

Identification of Medically Important Fungi.

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Notes on the Identification of Zygomycetes.

Subcutaneous zygomycosis is generally caused by traumatic implantation and lesions vary considerably, but include plaques, pustules, ulcerations, deep abscesses and ragged necrotic patches. In the immunocompetent host, lesions usually remain localised around the site of inoculation and generally respond well to local debridement and Amphotericin B treatment. However, in the debilitated patient, zygomycosis is the most acute and fulminate fungal infection known. The disease typically involves the rhino-facial-cranial area, lungs, gastrointestinal tract, skin, or less commonly other organ systems. It is often associated with acidosis diabetes, malnourished children, severely burned patients and other diseases such as leukemia and lymphoma, immunosuppressive therapy, or use of cytotoxins and corticosteroids. The infecting fungi have a predilection for invading vessels of the arterial system, causing embolization and subsequent necrosis of surrounding tissue.

Two orders, the Mucorales and the Entomophthorales contain genera and species of medical importance. In general, fungi in the order Mucorales cause the more severe forms of disease with most species belonging to the genera *Rhizopus*, *Absidia*, *Rhizomucor*, *Mucor* and *Apophysomyces*. *Rhizopus oryzae* is the most frequent infectious agent reported, followed by *R. microsporus* var. *rhizopodiformis*, *Absidia corymbifera* and *Rhizomucor pusillus*. These four species account for more than 80% of culture proven cases of zygomycosis. Other mucoraceous species reported less frequently include *R. microsporus* var. *microsporus*, *Mucor ramosissimus*, *M. circinelloides*, *M. indicus*, *Apophysomyces elegans*, *Cunninghamella bertholletiae* and *Saksenaia vasiformis*. *Basidiobolus ranarum* and *Conidiobolus coronatus* are the principle species of the Entomophthorales that cause human disease, predominantly of the nasal mucosa and subcutaneous tissue.

Key features for identification include:

- Coenocytic, mostly non-septate hyphae.
- Zygosporangium morphology in homothallic strains, however most isolates are heterothallic, ie zygosporangia are absent, therefore identification is based primarily on sporangial morphology. This includes the following:
 1. Arrangement and number of sporangiospores.
 2. Sporangial shape and colour.
 3. Presence or absence of columellae and apophyses.
 4. Arrangement of sporangiophores.
 5. Presence or absence of rhizoids.
- Growth temperature studies are also useful ie 35, 40, 45°C.
- 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 *Saksenaia vasiformis* and *Apophysomyces elegans* by using Czapek-dox agar or water agar.

Absidia corymbifera. Common human pathogen causing pulmonary, rhinocerebral, disseminated, CNS or cutaneous zygomycosis. World-wide mostly in association with soil and decaying plant debris. Small pyriform shaped sporangia with a characteristic conical shaped columellae and pronounced apophysis, rapid growth at 40°C.

Apophysomyces elegans. 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 sporangiophore, rapid growth at 42°C.

Basidiobolus ranarum. Causes chronic inflammatory or granulomatous subcutaneous lesions, generally restricted to the limbs, chest, back or buttocks; primarily in children with a predominance in males. Tropical distribution from decaying vegetable matter and from intestinal tract of amphibians. Numerous zygospores are produced and conidia are forcibly discharged from a sporophore.

Conidiobolus coronatus. Causes chronic inflammatory or granulomatous disease that is typically restricted to the nasal submucosa and characterised by polyps or palpable restricted subcutaneous masses. World-wide distribution, but mainly from tropical rain forests of Africa. Conidia with hair-like appendages (villae) and prominent papillae and are forcibly discharged.

Cunninghamella bertholletiae. Rare cause of zygomycosis in humans often associated with trauma and immunosuppression. Common soil fungus found throughout the temperate regions of the world. Produces single celled, echinulate sporangia on swollen terminal or lateral vesicles, rapid growth at 40°C.

Mucor. Rare cause of human zygomycosis. *M. indicus*, *M. ramosissimus* and *M. circinelloides* have all been implicated in human infection. Characteristic large, spherical, non-apophysate sporangia with pronounced columellae and conspicuous collarete following sporangiospore dispersal. Stolons and rhizoids are absent.

Rhizomucor pusillus. Rare human pathogen causing pulmonary, disseminated or cutaneous zygomycosis. World-wide distribution and is commonly associated with compost heaps. Rapid growth at 45°C, poorly developed stolons and rhizoids, branching sporangiophores with a septum below the sporangium, dark coloured sporangia without apophyses and smooth-walled sporangiospores.

Rhizopus oryzae. Most common causative agent of zygomycosis, accounting for 60% of reported culture positive cases and 90% of rhinocerebral cases. World-wide distribution from soil, decaying vegetation, foodstuffs and animal and bird dung. Rapid growth at 40°C, sporangiophores often >1 mm in height, formed opposite rhizoids, sporangia 100-200 µm in diameter with distinctive columellae and apophyses.

Saksenaea vasiformis. Rare human pathogen usually associated with invasive lesions following the traumatic implantation of the fungus through the skin. Soil fungus with a world-wide distribution. Characteristic “flask-shaped” sporangia with distinct venter and long-neck, columella and apophysis present, arising singly or in pairs from dichotomously branched, darkly pigmented rhizoids.

Notes on the Identification of Hyphomycetes

Key Features include Microscopic Morphology and Culture Characteristics:

Mandatory to see conidial characteristics to make an identification therefore you must have a good slide preparation [needle mounts, tape mounts, slide cultures]. May also need to stimulate sporulation by using different media, such as potato dextrose agar or cornmeal agar. If conidia are present then assess the following characters:

1. Conidial characteristics:

- Septation [amero, didymo, phragmo, dictyo].
- Shape [spherical, subspherical, pyriform, clavate, ellipsoidal etc].
- Size [need graduated eye piece, <10 µm etc].
- Colour [hyaline or darkly pigmented].
- Wall texture [smooth, rough, verrucose, echinulate etc].
- How many conidial types present? [micro and macro].

2. Arrangement of conidia as they are borne on the conidiogenous cells.

- Solitary [single, in balls, acropleurogenous].
- Catenulate [acropetal or basipetal].
- Botryose [synchronous or asynchronous].

3. Growth of the conidiogenous cell.

- Determinant.
- Proliferous [basauxic, percurrent or sympodial (narrow or broad base)].
- Retrogressive.

4. Type of conidiogenous cell present.

- Non-specialised.
- Phialide.
- Annelide.
- Porogenous.

5. Any additional features present.

- Origin of conidium wall [holoblastic or enteroblastic].
- Hyphal structures [clamps, spirals, nodular organs etc].
- Synnemata, Sporodochia, Chlamydoconidia, Pycnidia etc.

6. Culture Characteristics. Least reliable as the media and growth conditions play an important part.

Examine the following characteristics:

- Surface texture [glabrous, suede-like, powdery, granular, fluffy, downy, cottony etc].
- Surface topography [flat, raised, heaped, folded, domed, radial grooved].
- Surface pigmentation [white, cream, yellow, brown, green, grey, black etc].
- Reverse pigmentation [none, yellow, brown, red etc].
- Growth rate [eg colonies growing less than 5 mm in 14 days etc].
- Growth temperature studies are also often very useful [37°C, 40°C & 45°C].

Hyalohyphomycosis: A mycotic infection caused by a hyaline (lightly coloured) hyphomycete (conidial mould), where the tissue morphology of the causative organism is mycelial. Although this term also applies to *Aspergillus*, infections caused by members of this genus are usually covered under the heading Aspergillosis. Clinical manifestations range from harmless colonisation to acute invasive disease. Predisposing factors include prolonged neutropenia, especially in leukemia or bone marrow transplant patients, corticosteroid therapy, cytotoxic chemotherapy and patients with AIDS. Aetiological agents include *Acremonium*, *Beauveria*, *Fusarium*, *Geotrichum*, *Paecilomyces*, *Penicillium*, and *Scopulariopsis*. Currently, 47 species from 23 genera have been reported as causative agents of hyalohyphomycosis.

***Acremonium*.** Rare cause of mycetoma, arthritis, osteomyelitis, peritonitis, endocarditis and pneumonia. Species include *A. falciforme*, *A. kiliense*, *A. recifei*, *A. alabamensis*, *A. roseo-griseum* and *A. strictum*. World-wide, mostly from plant debris and soil. Solitary, erect, hyaline, awl-shaped phialides producing single-celled, globose to cylindrical conidia, mostly aggregated in slimy heads.

***Aspergillus fumigatus*.** Common human pathogen causing all forms of invasive and non-invasive aspergillosis. Cosmopolitan mould from soil and plant debris, but will grow on wide variety of substrates. Conidial heads typically columnar with a uniseriate row of phialides on the upper two thirds of the vesicle, producing long basipetal chains of conidia. Cultures are typically blue-green and rapid growth occurs at 45°C.

***Beauveria bassiana*.** Usually isolated as a saprophytic contaminant. World-wide, known as an insect parasite. Sympodial development of single-celled conidia on a narrow geniculate or zig-zag rachis emanating from a flask-shaped conidiophore.

***Fusarium*.** Rare opportunistic pathogen causing cutaneous and subcutaneous infections, mycotic keratitis, endophthalmitis, osteomyelitis, and arthritis following traumatic implantation. *F. oxysporum*, *F. solani* and *F. moniliforme* common soil fungi, with a world-wide distribution. Characteristic sickle-shaped multiseptate macro-conidia and smaller 1- to 2-celled microconidia from phialides on branched or unbranched conidiophores.

***Geotrichum candidum*.** Rare opportunistic pathogen causing bronchial and pulmonary infections, oral, vaginal, cutaneous and gastrointestinal infection reported. Common fungus with a world-wide distribution. Species produce chains of hyaline, smooth, single celled, cylindrical arthroconidia by the fragmentation of undifferentiated hyphae.

***Paecilomyces*.** Common environmental fungi, rarely causing mycotic keratitis, peritonitis, endophthalmitis, endocarditis, pyelonephritis, sinusitis and cutaneous lesions in immunosuppressed patients. *P. variotti*, *P. marquandii* and *P. lilacinus*. Similar to *Penicillium* but with long slender divergent phialides and the culture pigmentation is never green.

***Penicillium marneffeii*.** Causes a non-specific, subacute febrile illness with anaemia, weight loss and with diffuse papular lesions mostly on the head, face and upper body in HIV patients with a travel history to endemic area. Dimorphic fungus restricted to southeast Asia. Characteristic flask-shaped phialides arranged in groups from metulae forming a penicillus. This species produces a typical red diffusible pigment and cultures are usually greyish-pink to brown.

Scopulariopsis. Most members of the genus are soil fungi, however *S. brevicaulis* has been reported as a causative agent of onychomycosis and of hyalohyphomycosis in immunosuppressed patients. Conidia often shaped like light globes, produced in chains arising from annellides. Cultures fast growing varying in colour from white to buff-brown.

Phaeohyphomycosis: A mycotic infection caused by a dematiaceous (brown-pigmented) hyphomycete (conidial mould), 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). Clinical manifestations range from localised superficial infections (tinea nigra) to subcutaneous cysts to invasion of the brain. Subcutaneous lesions due to traumatic implantation are mostly due to *Exophiala* and *Wangiella*. Paranasal sinus infections are mostly due to *Bipolaris*, *Exserohilum*, *Curvularia* and *Alternaria*. Cerebral infections are mostly due to *Cladophialophora bantiana*. Currently, 101 species from 57 genera have been reported as causative agents of phaeohyphomycosis.

Alternaria. Common saprophytic contaminants but are recognised as causative agents of mycotic keratitis. Most are plant parasites, but a few species are ubiquitous and are also frequently soil borne. Dematiaceous hyphomycete producing chains of darkly pigmented, ovoid to obclavate dictyoconidia, often with short conical or cylindrical beaks.

Aureobasidium pullulans. World-wide distribution, 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 CAPD. Produces hyaline blastoconidia simultaneously from the vegetative hyphae, which may also form chains of darkly pigmented, thick-walled arthroconidia.

Bipolaris. Rare cause of mycotic keratitis, subcutaneous, sinusitis, peritonitis in patients on CAPD, and cerebral and disseminated infections. *B. australiensis*, *B. hawaiiensis* and *B. spicifera* are well documented human pathogens. Dematiaceous hyphomycete producing sympodial, pseudoseptate, pale brown, straight, fusiform to ellipsoidal poroconidia, which are rounded at both ends.

Cladosporium. Common fungi with a world-wide distribution. *C. cladosporioides* reported from a cutaneous infection in an immunosuppressed patient. *C. carrionii* and *C. trichoides* (*Xylohypha bantiana*) now transferred to *Cladophialophora*. Dematiaceous hyphomycete forming branched acropetal chains of conidia, each with a distinct hilum and with characteristic “shield-shaped” conidia present.

Cladophialophora bantiana. This fungus is neurotropic and may cause brain abscess and subcutaneous lesions in both normal and immunosuppressed patients. Soil fungus with world-wide distribution. Dematiaceous hyphomycete, growth at 42°C, producing sparsely branched acropetal chains of blastoconidia without a distinct hilum.

Cladophialophora carrionii. Causative agent of chromoblastomycosis. Mainly from arid regions of tropical South America, South Africa and Australia. Dematiaceous hyphomycete, growth at 35-37°C, producing elongate conidiophores with branched acropetal chains of blastoconidia without a distinct hilum.

***Curvularia*.** Rare cause of subcutaneous, sinusitis, endocarditis, peritonitis and disseminated infection in immunosuppressed patients. *C. lunata*, *C. pallescens* and *C. geniculata* are well documented human pathogens. Dematiaceous hyphomycete producing sympodial, pale brown, cylindrical or slightly curved phragmoconidia, with one of the central cells being larger and darker.

***Exophiala*.** Rare cause of mycetoma, localised cutaneous infections, subcutaneous cysts, endocarditis and disseminated infections. Common environmental fungi often associated with decaying wood and soil enriched with organic wastes. *E. jeanselmei*, *E. moniliae* and *E. spinifera* are well documented human pathogens. Conidia formed in slimy balls on terminal or lateral “peg-shaped” annellides, often yeast-like cells and torulose hyphae are also present.

***Exserohilum*.** Rare cause of mycotic keratitis, subcutaneous, endocarditis, osteomyelitis and sinusitis. *E. rostratum*, *E. meginnisii* and *E. longirostratum* have all been reported as causative agents. Dematiaceous hyphomycete producing sympodial, transverse septate, ellipsoidal to fusiform conidia with a strongly protruding, truncate hilum.

***Phaeoannellomyces werneckii*.** Causative agent of tinea nigra. Common saprophytic fungus from soil, compost and wood in humid tropical regions. Dematiaceous hyphomycete producing two-celled, pale brown, cylindrical to spindle-shaped conidia with distinctive darkly pigmented septa. Conidia are produced from an annellide.

***Phialophora*.** Rare cause of chromoblastomycosis, osteomyelitis, arthritis and disseminated disease. *P. verrucosa*, *P. richardsiae*, *P. parasitica*, *P. repens* and *P. hoffmannii* have all been reported. Dematiaceous hyphomycete producing flask-shaped phialides with distinctive, often widely flaring, collarettes. Conidia produced in basipetal succession and usually aggregate in slimy heads.

Pseudallescheria boydii (anamorph: *Scedosporium apiospermum*). Usually classified under the term pseudallescheriasis; causing mycetoma, mycotic keratitis, arthritis and osteomyelitis due to traumatic implantation and invasive disseminated infections following inhalation of conidia such as sinusitis, pneumonia and brain abscesses. World-wide distribution from soil and plant debris. Homothallic cleistothecial ascomycete with a typical *Scedosporium* anamorph showing pale brown, single-celled, broadly clavate to ovoid conidia borne singly or in small groups on annellides. A *Graphium* synanamorph may also be present.

***Scedosporium prolificans*.** Rare cause of mycotic keratitis, septic arthritis and osteomyelitis due to traumatic implantation (80% of cases) and invasive disseminated infections following inhalation of conidia. World-wide distribution from soil or plant debris. Produces basally swollen (inflated), flask-shaped annellides forming single-celled ovoid to pyriform conidia, aggregated in balls.

***Sporothrix schenckii*.** A thermal dimorphic fungus and causative agent of sporotrichosis. It has a world-wide distribution, particularly in tropical and temperate regions and is commonly found in soil and on decaying vegetation. Conidia are formed in clusters on tiny denticles by sympodial proliferation of the conidiophore, their arrangement often suggestive of a flower.

***Wangiella (Exophiala) dermatitidis*.** Rare cause of mycetoma, subcutaneous cystic lesions, endocarditis and brain abscesses. Isolated from plant debris and soil. Dematiaceous hyphomycete producing cylindrical phialides without collarettes. Conidia usually aggregate in slimy heads and growth occurs at 42°C. Often an initial yeast-like *Phaeococcomyces* synanamorph is present.