The Practical Course for Yeast Science

349 MIC

Prepared by:

Bassam Alnafisi

1st	**Introduction to laboratory safety and biosafety**
2nd	**Introduction to Yeast Science**
3d	**Isolation of yeasts from natural environments**
4th	**Culture media used in mycology and yeast laboratories**
5th	**Cultural and microscopic examination, and commonly used stains for yeasts**
6th	**A– Ascomycetous yeasts: 1. *Saccharomyces cerevisiae* (Baker's yeast and fermentation)**
7th	**2. *Schizosaccharomyces pombe***
8th	**3. *Hansenula anomala* (*Pichia anomala*)**
9th	**4. *Candida* spp. (*C. albicans***
10th	**5. *Geotrichum* spp.**
11th	**B– Basidiomycetous yeasts: *Rhodotorula mucilaginosa***
12th	***Cryptococcus neoformans***
13th	***Malassezia* furfur**
14th	***Trichosporon spp.***

Practical Session 1

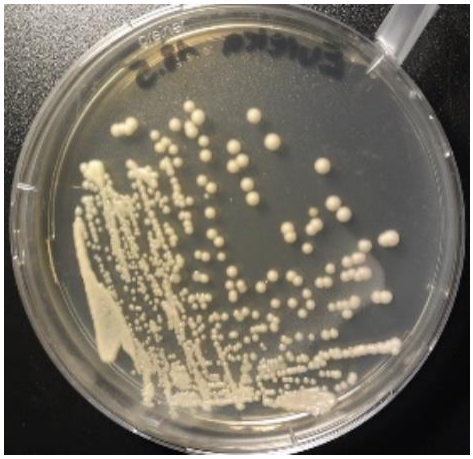
Introduction to Yeast Science

Mycology: It is the science that deals with studying the structure, classification, and reproductive methods of different types of fungi, in addition to their economic and medical importance.

Fungi are classified according to morphology into two types:

1-Molds:

2-yeast:



Fungi are classified according to their medical and pathogenic importance into three forms:

1. **Molds 25C**
2. **Yeasts 37C**
3. **Dimorphic fungi** :In dimorphic fungi, the organism appears in yeast form at 37°C (Infection mode) and in Molds form at 25°C.

In this practical course, we will study the most important experiments in Yeast Science.

Yeast Science (Mycology of Yeasts): It is the science concerned with studying yeasts, their methods of reproduction, and their economic, industrial, and medical importance.

Yeasts are microscopic, unicellular, eukaryotic organisms that do not form true mycelium like molds.

They may appear as chains of cells due to repeated budding and the failure of daughter cells to separate from each other.
Yeast cells range from round to oval, have a distinct cell wall, and contain a nucleus. Most yeasts belong to the Ascomycota (ascomycetous yeasts), and a smaller number belong to the Basidiomycota (basidiomycetous yeasts).

General Characteristics of Yeasts

• Yeasts do not differ greatly in their external morphology and structure, but they show significant differences in physiological and cultural characteristics.
• Humans use yeast in many industries, the most important of which are:
– Bread, beer, vinegar, and alcoholic beverages
– Production of single-cell protein (SCP) used as animal feed and as a meat substitute (e.g., in burger restaurants), resembling red beef
– Production of some vitamins such as B-complex, C, and D (in yeasts exposed to UV light)
– Some species produce large amounts of lipids.
• Yeasts are chemo-organotrophic, using organic compounds as an energy source.
– They obtain carbon mainly from sugars such as glucose, fructose, and maltose.
• Yeast cells require oxygen to carry out aerobic respiration for energy production (facultative aerobes).
– They can also respire anaerobically, but they retain aerobic pathways for energy production.
– Unlike some bacteria, yeasts cannot grow under strictly anaerobic (obligate anaerobic) conditions.
• Yeasts prefer to grow in acidic environments with low pH.

• The optimal growth temperature varies among species, but most grow well between 28°C and 37°C.

• Yeasts are widespread in nature but less abundant than bacteria.

– They can be isolated from sugar-rich materials such as nectar, fruits (grapes, apples), soil, and insects.

Yeast Reproduction

• Yeasts reproduce asexually, and their reproduction is rapid, especially in sugar-containing media.

• The most common method is budding, where a new bud (daughter cell) forms from the parent cell.

– A portion of the cell wall swells to form the bud.

– The bud eventually separates to form a new cell.

• A small number of yeasts reproduce asexually by binary fission (e.g., *Schizosaccharomyces*).

• Some species form endospores (chlamydospores) under harsh conditions, e.g., *Candida albicans*

• Some yeasts do not form spores, such as *sacharomyces* spp.

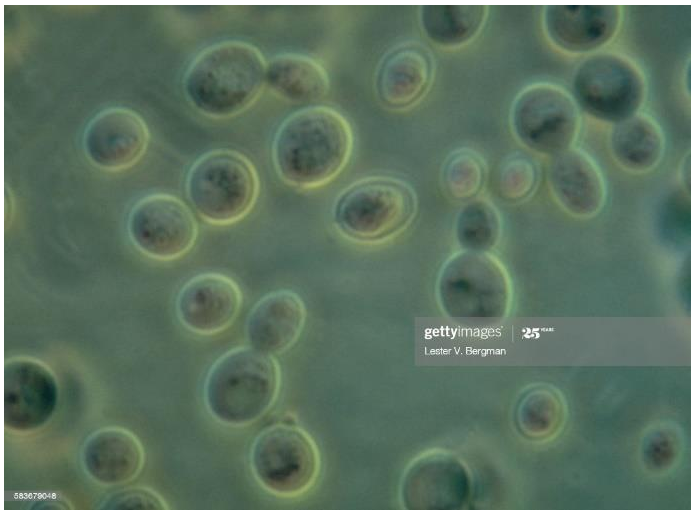
• Sexual reproduction is uncommon in yeasts.

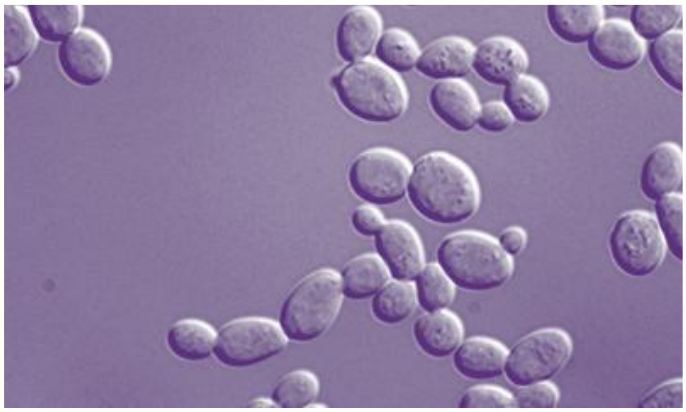
– It involves fusion of the nuclei of two cells to form a diploid nucleus,

– which then divides three times to form eight nuclei,

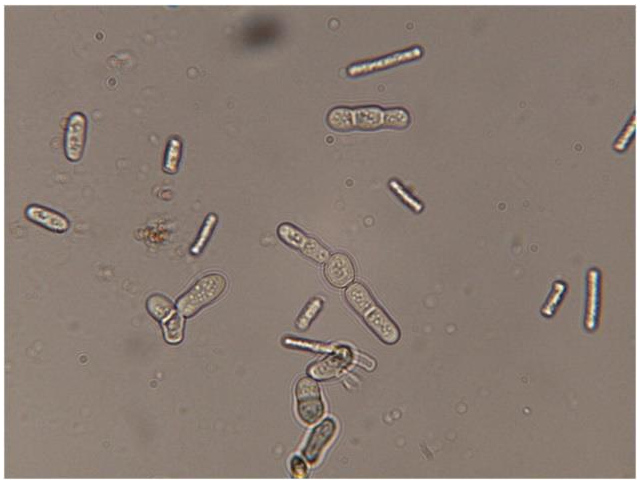
– producing an ascus containing eight ascospores.

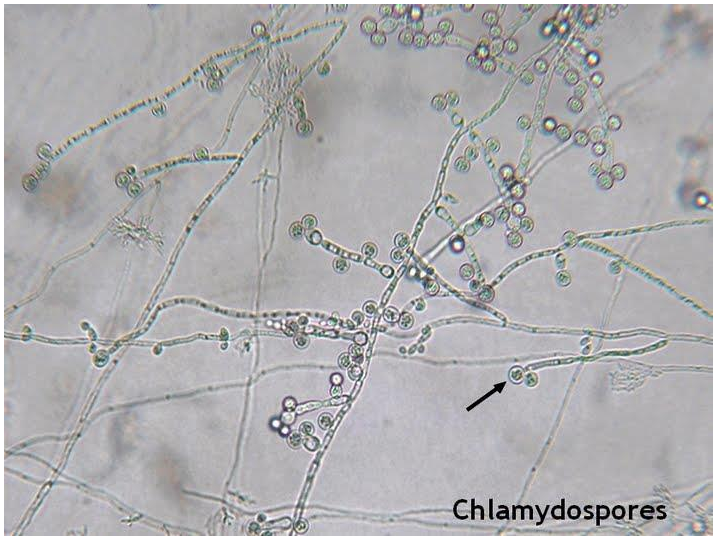
1)Budding yeast

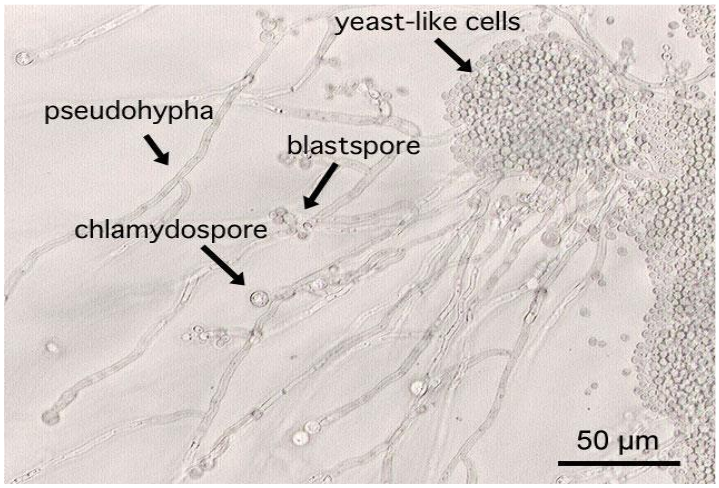


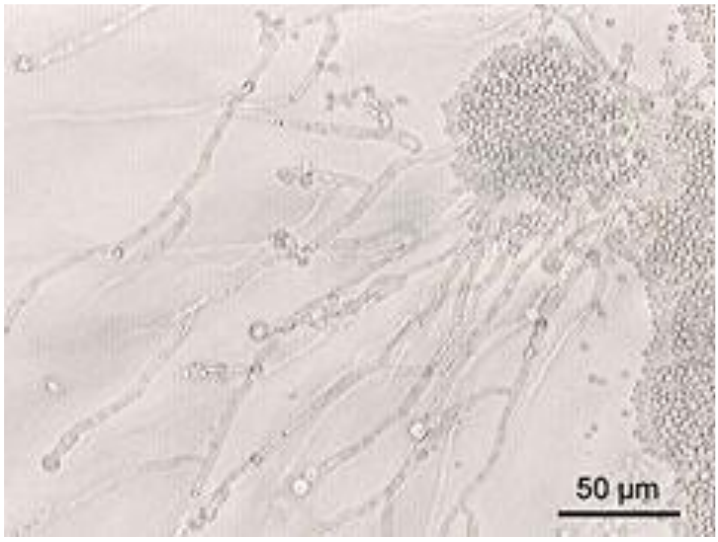


2) Binary fission in *schizosaccharomyces pombe*









3) Chlamydospores and Blastospores and pseudo hyphae in candida albicans

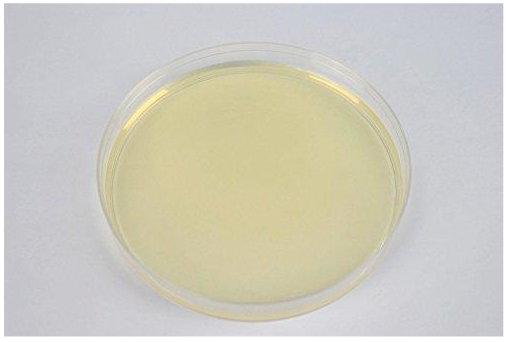
Practical session 2

Media Used in Fungi and Yeast Laboratories

Media Used for Yeasts:

1. Potato Dextrose Agar (PDA)

A general medium for cultivating both pathogenic and non-pathogenic yeasts.
Rose Bengal is often added to inhibit bacterial growth.



2. Malt Extract Agar (MEA)

A general medium for cultivating pathogenic and non-pathogenic yeasts.
It is also used for isolating fungi responsible for food spoilage.

3**. Sabouraud Dextrose Agar (SDA)**

A selective acidic medium used specifically for isolating pathogenic Fungi, which prefer acidic conditions.

4. Mycological Media Slant Agar

A specialized medium used for isolating molds from clinical specimens.
Prepared in slanted agar tubes.

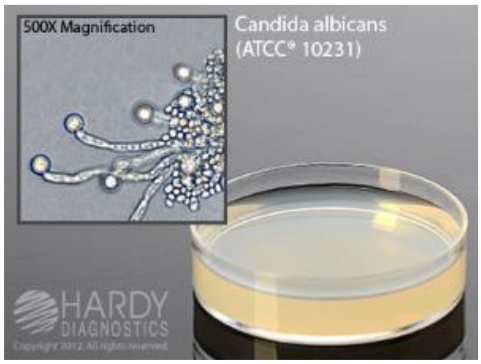
5. Czapek–Dox Medium

A general fungal medium used for isolating fungi that utilize sodium nitrate as a nitrogen source.

6. Corn Meal Agar (CMA)

A special medium used for identifying pathogenic yeasts that produce:

- Arthroconidia
- Chlamydospores
- Pseudohyphae



7. Bird Seed Agar (Niger Seed Agar)

A differential medium for cultivating *Cryptococcus neoformans*.

• Positive result: Creamy, dark-brown to black colonies.

Cultural Characteristics of Yeasts

Yeasts appear on culture plates as colonies that are typically:

- White and creamy
- White and dry
- Creamy pink
- Creamy red or orange
- Some produce pseudohyphae, resembling mold-like growth

Practical Session 3

Isolation of Yeasts from Natural Environments

Experiment Title: Isolation of yeasts from natural sources

Objective of the Experiment:

To isolate yeasts, culture them on selective media, and study their microscopic and cultural characteristics.

Materials:

- Soil sample
- Fermented barley sample
- Sugary solutions from fruits
- Nasal swab sample
- Oral swab sample
- Sterile cotton swabs
- Potato Dextrose Agar (PDA) medium
- Sterile Petri dishes
- Inoculating loop (wire loop)

Procedure:

1. Prepare the PDA medium and sterilize it in the autoclave at 121°C for 20 minutes.

2. After sterilization, pour the medium aseptically into Petri dishes and allow it to solidify.

3. Once the medium has solidified, proceed with inoculation:

A. Soil Sample

1. Place the soil in sterile tap water.
2. Weigh 1 g of soil, transfer it into a 100 mL flask, shake well.

3. Allow it to sit for 5–10 minutes.
4. Transfer 1 mL of the supernatant onto the surface of the PDA plate.
5. Perform three- or four-quadrant streaking.

B. Fermented Barley Sample

- Place 1 mL of the sample onto the PDA plate surface.
- Perform three- or four-quadrant streaking using an inoculating loop.

C. Nasal Swab Sample

- Swab the surface of the PDA plate with the cotton swab.
- Inoculate the center of the plate by pressing the swab.
- Perform three- or four-quadrant streaking using the inoculating loop.

D. Oral Swab Sample

- Swab the surface of the PDA plate with the cotton swab.
- Inoculate the center of the plate.
- Perform three- or four-quadrant streaking using the inoculating loop.

E. Sugary Fruit Solution Sample

- Place 1 mL of the sample onto the PDA plate.
- Perform three- or four-quadrant streaking using the inoculating loop.

4. Incubate the plates inverted:At 37°C for 48 hours, orAt room temperature for 3 days and record all observations.

Results:

Yeast colonies appear on the plates.
Afterward, the colonies are subcultured onto Sabouraud Dextrose Agar (SDA), especially those isolated from the mouth and nose.

For studying spores and pseudohyphae, the isolates are then cultured on specialized media such as: *e.g., Corn Meal Agar with Tween 80.)*

Practical Session 4

Microscopic Examination and Preferred Stains for Yeast Observation

• Preliminary examination of yeasts

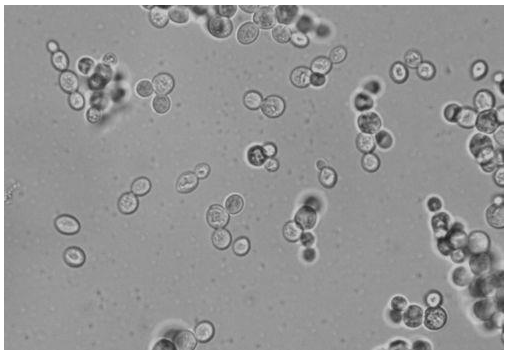
Performed using the wet mount (wet prep slide) technique.

• Preferred stains for microscopic examination of yeasts:

- Safranin and Methylene Blue are commonly used for staining most yeasts.
- For *Cryptococcus neoformans*, the preferred stain is India Ink, used to visualize the capsule.
- When stained with Gram stain, yeasts usually appear Gram-positive (purple) due to their thick cell wall.

• Microscopic examination

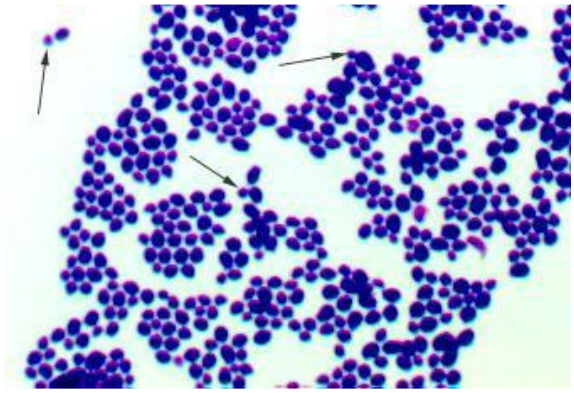
Yeasts are typically examined under the microscope using 40x – 60x objective lenses.



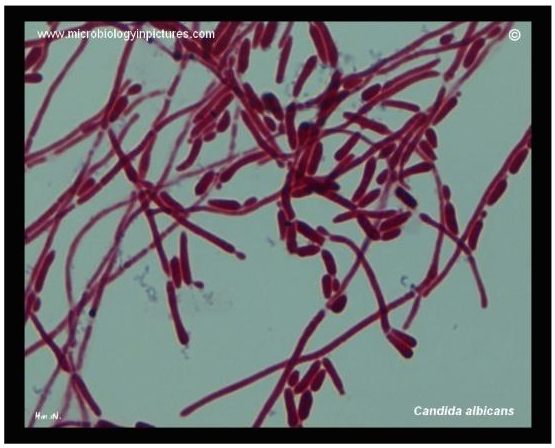
Wet prep slide of yeast

Safranin

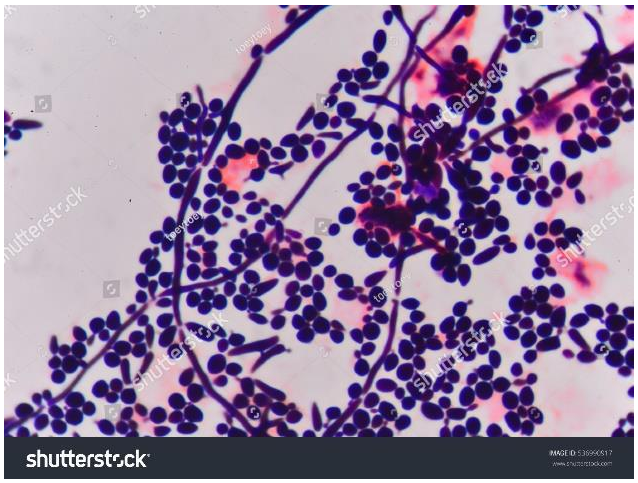
Methylene blue



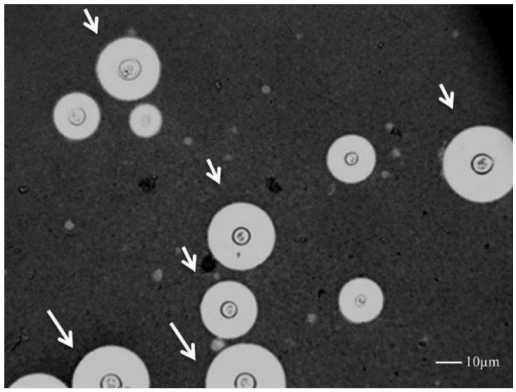
Blue methylene yeast



Safranin yeast



Gram positive yeast



Indian ink capsulated yeast

Practical session 5

A.Ascomycota

Saccharomyces cerevisiae

It is a true yeast belonging to the Ascomycota (Ascomycetous fungi), and it is one of the most important genera in the *Saccharomycetaceae family*. It has the ability to ferment sugars and is used in the production of baked goods. It converts simple sugars into alcohol with a concentration of 10–20% and contains vitamin A.

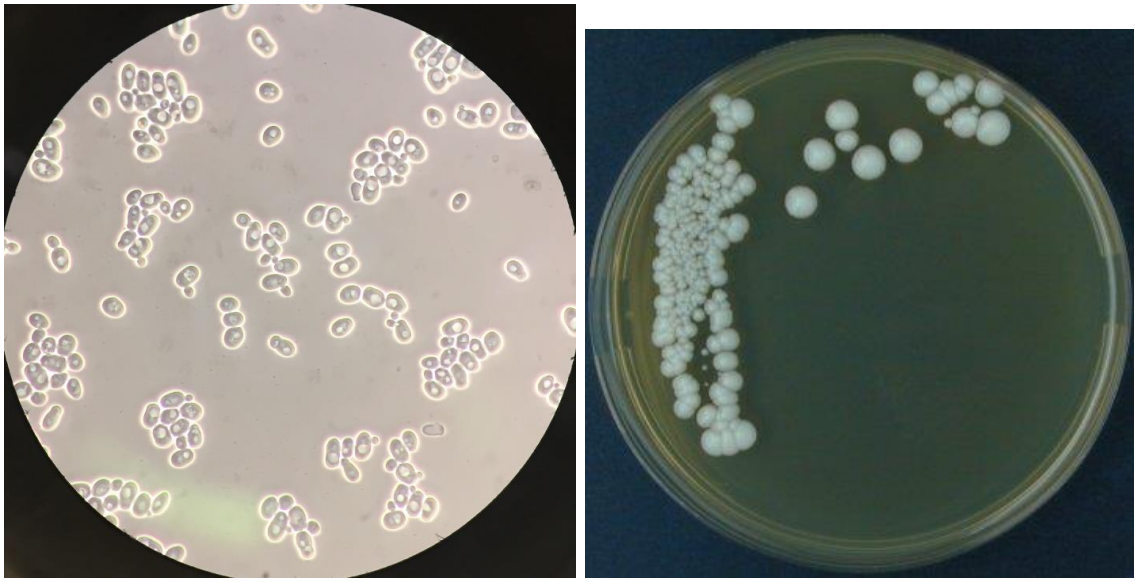
This yeast is found in flower nectar, tree exudates, leaf surfaces, fruits, bread, and soil. Microscopically, it appears as single cells, and on agar plates it forms white, creamy or pasty colonies. It produces ascospores and reproduces asexually by budding.

It is used in the production of bread and alcoholic beverages. It has no medical significance, but it has caused pathogenic effects when used as a probiotic, leading to fungemia (bloodstream infection).

Scientific Classification (Taxonomy)

For the yeast described (this is Saccharomyces cerevisiae):

Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Saccharomycotina
Class: Saccharomycetes
Order: Saccharomycetales
Family: Saccharomycetaceae
Genus: Saccharomyces
Species: Saccharomyces cerevisiae



Saccharomyces cerevisiae under microscope and culture morphology

Fermentation

is a chemical process in which a sugar compound is broken down by enzymes to obtain energy, producing ethyl alcohol and carbon dioxide as the main end products. Fermentation is considered a form of anaerobic respiration.

• The main sugars fermented by yeasts

Yeasts primarily ferment:

- Simple sugars: glucose and fructose
- Disaccharides: sucrose

• Importance of yeast fermentation

Fermentation by yeast plays a major role in the production of:

- Bread
- Alcoholic beverages

Fermentation in Bread & Mechanism of Bread Fermentation

When bread ingredients are mixed, yeast becomes active.
The fermentation process begins with the **hydrolysis of starch** in the flour into the disaccharide maltose by enzymes naturally present in the flour.

Then:

1. **Maltase enzyme** secreted by yeast converts **maltose → glucose**.
2. **Zymase enzyme** secreted by yeast breaks down **glucose**, producing:
    - **Carbon dioxide (CO_2)**
    - **Ethanol (ethyl alcohol)**

To increase the speed of fermentation, **sugar** is added to the flour.
The yeast enzyme **invertase** converts sucrose into:

- Glucose
- Fructose

These simple sugars are then converted by **zymase** into CO_2 and ethanol.

- **Carbon dioxide** inflates the dough, giving bread its rise.
- **Ethanol** evaporates during baking.
- Yeast cells **die** from exposure to the high temperature in the oven.

Laboratory Detection of Fermentation

To test fermentation in the laboratory:

A mixture is prepared containing:

- Yeast
- Glucose or sucrose
- Warm water (30°C–45°C)

The mixture is kept under **anaerobic conditions** (absence of oxygen) and allowed to stand for a period to permit fermentation to occur.

Baking Yeast Powder (Baker's Yeast)

It is a ready-to-use form of baker's yeast containing **Saccharomyces cerevisiae**.
When warm water is added and left for about **15 minutes**, the yeast becomes activated.
Then flour (which contains the protein **gluten**, as well as ammonium and phosphate salts that support yeast growth) and sugar are added so that the yeast can ferment the sugar and produce **gas and alcohol**.

• Baking Powder

Baking powder is a mixture of **chemical ingredients** that produce gas when mixed with dough.
It typically contains:

- **Acidic potassium tartrate (cream of tartar)**
- **Sodium bicarbonate (baking soda)**

When moisture is added, these components react and release **carbon dioxide gas**, which causes the dough to rise.

• Invertase Enzyme

This enzyme is secreted by **Saccharomyces cerevisiae**, and it breaks down **sucrose** into:

- **Glucose**

- **Fructose**

Invertase is used in:

- The confectionery (sweets) industry
- Production of **artificial honey**
- Making **invert sugar** (a sweeter, more soluble form of sugar)

Yeast Fermentation & CO_2 Production Table

Yeast	Fermentation Ability	CO_2 Production
Saccharomyces cerevisiae	Yes – strong fermenter (glucose, fructose, maltose, sucrose)	Yes – produces CO_2 (used in bread rising, alcohol production)
Schizosaccharomyces pombe	Yes – ferments sugars (including sucrose)	Yes – produces CO_2, but less foam than Saccharomyces
Hansenula (Wickerhamomyces anomalus / Pichia anomala)	Weak / limited fermentation	Low CO_2 production
Rhodotorula mucilaginosa	No fermentation (non-fermentative yeast)	Does NOT produce CO_2
Candida albicans	Weak fermenter (ferments glucose only)	Low CO_2 production
Candida tropicalis	Ferments glucose	Produces small amount of CO_2
Candida krusei	Poor fermenter (limited sugars)	Very low CO_2
Candida glabrata	Weak fermenter	Minimal or no CO_2
Geotrichum candidum	Non-fermentative	No CO_2 production
Cryptococcus neoformans	Non-fermentative	No CO_2 production
Malassezia furfur	Non-fermentative (lipid-dependent)	No CO_2 production
Trichosporon spp.	Non-fermentative	No CO_2 production

Prcatical 5

Schizosaccharomyces pombe

Schizosaccharomyces pombe is a yeast belonging to the Ascomycota (Ascomycetous fungi).
It reproduces asexually only by binary fission (not by budding).

It is known as “bottom-fermenting yeast” because it settles at the bottom of wooden fermentation vats used for fermenting fruits.
Its first isolation occurred from the bottom of a fermented fruit vat, which is why it is sometimes called “wine yeast.”

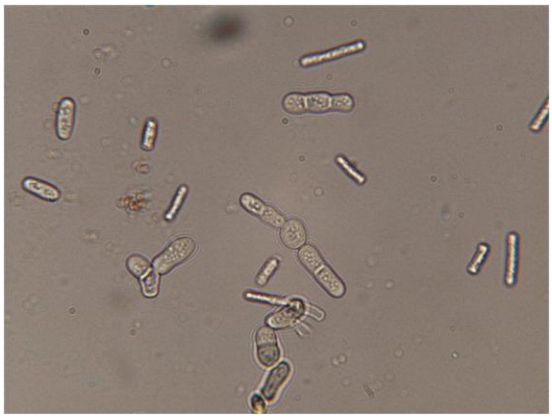
It has also been isolated from:

- Sugar molasses
- Grape juice

It is capable of fermenting sucrose.

Schizosaccharomyces pombe is widely used in biomedical research, especially in studying:

- Cell cycle regulation
- DNA repair
- Cellular aging
- Mechanisms of antimicrobial resistance in yeasts



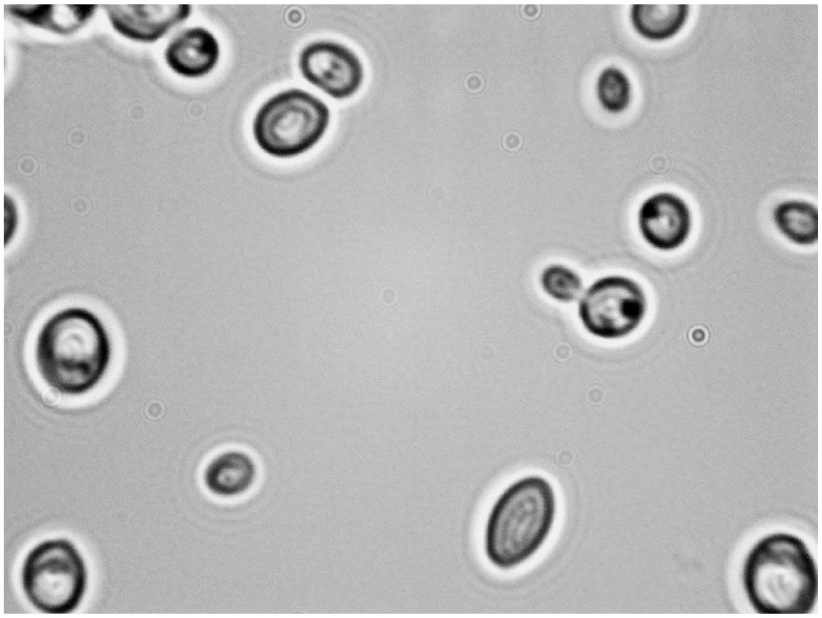
Taxonomic Classification of Schizosaccharomyces pombe

Rank	***Classification***
Kingdom	*Fungi*
Phylum	*Ascomycota*
Subphylum	*Taphrinomycotina*
Class	*Schizosaccharomycetes*
Order	*Schizosaccharomycetales*
Family	*Schizosaccharomycetaceae*
Genus	*Schizosaccharomyces*
Species	*Schizosaccharomyces pombe*

Prcatical session 6

Pichia anomala (Hansenula anomala)

(= Wickerhamomyces anomalus)



Pichia anomala (formerly known as *Hansenula anomala*) is a yeast belonging to the Ascomycota.
It reproduces asexually by budding.

It is used in the production of alcoholic beverages, but it can also cause faults in wine, producing a characteristic ester-like flavor and aroma.
On culture plates it forms creamy colonies.

This yeast is saprophytic in the external environment and is commonly found in:

- Milk
- Cheese
- Yogurt

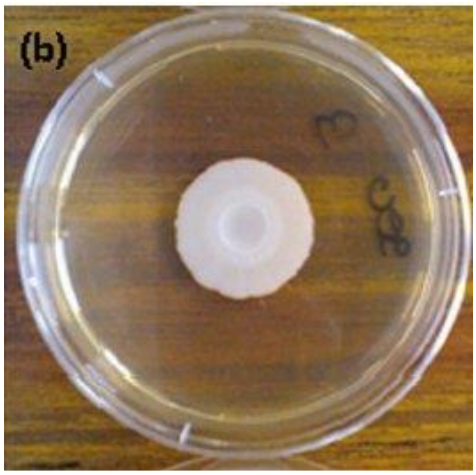
It is generally considered non-pathogenic, but it has been associated with hospital outbreaks (nosocomial infections) in newborn infants, causing:

- Fungemia (bloodstream infection)

- Pulmonary dysfunction
- Myocarditis
- Enteritis (intestinal inflammation)

Taxonomic Classification

(Current accepted classification for *Wickerhamomyces anomalus*)



Rank	Classification
Kingdom	Fungi
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Order	Saccharomycetales
Family	Pichiaceae
Genus	*Wickerhamomyces*
Species	*Wickerhamomyces anomalus* (synonyms: *Pichia anomala*, *Hansenula anomala*)

Geotrichum spp.

Are species are Ascomycetous fungi (phylum Ascomycota).
They are commonly found in water, air, soil, and sewage.
They also naturally occur in feces and sputum.

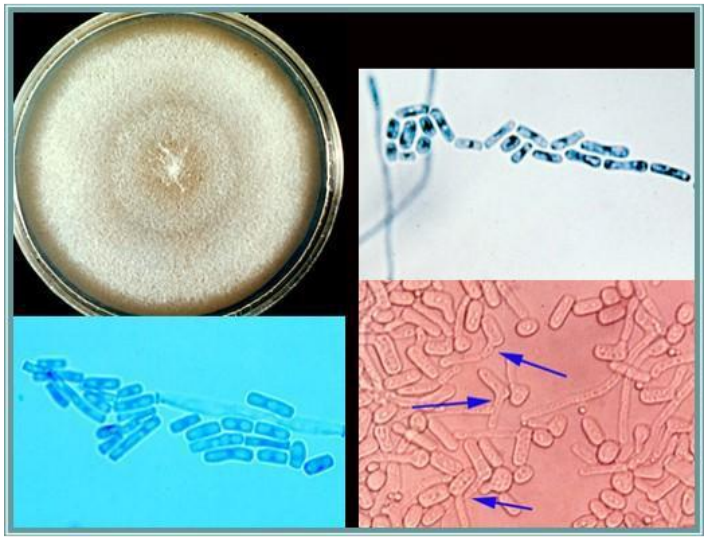
The most well-known species is ***Geotrichum candidum***, which is used in the **cheese-ripening industry** (cheese rind formation) and in **fermented milk products**, especially in goat milk.

Although often environmental or part of normal flora, *Geotrichum* species can cause disease in:

- Immunocompromised individuals
- Cancer patients
- Leukemia patients

The infection is known as Geotrichosis, which may involve:

- Lungs
- Oral cavity
- Skin
- Gastrointestinal tract



Taxonomic Classification of *Geotrichum candidum*

Rank	Classification
Kingdom	Fungi
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Order	Saccharomycetales
Family	Dipodascaceae
Genus	*Geotrichum*
Species	*Geotrichum candidum*

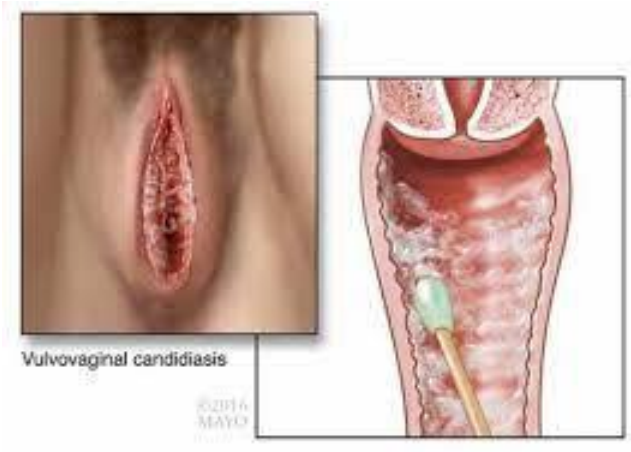
Practical Session 7

Candida albicans

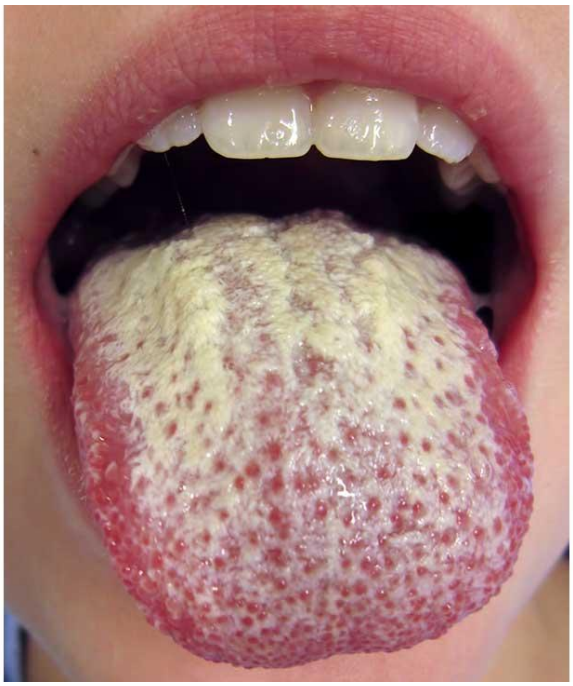
Candida albicans is a yeast belonging to the Ascomycota (ascomycetous fungi).

It is an opportunistic pathogen that causes many diseases, especially in:

- **Women** → *Vaginal thrush*



- **Oral cavity** → *Mouth thrush (oral candidiasis)*



The disease is called **Candidiasis**.

It commonly affects:

- The elderly
- Immunocompromised patients

It can also cause urinary tract infections (UTIs).

Candida albicans normally lives as part of the flora of:

- The mouth
- The gastrointestinal tract

It can produce:

- Blastoconidia (budding cells)
- Chlamydospores
- Pseudohyphae under stressful conditions (e.g., on Corn Meal Agar)

Diagnosis can be performed using several methods, including CHROMagar Candida.

Taxonomic Classification of *Candida albicans*

Rank	Classification
Domain	Eukaryota
Kingdom	Fungi
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Genus	*Candida*

Rank	Classification
Species	*Candida albicans*

1. CHROMagar Candida Medium

CHROMagar Candida is a differential medium used to distinguish between *Candida* species based on colony color.

Principle and Interpretation

Berry and Miller reported that *Candida albicans* produces the enzyme α-acetyl-N-galactosaminidase.
According to Rousselle et al., incorporating chromogenic or fluorogenic hexosaminidase substrates into the medium helps identify *C. albicans* isolates.

HiCrome Candida Differential Agar is a selective and differential medium that allows:

- Rapid isolation of yeasts from mixed cultures
- Differentiation between common *Candida* species

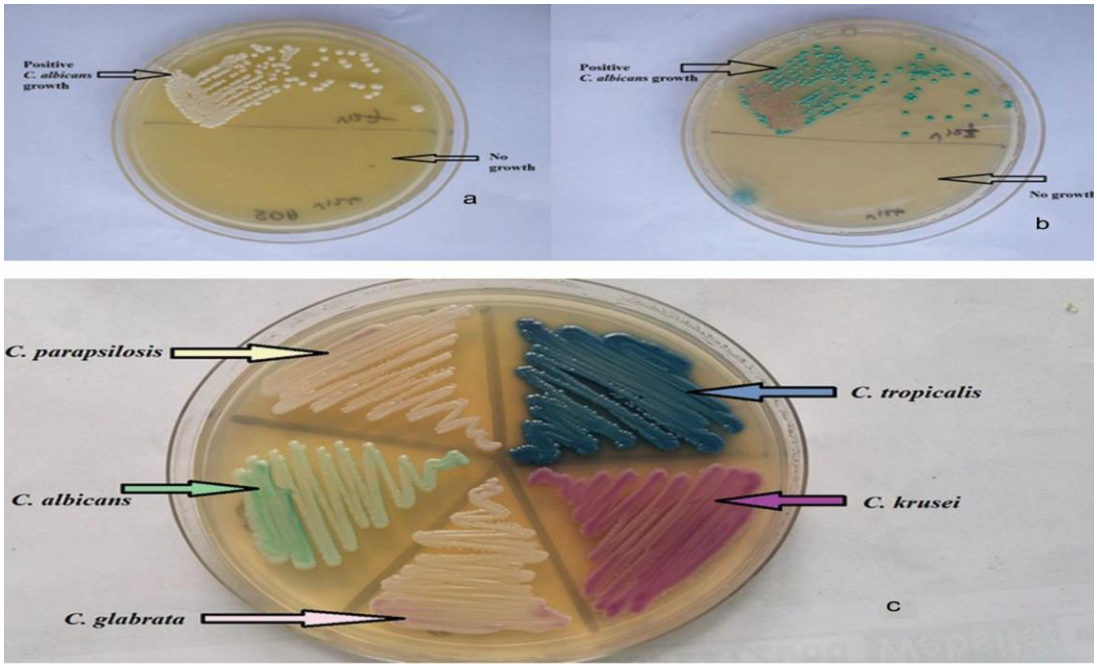
Results appear within 48 hours, making it useful for rapid identification in clinical microbiology laboratories.

Nutritional Composition and Selectivity

- Special peptones and yeast extract provide carbon, nitrogen, and essential nutrients.
- Phosphate buffer enriches the medium.
- Chloramphenicol inhibits accompanying bacterial flora.

Color Appearance of Candida Species on CHROMagar Candida

Species	Colony Appearance
C. albicans	Smooth, **light green** colonies
C. tropicalis	Raised colonies, **blue to metallic blue**
C. glabrata	Cream-colored to **soft white** colonies
C. krusei	Fuzzy, **pink to mauve (purple-hazy)** colonies



2. Germ Tube Test

The Germ Tube Test is used to differentiate Candida albicans from other *Candida* species. The germ tube represents an early stage of pseudohyphal formation.

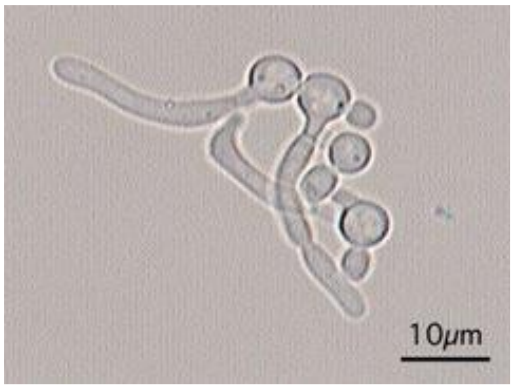
The test is performed in a tube containing human serum, which is the liquid portion of blood after clotting factors have been removed.

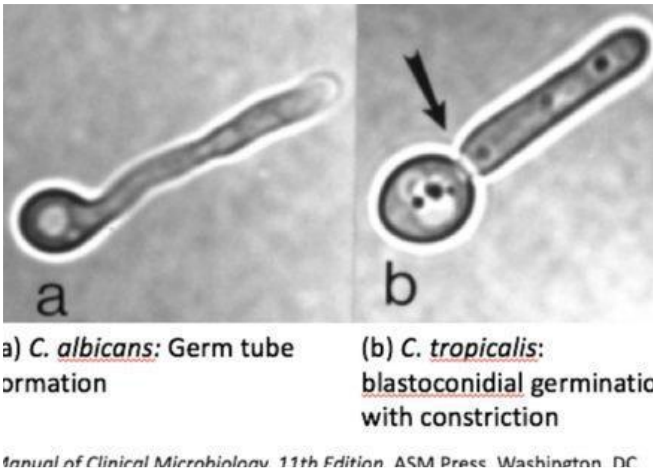
Procedure:

1. Inoculate the yeast sample into the serum tube.
2. Incubate at **37°C for 2 hours**.
3. Examine the result using a wet preparation slide (wet mount).

Positive Result (for *Candida albicans*):

- *Candida albicans* forms germ tubes, which appear as tube-like extensions emerging from the yeast cell without constriction at the point of origin.



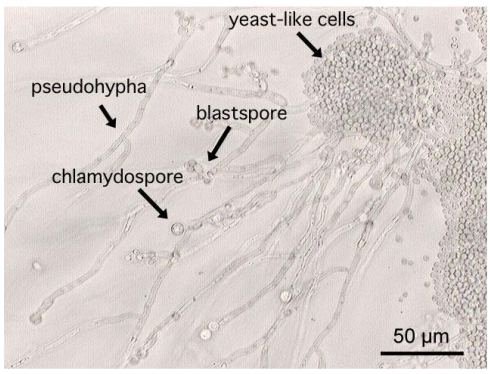


Positive germ tube of *c.albicans* a)positive b)false

3- Corn Meal Agar:

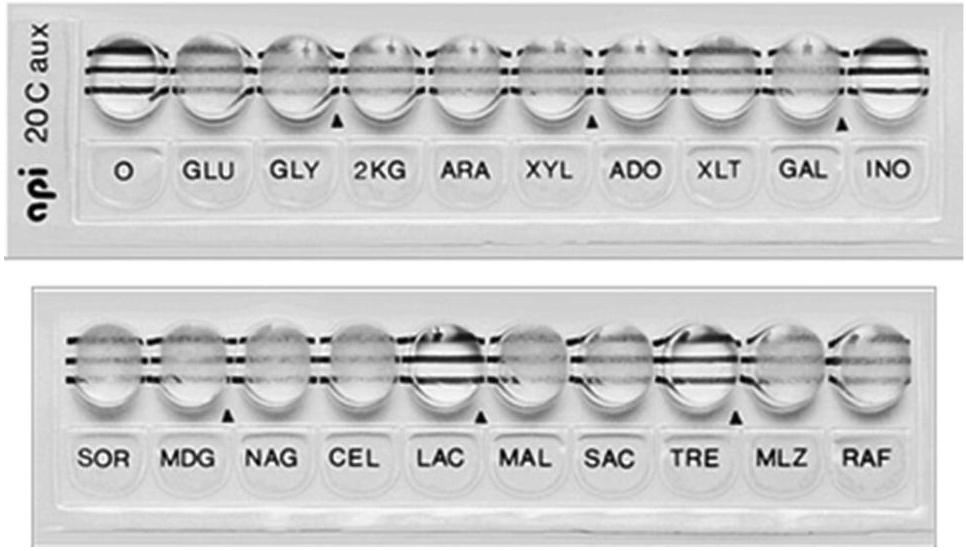
The yeast is cultured on this medium and examined microscopically after 24–48 hours of incubation.

The results are read based on the production of pseudohyphae, chlamydospores, and blastoconidia.



4- Carbohydrate Fermentation Test (API 20C)

This test is used to determine the ability of yeasts to **ferment different carbohydrates**.



Species	Xyl	Lac	Suc	Mal	Mel	Cel	Tre
C. albicans	+	+	+	_	+	+	+
C. tropicalis	+	_	+	+	_	+	+
C. glabarata	+	_	_	_	_	_	+
C. parapsilosis	+	_	+	+	_	_	+
C. krusei	+	_	_	_	_	_	_
C. dublinensis	+	+	+	_	+	+	+

Candida species Identification

		C. albicans	C. dubliniensis	C. glabrata	C. krusei	C. kefyr	C. parapsilosis	C. tropicalis	C. guilliermondii	C. famata	C. lipolytica
Chlamydospore		+	+	-	-	-	-	-	-	-	-
Germ tube		+	+	-	-	-	-	-	-	-	-
Pellicle On broth					+						+
Urease					+						+
Fermentation	Glu	F	F	F	F	F	F	F	F	W	-
	Mal	F		-	-	-	-	F	-	-	-
	Suc	-	-	-	-	F	-	F	F	W	-
	Lac	-	-	-	-	F	-	-	-	-	-
	Gal	F	F	-	-	F	-	F	F	-	-
	Tre	F	F	F	-	-	-	F	F	W	-
Assimilation	Glu	+	+	+	+	+	+	+	+	+	+
	Mal	+	+	-	-	-	+	+	+	+	-
	Suc	+	-	-	-	+	+	+	+	+	-
	Lac	+	-	-	-	+	-	-	-	+	-
	Tre	+	-	+	-	-	+	+	+	+	-

B-Basidiomycetes

1-Rhodotorula mucilaginosa

Rhodotorula mucilaginosa is a yeast belonging to the Basidiomycota.
It reproduces asexually, and its colonies appear creamy to mucoid with an orange color.
Some strains produce red pigments, giving the colonies an orange-red appearance.

It is commonly found in:

- Air
- Soil
- Milk
- Water
- Fruit juices

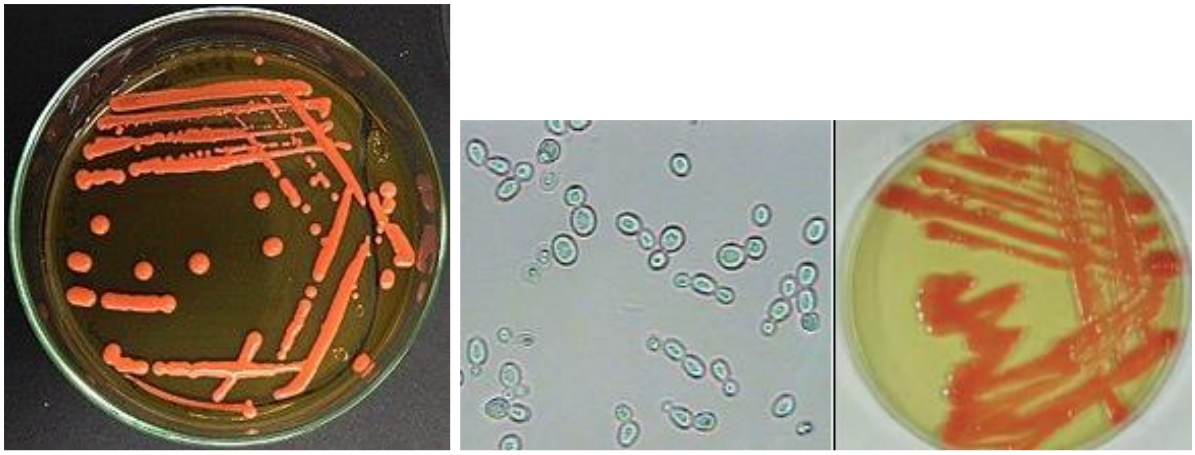
Earlier, it was not considered pathogenic, but it is now classified as an opportunistic pathogen.
It has been associated with:

- Fungemia (bloodstream infection), often through catheter tubes
- Endocarditis (heart infection)
- Meningitis

It can also cause disease in animals such as:

- Goats
- Sheep
- Marine animals
- Skin infections in poultry

Additionally, *Rhodotorula* plays an important role in bioremediation of water.



Taxonomic Classification of *Rhodotorula mucilaginosa*

Rank	
Domain	Eukaryota
Kingdom	Fungi
Phylum	Basidiomycota
Subphylum	Pucciniomycotina
Class	Microbotryomycetes
Order	Sporidiobolales
Family	Sporidiobolaceae
Genus	*Rhodotorula*
Species	*Rhodotorula mucilaginosa*

Rank

Practical Session 8

2.Cryptococcus neoformans

Cryptococcus neoformans is a yeast belonging to the Basidiomycota.
It is a highly pathogenic yeast that causes:

- Severe pneumonia
- Meningitis (cryptococcal meningitis)

Diagnosis is commonly performed by examining a sample of cerebrospinal fluid (CSF).

Microscopic identification is done using a negative staining technique, specifically India Ink stain, which reveals the capsule that surrounds the yeast cell.
The capsule is the major virulence factor, and under India Ink staining it appears as a clear halo around each cell.

Its colonies on culture media appear creamy and mucoid, due to the presence of the capsule.

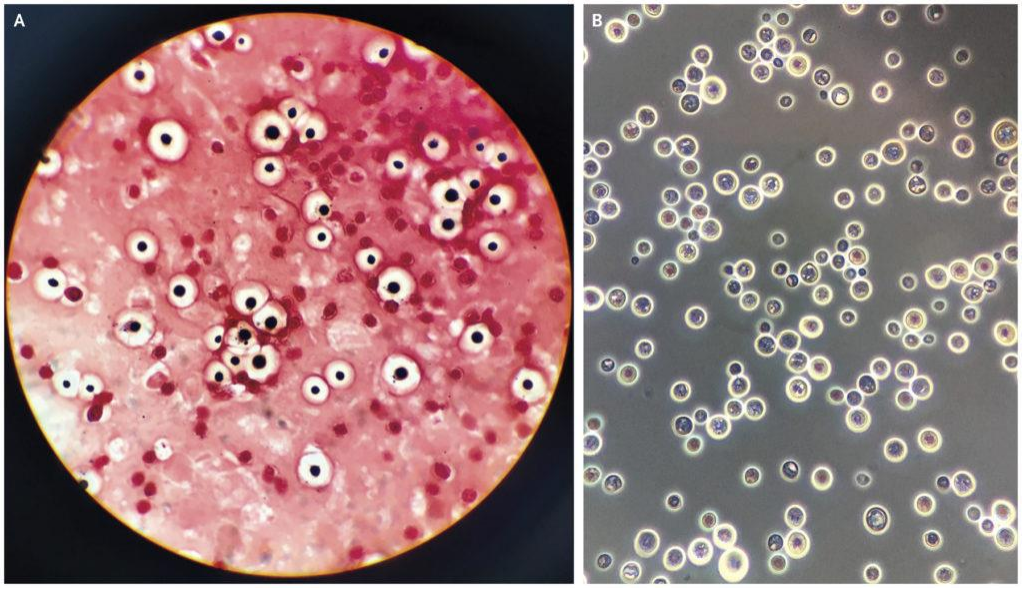
For laboratory identification, Bird Seed Agar (Niger Seed Agar) is used, where the colonies appear brown to black because of melanin production.

Taxonomic Classification of *Cryptococcus neoformans*

Rank	Classification
Domain	Eukaryota
Kingdom	Fungi
Phylum	Basidiomycota
Subphylum	Agaricomycotina

Rank

Class	Tremellomycetes
Order	Tremellales
Family	Tremellaceae
Genus	*Cryptococcus*
Species	*Cryptococcus neoformans*



Gram stain of cryptococcus neoformans Indian ink of cryptococcus neoformans

Left Image (A): India Ink Stain – Negative Staining

- This is the classic India Ink (Nigrosin) preparation.
- Background is dark, and the capsule appears as a clear halo around the yeast cell.
- This stain is used specifically for Cryptococcus neoformans because of its thick capsule.

Key features visible:

- White or clear halos = capsule
- Dark background = India ink
- Round budding yeast cells in the center
- Typical appearance in CSF samples

Right Image (B): Gram Stain (Gram-positive Yeast)

- Yeasts appear Gram-positive (purple).
- Capsule does not take the stain, so a faint halo may remain.
- Background is lighter because Gram stain uses crystal violet + safranin.

Key features visible:

- Round, purple-stained cells
- Some budding
- Clear capsule outline
- No dark background (not India ink)

****Bird Seed Agar**

(for the isolation of *Cryptococcus neoformans*)**

Bird Seed Agar, also called Niger Seed Agar or Staib Agar, is a selective and differential medium used specifically to isolate and identify *Cryptococcus neoformans*.

Why does *Cryptococcus neoformans* turn brown or black?

The seeds in this agar (usually Guizotia abyssinica or Niger seeds) contain:

- Caffeic acid
- Phenolic compounds

C. neoformans possesses the enzyme phenol oxidase (a type of laccase).

Phenol oxidase + caffeic acid = melanin

This reaction produces melanin pigment, which accumulates in the cell wall of the yeast.

→ Result: colonies turn brown to black.

Interpretation of the Image

Left plate

You see:

- Dark brown/black colonies
  → Positive for *Cryptococcus neoformans*
- Color change due to melanin production

Right plate

Shows:

- Cream to yellow colonies (non-pigmented)
- These represent other yeasts that do NOT produce melanin
  → Negative for *C. neoformans*

Summary

Feature	Bird Seed Agar
Selective for	*Cryptococcus neoformans*
Differential for	Melanin production
Positive result	Brown/black colonies
Negative result	Cream/yellow colonies
Enzyme detected	Phenol oxidase (laccase)
Substrate	Caffeic acid from Niger seeds

Why is this test important?

Cryptococcus neoformans is a major cause of:

- Cryptococcal meningitis
- Severe pneumonia
  Especially in immunocompromised patients (HIV/AIDS, transplant, cancer patients).

Bird Seed Agar helps quickly differentiate it from other yeasts.

On **Bird Seed Agar**, a **negative result** means the organism **does NOT produce melanin** from the caffeic acid in the medium.

Negative Result on Bird Seed Agar

Colony Color:

- Cream,
- White,
- Yellow, or
- Pale colonies

Interpretation:

- Organism does NOT have phenol oxidase (laccase)
- Organism cannot convert caffeic acid → melanin
- Therefore, pigmentation does NOT appear

Typical Negative Yeasts:

- *Candida albicans*
- *Candida tropicalis*
- *Candida glabrata*
- *Candida krusei*
- *Rhodotorula spp.*
- *Trichosporon spp.*
- *Malassezia spp.*
- *Other non-melanized yeasts*

So, a negative result = NOT Cryptococcus neoformans

Because *Cryptococcus neoformans* is the only common yeast that produces the brown–black melanin pigment, giving a positive result.

3.Malassezia furfur

Malassezia furfur is a basidiomycetous yeast.
It is commensal on humans and naturally lives on human skin and hair.
It is also an opportunistic pathogen that causes several skin diseases.
It is the most common cause of dandruff and seborrheic dermatitis.

Because *Malassezia* species are lipophilic (lipid-dependent), they require fatty acids for growth.
Therefore, when isolating them, animal or plant oils are added to the culture media.

Malassezia furfur grows best when incubated at 32°C for 5 days.

Taxonomic Classification of *Malassezia furfur*

Rank	Classification
Domain	Eukaryota
Kingdom	Fungi
Phylum	Basidiomycota
Subphylum	Ustilaginomycotina
Class	Malasseziomycetes
Order	Malasseziales
Family	Malasseziaceae
Genus	*Malassezia*
Species	*Malassezia furfur*

Media Used for *Malassezia* Isolation

Because *Malassezia* is lipid-dependent, it does NOT grow well on routine fungal media like SDA.
It requires lipid-enriched media.

Common media used:

1. Dixon's Agar / Modified Dixon Agar
    - Contains lipids, Tween, and ox bile
    - Most widely used for *Malassezia* species
2. Leeming–Notman Agar
    - Contains milk, lipids, glycerol, Tween 60, and ox bile
3. Sabouraud Dextrose Agar + Olive oil overlay
    - When SDA is used, a drop of olive oil must be added for growth
    - Because *Malassezia* is lipophilic
4. MEA + lipid supplements
    - Malt extract agar supplemented with fatty acids

Infections Caused by *Malassezia furfur*

Malassezia furfur causes several common superficial fungal infections, including:

1. Pityriasis versicolor (Tinea versicolor)

- Most common infection
- Hypo- or hyperpigmented patches on chest, back, shoulders
- Caused by yeast-to-hyphal change

2. Dandruff (Pityriasis capitis)

- Flaking of scalp
- Most common cause of dandruff worldwide

3. Seborrheic dermatitis

- Affects areas rich in sebaceous glands
- Scalp, face, eyebrows, chest, ears

4. Folliculitis (Malassezia folliculitis)

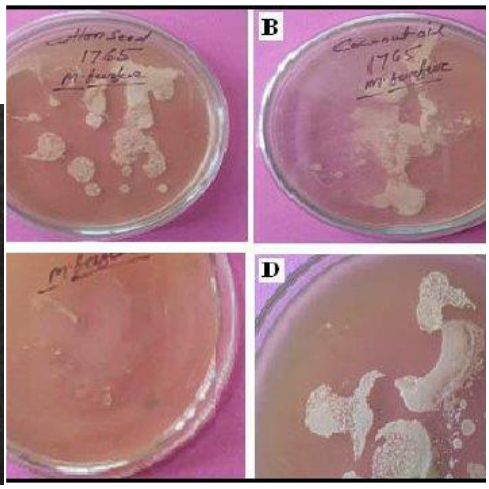
- Acne-like eruptions on chest, back
- Common in humid climates

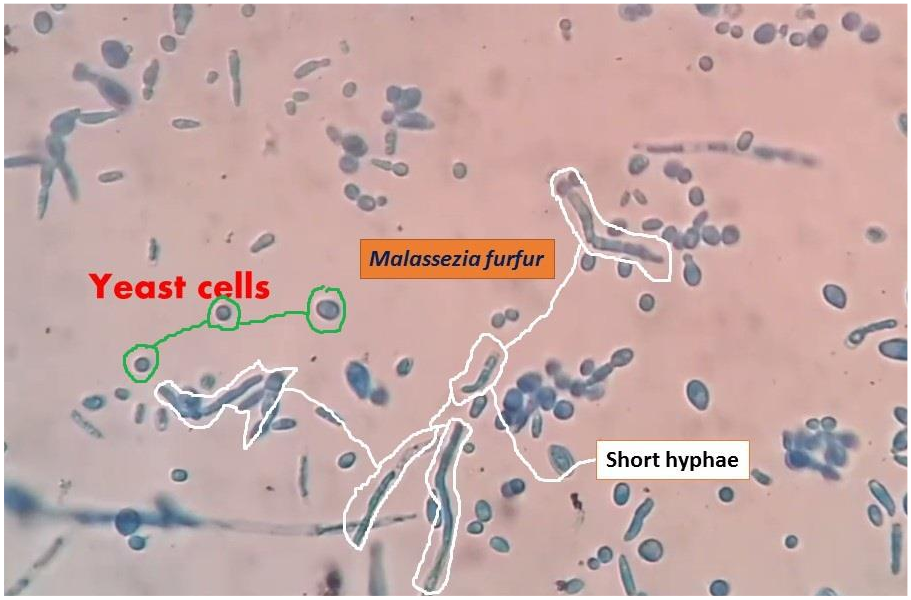
5. Opportunistic fungemia (rare)

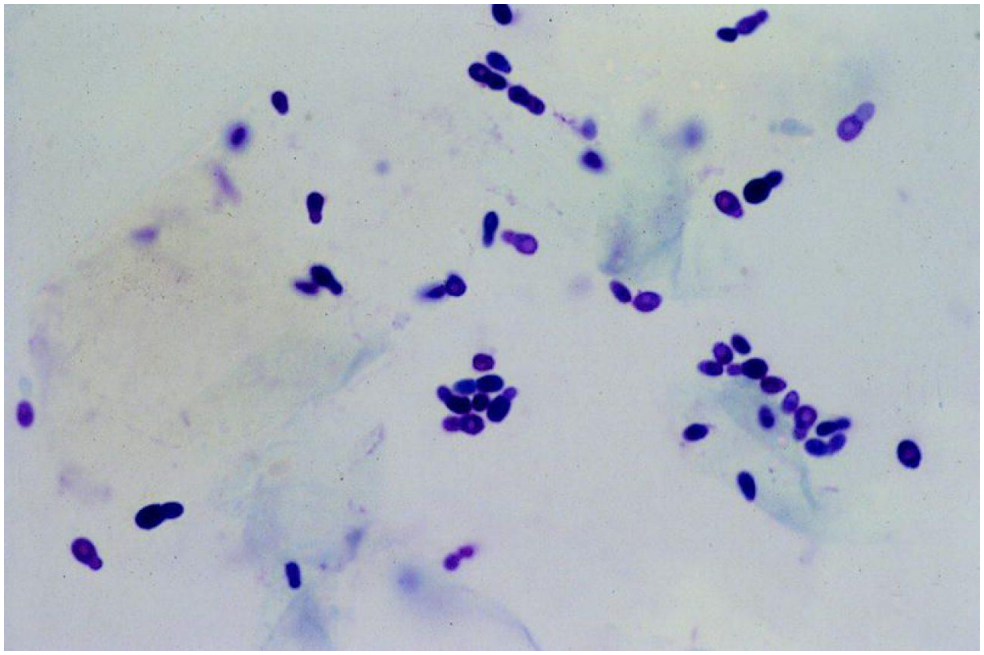
- In neonates receiving lipid-rich TPN (total parenteral nutrition)
- Because *Malassezia* thrives in lipid environments

Growth Characteristics

- Requires lipids for growth
- Incubation: 32°C
- Time: 5 days
- Microscopy shows:
    - Round budding yeast
    - Sometimes "spaghetti and meatballs" appearance (in tinea versicolor: hyphae + spores)


B
D


Yeast cells
Malassezia furfur
Short hyphae



Dandruff of malessizia furfur

4- Trichosporon spp.

Trichosporon species are basidiomycetous fungi that reproduce asexually.
They are found in soil and also live naturally on human skin.
They are opportunistic pathogens and can cause White Piedra, a superficial fungal infection of the hair.

The most well-known species is Trichosporon beigelii (old name), now called Trichosporon cutaneum.

Taxonomic Classification of *Trichosporon* spp.

Rank	Classification
Domain	Eukaryota
Kingdom	Fungi
Phylum	Basidiomycota
Subphylum	Agaricomycotina
Class	Trichosporonales
Order	Trichosporonales
Family	Trichosporonaceae
Genus	*Trichosporon*
Species	Several species (see below)

Famous / Clinically Important Species

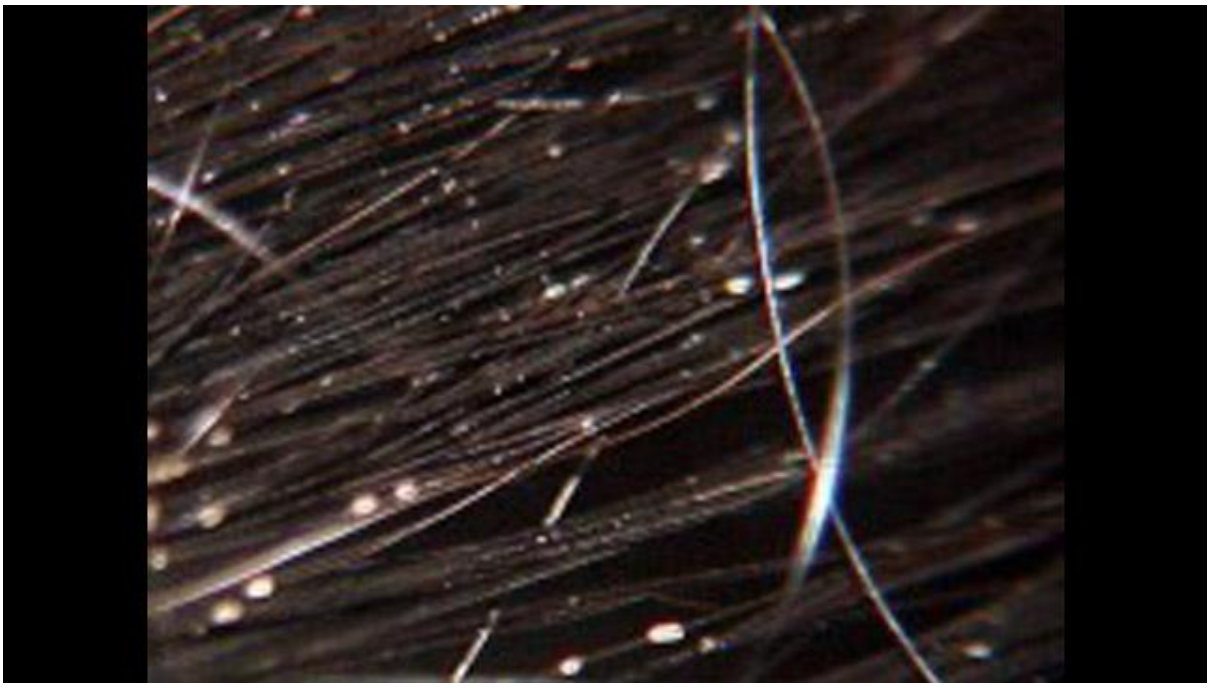
Species	Notes
Trichosporon asahii	Most common cause of systemic infections / fungemia
Trichosporon mucoides	Causes invasive disease in immunocompromised patients
Trichosporon cutaneum (formerly *T. beigelii*)	Classic cause of White Piedra
Trichosporon ovoides	Involved in White Piedra of scalp
Trichosporon inkin	White Piedra in pubic and axillary hair
Trichosporon asteroids	Rare but pathogenic
Trichosporon loubieri	Rare systemic disease

Infections Caused by *Trichosporon* spp.

1. White Piedra (Main Infection)

A superficial fungal infection of the hair shaft, characterized by:

- White or light-colored nodules around hair
- Affects scalp, beard, mustache, pubic hair, axillary hair

- 

2. Opportunistic Systemic Infections

Usually in immunocompromised patients:

- Trichosporonosis / fungemia
- Pneumonia
- Urinary tract infection
- Disseminated infection
- Seen in:
    - Leukemia patients
    - Bone marrow transplant
    - Neutropenic patients
    - ICU patients

3. Skin and soft-tissue infections

Rare but possible.

Media Used for Isolation of *Trichosporon*

Trichosporon grows well on general fungal media:

1. Sabouraud Dextrose Agar (SDA)

- Standard medium
- Colonies are white, wrinkled, creamy to dry

2. Corn Meal Agar

- Shows:
    - Arthroconidia (square/rectangular spores)
    - Hyphae + pseudohyphae

3. CHROMagar Candida

- *Trichosporon* produces characteristic blue-green colonies
  (helps differentiation in mixed cultures)

Optional Enrichment

- SDA with antibiotics (chloramphenicol) to suppress bacteria

Microscopic Features

On Corn Meal Agar:

- Hyphae
- Pseudohyphae
- Arthroconidia (rectangular spores)
  These are diagnostic for *Trichosporon*.

- 
- 

Summary

Trichosporon spp.

- Basidiomycetous yeasts
- Opportunistic
- Cause White Piedra
- Also cause severe infections in immunocompromised patients
- Grow on SDA, Corn Meal Agar, CHROMagar Candida
- Show arthroconidia + hyphae

References

عبدالله ناصر الرحمة, أساسيات علم الفطريات ,دار جامعة الملك سعود للنشر 1998, ردمك:9789960558745

عبدالله بن صالح الخليل ، حميد احمد محمد جوله ، جمال محمد علي خالد , علم الفطريات الطبية , دار جامعة الملك سعود للنشر 2011 , ردمك:9789960558110

McGinnis MR, Tyring SK. Introduction to Mycology. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8125/

Fleet, G.H. (1985). Composition and Structure of Yeast Cell Walls. In: McGinnis, M.R. (eds) Current Topics in Medical Mycology. Current Topics in Medical Mycology, vol 1. Springer, New York, NY. https://doi.org/10.1007/978-1-4613-9547-8_2