# **Phenotypic Identification** of Zygomycetes

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# Zygomycetes

- Pin or bread moulds.
- Fast growing, cosmopolitan (870 species).
- Primitive coenocytic

   [infrequently septate]
   hyphae.



- Asexual reproduction by sporangiospores formed in sporangia.
- Key genera: *Rhizopus, Rhizomucor, Lichtheimia, Cunninghamella, Saksenaea* and *Aphophyomyces.*





### A thick-walled sexual spore formed by the fusion of two similar gametangia; characteristic of the Zygomycetes.





Coenocytic hyphae are infrequently septate, multi-nucleate hyphae as in the Zygomycetes.





Haematoxylin and eosin (H&E) stained section of lung tissue showing the broad, infrequently septate, thin-walled hyphae of Lichtheimia (Absidia) corymbifera.





# *Rhizopus oryzae* – culture showing masses of sporangia ("pin heads").







Sporangium of *Rhizopus* showing sporangiospores sitting on the columella awaiting dispersal, the sporangial wall has undergone autolysis which leaves a "head" of closely packed sporangiospores.





# **Zygomycota Sporangia Characters**

- Arrangement of sporangiospores [multispored, sporangiola, merosporangium]
- Arrangement of sporangiophores [unbranched often in groups or frequently branched]
- Sporangium shape [pyriform, spherical, flask-shaped etc]
- Sporangium size [<100 µm diam. or >100 µm diam.]
- Columella [Present or Absent]
- Apophyses [Present or Absent]
- Sporangiophore height [<0.8 mm or >1 mm]
- Rhizoids [Present or Absent] (look in the agar)
- Sporangiospore size [<6 µm or >6 µm]





# Identification of Zygomycetes

## Growth temperature studies ie. 35, 40, 45C.



Tease mounts are best, use a drop of 95% alcohol as a wetting agent to reduce air bubbles.

May need to induce sporulation in isolates of Saksenaea and Apophysomyces.





### Mucor Lichtheimia (Absidia)

## **Mutispored sporangia with columella**, <u>+</u> apophysis







# **Rhizomucor pusillus**

### Multispored sporangia, columella, no-apophysis.







# Rhizopus

# Multispored sporangia, columella, apophysis, sporangiophores in clusters with rhizoids.







# Apophysomyces complex

Multispored sporangia, funnel-shaped apophysis and conspicuous pigmented sub-apical thickening below the apophysis.







# Saksenaea vasiformis

Flask shaped multispored sporangium, columella, apophysis and rhizoids.







## **Cunninghamella bertholletiae** Sporangiola "single celled conidia" borne on swollen denticles on a terminal vesicle.









## **Syncephalestrum racemosum** Terminal vesicle and merosporangia.





# Mortierella wolfii

# Sporangia with acrotonous (terminal) branches, no columella, no apophysis and rhizoids.





# Unknown









Cultures fast growing, white cottony at first, becoming grey-black with sporulation, No growth at 45C.

Unbranched sporangiophores arising from rhizoids Multispored sporangium, large >100 um, spherical, columella and apophysis present.



- 6a. Sporangia cylindrical, with spores in rows
- 6b. Sporangia of other shape
- 7a. Sporangia mostly multi-spored
- 7b. Sporangia are few-spored sporangiola on recurved stalks
- 8a. Sporangia flask-shaped
- 8b. Sporangia spherical or pyriform
- 9a. Sporangia 1-celled; sporangiola, borne on the surface of large swellings
- 9b. Sporangia otherwise
- 10a. Sporangia usually pyriform
- 10b. Sporangia spherical
- 11a. Dark, septate chlamydospores formed in aerial mycelium
- 11b. Dark, septate chlamydospores absent
- 12a. Apophysis funnel-shaped
- 12b. Apophysis vase-shaped
- 13a. Sporangia without columella
- 13b. Sporangia with columella
- 14a. Sporangia with apophyses
- 14b. Sporangia without apophyses
- 15a. Rhizoids present
- 15b. Rhizoids absent

Syncephalastrum 8 Cokeromyces Saksenaea 9 Cunninghamella 1011 13 Chlamydoabsidia 12 Absidia Apophysomyces Mortierella 14 Rhizopus 15 Rhizomucor Mucor



### Zygomycota, Mucorales, Mucoraceae. Genus: RHIZOPUS

Generic description. Colonies expanding, hairy. Stolons and rhizoids present. Sporangiophores arising from swellings just above rhizoids. Sporangia terminal, multi-spored, with apophysis and columella; columellae subspherical to slightly ellipsoidal. Sporangiospores angular or (sub)spherical, ornamented. Zygospores reddish, between unequal, spherical suspensors.

General remarks. Experimental infection was performed by van Cutsem et al. (1988). The GC% of DNA of medical species were listed by Frye & Reinhardt (1993).

References. Domsch et al. (1980), Schipper (1984), Schipper & Stalpers (1984).

R. microsporus varieties not supported by molecular data Key to the treated species of Rhizopus:

1a.	Azygospores abundant
1b.	Azygospores infrequent $\rightarrow 2$
2a.	Sporangiophores mostly not exceeding 0.8 mm in height; sporangia up to 100 µm diam;
	growth at 45°C, though sometimes poor $\rightarrow 3$
2b.	Sporangiophores often higher than 1 mm; sporangia mostly over 100 µm diam; no grow
	at $45^{\circ}C \rightarrow 7$
3a.	Sporangiospores angular R. microsporus
3b.	Sporangiospores broadly ellipsoidal to subspherical $\rightarrow 4$
4a.	Sporangiospores rarely exceeding 6 $\mu$ m in diam and 6.5 $\mu$ m in length $\rightarrow$ 5
4b.	Sporangiospores often exceeding 6 µm in diam and 9 µm in length R. microsporus va
5a.	Sporangiospores distinctly striate $\rightarrow 6$
5b.	Sporangiospores minutely spinulose R. microsporus var. r.
6a.	Colonies hairy, dark greyish-brown; good growth at 45°C R. microsporus va
6b.	Colonies thinly floccose, greyish-white; restricted growth at 45°C
7a.	Sporangia up to 275 µm diam; no growth at 37°C
7b.	Sporangia not exceeding 250 µm diam; growth at 37°C

R. azygosporus (102)

R. microsporus th

var. chinensis (105)

ar. oligosporus (105)

hizopodiformis (105) r. microsporus (104) R. schipperae (108) R. stolonifer (110) R. oryzae (106)



### Rhizopus microsporus v. Tiegh

Synonymy:Rhizopus azygosporus Yuan & Jong.<br/>Rhizopus microsporus var. microsporus Tiegh.<br/>Rhizopus microsporus var. oligosporus (Saito) Schipper & Stalpers.<br/>Rhizopus microsporus var. rhizopodiformis (Cohn) Schipper & Stalpes.<br/>Rhizopus microsporus var. chinensis (Saito) Schipper & Stalpers.

### RG-2 organism.

**Morphological Description:** Colonies are dark greyish-brown, up to 10 mm high producing simple rhizoids. Sporangiophores are brownish, up to 400 µm high and 10 µm wide, and may be produced in groups of one to four, usually in pairs. Sporangia are greyish-black, spherical, up to 100 µm in diameter. Columellae are subglobose to globose to conical comprising 80% of the sporangium. Sporangiospores are angular to broadly ellipsoidal or subglobose, up to 5-9 µm in length and are distinctly striate. Chlamydospores may be present. Zygospores are dark red–brown, spherical, up to 100 µm in diameter, with stellate projections and unequal suspensor cells. Some strains may be homothallic and produce azygospores. There is good growth at  $45^{\circ}$ C, with a maximum of  $50-52^{\circ}$ C.



Rhizopus microsporus sporangia showing sporangiospores, columellae, sporangiophores and rhizoids.

### Rhizopus arrhizus Fischer

Synonymy: Rhizopus oryzae Went & Prinsen Geerligs.

### RG-2 organism.

**Morphological Description:** 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. Sporangiophores up to 1500  $\mu$ m in length and 18  $\mu$ m in width, smooth-walled, non-septate, simple or branched, arising from stolons opposite rhizoids usually in groups of three or more. Sporangia are globose, often with a flattened base, greyish black, powdery in appearance, up to 175  $\mu$ m in diameter and many spored. Columellae and apophysis together are globose, subglobose or oval, up to 130  $\mu$ m in height collapsing to an umbrella-like form after spore release. Sporangiospores are angular, subglobose to ellipsoidal, with striations on the surface, and up to 8  $\mu$ m in length. No growth at 45°C; good growth at 40°C.



*Rhizopus arrhizus* (a) culture, (b) columellae and (c) sporangia showing sporangiospores, sporangiophores and rhizoids.







Multispored sporangium, spherical, <100 um diam. unbranched sporangiophore

Columella present, apophysis absent, spores spherical



### Zygomycota, Mucorales, Mucoraceae. Genus: MUCOR

Generic description. Colonies fast-growing, whitish to greyish, often several cm high, usually forming a thick mat due to abundant production of erect sporangiophores. Sporangiophores without basal rhizoids, not originating from stolons, unbranched or irregularly branched, terminally bearing multi-spored sporangia. Sporangia spherical, without apophyses, with large columellae; wall deliquescent or persistent and rupturing at maturity, often with adhering calcium oxalate crystals. Zygospores without appendages on suspensors. General remarks. *Mucor* species are rather frequently isolated as culture contaminants. Only few, thermolerant species may have clinical significance.

References. Zycha et al. (1969), Schipper (1978a), Domsch et al. (1980).

### Key to the treated species of Mucor:

- 1a. Sporangiophores unbranched or weakly sympodially branched
- 1b. Sporangiophores repeatedly branched
- 2a. Sporangiospores spherical, 3.5-5.5 µm diam
- 2b. Sporangiospores ellipsoidal, 5.7-8.7 x 2.7-5.4 μm
- 3a. Growth at 40°C
- 3b. Poor or no growth at 37°C
- 4a. Sporangiospores mostly about 3.5-6 µm diam; chlamydospores in hyphae rare
- 4b. Sporangiospores up to 8-10 μm diam; chlamydospores in hyphae abundant, occasionally in the columella
- 5a. Colonies with restricted growth; columellae applanate; no assimilation of ethanol
- 5b. Colonies with expanding growth; columellae spherical to ellipsoidal; assimilation of ethanol

M. amphibiorum (256) M. hiemalis (258) M. indicus (260) 4

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M. racemosus (262) M. ramosissimus (264) M. circinelloides (50)



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### Mucor circinelloides v. Tiegh

M. circinelloides is a common and variable species that includes four formae: circinelloides, lusitanicus, griseocyanus and janssenii (Schipper 1978, Scholer et al. 1983).

### RG-1 organism.

Morphological Description: Colonies are floccose, pale greyish-brown and grow poorly at 37°C (maximum growth temperature 37°C). Sporangiophores are hyaline and mostly sympodially branched with long branches erect and shorter branches becoming circinate (coiled). 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, smoothwalled, ellipsoidal, and 4.5-7 x 3.5-5 µm in size. Chlamydospores are generally absent. Zygospores are only produced in crosses of compatible mating types and are reddishbrown to dark-brown, spherical with stellate spines, up to 100 µm in diameter and have equal to slightly unequal suspensor cells.

Comment: M. circinelloides differs from other species of Mucor in its formation of short circinated, branched sporangiophores bearing brown sporangia and its ability to assimilate ethanol and nitrates (Schipper 1976, Scholer et al. 1983, Samson et al. 1995, de Hoog et al. 2000, Schipper and Stalpers 2003).

Antifungal Susceptibility: <i>M. circinelloides</i> (Espinel-Ingroff <i>et al.</i> 2015a, Australian National data); MIC μg/mL.												
	No.	<u>&lt;</u> 0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	<u>&gt;</u> 16
AmB	123		1	4	14	42	44	18				
POSA	120			2	2	9	21	49	26	5	2	4
ITRA	48					4	3	7	12	15	5	3



### Mucor circinelloides culture.

Mucor Micheli ex Staint-Amans



Mucor spp. showing sporangia, columella with inconspicuous collarette (arrow) and sporangiospores.

### Mucor amphibiorum Schipper

### RG-2 organism.

Morphological Description: 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. Zygospores, when formed by compatible mating types, are spherical to slightly compressed, up to 70 x 60 µm in diameter, with stellate projections.

Comment: Mucor amphibiorum is distinguished by poor branching of the sporangiophores and by globose sporangiospores. Ethanol and nitrates are not assimilated (Schipper 1978, Scholer et al. 1983, Hoog et al. 2000, 2015).

Antifungal Susceptibility: M. amphibiorum very limited data (Australian National data); MIC µg/mL.

	No.	<u>&lt;</u> 0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	<u>&gt;</u> 16
AmB	1						1					
POSA	1						1					
ITRA	1							1				

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# Its' not always that easy!

- Many uncommon moulds may present (especially from non-sterile sites) so remember growth at 37C is an important selector for medical fungi.
- Often can not see the essential identification characters.
- Can not find a suitable key or reference.
- Non-sporulating moulds (now use ITS + sequencing).









# **Mould identification**

1. Need to see the required characters

- sporulating culture
- slide culture + good microscope
- careful observation

2. Learn mycology terminology and keys 3. Practice and reference books







