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Zygomycoses: biologie, épidémiologie et diagnostic microbiologique

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Zygomycetes

- Definition

Division	Class	Order
Ascomycota		
Basidiomycota		
Zygomycota	Zygomycetes	Mucorales
		Mortierellales
		Entomophthorales

(de Hoog, Guarro, et. al Atlas of Clinical fungi)

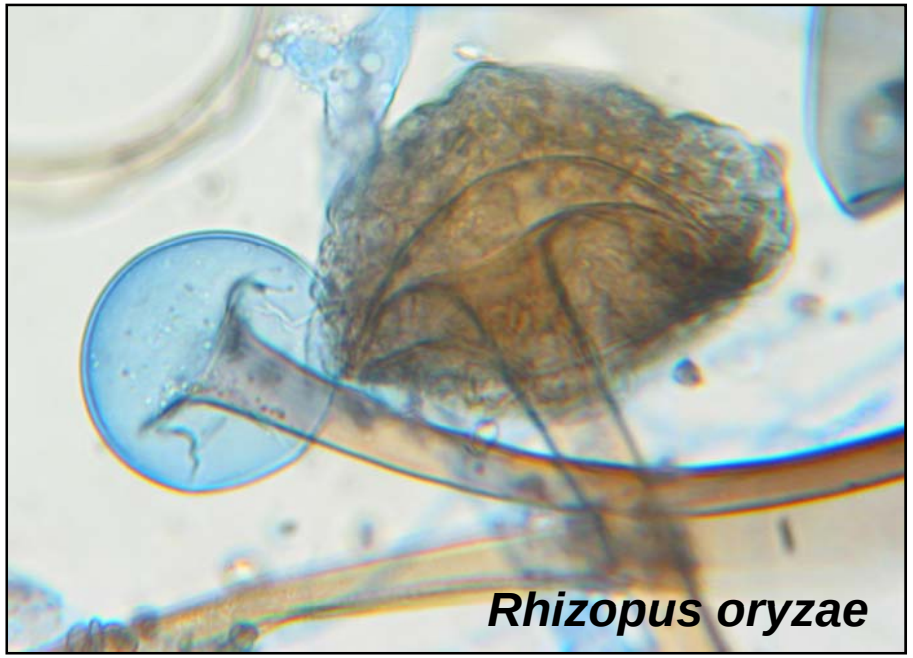
- Cosmopolites - Environnement
- De l'Antarctique[1] aux sources géothermale[2]
- Quelques espèces restreintes aux régions tropicales / subtropicales : *Apophysomyces* and *Saksenaea*

1. Lawley B., et al. 2004. *Appl. Environ. Microbiol.* **70:** 5963.
2. Redman RS., et al. 1999. *Appl. Environ. Microbiol.* **65:** 5193.

Écologie, espèces pathogènes

✓ Groupe de champignons filamenteux caractérisés par mycélium non cloisonné (coenocytique)

- Saprophytes
  - sol
  - matières en décomposition
  - fruits
  - compost
  - graines de céréales


Rhizopus oryzae

- Sporulation ++, spores aéroportées

→ Fréquemment isolés comme contaminant

Espèces pathogènes : ca. 20 espèces / 10 genres

Order	Family	Genus	Species
Mucorales	Mucoraceae	*Rhizopus*	*R. oryzae*
			R. microsporus
			R. azygosporus
			R. schipperae
		Mucor	*M. circinelloides*
			M. hiemalis
			M. racemosus
			M. ramosissimus
			M. indicus
		Rhizomucor	*R. pusillus*
	Mycocladiaceae[1]	*Mycocladus (Absidia)*	*M. corymbifer*

1. **Hoffmann, K., et al.** 2007. Mycol Res **111:**1169-1183.

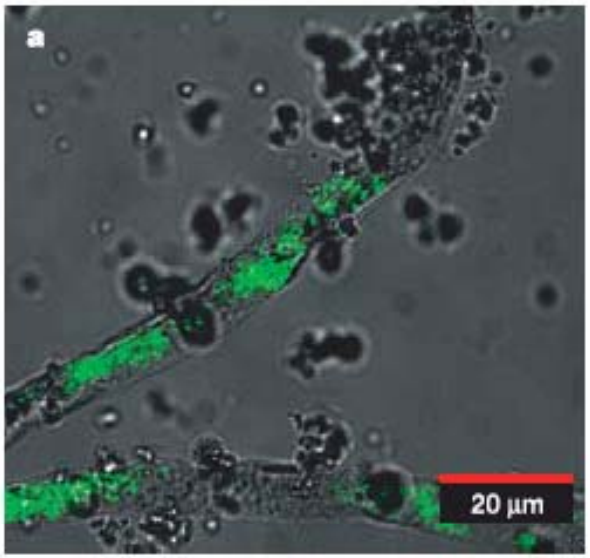
Espèces pathogènes

Order	Family	Genus	Species
Mucorales	Cunninghamellaceae	*Cunninghamella*	*C. bertholletiae*
	Saksenaeaceae	*Saksenaea*	*S. vasiformis*
	Syncephalastraceae	*Syncephalastrum*	*S. racemosum*
	Thamnidiaceae	*Cokeromyces*	*C. recurvatus*
Mortierellales			
Entomophthorales			

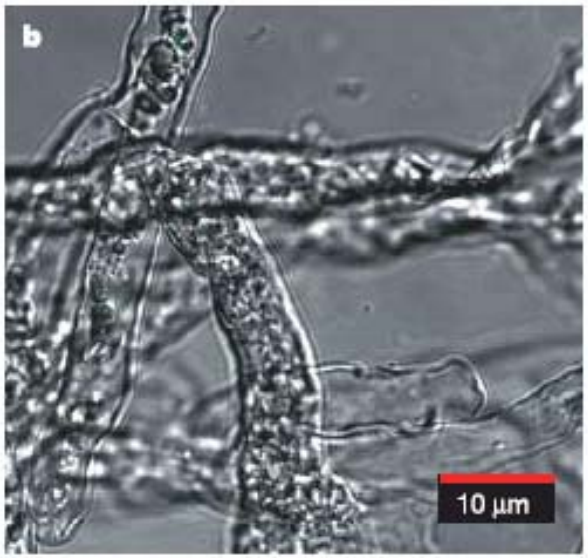
- Problèmes actuels
  - Biologie / Facteurs de virulence
  - Epidémiologie
  - Diagnostic microbiologique:
    - Identification des espèces en culture
    - Identification des espèces à partir des tissus (cultures négatives)

The Zygomycetes, p. 159-183. *In* **K. Kavanagh** (ed.), 2007, New insights in fungal pathogenicity. Springer.

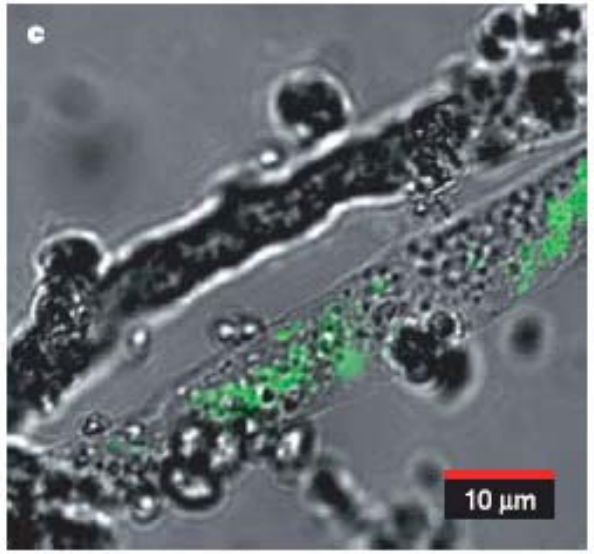
Endosymbiont bacteria in *Rhizopus microsporus*



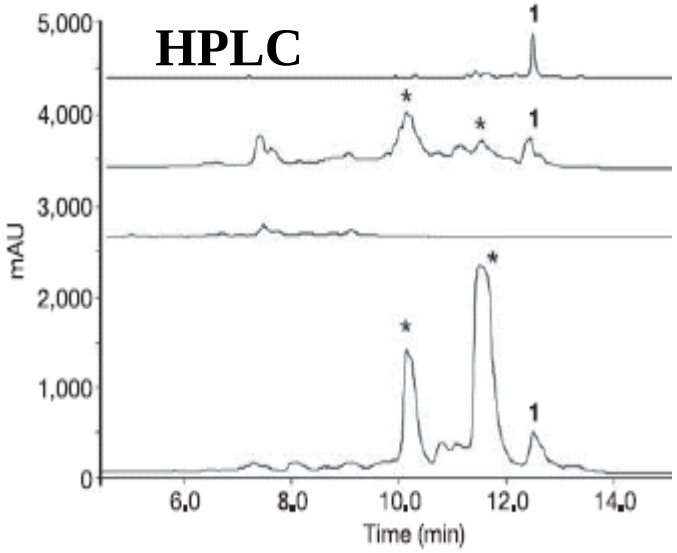
endosymbionts in ATCC 62417



Symbiont-free ATCC 62417 (treated /ciprofloxacin)



ATCC 62417 after reinfection



Rhizoxin reference (12.5 mg.l^{-1})

ATCC 62417

Symbiont-free ATCC 62417 (treated /ciprofloxacin)

Burkholderia sp. isolated from ATCC 62417

Partida-Martinez, Nature 2005, 437, 884

Endosymbiont bacteria in *Rhizopus microsporus*

- **Hypothesis**: endosymbiotic toxin-producing bacteria may significantly enhance the virulence of *Rhizopus* species and explain their predominant role as human pathogens over other Zygomycetes species[1]

- Bacterial endosymbiosis detected[2] or not[3] in clinical isolates
  - No difference in virulence between bacteria-free zygomycetes and their parent strains[2]

1. Chamilos G. et al. 2007, Fungal Genet Biol, **44**, 88
2. Ibrahim AS. et al. 2008 JID **198**:1083
3. Partida-Martinez L. et al. 2008, Mycoses, **51**:266

Zygomycètes : épidémiologie

- ✓ Pathogène chez l'immunodéprimé
- ✓ Facteurs favorisants
  - Diabète (acidocétose)
  - Neutropénie, corticothérapie
  - Trauma, brûlures
  - Dénutrition, toxicomanie IV
  - Deferoxamine
- ✓ Formes cliniques
  - Rhinocérébrales
  - Pulmonaires
  - Cutanées
  - Gastro-intestinales
  - Disséminées

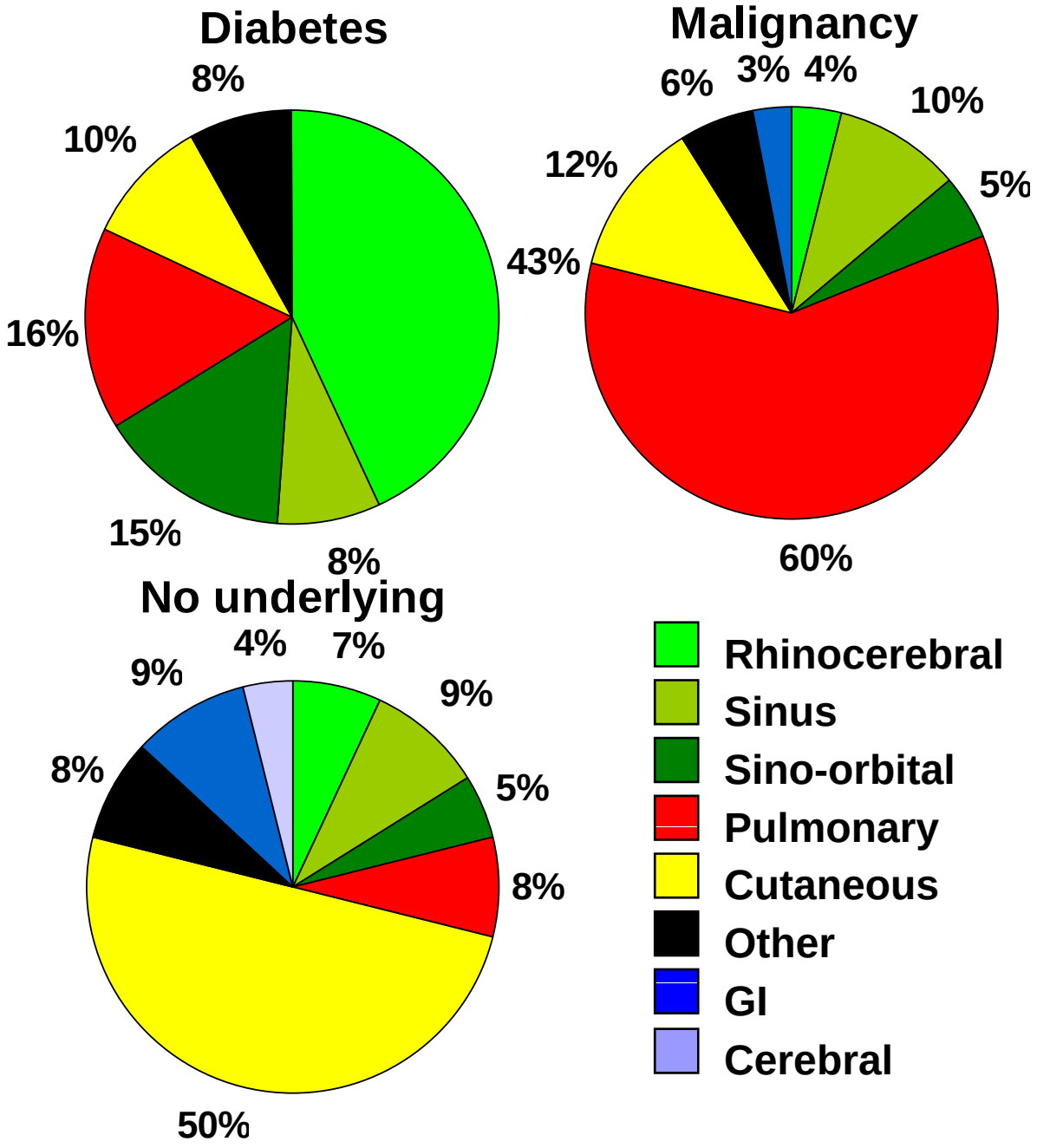
Epidemiology

Clinical forms

- Rhino-cerebral
- Pulmonary
- Cutaneous
- Gastro-intestinal
- Disseminated

Predisposing factors

- Diabetes
- Malignancy
- BMT - SOT
- Deferoxamine
- IVDU
- No underlying condition



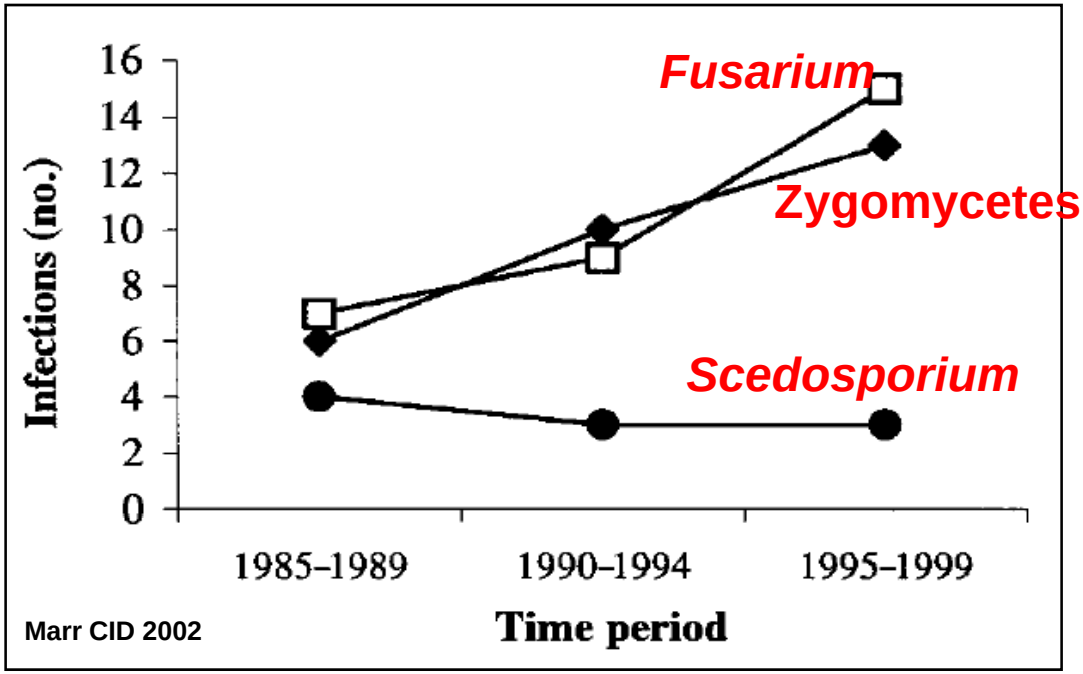
Roden MM. et al. 2005 CID **41**, 634

Zygomycètes

- Pathologies rares
  - 929 cas dans la littérature de 1940 à 2003[1]
  - Incidence des zygomycoses mal connues
    - 1.7 cas / million / an[2]
  - TRANSNET: zygomycoses représentent
    - 2% des IFI / SOT[3]
    - 8% des IFI / HSCT[4]

1. Roden MM. et al. 2005. CID **41:**634.
2. **Rees JR. et al.** 1998. CID **27:**1138.
3. **Pappas PG. et al.** 2007. ICAAC M-1195
4. **Kontoyiannis DP. et al.** 2007. ICAAC M-1196

- Pathologies émergentes
  - En particulier chez patients d'hématologie[1]
  - Infections chez patients sous voriconazole[2-4]

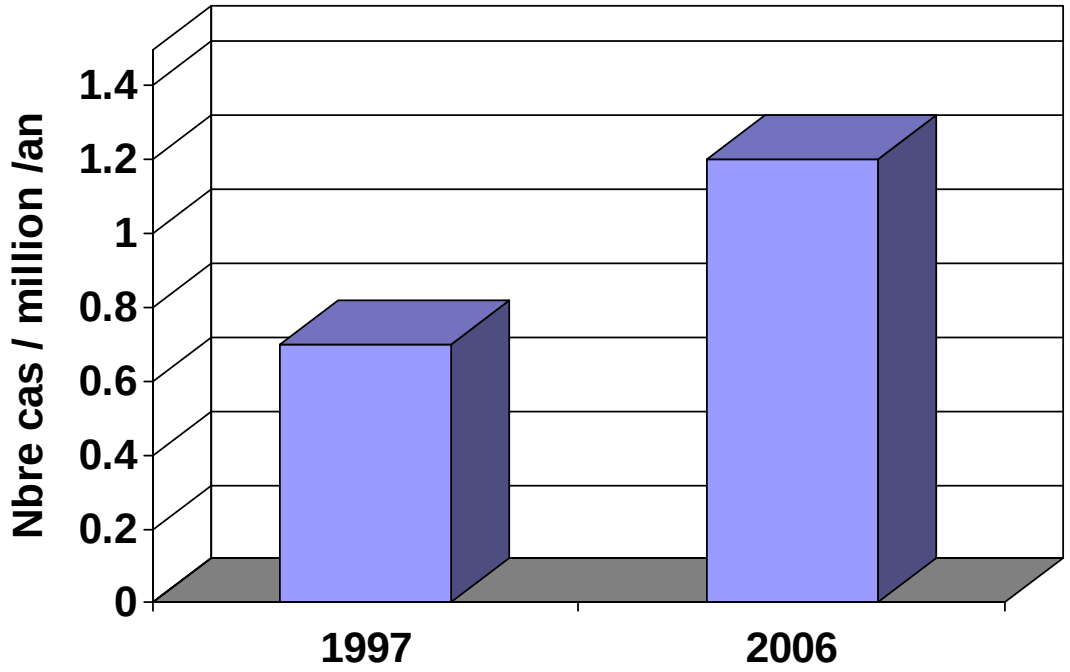


1. Marr KA. et al. 2002. CID **34:**909.
2. Vigouroux CID 2005, **40**, e35.
3. Kontoyiannis DP. et al. JID 2005, **191**, 1350
4. Trifilio SM. et al. 2007. BMT 39:425.

- Pathologies émergentes

  - Etude InVS – Incidence des zygomycoses en France métropolitaine, 1997 - 2006

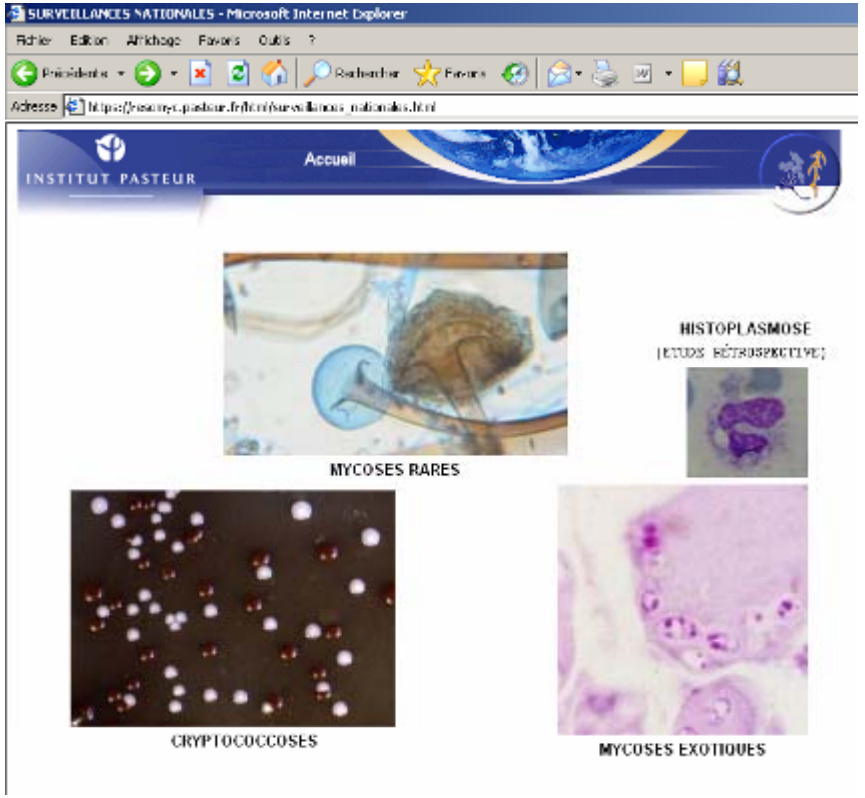
  - 547 cas, taux d'incidence moyen: 0.9 / million /an



Bitar D, Van Cauteren D, Lanternier F. et al. JNI 2008- COL6-01

Nationwide survey of rare mycoses in France

- Starting 2003
  - Epidemiology and microbiology
  - Web-based notification of cases (Voozanoo)



Ongoing study: RETROZYGO

- Nationwide epidemiological retrospective study of zygomycosis (starting 2008)
  - Analysis of 3 years: 2005-2007
  - Analysis of all cases in France

Le diagnostic mycologique

✓ Prélèvements : AB, LBA, biopsie…, en fonction de la localisation

✓ Examen direct : importance ++

✓ Mise en culture : attention aux faux positifs (contaminant)
- Poussent sur nbreux milieux, mais cultures souvent neg
- Incubation 37°C, 30°C
- Biopsies : pas d'homogénéisation +++, détruit le mycélium

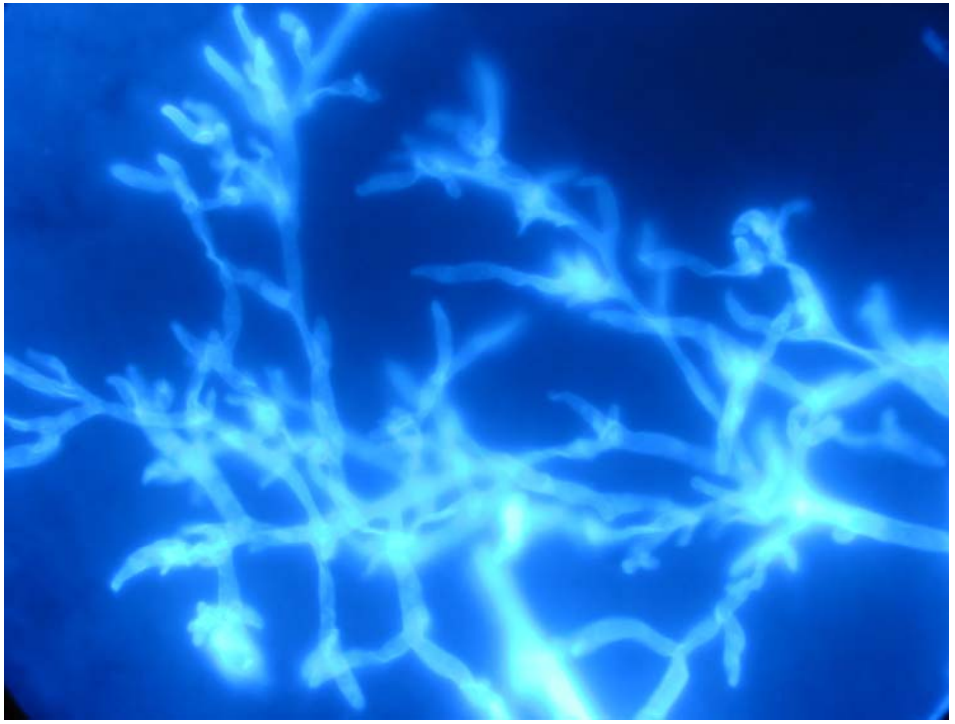
✓ Anatomopathologie
- diagnostic de certitude
- pas de diagnostic d'espèce

✓ Pas de sérologie, pas de détection d'antigène, pas de PCR

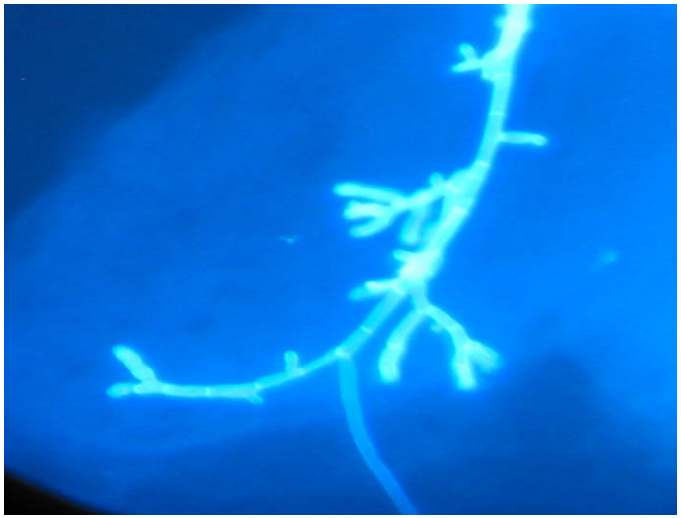
***Schwarz** JCM 2006, **44,**340 ****Machouart** JCM 2006, **44,**805

Zygomycètes : diagnostic

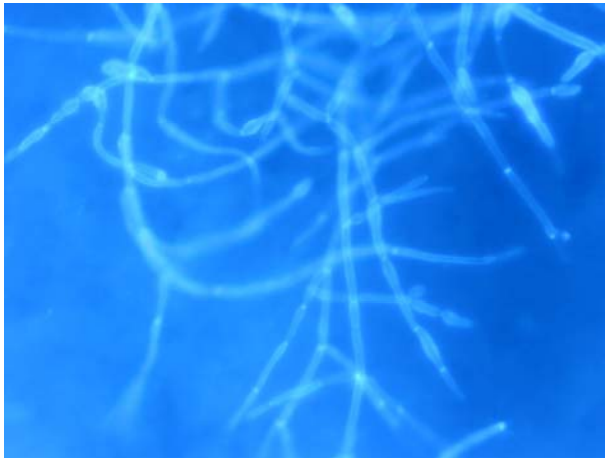
Zygomycètes : mycélium large, rubané, non septé, de diamètre irrégulier. Branchements à angles droits



Aspergillus : mycélium fin, septé, de diamètre régulier. Branchements à angles aigus.

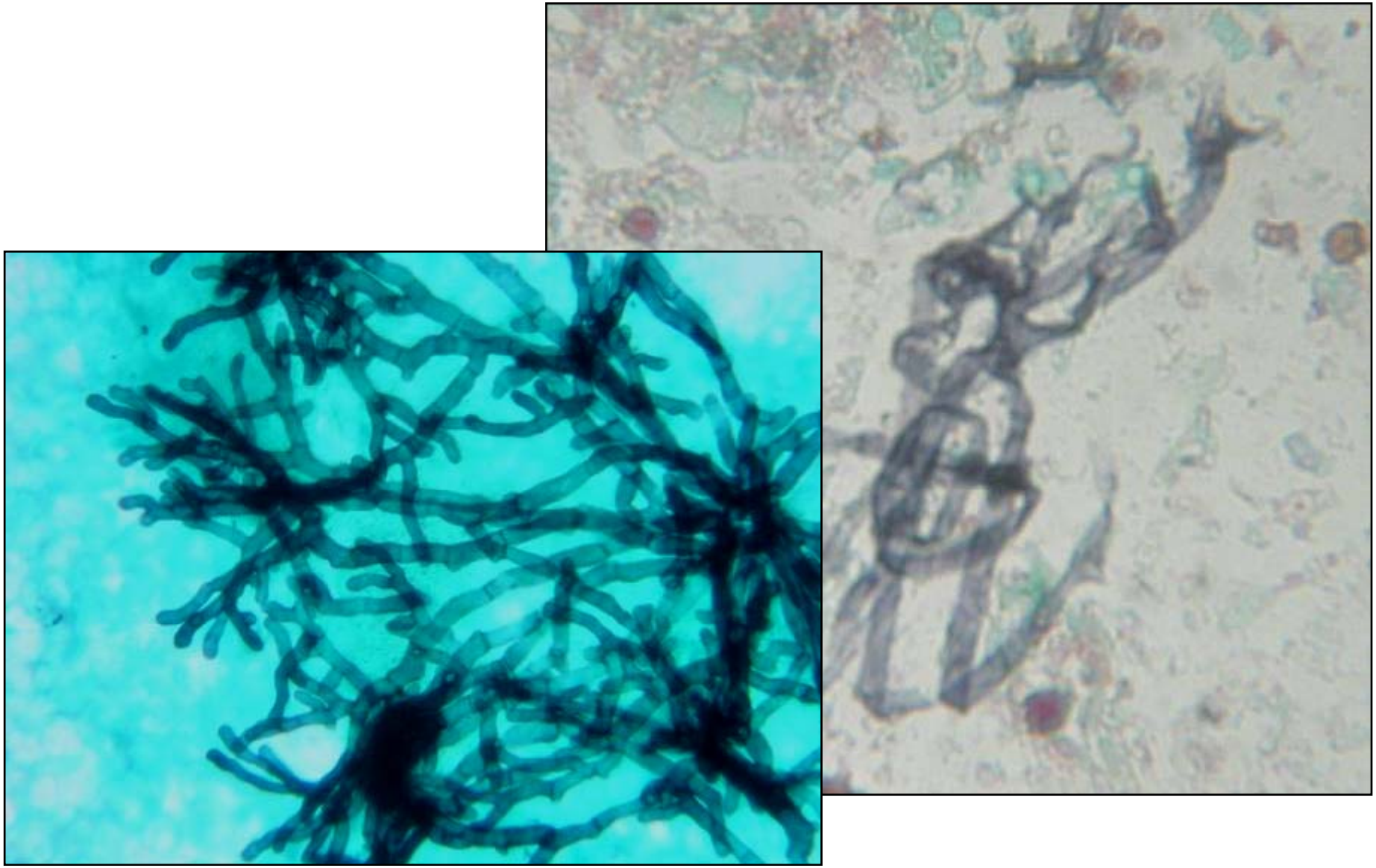


Candida : pseudo-mycélium fin, avec blastospores, de diamètre régulier. Branchement à angles aigus.



Morphologie générale des zygomycètes

- ED (différentiation avec *Aspergillus*)

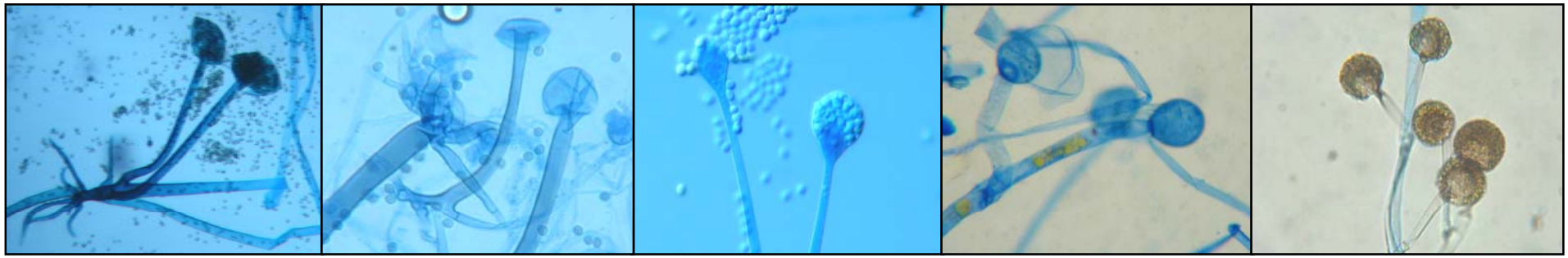


Identification of Zygomycetes from culture

The classical way

- Phenotypic identification
- Mainly based on morphological characteristics
- Remains difficult and sometimes needs expertise of a reference laboratory

R. oryzae ***R. microsporus*** ***Mycocladus*** ***Mucor*** ***Rhizomucor***



- Some species (*Apophys., Saksenaea*) fail to sporulate on routine media

- Morphological-based identification erroneous in > 20% of the cases[1]

→ **New identification methods are needed**

1. Kontoyiannis, D. P., et al. 2005. JID **191:**1350.

Zygomycetes : molecular identification of species

- Reliable identification (DNA barcoding) needs[1]
  - An informative DNA target
    - Working for all species in the group
    - Low intra-specific and high inter-specific variability
  - A validated sequence database for comparison (public, commercial)
    - MicroSeq D2 large-subunit DNA sequencing kit: misidentification of Zygo in ~50%[2]

1. **Balajee A. et al**. 2007. Med. Mycol **45**:475.

2. **Hall L. et al,** 2004. JCM **42:**622.

Current guidelines

- The Clinical Laboratory Standards Institute (CLSI) proposed guidelines for fungal identification by DNA Target Sequencing

Microorganism or Group: *Zygomycetes*

ITS Target	Comments for ITS Target	Alternative DNA Target
Resolution to genus and usually to species	Good discrimination of genera; interspecies variation 0- 40%; intraspecies differences 0-2%. Five species of *Rhizopus* can be distinguished.	D1/D2 region may provide resolution to species

CLSI. Interpretive Criteria for Microorganism Identification by DNA Target Sequencing; Approved Guideline. Document MM18-A, 2008.

Diagnosis of zygomycosis in FRESH tissues

- Good sensitivity and specificity in experimental model[1]
- PCR + sequencing (ITS1) in samples from patients with proven zygomycosis[2]
- Several case-reports[3 -6]
  - Various infections
  - Various species
  - Various techniques and targets

→ **Overall: good results**

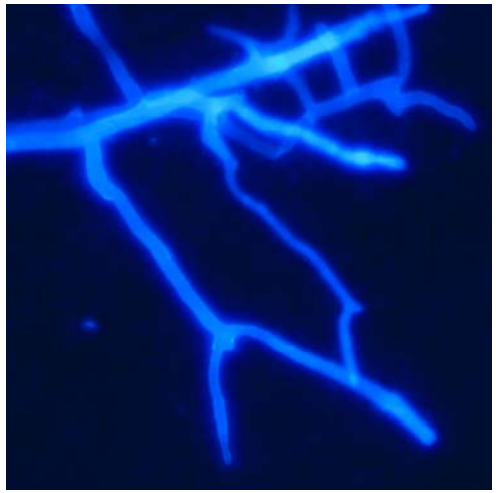
1. Schwarz P. et al. 2006. JCM **44:**340.
2. Lau A. et al. 2007. JCM **45:**380
3. Kobayashi M. et al. 2004. Respirology **9:**397.
4. Larché J. et al. 2005. CID **41:**1362.
5. Machouart M. et al. 2006. JCM **44:**805.
6. Iwen PC. et al. 2005. JCM **43:**5819.

Molecular identification in tissues: a clinical case

- healthy 14-year-old patient
- cutaneous zygomycosis
- scorpion sting

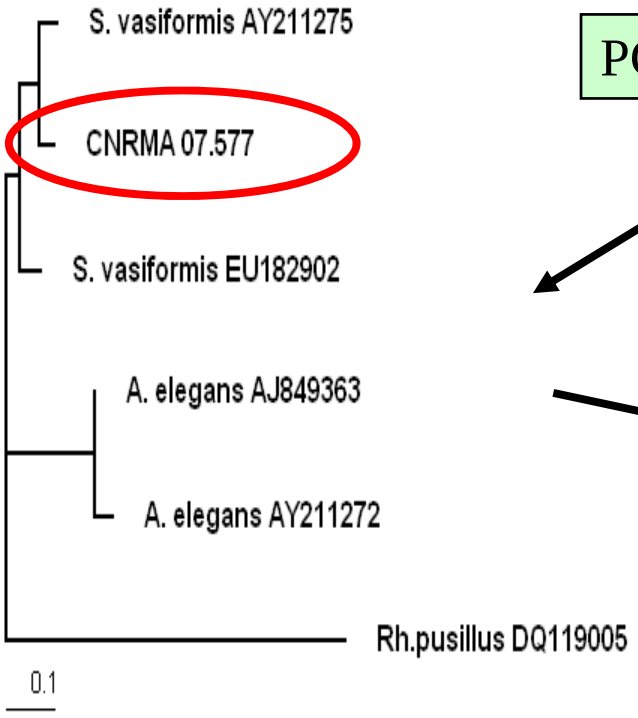
cutaneous biopsy → Non sporulating Zygomycete
Culture non-viable, lost

cutaneous biopsy (frozen)



Direct examination of the tissue sample (calcofluor)

PCR + sequencing ITS / 28S



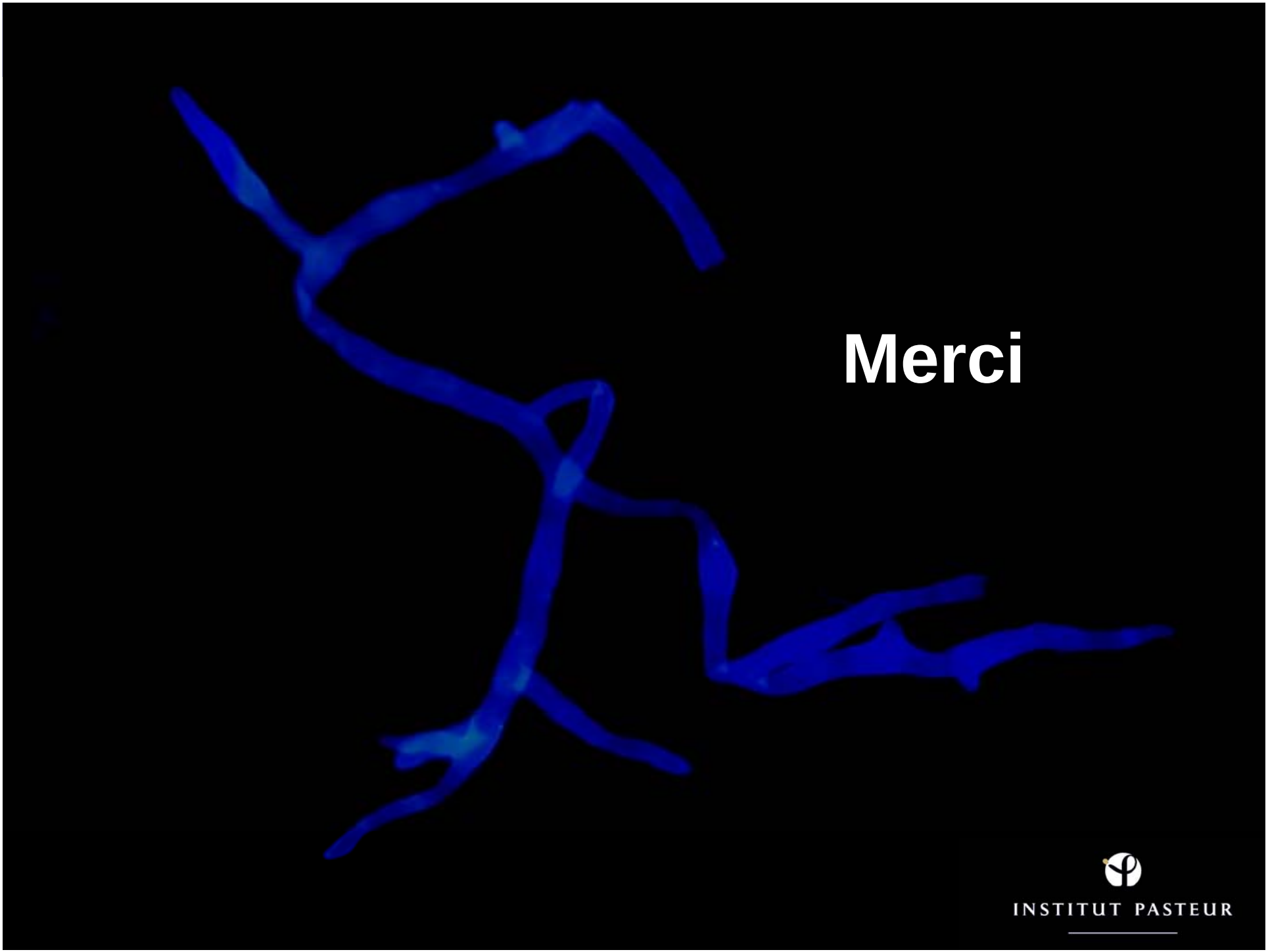
Saksenaea vasiformis

Lechevalier P. et al. JCM. 2008 **46:**3169

Diagnosis of zygomycosis in PE tissues

- Different molecular approaches
  - Good specificity, in experimental model[1], sensitivity to be improved
  - Panfungal PCR[2] (ITS region): PCR+ in 5/9 samples (all histo+)
  - 18S-targeted semi-nested PCR specific for zygo
    - PCR+ in 14/23 samples[3]
    - PCR+ in 5/5 samples[4]
    - Prospectively evaluated[5]: PCR assay superior to culture in histo+ samples

1. ESCMID Fungal Infection Study Group. 2007 ICAAC M-567
2. Lau A. et al. 2007. JCM **45:**380.
3. Bialek et al. JCP 2005, **58**, 1180.
4. Rickerts V. et al. 2006. EJCMID **25**:8
5. Rickerts V. et al. 2007. CID 44:1078.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