College of Sciences

Department of Botany and Microbiology

1. **Course no. & code:** MBI 349 (32829)
2. **Course name:** Yeast
3. **Experiment no.:** 1
4. **Experiment title:** Introduction to Yeast and Biohazards, Biosafety Precaution.
5. **Aim of the Experiment:** Introduction to the yeast laboratory and reviewing the safety precaution.
6. **Brief introduction:** This section gives the awareness about the Yeast to the student and precautionary measures for the laboratory
7. **Materials, Methods & Equipment:** General methods will be discussed with students about yeast
8. **Procedures:** Standard microbiological methods
9. **Results & Observations:**
   Student will assess about the discussion for yeast.
10. **Conclusion:** Discussion will be useful for the next experiments.
11. **References:**

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 2

4. **Experiment title:** Isolation and purification of Yeast.

5. **Aim of the Experiment:** Isolation strategies for the student.

6. **Brief introduction:** This session will enable the student with isolation strategies for yeast.

7. **Materials, Methods & Equipment:**

Malt Extract Agar Composition

Ingredients	Gms / Litre
Malt extract	30.000
Mycological peptone	5.000
Agar	15.000
Final pH (at 25°C)	5.4±0.2

Tools:

- Microbial loops.
- Plates to cultivate our yeasts.
- Alcohol for the sterilization.
- Bunsen burner.
- 500 ml flasks with distilled water.

8. **Procedures:**

Media preparation & Yeast Inoculation:

1- Malt Extract Agar is the general media used for yeast cultivation, weight the right amount of the powder for 5 plates.

2- Suspend the powder media in distilled water, shake gently to dissolve the powder.

3- Sterilize the media in the autoclave under 1- 1.5 atmosphere pressure and 121 C for 15-20 minutes.

4- Once the sterilization finished, carefully open the autoclave and wait until the temperature comes down.
5- Prepare the petri dishes and sterilize the area where you will work, then pour the media to the dishes and wait for the solidification.
6- Each yeast inoculated to the plates and incubated.
7- Zigzag streaking for the yeast.

9. Results & Observations:
After inoculation the sample must be kept in the incubator for 4-5 days at 25 C.

10. Conclusion: These yeasts will be used for the next experiments

11. References:

College of Sciences

Department of Botany and Microbiology

1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 3

4. **Experiment title:** Isolation and purification of Yeast.

5. **Aim of the Experiment:** Purification of the yeast

6. **Brief introduction:** This session will enable the student with strategies for purification of yeast

7. **Materials, Methods & Equipment:**

Malt Extract Agar Composition

Ingredients	Gms / Litre
Malt extract	30.000
Mycological peptone	5.000
Agar	15.000
Final pH (at 25°C)	5.4±0.2

Tools:

- Microbial loops.
- Plates to cultivate our yeasts.
- Alcohol for the sterilization.
- Bunsen burner.
- 500 ml flasks with distilled water.

8. **Procedures:**
Standard microbiological methods will be employed for the purification of yeast

9. **Results & Observations:** Obtained culture will be assessed for its purity in the laboratory

10. **Conclusion:**
Purified yeast will be stored for further experimentation

11. **References:**

College of Sciences

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 4

4. **Experiment title:** Morphology & Colonial Characteristics of Yeast

5. **Aim of the Experiment:** To study the colonial characteristics of the yeast.

6. **Brief introduction:** This session allows student to explore the morphological features and colony characteristics for various yeast

7. **Materials, Methods & Equipment:**
   Student bring the previously cultivated culture and identify the characteristics of the yeasts.
   Tools:

- Microbial loops.
- Alcohol for the sterilization.
- Bunsen burner.

8. **Procedures:**

Standard microbiological methods will be employed

9. **Results & Observations:**

Characteristics:

- Colony color: Yellow, White, Orange, Creamy, etc…
- Colony Size: less than 5 mm.
- Colony elevation: Concave, Convex, Flat, Raised, etc…
- Colony shape: Circular, Filamentous, irregular, etc…
- Colony maragin: Entire, labulate, etc…

10. **Conclusion:**
    Isolated, purified and morphological identified yeast will be stored for further experiments.
11. **References:**

College of Sciences

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 5

4. **Experiment title:** Microscopic characteristics of isolated yeast

5. **Aim of the Experiment:** To study the cellular characteristics of yeast and Ascospores formation.

6. **Brief introduction:** Microscopic examination of the isolated yeast

7. **Materials, Methods & Equipment:**
   Media preparation & Yeast Inoculation:

1- Malt Extract Agar is the general media used for yeast cultivation, weight the right amount of the powder for 5 plates.
2- Suspend the powder media in distilled water, shake gently to dissolve the powder.
3- Sterilize the media in the autoclave under 1- 1.5 atmosphere pressure and 121 C for 15-20 minutes.
4- Once the sterilization finished, carefully open the autoclave and wait until the temperature comes down.
5- Prepare the petri dishes and sterilize the area where you will work, then pour the media to the dishes and wait for the solidification.

Tools:

- Microbial loops.
- Alcohol for the sterilization.
- Bunsen burner.
- Flasks with 250 distilled water.
- Simple Stain.

8. **Procedures:**
   Isolated yeast will be inoculated to the plates and incubated for microscopic examination.

9. **Results & Observations:**

After inoculation the sample must be kept in the incubator for 3-5 days at 25 C.

After incubation period yeasts are stained and examined under the microscope.

Dry preparation	Wet preparation
No cover slip	Cover slip
Examine under: 4X, 10X, 40X, 100X	4X, 10X, 40X

Neutral Red 1% is used to stain volutine granule.

10. **Conclusion:**

Isolated, purified, morphological and microscopic identified yeast will be stored for further experiments.

11. **References:**

1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 6

4. **Experiment title:** Formation of Pseudomycelium and detection of chlamydospore formation.

5. **Aim of the Experiment:** To study the ability of yeast to produce pseudomycelium and chlamydospore.

6. **Brief introduction:**
   Pseudomycelium: a cellular association occurring in various higher bacteria and yeasts in which cells cling together in chains resembling small true mycelia

   Chlamydospore: formation is a characteristic of many fungal species, among them the closely related human-pathogenic dimorphic yeasts Candida albicans and C. dubliniensis

7. **Materials, Methods & Equipment:**
   Media used:
   Corn Meal Tween 80 Agar

Corn meal infusion	2 gm
Tween 80	7 ml
Agar	15 gm
Distilled Water	1000 ml

Tools:

- Microbial loops.
- Alcohol for the sterilization.
- Bunsen burner.
- Flasks with 250 distilled water.
- Simple Stain.

8. **Procedures:**

<u>Media preparation & Yeast Inoculation:</u>

1- Corn meal tween 80 Agar is used for pseudohyphae detection, weight the right amount of the powder for 5 plates.
2- Suspend the powder media in distilled water, shake gently to dissolve the powder.
3- Sterilize the media in the autoclave under 1- 1.5 atmosphere pressure and 121 C for 15-20 minutes.
4- Once the sterilization finished, carefully open the autoclave and wait until the temperature comes down.
5- Prepare the petri dishes and sterilize the area where you will work, then pour the media to the dishes and wait for the solidification. Each yeast inoculated to the plates and incubated.
6- This media is used for the cultivation of fungi and for the inducement of **chlamydospores** formation.

9. **Results & Observations:**

After the incubation period, student can analyse the results.

10. **Conclusion:**

Pseudomycelium and chlamydospore formation in yeast

1. **References:**

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 7

4. **Experiment title:** Germ tube formation test.

5. **Aim of the Experiment:** To study the Germ tube formation in yeast

6. **Brief introduction:** Germ Tube Test is a screening test which is used to differentiate Candida albicans from other yeast. Germ tube (GT) formation was first reported by Reynolds and Braude in 1956.

7. **Materials, Methods & Equipment:**
   Sheep serum, Fetal bovine serum, Pasteur pipette
   Standard microbiological method

8. **Procedures:**
   <u>Procedure of Germ Tube Test</u>

1. Put 0.5 ml of sheep or human serum into a small tube.
   Note: Fetal bovine serum can also be used instead of human serum.
2. Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum.
   Note: Too large of an inoculum will inhibit germ tube formation.
3. Incubated the tube at 37°C for 2 to 4 hours.
4. Transfer a drop of the serum to a slide for examination.
5. Coverslip and examine microscopically under 40x.

9. **Results & Observations:**
   After the incubation period, student can analyse the results.

10. **Conclusion:**
    Germ tube formation in yeast

11. **References:**

College of Sciences

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 8

4. **Experiment title:** Fermentation Test.

5. **Aim of the Experiment:** To study the ability of yeast to ferment sugars.

6. **Brief introduction:**
   During alcoholic fermentation, yeast converts sugars into carbon dioxide (CO_2) and ethanol

7. **Materials, Methods & Equipment:**
   Materials & Methods

Carbohydrates	10 gm
Pepton	5 gm
Bromocresol purple	0.04 gm
Distilled Water	1000 ml

Media preparation & Yeast Inoculation:

1- Carbohydrates broth media added for fermentation test prepare the right amount of the media for 5 plates.
2- Suspend the powder media in distilled water, shake gently to dissolve the powder.
3- Sterilize the media in the autoclave under 1- 1.5 atmosphere pressure and 121 C for 15-20 minutes.
4- Once the sterilization finished, carefully open the autoclave and wait until the temperature comes down.
5- Prepare the petri dishes and sterilize the area where you will work, then pour the media to the dishes and wait for the solidification.
6- Each yeast inoculated to the plates and incubated.

Tools:

- Microbial loops.
- Alcohol for the sterilization.
- Bunsen burner.

- Flasks with 250 distilled water.
- Simple Stain.
- Durhams tumes.
- 12 test tubes.
- Our fermentation indicator.

8. **Procedures:**
   Procedures:
   - A set of three sugars (mono, DI and poly) are prepared, each with four tubes.
   - After sterilization, media poured into 12 test tubes.
   - Insert one Durhams tubes into each of the test tubes.
   - Brolmocresole purple, then mixed with the media.
   - Inoculate each tube with one yeast.

9. **Results & Observations:**
   After the incubation period, student can analyse the results.

10. **Conclusion:**
    Fermentation test in yeast

11. **References:**

College of Sciences

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 9

4. **Experiment title:** Detection of osmophilic yeast.

5. **Aim of the Experiment:** To study the ability of yeast to grow under high osmotic pressure.

6. **Brief introduction:**

   What is the osmotic pressure?

   It is the pressure resulted from the movement of water from low concentration to higher concentration through a semi-permeable membrane.

7. **Materials, Methods & Equipment:**

   Material & Methods

Sucrose	400 gm
Malt Extract	12gm
Agar	15 gm
Distilled Waster	1000 ml

   Media preparation & Yeast Inoculation:

   1- Preparation of Malt extract agar media supplemented with different sucrose (sugar) concentrations (20, 40, 60 gm/l) as osmotic pressure media in this test.
   2- Suspend the powder media in distilled water, shake gently to dissolve the powder.
   3- Sterilize the media in the autoclave under 1- 1.5 atmosphere pressure and 121 C for 15-20 minutes.
   4- Once the sterilization finished, carefully open the autoclave and wait until the temperature comes down.
   5- Prepare the petri dishes and sterilize the area where you will work, then pour the media to the dishes and wait for the solidification.
   6- Each yeast inoculated to the plates and incubated.

Tools:

- Microbial loops.
- Alcohol for the sterilization.
- Bunsen burner.
- Flasks with 250 distilled water.
- Media in petri dishes.

8. **Procedures:**
   Standard microbiology protocol

9. **Results & Observations:**

   After the incubation period, student can analyse the results.

Solutions

- Hypertonic: the conc is higher than cell. Cell shrink
- Hypotonic: the conc is lower than cell. Cell explosion
- Isotonic: the conc is equal to the cell. No effect

10. **Conclusion:**
    Osmophilic yeast will be screened and identified.

11. **References:**

College of Sciences

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1. **Course no. & code:** MBI 349 (32829)

2. **Course name:** Yeast

3. **Experiment no.:** 10

4. **Experiment title:** Detection of halophilic yeast.

5. **Aim of the Experiment:** To study the ability of yeast to grow under high salinity.

6. **Brief introduction:**
   A halophile (from the Greek word for 'salt-loving') is an extremophile that thrives in high salt concentrations.

7. **Materials, Methods & Equipment:**
   Material & Methods
   Media used
   Malt extract agar supplemented with different concentrations of Nacl (5, 15, 25 g/l)

Malt Extract	12gm
Agar	15 gm
Distilled Waster	1000 ml

   Media preparation & Yeast Inoculation:
   1. Preparation of Malt extract agar media supplemented with different sucrose (Nacl) concentrations (5, 15, 25 gm/l) as osmotic pressure media in this test.
   2. Suspend the powder media in distilled water, shake gently to dissolve the powder.
   3. Sterilize the media in the autoclave under 1- 1.5 atmosphere pressure and 121 C for 15-20 minutes.
   4. Once the sterilization finished, carefully open the autoclave and wait until the temperature comes down.
   5. Prepare the petri dishes and sterilize the area where you will work, then pour the media to the dishes and wait for the solidification.
   6. Each yeast inoculated to the plates and incubated.

Tools:

- Microbial loops.
- Alcohol for the sterilization.
- Bunsen burner.
- Flasks with 250 distilled water.
- Media in petri dishes.

8. **Procedures:**
   Standard microbiology protocol

9. **Results & Observations:**
   After the incubation period, student can analyse the results.

10. **Conclusion:**
    Halophilic yeast will be screened and identified.

1. **References:**