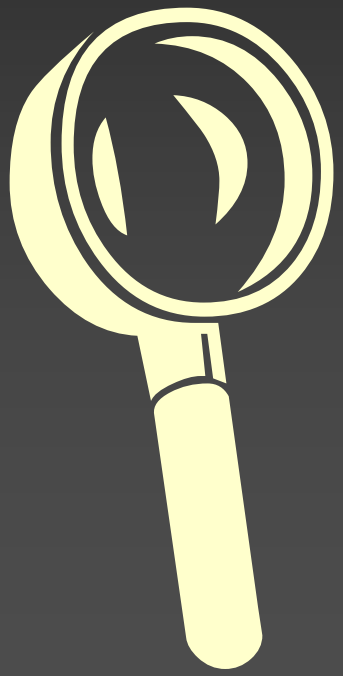
Institute for microbiology shows

TRACING THE CRIMINAL



Part ten:
Criminals – fungi

Instead of an introduction

Survey of topics

Characteristics of fungi

Examples of mycotic diseases

Morphology and physiology of fungi

Diagnostics of mycoses

Pictures of fungi

Characteristics of fungi

Common characteristics of fungi

- Fungi are eukaryotic organisms, unlike procaryotic bacteria
- Their cell wall is formed by polysacharids, it has a different composition than that of G+ bacteria. Neverthreless, it stains violet („gram-positive“)
- Fungi use to have a slower cell cycle than bacteria → infections use to be prolonged
- Majority of antibacterial agents is not effective, so we have to use special drugs – antimycotics, that are not effective againts bacterial infections

Fungi and health

- In the practical we are going to speak about microscopical fungi. But we shoud not forget about fungi with macroscopical fruits
- Fruits of macroscopical fungi (*Amanita phalloides, Inocybe patouillardi, Amanita panterina, Entoloma eulividum*, halucinogenous fungi) cause diseases and death of tens of persons every year (especially in countries like Czechia, where fungi picking is a very common hobby). In case of *Amanita phalloides*, the result is often death.

Some toxical mushrooms

cs.wikipedia.org/wiki/Otrava_houbami

Do you know?

1 *Amanita phaloides*

2 *Inocybe Patouillardi*

3 *Amanita panterina*

4 *Entoloma eulividum*

http://www.micologia.net/g3/Amanita-panterina/Amanita_pantherina_001

http://www.houbar.cz/default.aspx?show=3&text=3

cs.wikipedia.org/wiki/Z%C3%A1vojenka_olovov%C3%A

Medically important fungi

- Microscopical fungi may cause in human body
  - Mycoses – fungal inflammations
  - Mycotoxicoses – toxical action
  - Mykoalergoses – allergy to fungi
  - Mycetisms – fungus present in the body, acting only in form of pressure to surronding tissues
- The most imporant are mycoses, that may be subclassified into
  - surface (skin and mucosal) mycoses and
  - organ and systemic mycoses

Examples of mycotic diseases

Story one

- Mrs. Udder came to dpt. of professional medicine with **„fungus“ on her hand.**
- She wanted her disease to be accepted as **proffessional disease**, as she works with cattle on a farm. Good luck for mrs. Udder: the causative agent of the disease was found to be *Epidermophyton floccosum*, that is supposed to by rather antropofilic species of so named dempatophyta, so it is transmitted rather person-to-person than from animals; nevertheless, actual guidlines say that mere professional exposition is sufficient for considering such case professional, not regarding the species diagnostics

Dermatophytes

- So named **dermatophytes** belong among the most common agents of skin mycoses (including mycoses of skin adnexa, i. e. hair, hairs and nails)
- Among dermatophytes there are genera ***Trichophyton***, ***Epidermophyton*** **and** ***Microsporum***
- According to the most common ways of transmission, they are **anthropophilic** (person-to-person), **zoophilic** (from animals) and **geophilic** (from environment)
- The disease have various names in relation with their localisation (**tinea manus, pedis, barbae** etc.).
- **Treatment** is usually local (ointments, shampoo). The mostly used drugs are nystatin, clotrimazol, ketokonazol etc.

Dermatomycoses of various parts of body

www.mycolog.com/chapter23.htm

Tinea barbae

www.emedicine.com

Tinea pedis

www.itg.be

Onychomycosis

www.itg.be

A severe infection of *Epidermophyton floccosum* before and after treatment

www.mycolog.com/chapter23.htm

Story two

- Mr. Leopold worked for an archive. All days he spent in the **wet and dusty archive.** Step by step he started to **cough**. For a moment, he was affraid of TB, but if was not TB. After assessment of the true reason and after the proper treatment Leopold problems started to disapear – again, slowly, step by step.

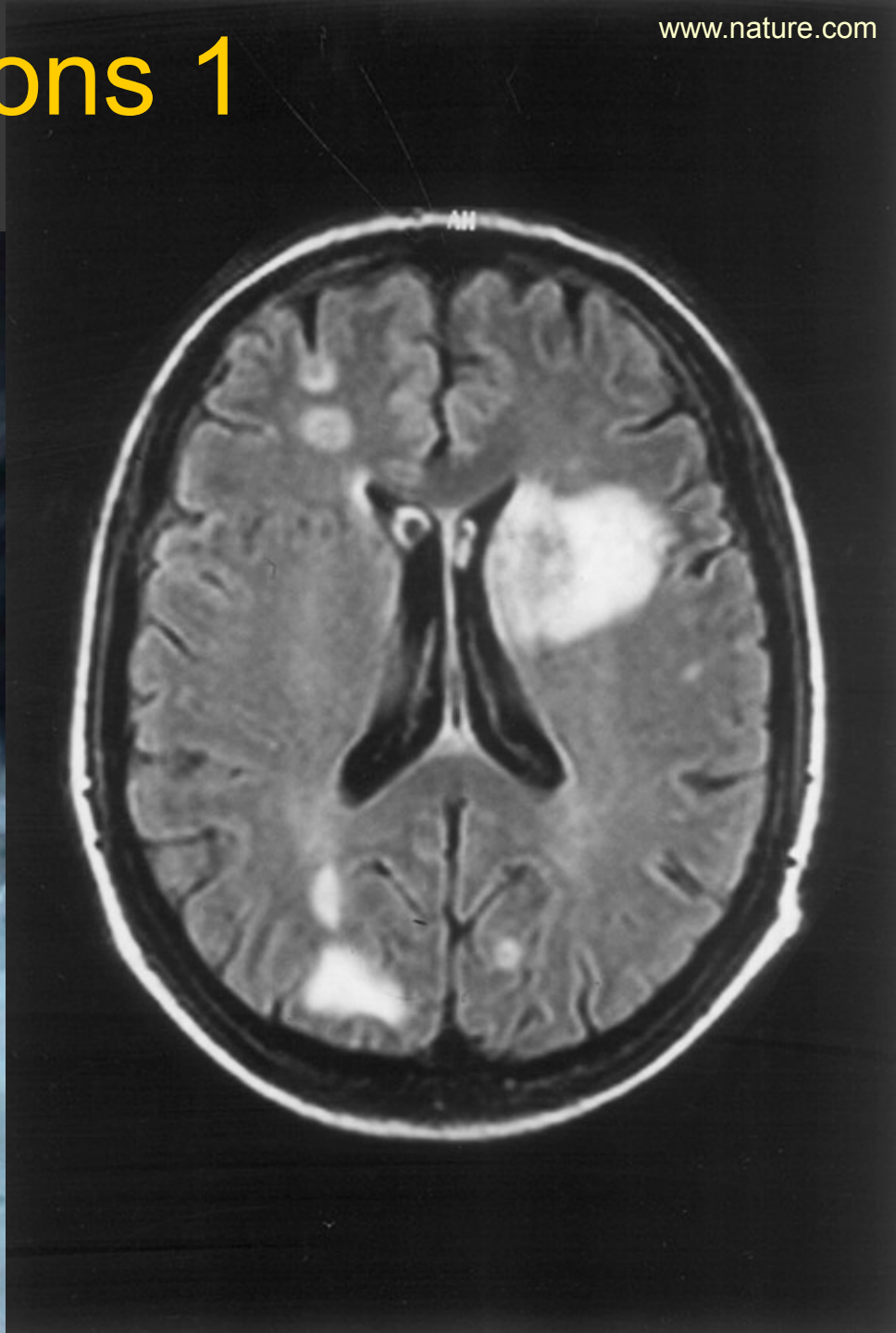
Causative agent was

- ***Aspergillus niger***
- Aspergilli usually attack diseased people, nevertheless, they are able to attact a heathy one, too.
- Aspergillosis is often a **professional disease** of persons working in wet, dusty places, full of mold spores
- Aspergillus disease is an example of **organ or systemic mycoses**

Aspergillus infections 1

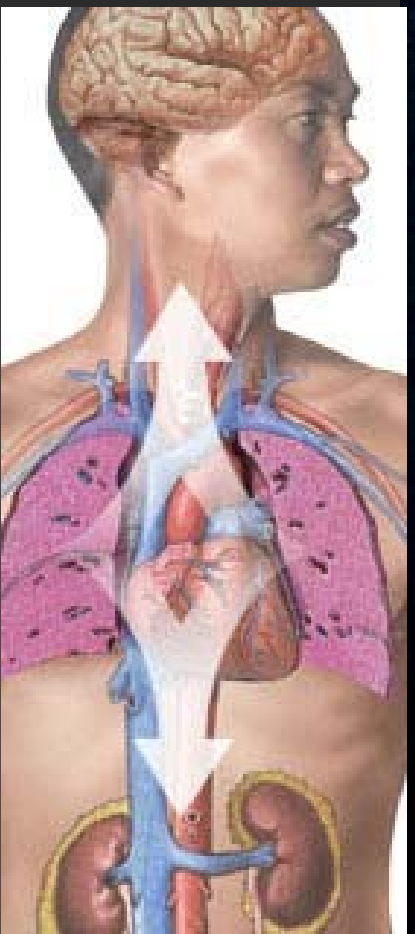
aapredbook.aappublications.org

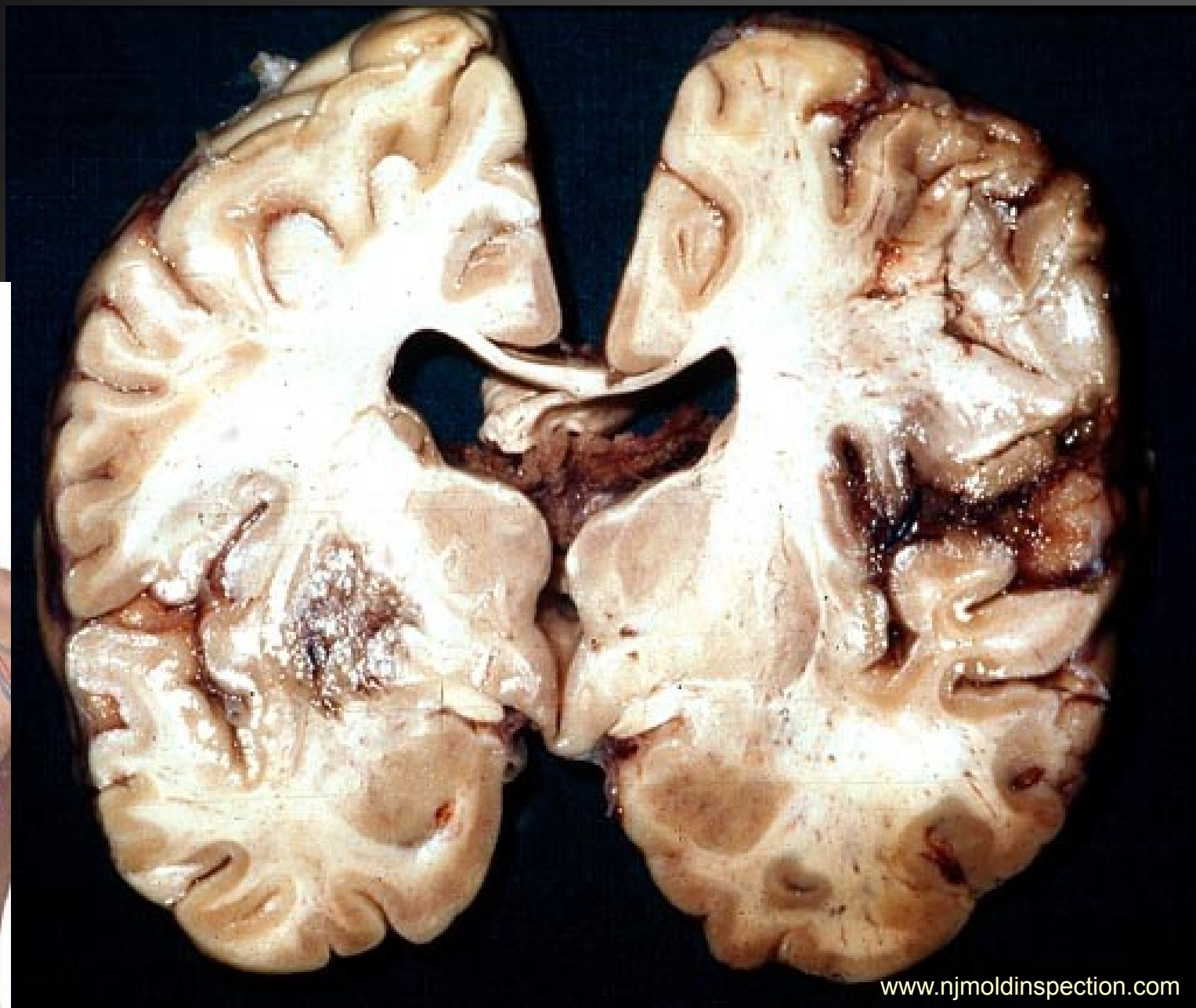
www.nature.com



Aspergillus infections 2

www.nlm.nih.gov





www.njmoldinspection.com

A real case: aspergillosis as an influenza complication in a 38 year old woman

- A female, born 1970, Brno-city, primary infection of upper and lower respiratory ways, as a causative agent proven **influenza type B** and *Staphylococcus aureus*. Death as a result of a heavy mycotic – ***Aspergillus* pneumonia and septicemia, with lung** and tracheobronchial lymphonodes **anthracosis**, exitus 26th Mar 2008. No risk factors in anamnesis, only about **15 years of smoking 15–20 cigarettes daily.** (From regional public health office of South Moravia)

Systemic mycoses

- They attack more organs, often the whole body
- Usually they follow after a **primary disease:**
  - Diabetes mellitus
  - Immunity defects, WBC tumors etc.
  - Transplanted pacients
- **Caused by:** *Candida, Penicillium, Aspergillus, Histoplasma, Pneumocystis* and other
- **Treatment:** strong, broad-spectered and highly effective antimycotics are used (amphotericin B, voriconazole, itraconazole, flucytosin)

Zygomycets

- Zygomycets – true molds form non-septed hyphae. They produce a strong growth, they are even able to pull the lid of the Petri dish up.
- Infections are **rare**, but they occur more and more e. g. in diabetics. Normally they live as saprophytes, e. g. on fruits. They are able to grow quickly, e. g. through wall of large vessels. They may cause even so called **living trombus** with a quick death of such a patient.
- Another typical feature is **quick growth from nasal cavity to brain,** even during a few hours
- The most important genera: ***Rhizopus*** & ***Mucor***

Dimorph fungi

- Cause mycoses in immunodeficient patients
- ***Coccidioides immitis*** grows more quickly than the others. In patients with small immune defficiencies the infection is asymptomatical or with small symptomas only. It is worse in persons with a developped AIDS, where you can see primary lung infections etc.
- ***Histoplasma capsulatum*** is seen mostly in the USA, but also in Africa.
- More genera: ***Blastomyces, Paracoccidiodioides, Sporothrix*** and other

Blastomycosis

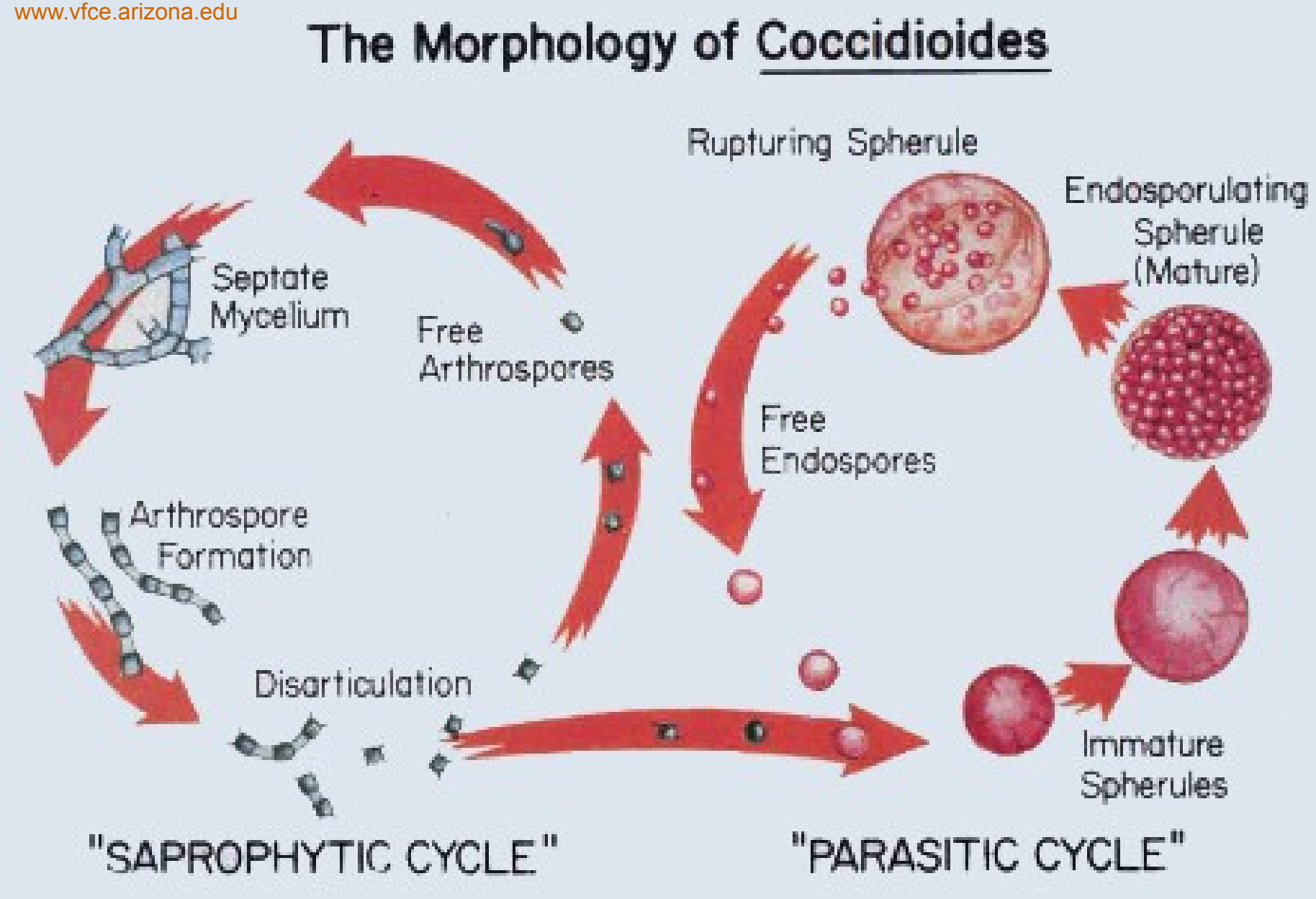
www.mycolog.com/chapter23.htm

Coccidioides immitis

http://www.mycology.adelaide.edu.au/gallery

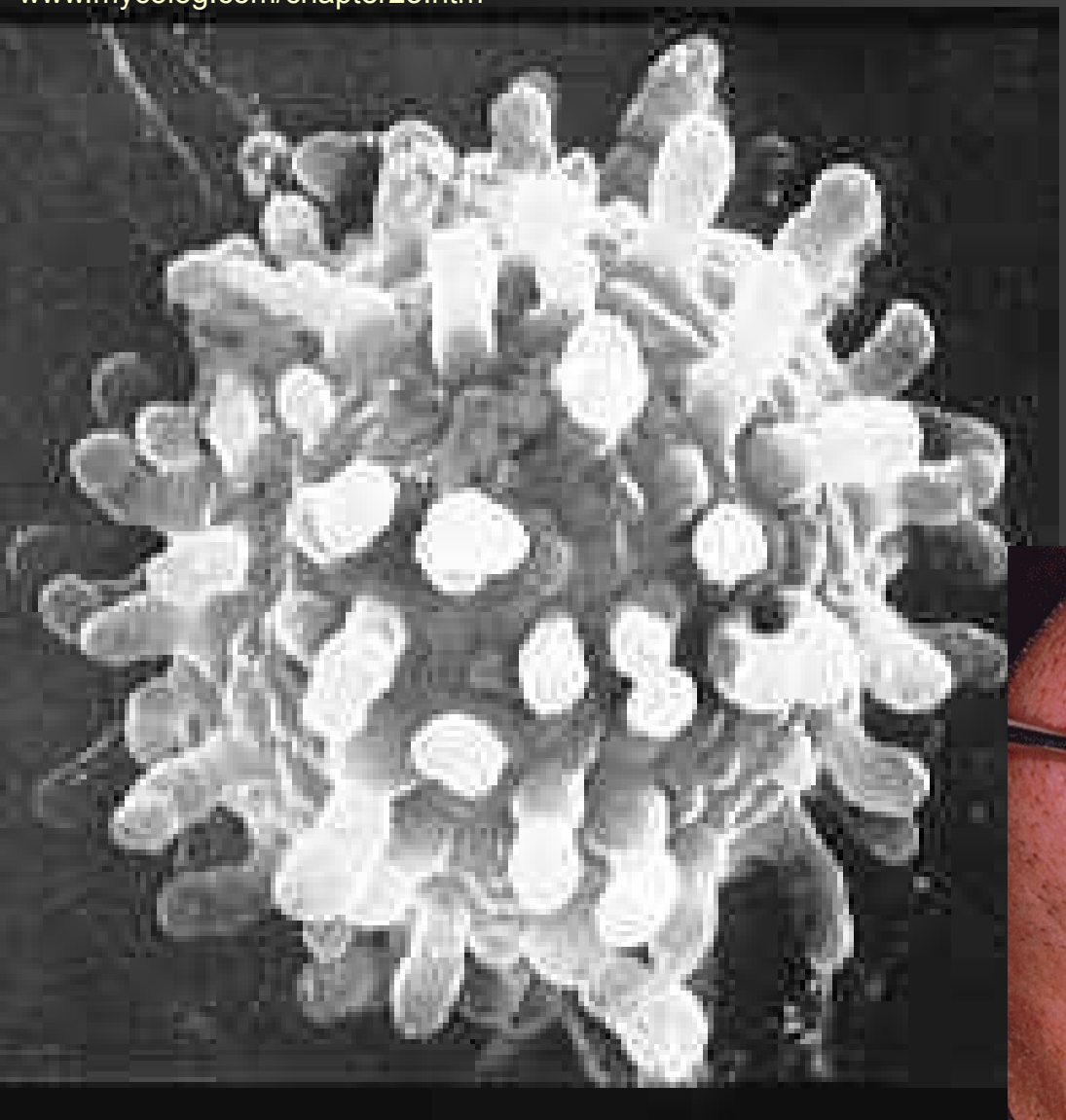
Coccidioides immitis: „strange fungus“

www.vfce.arizona.edu



Histoplasma capsulatum

www.mycolog.com/chapter23.htm



http://www.mycology.adelaide.edu.au/gallery

Story three

Ellen was scared. She loved her boyfried very much, but the intime moments were complicated by **vaginal pruritus**.

Well, she allready visited her gynecologist, and she got **vaginal suppositoria**. They helped always for a moment only.

- Ellen was really angry. She changed her gynecologist. The new gynecologist understood, that local treatment will not be sufficient in this specific case. **Systemic treatment was able to destroy the causative agent both in vagina and intestinal reservoir**. So her problems finished.

Causative agent was

- *Candida albicans*, the most common among medically important yeasts. Vaginal mycoses (mostly candidoses) are very unpleasant and difficult.
- The vaginal candidoses are multifactorial. Important are dietary influences (yeasts love sweet, so if does so their host, they would enjoy it), but also hormonal influences, pregnancy, diabetes and others.
- The reservoir of the infection is the intestine. Recidivating infections should not be treated only localy (suppositoria) but by combination of a local and systemic treatment.
- Vaginal mycosis of course should not be solved without thinking about the total status of the organism.

Candida treatment

- Candidosis **may be both surface (skin, mucosal) and systemic.**
- Among **mucosal candidoses,** beside vaginal mycoses also oral mycosis is seen (in sucklings and people with diseased immunity)
- **Skin candidoses** are common, too (for example „diaper dermatitis“ in sucklings)
- **Systemic infections** are present mostly in immunodefficient persons and persons treated by combination of broad-spectered antibiotics
- The most common is ***Candida albicans***, also *C. tropicalis, C. glabrata, C. krusei, C. parapsillosis* etc.
- In some of them, we can see **natural resistances** (e. g. *C. krusei* for fluconazole)

Genital candidosis

Oral candidosis

ww.asnanak.net/ar/article.php?sid=62.

Intertrigo and diaper dermatitis

Intestinal candidosis

http://george-eby-research.com/html/depression-anxiety.html

Other yeasts and yeast-like organisms

- Very dangerous is ***Cryptococcus neoformans***, in immunodeficient persons it may cause **pneumonia, meningitis, sepsis**
- ***Pneumocystis jiroveci*** is a very strange fungus, some time ago it was supposed to be a protozoon (for example a stage of trypanosomas)
- Genus ***Saccharomyces*** contains wine and bear yeasts. It was supposed to be non-pathogenous, but some studies say that 8 % of vaginal mycoses may be caused by this agent.


PRINCIPAL
GET THE MOLD OUT OF OUR SCHOOL NOW!
HOW LONG DO YOU THINK IT WILL TAKE BEFORE SHE ADMITS WE'RE NOT MAKING THIS UP?
MOLD IS MAKING US SICK!
KONOPACKI
©2005
HUCK/KONOPACKI TEACHER CARTOONS
WWW.SOLIDARITY.COM/HKCARTOONS - OCT

Morphology and physiology of fungi

Morphology of fungi (micromycetes)

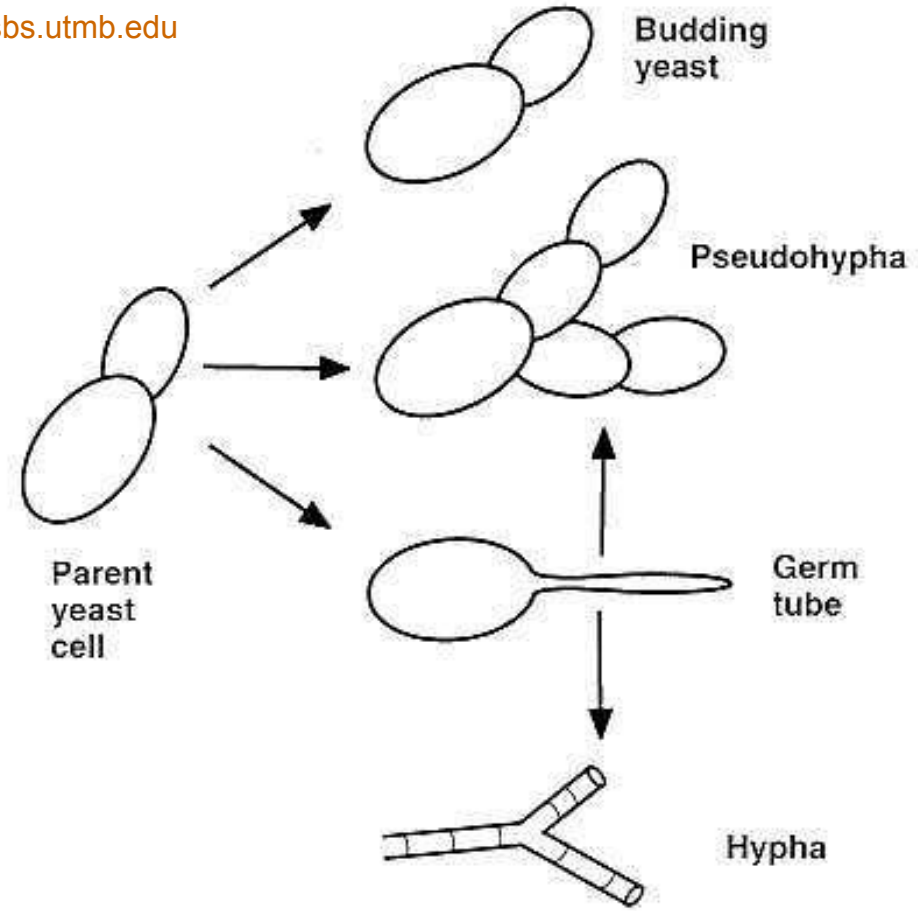
- A **blastoconidia** is an oval or round cell, characteristic for yeasts. Often we see budding blastoconidia (blastospores).
- A **hypha** is a fiber. It may be branched, septed or not septed. A sample of hyphae is called **mycelium**, that may be
  - **vegetative**, anchoring the fungus in the substrate
  - **generative** or air mycelium, bearing generatory organs of the fungus

Multiplication of fungi

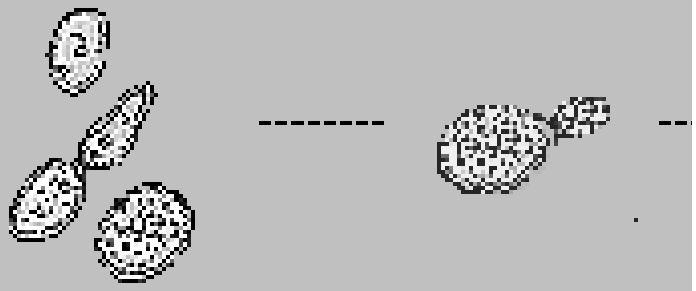
- **Multiplication of fungi may be sexual or asexual.** It is simillar like in plants, here, too, we have both asexual and sexual multiplication methods. It is recomended to use terminology like that:
  - for **sexual** multiplication particles use term **spore** (do not confuse with bacterial spores!)
  - for asexual, **vegetative** reproductory particles to use term **conidia**

Some morphological features in fungi

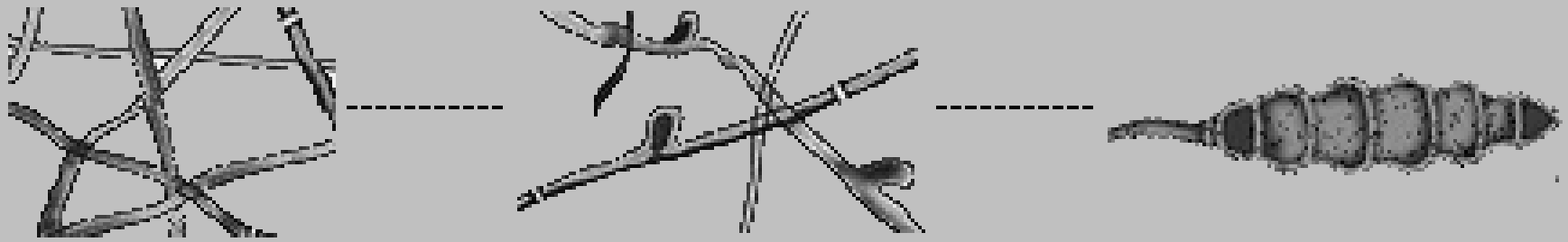
gsbs.utmb.edu



- yeast cells---------- budding----

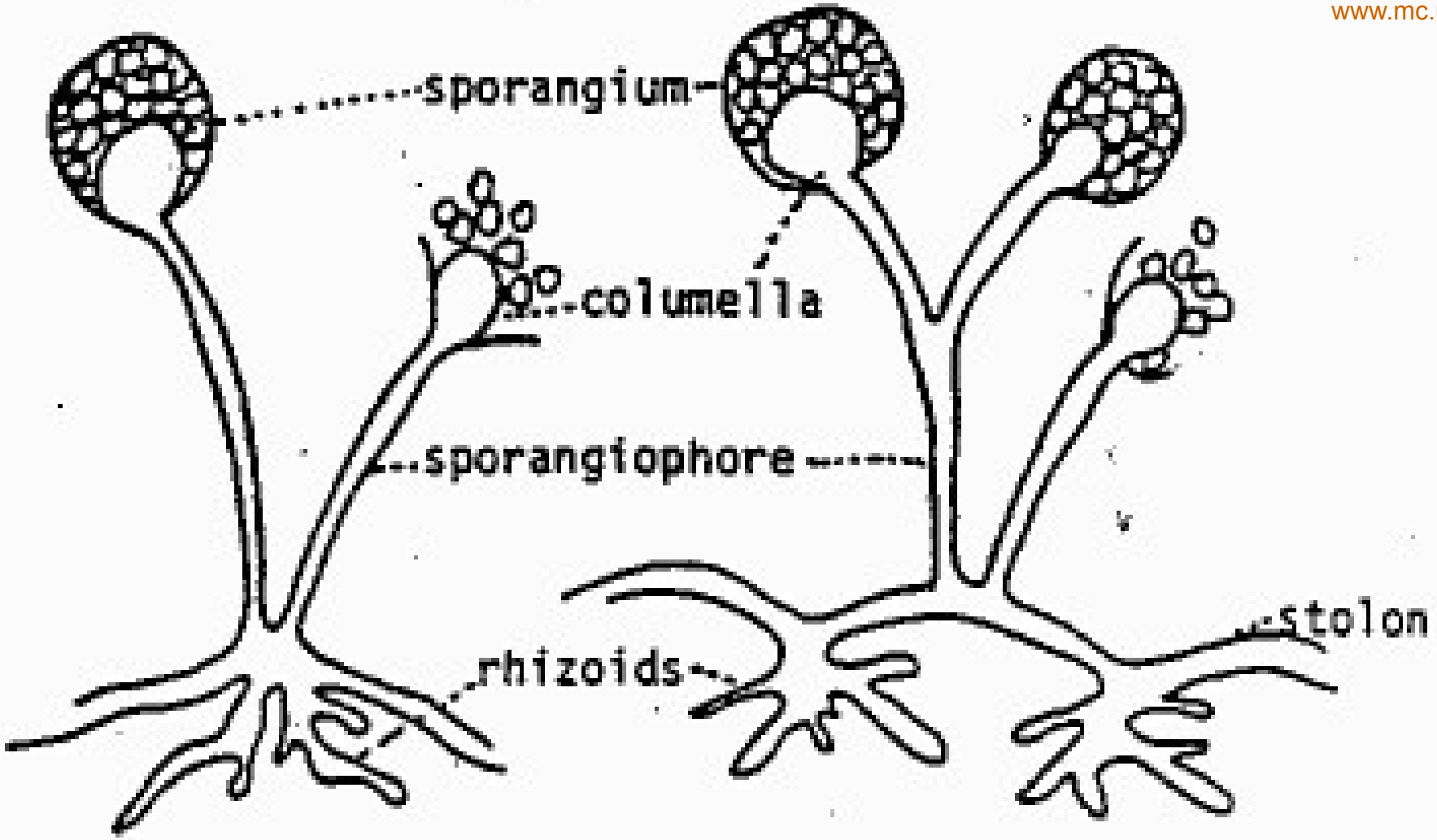


- mycelium----------microconidia----------macroconidium

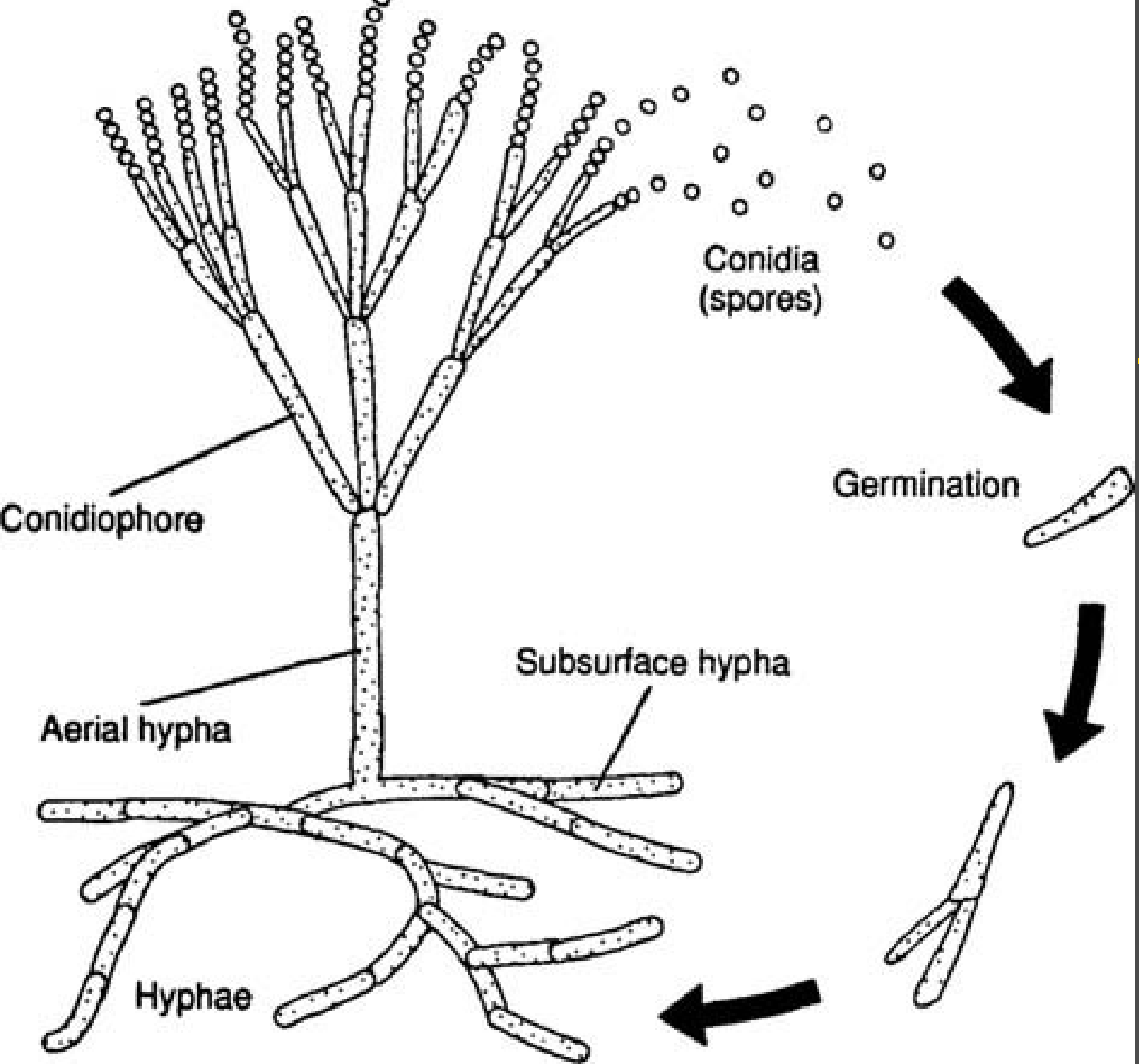


education.med.nyu.edu

How individual parts of a fungus are called

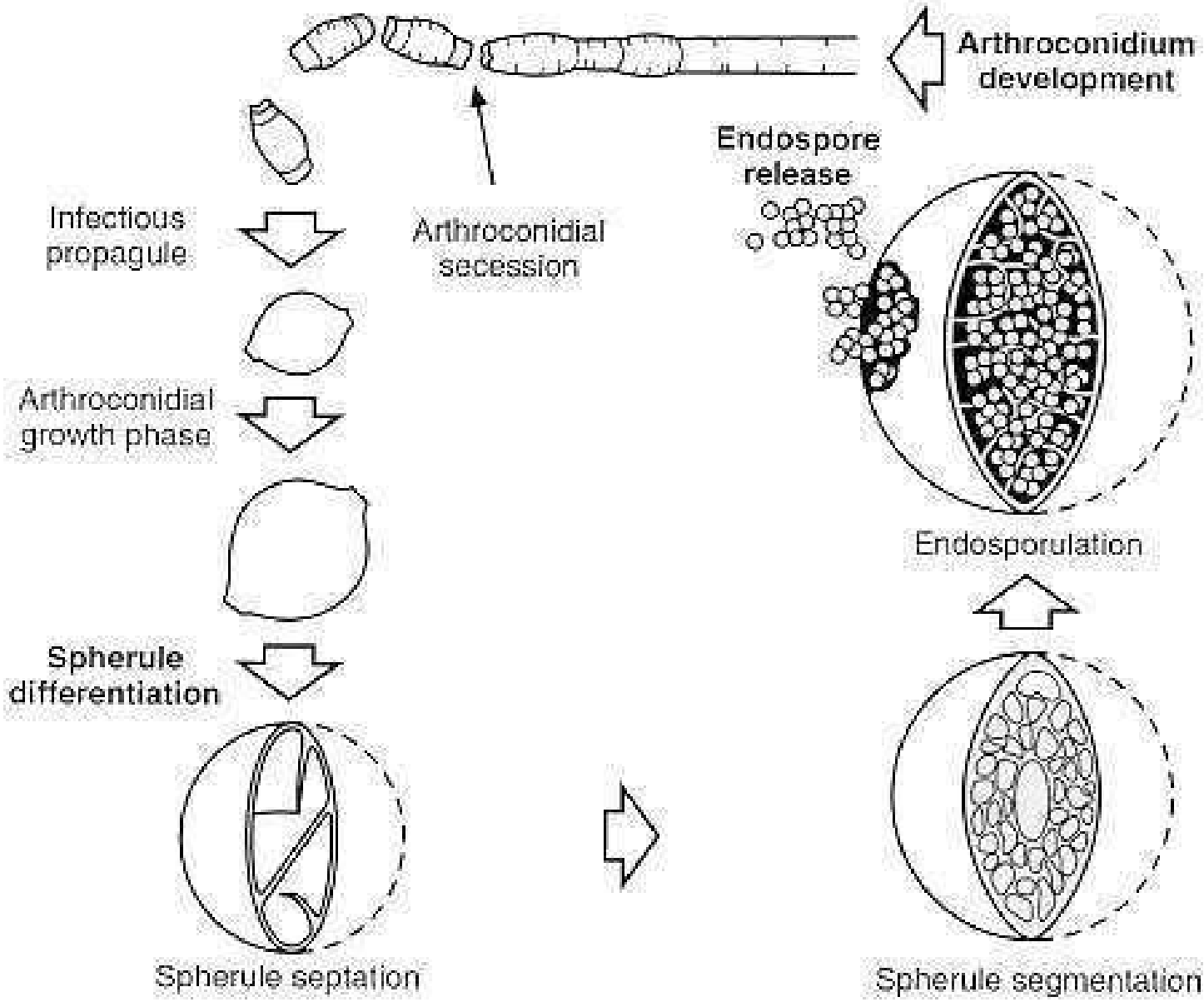


Life cycle of a fungus



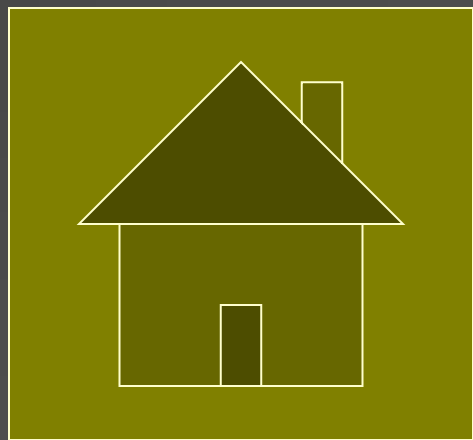
/media.wiley.com

How an arthroconidia are formed



Physiology of micromycetes

- Fungi multiply **usually more slowly** than bacteria, but there are big differences. They grow easily even on poor media.
- Majority of medically important media grow well even at **lower temperature**. We culture ther at 30 °C rather than at 37 °C. Another way is a **parallel culture** at 22 °C and 37 °C, suitable for dimorph fungi
- **Biochemical activity** is rich especially in yeasts



Diagnostics of mycoses

Sampling an diagnostics in surface mycoses

- **Sampling:** particles of skin, parts of nails, hairs etc; always the specimen should contain the site where the inflammation is active, and not to catch contamination; even surface disinfection is recommended (to destroy contaminants from skin surface)
- **Proper diagnostics:** microscopical (files in tissue) and culture. Microscopy is more important – even contamination may be cultured, but hyphae growing through an epitelium are a clear sign of an infection

Sampling for dermatomycoses

- Main rules for sampling:
  - do not send swab only, send several particles of skin (nail, hair, hairs etc.)
  - perform surface disinfection
  - superficial layer should be discarded, not used
  - in larger infections sample from margins (here the fungus is active)

Diagnostics of systemic mycoses

Not only the proper mycosis diagnostic is to be performed. It is also always necessary to find what is primary cause of the disease (if we do not know): immunodefficienty, diabetes, tumor etc.

- Diagnostics:
  - for direct diagnostics any relevant material: blood for blood culture, punctates, excisions etc.
  - modern methods enable e. g. direct detection of antigens in blood (mannans, glucans)
  - indirect detection – serum antibodies (aspergilli)

Sampling in candidosis

- In skin and mucosal form we use swabs mostly in transport medium FungiQuick or (in genital swabs only) C. A. T.
- In systemic form swabs, too, or blood, punctate etc.

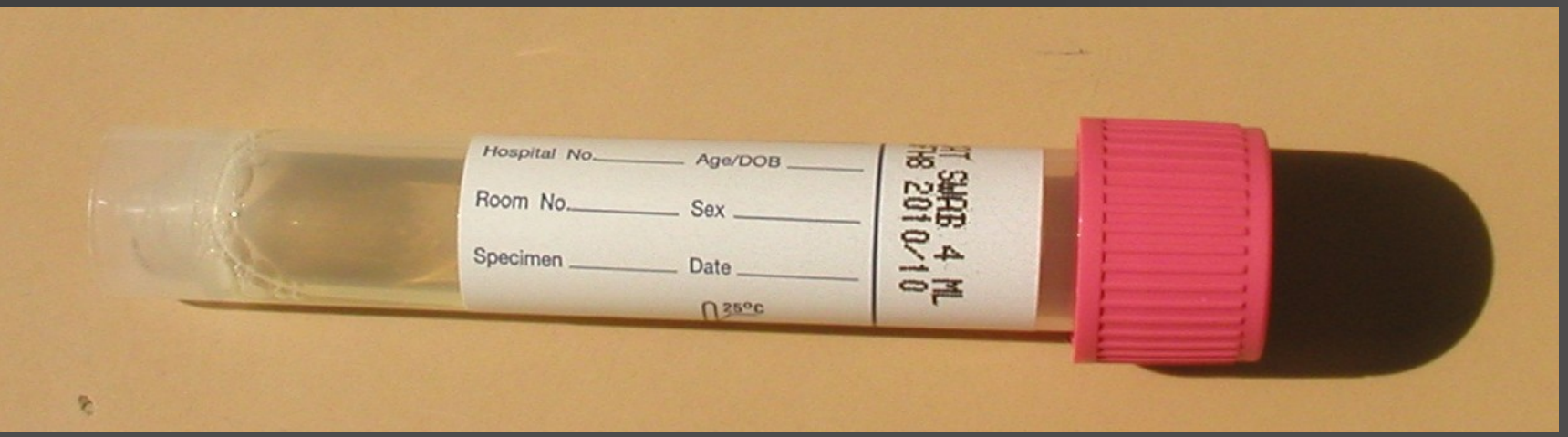


Foto O. Z.

C. A. T.

Diagnostics of candidoses

- The basic is **culture**. For identification of candida we use chromogenous media and biochemical methods (mutual differences in metabolism between candida)
- **Microscopically** in a wet mount (C. A. T.), in Gram and Giemsa stain we can see oval cells, often budding, sometimes even so called pseudomyelia
- It is also possible to test **in vitro susceptibility**, but tests are less reliable than in bacteria
- A modern method is the **direct detection of mannan antigens in blood**

Fungi on bacteriological media

- Although we use special media for fungi, many fungi grow on bacteriological media, too. And not only this: some of them, mostly Candida, have often feature very simillar to bacterial colonies.
- To differenciate colonies of Candida from colonies of staphylococci is often difficult. Smell may help (bread, yeast); when nothing other helps, smear is useful.

A selective medium for fungi

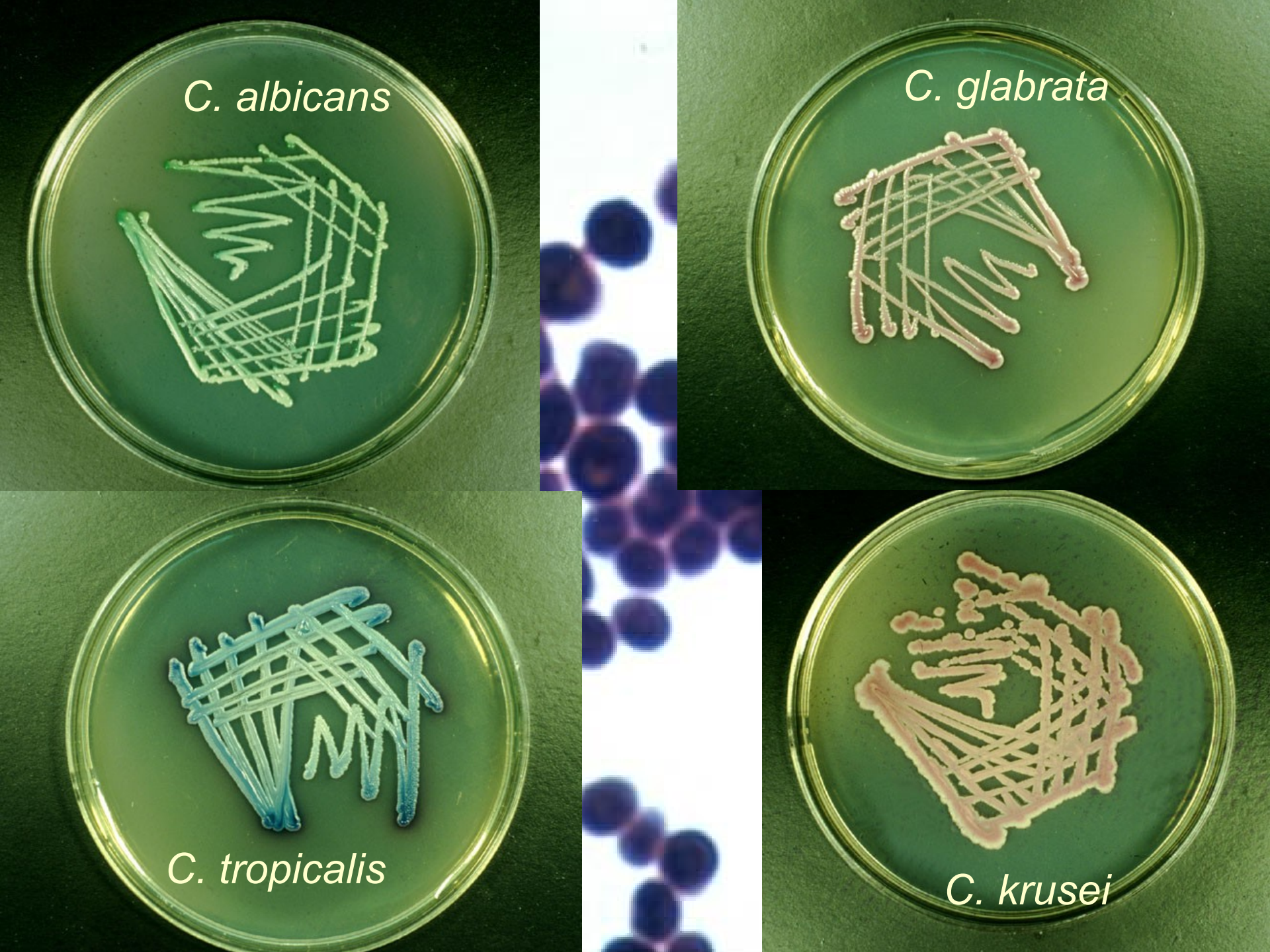
- The typical medium for yeasts, Sabouraud agar, is not selective itself, and many bacteria could grow on it
- For culture of mycoorganisms we use Sabouraud agar with antibiotics, that nearly excludes growth of bacteria. *(In practice, nevertheless, we often meet very resistant strains of Pseudomonas, that grow where they want ☺)*

Chromogenic media – principle *(Review from spring term)*

- CHROMOGENIC media contain a stuff that is originally colourless (a chromogene)
- Only in presence of a specific reaction they become coloured (splitting of a substrate)
- The medium may contain more chromogenes with bound substrates for warious bacteria or fungi
- FLUOROGENIC media are principially simillar, but with a fluorescent stain

Chromogenic medium at diagnostics of Candida

- We use various chromogenic media. Some differenciate *Candida albicans* from other media only, some other differenciate mutually several species of Candida.
- On the medium used in our Task 2c, *C. albicans* is green, *C. tropicalis* blue, *C. glabrata* smooth pink and *C. krusei* rough pink.
- If a strain is not determined using this medium, we have to use another test (e. g. biochemical test)


C. albicans
C. glabrata
C. tropicalis
C. krusei

Biochemical identification of yeasts

- Like bacteria, fungi, too (but not filamentous fungi) may be identified biochemically. (Also use of a chromogenic medium is based on selective splitting of various substrates.)
- One of commonly used test is Auxacolor, that replaced ancient sets of „auxanograms“ (testing use of sugars) and „zymograms“ (testing breakdown of sugars)

Diffusion disc test of susceptibility to antimicromial agents

- With some exceptions it is valid, that antibacterial agents are useless in mycotic diseases.
- Similarly, antimycotics do not act to majority of bacterial agents
- Fungi cannot be cultured on MH, they need Sabouraud agar

To reading of antimycotic tests

- In amphotericin B a strain is considered to be susceptible even in small zone, but there should be no colonies inside the zone
- In azolic antimycotics (the names ending „-conazol“) the zone should be large enough, but „something“ may be present inside the zone, if this „sometning“ is not more than 20 % of intensity of growth inside the zone

Microscopy of filamentous fungi

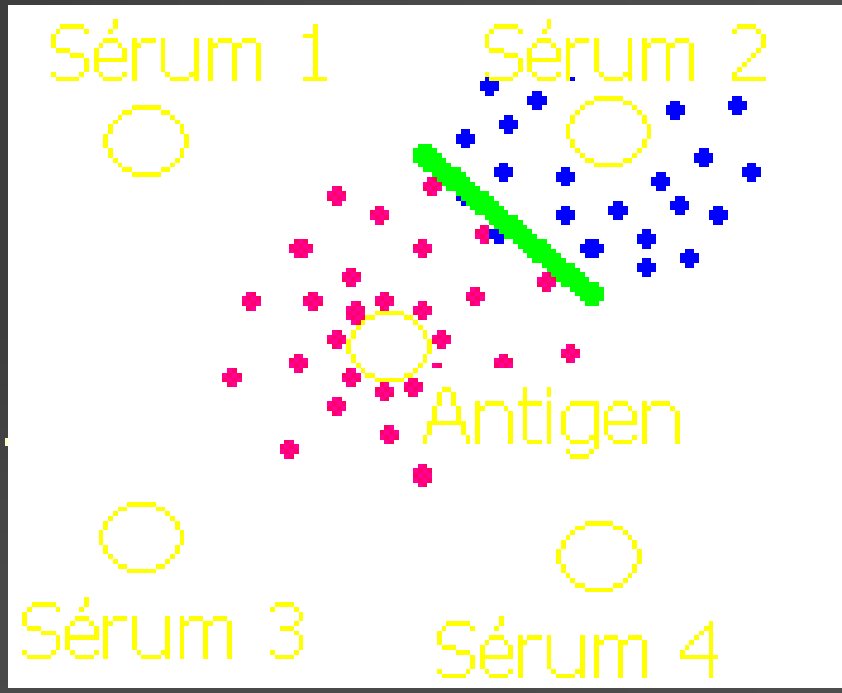
- Also microscopy is different than that of yeasts. It is more important here. We can observe various types of spores and conidiae.
- We observe without immersion, objective multiplying 4× or 10×, eventually 40 ×

Culture of filamentous fungi

- Results of culture in filamentous fungi are different from yeast, both on Sabouraud agar and eventually blood agar.
- *Some of them, especially dermatophytes, grow very slowly. This is because of them, why Sabouraud agar is poured into test tubes.*
- *Biochemical differenciation is usually not performed here, unlike the situation in yeasts.*

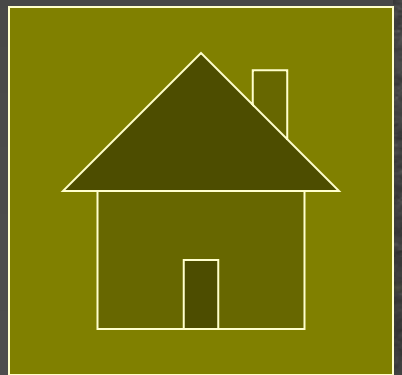
Example of indirect diagnostics of fungi: microprecipitation in agar

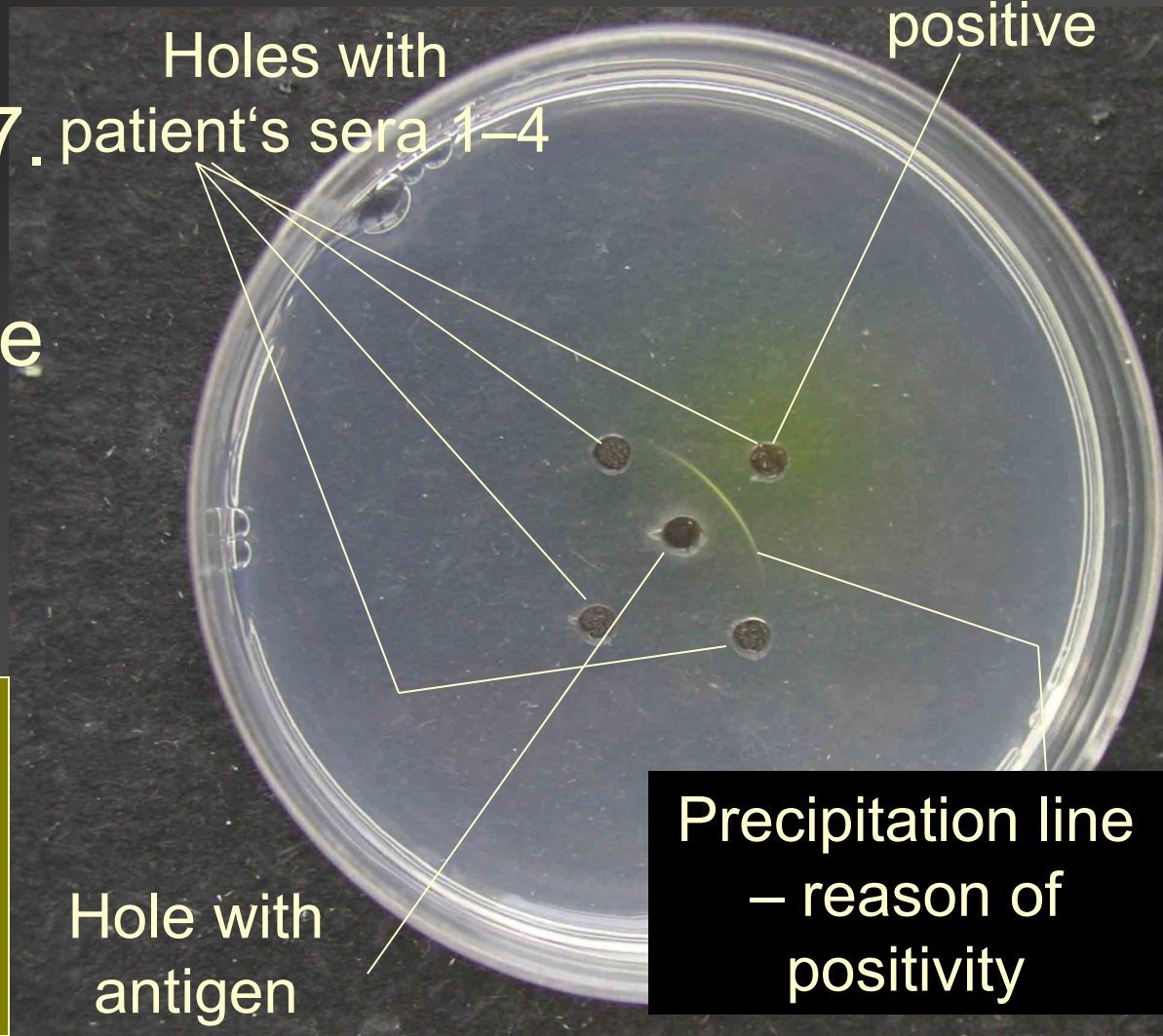
- From the middle hole, antigen diffunds (marked red)
- From the positive hole with serum No. 2 the antibody diffunds (blue)
- From negative holes (sera No. 1, 3, 4) of course nothing diffunds.
- In place of meeting of antigen and antibody, precipitation line is formed (green)



Example of indirect diagnostics of fungi: microprecipitation in agar

The test is a repeating from J 07. Precipitation line is formed between the hole with antigen and the hole with antibody



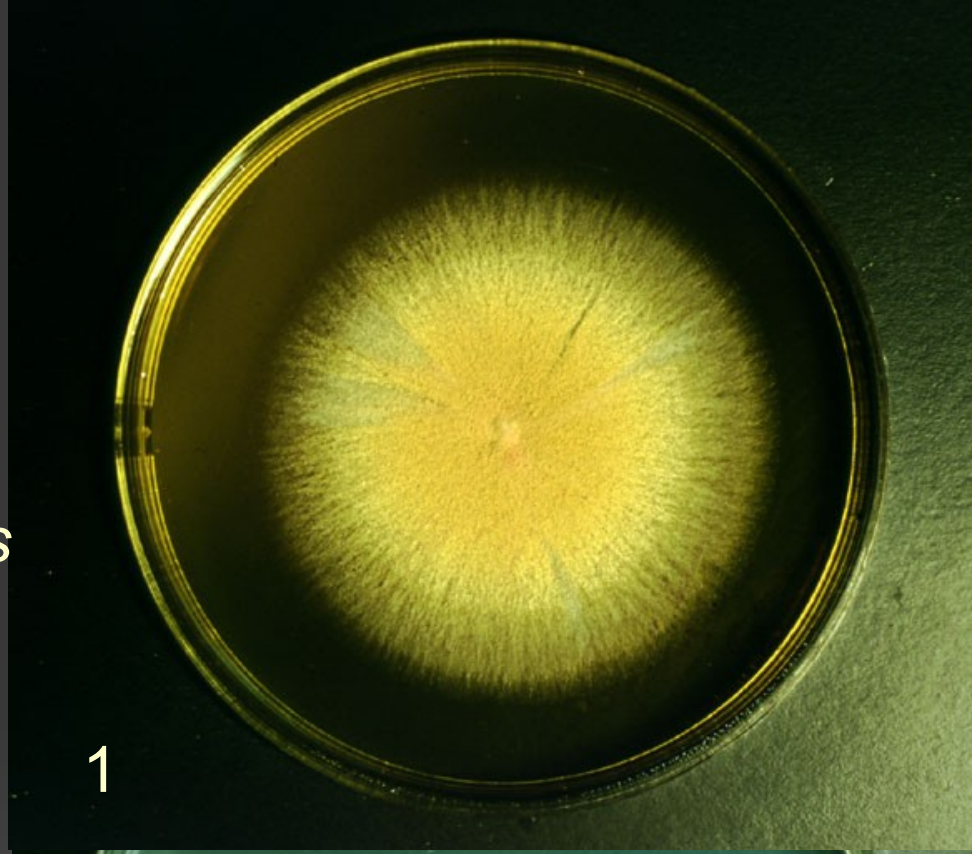


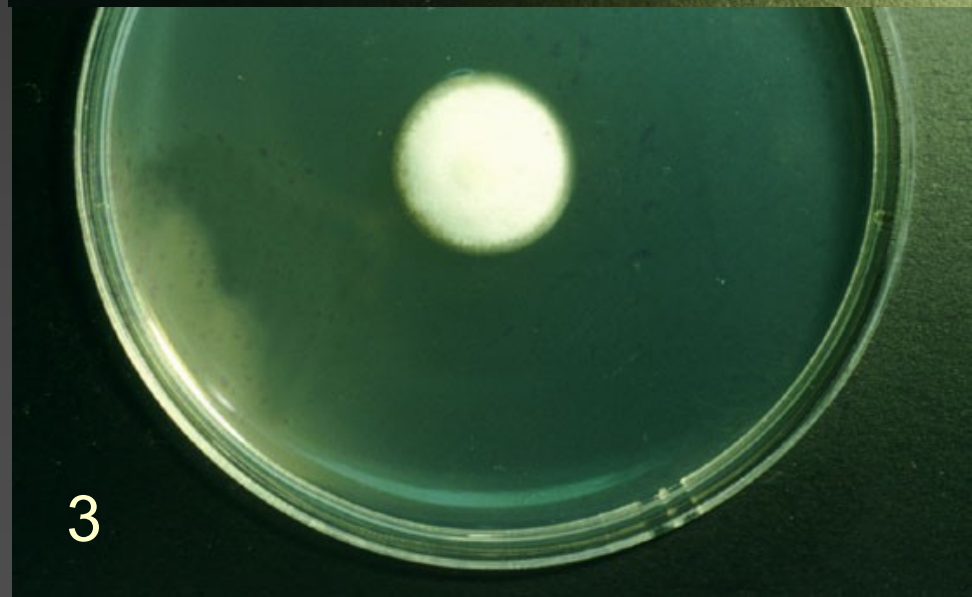
Pictures of fungi

Dermatophytes

3× www.medmicro.info

1 *Epidermophyton floccosum*
2 *Trichophyton rubrum*
3 *Trichophyton mentagrophytes*

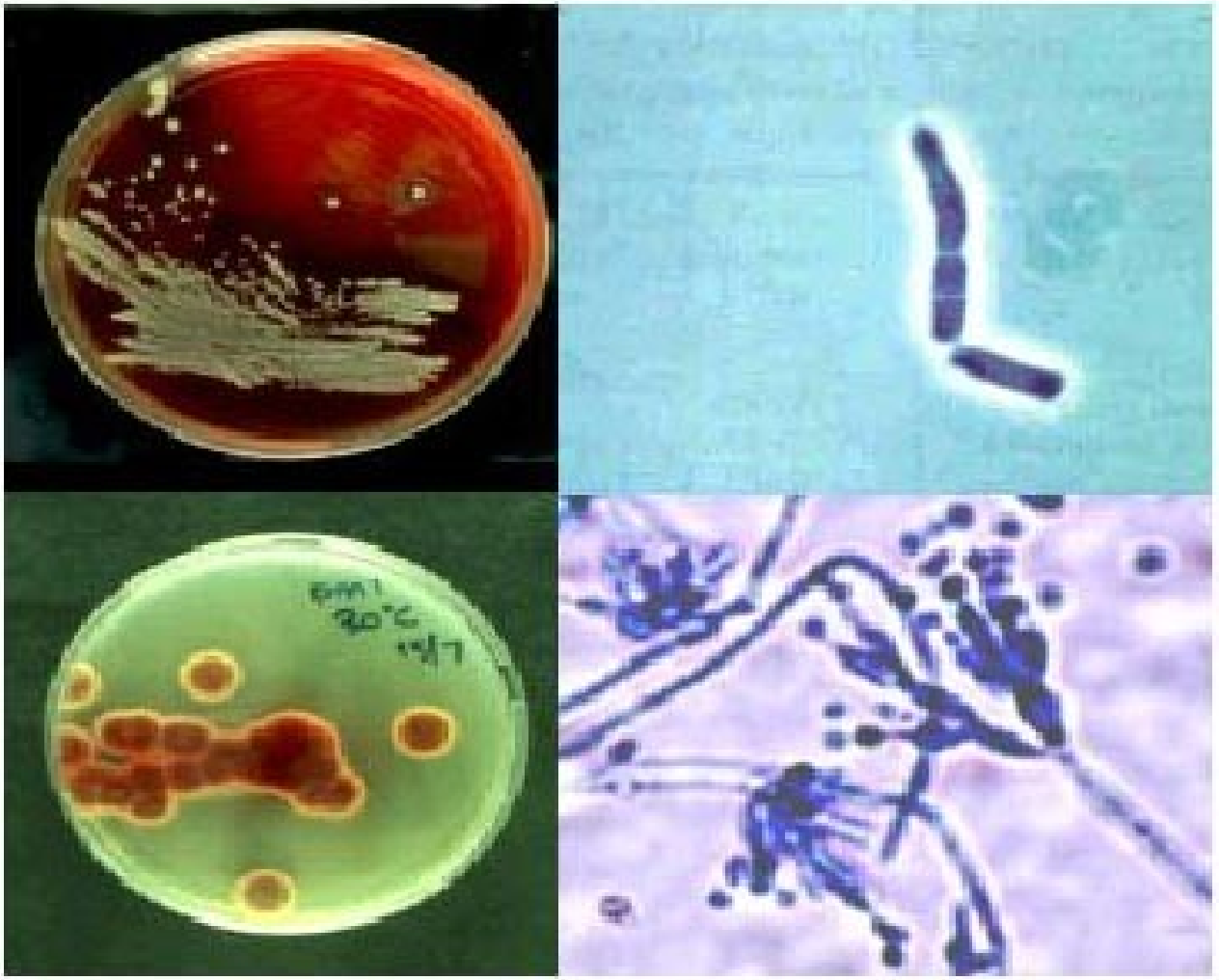




Penicillium marneffei

37°C
BHI blood

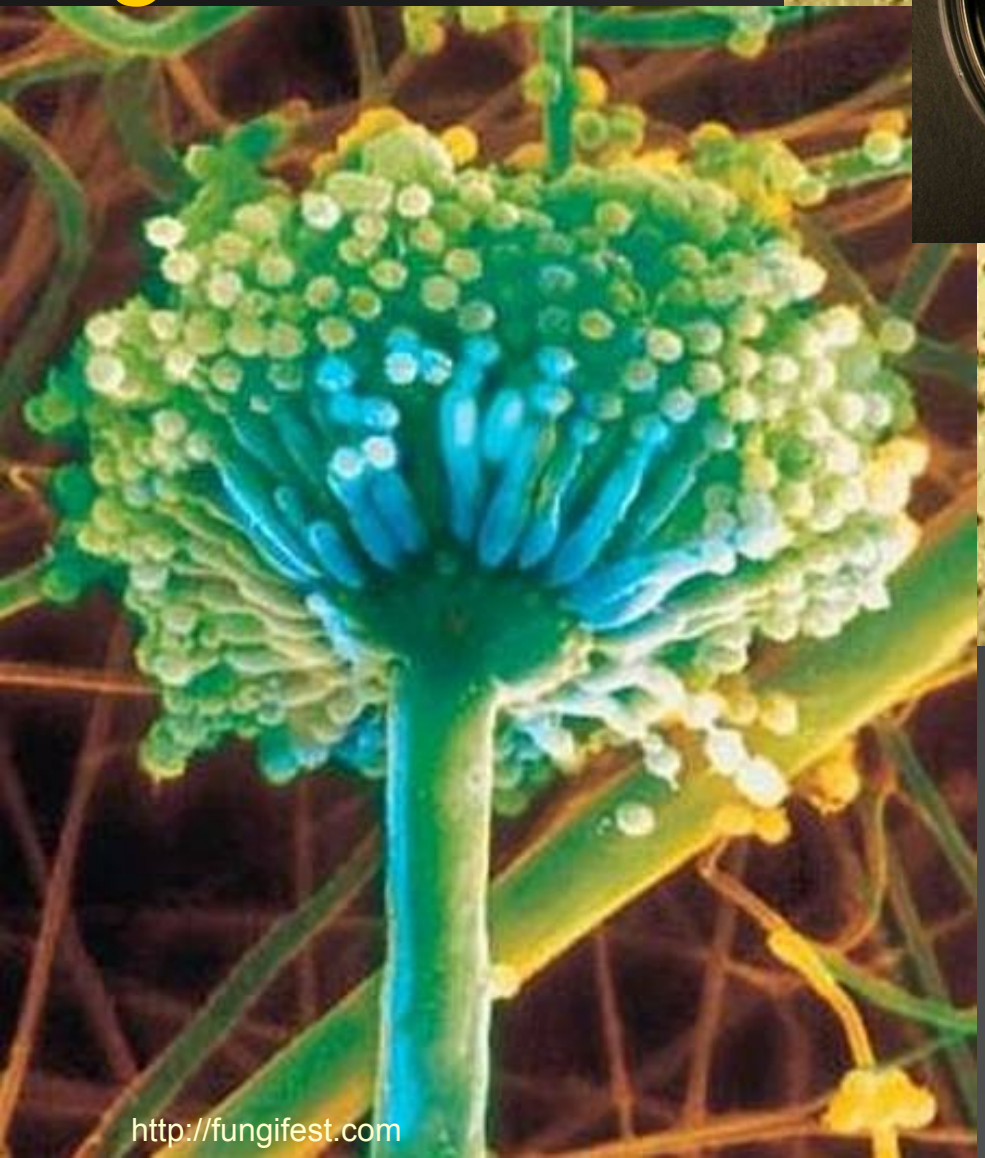
25°C
Sabouraud

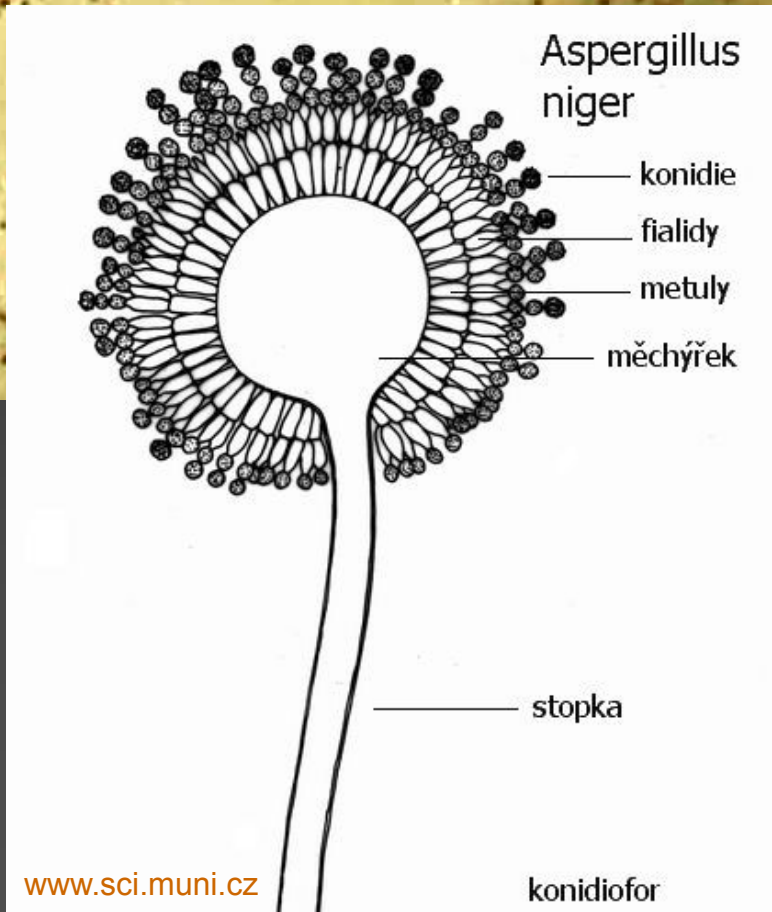


Segretain, IP, 1959: *Penicillium marneffei*

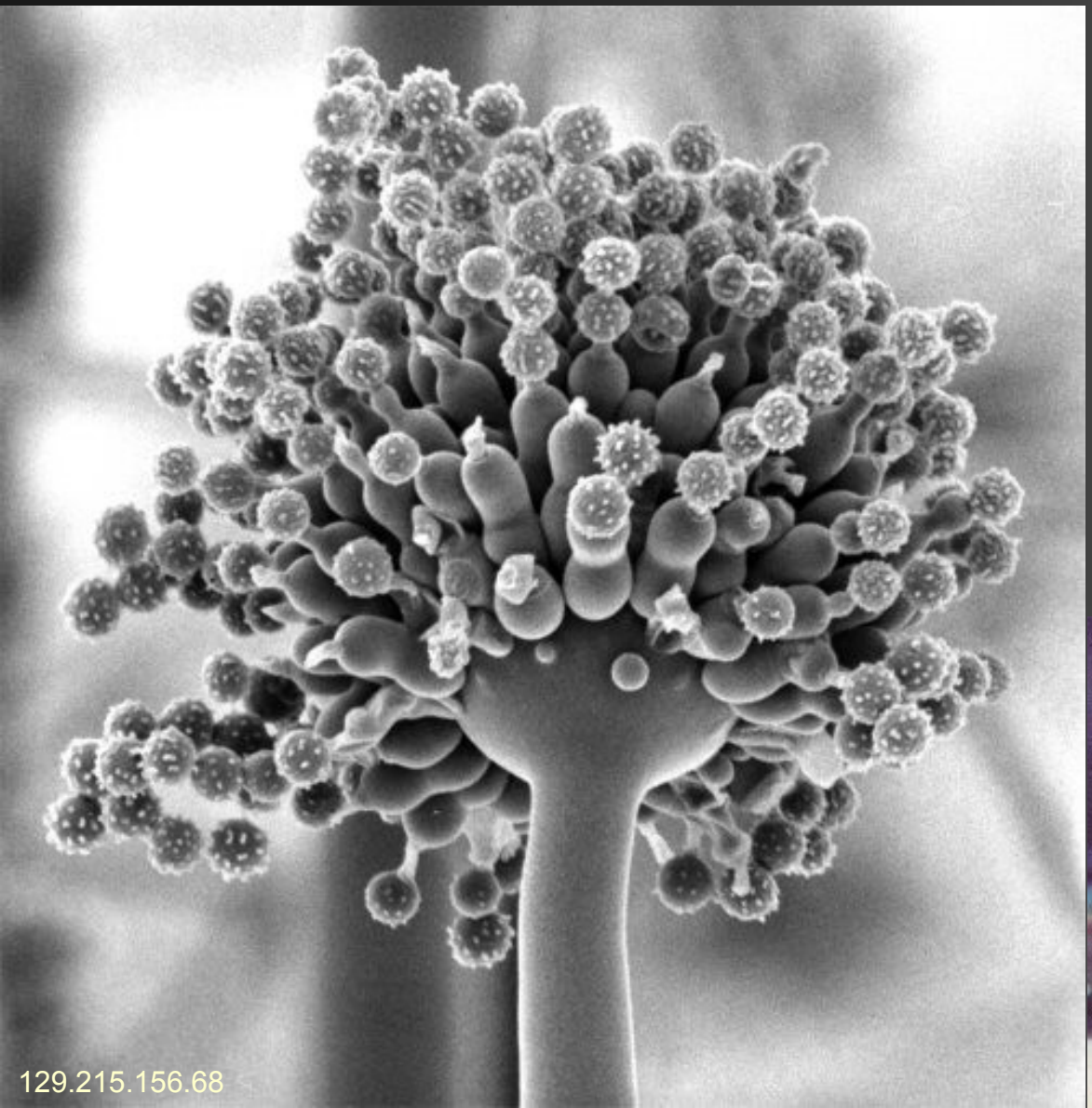
Photo from Prof K Y Yuen

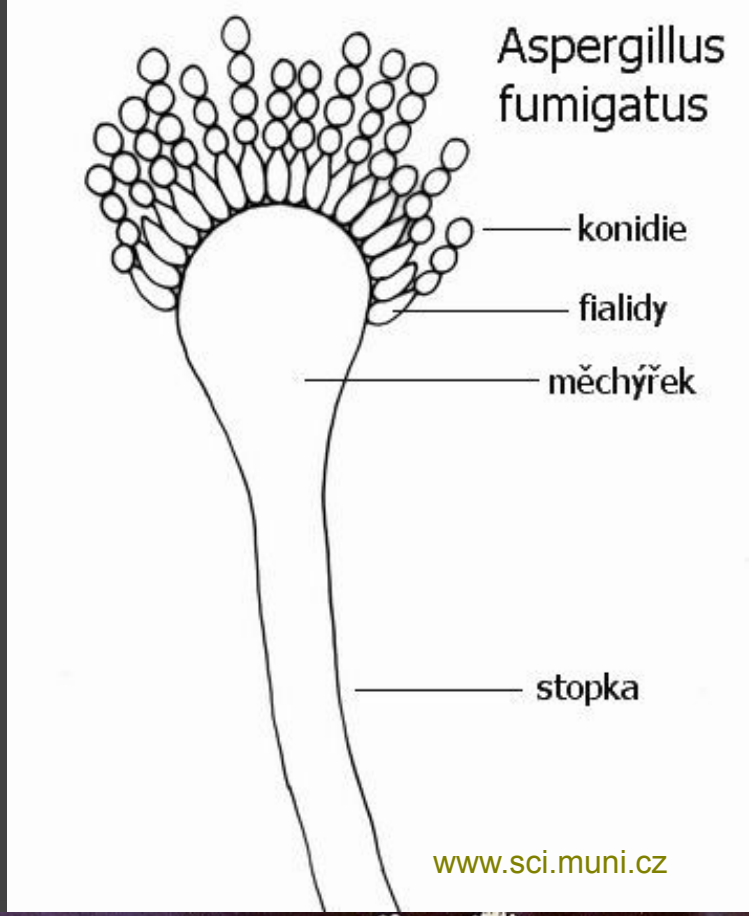
Aspergillus niger



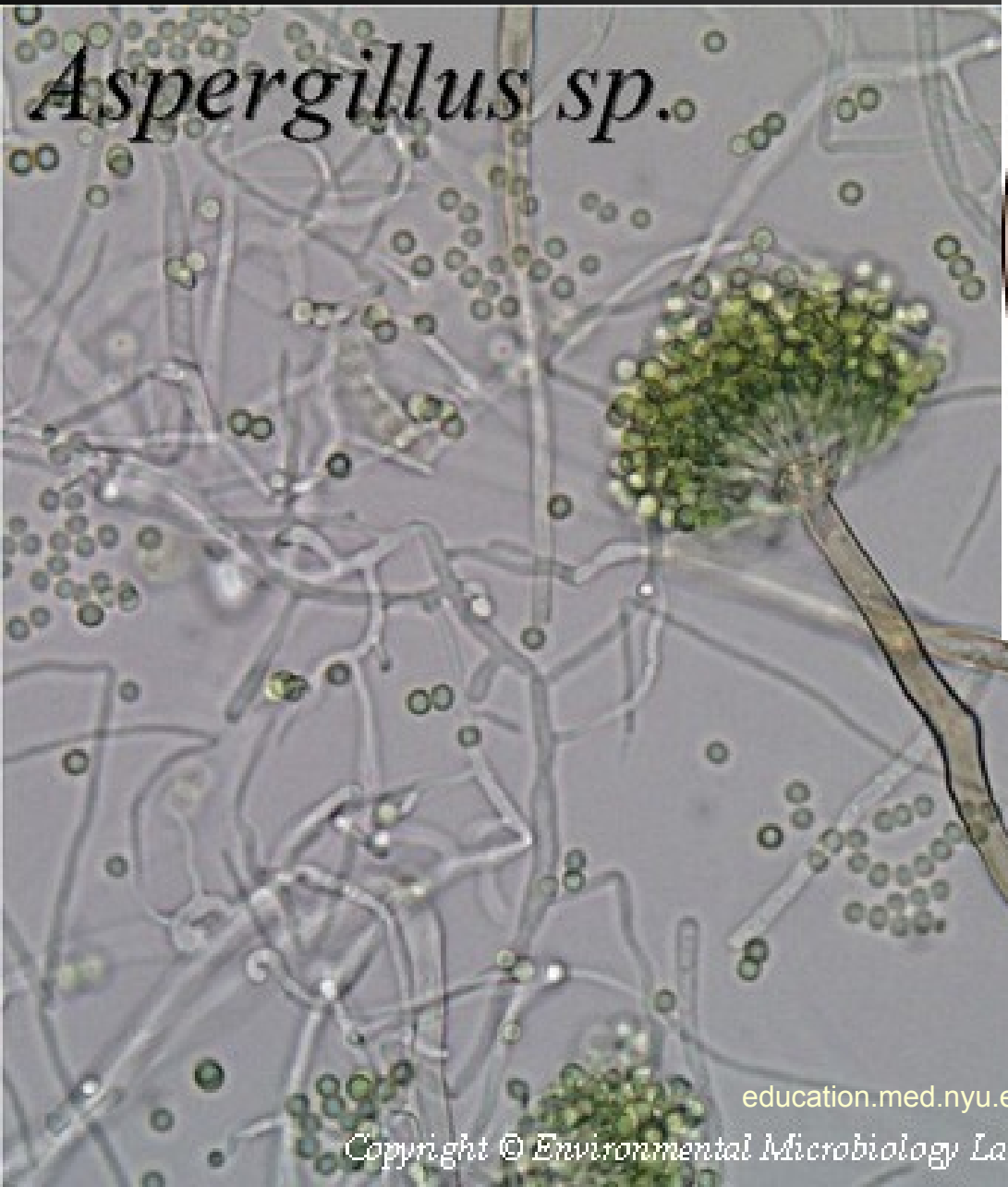


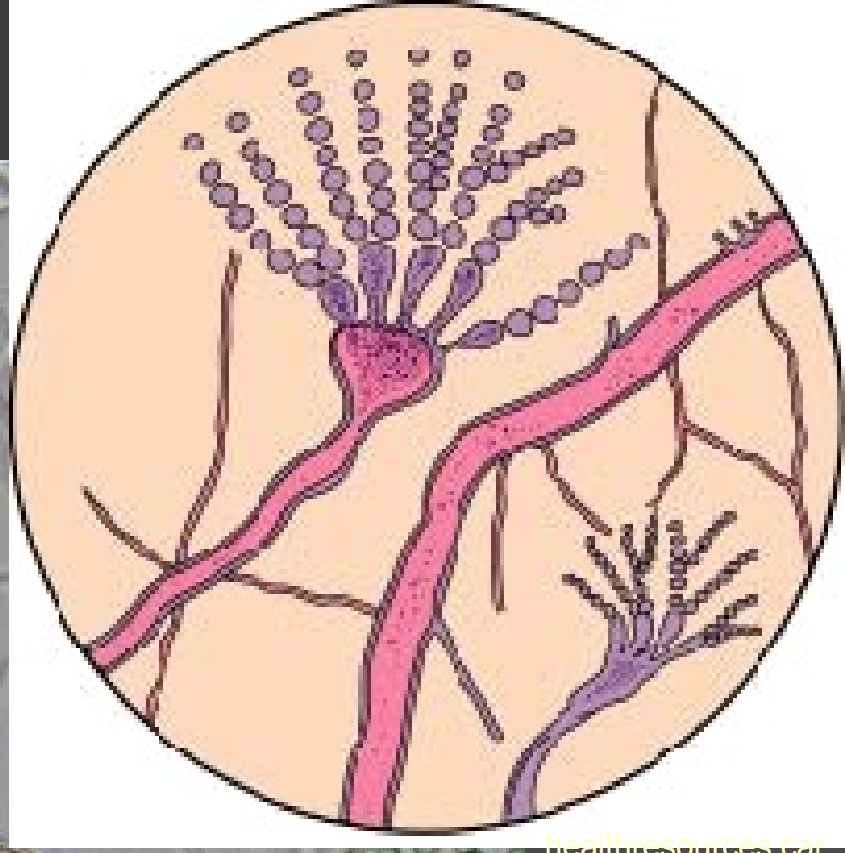
Aspergillus sp.





Aspergillus sp.





healthresources.cardemark.com

Mucor

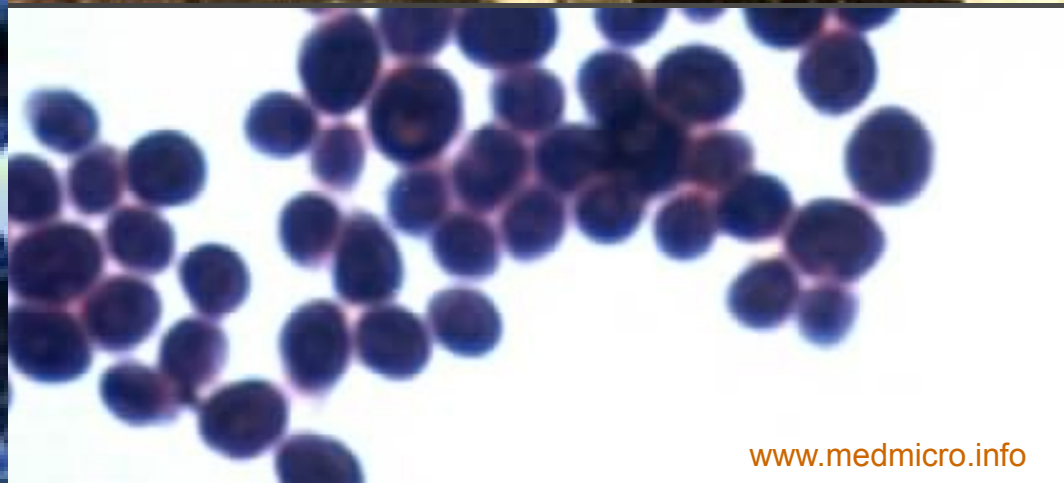
Mucor sp.

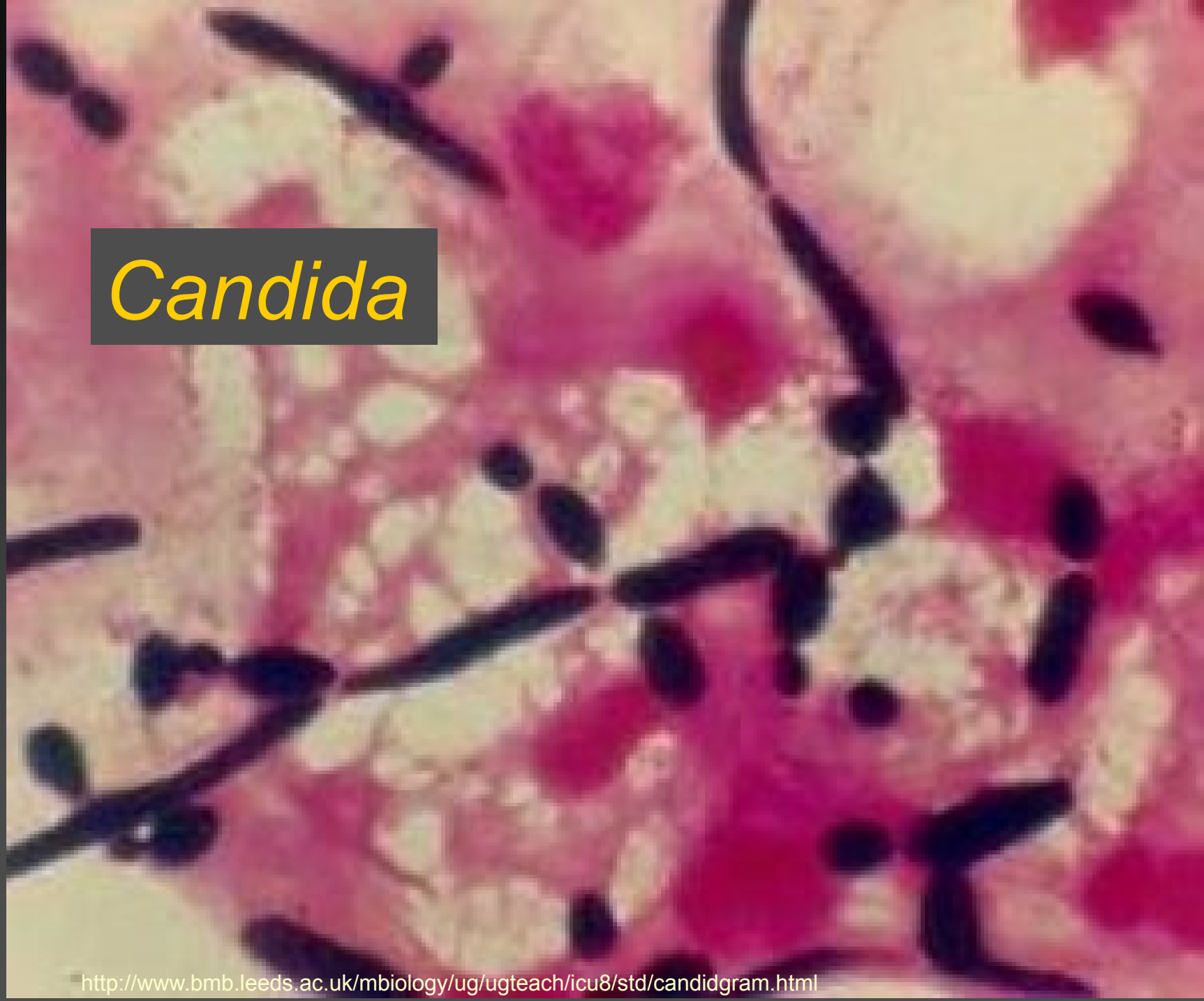
http://www.mycology.adelaide.edu.au/gallery


www.zsdukla.cz/nature/article86.php

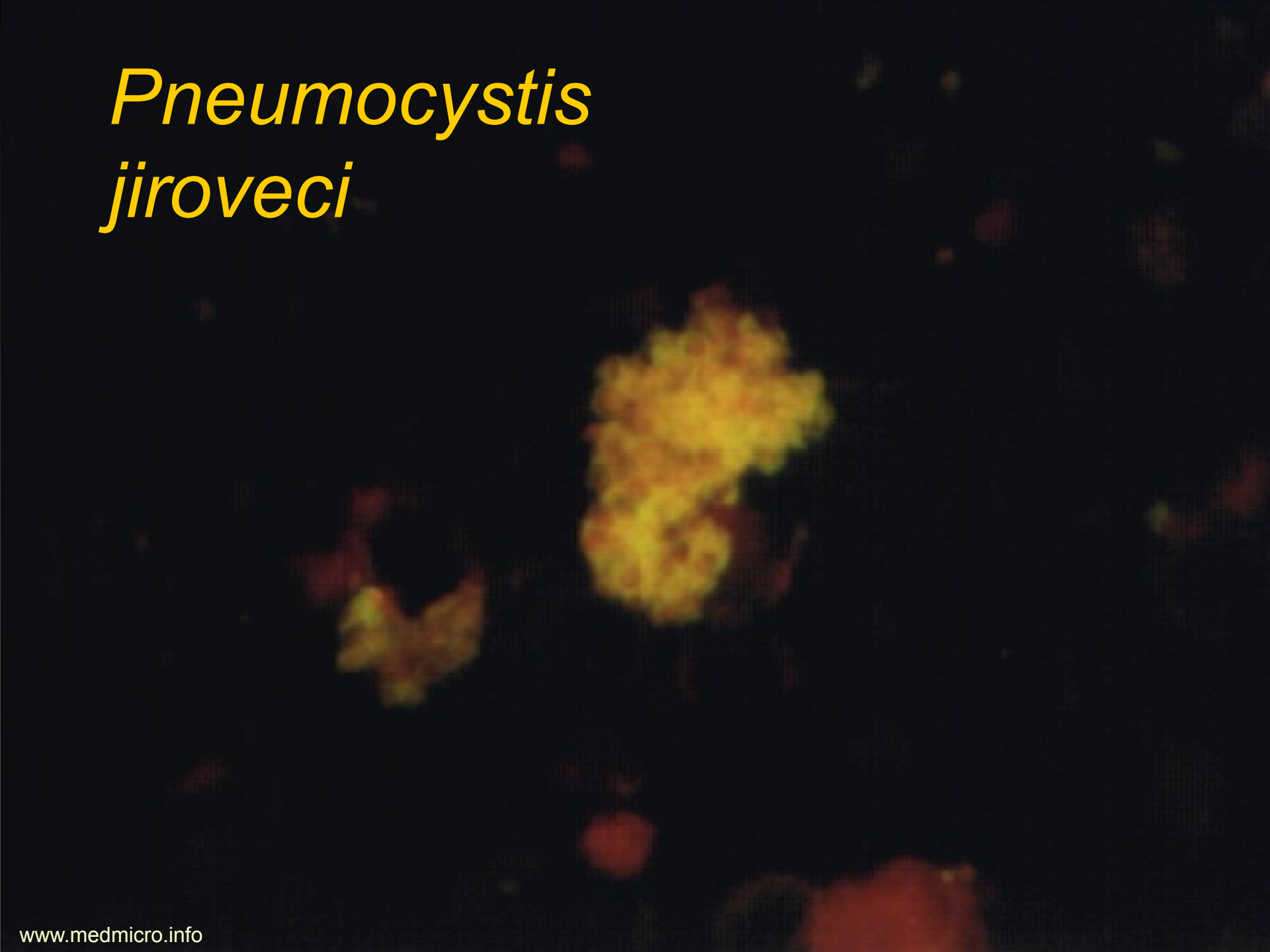
Causative agents: *Candida albicans*

Candida albicans

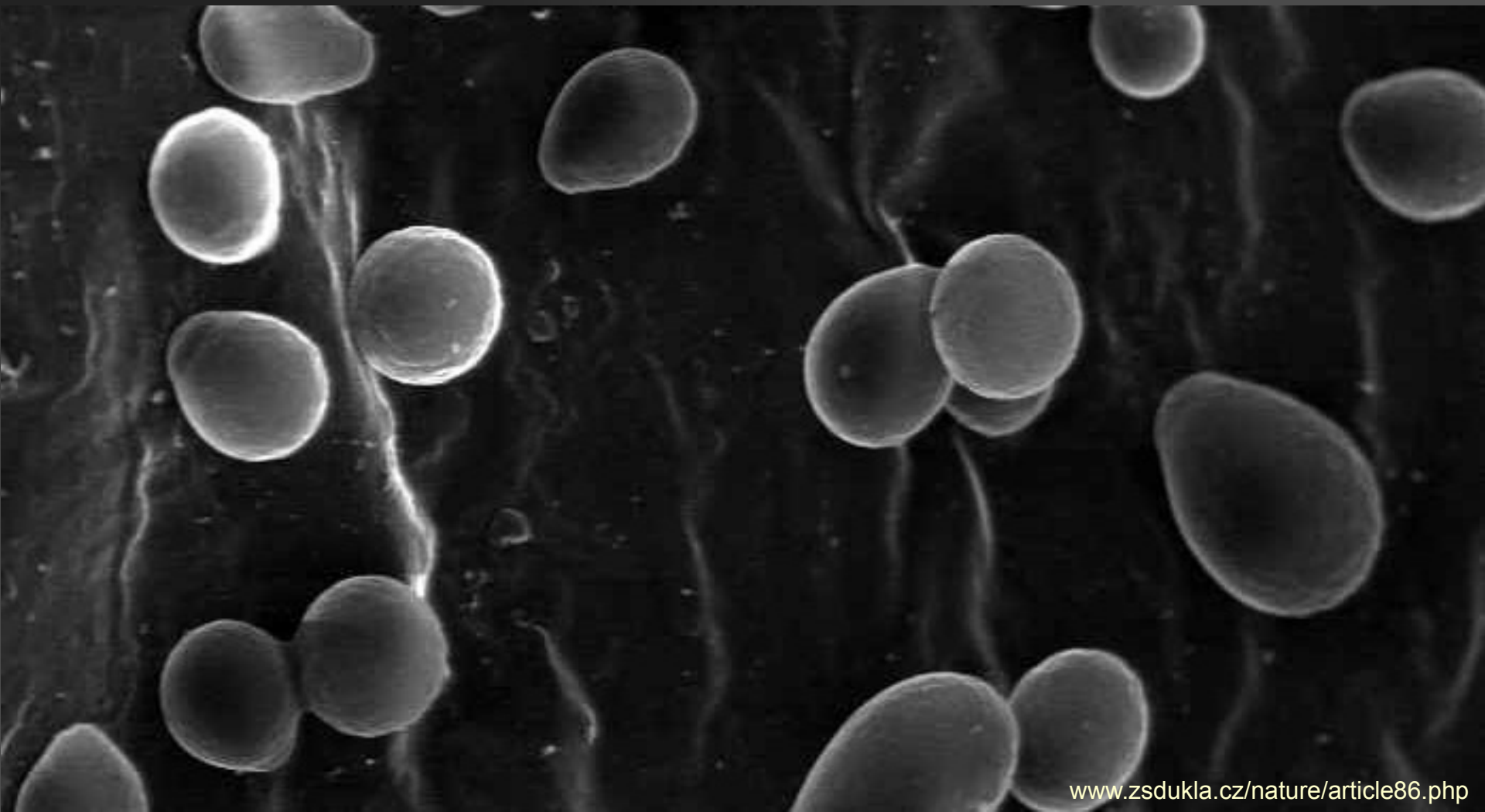




http://www.bmb.leeds.ac.uk/mbiology/ug/ugteach/icu8/std/candidgram.html

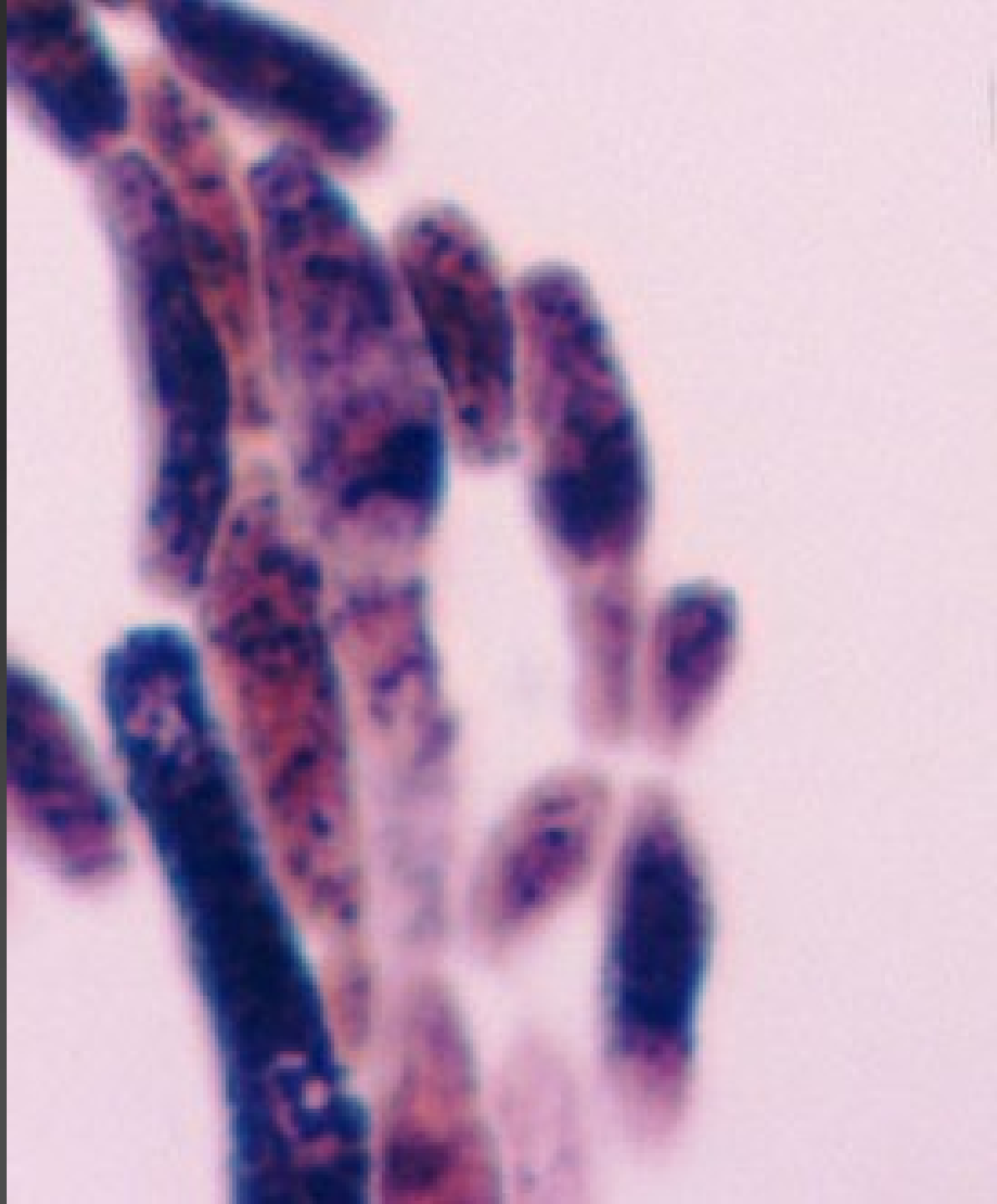


Saccharomyces cerevisiae



www.zsdukla.cz/nature/article86.php

Geotrichum candidum



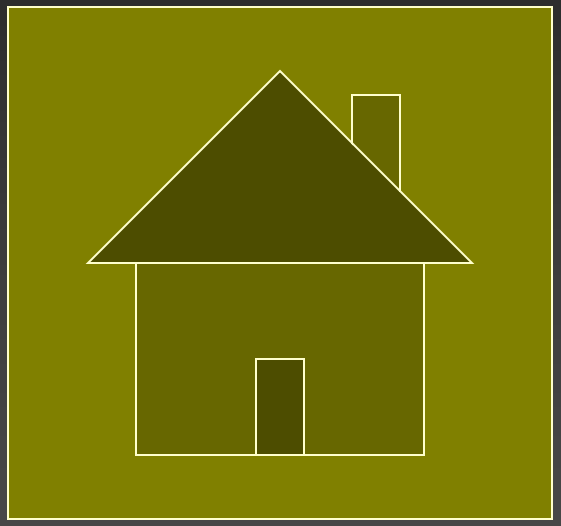
Rhodotorula rubra

Cryptococcus neoformans

http://www.higiene.edu.uy/ciclipa/parasito/Cryptococcus.jpg

http://www.mycology.adelaide.edu.au/gallery

The End



http://www.jiricisar.com/blog/photo/20050824_kremenac.jpg