



Koneru Lakshmaiah Education Foundation

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KL COLLEGE OF AGRICULTURE

MycoMarvels MUSHROOM CULTIVATION CLUB

Name of the Event: Isolation of Button Mushroom using tissue isolation method.

Date: 17/12/2024

Venue: Plant Pathology Lab- S316

Number of students participated: 30 members

Objective of the event :

The main objective is to isolate the Button mushroom culture using tissue isolation method.

Isolation of Button Mushroom (*Agaricus bisporus*) on Potato Dextrose Agar (PDA) medium involves several key steps to obtain pure mycelial culture for further study or commercial purposes. Here's a step-by-step guide:

Description of the Procedure:

1. Preparation of PDA Medium

- Boil 200 g of peeled potatoes in 1 L of distilled water for 30 minutes.
- Filter the extract and add 20 g of dextrose and 15-20 g of agar.
- Autoclave at 121°C for 15 minutes.
- Pour the sterilized PDA into sterile Petri dishes under laminar airflow and allow it to solidify.

2. Sample Collection

- Take a fresh Button Mushroom and clean the surface using 70% ethanol.
- Using a sterile scalpel, carefully cut a small piece (5-10 mm) from the inner tissue of the mushroom cap or stem base (avoid contaminated outer layers).

3. Inoculation on PDA Medium

- Transfer the tissue piece to the center of a PDA plate using sterile forceps.
- Gently press it onto the agar surface without embedding it completely.
- Seal the Petri dish with parafilm to prevent contamination.

4. Incubation

- Incubate the Petri dishes at 25°C–28°C in a dark or dimly lit environment.
- After 3-7 days, white mycelial growth should appear around the tissue.

5. Subculturing for Pure Culture

- Once mycelium develops, transfer a small portion of it to a fresh PDA plate using a sterile scalpel.
- Repeat the process until a pure culture of *Agaricus bisporus* is obtained.

6. Storage of Pure Culture

- Store the culture at 4°C for short-term use.
- For long-term storage, mycelium can be preserved in liquid nitrogen or on sterile wheat grains at lower temperatures.

Observations:

- Day 1-2: No visible growth.
- Day 3-5: White, cottony mycelium starts to appear.
- Day 7-10: Well-developed mycelium spreads over the PDA surface.

Activity Photographs



Fig: Isolation of Button Mushroom using tissue isolation method