times$  3-4  $\mu\text{m}$ , usually with an acuminate base and unpigmented scars. RG-1 organism.

For descriptions of species, keys to taxa and additional information see de Hoog (1977), Rippon *et al.* (1985) and de Hoog *et al.* (2000).



Conidiophores showing sympodial development of conidia of *R. schulzeri*.

## ***Rhinocladiella* Nannfeldt**

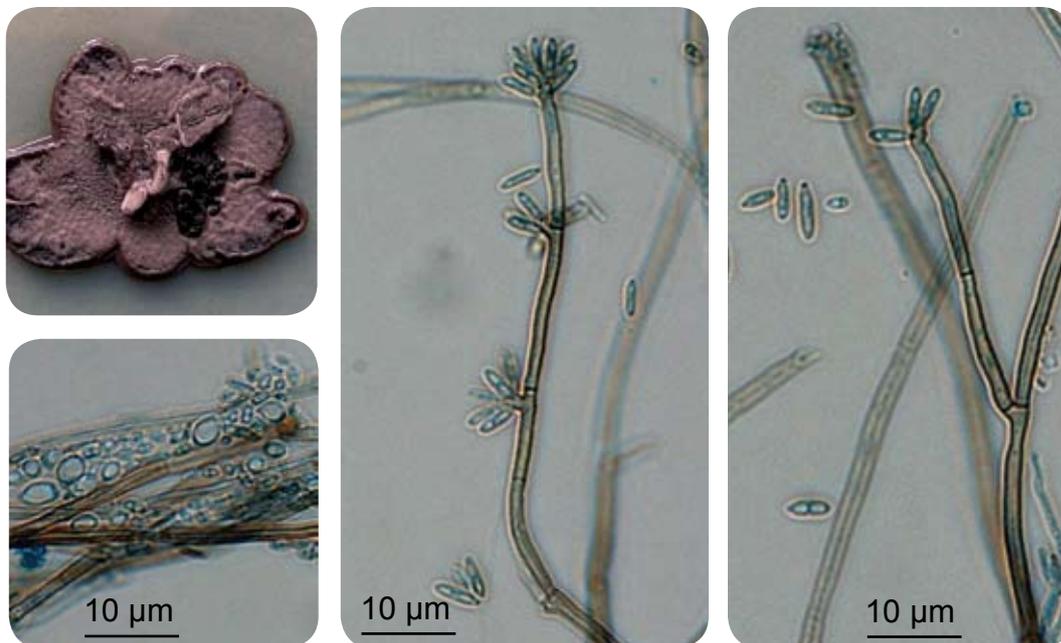
Colonies are restricted, velvety, lanose or nearly smooth, grey to olivaceous-brown. Hyphae pale olivaceous. Conidiophores are slightly differentiated, sub-erect, usually branched, pale to dark brown. Conidiogenous cells are intercalary or free, cylindrical, in the apical part with conidium bearing denticles with unpigmented scars. Conidia are hyaline to subhyaline, one-celled and smooth-walled. Budding cells and an accompanying *Exophiala* state may be present.

*Rhinocladiella* contains 6-8 species, with two species of medical interest; *R. atrovirens* and *R. aquaspersa*.

### ***Rhinocladiella atrovirens* Nannfeldt**

Colonies are restricted, velvety or lanose, olivaceous, often slightly mucoid at the centre; reverse dark olivaceous green to blackish. Conidiophores are short, brown, thick-walled. Conidiogenous cells are cylindrical, intercalary or free, 9-19 × 1.6-2.2 µm; denticulate rachis up to 15 µm long, with crowded, flat or butt-shaped, unpigmented conidial denticles. Conidia are hyaline, thin- and smooth-walled, short-cylindrical, with truncate basal scars, 3.7-5.5 × 1.2-1.8 µm. Budding cells, if present, are hyaline, thin-walled, broadly ellipsoidal, 3.0-4.3 × 1.7-2.5 µm. Germinating cells are inflated, spherical to subspherical, 4.5-6.0 µm. An annellidic *Exophiala* synanamorph may be present. RG-1 organism.

For descriptions of species, keys to taxa and additional information see de Hoog (1977, 1983), Schell *et al.* (1983) and de Hoog *et al.* (2000).



Culture, conidiophores showing a terminal denticulate rachis, conidia and budding yeast cells of *Rhinocladiella atrovirens*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	0.03-0.25	Itraconazole	0.03-0.06	Voriconazole	0.03-0.5

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998), Espinel-Ingroff *et al.* (2001) and WCH in-house data.

## ***Rhizomucor* Lucet & Costantin**

The genus *Rhizomucor* is distinguished from *Mucor* by the presence of stolons and poorly developed rhizoids at the base of the sporangiophores and by the thermophilic nature of its 3 species: *R. miehei*, *R. pusillus* and *R. tauricus*. All 3 of these species are potential human and animal pathogens and were originally classified in the genus *Mucor*. *Rhizomucor variabilis* as described by de Hoog *et al.* (2000) is not thermophilic and is probably a degenerate culture of *Mucor hiemalis* (Voigt *et al.*, 1999). *Rhizomucor pusillus* is cosmopolitan and both *R. miehei* and *R. pusillus* have been reported as pathogens to humans and animals, the latter to a greater extent.

For descriptions of species, keys to taxa and additional information see Cooney and Emerson (1964), Schipper (1978), Domsch *et al.* (1980), McGinnis (1980), Ellis and Keane (1981), Scholer *et al.* (1983), Hoog *et al.* (2000), Schipper and Staplers (2003) and Ellis (2005b).

Remember, identification of most zygomycetes is based primarily on the morphology of the sporangia; i.e. arrangement and number of sporangiospores, shape, colour, presence or absence of columellae and apophyses, as well as the arrangement of the sporangiophores and the presence or absence of rhizoids. Growth temperature tests can also be especially helpful in identifying and differentiating members of the genera *Rhizomucor*, *Rhizopus* and *Absidia*.

### ***Rhizomucor miehei* (Cooney and Emerson) Schipper**

**Synonym:** *Mucor miehei* Lindt

This species has been reported as a rare cause of bovine mastitis (Scholer *et al.* 1983) and is similar in most respects to *R. pusillus*. However, all strains are homothallic forming numerous zygospores, which are reddish-brown to blackish-brown, globose to slightly compressed, up to 50 µm in diameter, with stellate warts and equal suspensor cells. Colony colour is a dirty grey rather than brown, and sporangia have spiny walls, are up to 50-60 µm in diameter, with columellae rarely larger than 30 µm in diameter. Growth is stimulated by thiamine, with no assimilation of sucrose and maximum temperature of growth is 54–58°C. RG-1 organism.

**Key Features:** growth at 45°C, the formation of numerous zygospores, a dirty grey culture colour and a partial growth requirement for thiamine.

### ***Rhizomucor pusillus* (Lindt) Schipper**

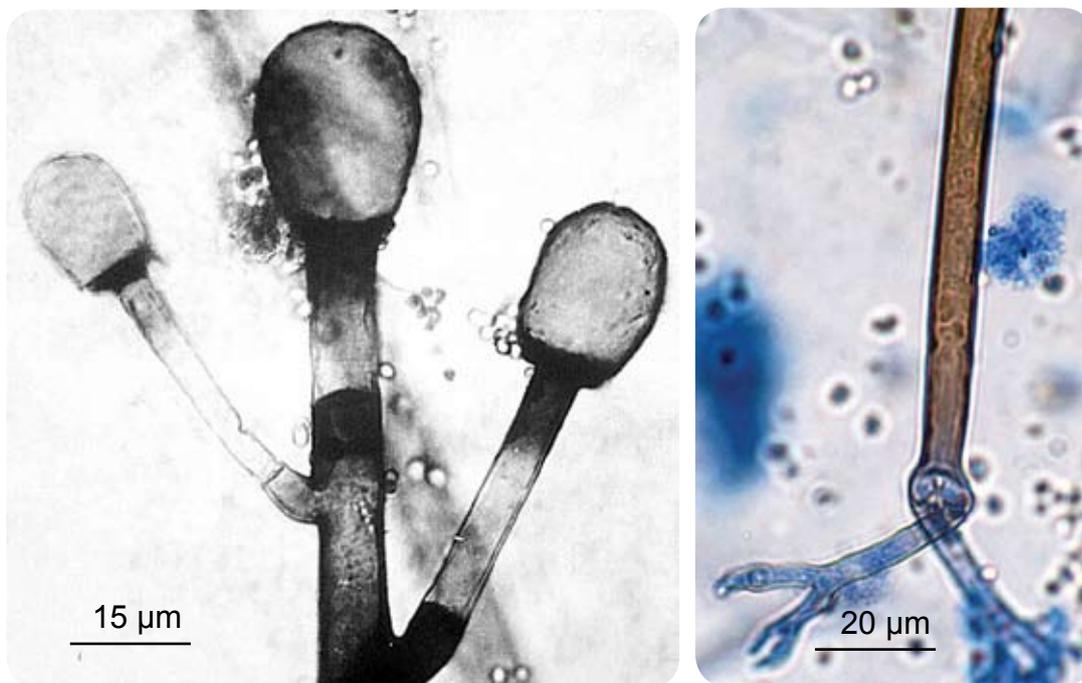
**Synonym:** *Mucor pusillus* Lindt

This species is a rare human pathogen. It has been reported from cases of pulmonary, disseminated and cutaneous types of infection. It is more often associated with animal disease, especially bovine abortion. *Rhizomucor pusillus* has a world-wide distribution and is commonly associated with compost heaps.

### ***Rhizomucor pusillus* (Lindt) Schipper**

This thermophilic zygomycete is readily recognizable by its characteristic compact, low growing (2-3 mm high), grey to greyish brown-coloured mycelium and by the development of typical sympodially branched, hyaline to yellow-brown sporangiophores (8-15  $\mu\text{m}$  in diameter), always with a septum below the sporangium. Sporangia are globose (40-60  $\mu\text{m}$  in diameter), each possessing an oval or pear-shaped columella (20-30  $\mu\text{m}$ ), often with a collarete. Sporangiospores are hyaline, smooth-walled, globose to subglobose, occasionally oval (3-5  $\mu\text{m}$ ), and are often mixed with crystalline remnants of the sporangial wall. Chlamydospores are absent. Zygosporangia are rough-walled, reddish brown to black, 45-65  $\mu\text{m}$  in diameter and may be produced throughout the aerial hyphae in matings between compatible isolates. Temperature growth range: minimum 20-27°C; optimum 35-55°C; maximum 55-60°C. There is positive assimilation of sucrose and no thiamine dependence. RG-2 organism.

**Key Features:** zygomycete, growth at 45°C (thermophilic), poorly developed stolons and rhizoids, branching sporangiophores with a septum below the sporangium, dark-coloured sporangia without apophyses and smooth-walled globose to subglobose sporangiospores.



Sporangiophores, collumellae and primitive rhizoids of *R. pusillus*.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range
Fluconazole	>64	Amphotericin B	0.06-0.25
Itraconazole	0.03-0.25	Flucytosine	>256
Posaconazole	0.06-0.25	Voriconazole	2-16

Very limited data, antifungal susceptibility testing of individual strains is recommended. Dannaoui *et al.* (2003), Singh *et al.* (2005) and WCH in-house data.

## ***Rhizopus* Ehrenberg ex Corda.**

The genus *Rhizopus* is characterised by the presence of stolons and pigmented rhizoids, the formation of sporangiophores, singly or in groups from nodes directly above the rhizoids, and apophysate, columellate, multispored, generally globose sporangia. After spore release the apophyses and columella often collapse to form an umbrella-like structure. Sporangiospores are globose to ovoid, one-celled, hyaline to brown and striate in many species. Colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	>64	>64	Amphotericin B	0.03-4	2
Itraconazole	0.25-8	4	Flucytosine	>256	>256
Posaconazole	0.03-8	2	Voriconazole	4->64	>64

Very limited data, antifungal susceptibility testing of individual strains is recommended. Sun *et al.* (2002), Dannaoui *et al.* (2003), Espinel-Ingroff (2001, 2003), Singh *et al.* (2005), Sabatelli *et al.* (2006) and WCH in-house data.

In the past, numerous attempts have been made to clarify the species concepts of the genus *Rhizopus*. Recently, 3 excellent revisions with easy to use keys have been produced by Schipper (1984), Ellis (1985, 1986) and Schipper and Stalpers (2003). Basically, 3 groups have been recognised: the 'stolonifer' group, the 'oryzae' group and the 'microsporus' group. The G-C values of the 3 groups have been defined by Frye and Reinhardt (1993), and temperature growth studies at 30°C, 36°C and 45°C are characteristic for each of the groups.

The 'stolonifer' group has sporangia up to 275 µm in diameter and grows at 30°C, but has a maximum growth temperature of 36°C. Species in this group include *R. sexualis* and *R. stolonifer*. The latter has been unconvincingly implicated in human infection (Ferry and Abedi 1983), although with a maximum growth temperature of only 32°C its pathogenicity is thus questionable.

The 'oryzae' group has been reduced to a single species that is able to grow at 40°C but not at 45°C, and has sporangia not exceeding 240 µm in diameter. There is no doubt that *R. oryzae* and *R. arrhizus* are synonymous, the contentious issue being which species name to use. The taxonomic treatment of Schipper and Stalpers (2003) will be used in this book; however, the synonym *R. arrhizus* is commonly used in the medical literature. *Rhizopus oryzae* is an important human pathogen.

The 'microsporus' group has simple rhizoids, and smaller sporangia up to 100 µm in diameter and grows at both 40 and 45°C. This group contains 4 species: *R. homothallicus*, *R. azygosporus*, *R. schipperae* and *R. microsporus* with the later subdivided into 3 varieties, namely *R. microsporus* var. *microsporus*, *R. microsporus* var. *oligosporus* and *R. microsporus* var. *rhizopodiformis*. All are thermophilic and *R. microsporus* is a well-recognised pathogen of humans and animals.

## ***Rhizopus Ehrenberg ex Corda.***

Differentiation of pathogenic *Rhizopus microsporus* group isolates.

Species	Growth at 45°C	Growth at 50°C	Main species characteristics
<i>R. azygosporus</i>	Good	No	Abundant azygospores
<i>R. microsporus</i> var. <i>microsporus</i>	Good	No	Sporangiospores angular to ellipsoidal and distinctly striate, up to 5–6 µm diam
<i>R. microsporus</i> var. <i>oligosporus</i>	Restricted	No	Sporangiospores globose, up to 9 µm diameter or more, heterogeneous
<i>R. microsporus</i> var. <i>rhizopodiformis</i>	Good	Good	Sporangiospores globose rarely over 5 µm in diameter minutely spinulose
<i>R. schipperae</i>	Good	No	Abundant chlamydospores and restricted sporulation

For descriptions of species, keys to taxa and additional information, see Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Scholer *et al.* (1983), Schipper (1984), Schipper and Stalpers (1984, 2003), Ellis (1985, 1986), Rippon (1988), Kwon-Chung and Bennett (1992), Samson *et al.* (1995); Hoog *et al.* (2000) and Ellis (2005b).

### ***Rhizopus azygosporus* Yuan & Jong**

*Rhizopus azygosporus* is closely related to *R. microsporus* (Yuan and Jong 1984) and has been reported as the causative agent of 3 fatal cases of gastrointestinal infection in premature babies (Woodward *et al.* 1992, Schipper *et al.* 1996). Previously, this fungus was only known from its type culture, which has been isolated from tempeh, a solid fermented soybean food from Indonesia (Yuan and Jong 1984). Colonies are whitish to grey-black, producing pale-brown simple rhizoids. Sporangiohores are brownish, up to 350 µm high and 6–14 µm wide. Sporangia are greyish-black, spherical and 50–100 µm in diameter. Columellae are subglobose to globose. Sporangiospores are ovoid to ellipsoidal 4–5 to 6–7 µm in diameter with faint striations. Azygospores are pale to dark-brown, spherical to subglobose, 30–70 µm in diameter, with coarse conical projections. All strains produce abundant azygospores in unmated isolates as a species characteristic. There is good growth at 45°C with a maximum of 46–48°C. RG-2 organism.

### ***Rhizopus microsporus* var. *microsporus***

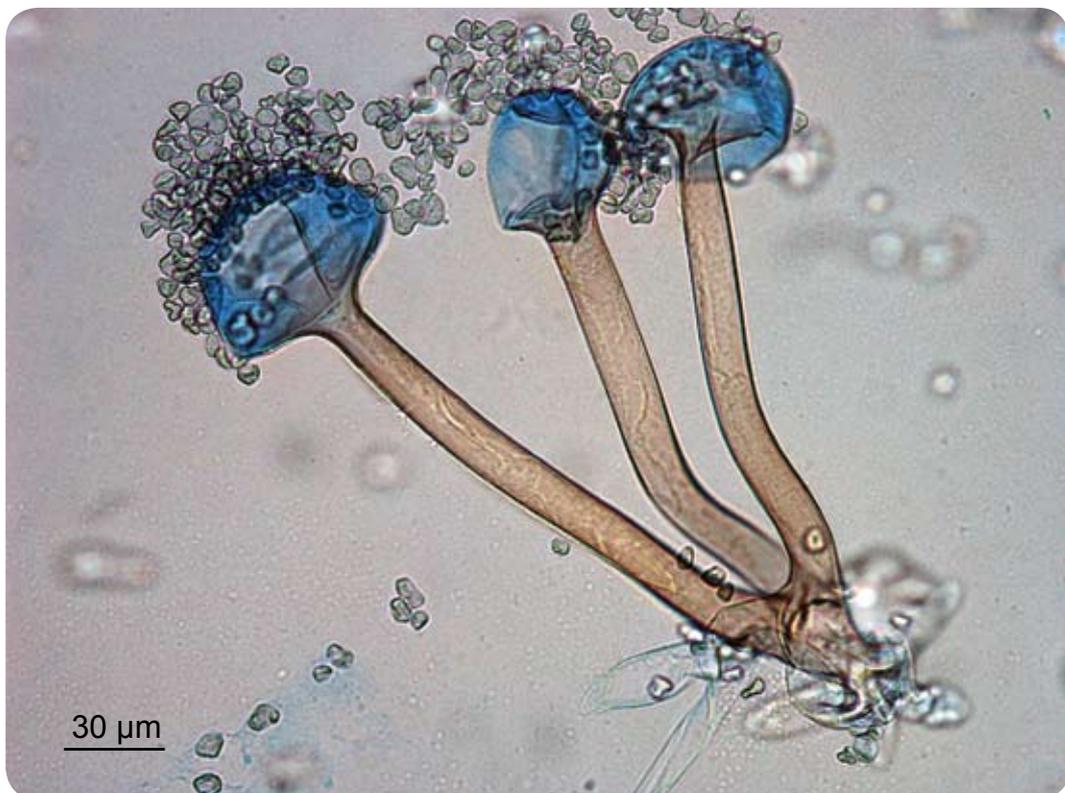
*Rhizopus microsporus* var. *microsporus* is a rare cause of human infection (Kerr *et al.* 1988, Kwon-Chung and Bennett, 1992). Colonies are pale brownish-grey producing simple rhizoids. Sporangiohores are brownish, up to 400 µm high and 10 µm wide, but most are smaller and are produced in pairs. Sporangia are greyish-black, spherical, up to 80 µm in diameter. Columellae are subglobose to globose to conical. Sporangiospores are angular to broadly ellipsoidal to lemon-shaped, quite equal in size, up to 5–6 µm in diameter and are distinctly striate. Zygosporangia, formed by crosses of compatible mating strains, are dark red–brown, spherical, up to 100 µm in diameter, with stellate projections and unequal suspensor cells. There is good growth at 45°C, with a maximum of 46–48°C. RG-2 organism.

***Rhizopus microsporus* var. *oligosporus* (Saito) Schipper & Stalpers**

*Rhizopus microsporus* var. *oligosporus* is a rare cause of human zygomycosis (Tintelnot and Nitsche 1989). Colonies are pale yellowish-brown to grey and sporulation is often poor. Rhizoids are subhyaline and simple. Sporangioophores are brownish, up to 300  $\mu\text{m}$  high and 15  $\mu\text{m}$  wide, with 1-3 produced together. Sporangia are black, spherical, up to 100  $\mu\text{m}$  in diameter. Columellae are subglobose to somewhat conical. Sporangiospores are subglobose to globose, up to 9  $\mu\text{m}$  in diameter, almost smooth, with larger spores often irregular in shape. Chlamydospores are abundant, hyaline, single or in chains, spherical, ellipsoidal or cylindrical, 7-35  $\mu\text{m}$  in diameter. Zygospores are not known. There is growth at 45°C with a maximum of 46–48°C. RG-2 organism.

***Rhizopus microsporus* var. *rhizopodiformis* (Cohn) Schipper & Stalpers**

*Rhizopus microsporus* var. *rhizopodiformis* is the second most frequently isolated zygomycete, accounting for between 10% and 15% of reported human cases (Scholer *et al.* 1983; Kwon-Chung and Bennett 1992). Colonies are dark greyish-brown, up to 10 mm high with simple rhizoids. Sporangioophores are brownish, up to 500  $\mu\text{m}$  high and 8  $\mu\text{m}$  wide, with 1-4 produced together. Sporangia are bluish to greyish-black, spherical and up to 100  $\mu\text{m}$  in diameter. Columellae are pyriform comprising 80% of the sporangium. Sporangiospores are subglobose to globose, quite equal in size, up to 6  $\mu\text{m}$  in diameter and minutely spinulose. Zygospores, when formed by crosses of compatible mating strains, are reddish-brown, spherical, up to 100  $\mu\text{m}$  in diameter, with stellate projections and unequal suspensor cells. There is good growth at 45°C with a maximum of 50–52°C. RG-2 organism.



Sporangia showing sporangiophores, columellae, sporangiospores and rhizoids of *R. microsporus* var. *oligosporus*.

## ***Rhizopus oryzae* Went & Prinsen Geerligs**

**Synonym: *Rhizopus arrhizus* Fischer**

*Rhizopus oryzae* is the most common causative agent of zygomycosis, accounting for some 60% of the reported culture positive cases, and nearly 90% of the rhinocerebral form of infection. Colonies are very fast growing, about 5-8 mm high, with some tendency to collapse, white cottony at first becoming brownish grey to blackish-grey depending on the amount of sporulation. Sporangioophores up to 1500  $\mu\text{m}$  in length and 18  $\mu\text{m}$  in width, smooth-walled, non-septate, simple or branched, arising from stolons opposite rhizoids usually in groups of 3 or more. Sporangia are globose, often with a flattened base, greyish black, powdery in appearance, up to 175  $\mu\text{m}$  in diameter and many spored. Columellae and apophysis together are globose, subglobose or oval, up to 130  $\mu\text{m}$  in height and soon collapsing to an umbrella-like form after spore release. Sporangiospores are angular, subglobose to ellipsoidal, with ridges on the surface, and up to 8  $\mu\text{m}$  in length. No growth at 45°C; good growth at 40°C. RG-2 organism



Culture, sporangia, sporangioophores and rhizoids of *R. oryzae*.

## ***Rhodotorula Harrison***

The genus *Rhodotorula* is characterised by the combination of red or yellow cultures due to the presence of carotenoid pigments, the inability to assimilate inositol and the absence of fermentation. The basidiomycetous nature of yeasts is usually indicated by a positive urease test. The genus *Cryptococcus* is similar to *Rhodotorula* both in production of carotenoid pigments and the presence of capsulated blastoconidia. The distinctive difference between the two is the assimilation of inositol, which is positive in *Cryptococcus*.

*Rhodotorula mucilaginosa* is a common airborne contaminant of skin, lungs, urine and faeces. *R. mucilaginosa* is a known cause of fungal peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). This is usually due to saprophytic colonisation of catheters or dialysis machinery and removal of the source of contamination usually leads to clearing of the symptoms.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Barnett *et al.* (1983), Kreger-Van Rij (1984), Rippon (1988), Kurtzman and Fell (1988) and de Hoog *et al.* (2000).



Culture of *Rhodotorula mucilaginosa*.

## ***Rhodotorula glutinis* (Fresenius) Harrison**

**Culture:** Colonies (SDA) are coral red to salmon-coloured or slightly orange, smooth to wrinkled, highly glossy to semi-glossy. Mucoid to pasty to slightly tough, yeast-like colonies.

**Microscopy:** Ovoidal to globose or more elongate budding yeast-like cells or blastoconidia, 2.3-5.0 x 4.0-10.0 µm.

**India Ink Preparation:** Small capsules present.

**Dalmau Plate Culture on Cornmeal and Tween 80 Agar:** Budding blastoconidia only. No pseudohyphae are formed.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
<b>Fermentation</b>		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	v
Glucose	-	Maltose	+	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	v	L-Rhamnose	v	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	-	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	v
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	v
Trehalose	-	Raffinose	v	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	+	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	-	Galactitol	v	0.1% Cycloheximide	v
Galactose	v	D-Xylose	v	D-Mannitol	v	Growth at 37°C	v

**Key Features:** germ tube negative yeast and sugar assimilation pattern. Common saprophyte however cases of fungemia have been reported. RG-1 organism.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	>64	Amphotericin B	0.06-0.25
Itraconazole	0.5-1	Flucytosine	0.03-0.06
Voriconazole	1-4	Caspofungin	0.25

Very limited data, antifungal susceptibility testing of individual strains is recommended. WCH in-house data only.

## ***Rhodotorula mucilaginosa* (Jorgensen) Harrison**

**Synonym:** *Rhodotorula rubra* (Demme) Lodder.

**Culture:** Colonies (SDA) are coral pink, usually smooth, sometimes reticulate, rugose or corrugated, moist to mucoid, yeast-like colonies.

**Microscopy:** Spherical to elongate budding yeast-like cells or blastoconidia, 2.5-6.5 x 6.5-14.0 µm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmay Plate Culture on Cornmeal and Tween 80 Agar:** Budding blastoconidia only. No pseudohyphae are formed.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	v	L-Arabinose	v	D-Glucitol	v
<b>Fermentation</b>		Sucrose	+	D-Arabinose	v	α-M-D-glucoside	v
Glucose	-	Maltose	v	D-Ribose	v	D-Gluconate	+
Galactose	-	Cellobiose	v	L-Rhamnose	v	DL-Lactate	v
Sucrose	-	Trehalose	+	D-Glucosamine	v	myo-Inositol	-
Maltose	-	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	v
Lactose	-	Melibiose	-	Glycerol	v	D-Glucuronate	-
Trehalose	-	Raffinose	+	Erythritol	v	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	v	Urease	+
Glucose	+	Soluble Starch	-	Galactitol	v	0.1% Cycloheximide	-
Galactose	v	D-Xylose	+	D-Mannitol	v	Growth at 40°C	+

**Key Features:** germ tube negative yeast and sugar assimilation pattern. Common saprophyte however cases of peritonitis and fungemia have been reported. RG-1 organism.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.5->64	>64	Amphotericin B	0.03-1	0.5
Itraconazole	0.25-4	2	Flucytosine	0.03-0.25	0.25
Voriconazole	0.25-4	2	Caspofungin	16	>16

Very limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Cuenca-Estrella *et al.* (2006) and WCH in-house data.

## ***Saccharomyces cerevisiae* Meyen ex Hansen**

The genus *Saccharomyces* is characterised by strong fermentation of at least glucose and the presence of rather large globose to ellipsoidal cells with multilateral budding. Pseudohyphae can be formed, but never true hyphae. Ascospores are globose to ellipsoidal, with a smooth wall, usually one to four per ascus. Lactose and nitrate are not utilised. The species *S. cerevisiae*, commonly known as Baker's yeast, is the most important representative.

**Culture:** Colonies (SDA) are white to cream, smooth, glabrous yeast-like colonies.

**Microscopy:** Large globose to ellipsoidal budding yeast-like cells or blastoconidia, 3.0-10.0 x 4.5-21.0 µm.

**India Ink Preparation:** Negative - No capsules present.

**Dalmu Plate Culture on Cornmeal and Tween 80 Agar:** Usually budding blastoconidia only, however pseudohyphae may be formed rarely.

<b>Physiological Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Germ Tube	-	L-Sorbose	-	L-Arabinose	-	D-Glucitol	-
<b>Fermentation</b>		Sucrose	+	D-Arabinose	-	α-M-D-glucoside	v
Glucose	+	Maltose	+	D-Ribose	-	D-Gluconate	-
Galactose	v	Cellobiose	-	L-Rhamnose	-	DL-Lactate	v
Sucrose	+	Trehalose	+	D-Glucosamine	-	myo-Inositol	-
Maltose	v	Lactose	-	N-A-D-glucosamine	-	2-K-D-gluconate	-
Lactose	-	Melibiose	v	Glycerol	-	D-Glucuronate	-
Trehalose	-	Raffinose	+	Erythritol	-	Nitrate	-
<b>Assimilation</b>		Melezitose	v	Ribitol	-	Urease	-
Glucose	+	Soluble Starch	-	Galactitol	-	0.1% Cycloheximide	-
Galactose	v	D-Xylose	-	D-Mannitol	-	Growth at 37°C	v

**Key Features:** germ tube negative yeast and sugar assimilation pattern. Common food and environmental saprophyte. RG-1 organism.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Barnett *et al.* (1983), Kreger-Van Rij (1984), Rippon (1988), Kurtzman and Fell (1988) and de Hoog *et al.* (2000).

<b>Antifungal</b>	<b>MIC µg/mL</b>		<b>Antifungal</b>	<b>MIC µg/mL</b>	
	<b>Range</b>	<b>MIC<sub>90</sub></b>		<b>Range</b>	<b>MIC<sub>90</sub></b>
Fluconazole	0.125-16	8	Amphotericin B	0.06-2	1
Itraconazole	0.03-4	0.5	Flucytosine	0.03-0.5	0.25
Voriconazole	0.06-0.25	0.125	Caspofungin	1	1

Very limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Cuenca-Estrella *et al.* (2006) and WCH in-house data.

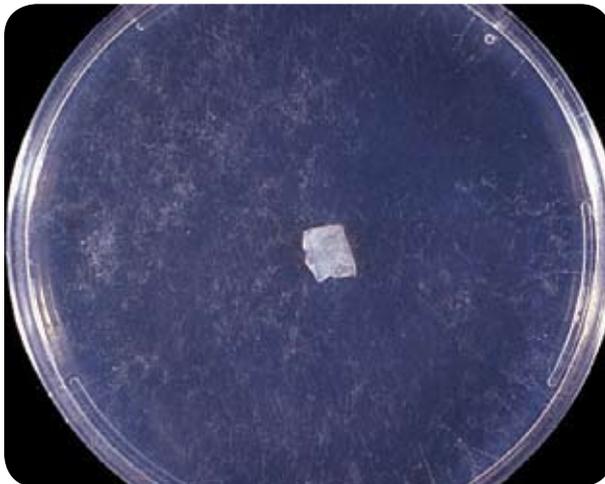
## ***Saksenaea vasiformis* Saksena**

The genus *Saksenaea* is characterised by the formation of flask-shaped sporangia with columellae and simple, darkly pigmented rhizoids. *Saksenaea vasiformis* is the only known species and appears to have a world-wide distribution in association with soil. It is an emerging human pathogen (Holland, 1997) that is most often associated with cutaneous or subcutaneous lesions after trauma. Colonies are fast growing, downy, white with no reverse pigment made up of broad, non-septate hyphae typical of a zygomycetous fungus. Sporangia are typically flask-shaped with a distinct spherical venter and long-neck, arising singly or in pairs from dichotomously branched, darkly pigmented rhizoids. Collumellae are prominent and dome-shaped. Sporangiospores are small, oblong, 1-2 x 3-4 µm, and are discharged through the neck following the dissolution of an apical mucilaginous plug. RG-2 organism.

**Key Features:** zygomycete, unique flask-shaped sporangia, failure to sporulate on primary isolation media.

Laboratory identification of this fungus may be difficult or delayed because of the mould's failure to sporulate on the primary isolation media or on subsequent subculture onto potato dextrose agar. Sporulation may be stimulated by the use of nutrient deficient media, like cornmeal-glucose-sucrose-yeast extract agar, Czapek Dox agar, or by using the agar block method described by Ellis and Ajello (1982), Ellis and Kaminski (1985) and Padhye and Ajello (1988).

For descriptions of species, keys to taxa and additional information see Saksena (1953), Ellis and Hesseltine (1966), Ajello *et al.* (1976), Ellis and Ajello (1982), Ellis and Kaminski (1985), Pritchard *et al.* (1986), Padhye *et al.* (1988), Padhye and Ajello (1988), Goldschmied-Reouven *et al.* (1989), de Hoog *et al.* (2000) and Ellis (2005b).



The agar block method to induce sporulation of *Saksenaea vasiformis* and *Apophysomyces elegans*.

A small block of agar is cut from a well established culture grown on PDA and is placed in the centre of petri dish containing 1% agar in distilled water. After 21 days at 26°C look for sporangium formation at the periphery of the petri dish.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range
Fluconazole	>64	Amphotericin B	0.125-2
Itraconazole	0.015-0.3	Flucytosine	>256
Posaconazole	0.015-0.25	Voriconazole	0.5-4

Very limited data, poor sporulation, antifungal susceptibility testing of individual strains is recommended. Sun *et al.* (2002) and WCH in-house data.

**Saksenaea vasiformis** Saksena



Sporangium of *Saksenaea vasiformis*.

## ***Scedosporium apiospermum/aurantiacum* Complex**

Recent genetic studies have shown *Scedosporium apiospermum* to be a species complex and two species of medical importance have now been recognised: *S. apiospermum* and *S. aurantiacum* (Gilgado *et al.* 2005).

### ***Scedosporium apiospermum* (Saccardo) Castellani and Chalmers**

Colonies are fast growing, greyish-white, suede-like to downy with a greyish-black reverse. Numerous single-celled, pale-brown, broadly clavate to ovoid conidia, 4-9 x 6-10 µm, rounded above with truncate bases are observed. Conidia are borne singly or in small groups on elongate, simple or branched conidiophores or laterally on hyphae. Conidial development can be described as annellidic, although the annellations (ring-like scars left at the apex of an annellide after conidial secession) are extremely difficult to see. Optimum temperature for growth is 30-37°C. RG-2 organism.

Ascocarp formation may be stimulated on cornmeal agar or other nutrient deficient media, however it should be noted that many isolates may fail to produce cleistothecia. Cleistothecia (non-ostiolate ascocarps) are yellow-brown to black, spherical, are mostly submerged in the agar and are composed of irregularly interwoven brown hyphae. When crushed cleistothecia release numerous, faintly brown, ellipsoidal ascospores, 4-5 x 7-9 µm in size. A *Graphium* synanamorph may also be present.

The teleomorph is currently referred to as *Pseudallescheria boydii*, however as all species of *Pseudallescheria* have *Scedosporium* anamorphs, it is presumptive to use the teleomorph name to describe this fungus without seeing cleistothecia (ascocarps). Until the taxonomy of *Pseudallescheria* is resolved, it is recommended that laboratories use the anamorphic name *Scedosporium apiospermum* when describing this fungus.

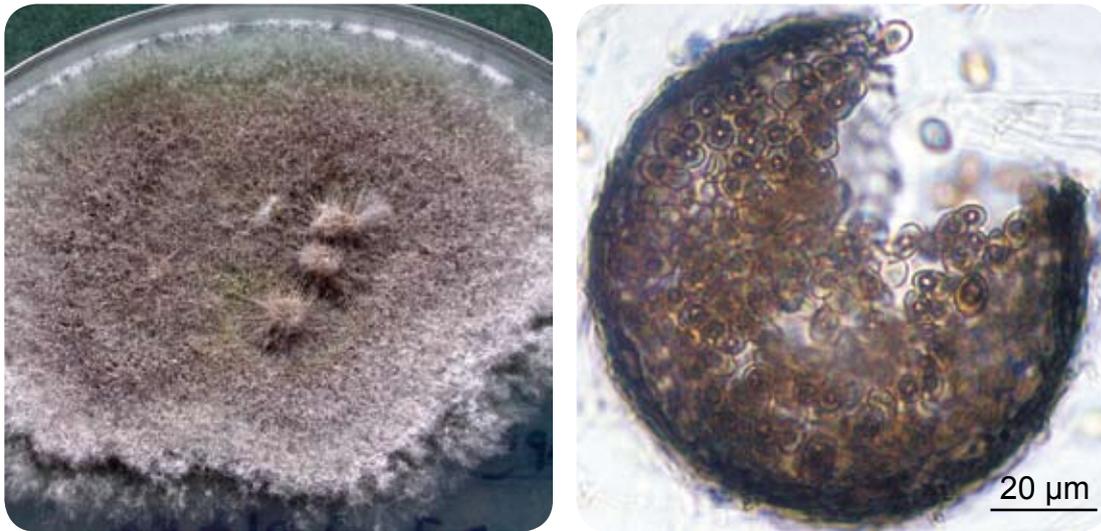
### ***Scedosporium aurantiacum* Gilgado *et al.***

All isolates produce a light yellow diffusible pigment on PDA after a few days incubation. Conidiogenous cells and conidia are similar in shape and size to *S. apiospermum*, and the two can best be distinguished by genetic analysis. Conidiogenous cells arising from undifferentiated hyphae are cylindrical to slightly flask-shaped, producing slimy heads of one-celled, smooth-walled, subhyaline, obovoid or sub-cylindrical conidia. 5-14 x 2-5 µm. Erect synnemata (a *Graphium* synanamorph) may be present in some isolates, but the teleomorph is unknown. Optimum temperature for growth 37-40°C, max 45°C. RG-2 organism.

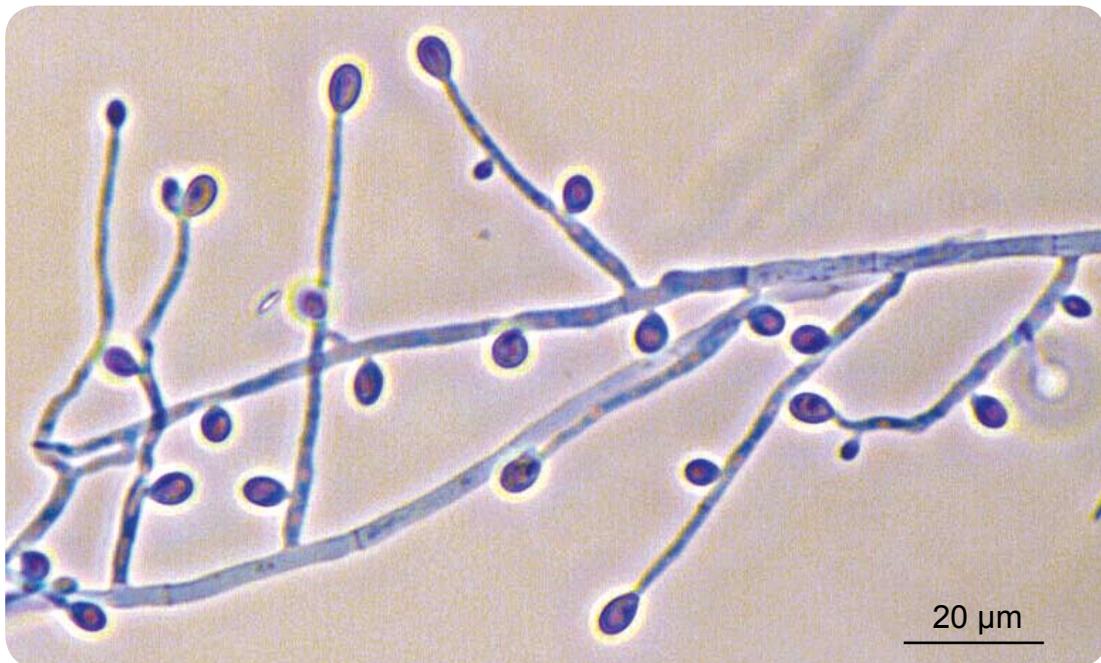
*S. apiospermum* and *S. aurantiacum* appear to be common soil fungi capable of causing a spectrum of diseases similar in terms of variety and severity to those caused by *Aspergillus* collectively referred to as Pseudallescheriasis. The vast majority of infections are mycetomas, the remainder include infections of the eye, ear, central nervous system, internal organs and more commonly the lungs.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Domsch *et al.* (1980), McGinnis *et al.* (1982), Campbell and Smith (1982), Rippon (1988), de Hoog *et al.* (2000) and Gilgado *et al.* (2005).

***Scedosporium apiospermum* (Saccardo) Castellani and Chalmers**



Culture and a cleistothecium of *Pseudallescheria boydii*.



Conidiophores (annellides) and conidia of *Scedosporium apiospermum*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.06-4	2	Amphotericin B	1-16	16
Voriconazole	0.03-1	0.5	Posaconazole	0.5-2	1

Good data available. McGinnis and Pasarell (1998), Espinel-Ingroff (2001, 2003), Espinel-Ingroff *et al.* (2001) and Cuenca-Estrella *et al.* (2006). MIC<sub>90</sub>s from the Australian *Scedosporium* Study.

***Scedosporium aurantiacum* Gilgado et al.**



Culture reverse (PDA) of *S. apiospermum* (left) and *S. aurantiacum* (right) showing the production of a light yellow diffusible pigment that is typical of *S. aurantiacum*.



Conidiophores (annellides) and conidia of *Scedosporium aurantiacum*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	0.25-2	1	Amphotericin B	2-16	16
Voriconazole	0.03-0.5	0.25	Posaconazole	0.125-1	0.5
Good data available. Australian <i>Scedosporium</i> Study.					

## ***Scedosporium prolificans* (Hennebert & Desai) Gueho & de Hoog**

**Synonym:** *Scedosporium inflatum* Malloch & Salkin

Colonies are rapid growing, flat, spreading, olive-grey to black and have a suede-like to downy surface texture. Conidia are borne in small groups on distinctive basally swollen, flask-shaped annellides, which occur singly or in clusters along the vegetative hyphae. Conidia are single-celled, hyaline to pale-brown, ovoid to pyriform, 2-5 x 3-13  $\mu\text{m}$  (average 3.4 x 5.3  $\mu\text{m}$ ), and have smooth thin walls. RG-2 organism.

**Key Features:** dematiaceous hyphomycete with initial black pasty colony, basally swollen (inflated) annellides and no growth on media containing cycloheximide (actidione).

For descriptions of species, keys to taxa and additional information see Malloch and Salkin (1984), Salkin *et al.* (1988), Rippon (1988), Wilson *et al.* (1990), Gueho and de Hoog (1991) and de Hoog *et al.* (2000).



Conidiophores (annellides) and conidia of *Scedosporium prolificans*.

Antifungal	MIC $\mu\text{g/mL}$		Antifungal	MIC $\mu\text{g/mL}$	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Itraconazole	1-32	>8	Amphotericin B	1-16	>8
Voriconazole	0.5-32	>8	Posaconazole	>8	>8

Good data available. McGinnis and Pasarell (1998), Espinel-Ingroff (2001, 2003), Espinel-Ingroff *et al.* (2001), Sabatelli *et al.* (2006), Cuenca-Estrella *et al.* (2006) and the Australian *Scedosporium* Study.

## ***Schizophyllum commune* Fries**

Colonies on 2% malt extract agar are spreading, woolly, whitish to pale greyish-brown, soon forming macroscopically visible fruiting bodies. Although some isolates may take up to 12 weeks to form fruiting bodies. Fruit bodies are sessile, kidney-shaped, lobed with split gills on the lower side. Hyphae are hyaline, wide and have clamp connections (although many primary clinical isolates are monokaryotic and will therefore not produce clamp connections). Basidia bear 4 basidiospores on erect sterigmata. Basidiospores hyaline, smooth-walled, elongate with lateral scar at lower end, 6-7 x 2-3  $\mu\text{m}$ .

*Schizophyllum* is a common bracket fungus on rotten wood, and is an occasional human pathogen, principally associated with sinusitis, allergic bronchopulmonary mycosis and as a contaminant from respiratory specimens. RG-1 organism.

**Note:** many clinical isolates of *S. commune* are monokaryotic and therefore do not show clamp connections, therefore any white, rapidly growing, sterile isolate showing good growth at 37°C with tolerance to benomyl, susceptibility to cycloheximide, and a pronounced odour should be suspected of being *S. commune* (Sigler *et al.*, 1995).

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Rippon (1988), Sigler *et al.* (1995) and de Hoog *et al.* (2000).



Basidiocarps of *Schizophyllum commune* on malt extract agar.

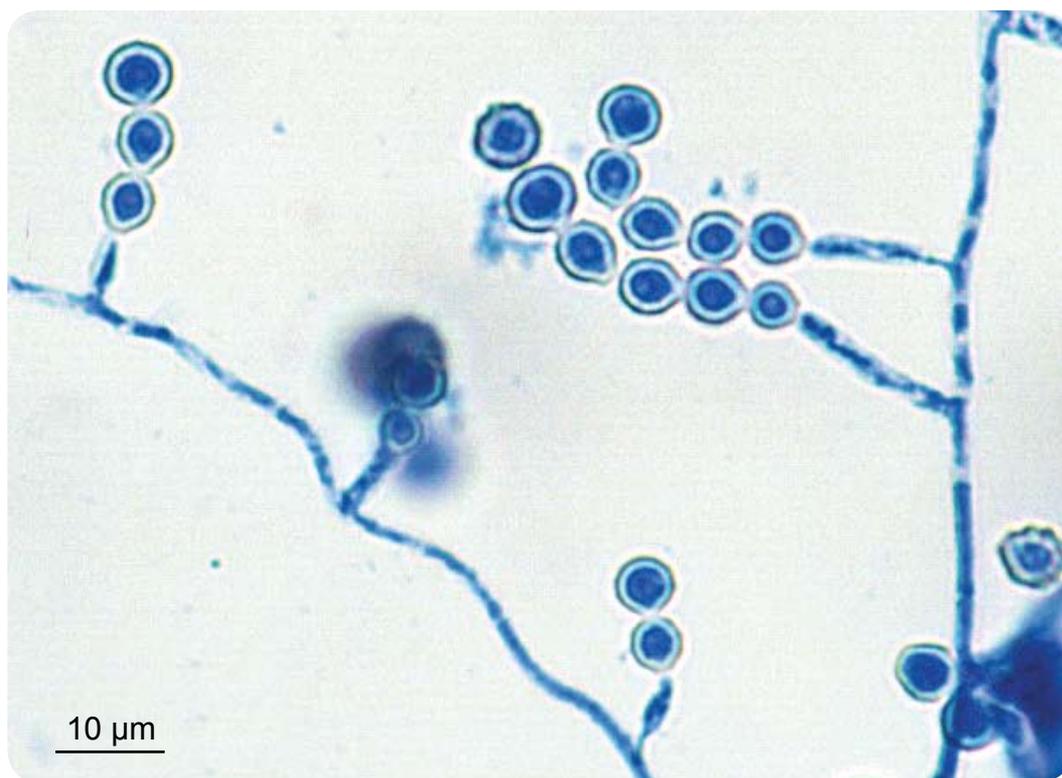
## Scopulariopsis Bain

Colonies are fast growing, varying in colour from white, cream, grey, buff to brown, black, but are predominantly light brown. Microscopic morphology shows chains of single-celled conidia produced in basipetal succession from by a specialised conidigenous cell called an annellide. Once again, the term basocatenate can be used to describe such chains of conidia where the youngest conidium is at the basal end of the chain. In *Scopulariopsis*, annellides may be solitary, in groups, or organised into a distinct penicillus. Conidia are globose to pyriform, usually truncate, with a rounded distal portion, smooth to rough, and hyaline to brown in colour.

Most members of the genus *Scopulariopsis* are soil fungi, however a few, in particular *S. brevicaulis*, have been reported as causative agents of onychomycosis and hyalohyphomycosis. RG-2 for species isolated from humans.

**Key Features:** hyphomycete, conidia often shaped like light globes, basocatenate arising from annellides.

For descriptions of species, keys to taxa and additional information see Morton and Smith (1963), Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), Samson *et al.* (1995) and de Hoog *et al.* (2000).



Conidiophores (annellides) and conidia of *Scopulariopsis brevicaulis*.

Antifungal	MIC µg/mL	Antifungal	MIC µg/mL	Antifungal	MIC µg/mL
	Range		Range		Range
Amphotericin B	2-16	Itraconazole	32	Voriconazole	2-8

Very limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis and Pasarell (1998) and WCH in-house data.

## ***Sepedonium* Link ex Greville**

Colonies are moderately fast growing, usually white to golden yellow, suede-like to downy, becoming fluffy with age. Conidiophores are hyaline and non-specialised, resembling short branches of the vegetative hyphae. Conidia are terminal, solitary, or in clusters, one-celled, globose to ovoid, 7 to 17  $\mu\text{m}$ , hyaline to amber, smooth to verrucose and usually with a thick wall.

The microscopic morphology of *Sepedonium* isolates resembles that of *Histoplasma capsulatum*. Therefore, positive identification of the latter, requires conversion of the mould form to the yeast phase by growth at 37°C on enriched media or by exoantigen test. RG-1 organism.

**Key Features:** hyphomycete, producing large, thick-walled, one-celled, verrucose, globose, terminal macroconidia from non-specialised conidiophores, resembling *Histoplasma capsulatum*.

For descriptions of species, keys to taxa and additional information see McGinnis (1980) and Rippon (1988).



Conidia of *Sepedonium*.

## ***Sporothrix schenckii* Hektoen & Perkins**

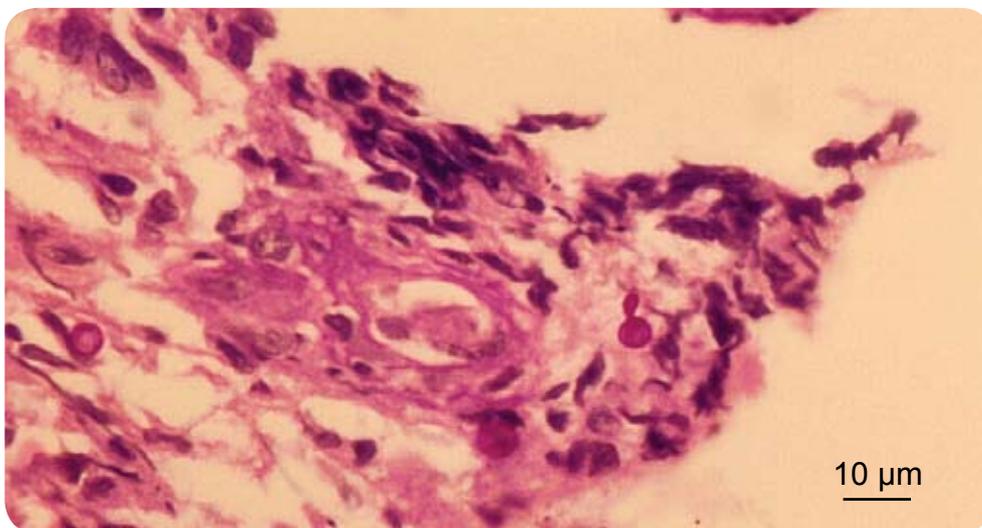
Colonies (SDA) at 25°C, are slow growing, moist and glabrous, with a wrinkled and folded surface. Some strains may produce short aerial hyphae and pigmentation may vary from white to cream to black. Conidiophores arise at right angles from thin septate hyphae and are usually solitary, erect and tapered toward the apex. Conidia are formed in clusters on tiny denticles by sympodial proliferation at the apex of the conidiophore, their arrangement often suggestive of a flower. As the culture ages, conidia are subsequently formed singly along the sides of both conidiophores and undifferentiated hyphae. Conidia are ovoid or elongated, 3-6 × 2-3 µm, hyaline, one-celled and smooth-walled. In some isolates, solitary, darkly-pigmented, thick-walled, one-celled, obovate to angular conidia may also be observed along the hyphae.

On brain heart infusion agar containing blood at 37°C, colonies are glabrous, white to greyish-yellow and yeast-like consisting of spherical or oval budding yeast cells.

*Sporothrix schenckii* is a dimorphic fungus and has a world-wide distribution, particularly in tropical and temperate regions. It is commonly found in soil and on decaying vegetation and is a well known pathogen of humans and animals. Sporotrichosis is primarily a chronic mycotic infection of the cutaneous or subcutaneous tissues and adjacent lymphatics characterised by nodular lesions which may suppurate and ulcerate. Infections are caused by the traumatic implantation of the fungus into the skin, or very rarely, by inhalation into the lungs. Secondary spread to articular surfaces, bone and muscle is not infrequent, and the infection may also occasionally involve the central nervous system, lungs or genitourinary tract. RG-2 organism.

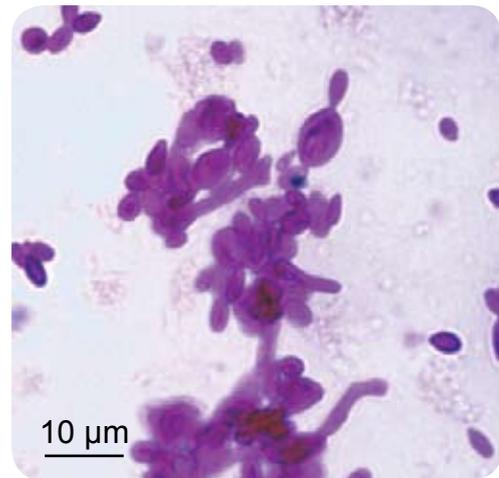
**Key Features:** hyphomycete characterised by thermal dimorphism and clusters of ovoid, denticulate conidia produced sympodially on short conidiophores.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Domsch *et al.* (1980), de Hoog *et al.* (1985), Rippon (1988) and de Hoog *et al.* (2000).

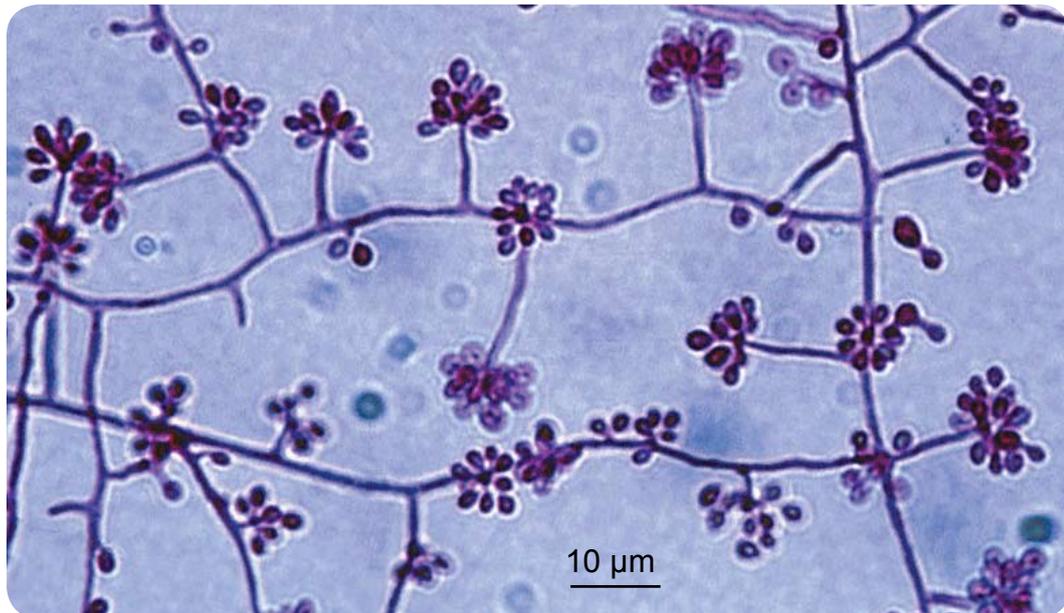


Periodic Acid-Schiff (PAS) stained tissue section showing budding yeast-like cells of *S. schenckii*.

### *Sporothrix schenckii* Hektoen & Perkins



Culture on SDA at 25°C and budding yeast cells in BHI at 37°C.



Conidiophores and conidia of *Sporothrix schenckii* on SDA at 25°C.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	32->64	>64	Amphotericin B	0.06->16	>16
Itraconazole	0.03->16	0.5 (4)	Flucytosine	4->64	>64
Posaconazole	0.125-4	2	Caspofungin	1->8	nd
Voriconazole	0.125->16	4 (16)	Anidulafungin	0.25->8	nd

Limited data, antifungal susceptibility testing of individual strains is recommended. McGinnis *et al.* (2001), Espinel-Ingroff *et al.* (2001), Espinel-Ingroff (2003), Gonzales *et al.* (2005) and Alvarado-Ramirez and Torres-Rodriguez (2007). (nd = not done).

## ***Stemphylium* Wallroth**

Colonies are rapid growing, brown to olivaceous-black or greyish and suede-like to floccose. Microscopically, solitary, darkly pigmented, terminal, multicellular conidia (dictyoconidia) are formed on a distinctive conidiophore with a darker terminal swelling. Note: the conidiophore proliferates percurrently through the scar where the terminal conidium (poroconidium) was formed. Conidia are pale to mid-brown, oblong, rounded at the ends, ellipsoidal, obclavate or subspherical and are smooth or in part verrucose. *Stemphylium* should not be confused with *Ulocladium* which produces similar dictyoconidia from a sympodial conidiophore, not from a percurrent conidiogenous cell as in *Stemphylium*. RG-1 organism.

**Key Features:** dematiaceous hyphomycete producing darkly pigmented, dictyoconidia from the swollen end of a percurrent conidiophore.

For descriptions of species, keys to taxa and additional information see Ellis (1971 and 1976), Rippon (1988) and de Hoog *et al.* (2000).



Conidiophores and conidia of *Stemphylium*.

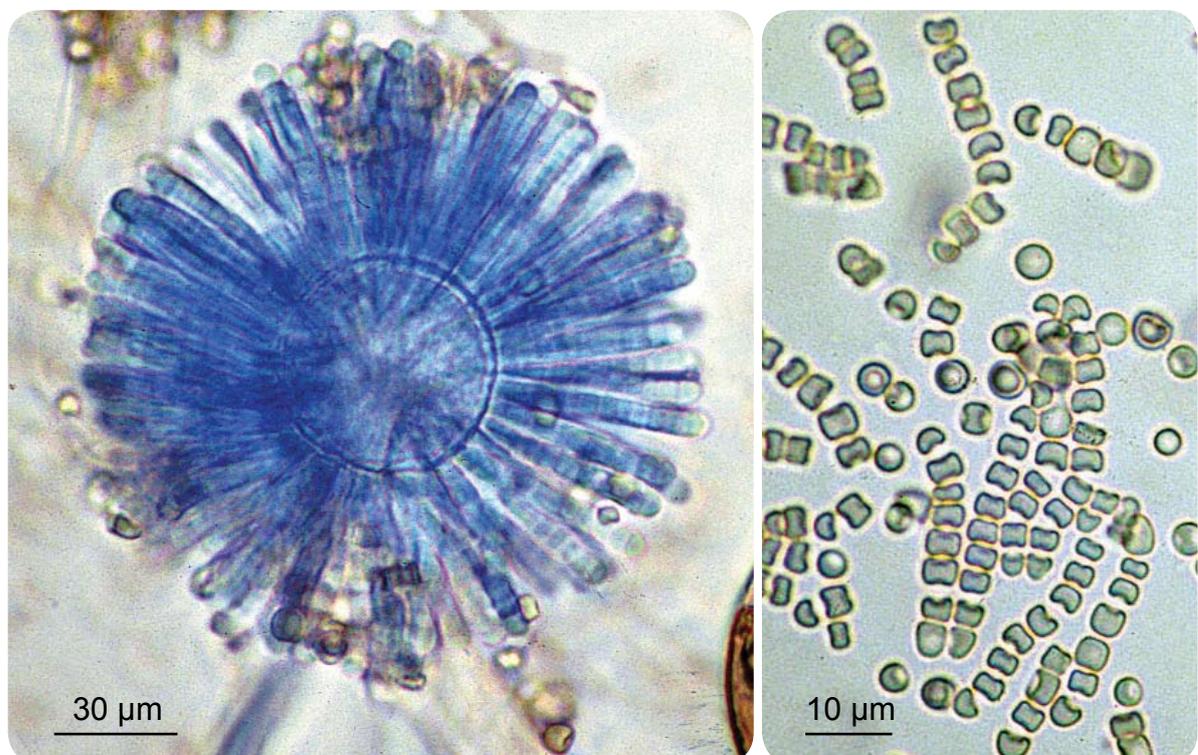
## *Syncephalastrum* Schröter

The genus *Syncephalastrum* is characterised by the formation of cylindrical merosporangia on a terminal swelling of the sporangiophore. Sporangiospores are arranged in a single row within the merosporangia. *Syncephalastrum racemosum* is the type species of the genus and a potential human pathogen; however, well-documented cases are lacking. It is found mainly from soil and dung in tropical and subtropical regions. It can also be a difficult laboratory aerial contaminant. The sporangiophore and merosporangia of *Syncephalastrum* species may also be mistaken for an *Aspergillus* species, if the isolate is not looked at carefully.

Colonies are very fast growing, cottony to fluffy, white to light grey, becoming dark grey with the development of sporangia. Sporangiophores are erect, stolon-like, often producing adventitious rhizoids, and show sympodial branching (racemose branching) producing curved lateral branches. The main stalk and branches form terminal, globose to ovoid vesicles which bear finger-like merosporangia directly over their entire surface. At maturity, merosporangia are thin-walled, evanescent and contain 5-10(18) globose to ovoid, smooth-walled sporangiospores (merospores). Optimum growth temperature 20-40°C. RG-2 organism.

**Key Features:** zygomycete producing sympodially branching sporangiophores with terminal vesicles bearing merosporangia.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980), Onions *et al.* (1981), Rippon (1988), Samson *et al.* (1995), Hoog *et al.* (2000) and Ellis (2005b).



Terminal vesicle, merosporangia and merospores of *S. racemosum*.

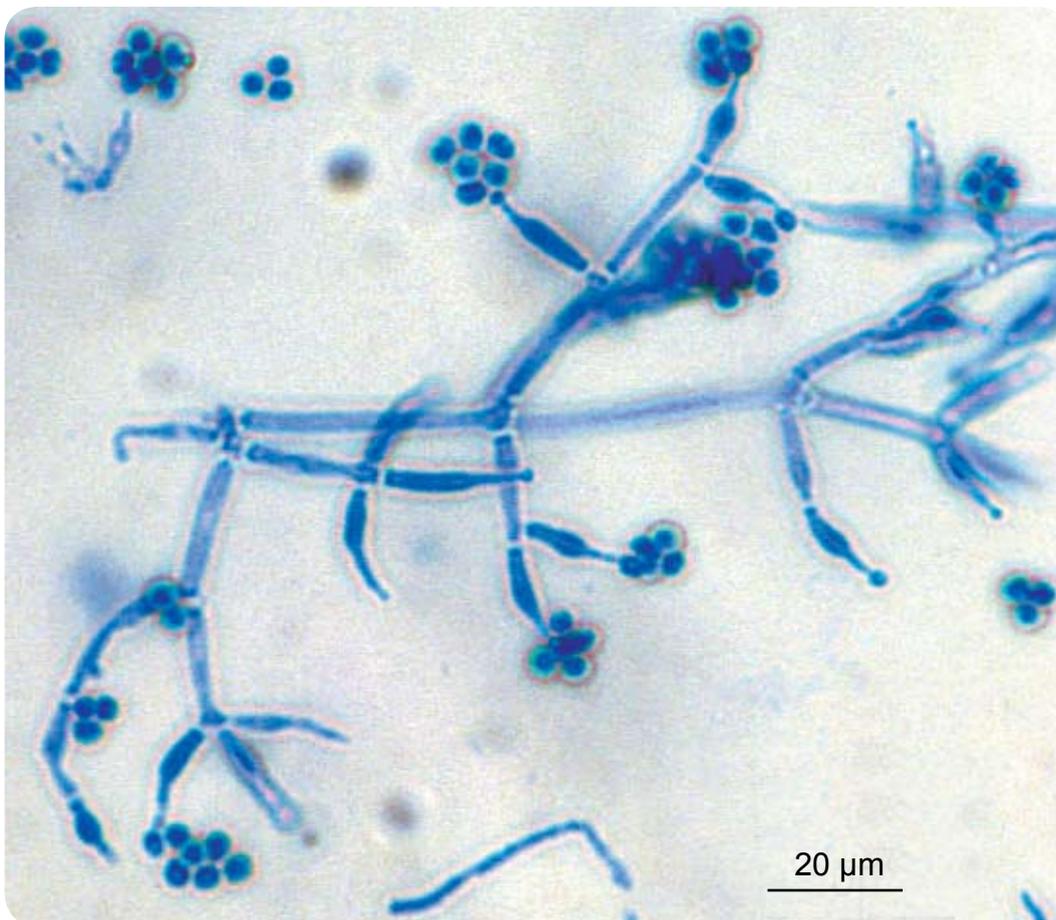
## ***Trichoderma* Persoon ex Grey**

Colonies are fast growing, at first white and downy, later developing yellowish-green to deep green compact tufts, often only in small areas or in concentric ring-like zones on the agar surface. Conidiophores are repeatedly branched, irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask-shaped phialides. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of the phialides.

*Trichoderma* is a very common genus especially in soil and decaying wood. *Gliocladium* (with strongly convergent phialides) and *Verticillium* (with straight and moderately divergent phialides) are closely related genera. *Trichoderma* is an RG-1 organism.

**Key Features:** hyphomycete with repeatedly branched conidiophores bearing clusters of divergent, flask-shaped phialides.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), Samson *et al.* (1995), de Hoog *et al.* (2000).



Phialides and conidia of *Trichoderma harzianum*.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range		Range
Amphotericin B	0.5-2	Itraconazole	2-16	Voriconazole	0.25-2

Very limited data, antifungal susceptibility testing of individual strains is recommended. Espinel-Ingroff (2001) and WCH in-house data.

## ***Trichophyton* Malmsten**

The genus *Trichophyton* is characterised by the development of both smooth-walled macro- and microconidia. Macroconidia are mostly borne laterally directly on the hyphae or on short pedicels, and are thin- or thick-walled, clavate to fusiform, and range from 4-8 x 8-50 µm in size. Macroconidia are few or absent in many species. Microconidia are spherical, pyriform to clavate or of irregular shape and range from 2-3 x 2-4 µm in size. The presence of microconidia distinguishes this genus from *Epidermophyton* and the smooth-walled, mostly sessile macroconidia separate it from *Microsporum*. Twenty species have been recognised, however only the more common species are included in these descriptions.

In practice, two groups may be recognised on direct microscopy:

1. Those species that usually produce microconidia, macroconidia may or may not be present i.e. *T. rubrum*, *T. interdigitale*, *T. mentagrophytes*, *T. equinum*, *T. erinacei*, *T. tonsurans*, *T. terrestre* and to a lesser extent *T. verrucosum*, which may produce conidia on some media; and
2. Those species that usually do not produce conidia. Chlamydoconidia or other hyphal structures may be present, but microscopy is generally non-diagnostic; i.e. *T. verrucosum*, *T. violaceum*, *T. concentricum*, *T. schoenleinii* and *T. soudanense*.

Many laboratories seem to have difficulty in distinguishing between species of *Trichophyton*, especially isolates of *T. rubrum*, *T. interdigitale*, *T. mentagrophytes* and *T. tonsurans*. Basically, the laboratories which consistently identify these fungi correctly do more work and use additional media and/or confirmatory tests. However, it must be stressed that no one single test is infallible, dermatophyte species are very variable organisms and many characteristics either overlap or are inconsistent.

The Mycology Unit at the Adelaide Women's and Children's Hospital uses a dermatophyte identification scheme, devised by the late Geraldine Kaminski, comprising 6 different media to help identify and differentiate the various species and strains of *Trichophyton*. The media in this scheme are Littman Oxgall agar, Lactrimel agar, Sabouraud's agar with 5% NaCl, 1% Peptone agar, *Trichophyton* agar No. 1, and hydrolysis of urea (see appendix for details).

**Note:** species concepts in dermatophytes are currently in a state of flux. Recent molecular studies have shown that many species appear to be clonal and that there is little correlation between "genetic" and "phenotypic" species (Graser *et al.* 2006). The descriptions and species concepts provided in this publication are based on traditional morphological criteria which may not correspond to molecular identification results.

For description of species, keys to taxa and additional information see Rebell and Taplin (1970), Ajello (1972), Vanbreusegham *et al.* (1978), Rippon (1988), McGinnis (1980), Domsch *et al.* (1980), Kane *et al.* (1997) and de Hoog *et al.* (2000).

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Griseofulvin	0.06-4	1-2	Amphotericin B	0.03-16	0.5-1
Itraconazole	0.01-8	0.25-0.5	Fluconazole	0.06->64	32
Terbinafine	0.01-16	0.06	Voriconazole	0.007-8	0.25
Fernandez-Torres <i>et al.</i> (2001), Sabatelli <i>et al.</i> (2006), Santos and Hamdan (2006).					

## *Trichophyton ajelloi* (Vanbreuseghem) Ajello

**Teleomorph:** *Arthroderma uncinatum* Dawson and Gentles

Colonies are usually flat, powdery, cream to tan to orange-tan in colour, with a blackish-purple submerged fringe and reverse. Macroconidia are numerous, smooth, thick-walled, elongate, cigar-shaped, 29-65 x 5-10  $\mu\text{m}$ , and multiseptate with up to 9 or 10 septa. Microconidia are usually absent, but when present are ovate to pyriform in shape. RG-1 organism.

*Trichophyton ajelloi* is a geophilic fungus with a world-wide distribution which may occur as a saprophytic contaminant on humans and animals. Infections in man and animals doubtful. Not known to invade hair *in vivo*, but produces hair perforations *in vitro*.

**Key Features:** culture characteristics, macroconidial morphology, urease positive and good growth on Sabouraud's 5% salt agar.



Culture and macroconidia of *Trichophyton ajelloi*.

## ***Trichophyton concentricum* Blanchard**

**Teleomorph:** *Arthroderma benhamiae* Ajello & Chang.

Colonies (SDA) are slow growing, raised and folded, glabrous becoming suede-like, mostly white to cream-coloured, but sometimes orange-brown-coloured, often deeply folded into the agar which may produce splitting of the medium in some cultures. Reverse is buff to yellow-brown to brown in colour. Cultures consist of broad, much-branched, irregular, often segmented, septate hyphae which may have “antler” tips resembling *T. schoenleinii*. Chlamydospores are often present in older cultures. Microconidia and macroconidia are not usually produced, although some isolates will produce occasional clavate to pyriform microconidia. Note: hyphal segments may artificially resemble macroconidia. RG-2 organism.

### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** Small, heaped and folded, blue-grey, suede to glabrous colonies with no reverse or diffusible pigment.

**Lactritmel Agar:** Colonies tend to be flatter, dull-white, suede-like, almost glabrous with a yellow-brown reverse pigment. Microscopic morphology is similar to that described above, however occasional clavate to pyriform microconidia may be present. Note: occasional, small, slender, smooth thin-walled macroconidia with 2-3 septa have been reported on specialised media like lactritmel agar, bean pod agar and hay infusion agar.

**Sabouraud's Dextrose Agar with 5% NaCl:** Small, stunted, heaped and folded, cream to buff, glabrous colonies with no reverse pigment.

**1% Peptone Agar:** Flat, white, suede-like colony with a pale yellow-brown reverse.

**Hydrolysis of Urea:** Negative after 7 days

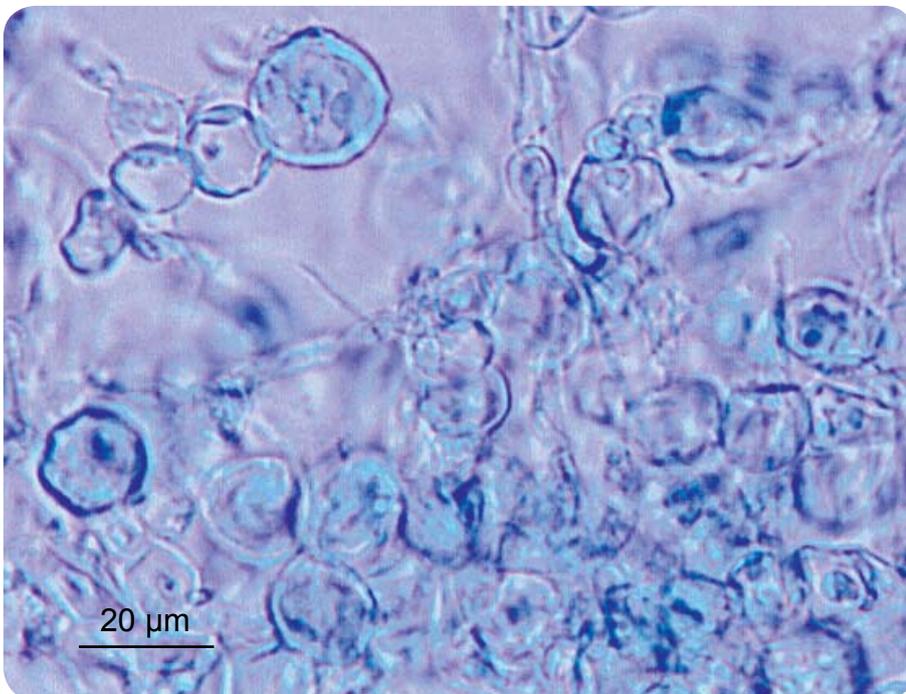
**Vitamin Free Agar (Trichophyton Agar No.1):** Growth occurs on vitamin free agar (T1) but is usually slightly better on media containing thiamine i.e. T3 = T1 + thiamine and inositol, and T4 = T1 + thiamine. The slight enhancement of growth in the presence of thiamine helps to distinguish *T. concentricum* from *T. schoenleinii*, although this does not occur in all strains.

**Hair Perforation Test:** Negative at 28 days.

*Trichophyton concentricum* is an anthropophilic fungus which causes chronic widespread non-inflammatory tinea corporis known as tinea imbricata because of the concentric rings of scaling it produces. It is not known to invade hair. Infections among Europeans are rare. Distribution is restricted to the Pacific Islands of Oceania, South East Asia and Central and South America.

**Key Features:** hyphomycete (dermatophyte), clinical disease, geographical distribution and culture characteristics.

### *Trichophyton concentricum* Blanchard



*T. concentricum* on mycobiologic agar showing a typical slow growing, heaped and folded, glabrous to suede like colony. Microscopic morphology of *T. concentricum* showing the formation of typical “balloon-shaped” chlamydospores. Note: microconidia and macroconidia are usually not produced.

## *Trichophyton equinum* (Matruchot & Dassonville) Geddoelst

**Teleomorph:** *Arthroderma vanbreuseghemii* Takashio

Colonies (SDA) are usually flat, but some may develop gentle folds or radial grooves, white to buff in colour, suede-like to downy in texture, and are similar to *T. mentagrophytes*. Cultures usually have a deep-yellow submerged fringe and reverse which later becomes dark red in the centre. Microscopically, abundant microconidia which may be clavate to pyriform and sessile or spherical and stalked are formed laterally along the hyphae. Macroconidia are only rarely produced, but when present are clavate, smooth, thin-walled and of variable size. Occasional nodular organs may be present and the microconidia often undergo a transformation to produce abundant chlamydospores in old cultures. RG-2 organism.

### Kaminski's Dermatophyte Identification Scheme

**Littman Oxgall Agar:** Compact, raised velvety white surface with yellowish reverse and diffusing yellow pigment.

**Lactrimel Agar:** Flat spreading, white to cream-coloured, powdery to granular surface with a central downy papilla, and deep brownish red reverse. Microscopic morphology as described above for the primary culture.

**Sabouraud's Dextrose Agar with 5% NaCl:** An extremely stunted, slow growing dark brown colony with a few submerged brown hyphae.

**1% Peptone Agar:** Flat white to cream, suede-like surface with raised white downy centre and pale yellow reverse.

**Hydrolysis of Urea:** Positive in 4-5 days.

**Nutritional Tests on Trichophyton Agars:** Most strains require nicotinic acid (Fig. 1) for growth except those from Australia and New Zealand, which are autotrophic (Fig. 2). T1 = vitamin free agar, T5 = vitamin free + nicotinic acid agar.

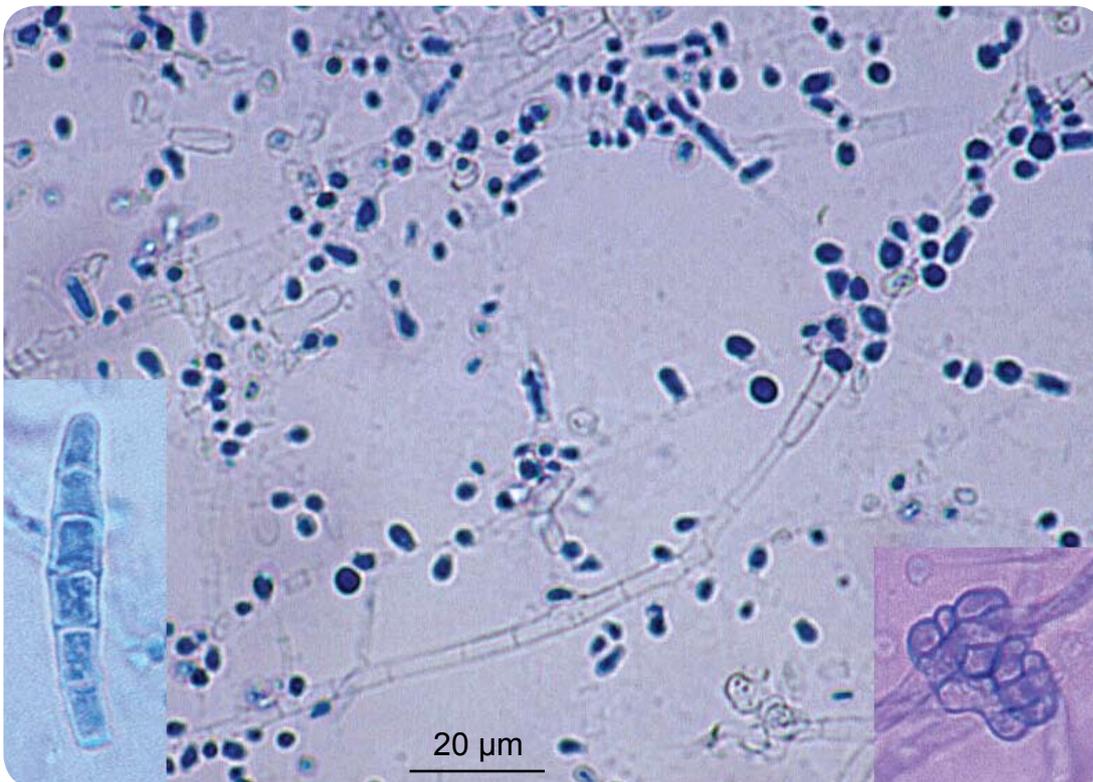
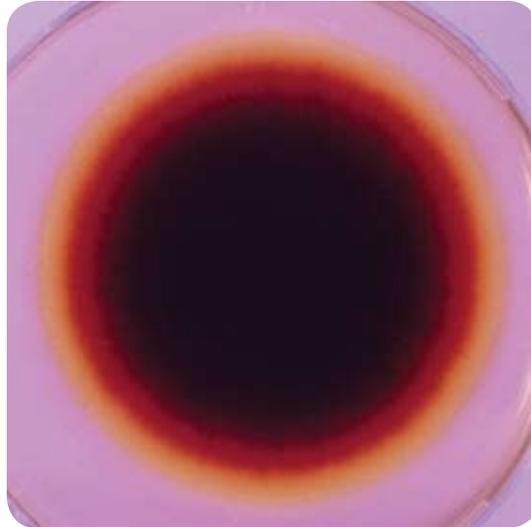


**Hair Perforation Test:** Negative; but positive for the *autotrophicum* strains.

**Key Features:** microscopic morphology, culture characteristics, nicotinic acid requirement and clinical lesions in horses.

## ***Trichophyton equinum* (Matruchot & Dassonville) Gedoelst**

*Trichophyton equinum* is a zoophilic fungus causing ringworm in horses and rare infections in humans. It has a world-wide distribution except for the *autotrophicum* strain which is restricted to Australia and New Zealand. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light.



Culture, microconidia, macroconidia and nodular organs of *T. equinum*.

## ***Trichophyton erinacei* (Smith & Marples) Quaife**

**Teleomorph:** *Arthroderma benhamiae* Ajello & Chang.

Colonies (SDA) are white, flat, powdery, sometimes downy to fluffy with a brilliant lemon-yellow reverse. Numerous large clavate microconidia are borne on the sides of hyphae. Macroconidia are smooth-walled, two- to six-celled, clavate, variable in size, and may have terminal appendages. Macroconidia are much shorter than those seen in *T. mentagrophytes*. RG-2 organism.

### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** White, downy colony with yellowish-green diffusible pigment.

**Lactritmel Agar:** White suede-like to powdery colony with brilliant yellow reverse. Numerous large slender clavate microconidia.

**Sabouraud's Dextrose Agar with 5% NaCl:** White folded suede-like to powdery colony with no reverse pigment.

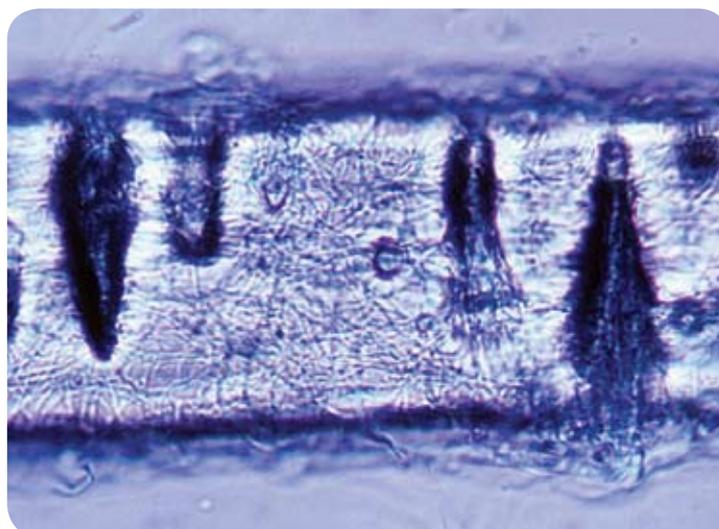
**1% Peptone Agar:** White, suede-like to powdery colony with pale yellow reverse.

**Hydrolysis of Urea:** Negative at 7 days.

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements. Colonies are white suede-like to powdery with no reverse pigment.

**Hair Perforation Test:** Positive

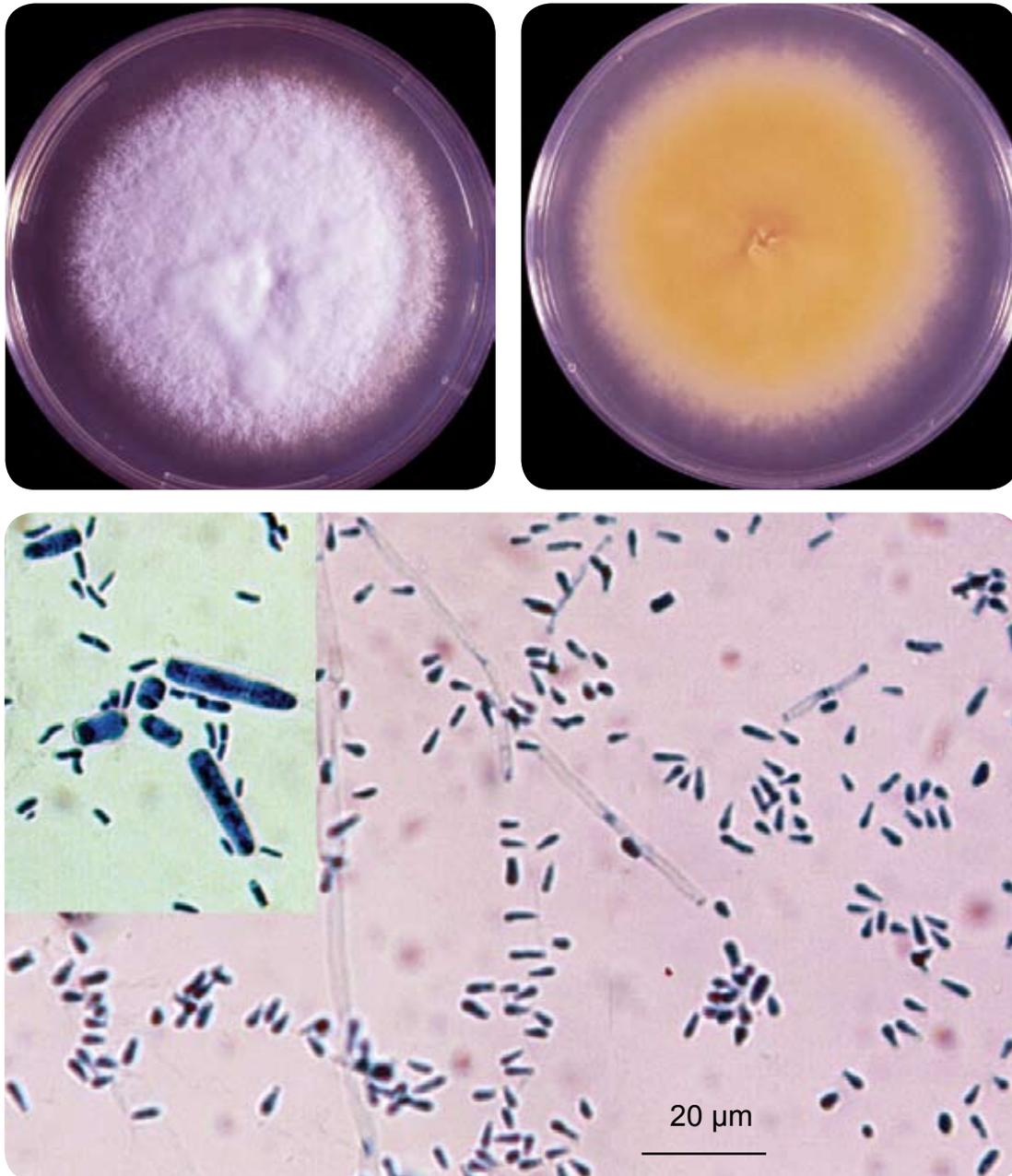
*Trichophyton erinacei* is a zoophilic fungus associated with hedgehogs and the epidermal mites which they often harbour. Human infections occur most frequently on the exposed parts of the body, but tinea of the scalp and nails can also occur. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light. The distribution of this fungus is New Zealand and Europe.



Positive "*in vitro*" hair perforation test.

## *Trichophyton erinacei* (Smith & Marples) Quaife

**Key Features:** culture characteristics, microscopic morphology, geographical distributions and negative urease test.



Culture, microconidia and macroconidia of *Trichophyton erinacei*.

*Trichophyton erinacei* is generally distinguished from *T. mentagrophytes* by (a) its microscopic morphology showing numerous large slender clavate microconidia borne on the sides of hyphae and its smooth, thin-walled clavate macroconidia; (b) its brilliant lemon yellow reverse pigment on plain Sabouraud's agar and Lactritmel agar; (c) its lack of reverse pigment on Sabouraud's salt agar; and (d) its negative hydrolysis of urea.

## ***Trichophyton interdigitale* Priestley**

**Teleomorph:** *Arthroderma vanbreuseghemii* Takashio

Colonies (SDA) are usually flat, white to cream in colour with a powdery to suede-like surface and yellowish and pinkish brown reverse pigment, often becoming a darker red-brown with age. Numerous subspherical to pyriform microconidia, occasional spiral hyphae and spherical chlamydospores are present, the latter being more abundant in older cultures. Occasional slender, clavate, smooth-walled, multiseptate macroconidia are also present in some cultures. RG-2 organism.

### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** raised white downy colony with no reverse pigment.

**Lactritmel Agar:** Macroscopic and microscopic features as described for the primary culture.

**Sabouraud's Dextrose Agar with 5% NaCl:** Heaped and folded, buff-coloured suede-like surface with a dark reddish-brown submerged fringe and brown reverse.

**1% Peptone Agar:** Flat, white to cream, suede-like surface with raised white downy centre. No reverse pigment.

**Hydrolysis of Urea:** Positive within 5 days.

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements, flat cream powdery surface with central downy tuft. Reverse pale pinkish-brown.

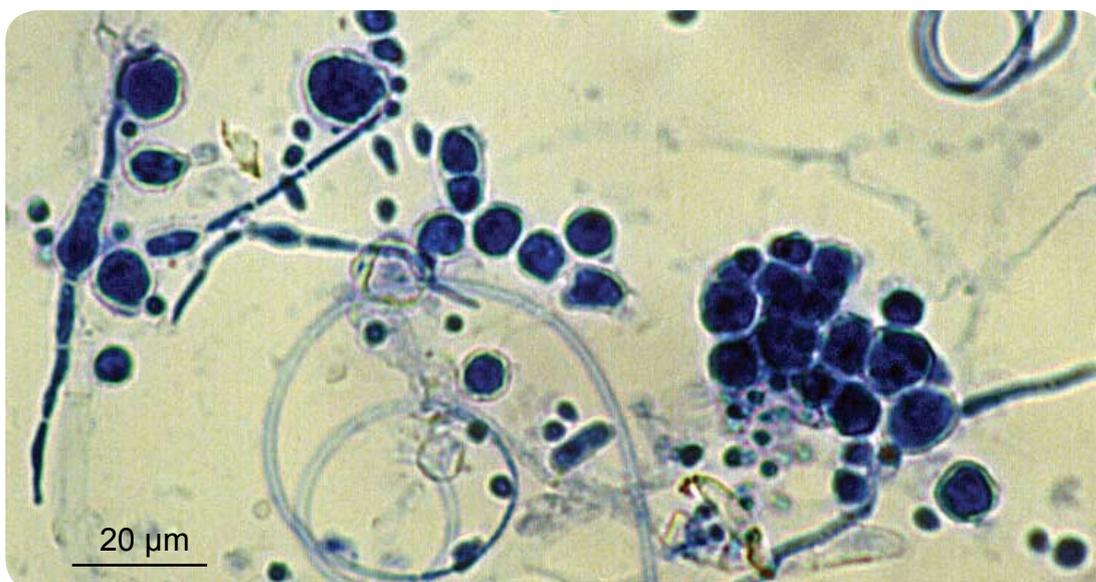
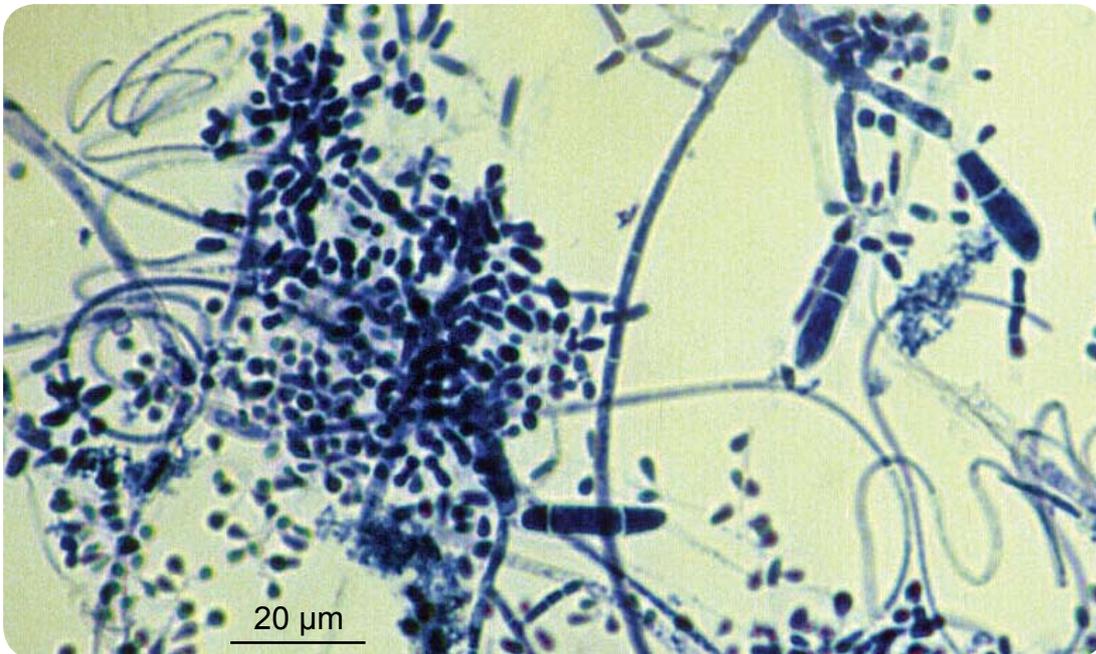
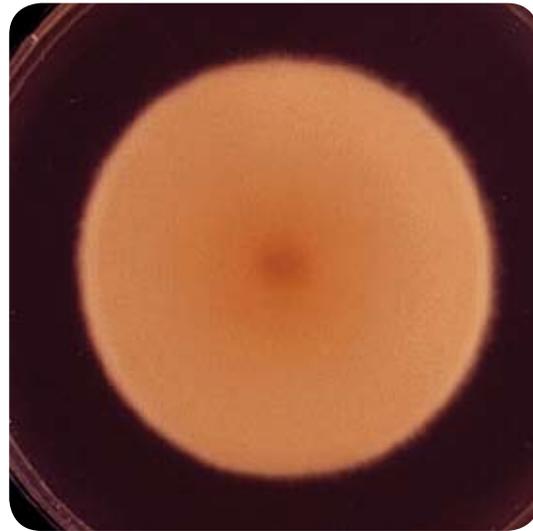
**Hair Perforation Test:** Positive.

**Key Features:** culture characteristics, microscopic morphology and *in vitro* perforation of human hair.

*Trichophyton interdigitale* can be distinguished from *T. rubrum* and *T. mentagrophytes* by (a) its culture characteristics and microscopic morphology on Sabouraud's dextrose agar and/or lactritmel agar; (b) its growth and colony morphology on Sabouraud's salt agar (colonies of *T. interdigitale* and *T. mentagrophytes* unlike *T. rubrum*, grow very well on this medium and usually produce a distinctive dark reddish-brown reverse pigment); (c) a positive urease test (within 7 days), a positive hair perforation test and the production of a yellow-brown to pinkish-brown reverse pigment on pigment stimulation media like lactritmel and Trichophyton No.1 agars; and (d) on 1% peptone agar *T. interdigitale* has a suede-like to downy surface whereas *T. mentagrophytes* has a characteristic granular appearance.

*Trichophyton interdigitale* is an anthropophilic fungus which is a common cause of tinea pedis, particularly the vesicular type, tinea corporis, and sometimes superficial nail plate invasion. It is not known to invade hair *in vivo* but produces hair perforations *in vitro*. Distribution is world-wide.

***Trichophyton interdigitale* Priestley**

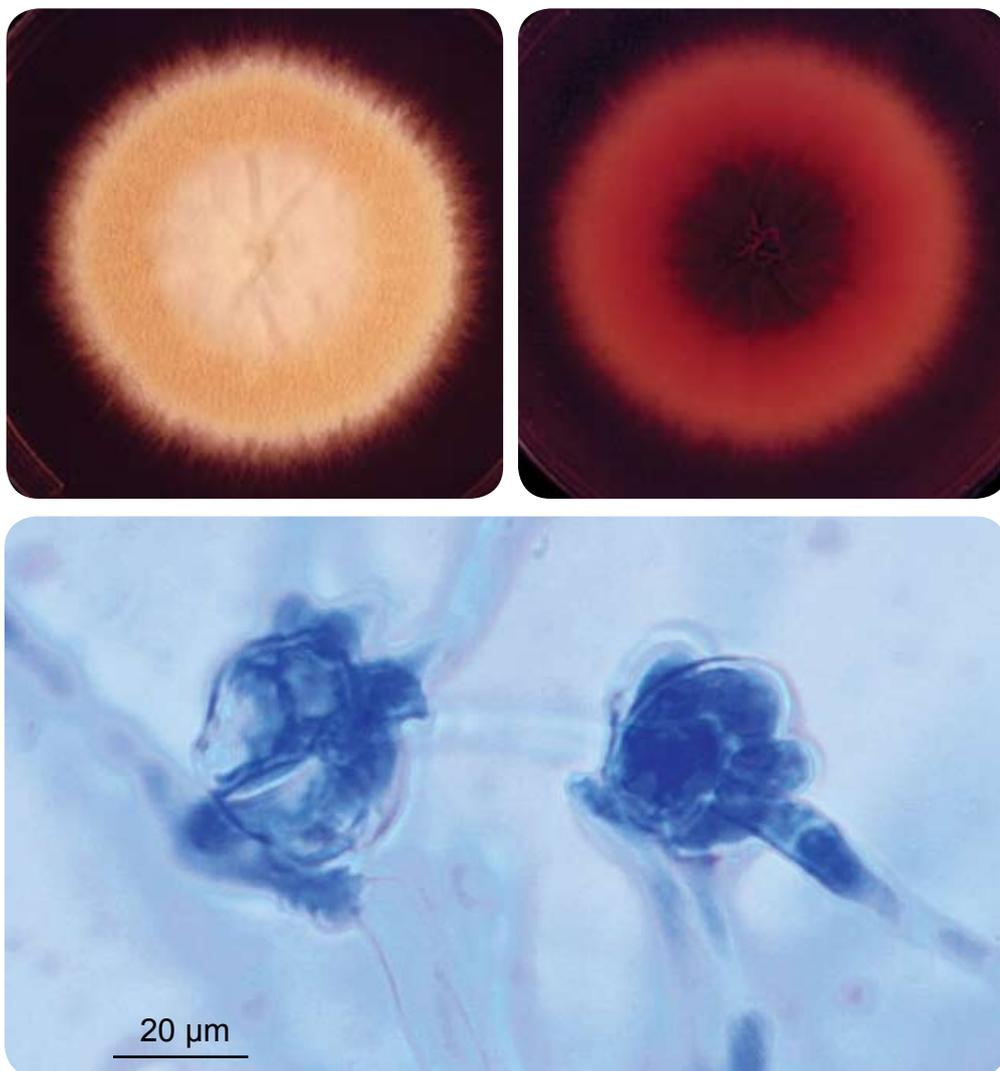


Culture, microconidia, macroconidia, chlamydospores and spiral hyphae in *Trichophyton interdigitale*.

### ***Trichophyton interdigitale* var. *nodulare***

Supplementary description for *Trichophyton interdigitale* var. *nodulare*, a dysgonic variant of *T. interdigitale* with distinctive bright yellow to apricot-coloured colonies with a suede-like to powdery surface and a bright yellow-brown to orange reverse. On primary isolation, colonies are often glabrous with minimal surface mycelium. Microscopically characteristic “nodular organs” are observed in the vegetative hyphae. Usually, no conidia are seen but some isolates, especially with subculture, may produce subspherical to pyriform microconidia similar to those of *T. interdigitale*. RG-2 organism.

*T. interdigitale* var. *nodulare* is an unusual cause of tinea pedis. It has a world-wide distribution. It is not known to invade hair *in vivo*, but produces hair perforations *in vitro*.



Culture and “nodular organs” *T. interdigitale* var. *nodulare*.

## ***Trichophyton mentagrophytes* (Robin) Blanchard**

**Teleomorph:** *Arthroderma simii* Stockdale *et al.*

Colonies (SDA) are generally flat, white to cream in colour, with a powdery to granular surface. Some cultures show central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation is usually a yellow-brown to reddish-brown colour. Numerous single-celled microconidia are formed, often in dense clusters. Microconidia are hyaline, smooth-walled, and are predominantly spherical to subspherical in shape, however occasional clavate to pyriform forms may occur. Varying numbers of spherical chlamydo spores, spiral hyphae and smooth, thin-walled, clavate-shaped, multi-celled macroconidia may also be present. RG-2 organism.

### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** Raised greyish-white, suede-like to downy colony. Some cultures may show a diffusible yellow to brown pigment.

**Lactritmel Agar:** Cultures are flat, white to cream in colour, with a powdery to granular surface. Some cultures develop a raised central tuft or pleomorphic downy areas. Reverse pigmentation is yellow-brown to pinkish-brown to red-brown. Microscopic morphology similar to that described above, with predominantly spherical microconidia, often forming in dense clusters and varying numbers of spherical chlamydo spores, spiral hyphae and smooth, thin-walled, clavate, multiseptate macroconidia.

**Sabouraud's Dextrose Agar with 5% NaCl:** Cultures are heaped and folded, buff to brown in colour, with a suede-like surface texture and characteristically have a very dark reddish-brown submerged peripheral fringe and reverse pigmentation.

**1% Peptone Agar:** Flat, cream-coloured, powdery to granular colony with no reverse pigment.

**Hydrolysis of Urea:** Positive within 7 days (usually 3 to 5 days).

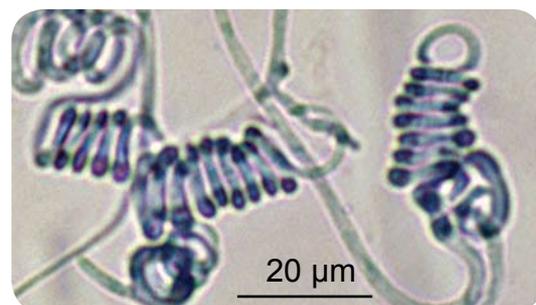
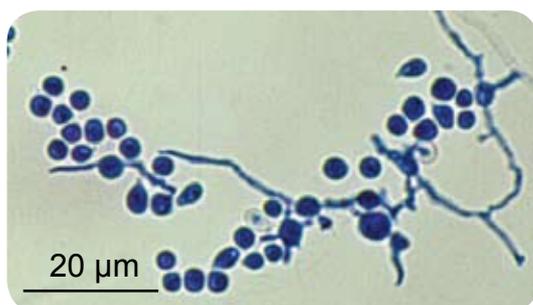
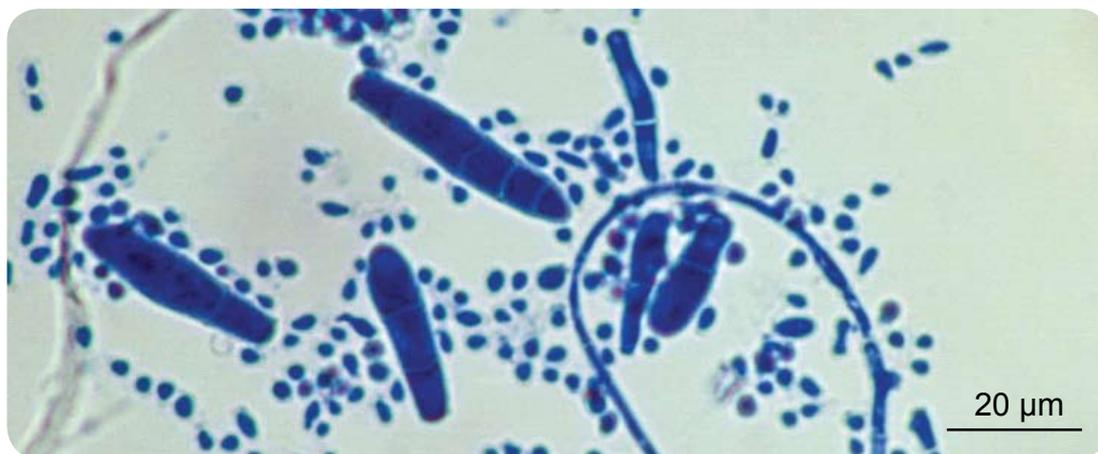
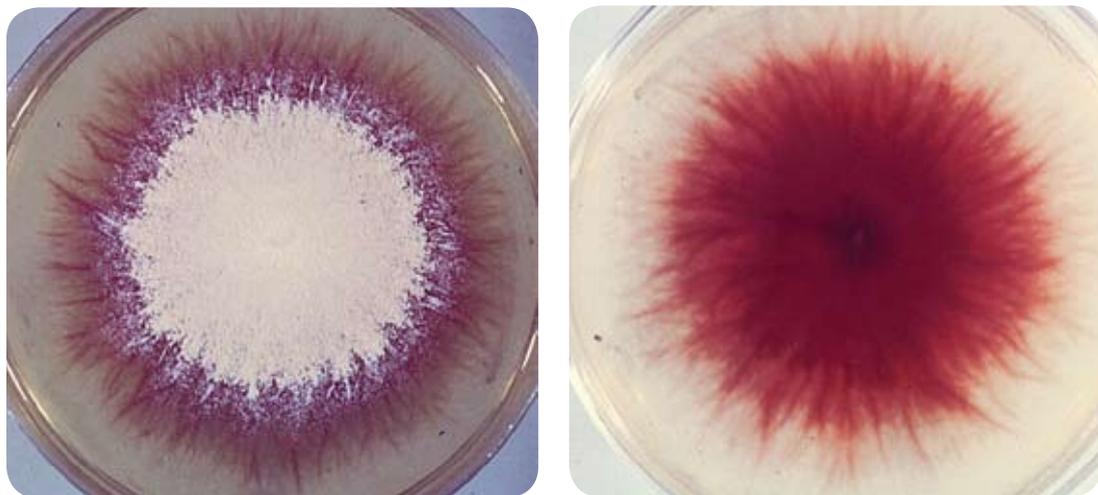
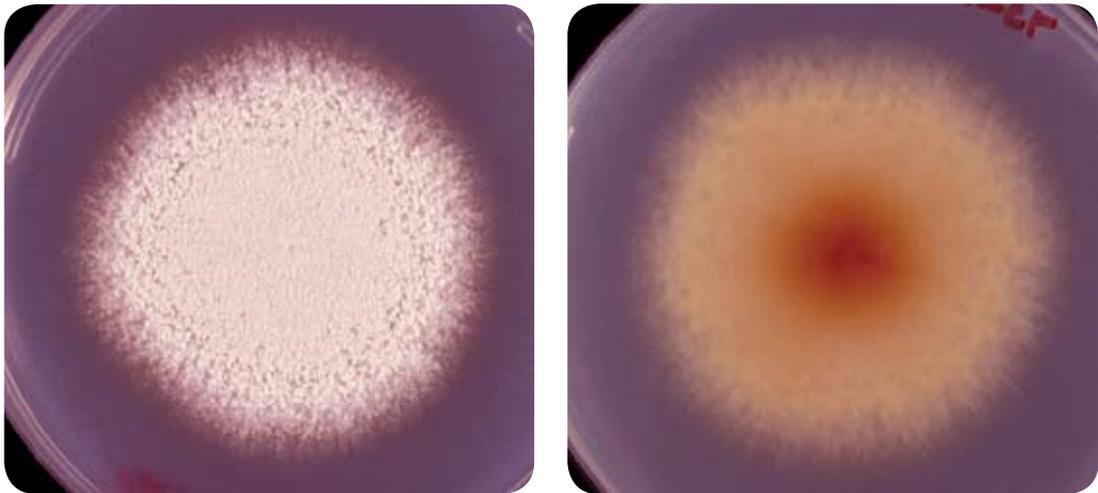
**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements. Cultures are flat, cream-coloured, with a powdery to suede-like surface, and have a reddish-brown reverse pigmentation.

**Hair Perforation Test:** Positive within 14 days.

**Key Features:** culture characteristics, microscopic morphology and clinical disease with known animal contacts. *T. mentagrophytes* can be distinguished from *T. interdigitale* by (a) its granular appearance on 1% Peptone agar, (b) its microscopic morphology of more spherical microconidia and generally greater numbers of macroconidia and (c) a yellow to brown diffusible pigment is often seen on Littman Oxgall agar.

*T. mentagrophytes* is the zoophilic form of *T. mentagrophytes* with a world-wide distribution and a wide range of animal hosts including mice, guinea-pigs, kangaroos, cats, horses, sheep and rabbits. Produces inflammatory skin or scalp lesions in humans, particularly in rural workers. Kerion of the scalp and beard may occur. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light.

***Trichophyton mentagrophytes* (Robin) Blanchard**



Cultures, microconidia, macroconidia, chlamydospores and spiral hyphae in *Trichophyton mentagrophytes*.

***Trichophyton mentagrophytes* var. *quinckeanum* (Zopf) MacLeod & Muende**

Colonies (SDA) are flat or slightly raised and folded, white to cream, suede-like in texture with a pale yellow-brown to pinkish brown reverse. A characteristic pungent “mousy” odour may be present. Numerous microconidia, predominantly slender clavate when young, are borne laterally along the sides of hyphae. With age the microconidia become broader and pyriform, and some subspherical forms are present. Occasional to moderate numbers of smooth-walled, multiseptate, clavate macroconidia may be present. RG-2 organism.

**Kaminski’s Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** Raised, dome-like bluish-grey, suede-like colony with a narrow flat, greyish-white, suede-like border. No diffusible or reverse pigment should be present.

**Lactritmel Agar:** Flat, white to cream, suede-like to powdery colony with either no reverse pigment or a pale yellow-brown to pinkish-brown reverse. Numerous slender clavate to pyriform (depending on age of sub-culture) microconidia and occasional to moderate numbers of smooth, thin-walled, clavate macroconidia are present.

**Sabouraud’s Dextrose Agar with 5% NaCl:** Heaped up and much folded white suede-like colony with very pale yellow-brown reverse. No submerged fringe.

**1% Peptone Agar:** Raised white suede-like to downy colony with no reverse pigment.

**Hydrolysis of Urea:** Positive within 7 days (usually very rapid 2-3 days).

**Vitamin Free Agar (Trichophyton Agar No.1):** Flat, white to cream, suede-like colony with either no reverse pigment or a pale yellow-brown reverse. i.e. no special nutritional requirements.

**Hair Perforation Test:** Positive in 7 to 10 days.

**Key Features:** culture characteristics, microscopic morphology, contact with mice, odour and rapid urease test.

*T. mentagrophytes* var. *quinckeanum* may be distinguished from *T. mentagrophytes* by (a) its characteristic culture appearance on Littman Oxgall agar (i.e. raised, dome-like, bluish-grey suede-like colony with a narrow flat, greyish-white, suede-like border and no diffusible or reverse pigment); and on Sabouraud’s salt agar (typically heaped and folded white suede-like colony, but with no distinctive dark reddish-brown submerged fringe and reverse pigment as seen in *T. mentagrophytes*); (b) microscopic morphology showing numerous slender clavate with some pyriform microconidia and moderate numbers of smooth thin-walled, clavate macroconidia; (c) a rapid urease test, usually within 2 to 3 days; and (d) cultures often have a characteristic pungent “mousy” odour.

***Trichophyton mentagrophytes* var. *quinckeanum* (Zopf) MacLeod & Muende**

*Trichophyton mentagrophytes* var. *quinckeanum* causes “mouse favus” on mice, and this is seen as thick, yellow, saucer-shaped crusted lesions up to 1 cm in diameter called scutula. Invaded hairs are rarely seen but they may show either ectothrix or endothrix infection. Infected human hairs do not fluoresce under Wood’s ultra-violet light, but very occasional hairs from experimental lesions in guinea pigs may show a pale yellow fluorescence. The geographical distribution of this dermatophyte is difficult to establish, but it is probably world-wide. It is often associated with mice plagues in the Australian Wheat Belt.



Culture, microconidia and macroconidia of *T. mentagrophytes* var. *quinckeanum*.

### ***Trichophyton rubrum* (Castellani) Semon**

Many strains and varieties of *T. rubrum* have been described and opinion differs between mycologists as to the exact validity of many of these. For practical purposes we will distinguish two types: *T. rubrum* downy type and *T. rubrum* granular type.

Microscopically, the downy type is characterised by the production of scanty to moderate numbers of slender clavate microconidia and no macroconidia. It frequently causes chronic infections of skin and nails. Granulomatous lesions may sometimes occur.

Microscopically, the granular type is characterised by the production of moderate to abundant numbers of clavate to pyriform microconidia and moderate to abundant numbers of thin-walled, cigar-shaped macroconidia. The macroconidia may or may not have terminal appendages.

*Trichophyton rubrum* granular strain is a frequent cause of tinea corporis in South East Asia and in Aborigines living in the Northern Territory of Australia. However, since the Vietnam War, it has been spread throughout the world, especially to those countries with returning troops or to those receiving refugees, where it has often been described as a new species. The granular strain of *T. rubrum* represents the parent strain of *T. rubrum* downy type; the latter evolved by establishing a niche in feet (tinea pedis) when the former was imported into Europe and North America at the beginning of the 20th century. It should be stressed that intermediate strains between the two types do occur and that many culture and morphological characteristics overlap.

### ***Trichophyton rubrum* typical downy strain**

Colonies (SDA) are flat to slightly raised, white to cream, suede-like to downy, with a yellow-brown to wine-red reverse. Most cultures show scanty to moderate numbers of slender clavate to pyriform microconidia. Macroconidia are usually absent, however closterospore-like projections may be present in some mounts. Note: on primary isolation some cultures may lack reverse pigmentation and fail to produce microconidia. These will need to be subcultured onto media like Lactrimel agar or potato dextrose agar which stimulate pigmentation and sporulation. If sporulation still fails subculture the fungus onto Trichophyton Agar No.1. RG-2 organism.



Culture of *Trichophyton rubrum* downy strain.

## ***Trichophyton rubrum* (Castellani) Semon**

### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** Raised, greyish-white, suede-like to downy colony with no reverse pigment. Some cultures may have showed a faint greenish-yellow diffusible pigment.

**Lactritmel Agar:** Flat, white, downy colonies with a deep wine-red reverse pigment. Microscopically, cultures show the typical downy type morphology of pyriform to slender clavate microconidia.

**Sabouraud's Dextrose Agar with 5% NaCl:** Very stunted, white downy colony with a pale yellow-brown reverse pigment.

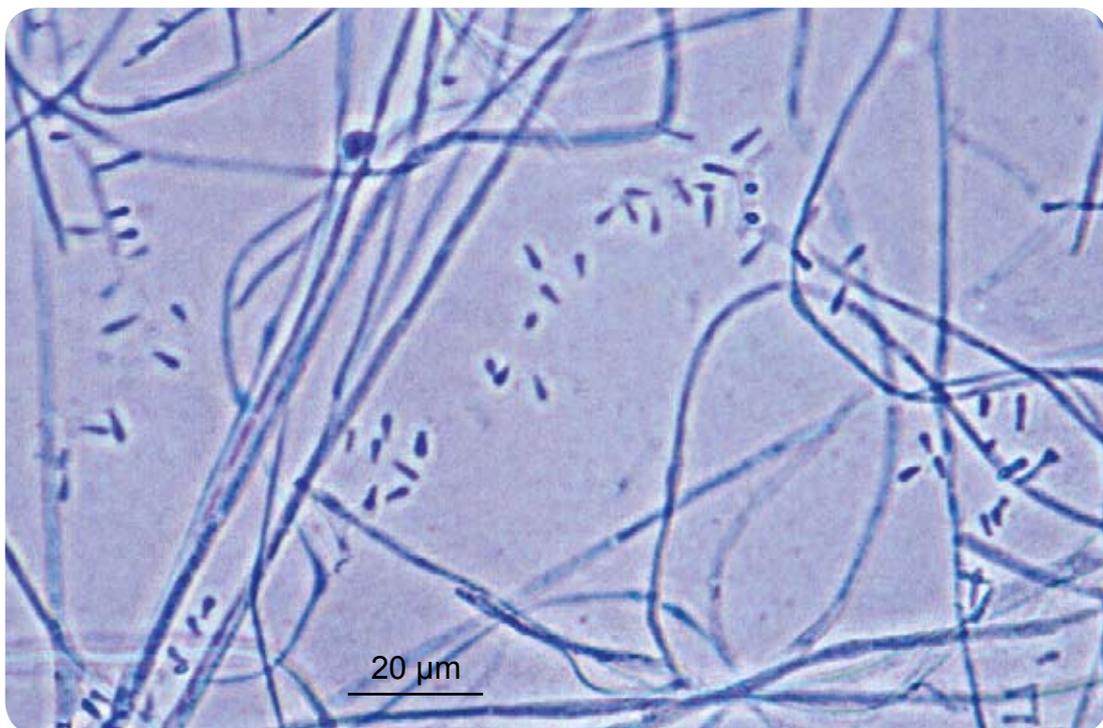
**1% Peptone Agar:** Flat, white to cream, downy colony often with a raised centre. No reverse pigment produced.

**Hydrolysis of Urea:** Negative at 7 days.

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special vitamin requirements. Colonies are flat, white to cream, suede-like to downy with a deep wine-red reverse pigment.

**Hair Perforation Test:** Negative at 28 days.

**Key Features:** culture characteristics, microscopic morphology and failure to perforate hair "*in vitro*".



Typical slender clavate microconidia of *Trichophyton rubrum* downy type.

### ***Trichophyton rubrum* granular type**

Colonies (SDA) are flat to slightly raised, white to cream, suede-like with a pinkish-red reverse. Microscopically, most cultures have numerous clavate to pyriform microconidia and moderate numbers of smooth, thin-walled multiseptate, slender cylindrical macroconidia. Older cultures may show numerous chlamydospores with few clavate to pyriform microconidia.

#### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** Raised, greyish, suede-like colonies with some radial folding and a greenish-yellow diffusible pigment.

**Lactritmel Agar:** Flat, white to rose pink, suede-like to granular colonies with a pinkish to wine-red reverse. Numerous broad clavate to pyriform microconidia and moderate numbers of smooth, thin-walled, slender cylindrical macroconidia are present. A few chlamydospores may be present in older cultures.

**Sabouraud's Dextrose Agar with 5% NaCl:** A very stunted, heaped and folded, glabrous, cream thallus, later developing a dark red central spot. Reverse is a brownish-yellow colour.

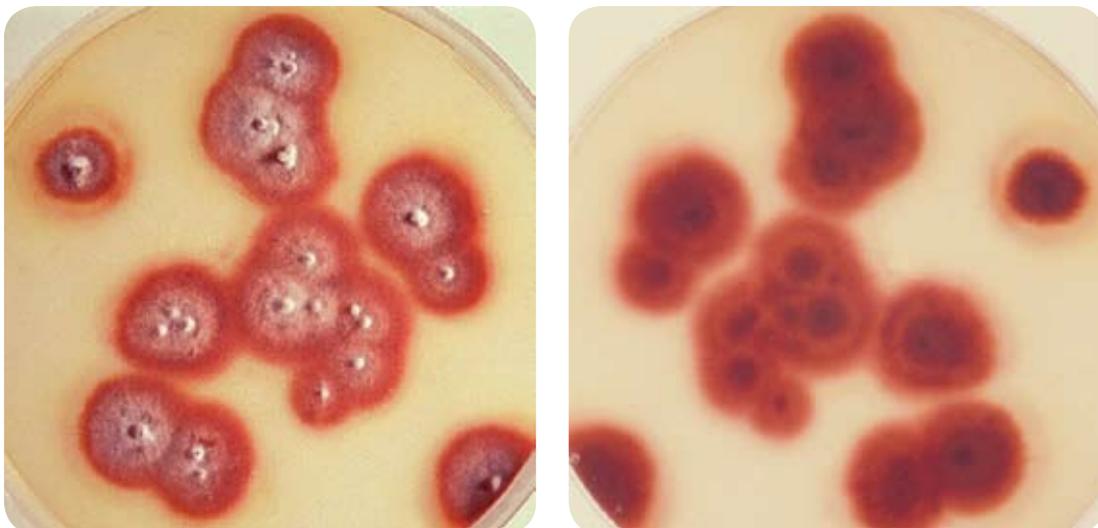
**1% Peptone Agar:** Flat, white to cream, glabrous to suede-like colony with no reverse pigment.

**Hydrolysis of Urea:** Positive at 7 days

**Vitamin Free Agar (Trichophyton Agar No.1):** Good growth indicating no special nutritional requirements. White to cream suede-like colonies with a pinkish-red to wine-red reverse.

**Hair Perforation Test:** Positive.

**Key Features:** culture characteristics and microscopic morphology.



Culture of *Trichophyton rubrum* granular strain.

***Trichophyton rubrum* granular type**

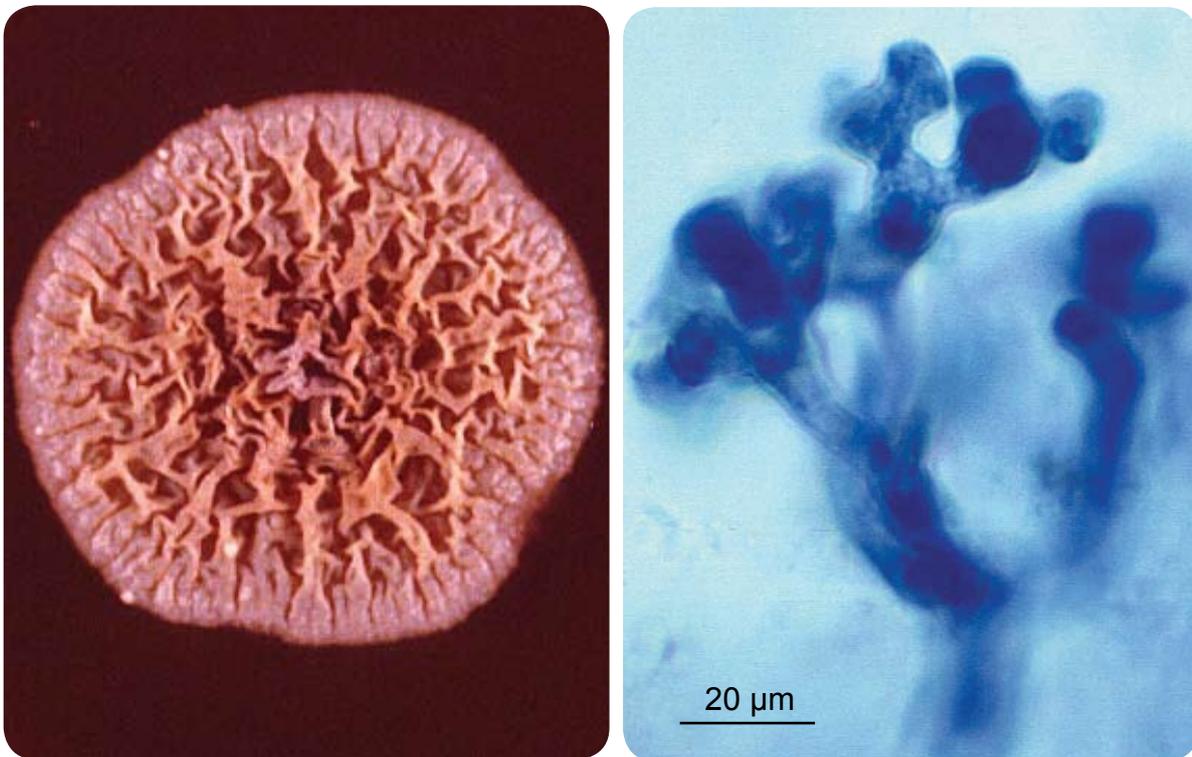


Macroconidia and microconidia of *Trichophyton rubrum* granular type.

### ***Trichophyton schoenleinii* (Lebert) Langeron & Milochevitch**

Colonies (SDA) are slow growing, waxy or suede-like with a deeply folded honey-comb-like thallus and some sub-surface growth. The thallus is cream-coloured to yellow to orange brown. Cultures are difficult to maintain in their typical convoluted form, and rapidly become flat and downy. No reverse pigmentation is present. No macroconidia and microconidia are seen in routine cultures, however numerous chlamydospores may be present in older cultures. However, characteristic antler “nail head” hyphae also known as “favic chandeliers” may be observed. A few distorted clavate microconidia may be formed by some isolates when grown on polished rice grains. RG-2 organism

**Key Features:** clinical history, culture characteristics and microscopic morphology showing favic chandeliers.



Culture and “favic chandeliers” of *Trichophyton schoenleinii*.

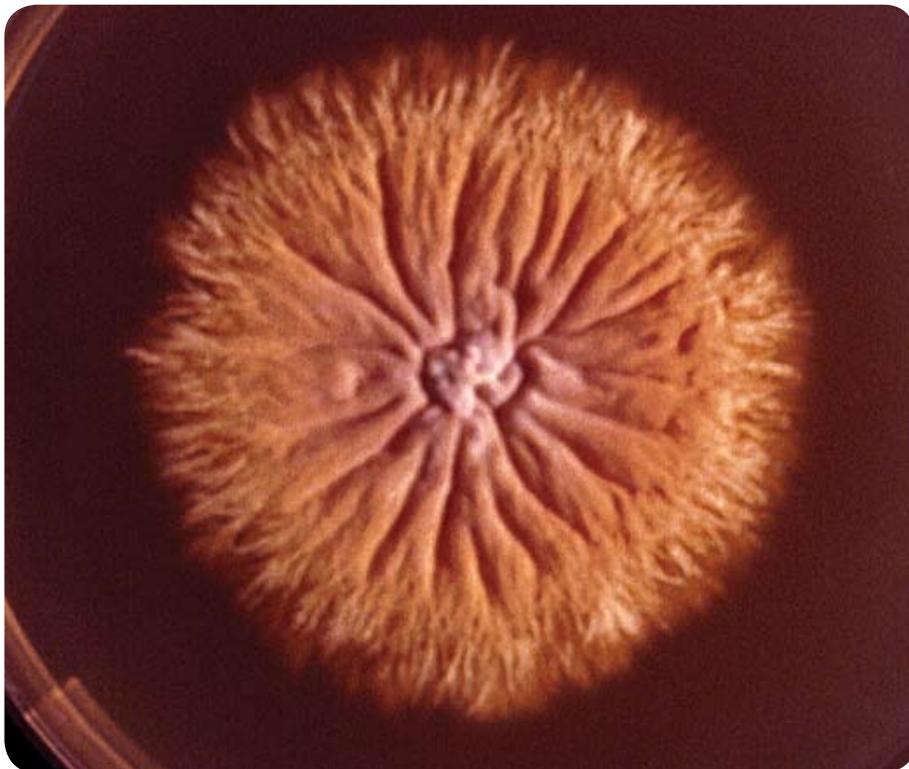
*Trichophyton schoenleinii* is an anthropophilic fungus causing favus in humans. Favus is a chronic, scarring form of tinea capitis characterised by saucer-shaped crusted lesions or scutula and permanent hair loss. Invaded hairs remain intact and fluoresce a pale greenish yellow under Wood’s ultra-violet light. Favus was once common in Eurasia and North Africa, however its incidence is now in decline.

### ***Trichophyton soudanense* Joyeux.**

Colonies (SDA) are slow-growing with a flat to folded, suede-like surface. Often there is a broad fringe of submerged growth. The surface mycelium and reverse pigment are characteristically a deep apricot-orange in colour. Microscopically, the hyphae often show reflexive or right-angle branching. Pyriform microconidia may occasionally be present and numerous chlamydoconidia are often found in older cultures. On BCP-milk solids agar, a thin halo of clearing usually appears in the milk solids around the colony edge at 7-10 days. RG-2 organism.

*T. soudanense* appears to be genetically related to *Trichophyton rubrum* and *T. violaceum* (Graser *et al.* 2007), however we have maintained the current description until the taxonomy is clarified.

**Key Features:** clinical history, culture characteristics and microscopic morphology showing reflexive hyphal branching and endothrix invasion of hair.



Culture and “reflexive” hyphal branching in *Trichophyton soudanense*.

*Trichophyton soudanense* is an anthropophilic fungus which is a frequent cause of tinea capitis in Africa. Invaded hairs show an endothrix infection but do not fluoresce under Wood’s ultra-violet light. Distribution is mainly in Africa with imported cases now reported from Europe, Brazil, Australia and USA.

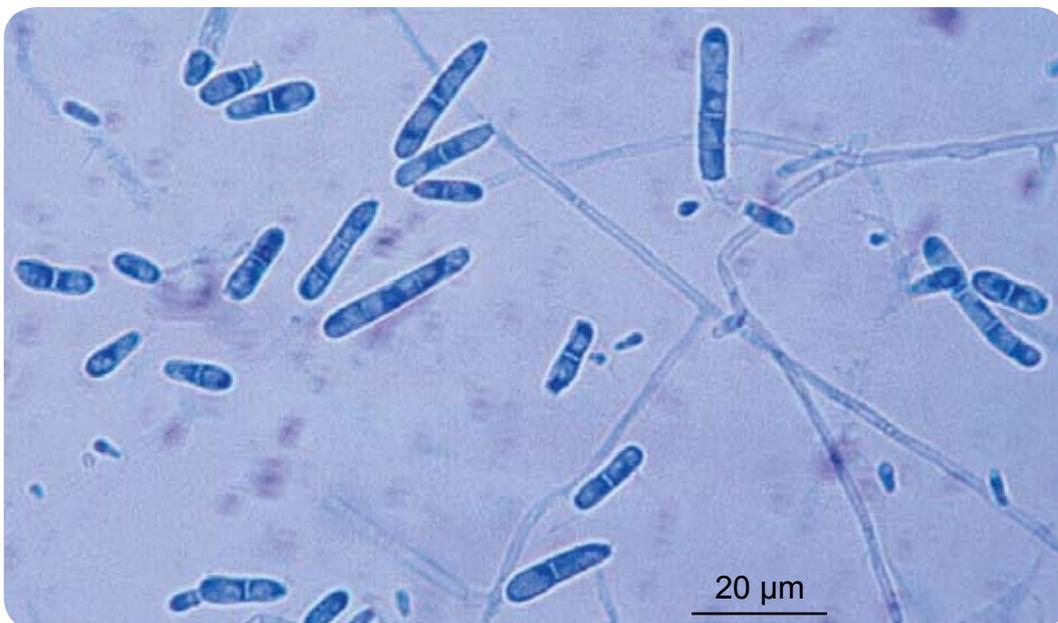
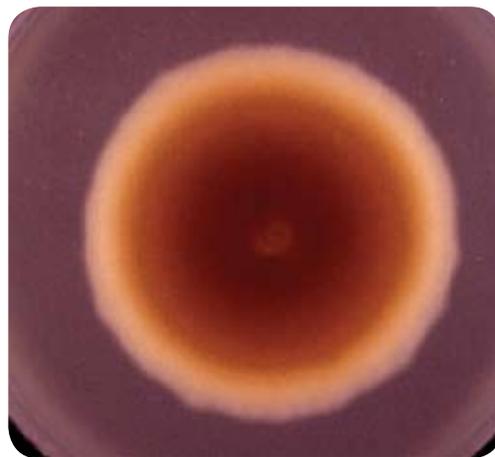
## *Trichophyton terrestre* Durie and Frey

Teleomorphs: *Arthroderma insingulare* Padhye and Carmichael  
*Arthroderma lenticulare* Pore, Tsao and Plunkett  
*Arthroderma quadrifidum* Dawson and Gentles

Colonies (SDA) are usually flat to downy with a suede-like to granular texture resembling *T. mentagrophytes*. The surface colour may range from white to cream, buff to yellow, or greenish-yellow. Reverse pigmentation is usually yellowish-brown although some variants have a deep rose-red reverse. Microconidia are large, clavate or pedicellate, usually exhibiting transition forms to more or less abundant lateral macroconidia. Macroconidia are clavate to cylindrical with rounded ends, smooth and thin-walled, and are two- to six-celled. Chlamydo spores, hyphal spirals, racquet mycelium and antler hyphae may also be present. No growth at 37°C. RG-1 organism.

*Trichophyton terrestre* is a geophilic fungus of world-wide distribution which may occur as a saprophytic contaminant on humans and animals. It is not known to invade hair *in vivo*, but produces hair perforations *in vitro*.

**Key Features:** culture characteristics and microscopic morphology.



Culture and macroconidia of *Trichophyton terrestre*.

## ***Trichophyton tonsurans* Malmsten**

Colonies (SDA) show considerable variation in texture and colour. They may be suede-like to powdery, flat with a raised centre or folded, often with radial grooves. The colour may vary from pale-buff to yellow, (the sulfureum form which resembles *Epidermophyton floccosum*), to dark-brown. The reverse colour varies from yellow-brown to reddish-brown to deep mahogany. Hyphae are relatively broad, irregular, much branched with numerous septa. Numerous characteristic microconidia varying in size and shape from long clavate to broad pyriform, are borne at right angles to the hyphae, which often remain unstained by lactophenol cotton blue. Very occasional smooth, thin-walled, irregular, clavate macroconidia may be present on some cultures. Numerous swollen giant forms of microconidia and chlamydospores are produced in older cultures. RG-2 organism.

### **Kaminski's Dermatophyte Identification Scheme**

**Littman Oxgall Agar:** Restricted colony with cream, sometimes greyish, suede-like folded surface with no reverse pigment.

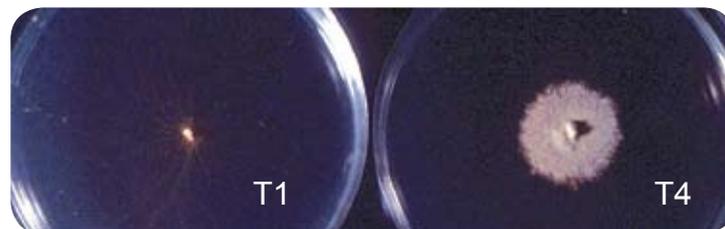
**Lactritmel Agar:** Macroscopic and microscopic features as described above for the primary culture.

**Sabouraud's Dextrose Agar with 5% NaCl:** Very stunted slow growing colony with dark brown surface and reverse.

**1% Peptone Agar:** Flat, white to cream suede-like surface with raised centre. No reverse pigment.

**Hydrolysis of Urea:** positive at 5 days

**Nutritional Tests on Trichophyton Agars:** results demonstrate a partial requirement for thiamine. T1 = vitamin free agar, T4 = vitamin free + thiamine agar.

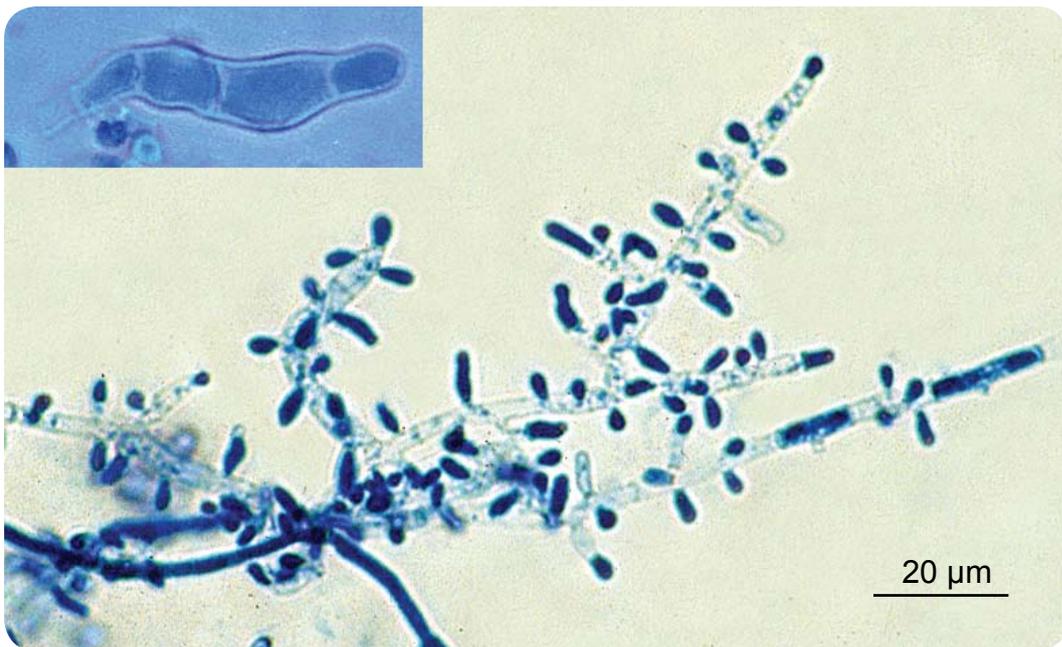


**Hair Perforation Test:** Positive within 14 days.

**Key Features:** microscopic morphology, culture characteristics, endothrix invasion of hairs and partial thiamine requirement.

*Trichophyton tonsurans* is an anthropophilic fungus with a world wide distribution which causes inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails and scalp. It is a common cause of tinea capitis in the Australian Aborigine and African Americans. Invaded hairs show an endothrix infection and do not fluoresce under Wood's ultra-violet light.

### *Trichophyton tonsurans* Malmsten



Colonies, hyphae, microconidia and macroconidia of *Trichophyton tonsurans*.

## ***Trichophyton verrucosum* Bodin**

**Teleomorph:** *Arthroderma benhamiae* Ajello & Chang.

Colonies (SDA) are slow growing, small, button or disc-shaped, white to cream-coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth. Reverse pigment may vary from non-pigmented to yellow. Broad, irregular hyphae with many terminal and intercalary chlamydospores are present. Chlamydospores are often in chains. The tips of some hyphae are broad and club-shaped, and occasionally divided, giving the so-called “antler” effect. When grown on thiamine-enriched media, occasional strains produce clavate to pyriform microconidia borne singly along the hyphae. Macroconidia are only rarely produced, but when present have a characteristic tail or string-bean shape. RG-2 organism.

### **Confirmatory Tests:**

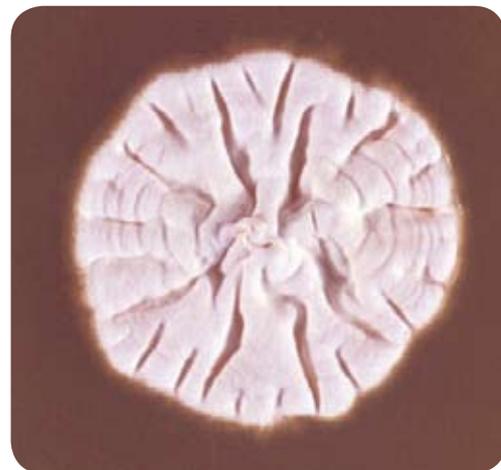
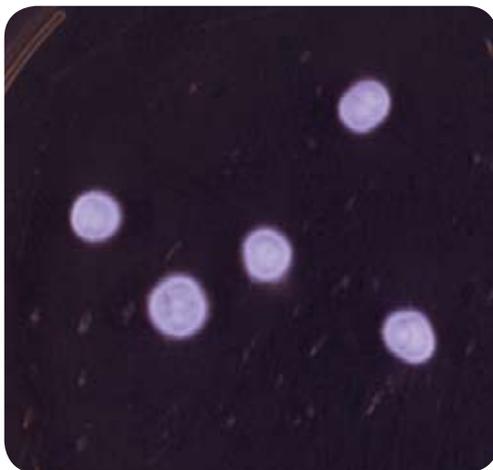
Growth at 37°C: unlike other dermatophytes growth is enhanced at 37°C

**Nutritional Requirements:** all strains require thiamine and approximately 80% require thiamine and inositol. There is no growth on casein vitamin free agar (T1), minimal submerged growth on T1 + inositol (T2), good growth on T1 + inositol and thiamine (T3) and good growth on T1 + thiamine only (T4).

All strains produce typical chains of chlamydospores, often referred to as “chains of pearls”, especially when grown on BCP milk solids glucose agar at 37°C. Also when grown at 25°C on milk solids glucose agar a halo of peripheral clearing of milk solids occurs within 7 days.

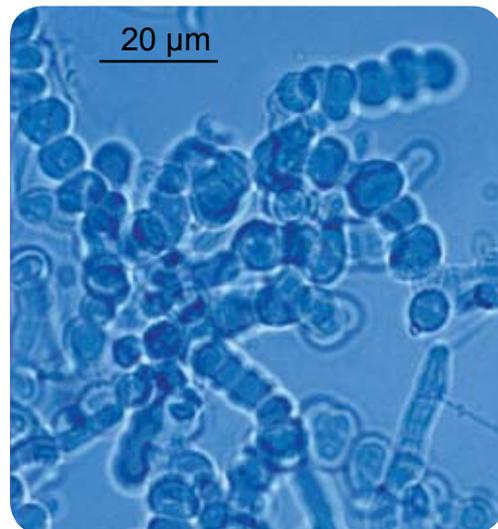
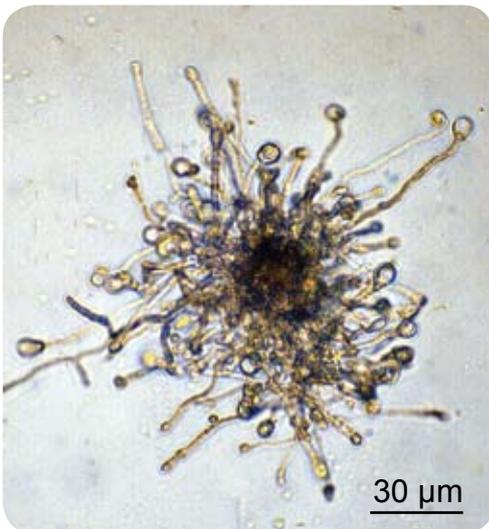
Microscopic examination of young 4 to 5 day old colonies, grown from a very small inoculum, on Sabouraud’s dextrose agar containing 0.5% yeast extract and incubated at 30°C, show characteristic terminal vesicles (not chlamydospores) at the tips of hyphae. The number of vesicles produced is greater from primary inoculations of skin scrapings or hairs.

**Key Features:** culture characteristics and requirements for thiamine and inositol, large ectothrix invasion of hair, clinical lesions and history.



*Trichophyton verrucosum* is a zoophilic fungus causing ringworm in cattle. Infections in humans result from direct contact with cattle or infected fomites and are usually highly inflammatory involving the scalp, beard or exposed areas of the body (ie. nails, skin). Invaded hairs show an ectothrix infection and fluorescence under Wood’s ultraviolet light has been noted in cattle but not in humans. Geographic distribution is world-wide.

### *Trichophyton verrucosum* Bodin



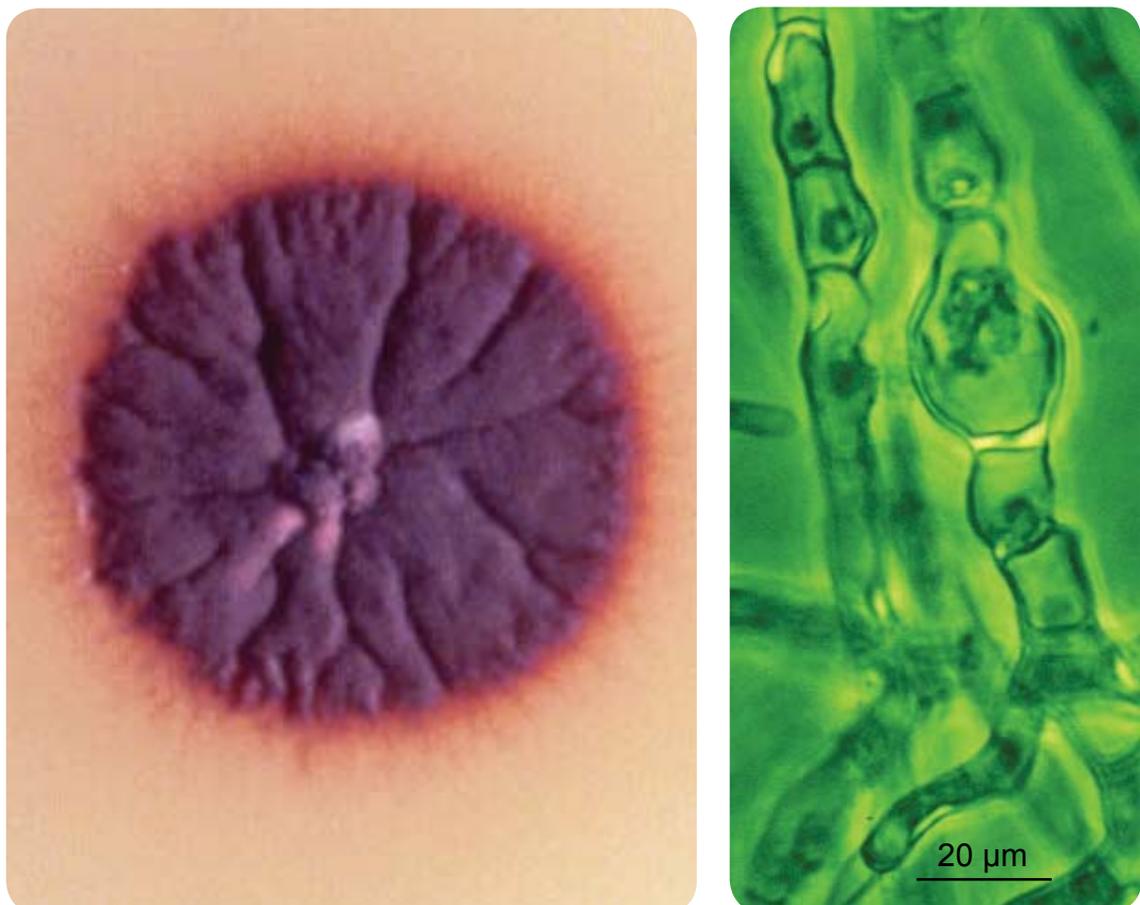
*Trichophyton verrucosum* showing clavate to pyriform microconidia, characteristic rat tail or string bean-shaped macroconidia, terminal vesicles at the tips of hyphae in young colonies and chains of chlamydospores.

### ***Trichophyton violaceum* Sabouraud apud Bodin**

Colonies (SDA) are very slow growing, glabrous or waxy, heaped and folded and a deep violet in colour. Cultures often become pleomorphic, forming white sectors and occasional non-pigmented strains may occur. Hyphae are relatively broad, tortuous, much branched and distorted. Young hyphae usually stain well in lactophenol cotton blue, whereas older hyphae stain poorly and show small central fat globules and granules. No conidia are usually seen, although occasional pyriform microconidia have been observed on enriched media. Numerous chlamydospores are usually present, especially in older cultures. RG-2 organism.

**Nutritional Requirements:** *T. violaceum* has a partial nutrient requirement for thiamine. There is minimal growth on casein vitamin-free agar (Trichophyton Agar No. 1), and slightly better growth on vitamin-free agar plus thiamine (Trichophyton Agar No. 4). The partial requirement for thiamine separates this organism from *T. gourvillii*, *T. rubrum*, and other species that may produce purple pigmented colonies.

**Key Features:** culture characteristics, partial thiamine requirement and endothrix hair invasion.



Culture and chlamydospores of *Trichophyton violaceum*.

*Trichophyton violaceum* is an anthropophilic fungus causing inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails, beard and scalp, producing the so-called “black dot” tinea capitis. Distribution is world-wide, particularly in the Near East, Eastern Europe, USSR and North Africa. Invaded hairs show an endothrix infection and do not fluoresce under Wood’s ultra-violet light.

## ***Trichosporon* Behrend**

The genus *Trichosporon* is characterised by the development of hyaline, septate hyphae that fragment into oval or rectangular arthroconidia. Some blastoconidia are also seen. The colonies are usually raised and have a waxy appearance, which develop radial furrows and irregular folds.

Following recent molecular studies, the genus has undergone major revision (Gueho *et al.* 1992, de Hoog *et al.* 2000, Rodriguez-Tudela *et al.* 2005) and 6 species of medical importance are described below. In particular, the name *Trichosporon beigelii* is now obsolete, and previously described infections reported in the literature under this name could in fact be due to any one of the species listed below.

*Trichosporon* species are a minor component of normal skin flora, and are widely distributed in nature. They are regularly associated with the soft nodules of white piedra, and have been involved in a variety of opportunistic infections in the immunosuppressed patient. Disseminated infections are most frequently caused by *T. asahii* and have been associated with leukaemia, organ transplantation, multiple myeloma, aplastic anaemia, lymphoma, solid tumours and AIDS. Disseminated infections are often fulminate and widespread, with lesions occurring in the liver, spleen, lungs and gastrointestinal tract. Infections in non-immunosuppressed patients include endophthalmitis after surgical extraction of cataracts, endocarditis usually following insertion of prosthetic cardiac valves, peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD), and intravenous drug abuse.

For descriptions of species, keys to taxa and additional information see Kurtzman and Fell (1988), Gueho *et al.* (1992), de Hoog *et al.* (2000), Rodriguez-Tudela *et al.* (2005).

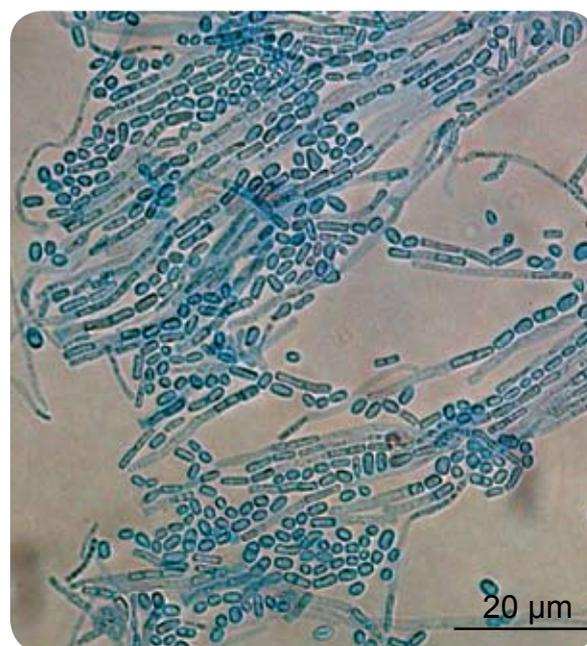
### **Key to medically important species (de Hoog *et al.* 2000).**

- |  |   |                      |
|--|---|----------------------|
| 1. Growth with melibiose   | 2 |                      |
| No growth with melibiose   | 3 |                      |
| 2. Tolerant to cycloheximide   |   | <i>T. mucoides</i>   |
| Not tolerant to cycloheximide  |   | <i>T. cutaneum</i>   |
| 3. Growth with <i>myo</i> -inositol, no growth with L-arabinose                                |   | <i>T. inkin</i>      |
| No growth with <i>myo</i> -inositol, growth with L-arabinose                                   | 4 |                      |
| 4. Colony with very slow growth; thallus consisting of clumps of meristematic cells (sarcinae) |   | <i>T. asteroides</i> |
| Colonies and microscopy otherwise  | 5 |                      |
| 5. Appressoria present in slide cultures   |   | <i>T. ovoides</i>    |
| Appressoria absent in slide cultures   | 6 |                      |
| 6. Arthroconidia barrel-shaped; thallus not meristematic                                       |   | <i>T. asahii</i>     |
| Arthroconidia elongate, or thallus meristematic  |   | <i>T. asteroides</i> |

### *Trichosporon asahii* Akagi ex Sugita *et al.*

Colonies (SDA) are white to cream-coloured, powdery, suede-like to farinose with radial furrows and irregular folds. Budding cells and lateral conidia are absent. Arthroconidia are barrel-shaped. Appressoria absent. This species assimilates L-arabinose but not melibiose. Growth at 37°C. Most common species, especially from invasive infections. RG-2 organism.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	+	D-Glucitol	v
Galactose	+	Raffinose	-	D-Glucosamine	+	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	v	N-A-D-glucosamine	+	D-Gluconate	+
Sucrose	v	Soluble Starch	v	Glycerol	v	DL-Lactate	v
Maltose	+	D-Xylose	v	Erythritol	+	myo-Inositol	v
Cellobiose	+	L-Arabinose	+	Ribitol	v	Nitrate	-
Trehalose	+	D-Arabinose	+	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	v	D-Glucuronate	+



Culture, hyphae and arthroconidia of *Trichosporon asahii*.

Antifungal	MIC µg/mL		Antifungal	MIC µg/mL	
	Range	MIC <sub>90</sub>		Range	MIC <sub>90</sub>
Fluconazole	0.25-16	8.0	Amphotericin B	0.25-16	8.0
Itraconazole	0.03-16	0.5	Flucytosine	2-128	16
Posaconazole	0.06-16	1.0	Caspofungin	>8	>8
Voriconazole	0.03-16	0.25	Anidulafungin	>8	>8

MIC data for *T. asahii*. Antifungal susceptibility may vary between species and resistant strains have been reported. Therefore, antifungal susceptibility testing of individual strains is recommended. Paphitou *et al.* (2002), Espinel-Ingroff (2003), Rodriguez-Tudela *et al.* (2005), Metin *et al.* (2005) and WCH in-house data.

### ***Trichosporon asteroides* (Rischin) Ota**

Colonies (SDA) are restricted, dry, cream-coloured, cerebriform, with radial furrows and irregular folds. The meristematic form is punctiform, brownish and consists of hyphae which swell and become multiseptate which may fall apart into smaller packets. Budding cells and lateral conidia are absent. Arthroconidia are elongate and hyphae are often present. Appressoria absent. This species assimilates L-arabinose but not myo-inositol. Growth at 37°C is variable. Uncommon species usually associated with superficial infections. RG-2 organism.

<b>Assimilation Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	+	D-Glucitol	v
Galactose	+	Raffinose	-	D-Glucosamine	v	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	+	N-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	+	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	myo-Inositol	-
Cellobiose	+	L-Arabinose	+	Ribitol	v	Nitrate	-
Trehalose	+	D-Arabinose	+	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	v	D-Glucuronate	+

### ***Trichosporon cutaneum* (de Beurmann *et al.*) Ota**

Colonies (SDA) are cream-coloured, cerebriform, glabrous, with radial furrows and irregular folds. Budding cells abundant in primary cultures; hyphae developing in older cultures. Arthroconidia are cylindrical to ellipsoidal. Appressoria absent. This species assimilates melibiose; not tolerant to 0.1% cycloheximide. No growth at 37°C. Uncommon species usually associated with superficial infections. RG-2 organism.

<b>Assimilation Tests:</b> + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	+	L-Rhamnose	+	D-Glucitol	+
Galactose	+	Raffinose	+	D-Glucosamine	v	α-M-D-glucoside	+
L-Sorbose	v	Melezitose	+	N-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	+	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	myo-Inositol	+
Cellobiose	+	L-Arabinose	+	Ribitol	+	Nitrate	-
Trehalose	+	D-Arabinose	v	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	+	D-Glucuronate	+

### ***Trichosporon inkin* (Oho *ex* Ota) do Carmo-Sousa & van Uden**

Colonies (SDA) are restricted, white, finely cerebriform with a granular covering, without marginal zone, often cracking the media. Budding cells and lateral conidia absent. Arthroconidia are long cylindrical. Appressoria present in slide cultures. Sarcinae present on media with high sugar-content. This species assimilates myo-inositol but not melibiose. Growth at 37°C. Usually associated with white piedra on pubic hairs. RG-2 organism.

### *Trichosporon inkin* (Oho ex Ota) do Carmo-Sousa & van Uden

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	-	D-Glucitol	-
Galactose	v	Raffinose	-	D-Glucosamine	v	$\alpha$ -M-D-glucoside	+
L-Sorbose	v	Melezitose	+	N-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	v	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	<i>myo</i> -Inositol	+
Cellobiose	+	L-Arabinose	v	Ribitol	-	Nitrate	-
Trehalose	+	D-Arabinose	v	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	v	D-Glucuronate	+

### *Trichosporon mucoides* Gueho & M.Th. Smith

Colonies (SDA) are moist and glabrous, white, cerebriform, heaped and folded. Budding cells present in primary cultures. Broadly clavate, terminal or lateral blastoconidia often present, becoming thick-walled with age. Arthroconidia are barrel-shaped. Appressoria absent. This species assimilates melibiose and is tolerant to 0.1% cycloheximide. Growth at 37°C. Common species associated with superficial infections, white piedra and onychomycosis. RG-2 organism.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	+	L-Rhamnose	+	D-Glucitol	+
Galactose	+	Raffinose	+	D-Glucosamine	+	$\alpha$ -M-D-glucoside	+
L-Sorbose	+	Melezitose	+	N-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	+	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	<i>myo</i> -Inositol	+
Cellobiose	+	L-Arabinose	+	Ribitol	+	Nitrate	-
Trehalose	+	D-Arabinose	+	Galactitol	+	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	+	D-Glucuronate	+

### *Trichosporon ovoides* Behrend

Colonies (SDA) are restricted, white, granular, folded at the centre, with a flat marginal zone. Budding cells and lateral conidia absent. Arthroconidia are cylindrical. Appressoria present in slide cultures. This species does not assimilate melibiose, but tolerates 0.1% cycloheximide. Growth at 37°C is variable. Uncommon species usually associated with superficial infections, like white piedra. RG-2 organism.

Assimilation Tests: + Positive, - Negative, v Variable, w Weak, s Slow							
Glucose	+	Melibiose	-	L-Rhamnose	+	D-Glucitol	v
Galactose	+	Raffinose	v	D-Glucosamine	v	$\alpha$ -M-D-glucoside	+
L-Sorbose	v	Melezitose	v	N-A-D-glucosamine	+	D-Gluconate	+
Sucrose	+	Soluble Starch	+	Glycerol	v	DL-Lactate	+
Maltose	+	D-Xylose	+	Erythritol	+	<i>myo</i> -Inositol	+
Cellobiose	+	L-Arabinose	v	Ribitol	-	Nitrate	-
Trehalose	v	D-Arabinose	v	Galactitol	-	2-K-D-gluconate	+
Lactose	+	D-Ribose	+	D-Mannitol	+	D-Glucuronate	+

## *Trichothecium roseum* (Persoon) Link ex Gray

Colonies are moderately fast growing, flat, suede-like to powdery, initially white but becoming rosy, pink or orange with age. The conidiophores are indistinguishable from the vegetative hyphae until the first conidium is produced. They are erect, unbranched, often septate near the base, more or less rough-walled, bearing basipetal zig-zag (alternating) chains of conidia at the apex. Note: the conidiophore is progressively shortened with the formation of each conidium i.e. retrogressive conidial development. Conidia are two-celled ellipsoidal to pyriform, with an obliquely truncate basal scar, hyaline, smooth to delicately roughened and thick-walled.

*Trichothecium roseum* has a world-wide distribution and is often isolated from decaying plant substrates, soil, seeds of corn, and food-stuffs (especially flour products). It is occasionally isolated as a saprophyte in the clinical laboratory. RG-1 organism.

*Trichothecium roseum* should not be confused with *Microsporium nanum*. Colonies of the latter may be pinkish-buff in colour and also produce ovoid to pear-shaped, mostly two-celled macroconidia with thin, verrucose walls. However, *M. nanum* usually produces a red-brown reverse pigment and the two-celled macroconidia are sessile and formed singly, sometimes on stalks, on undifferentiated conidiophores which do not undergo further change or produce secondary conidia. Note: conidia are not produced in basipetal chains as in *T. roseum*. Finally, *M. nanum* will perforate hair *in vitro*.

**Key Features:** hyphomycete, basipetal zig-zag chains of two-celled conidia showing retrogressive development where the conidiophore becomes progressively shorter.

For descriptions of species, keys to taxa and additional information see McGinnis (1980), Domsch *et al.* (1980), Rippon (1988) and Samson *et al.* (1995).



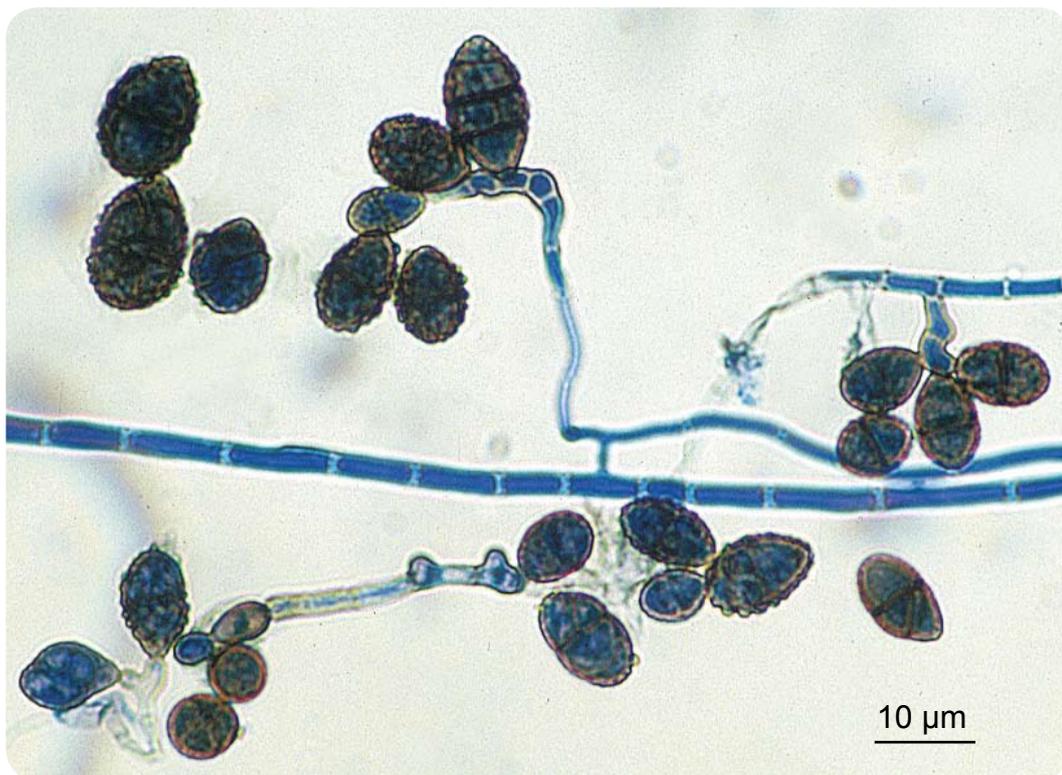
Conidiophores of *T. roseum* demonstrating retrogressive conidial development.

### ***Ulocladium* Preuss**

Colonies are rapid growing, brown to olivaceous-black or greyish and suede-like to floccose. Microscopically, numerous, usually solitary, multi-celled conidia (dictyocoonidia) are formed through a pore (poroconidia) by a sympodially elongating geniculate conidiophore. Conidia are typically obovoid (narrowest at the base), dark brown and often rough-walled. Seven species have been described, all being saprophytes. RG-1 organism.

Species of *Ulocladium* should not be confused with other poroconidial genera such as *Stemphylium*, *Alternaria*, *Bipolaris*, *Exserohilum*, *Dreschlera* and *Curvularia*.

For descriptions of species, keys to taxa and additional information see Ellis (1970 and 1976), Domsch *et al.* (1980), Rippon (1988), Samson *et al.* (1995) and de Hoog *et al.* (2000).



Conidia of *Ulocladium*.

Antifungal	MIC $\mu\text{g/mL}$	Antifungal	MIC $\mu\text{g/mL}$
	Range		Range
Fluconazole	8->64	Amphotericin B	1->16
Itraconazole	0.06->16	Flucytosine	>128
Voriconazole	0.25		

Very limited data. Antifungal susceptibility testing of individual strains is recommended. Pujol *et al.* (2000) and WCH in-house data.

### ***Veronaea botryosa* Ciferri & Montemartini**

Colonies grow rapidly and are suede-like to downy, greyish-brown to blackish-brown. Conidiophores are erect, straight or flexuose, occasionally branched and are usually geniculate, due to the sympodial development of the conidia. They are smooth-walled, pale to medium olivaceous-brown, up to 250  $\mu\text{m}$  long and 2-4  $\mu\text{m}$  wide. Conidia are pale brown, two-celled, cylindrical with a truncated base, smooth-walled or slightly verrucose, 5-12  $\times$  3-4  $\mu\text{m}$ . RG-1 organism.

This genus is very similar to *Rhinocladiella*, however the conidia are typically two-celled. Occasional skin infections have been reported from humans.

For descriptions of species, keys to taxa and additional information see Ellis (1971) and de Hoog *et al.* (2000).



Conidiophores and conidia of *Veronaea botryosa*.

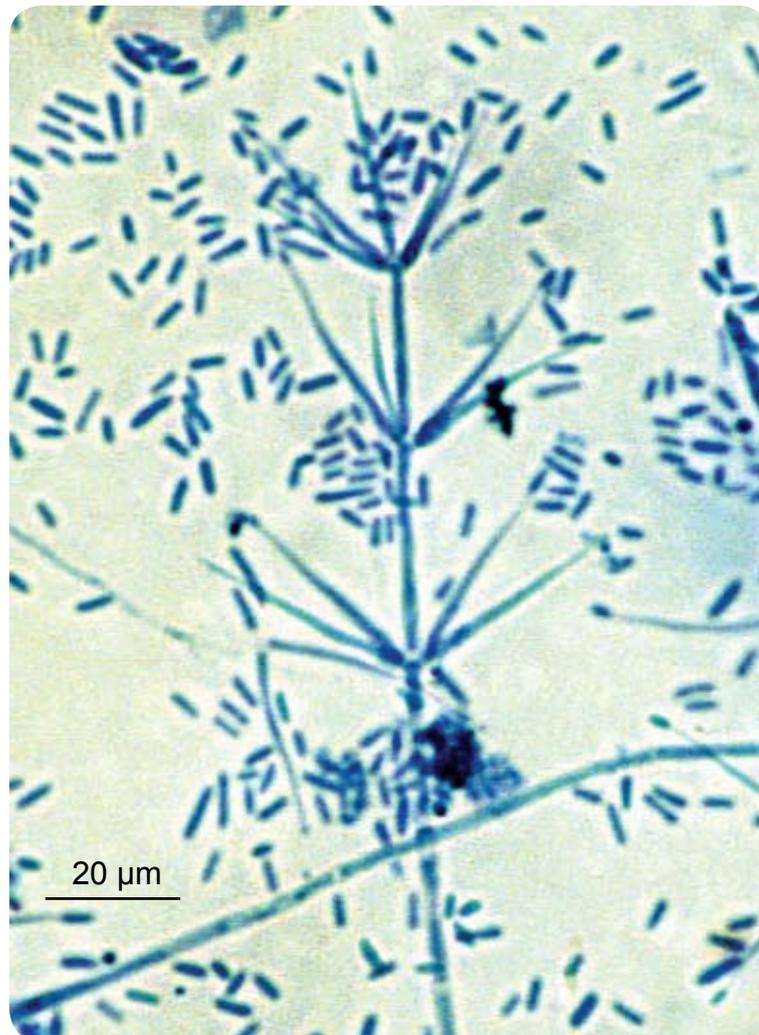
### ***Verticillium* Nees ex Link**

Colonies are fast growing, suede-like to downy, white to pale yellow in colour, becoming pinkish brown, red, green or yellow with a colourless, yellow or reddish brown reverse. Conidiophores are usually well differentiated and erect, verticillately branched over most of their length, bearing whorls of slender awl-shaped divergent phialides. Conidia are hyaline or brightly coloured, mostly one-celled, and are usually borne in slimy heads (glioconidia).

Members of this genus are often isolated from the environment. It has been reported as a rare agent of mycotic keratitis. RG-1 organism.

**Key Features:** hyphomycete, verticillate branched conidiophores bearing whorls of awl-shaped, divergent phialides.

For descriptions of species, keys to taxa and additional information see Domsch *et al.* (1980), McGinnis (1980), Rippon (1988), Samson *et al.* (1995), de Hoog *et al.* (2000).



Conidiophores, phialides and conidia of *Verticillium*.

## MICROSCOPY STAINS & TECHNIQUES

### Calcofluor White with 10% KOH.

For the direct microscopic examination of skin scrapings, hairs, nails and other clinical specimens for fungal elements. This as a very sensitive method, however, a fluorescence microscope with the correct ultraviolet filters is required (Hageage and Harrington, 1984; Hollander *et al.*, 1984; Monheit *et al.*, 1984).

#### Solution A: Potassium hydroxide reagent.

Potassium hydroxide	10 g
Glycerine	10 mL
Distilled water	80 mL

#### Solution B: Calcofluor white reagent.

Calcofluor white	0.5 g
Evans blue	0.02 g
Distilled water	50 mL
Mix one drop of each solution on the centre of a clean microscope slide. Place the specimen in the solution and cover with a coverslip.	

### Potassium Hydroxide (KOH) with Chlorazol Black.

For the direct microscopic examination of skin scrapings, hairs, nails and other clinical specimens for fungal elements. Note: Parker Quink ink is no longer available.

Potassium hydroxide	10 g
Coral Azole E Black (0.1% - 100mg in 100ml)	10 mL
Glycerol	10 mL
Distilled water	80 mL
Using sterile technique, remove a small portion of the specimen with an inoculation needle and mount in a drop of KOH on a clean microscope slide. Cover with a coverslip, squash the preparation with the butt of the inoculation needle and then blot off the excess fluid.	

### Indian Ink Mounts.

For the direct microscopic examination of CSF for *Cryptococcus* species. Place a drop of Indian Ink on the specimen, mix well with a sterilised loop, and cover with a coverslip. Best brands to use are "Pelikan" or "Talons" Indian Ink.

## MICROSCOPY STAINS & TECHNIQUES

### Lactophenol Cotton Blue (LPCB).

For the staining and microscopic identification of fungi.

Cotton Blue (Aniline Blue)	0.05 g
Phenol Crystals (C <sub>6</sub> H <sub>5</sub> O <sub>4</sub> )	20 g
Glycerol	40 mL
Lactic acid (CH <sub>3</sub> CHOH COOH)	20 mL
Distilled water	20 mL

**This stain is prepared over two days.**

1. On the first day, dissolve the Cotton Blue in the distilled water. Leave overnight to eliminate insoluble dye.
2. On the second day, wearing gloves add the phenol crystals to the lactic acid in a glass beaker. Place on magnetic stirrer until the phenol is dissolved.
3. Add the glycerol.
4. Filter the Cotton Blue and distilled water solution into the phenol/glycerol/lactic acid solution. Mix and store at room temperature.

### Direct Microscopic Mounts or Squash Preparations.

Using sterile technique, remove a small portion of the colony with an inoculation needle and mount in a drop of Lactophenol Cotton Blue on a clean microscope slide. Cover with a coverslip, squash the preparation with the butt of the inoculation needle and then blot off the excess fluid.

### Cellotape Flag Preparations.

An excellent technique for the rapid mounting of sporulating fungi because it keeps more of the reproductive structures intact.

1. Using clear 2cm wide cellotape and a wooden applicator stick (orange stick) make a small cellotape flag (2 x 2 cm).
2. Using sterile technique, gently press the sticky side of the flag onto the surface of the culture.
3. Remove and apply a drop of 95% alcohol to the flag, this acts as a wetting agent and also dissolves the adhesive glue holding the flag to the applicator stick.
4. Place the flag onto a small drop of Lactophenol cotton blue on a clean glass slide, remove the applicator stick and discard, add another drop of stain, cover with a coverslip, gently press and mop up any excess stain.

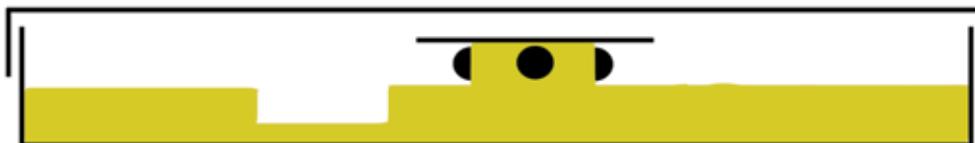
## MICROSCOPY STAINS & TECHNIQUES

### Slide Culture Preparations.

In order to accurately identify many fungi it is essential to observe the precise arrangement of the conidiophores and the way in which spores are produced (conidial ontogeny). Riddel's simple method of slide culturing (*Mycologia* 42:265, 1950) permits fungi to be studied virtually *in situ* with as little disturbance as possible. A simple modification of this method using a single agar plate is described below.

One plate of nutrient agar; potato dextrose is recommended, however, some fastidious fungi may require harsher media to induce sporulation like Cornmeal agar or Czapek Dox agar.

1. Using a sterile blade cut out an agar block (7 x 7 mm) small enough to fit under a coverslip.
2. Flip the block up onto the surface of the agar plate.
3. Inoculate the four sides of the agar block with spores or mycelial fragments of the fungus to be grown.
4. Place a flamed coverslip centrally upon the agar block.
5. Incubate the plate at 26°C until growth and sporulation have occurred.
6. Remove the cover slip from the agar block.
7. Apply a drop of 95% alcohol as a wetting agent.
8. Gently lower the coverslip onto a small drop of Lactophenol cotton blue on a clean glass slide.
9. The slide can be left overnight to dry and later sealed with fingernail polish.
10. When sealing with nail polish use a coat of clear polish followed by one coat of red-coloured polish.



Simple agar block method, inoculated on four sides with cover slip on top. Make at least 2 slides per culture.

## SPECIALISED CULTURE MEDIA

### Bird Seed Agar (Staib, 1987).

for selective isolation of *Cryptococcus neoformans* and *C. gattii*.

<i>Guizotia abyssinica</i> (niger seed)	50 g	Glucose	1 g
KH <sub>2</sub> PO <sub>4</sub> (potassium dihydrogen orthophosphate)	1 g	Creatinine	1 g
Bacto Agar (BD 214010)	15 g	Distilled water	1000 mL
Penicillin G (20 units/mL)	1 mL	Gentamicin (40 mg/mL)	1 mL

1. Grind seeds of *Guizotia abyssinica* as finely as possible with an electric mixer and add to 1000 mL distilled water in a stainless steel jug.
2. Boil for 30 minutes, pass through filter paper and adjust volume to 1000 mL.
3. Add remaining ingredients except Bacto Agar to filtrate and dissolve.  
If required: Cool to room temperature and adjust pH to 5.5.  
Dispense into 500 mL bottles.
4. Add 7.5 g Bacto Agar to each 500 mL reagent bottle.
5. Autoclave 110°C for 20 minutes.
6. Cool to 48°C and add 0.5 mL Penicillin G and 0.5 mL Gentamicin to each 500 mL of Bird Seed Agar.
7. Mix gently and pour into 90 mm plastic petri dishes.

### Bromcresol Purple Milk Solids Glucose Agar (BCP-MS-G).

for the differentiation of *Trichophyton* species (Kane *et al.* 1977).

#### Solution A:

Distilled water	1000 mL
Skim milk powder (Carnation Brand)	80 g
Bromcresol (or bromocresol) purple (1.6% solution in alcohol)	2 mL
Dissolve in 2 litre flask and autoclave 10 psi/15 minutes.	

#### Solution B:

Glucose	40 g	Distilled water	200 mL
Dissolve and autoclave at 10 psi/8minutes.			

#### Solution C:

Bacto Agar (BD 214010)	30 g	Distilled water	800 mL
Soak for 15 minutes in 3 litre flask; autoclave at 15 psi/15 minutes			

To make media; add solution A and B to solution C. Adjust final pH to 6.6. Aseptically dispense for slopes (7 mL amounts into 30 mL disposable bottles).

**Caution:** Do not substitute casein for skim milk. Check pH is 6.6.

## SPECIALISED CULTURE MEDIA

### **CDBT (Creatinine dextrose bromothymol blue thymine agar).**

for differentiation of *Cryptococcus neoformans* var. *neoformans* and *Cryptococcus neoformans* var. *grubii* (Irokanulo *et al.* 1994).

#### **Solution A:**

Creatinine	1 g	Dextrose	0.5 g
KH <sub>2</sub> PO <sub>4</sub>	1 g	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5 g
Thymine	0.1 g	Distilled water	980 mL
1. Dissolve ingredients in small beaker and adjust pH to 5.6			
2. Store in refrigerator.			

#### **Solution B (Aqueous Bromothymol Blue):**

Bromothymol blue	0.4 g	0.01N NaOH	64 mL
Distilled water	36 mL		
1. Dissolve the Bromothymol Blue in the NaOH			
2. Add to the water.			

#### **To prepare medium (1 litre for plates):**

Solution A	980 mL	Solution B	20 mL
Bacto Agar (BD 214010)	20 g		
Autoclave to 121°C for 15 minutes, cool to 48°C and pour plates.			

### **CGB (L-Canavanine glycine bromothymol blue agar).**

for differentiation of *Cryptococcus neoformans* and *Cryptococcus gattii* (Kwon-Chung *et al.* 1982).

#### **Solution A:**

Glycine Univar	10 g	KH <sub>2</sub> PO <sub>4</sub>	1 g
MgSO <sub>4</sub>	1 g	Thiamine HCl	1 mg
L-canavanine sulphate	30 mg	Distilled water	100 mL
1. Dissolve ingredients in small beaker and adjust pH to 5.6			
2. Filter sterilise solution using 0.22 µm filter.			
3. Store in refrigerator.			

#### **Solution B (Aqueous Bromothymol Blue):**

Bromothymol blue	0.4 g	0.01N NaOH	64 mL
Distilled water	36 mL		
1. Dissolve the Bromothymol Blue in the NaOH			
2. Add to the water.			

#### **To prepare medium (1 litre for plates):**

Distilled water	980 mL	Solution B	20 mL
Bacto Agar (BD 214010)	20 g		
1. Autoclave to 121°C for 15 minutes, cool to 48°C.			
2. For plates add 100 mL of the filtered solution A and mix. Dispense in plates.			

## SPECIALISED CULTURE MEDIA

### Cornmeal Agar.

for routine cultivation and identification of fungi.

Cornmeal agar (Oxoid CM 0103)	8.5 g
Distilled water	500 mL
<ol style="list-style-type: none"> <li>1. Mix dry ingredients into 100 mL H<sub>2</sub>O, boil remaining water.</li> <li>2. Add boiling water to mixture and bring to boil.</li> <li>3. Autoclave for 10 minutes at 120°C, then slope on racks.</li> </ol>	

### Cornmeal Glucose Sucrose Yeast Extract Agar.

for zygomycete sporulation

Cornmeal agar (Oxoid CM 0103)	17 g
Dextrose (Glucose)	2 g
Sucrose	3 g
Yeast extract	1 g
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Mix dry ingredients into 100 mL H<sub>2</sub>O, boil remaining water.</li> <li>2. Add boiling water to mixture and bring to boil.</li> <li>3. Dispense for slopes.</li> <li>4. Autoclave for 10 minutes at 120°C, remove and slope.</li> </ol>	

### Czapek Dox Agar.

for routine cultivation of fungi, especially *Aspergillus*, *Penicillium*, and non-sporulating moulds.

Czapek Dox Agar (Oxoid CM97)	45.4 g
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Soak the ingredients in small amount of water.</li> <li>2. Bring remaining water to boil, add to soaking ingredients and bring to the boil again, stirring continuously.</li> <li>3. Dispense for slopes as required.</li> <li>4. Autoclave at 121°C for 10 minutes, remove and slope or pour for plates as required.</li> </ol>	

### Dixon's Agar (modified).

for primary isolation and cultivation of *Malassezia* species.

Malt extract (Oxoid L39)	9 g	Bacto Tryptone	1.5 g
Ox-bile Desiccated (Oxoid L50)	5 g	Tween 40	2.5 mL
Oleic acid	0.5 g	Glycerol	0.5 mL
Bacto Agar	3 g	Distilled water	250 mL
<ol style="list-style-type: none"> <li>1. Soak ingredients in a little of the water.</li> <li>2. Bring remaining water to boil, add to the soaking ingredients and bring to the boil again constantly stirring.</li> <li>3. Dispense for slopes (7 mL amounts into 30 mL disposable bottles).</li> <li>4. Autoclave at 121°C for 10 minutes and then slope.</li> </ol>			

## SPECIALISED CULTURE MEDIA

### Hair Perforation Test.

for the differentiation of *Trichophyton* species.

Blonde pre-pubital hair cut into short pieces (1 cm)	10-20 hairs
Distilled water	5 mL
<ol style="list-style-type: none"> <li>1. Autoclave hair at 121°C for 10 minutes and store in sterile container.</li> <li>2. Place 10-20 short pieces of hair in 5 mL water in vial.</li> <li>3. Inoculate with small fragments of the test fungus.</li> <li>4. Incubate at room temperature.</li> <li>5. Individual hairs are removed at intervals up to 4 weeks and examined microscopically in lactophenol cotton blue. Isolates of <i>T. mentagrophytes</i> produce marked localised areas of pitting and marked erosion whereas those of <i>T. rubrum</i> do not.</li> </ol>	

### Lactritmel Agar.

for the production of pigment by *Trichophyton* species.

Skimmed milk powder (use only Dutch Jug skimmed milk powder)	7 g
Honey	10 g
Cornmeal agar (Oxoid CM 0103)	17 g
Chloramphenicol	1 x 250 capsule
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Weigh skimmed milk into stainless steel jug. Slowly add some water, mixing milk into smooth paste. Continue adding small quantities of water until powder is dissolved (about 150 mL).</li> <li>2. Weigh other ingredients into skimmed milk and allow to soak.</li> <li>3. Boil remaining water, and with it wash out honey from beaker.</li> <li>4. Add to other ingredients and boil.</li> <li>5. Dispense for slopes (7 mL).</li> <li>6. Autoclave for 10 minutes at 115°C.</li> <li>7. On removal from autoclave allow to stand 5 minutes then shake and slope on racks.</li> </ol> <p><b>Note:</b> Do not filter or adjust pH in any way</p>	

### Littman Oxgall Agar.

for the differentiation of *Trichophyton* species.

Littman Oxgall Agar (US Biological L3025)	27.5 g
Distilled water	500 mL
<ol style="list-style-type: none"> <li>1. Soak agar in 100 mL of water in stainless steel jug. Boil remaining 400mL in a separate jug.</li> <li>2. When water has boiled add to soaking agar and reboil, stirring constantly.</li> <li>3. Dispense for slopes.</li> <li>4. Autoclave for 10 minutes at 121°C, remove and slope.</li> </ol>	

## SPECIALISED CULTURE MEDIA

### Malt Extract Agar.

for routine cultivation and identification of fungi.

Oxoid Malt Extract (L39)	20 g
Bacto Agar (BD 214010)	20 g
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Dissolve malt extract in a plastic beaker and pH the solution to pH 6.5 with NaOH.</li> <li>2. Soak agar in small quantity of solution. Bring remaining solution to the boil, stirring constantly.</li> <li>3. Add to soaking agar. Bring to boil, stirring constantly.</li> <li>4. Dispense for slopes as required.</li> <li>5. Autoclave at 121°C for 10 minutes, remove and slope or pour for plates as required.</li> </ol>	

### 1% Peptone Agar.

for the differentiation of *Trichophyton* species.

Tryptone Peptone (BD 211705)	5 g
Bacto Agar (BD 214010)	10 g
Distilled water	500 mL
<ol style="list-style-type: none"> <li>1. Soak agar and peptone in about 50 mL of water.</li> <li>2. Boil remaining water, add this to soaking ingredients and bring to boil again.</li> <li>3. Dispense for slopes (7 mL).</li> <li>4. Autoclave for 10 minutes at 121°C, then slope on racks.</li> </ol>	

### Potato Dextrose Agar.

for routine cultivation and identification of fungi.

Potato Dextrose Agar (Oxoid CM139)	39 g
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Soak potato dextrose agar in small amount of the water in a stainless steel jug.</li> <li>2. Boil remaining water, add to soaking ingredients, bring to the boil, stirring constantly.</li> <li>3. Dispense for slopes as required.</li> <li>4. Autoclave at 121°C for 15 minutes. Remove and slope or pour for plates as required.</li> </ol>	

### Rice Grain Slopes.

to induce sporulation and for differentiation of *M. audouinii* and *M. canis*.

Polished rice grains	Distilled water
<ol style="list-style-type: none"> <li>1. Place ~ 1/2 teaspoon rice grains into wide neck 20 mL glass vials.</li> <li>2. Add 8 mL distilled water to each vial.</li> <li>3. Lid, then slope on racks ensuring rice grains are evenly distributed.</li> <li>4. Autoclave racks at 121°C for 15 minutes.</li> </ol>	

## SPECIALISED CULTURE MEDIA

### **Sabouraud Dextrose Agar with Cycloheximide, Chloramphenicol, Gentamicin and Yeast Extract.**

for the primary isolation and cultivation of dermatophytes.

Sabouraud Dextrose Agar (Oxoid CM41)	65 g
Cycloheximide (Actidione)	0.5 g
Chloramphenicol	1 x 250 capsule
Gentamicin (40mg/mL)	0.56 mL
Yeast extract	5 g
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Soak all ingredients, except Gentamicin, in 100 mL water.</li> <li>2. Boil remaining water, add to soaking ingredients, and bring to boil to dissolve, stirring well to prevent from charring.</li> <li>3. Add the Gentamicin. Mix well.</li> <li>4. Dispense for slopes as required.</li> <li>5. Autoclave at 121°C for 10 minutes. Remove and slope, or pour for plates as required.</li> </ol>	

### **Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin.**

for primary isolation and routine culture of yeasts and moulds.

Sabouraud Dextrose Agar (Oxoid CM41)	65 g
Chloramphenicol	1 x 250 capsule
Gentamicin (40mg/mL)	0.56 mL
Distilled water	1000 mL
See above method for Sabouraud Dextrose Agar with Cycloheximide, Chloramphenicol, Gentamicin and Yeast Extract.	

### **Sabouraud Dextrose Agar with 5% Salt.**

for the differentiation of *Trichophyton* species.

Sabouraud Dextrose Agar (Oxoid CM41)	32.5 g
Sodium Chloride NaCl (Univar 465)	25 g
Distilled water	500 mL
<ol style="list-style-type: none"> <li>1. Soak ingredients in approximately 100 mL water.</li> <li>2. Bring remaining water to boil, add to soaking ingredients.</li> <li>3. Dispense for slopes (7 mL).</li> <li>4. Autoclave at 118°C for 10 minutes, then slope on racks.</li> </ol>	

## SPECIALISED CULTURE MEDIA

### Tap Water Agar.

for the stimulation of sporulation in *Apophysomyces* and *Saksenaea* isolates.

Bacto Agar (BD 214010)	15 g
Distilled water	1000 mL
<ol style="list-style-type: none"> <li>1. Add agar to water in stainless steel jug, allow to soak.</li> <li>2. Dispense for slopes.</li> <li>3. Autoclave at 118°C for 10 minutes, remove and slope.</li> </ol>	

### Urease Agar Slopes with 0.5% Glucose.

for the differentiation of Urease producing organisms.

#### Urease glucose broth base:

Urea, broth base (Oxoid CM71)	0.9 g
Glucose	5 g
Distilled water	450 mL
<ol style="list-style-type: none"> <li>1. Add the Urea broth base and glucose to the distilled water in a 500mL beaker.</li> <li>2. Dispense in 5 X 90 mL amounts.</li> <li>3. Autoclave at 115°C for 20 mins.</li> <li>4. When cool, label and store in the fridge.</li> </ol>	

#### Method to make slopes:

40% Urea Solution (Oxoid SR 20)	10 mL
Bacto Agar (BD 214010)	3 g
Distilled water	100 mL
<ol style="list-style-type: none"> <li>1. Add 3.0 grams of agar to 100 mL of distilled water in a 250 mL pyrex bottle.</li> <li>2. Autoclave at 121°C for 15 minutes and place in 50°C water bath.</li> <li>3. When cool add 90 mL of the Urease broth with glucose and the 10 mL of 40% urea solution to agar and dispense in 3 mL aliquots and slope on racks.</li> </ol>	

### Vitamin Free Agar (Trichophyton Agar No.1).

for the differentiation of *Trichophyton* species.

Trichophyton Agar No. 1 (BD 287710)	11.8 g
Distilled water	200 mL
<ol style="list-style-type: none"> <li>1. Add agar to water in stainless steel jug, allow to soak.</li> <li>2. Bring to boil to dissolve, stirring constantly.</li> <li>3. Once boiled remove immediately to avoid discolouration.</li> <li>4. Dispense for slopes.</li> <li>5. Autoclave at 118°C for 10 minutes, remove and slope.</li> </ol>	

## REFERENCES

- Adam, R.D., M.L. Paquin, E.A. Petersen et al.** (1986). Phaeohyphomycosis caused by the fungal genera *Bipolaris* and *Exserohilum*. A report of 9 cases and review of the literature. *Medicine*. 65:203-217.
- Ajello, L.** 1957. *Coccidioides immitis*: Isolation procedures and diagnostic criteria. Proceedings of symposium on Coccidioidomycosis. Public Health Publication No. 575, CDC Atlanta, USA.
- Ajello, L.** 1977. Taxonomy of the dermatophytes: a review of their imperfect and perfect states. In "Recent Advances in Medical and Veterinary Mycology" (K. Iwata, ed.), pp. 289-297. University Park Press, Baltimore, Maryland, USA.
- Ajello, L., D.F. Dean and R.S. Irwin.** 1976. The zygomycete *Saksenaea vasiformis* as a pathogen of humans with a critical review of the etiology of zygomycosis. *Mycologia*. 68:52-62.
- Alcorn, J.L.** 1983. Genetic concepts in *Drechslera*, *Bipolaris* and *Exserohilum*. *Mycotaxon*. 17:1-86.
- Al-Mohsen, I.Z., D.A. Sutton, L. Sigler et. al.** 2000. *Acrophialophora fuispora* brain abscess in a child with acute lymphoblastic leukaemia: review of cases and taxonomy. *J. Clin. Microbiol.* 38:4569-4576.
- Alvarado-Ramirez, E., J.M. Torres-Rodriguez.** 2007. *In vitro* susceptibility of *Sporothrix schenckii* to six antifungal agents using three different methods. *Antimicrob. Agents Chemother.* Apr 16 (Epub).
- Ames, L.M.** 1963. A monograph of the Chaetomiaceae. U.S. Army Research and Development Serial. 2:1-125.
- Barnett, J.A., R.W. Payne and D. Yarrow.** 1983. Yeasts: characteristics and identification. Cambridge University Press, London, UK.
- Barron, G.L.** 1968. The genera of hyphomycetes from soil. Williams & Wilkins Co. Baltimore, USA.
- Booth, C.** 1966. The genus *Cylindrocarpon*. *Mycol. Pap.* 104:1-56.
- Booth, C.** 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England.
- Booth, C.** 1977. *Fusarium*: laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, England.
- Buchta, V. and M. Otcenasek.** 1988. *Geotrichum candidum* - an opportunistic agent of mycotic diseases. *Mycoses*. 31:363-370.
- Burges, G.E., C.T. Walls and J.C. Maize.** 1987. Subcutaneous phaeohyphomycosis caused by *Exserohilum rostratum* in an immunocompetent host. *Arch. Dermatol.* 123:1346-1350.
- Burgess, L.W. and C.M. Liddell.** 1983. Laboratory manual for *Fusarium* research. Fusarium Research Laboratory, Department of Plant Pathology and Agricultural Entomology. The University of Sydney.
- Campbell, C.K. and M.D. Smith.** 1982. Conidiogenesis in *Petriellidium boydii* (*Pseudallescheria boydii*). *Mycopathologia*. 78:145-150.
- Carmichael, J.W.** 1962. *Chrysosporium* and some aleuriosporic hyphomycetes. *Can. J. Bot.* 40:1137-1173.
- Casadevall, A. and J.R. Perfect.** 1988. *Cryptococcus neoformans*. ASM Press USA.
- Catanzaro, A.** 1985. *Coccidiomycosis*. In Fungal Diseases of the Lung, eds G.A. Sarosi and S.F. Davies. Grune and Stratton Inc.
- Cavalier-Smith, T.** 1998. A revised six-kingdom system of life. *Biol Rev Canm Philos Soc.* 73: 203-266.

## REFERENCES

- Chandler, F.W., W. Kaplan and L. Ajello.** 1980. A colour atlas and textbook of the histopathology of mycotic diseases. Wolfe Medical Publications Ltd.
- Cooney, D.H. and R. Emerson.** 1964. Thermophilic fungi. W.H. Freeman & Co.
- Cooter, R.T., I.S. Lim, D.H. Ellis et. al.** 1990. Burn wound zygomycosis caused by *Apophysomyces elegans*. J.Clin. Microbiol. 28: 2151-2153.
- Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado et. al.** 2006. Head-to-head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentous fungi. Antimicrob. Agents Chemother. 50:917-921.
- Dannaoui, E., J. Meletiadis, J.W. Mouton et. al.** 2003. *In vitro* susceptibilities of zygomycetes to conventional and new antifungals. J. Antimicrob. Chemother. 51:45-52.
- Davis, S.R., D.H. Ellis, P. Goldwater et. al.** 1994. First human culture-proven Australian case of entomophthoromycosis caused by *Basidiobolus ranarum*. J Med. Vet. Mycol. 32: 225-230.
- de Hoog, G.S.** 1972. The genera *Beauvaria*, *Isaria*, *Tritrachium* and *Acrodontium* Gen. Nov. Studies in Mycology, Centraalbureau voor Schimmelcultures, Baarn. 1:1-41.
- de Hoog, G.S.** 1977. *Rhinocladiella* and allied genera. Studies in Mycology, Centraalbureau voor Schimmelcultures, Baarn. 15:1-140
- de Hoog, G.S.** 1983. On the potentially pathogenic dematiaceous Hyphomycetes. In: D.H. Howard (ed). The fungi pathogenic to humans and animals. A:149-216.
- de Hoog, G.S.** 1985. The taxonomic structure of *Exophiala*. in Fungi pathogenic for humans and animals. Part B: Pathogenicity and detection: II. (ed. D. Howard). Marcel Dekker Inc.
- de Hoog, G.S., E. Gueho, F. Masclaux et. al.** 1995. Nutritional physiology and taxonomy of human-pathogenic *Cladosporium-Xylohypha* species. J. Med. Vet. Mycol. 33:339-347.
- de Hoog, G.S. and E.J. Hermanides-Nijhof.** 1977. The black yeasts and allied hyphomycetes. Studies in Mycology No. 15. Centraalbureau voor Schimmelcultures, The Netherlands.
- de Hoog, G.S., A.H. Rantio-Lehtimäki and M.TH. Smith.** 1985. *Blastobotryis*; *Sporothrix* and *Trichosporiella*; generic delimitation, new species, and a *Stephanoascus* teleomorph. Antonie van Leeuwenhoek. 51:79-109.
- de Hoog, G.S., V. Vincent, R.B. Caligiorne et. al.** 2003. Species diversity and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. J. Clin. Microbiol. 41:4767-4778.
- de Hoog, G.S., D. Attili, V.A. Vicente et. al.** 2004. Molecular ecology and pathogenic potential of *Fonsecaea* species. Med. Mycol. 42:405-416.
- de Hoog, G.S., J. Guarro, J. Gene and M.J. Figueras.** 2000. Atlas of Clinical Fungi (second edition). Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
- de Hoog, G.S., J.S. Zeng, M.J. Harrak and D.A. Sutton.** 2006. *Exophiala xenobiotica* sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons. Antonie Van Leeuwenhoek 90:257-268.
- Diekema, D.J., S.A. Messer, R.J. Hollis et. al.** 2003. Activities of caspofungin, itraconazole, posaconazole, ravuconazole, voriconazole, and amphotericin B against 448 recent clinical isolates of filamentous fungi. J. Clin. Microbiol. 41:3623-3626.

## REFERENCES

- Dixon, D.M. and A. Polak-Wyss.** 1991. The medically important dematiaceous fungi and their identification. *Mycoses*. 34:1-18.
- Domsch, K.H., W. Gams and T.H. Anderson.** 1980. Compendium of soil fungi. Volume 1. Academic Press.
- Dworzack, D.L., A.S. Pollock, G.L. Hodges et. al.** 1978. Zygomycosis of the maxillary sinus and palate caused by *Basidiobolus haptosporus*. *Arch. Intern. Med.* 138:1274-1276
- Ellis, D.H.** 1981. Ascocarp morphology and terminal hair ornamentation in thermophilic *Chaetomium* species. *Mycologia*. 73:755-773.
- Ellis, D.H.** 2005a. Subcutaneous Zygomycetes - Entomophthoromycosis. Chapter 17. In Topley and Wilson's Microbiology and Microbial Infections: medical Mycology, 10th edition, Hodder Arnold London. pp 347-355.
- Ellis, D.H.** 2005b. Systemic Zygomycetes - Mucormycosis. Chapter 33. In Topley and Wilson's Microbiology and Microbial Infections: Medical Mycology, 10th edition, Hodder Arnold London. pp 659-686.
- Ellis, D.H., and G. Kaminski** 1984. Laboratory identification of *Saksenaea vasiformis*: a rare cause of zygomycosis in Australia. *Sabouraudia: Journal of Medical and Veterinary Mycology*. 23:137-140.
- Ellis, D.H., and P.J. Keane.** 1981. Thermophilic fungi isolated from some Australian soils. *Aust. J. Bot.* 29:689-704.
- Ellis, J.J.** 1985. Species and varieties in *Rhizopus arrhizus* - *Rhizopus oryzae* group as indicated by their DNA complementarity. *Mycologia*. 77:243-247.
- Ellis, J.J.** 1986. Species and varieties in the *Rhizopus microsporus* group as indicated by their DNA complementarity. *Mycologia*. 78:508-510
- Ellis, J.J., and L. Ajello.** 1982. An unusual source of *Aphophysomyces elegans* and a method of stimulating sporulation of *Saksenaea vasiformis*. *Mycologia* 74:144-145.
- Ellis, J.J. and C.W. Hesseltine.** 1966. Two new families of Mucorales. *Mycologia*. 66:87-95.
- Ellis, J.J. and C.W. Hesseltine.** 1965. The genus *Absidia*: globose spored species. *Mycologia*. 57:222-235.
- Ellis, J.J. and C.W. Hesseltine.** 1966. Species of *Absidia* with ovoid sporangiospores. II. *Sabouraudia*. 5:59-77.
- Ellis, M.B.** 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.
- Ellis, M.B.** 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.
- Emmons, C.W. and C.H. Bridges.** 1977. *Entomophthora coronata*, the etiologic agent of a phycomycosis of horses. *Mycologia*. 53:307-312.
- Espinel-Ingroff, A., K. Boyle and D.J. Sheehan.** 2001. *In vitro* antifungal activities of voriconazole and reference agents as determined by NCCLS methods: review of the literature. *Mycopathologia* 150:101-115.
- Espinel-Ingroff, A.** 2001. *In vitro* fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi. *J. Clin. Microbiol.* 39:954-958.
- Espinel-Ingroff, A.** 2003. *In vitro* antifungal activities of anidulafungin and micafungin, licensed agents and the investigational triazole posaconazole as determined by NCCLS methods for 12,052 fungal isolates: review of the literature. *Rev. Iberoam. Micol.* 20:121-136.

## REFERENCES

- Espinel-Ingroff, A.** 2006. Comparison of three commercial assays and a modified disk diffusion assay with two broth microdilution reference assays for testing zygomycetes, *Aspergillus* spp., *Candida* spp., and *Cryptococcus neoformans* with posaconazole and amphotericin B. *J. Clin. Microbiol.* 44:3616-3622.
- Fernandez-Torres, B., A.J. Carrillo, E. Martin et al.** 2001. *In vitro* activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob. Agents Chemother.* 45:2524-2528.
- Ferry, A.P. and S. Abedi.** 1983. Diagnosis and management of rhino-orbitocerebral mucormycosis (phycomycosis). *Ophthalmology.* 90: 1096-1104.
- Fisher, M.C., G.L. Koenig, T.J. White and J.W. Taylor.** 2002. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognised as the non-California population of *Coccidioides immitis*. *Mycologia.* 94:73-84.
- Frankel, D.H. and J.W. Rippon.** 1989. *Hendersonula toruloidea* infection in man. *Mycopathologia.* 105:175-186.
- Franzot, S.P., I.R. Salkin and A. Casadevall.** 1999. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J. Clin. Microbiol.* 37:838-840.
- Frye, C.B. and J. Reinhardt.** 1993. Characterization of groups of the zygomycete genus *Rhizopus*. *Mycopathologia.* 124: 139-147.
- Gams, W.** 1971. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes). G. Fisher, Stuttgart, p.262.
- George, R.B. and R.L. Penn.** 1986. Histoplasmosis. In *Fungal diseases of the Lung*. eds Sarosi, G.A. and S.F. Davies. Grune and Stratton Inc.
- Gilgado, F., J. Cano, J. Gene and J. Guarro.** 2005. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. *J. Clin. Microbiol.* 43:4930-4942.
- Girmania, C., G. Pizzarelli, D. D'Antonio et al.** 2003. *In vitro* susceptibility testing of *Geotrichum capitatum*: comparison of the Etest, disk diffusion, and sensititre colorimetric methods with the NCCLS M27-A2 broth microdilution reference method. *Antimicrob. Agents Chemother.* 47:3985-3988.
- Goldschmied-Reouven, A., A. Shvoron, M. Topaz and C. Block.** 1989. *Saksenaia vasiformis* infection in a burn wound. *J. Med. Vet. Mycol.* 27:427-429.
- Gonzalez G.M., A.W. Fothergill, D.A. Sutton et al.** 2005. *In vitro* activities of new and established triazoles against opportunistic filamentous and dimorphic fungi. *Med. Mycol.* 43:281-284.
- Goodman, N.L. and M.G. Rinaldi.** 1991 Agents of zygomycosis. In Balows, A., Hausler, W.J., Herrmann, K.L. et al. (eds.), *Manual Clinical Microbiology* 5th edition. American Society for Microbiology Washington DC.
- Graser, Y., S. de Hoog and R.C. Summerbell.** 2006. Dermatophytes: recognising species of clonal fungi. *Med. Mycol.* 44:199-209.
- Greer, D.L. and L. Friedman.** 1966. Studies on the genus *Basidiobolus* with reclassification of the species pathogenic for man. *Sabouraudia.* 4:231-241.
- Guarro, J., W. Gams, I. Puiol and J. Gene.** 1997. *Acremonium* species: new emerging fungal opportunistic: *in vitro* antifungal susceptibilities and review. *Clin. Infect. Dis.* 25:1222-1229.
- Gueho, E.S.** 1979. Dexoyribonucleic acid base composition and taxonomy in the genus *Geotrichum* Link. *Antonie van Leeuwenhoek.* 45:199-210.
- Gueho, E. and G.S. de Hoog.** 1991. Taxonomy of the medical species of *Pseudallescheria* and *Scedosporium*. *J. Mycol. Med.* 118:3-9.

## REFERENCES

- Gueho, E., M.Th. Smith, G.S. de Hoog et al.** 1992. Contributions to a revision of the genus *Trichosporon*. *Antonie van Leeuwenhoek*. 61:289-316.
- Gueho, E., G. Midgley and J. Guillot.** 1996. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek*. 69:337-55.
- Guillot J. and E. Gueho.** 1995. The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie Van Leeuwenhoek*. 67:297-314.
- Guillot J., E. Gueho, M. Lesourd et al.** 1996. Identification of *Malassezia* species. *J. Mycol. Med.* 6:103-110.
- Guillot J., M. Deville, M. Berthelemy et al.** 2000. A single PCR-restriction endonuclease analysis for rapid identification of *Malassezia* species. *Lett. Appl. Microbiol.* 31:400-403.
- Gupta, A.K., C.B. Horgan-Bell and R.C. Summerbell.** 1998. Onychomycosis associated with *Onychocola canadensis*: ten case reports and a review of the literature. *J. A. Acad. Dermatol.* 39:410-407.
- Hajjeh, R.A., A.N. Sofair, L.H. Harrison et al.** 2004. Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population based active surveillance program. *J. Clin. Microbiol.* 42:1519-1527.
- Hermanides-Nijhof, E.J.** 1977. *Aureobasidium* and allied genera. *Studies in Mycology, Baarn*. 15:141-177.
- Hesseltine, C.W. and J.J. Ellis.** 1964. The genus *Absidia*: *Gongronella* and cylindrical-spored species of *Absidia*. *Mycologia*. 56:568-601.
- Hesseltine, C.W. and J.J. Ellis.** 1964. An interesting case of *Mucor*, *M. ramosissimus*. *Sabouraudia*. 3: 151-154.
- Hesseltine, C.W. and J.J. Ellis.** 1966. Species of *Absidia* with ovoid sporangiospores. I. *Mycologia*. 58:173-194.
- Hohl, P.E., H.P. Holley, E. Prevost et al.** 1983. Infections due to *Wangiella dermatitidis* in humans: Report of the first documented case from the United States and a review of the literature. *Reviews of Infectious Diseases*. 5:854-864.
- Holland, J.** 1997. Emerging zygomycosis of humans: *Saksenaea vasiformis* and *Apophysomyces elegans*. *Curr. Top. Med. Mycol.* 8: 27-34.
- Humber, R.A., C.C. Brown and R.W. Kornegay.** 1989. Equine zygomycosis caused by *Conidiobolus lampragues*. *J. Clin. Microbiol.* 27: 573-6.
- Irokanulo, E.A.O., C.O. Akueshi and A.A. Makinde.** 1994. Differentiation of *Cryptococcus neoformans* serotypes A and D using creatinine dextrose bromothymol blue thymine medium. *Br. J. Biomed. Sci.* 51:100-103.
- Jong, S.C. and F.M. Dugan.** 2003. Zygomycetes: The Order Entomophthorales. In Howard, D.H. (ed.), *Pathogenic Fungi in Humans and Animals*. 2<sup>nd</sup> edition, Marcel Dekker Inc., New York, 127-139.
- Kane, J., R. Summerbell, L. Sigler et al.** 1997. Laboratory handbook of dermatophytes. Star Publishing Co. Belmont, CA. USA.
- Kaplan, W.** 1977. Protothecosis and infections caused by morphologically similar green algae. The black and white yeasts. Proceedings of the Fourth International Conference on the Mycoses. Scientific Publication No. 356. Pan American Health Organization. Washington D.C. USA.
- Kaufman, L. and P.G. Standard.** 1987. Specific and rapid identification of medically important fungi by exoantigen detection. *Ann. Rev. Microbiol.* 41:209-225.

## REFERENCES

- Kerr, P.G., H. Turner, A. Davidson et. al.** 1988. Zygomycosis requiring amputation of the hand: an isolated case in a patient receiving haemodialysis. *Med. J. Aust.* **148**: 258-259.
- Khan, Z.U., N.A. Al-Sweih, S. Ahmad et. al.** 2007. Outbreak of fungemia among neonates caused by *Candida haemulonii* resistant to amphotericin B, itraconazole, and fluconazole. *J. Clin. Microbiol.* **45**:2025-2027.
- King, D.S.** 1976a. Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. I. Biology and cluster analysis. *Can J Bot* **54**: 45-46.
- King, D.S.** 1976b. Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. II. Taxonomic considerations. *Can J Bot* **54**: 1285-1296.
- King, D.S.** 1983. Entomophthorales. In: Howard DH, ed. Fungi pathogenic for humans and animals. Part A Biology. Marcel Dekker Inc. New York pp 61-73.
- Klich, M.A.** 2002. Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures, The Netherlands.
- Kreger-van Rij, N.J.W. (ed.).** 1984. The yeasts, a taxonomic study, 3<sup>rd</sup> edition. Elsevier Sci. Publ., Amsterdam, 1082 pp.
- Kucukates, E., Z. Erturan, S. Susever and Y. Yegenoglu.** 2005. *In vitro* susceptibility of yeast isolated from patients in intensive care units to fluconazole and amphotericin B during a 3-year period. *APMIS* **113**:278-283.
- Kurtzman and J.W. Fell.** 1998. The Yeasts: a taxonomic study. 4<sup>th</sup> Edition. Elsevier Science Publishers B.V. Amsterdam.
- Kwon-Chung K.J., Polacheck I. and Bennett J.E.** (1982): Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* Serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (Serotypes B and C). – *J. Clin. Microbiol.* **15**:535-537.
- Kwon-Chung, K.J. and J.W. Bennett.** 1992. Medical Mycology. Lea & Febiger, Philadelphia, 861pp.
- Lawrence, R.M., Snodgrass, W.T., Reichel, G.W. et. al.** 1986. Systemic zygomycosis caused by *Apophysomyces elegans*. *J. Med. Vet. Mycol.* **24**: 57-65.
- Lunn, J.A. and W.A. Shipton.** 1983. Re-evaluation of taxonomic criteria in *Cunninghamella*. *Trans. Br. Mycol. Soc.* **81**:303-312.
- Luttrell, E.S.** 1978. Biosystematics of *Helminthosporium*: impact on agriculture. In Biosystematics in Agriculture. eds. J.A. Romberger et al. Allanheld, Osmon & Co., N.J. USA.
- Mackenzie, D.W.R., W. Loeffler, A. Mantovani and T. Fujikura.** 1986. Guidelines for the prevention, preservation and control of dermatophytoses in man and animals. World Health Organization.
- Malloch, D. and I.F. Salkin.** (1984). A new species of *Scedosporium* associated with osteomyelitis in humans. *Mycotaxon.* **21**:247-255.
- Matsumoto, T., A.A. Padhye and L. Ajello.** 1987. Medical significance of the so-called black yeasts. *Eur. J. Epidemiol.* **3**:87-95.
- Matsumoto, T., A.A. Padhye, L. Ajello et. al.** 1984. Critical review of human isolates of *Wangiella dermatitidis*. *Mycologia.* **76**:232-249.
- McGinnis, M.R.** 1978. Human pathogenic species of *Exophiala*, *Phialophora*, and *Wangiella*. In the black and white yeasts. Proceedings of the fourth international conference on the mycoses. 1978. Scientific Publication No. 356. Pan American Health Organization. Washington D.C. USA. pp.37-59.
- McGinnis, M.R.** 1978. Taxonomy of *Exophiala jeanselmei*. *Mycopathologia.* **65**:79-87.

## REFERENCES

- McGinnis, M.R.** 1980. Laboratory handbook of medical mycology. Academic Press.
- McGinnis, M.R. and D. Borelli.** 1981. *Cladosporium bantianum* and its synonym *Cladosporium trichoides*. Mycotaxon. 13:127-136.
- McGinnis, M.R. and W.A. Schell and J. Carson.** 1985. *Phaeoannellomyces* and the Phaeococcomycetaceae, new dematiaceous blastomycete taxa. J. Med. Vet. Mycol. 23:179-188.
- McGinnis, M.R., D. Borelli, A.A. Padhye and L. Ajello.** 1986a. Reclassification of *Cladosporium bantiana* in the genus *Xylohypha*. J. Clin. Microbiol. 23:1148-1151.
- McGinnis, M.R., M.G. Rinaldi and R.E. Winn.** 1986b. Emerging agents of Phaeohyphomycosis: pathogenic species of *Bipolaris* and *Exserohilum*. J. Clin. Microbiol. 24:250-259.
- McGinnis, M.R. and A.A. Padhye.** 1977. *Exophiala jeanselmei*, a new combination for *Phialophora jeanselmei*. Mycotaxon. 5:341-352.
- McGinnis, M.R., A.A. Padhye and L. Ajello.** 1982. *Pseudallescheria* Negroni et Fischer, 1943 and its later synonym *Petriellidium* Malloch, 1970. Mycotaxon 9:94-102.
- McGinnis, M.R., N. Nordoff, R.K. Li et. al.** 2001. *Sporothrix schenckii* sensitivity to voriconazole, itraconazole and amphotericin B. Med. Mycol. 39:369-371.
- McGinnis, M.R. and L. Pasarelli.** 1998. *In vitro* testing of susceptibilities of filamentous ascomycetes to voriconazole, itraconazole, and amphotericin B, with consideration of phylogenetic implications. J. Clin. Microbiol. 36:2353-2355.
- Metin, D.Y., S. Hilmioglu-Polat, F. Hakim et. al.** 2005. Evaluation of the microdilution, Etest and disk diffusion methods for antifungal susceptibility testing of clinical strains of *Trichosporon* spp. J. Chemother. 17:404-408.
- Millner, P.D.** 1975. Radial growth responses to temperature by 58 *Chaetomium* species, and some taxonomic relationships. Mycologia. 69:492-502.
- Miranda, K.C., C.R. de Araujo, C.R. Costa et. al.** 2007. Antifungal activities of azole agents against the *Malassezia* species. Int. J. Antimicrob. Agents. 29:281-284.
- Misra, P.C., Srivastava, K.J. and Latas, K.** 1979. *Apophysomyces*, a new genus of the Mucorales. Mycotaxon. 8: 377-382.
- Mok, W.Y.** 1982. Nature and identification of *Exophiala werneckii*. J. Clin. Microbiol. 16:976-978.
- Montel, E., P.D. Bridge and B.C. Sutton.** 1991. An integrated approach to *Phoma* systematics. Mycopathologia. 115:89-103.
- Moore, M.K.** 1986. *Hendersonula toruloidea* and *Scytalidium hyalinum* infections in London, England. J. Med. Vet. Mycol. 24:219-230.
- Morton, F.J. and G. Smith.** 1963. The genera *Scopulariopsis* Bainier, *Microascus* Zukal, and *Doratomyces* Corda. Mycological Papers, No. 86. Commonwealth Mycological Institute, Kew, London.
- Nakamura, Y., R. Kano, T. Mural et. al.** 2000. Susceptibility testing of *Malassezia* species using the urea broth microdilution method. Antimicrob. Agents. Chemother. 44:2185-2186.
- Nishimura, K. and M. Miyaji.** 1983. Studies on the phylogenesis of pathogenic "black yeasts". Mycopathologia. 81:135-144.
- Nottebrock, H., H.J. Scholer and M. Wall.** 1974. Taxonomy and identification of mucormycosis causing fungi. 1. Synonymy of *Absidia ramosa* with *A. corymbifera*. Sabouraudia. 12:64-74.

## REFERENCES

- Nucci, M., T. Akiti, G. Barreiros et. al.** 2001. Nosocomial fungemia due to *Exophiala jeanselmei* var. *jeanselmei* and a *Rhinocladiella* species: newly described causes of bloodstream infection. *J. Clin. Microbiol.* 39:514-518.
- O'Donnell, K.L.** 1979. Zygomycetes in culture. *Palfrey Contributions in Botany* 2. University of Georgia. pp 257.
- Onions, A.H.S., D. Allsopp and H.O.W. Eggins.** 1981. Smith's introduction to industrial mycology. Edward Arnold.
- Padhye, A.A., and L. Ajello** 1988. Simple method of inducing sporulation by *Apophysomyces elegans* and *Saksenaea vasiformis*. *J. Clin. Microbiol.* 26:1861-1863.
- Padhye, A.A., G. Koshi, V. Anandi et. al.** 1988. First case of subcutaneous zygomycosis caused by *Saksenaea vasiformis* in India. *Diagn. Microbiol. Infect. Dis.* 9:69-77.
- Paphitou, N.I., L. Ostrosky-Zeichner, V.L. Paetznick et. al.** 2002. *In vitro* antifungal susceptibility of *Trichosporon* species. *Antimicrob. Agents. Chemother.* 46:1144-1146.
- Pfaller, M.A., F. Marco, S.A. Messer and R.N. Jones.** 1998. *In vitro* activity of two echinocandin derivatives, LY303366 and MK-0991 (L-743,792), against clinical isolates of *Aspergillus*, *Fusarium*, *Rhizopus*, and other filamentous fungi. *Diagn. Microbiol. Infect. Dis.* 30:251-255.
- Pfaller, M.A., S.A. Messer, R.J. Hollis et. al.** 2002a. Antifungal activities of posaconazole, ravuconazole and voriconazole compared with those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY antimicrobial surveillance program, 2000. *Antimicrob. Agents Chemother.* 46:1032-1037.
- Pfaller, M.A., S.A. Messer, R.J. Hollis et. al.** 2002b. *In vitro* activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of *Candida* spp. *Antimicrob. Agents Chemother.* 46:1723-1727.
- Pfaller, M.A., D.J. Diekema, S.A. Messer et. al.** 2003. *In vitro* activities of voriconazole, posaconazole, and four licensed systemic antifungal agents against *Candida* species infrequently isolated from blood. *J. Clin. Microbiol.* 41:78-83.
- Pfaller, M.A., L. Boyken, R.J. Hollis et. al.** 2006. *In vitro* susceptibility of *Candida* spp. to Caspofungin: four years of global surveillance. *J. Clin. Microbiol.* 44:760-763.
- Pfaller, M.A. and D.J. Diekema.** 2007. The epidemiology of invasive candidiasis: a persistent public health problem. *Clin. Microbiol. Rev.* 20:133-163.
- Pitt, J.I.** 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press.
- Pore, R.S.** 1985. Prototheca taxonomy. *Mycopathologia.* 129:129-139.
- Pritchard, R.C., D.B. Muir, K.H. Archer et. al.** 1986. Subcutaneous zygomycosis due to *Saksenaea vasiformis* in an infant. *Med. J. Aust.* 145:630-631.
- Pujol, I., C. Aguilar, J. Gene, J. Guarro.** 2000. *In vitro* antifungal susceptibility of *Alternaria* spp. and *Ulocladium* spp. *J. Antimicrob. Chemother.* 46:337.
- Punithalingam, E.** 1979. Sphaeropsidales in culture from humans. *Nova Hedwigia.* 31:119-158.
- Raper, K.B. and D.I. Fennell.** 1965. The genus *Aspergillus*. William & Wilkins Co., Baltimore.

## REFERENCES

- Raper, K.B. and C.H. Thom.** 1949. A manual of the penicillia. William & Wilkins Co., Baltimore.
- Ramirez, C.** 1982. Manual and atlas of the Penicillia. Elsevier Biomedical Press.
- Rebell, G., and D. Taplin.** 1970. The Dermatophytes. 2nd. revised edition. University of Miami Press, Coral Gables, Florida. USA.
- Richter, S.R., R.P. Galask, S.A. Messer et. al.** 2005. Antifungal susceptibility of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. J. Clin. Microbiol. 43:2155-2162.
- Rippon, J.W.** 1988. Medical Mycology. 3rd Edition. W.B. Saunders Co.
- Rippon, J.W., P.M. Arnow, R.A. Larson et. al.** 1985. "Golden tongue" syndrome caused by *Ramichloridium schulzeri*. Arch. Dermatol. 121:892-894.
- Rodero, L., M. Cuenca-Estrella, S. Cordoba et. al.** 2002. Transient fungemia caused by an amphotericin B-resistant isolate of *Candida haemulonii*. J. Clin. Microbiol. 40:2266-2269.
- Rodriguez-Tudela, J.L., T.M. Diaz-Guerra, E. Mellado et. al.** (2005). Susceptibility patterns and molecular identification of *Trichosporon* species. Antimicrob. Agents Chemother. 49:4026-4034.
- Sabatelli, F., R. Patel, P.A. Mann et al.** 2006. *In vitro* activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important moulds and yeasts. Antimicrob. Agents Chemother. 50:2009-2015.
- Saksena, S.B.** 1953. A new genus of Mucorales. Mycologia 45:426-436
- Salkin, I.F., M.R. McGinnis, M.J. Dykstra and M.G. Rinaldi.** 1988. *Scedosporium inflatum*, an emerging pathogen. J. Clin. Microbiol. 26:498-503.
- Samson, R.A.** 1969. Revision of the genus *Cunninghamella* (Fungi, Mucorales). Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, ser. C, 72:322-335.
- Samson, R.A.** 1974. *Paecilomyces* and some allied hyphomycetes. Studies in Mycology No. 6. Baarn, The Netherlands.
- Samson, R.A., E.S. Hoekstra, J.C. Frisvad and O. Filtenborg.** 1995. Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, P.O.Box 273, 3740 AG BAARN, The Netherlands.
- Samson, R.A. and J.I. Pitt.** 1990. Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York, USA.
- Santos D.A., and J.S. Hamdan.** 2006. *In vitro* antifungal oral drug and drug-combination activity against onychomycosis causative dermatophytes. Medical Mycology. 44:357-362.
- Schell, W.A., M.R. McGinnis and D. Borelli.** 1983. *Rhinocladiella aquaspora* a new combination for *Acrotheca aquaspersa*. Mycotaxon 17:341-348
- Schipper, M.A.A.** 1976. On *Mucor circinelloides*, *Mucor racemosus* and related species. Stud Mycol. 12: 1-40.
- Schipper, M.A.A.** 1978. 1. On certain species of *Mucor* with a key to all accepted species. 2. On the genera *Rhizomucor* and *Parasitella*. Studies in Mycology No.17. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
- Schipper, M.A.A.** 1984. A revision of the genus *Rhizopus* 1. The *Rhizopus stolonifer*-group and *Rhizopus oryzae*. Stud. Mycol. 25: 1-19.
- Schipper, M.A.A. and Stalpers, J.A.** 1984. A revision of the genus *Rhizopus* II. The *Rhizopus microsporus* group. Stud. Mycol. 25: 30-34.

## REFERENCES

- Schipper, M.A.A. and Stalpers, J.A.** 2003. Zygomycetes: The Order Mucorales. In Howard, D.H. (ed.), *Pathogenic Fungi in Humans and Animals*. 2<sup>nd</sup> edition, Marcel Dekker Inc., New York, 67-125.
- Schipper, M.A.A., M.M. Maslen, G.G. Hogg et. al.** 1996. Human infection by *Rhizopus azygosporus* and the occurrence of azygospores in Zygomycetes. *J. Med. Vet. Mycol.* **34**: 199-203.
- Scholer, H.J., E. Müller and M.A.A. Schipper.** 1983. Mucorales. In: Howard DH, ed. *Fungi pathogenic for humans and animals, Part A Biology*. Marcel Dekker Inc New York, pp 9-59.
- Serrano, M.C., D. Morilla, A. Valverde et. al.** 2003. Comparison of Etest with modified broth microdilution method for testing susceptibility of *Aspergillus* spp. to voriconazole. *J. Clin. Microbiol.* **41**:5270-5272.
- Seth, H.K.** 1970. A monograph of the genus *Chaetomium*. *Nova Hedwigia* **37**:1-134.
- Shipton, W.A. and P. Zahari.** 1987. Sporulation media for *Basidiobolus* species. *J. Med. Vet. Mycol.* **25**:323-327.
- Sigler, L., S.P. Abbott and A.J. Woodgyer.** 1994. New records of nail and skin infection due to *Onychocola canadensis* and description of its teleomorph *Arachnomycetes nodosetosus* sp. nov. *J. Med. Vet. Mycol.* **32**:275-285.
- Sigler, L. and H. Congly.** 1990. Toenail infection caused by *Onychocola canadensis* gen. et. sp. nov. *J. Med. Vet. Mycol.* **28**:405-417.
- Sigler, L., L.M. de la Maza, G. Tan et. al.** 1995. Diagnostic difficulties caused by a nonclamped *Schizophyllum commune* isolate in a case of fungus ball of the lung. *J. Clin. Micro.* **33**:1979-1983.
- Sigler, L. and J.W. Carmichael.** 1976. Taxonomy of *Malbranchea* and some other hyphomycetes with arthroconidia. *Mycotaxon.* **4**:349-488.
- Singh, J., D. Rimek and R. Kappe.** 2005. *In vitro* susceptibility of 15 strains of zygomycetes to nine antifungal agents as determined by the NCCLS M38-A microdilution method. *Mycoses.* **48**:246-250.
- Simmons, E.G.** 1967. Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. *Mycologia.* **59**:67-92.
- Sivanesan, A.** 1987. Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. *Mycological Paper No. 158*. CAB International, U.K.
- Staib F.** (1987). *Cryptococcus* in AIDS Mycological Diagnostic and Epidemiological Observations. *Aids Forshung (AIFO)*2, 363-382.
- Steele, T., G.W. Kaminski and D. Hansman.** 1977. A case of coccidioidomycosis in Australia. *Med. J. Aust* **1**:968-969.
- Sorrell, T. C.** 2001. *Cryptococcus neoformans* variety *gattii*. *Med, Mycol.* **39**:155-168.
- Strinivasan, M.C. and M.J. Thirumalachar.** 1965. *Basidiobolus* species pathogenic for man. *Sabouraudia.* **4**:32-34.
- Sugar, A.M. and X.P. Liu.** 1996. *In vitro* and *in vivo* activities of SCH 56592 against *Blastomyces dermatitidis*. *Antimicrob. Agents Chemother.* **40**:1314-1316.
- Sun, Q.N., A.W. Fothergill, D.I. McCarthy et. al.** 2002. In vivo activities of posaconazole, itraconazole, voriconazole, amphotericin B, and fluconazole against 37 clinical isolates of zygomycetes. *Antimicrob. Agents Chemother.* **46**: 1581-1582.

## REFERENCES

- Sutton, B.C.** 1980. The Coelomycetes, fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycology Institute, Kew, London.
- Sutton, B.C. and B.J. Dyko.** 1989. Revision of *Hendersonula*. Mycol. Res. 93:466-488.
- Tintelnot, K. and B. Nitsche.** 1989. *Rhizopus oligosporus* as a cause of mucormycosis in man. Mycoses. 32: 115-118.
- Trilles, L., B. Fernandez-Torres, M. dos Santos Lazera et. al.** 2004. *In vitro* antifungal susceptibility of *Cryptococcus gattii*. J. Clin. Microbiol. 42:4815-4817.
- Vanbreusegham, R, CH. de Vroey and M. Takashio.** 1978. Practical guide to medical and veterinary mycology. Mason Publishing USA, Inc.
- Van Oorschot, C.A.N.** 1980. A revision of *Chrysosporium* and allied genera. Studies in Mycology No.20. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
- Velegriaki, A., E.C. Alexopoulos, S. Kritikou et. al.** 2004. Use of fatty acid RPMI 1640 media for testing susceptibilities of eight *Malassezia* species to the new triazole posaconazole and six established antifungal agents by a modified NCCLS M27-A2 microdilution method and Etest. J. Clin. Microbiol. 42:3589-3593.
- Vitale, R.G. and G.S. de Hoog.** 2002. Molecular diversity, new species and antifungal susceptibilities in the *Exophiala spinifera* clade. Medical Mycology. 40:545-556.
- Voigt, K., E. Cigelnik and K. O'Donnell, K.** 1999. Phylogeny and PCR identification of clinically important zygomycetes based on nuclear ribosomal-DNA sequence data. J. Clin. Microbiol. 37: 3957-3964.
- Weitzman, I.** 1984. The case for *Cunninghamella elegans*, *C. bertholletiae* and *C. echinulata* as separate species. Trans. Br. Mycol. Soc. 83:527-528.
- Weitzman, I., M.R. McGinnis, A.A. Padhye and L. Ajello.** 1986. The genus *Arthroderma* and its later synonym *Nannizzia*. Mycotaxon. 25:505-505.
- Weitzman, I. and M.Y. Crist.** 1980. Studies with clinical isolates of *Cunninghamella*. II. Physiological and morphological studies. Mycologia. 72: 661-669.
- Wieden, M.A., Steinbronn, K.K., Padhye, A.A. et. al.** 1985. Zygomycosis caused by *Apophysomyces elegans*. J Clin Microbiol. 22: 522-526.
- Wilson, C.M., E.J. O'Rourke, M.R. McGinnis et. al.** 1990. *Scedosporium inflatum*: Clinical spectrum of a newly recognised pathogen. J. Infect. Dis. 161:102-107.
- Woodward, A., C. McTigue, G. Hogg et. al.** 1992. Mucormycosis of the neonatal gut: a new disease or a variant of necrotizing enterocolitis? J. Pediatr. Surg. 27: 737-740.
- Yarrow, D. and S.A. Meyer.** 1978. Proposal for the amendment of the diagnosis of the genus *Candida* Berkhout nom. cons. Int. J. Syst. Bacteriol. 28:611-615.
- Yuan, G.F. and S.C. Jong.** 1984. A new obligate azygosporic species of *Rhizopus*. Mycotaxon. 20: 397-400.
- Zycha, H., R. Siepmann and G. Linnemann.** 1969. *Mucorales*, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe. Cramer Lehre, 355p.