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Black mycelial fungal infections – Asian perspectives

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Black Mycelial Fungal Infection Asian Perspectives

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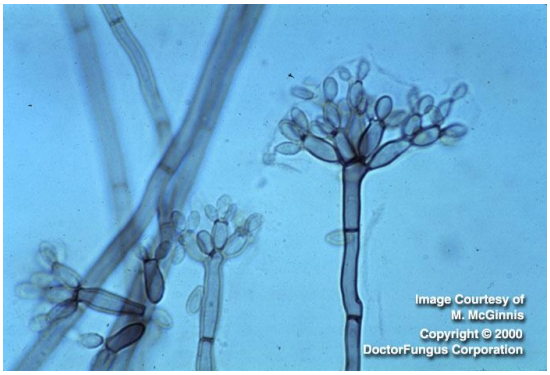
Dematiaceous fungi

- a heterogeneous group of fungi characterized by their production of melanin pigments in their hyphae & conidia.
- widely found in nature e.g. soil, wood, decomposing plant matter, & polluted water
- Rarely cause infection but has wide range of diseases especially in immuno-compromised patients (transplant recipients, HIV infected)



Brandt & Warnock J Chemother 2003; 15 - Suppl. 2 (36-47)
https://drfungus.org/knowledge-base/fonsecaea-species/

Clinical manifestations

Superficial infection *:* dermatomycosis & onychomycosis

Subcutaneous infection: chromoblastomycosis, sportrichosis, etc

Systemic infection: visceral organ incl. cerebral infection

Brandt & Warnock J Chemother 2003; 15 - Suppl. 2 (36-47)

Dermatomycosis

Tinea nigra

- *Hortaea werneckii*
- Chronic cutaneous infection, in the palm (>) & sole (<),
- Central & South America, Africa, & Asia
- Diagnosis:
- Treatment:
  - Terbinafine
  - Iodine & salycilic acid alcohol sol.

The fungus & clinical occurrence

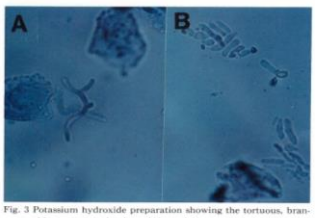


Fig. 3 Potassium hydroxide preparation showing the tortuous, branching, septate hyphae (A) and fragmented hyphae and spores (B).

Fig. 4 Culture of Exophiala werneckii on Sabouraud's agar showing greenish-black colony with peripheral areas of fluffy, gray aerial mycelium.

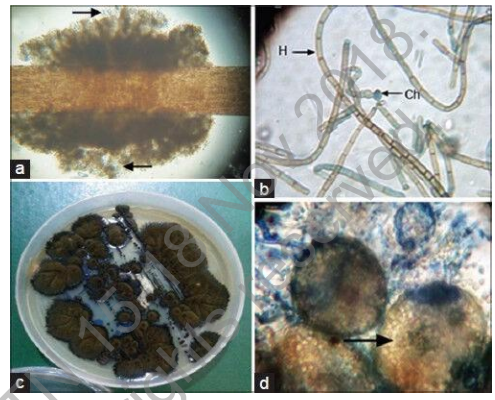
Kuo et al, Dermato Sinica 1993; 11:47-53
Guan et al. J Formos Med Assoc 2008; 107 : 21-9

Dermatomycosis

Black piedra

- *Piedraia hortae*
- Infect hair (scalp)
- humid tropical regions: South & Central America, Africa, Southeast Asia, Africa, & the South Pacific islands.
- Nature: soil?, still unknown
- young adults, men > women
- Spread: common use of combs, hairbrushes or other hair utensils

The fungus

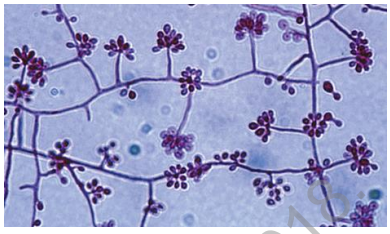


Khatu et al. Int J Trichology. 2013;5(4): 220–3

Subcutaneous infection

Sporotrichosis

- Subcutaneous infection
- Caused by dimorphic fungus S. schenckii
- Saprophyte in soil, sphagnum moss, decaying vegetation & hay
- Asia: mostly reported from India, Japan & Australia



Chakrabarti & Slavin Med Mycol 2011, 49, 337–44

Chromoblastomyosis

The causes & other characteristics

- The causes dematiceaous fungi e.g. *F. pedrosoi* species complex
- Microscopy of clinical specimen: round, thick-walled, brown cells, or sclerotic bodies
- Prevalent in Asia & Latin America

Clinical presentation

- common: erythematous & verrucous plaques, nodules
- Cicatricial & tumorous (less)

Yang et al. Medical Mycology, 2017, 0, 1–11
Agarwal et al. PLoS Negl Trop Dis 11 (8): e0005534. (CR & Rev)

Clinical presentation of chromoblastomycosis

Figure 1. Clinical manifestations of chromoblastomycosis (CBM) cases. (A–C) Uncommon locations of CBM, including the ear lobe (A), cheek (B), and areola (C). (D–E) Well-defined papuloplaque-type lesion, easily misdiagnosed as psoriasis or Bowen's disease. (F–H) The verrucous type was the most common presentation in our study, with a verruciform surface and black dots. (I–K) Cicatricial type of lesion with an active border and central clearance. (L) Targetoid type lesions are very rare and present with three zones, namely the central papule, clear area, and rimmed elevated border.

Yang et al Medical Mycology, 2017, 0, 1–11

Chromoblastomycoses: epidemiology

country	Cause (s)	demography	Ref
Japan	*F. nubica*		Yanagihara et al. JDA 2015 (CR)
India	*F. pedrosoi* & *C. tenuissimum*	7±81 y.o.; males> females; ratio (4.2:1).	Agarwal et al. PLoS Negl Trop Dis 11 (8): e0005534. (CR & Rev)
Thailand	? *F. pedrosoi*	male	McDaniel & Walsh. Am. J. Trop. Med. Hyg., 83(3), 2010, p. 448 Kullavanijaya & Rojanavanich. Internat J Dermatol 1995.
China	*C. carrionii* , *F. monophora*, *F. pedrosoi* & *Phialophora verucosa*	10-79 y.o., male>female (5.21:1) & farmers,	Lu et al Mycopathologia DOI 10.1007/s11046-012-9586-z
Indonesia	?	Adult, male>female, 3 cases	Yahya et al. J Gen Pro DVI. 2016;1(2):36-43
Taiwan	*F. monophora* & *F. nubica*	male>female (2.75:1); 29-90 y.o.	Yang et al. Med Mycol. 2017; 0, 1–11

Jpn. J. Med. Mycol.
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Review

Phaeohyphomycosis in Korea

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Abstract

Phaeohyphomycosis is a mycotic disease caused by dematiaceous fungi that produce brown yeast-like cells, pseudohyphae, and irregular true hyphae in tissues. Seven Korean cases of subcutaneous phaeohyphomycosis have been reported to date, four males and three females, ranging in age from 9-84 years (mean 50.4 years). Causative organisms of subcutaneous phaeohyphomycosis were 3 of *Exophiala jeanselmei*, 2 of *E. dermatitidis*, 1 of *Drechslera dematioidea* and 1 of *Phoma* sp. Four cases of abscess and 3 cases of verrucous plaque were present as skin lesions, and were all exposed areas of the body. Patients were treated with itraconazole, ketoconazole, fluconazole or amphotericin B.

A subcutaneous infection caused by dematiaceous fungi

JCM
Journals.ASM.org

Acrophialophora, a Poorly Known Fungus with Clinical Significance

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Acrophialophora fusispora **is an emerging opportunistic fungus capable of causing human infections. The taxonomy of the genus is not yet resolved and, in order to facilitate identification of clinical specimens, we have studied a set of clinical and environmental *Acrophialophora* isolates by morphological and molecular analyses. This set included the available type strains of *Acrophialophora* species and similar fungi, some of which were considered by various authors to be synonyms of *A. fusispora*. Sequence analysis of the large subunit (LSU) and internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA and a fragment of the β-tubulin (*Tub*) gene revealed that *Acrophialophora* belongs in the family *Chaetomiaceae* and comprises three different species, i.e., *A. fusispora*, *Acrophialophora levis*, and *Acrophialophora seudatica*; the latter was previously included in the genus *Ampullifera*. The most prevalent species among clinical isolates was *A. levis* (72.7%), followed by *A. fusispora* (27.3%), both of which were isolated mostly from respiratory specimens (72.7%), as well as subcutaneous and corneal tissue samples. In general, of the eight antifungal drugs tested, voriconazole had the greatest *in vitro* activity, while all other agents showed poor *in vitro* activity against these fungi.**

Systemic infection

Clinical manifestations & its causes

Cerebral & systemic infection

- Cerebral
  - *Cladophialophora bantiana*
  - *Ramichloridium mackenziei*
  - *Exophiala dermatitidis*
  - *Lomentospora prolificans*
- other systemic infection
  - Endocarditis
  - Osteomyelitis

Brandt & Warnock J Chemother 2003; 15 - Suppl. 2 (36-47)
Sudhadham et al. Studies in Mycology 2008; 61: 145–55.
Kelly et al. BMC Infectious Diseases. 2016; 16:36
Steinbach et al. JCM 2003; 41: 3981–5
Lang et al. BMC Infectious Diseases (2018) 18:255

Source in nature

Soil & decaying vegetation in tropical countries

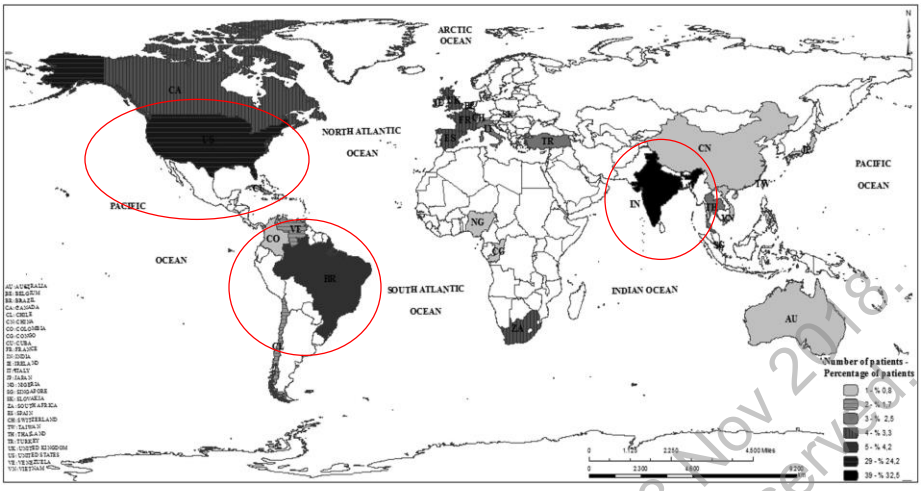
C. bantiana

A known neurotropic fungus

C. bantiana brain infection

- is a melanized hyphomycetous, highly virulent fungus & the most common cause of brain infection
- High mortality due many factors e.g.
  - Delay of diagnosis
  - (No) standardized treatment

Revankar & Sutton. Clin Microbiol Rev. 2010;23(4):884-928
Chakrabarty et al. Med Mycol, 2016, 54, 111–9

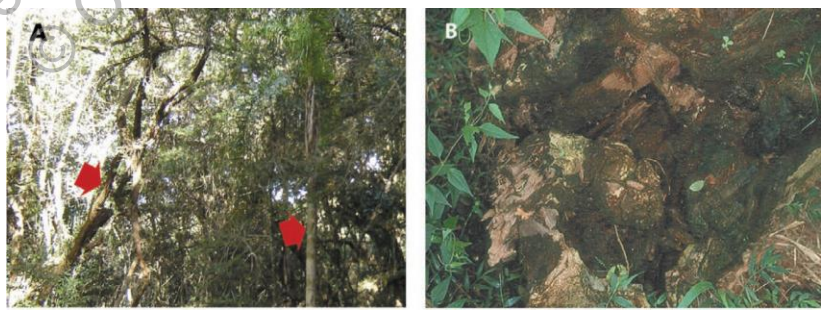


1. India (39 - 32.5%),
2. United States (29 - 24.2%)
3. Brazil (each 5 - 4.2%)

Kantarcioglu et al. Med Mycol 2016;5:579–604

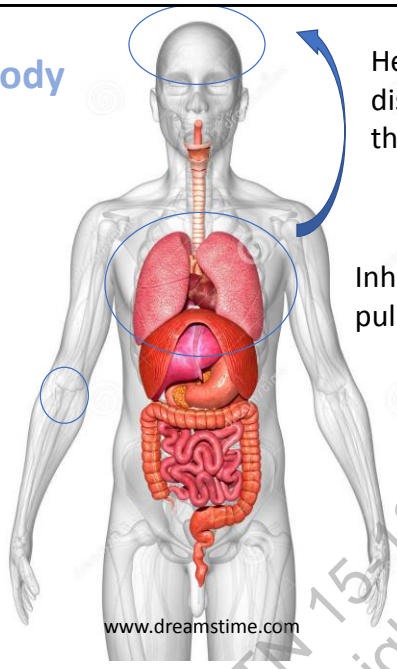
Source in nature

- soil saprophyte
- can be found in living or dead plants
- individuals who work with soil



Vicente et al. Studies Mycol. 2008; 61: 137–44.
Dixon et al. Mycopathologia. 1980; 70(3):153–61

Enter human body



Hematogenous dissemination to the brain

Inhalation: sinus or pulmonary infection

Skin trauma causes skin inoculation

Kantarcioglu et al. Med Mycol 2016;5:579–604
Dixon et al . Mycopathologia. 1980; 70153–61.

Risk factors

Immunocompetent
59.2% patients

Immunosuppressed
40.8% patients

Chakrabarti A, et al. Med Mycol 2016;54:111–9

Report from Asian Countries

- Other Asian countries: Japan, Vietnam, Thailand & Indonesia
- Immunocompetent patients with no clear risk factors except from Indonesia (IVDU)
- **Patients in Asia are generally young**

Japan: women aged around 20s with cerebral cladosporiosis

Indonesia: A brain infection in a IVDU young male

Chakrabarti A, et al. Med Mycol 2016;54:111–9;
Harris & Wahyuningsih unpublished

Indonesian case

- A 31 year old male, drug user (IVDU) complained of head ache, left side weakness & slurred in speech, which worsening from time to time
- By his family he was sent abroad & was diagnosed as brain abscess, caused by *Aspergillus*
- He was treated with voriconazole without any improvement & send back to Jakarta
- Since his condition deteriorate, MRI was conducted (irregular area enhancement at T1-T2) & LP was done & no organisms were isolated, later on surgical excision was conducted
- Material from brain abscess was sent to our lab. & *C. bantiana* was isolated & identified based on phenotypic method.

Indonesian case

- a possible risk factor was identified.
- Possibly the fungus enter the body via skin trauma by the injection of syringe (an IVDU)
- The wound caused by the usage of non-sterile needle is the entrance of the fungus into the body & subsequently disseminated to the brain

Walz et al. J Med Vet Mycol 1997; 35, 427-31

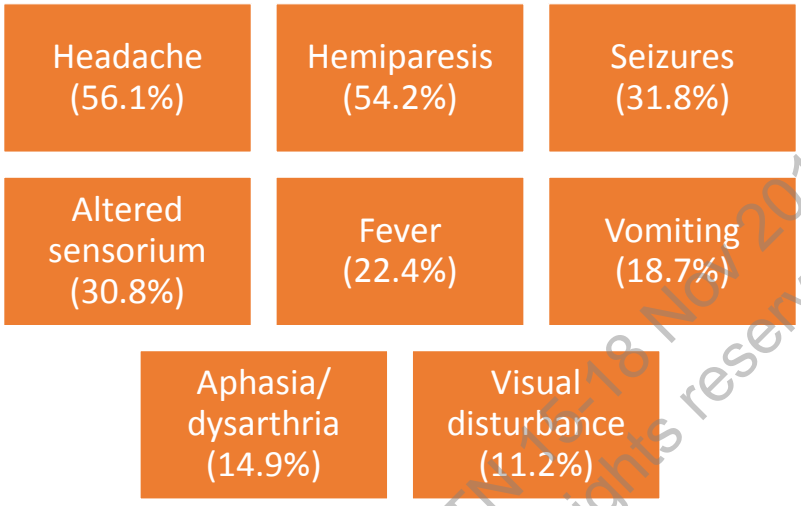
Diagnosis

Based on clinical presentation

Based on laboratory result: collection of clinical specimens

Imaging

Clinical manifestation - atypical



Chakrabarti A, et al. Medical Mycology 2016;54:111–9

Diagnosis

- Clinical symptoms – atypical – misdiagnosis
  - The mean duration of symptoms before diagnosis was ± 115 days
- Laboratory: hard to collect samples due to location in the brain
- Once the fungus is isolated:
  - Phenotypic identification – morphology
  - histopathology
  - nutritional studies
  - Genetic analysis based on sequencing of rDNA

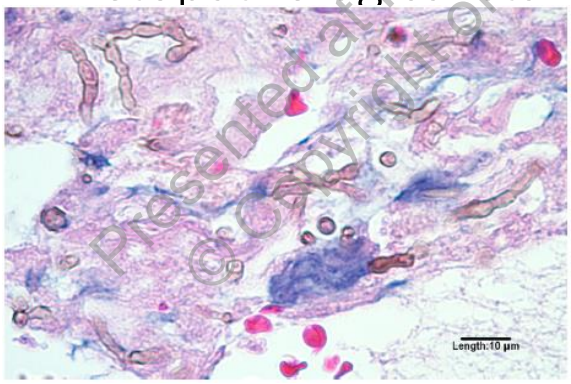
Chakrabarti A, et al. Med Mycol 2016;54:111–9

Morphology: macroscopy

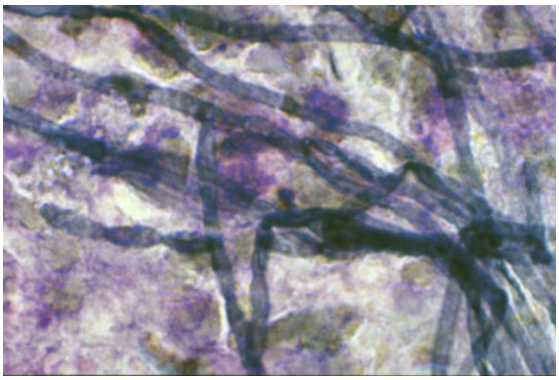
- Colony is black, velvety (RT & 37°C)
- Long, non-fragile chains of conidia

Revankar SG, Sutton DA. Clin Microbiol Rev. 2010;23(4):884-928
Levin TP, et al. J Clin Microbiol. 2004;42(9):4374-8.
Ajantha GS, et al. J Clin Diagn Res. 2011;5(6) Suppl 1: 1301-6

Histopathological stains



H&E stain of melanized, moniliform hyphal elements of *C. bantiana* from a brain abscess.



Giemsa stain of abcess specimens

Revankar & Sutton. CMRev. 2010;23(4):884-928;
Kantarcioglu et al, Med Mycol 2016; 5: 579-604

Genetic analysis & Nutritional physiology

- **Primary region:**
  Internal transcribed spacer (ITS) region using ITS1 and ITS4 primer
- Alternative region:
  Large subunit (LSU) region
  Small subunit (SSU) region
- Maximum temperature for growth 42°C.
- *Methyl-α-D-glucoside, meso-erythritol* & *ethanol* assimilated
- No or poor growth with L-arabinitol
- Good or weak growth with ribitol & galactitol

Singh A, et al. J CM. 2017;55(4):1090-103; Kuan et al PLOS ONE 2016;
De Hoog GS, et al. J Med Vet Mycol. 1995;33(5):339-47

Treatment

Surgical excision + Antifungal

- Overall mortality rate was 65%
- Higher in immunosuppressed patients (77.1%) than immunocompetent hosts (56.3%)

Chakrabarti A, et al. Med Mycol 2016;54:111–9
Kantarcioglu et al., Med Mycol 2016;5:579–604

Antifungal susceptibility testing

S. no	AmB	Flu	Vor	Itra	Posa	Casp	Ani	Mica
1	8	16	0.06	0.03	0.06	4	2	8
2	8	32	0.06	0.03	0.12	4	8	8
3	0.5	32	0.25	0.12	0.12	4	4	8
4	0.06	> = 64	0.25	0.12	0.03	1	8	8
5	0.06	> = 64	0.06	0.12	0.03	1	8	8
6	0.5	32	0.125	0.125	0.03	1	1	2
7	1	8	0.06	0.250	0.03	2	1	4
8	8	32	0.03	0.06	0.03	0.250	0.250	0.125
9	2	16	0.06	0.125	0.03	4	4	4
10	8	64	0.125	0.5	0.125	2	0.5	4
11	0.5	64	0.06	0.06	0.03	2	2	4
12	1	> = 64	0.06	0.250	0.03	2	0.5	2
13	4	64	0.250	0.125	0.03	4	1	4

Adapted from : Chakrabarti et al. Med Mycol 2016;54:111–9
Kuan et al PLOS ONE 2016; Kantarcioglu et al . Med Mycol 2016;

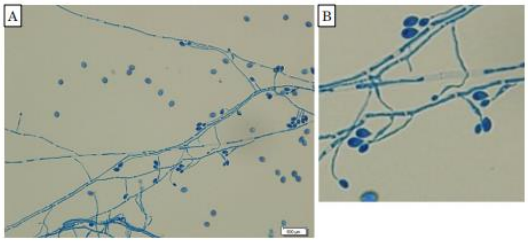
Antifungal

- standard guideline: ESCMID – ECMM for black fungi
- Personal experience
- Amphotericin B is the most widely used drug (70.4%), followed by flucytocine (34.6%)
- Recommendation: combination of amphotericin B and flucytocine

Chakrabarti , et al. Med Mycol 2016;54:111–9
Kantarcioglu et al., Med Mycol 2016;5:579–604
Chowdary et al Clin Microbiol Infect 2014; 20.

Lomentospora (Scedosporium) prolificans

- Rarely reported in Asia
- One case from Japan, a 68 yo woman with lung infection
- Treated with voriconazole



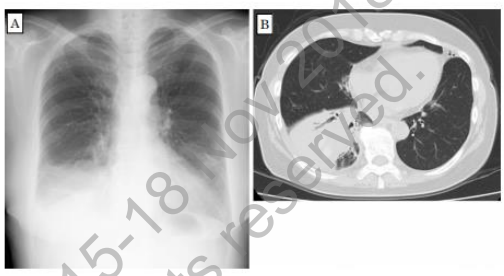


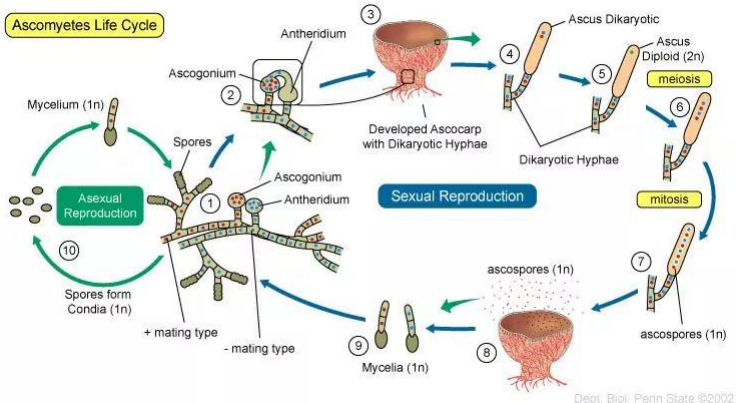
Figure 2. Chest radiograph (A) and computed tomography scan (B) taken on hospitalization, showing enlargement of lung consolidation with a high-density area and bronchiectasis.

Masukane et al. Intern Med 56: 973-977, 2017)

Infection caused by *E. rostratum*

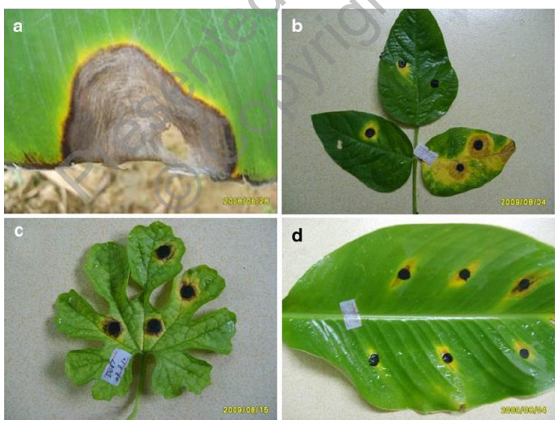
A newly recognized neurotropic mould

Life cycle of *Exerohilum rostratum*



http://www.dannyhaelewaters.com/exserohilum-rostratum-the-killing-fungus/
Mycology online

E. rostratum in nature



- A soil saprophytes
- Pathogen in plant – banana leaf & others, causes black spot disease
- biocontrol/herbicides agent against red sprangletop (*Leptochloa chinensi*), a pest to rice plant
- 2012 an outbreak of meningitis; a new insight for neurotropic characteristic

Lin et al. Australasian Plant Pathology 2011; 40(3):246-59
http://www.dannyhaelewaters.com/exserohilum-rostratum-the-killing-fungus/·

Exerohilum rostratum – neurotropism

- October 2012, US Federal official reported meningitis in 214 people, then 304 people after having injection of steroid to their epidural space for back pain
- The steroid was contaminated by *E. rostratum*
- This fungus was directly inoculated to the human tissue & induces response
- Causes severe inflammatory response marked by high concentration of leukocytes in LCS
- *E. rostratum*
  - black mould;
  - synthesize melanin - associated with virulence & resistance to both polyene- & echinocandin-type antifungal agents

Casadevall & Pirofski. Future Microbiol. 2013; 8(2): 135–7
Nosanchuk & Casadevall . AAC. 2006; 50(11):3519–28.

E. rostratum: susceptibility to Anti fungal

Antifungal Susceptibility: *Exserohilum rostratum* (Australian National data); MIC µg/mL										
	No	≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	≥8
AmB	8				4	2	2				
VORI	8			1		1	6				
POSA	6		5	1							
ITRA	8			4	4						

E. rostrum data from 34 isolates (da Cunha *et al.* 2012); MIC µg/mL			
AmB	Range <0.03-0.125: MIC_{90}= 0.03	VORI	Range 0.03-1: MIC_{90}= 0.25
ITRA	Range <0.03-0.125: MIC_{90}= 0.03	POSA	Range <0.03-0.5: MIC_{90}= 0.06

CDC suggestion is Voriconazole 6mg/Kg BW/daily

Mycology online

Bipolaris hawaiiensis

Culture of *B. hawaiiensis* in LPCB.

Bashir et al. Mycopathologia, 2008;167(1), 51–3.

B. hawaiiensis

- Prev. *Dreschlera hawaieensis*
- Optimum temperature is 25-35°C, but able to grow at 37°C
- A problem in the storage of crops (post harvest disease)
- Means able to grow in human tissue & causes infection

Almaguer et al. Aerobiologia DOI 10.1007/s10453-012-9257-z
Pic. Wikimedia

B. hawaiiensis: range of disease

- ABPA (allergy) in pediatric (Chowdary et al. Med Mycol. 2011;49:760-5)
- 14/17 developed endophthalmitis after intra vitreal of triamcinolone from the same lot. (Small et al. Ophthalmology 2014; 121:952-8)
- 8 eyes of 8 patients developed endophthalmitis after intra vitreal injection of bevacizumab-triamcinolone combination. (Sheyman et al. JAMA Ophthalmol. 2013;131(7):864-9)
- Cutaneous infection (Khan & Sohnle. Ref Mod Biomed Sci doi:10.1016/b978-0-12-801238-3.02398-9)

Susceptibility of *B. hawaiiensis* to antifungal agents

B. hawaiiensis (14)	Anidulafungin	<0.015–>8	0.07	0.125
	Amphotericin B	0.125–0.25	0.18	0.25
	Caspofungin	0.5–1	0.90	1
	Itraconazole	<0.03–0.5	0.32	0.5
	Fluconazole	2–32	8	16
	Flucytosine	>64	>64	>64
	Micafungin	<0.015–0.06	0.04	0.06
	Posaconazole	<0.03–0.5	0.14	0.25
	Voriconazole	0.25–2	0.67	1

Adapted from:
Espinel-Ingroff A. JCM 2001;39(3):954-8 &
da Cunha et al. JCM. 2012; 50: 4061–6

Damage response framework (DFR)

- Black mycelial fungi rarely cause disease, but once an infection occurs there will be severe damage to the host tissue. This can be explained by DFR.
- Tissue damage in infection is not merely due to microbial activity
- interaction between host- micro organism determine tissue damage which associate with pathological outcome.
- The damage is the result of microbial activity & host response
- In case of mycosis this theory applies e.g. *B. hawaiiensis* & *E. rostratum* were previously known as non pathogenic to human, but direct inoculation in epidural space & steroids allows changes that facilitate infection

My opinion
Casadevall & Pirofski. Future Microbiol. 2013; 8(2): 135–137

Asian perspective- conclusion

- Melanized fungi are able to cause infection in human with broad clinical spectra; from cutaneous to cerebral infection
- Mostly found in humid tropical countries such as many regions in Asia, but report from Asia is limited
- the humid climate in Asian countries is a fertile medium for fungal growth & the environment is an important source of infections.
- Climate change (deforestation, pollution etc.) allows fungi to adapt to high temperatures so that infections in humans are very likely to occur
- some fungi are clinically significant, some are unclear, but we need to be aware because their ability to adapt to the human body paves the way for infection
- both clinicians & laboratories must be prepared to deal with it

Thank you

Bunga bangkai or corpse flower