CTAB- Genomic DNA Isolation

Introduction

To isolate genomic DNA from fungus we used CTAB (Cetyl Tri-methyl Ammonium Bromide). Purifying DNA using CTAB exploits that polysaccharides and DNA have different solubilities in CTAB depending on the concentration of sodium chloride. At higher salt concentrations, polysaccharides are insoluble, while at lower concentrations DNA is insoluble. Consequently, by adjusting salt concentration in lysates containing CTAB, polysaccharides and DNA can be differentially precipitated. In order to purify DNA, insoluble particulates are removed through centrifugation while soluble proteins and other materials are separated through mixing with Chloroform: isoamyl alcohol and centrifugation. DNA must then be precipitated from the aqueous phase and washed thoroughly to remove contaminating salts using 70% alcohol. The purified DNA is then re-suspended and stored in TE buffer or sterile distilled water..

Materials

- CTAB buffer- 2% CTAB (Cetyl tri methyl ammonium bromide), 1M Tris (pH 8)-10ml, 0.5M EDTA(pH 8)-4ml, 5M NaCl- 28ml make up the volume to 100ml with sterile distilled water.
- Dried Mycelium
- Liquid Nitrogen
- Microfuge tubes
- Autoclaved Mortar and Pestle
- 70 % Ethanol (ice cold)
- Isopropanol
- Chloroform: Iso-Amyl Alcohol (24:1)
- Water (sterile)
- TE buffer (0.1 X)
- Water bath
- Float rack
- Spatula
- 1.5 mL Eppendorf tube
- 70% ethanol
- Tissue paper

Procedure

1. From -81°C directly transfer the sample(mycelium) to liquid nitrogen and put spatula also in liquid nitrogen.
2. Clean mortar & pestle, spatula, and needle with ethanol.

3. Take a sample and crush it properly, add liquid nitrogen again if required.
4. Transfer it to 1.5 ml eppendorf tube.
5. Add 400 µL CTAB buffer & then vortex it & then directly keep for 5- 8 minutes in a water bath at 65℃.
6. Allow it to cool.
7. Add an equal volume of 24:1 (Chloroform: Isoamyl alcohol) and mix properly so that it dissolves.
8. Centrifuge at 13000 rpm for 10 minutes at 4℃.
9. Transfer the aqueous phase to a new Eppendorf tube,by pipetting smaller volumes.
10. Add 2/3rd volume of ice-cold Isopropanol,and mix very gently (you can see DNA precipitating).
11. Incubate at room temperature for 20 minutes.
12. Centrifuge at 13000 rpm for 20 minute at 4℃.
13. Wash the pellet with 1mL 70% Ethanol, add gently mix up & down 3 times.
14. Air dry for less than 20 minutes, it should not be dried more.
15. Add 50 µL 0.1 X TE buffer and tap gently to mix.
16. You can store Genomic DNA in -21 degree fridge.

Filtration of mycelium

Introduction

Mycelium is filtered from PDB and dried to take it for genomic DNA isolation. The sample is dried very well so that any PDB remaining is removed. Dry sample is required for the easy and efficient isolation of genomic DNA after treatment with Liq. N2.

Materials

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- Beaker
- Funnels
- Conical flasks
- Forceps
- Eppendorf
- Eppendorf stands
- Inoculated sample
- Whatman filter paper No. 1
- Ice

Procedure

1. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
2. After 15 minutes, switched off the UV, wipe your hands and the working area with 70% ethanol.
3. Take Whatman filter paper No.1
4. Warm the neck of the funnel & conical flask.
5. Put filter paper on the funnel and put it on a conical flask (250mL)
6. Filter the mycelial beads from culture broth using Whatman No. 1 filter paper.
7. Let all the broth liquid pass through.
8. Wipe the forceps with ethanol and heat them.
9. Let it cool down
10. Collect the mycelium mat from filter paper dry it as much as you can and transfer it to the Eppendorf tube and keep it on ice.
11. Heat the neck of the conical flask and add bleach to it.

12. Autoclave it & then it can be discarded.

13. You can store dried mycelium in -81 degree.

FREEZARY STOCK PREPARATION

Introduction

Fungal samples glycerol stock is made and kept in -80 degree. This is done in order to store the sample for a long time. The addition of glycerol stabilises the cell, preventing damage to the cell membranes and keeping the cells alive. In case of fungal plates getting contaminated, this glycerol stock can be used to culture fungus and perform the remaining experiments.

Materials

- 80% glycerol
- Needle
- Cryo vial
- Spirit lamp
- 70% ethanol

Procedure

1. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
2. After 15 minutes, switched off the UV, wipe the work area with 70% ethanol.
3. Add 1mL of 80% glycerol in cryo vial.
4. Warm the needle wiped with 70% ethanol
5. Once the needle cools, transfer the sample with needle in cryo vial.
6. Store cryo vial in -80 degree.

FUNGAL SAMPLE COLLECTION

Introduction

Fungal samples are collected from different places in the IISER Thiruvananthapuram campus. This is done in order to identify the fungal species present inside the campus and also to culture them to conduct various experiments. This has to be done with utmost care and protection as the air near the places with fungal contamination will be contaminated with fungal spores, and certain species can cause harmful respiratory diseases. Individuals with allergic responses are advised to refrain from taking part in sample collection, and those who are involved in collecting fungal samples should follow proper protection rules. Wearing a mask will be helpful. Plates containing Potato Dextrose Agar (PDA), a common microbiological growth medium is ideal for culturing fungus. It is made up of potato infusion, and dextrose. The nutritionally rich base (potato infusion) encourages mold sporulation.

Materials

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- PDA Plate
- Parafilm
- Vial
- 70% ethanol
- Needle
- Spirit lamp
- Tissue paper

Procedure

1. Before beginning sample collection,wear a mask, lab coat, safety goggles, and gloves.
2. Sterilise the needle by wiping with 70% ethanol and then heating it.
3. Let needle cool down taking care that it does not touch anything.
4. Scrap the fungus from the wall and collect in the vial.
5. Before taking another sample, sanitise the hands with 70% ethanol.
6. Repeat the steps from 1 to 5 for each sample collection.
7. After collection, we come to the lab with the samples. Switch on the UV light in the biosafety cabinet for about 20 minutes to sterilize the area.
8. After 20 minutes, switch off the UV, and wipe the work area with 70% ethanol.
9. Transfer the sample collected in vial into the PDA (Potato dextrose agar) plate.
10. Seal the petri plates with parafilm.

11. Label the PDA plate with appropriate information.

12. Wipe the laminar airflow with 70% ethanol after transferring.

13. Incubate the cultures for 5-6 days at 28 ± 2ºC.

Inoculation from Slant PDA to PDB

Introduction

A fungi sample is inoculated in PDB to get mycellium beads/mat. PDB without the soldifying agent agar in it will act as a perfect liquid medium for the growth of fungal mycelium beads/mat. After inocculating fungus, this PDB will be kept in the incubator shaker for 3-4 days and thus spore formation can be avoided.

Materials

- PDB
- 70% ethanol
- Tissue paper
- Spirit lamp
- Slant PDA containing fungi
- Inocculating loop

Procedure

1. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
2. After 15 minutes, switched off the UV, wipe your hands and the working area with 70% ethanol.
3. Wipe the inoculating loop with 70% ethanol
4. Red hot the inoculating loop, hold it in your right hand, grasping it a little farther back than you would a pencil.
5. Hold the test tube containing PDA with left hand and remove cotton plug.
6. The cotton plug should not lay down or touch anything, hold it with right hand in between the fingers.
7. Transfer a small quantity of the culture from the Petri plate containing the sample to 25 mL PDB.
8. Sterilize the mouth of the tube and of the conical flask containing PDB by passing them through the flame several times.
9. Close the conical flask and keep it in an incubator cum shaker at 28ºC at 150 rpm for 3-4 days until you see mycelia.
10. Put the cotton plug back into slant PDA.
11. Switch on the UV light in the biosafety cabinet for about 20 minutes to sterilize the area.

PCR

Introduction

We can amplify the genomic DNA of fungus using ITS 4 & ITS 5 primers. PCR using ITS primers targets the Internal Transcribed Spacer (ITS) region of the nuclear ribosomal repeat. These ITS primers have improved coverage across diverse taxonomic groups of fungi and thus are helpful in identifying the species.

Materials

- hyb ITS5 primer (TAATACGACTCACTATAGGGGGAAGTAGAAGTCGTAACAAGG)
- hyb ITS4 primer (ATTAACCCTCACTAAAGTCCTCCGCTTATTGATATGC)
- Master mix(origin company)
- Ice
- DNAase free water
- Genomic DNA
- PCR tube

Procedure

1. Thaw all reagents on ice.
2. Assemble reaction mix into 20 µL volume in a PCR tube.
3. Minispin mastermix and primers before adding.
4. Add reagents in the following order: water, master mix, primers and then template.

PCR Reaction Mix(20 uL)

Master mix	10ul
(10uM)hyb ITS4 primer	0.5ul
(10uM)hyb ITS5 primer	0.5ul
Dna(100ng/ul)	1ul
DNAase free water	8ul

5. Gently mix by tapping the tube. Minispin to settle tube contents.
6. Prepare negative control reactions without template DNA.
7. Prepare a positive control reaction with a template of known size and appropriate primers.

Program the thermocycler for the PCR reaction using the following steps:

Initial denaturation step-	95ºC for 5min	
Denaturation step	94ºC 30 sec	*30
Annealing step	50ºC 30 sec	*30
Extension step	72ºC 1.30min	*30
Final Extension	72ºC 10 min	
Hold	4ºC	

PCR used had a total of 30 cycle repeats for the specific product amplification

8. Analyze the results of PCR reaction via gel electrophoresis.

9. You can store PCR product at -20 degree.

PCR Clean Up_QiAGEN KIT

Introduction

This is done to purify the PCR product. After PCR, the product contains remains of primers, enzymes, and dNTPs which were present in the mixture in excess. PCR clean up is performed inorder to remove these excess particles and recover pure DNA. This procedure is necessary to ensure clean and readable DNA sequences.

Materials

- QiAGEN PCR Clean up kit
- 1.5mL Eppendorf tubes
- Water bath

Procedure

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the colour of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The colour of the mixture will turn yellow.
2. Place a QIAquick column in a provided 2 ml collection.
3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60s.
4. .Discard flow-through and place the QIAquick column back in the same tube.
5. To wash, add 600 µl Buffer PE to the QIAquick column & centrifuge for 30–60 s.
6. Discard flow-through and place the QIAquick column back into the same tube.
7. Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
8. Place each QIAquick column in a clean 1.5 ml microcentrifuge tube.
9. To elute DNA, add 25 µl Buffer EB (10 mM Tris·Cl, pH 8.5) at 70 degrees Celsius or water (pH 7.0–8.5) to the centre of the QIAquick membrane let the column stand for 1 min in a water bath at 70 degrees and centrifuge the column for 1 min. For increased DNA concentration, add 25 µl elution buffer to the centre of the QIAquick membrane, let the column stand for 1 min and then centrifuge.

PDA Plate Preparation

Introduction

Potato Dextrose Agar (PDA) is a microbiological growth media used for growing fungi. It consists of agar, and dehydrated potato infusion and dextrose that encourage the growth of fungus. Agar act as a solidifying agent. 39g PDA (HI Media) is required to make 1L media. For a 9cm (diameter) petri plate, 15-20 mL of PDA would be enough. Antibiotic is added after autoclaving and this helps to prevent bacterial contamination.

Materials

- PDA
- Petri plate
- Spatula
- MiliQ water
- 1L glass bottle
- Glass rod
- Parafilm
- Antibiotic

Procedure

Making 500mL PDA Media

1. Weigh 19.5g PDA.
2. Add it in 1L glass bottle containing 500mL MiliQ water.
3. Mix with glass rod.
4. Autoclave (remeber to keep the cap loosen for autoclaving) for 20 mins at 120 degree.
5. Wait till it reaches around room temperature.

Making PDA plate

6. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
7. After 15 minutes, switched off the UV, wiped the work area with 70% ethanol.
8. Add antibiotic as per the working concentration and mix.

9. Pour about 15-20mL PDA containing antibiotic into a petri plate.
10. Wait for 20-25 mins for PDA to get solidified.
11. Seal the petri plates with parafilm.
12. Label the PDA plate.
13. Wiped the laminar airflow with 70% ethanol.
14. You can transfer the fungus sample to the plate and keep it in the incubator or keep the PDA plate in the fridge for future use.

pda prep

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

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Procedure

1.

PDB Preparation

Introduction

Potato Dextrose Broth is PDA without agar. Potato Dextrose Broth is a general-purpose broth fot yeasts and moulds. The low pH of this medium inhibits bacterial growth. Common organisms that can be cultured on PDB are yeasts such as *Candida albicans* and *Saccharomyces cerevisae* and molds such as *Aspergillus niger*. PDB, which is devoid of agar forms the perfect liquid medium for growing fungus.

Materials

- PDA
- Filter paper
- Spatula
- MiliQ water
- glass bottle
- Glass rod

Procedure

1. Weigh PDA and add it in required MiliQ water. (39g PDA is required for 1L solution).
2. Mix gently with glass rod.
3. Filter it with filter paper twice (so that agar can remain in it) and transfer it into glass bottle of volume double to that of volume of PDB.
4. Autoclave (remeber to keep the cap loosen for autoclaving) for 20 mins at 120 degree.
5. Wait till it reaches around room temperature.
6. You can store it in fridge and add antibiotic also at the time of using.

Sanger PCR

Introduction

Sanger PCR is done as per the protocol to carry out DNA sequencing by means of capillary electrophoresis (CE) using a Applied Biosystems 3500 Genetic Analyzer. Only a single primer is used while performing Sanger PCR. In Sanger PCR, all ddNTPs are mixed in a single reaction, and each of the four dNTPs has a unique fluorescent label.

Materials

- Sequence buffer
- Sequence mix Big dye
- Sequencing primer 10mM
- Template (purified)
- Deionized (MQ) H_2O
- PCR tube

Procedure

1. Thaw all reagents on ice.

2. Assemble reaction mix into 10 µL volume in a PCR tube.

Sequence Mix

Volume Per Reaction (µL)	1X
deionized (MQ) H2O	4.75 (adjust to final volume)
Sequence buffer	1.75
Sequence mix Big dye	0.5
Sequencing primer 10uM	1
Template (purified)	2 (adjusted to conc.)
Total	10

BigDye Cycling Condition

Incubate	96 degree 1 min
Denature	96 degree 10s
Anneal	50 degree 5s
Extend	60 degree 4min
Hold	4 degree

3. You can store product in -20 degree fridge.

Sequencing reaction Clean Up

Introduction

Ethanol/EDTA Precipitation method is used for sequencing reaction clean up. It is is done as per the protocol to carry out DNA sequencing by means of capillary electrophoresis (CE) using an Applied Biosystems 3500 Genetic Analyzer.

Materials

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- 100% ethanol
- 70% ethanol
- EDTA
- Nuclease free water
- 96-well reaction plate
- injection buffer (10µL HiDi Formamide)
- aluminium tape

Procedure

To precipitate 20µL sequencing reactions in 96-well reaction plates:

1. 10µL of nuclease-free water can be added to the PCR mixture for making the volume to 20µL.
2. Remove the 96-well reaction plate from the thermal cycler and briefly spin.
3. Add 5µL of 125 mM EDTA to each well. Note: Make sure the EDTA reaches the bottom of the wells.
4. Add 60µL of 100% ethanol to each well.
5. Seal the plate with aluminium tape and mix by inverting 4 times.
6. Incubate at room temperature for 15 min.
7. Spin in a plate centrifuge for 30 min at 3000g. (Alternatively, in case of limiting maximum speed, spin for 45 minutes at 2200 g)
8. Invert the plate and spin up to 185g for 1 minute, then remove from the centrifuge. (Provide a cushion of three-four tissue layers in the plate holder for absorbing the decanted ethanol)
   Note: Start timing when the rotor starts moving.
9. Add 60µL of 70% ethanol to each well
10. With the centrifuge set to 4°C, spin at 1650 g for 15 min.

11. Invert the plate and spin up to 185 × g for 1 min, then remove from the centrifuge. Note: Start timing when the rotor starts moving.

12. To continue, resuspend the samples in the injection buffer (10µL HiDi Formamide), cover with septa, denature(95 degrees for 2mins), snap chill(4 degrees) and proceed for electrophoresis/ Sanger sequencing.

13. To store, cover with aluminium foil, and store at 4 °C.

Slant PDA Preparation

Introduction

Potato Dextrose Agar(PDA) is a microbiological growth media used for growing fungi. It consists of dehydrated potato infusion and dextrose that encourage the growth of fungus. Agar act as a solidifying agent. 39g PDA(HiMedia) is required to make 1L media. Pure colony of fungus are maintained in slant PDA to reduce the flow of spores inside the biosafety cabinet while working on the fungi.

Materials

- PDA
- Autoclaved test tube with cotton plug
- Spatula
- MiliQ water
- 1L glass bottle
- Glass rod
- Autoclaved cotton plug
- Antibiotic

Procedure

Making 500mL PDA Media

1. Weigh 19.5g PDA.
2. Add it in 1L glass bottle containing 500mL MiliQ water.
3. Mix with glass rod.
4. Autoclave (remeber to keep the cap loosen for autoclaving) for 20 mins at 120 degree.\
5. Wait till it reaches around room temperature

Making slant PDA

6. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
7. After 15 minutes, switched off the UV, wiped the work area with 70% ethanol.
8. Add antibiotic as per the working concentration and mix.

9. Pour about 10-15mL PDA containing antibiotic into a test tube.
10. Keep test tube slanted and wait for 20-25 mins for PDA to get solidified.
11. Put the cotton plug on slant PDA.
12. Label the slant PDA.
13. Wiped the laminar airflow with 70% ethanol.
14. You can transfer the fungus sample to the plate and keep it in the incubator or keep the slant PDA in the fridge for future use.

Slide preparation of Fungal Samples

Introduction

Cotton blue in lactophenol stain is commonly used for making semi-permanent microscopic preparations of fungi. It stains the fungal cytoplasm and provides a light blue background, against which the walls of hyphae can readily be seen. The stain contains four constituents: (i) phenol, which serves as a fungicide; (ii) lactic acid, which acts as a clearing agent; and (iii) cotton blue, which stains the chitin of the fungus; and (iv) glycerine, which gives a semi-permanent preparation. A permanent preparation may be made by incorporating polyvinyl alcohol in place of glycerine into the mounting medium. Lactophenol alone (without cotton blue) may be used in the case of dark coloured fungi.

Materials

- Fungal Culture
- Lactophenol Blue (Cotton Blue) Stain
- Mounting needle
- Spirit lamp
- Cover Slips
- Compound Microscope
- Nail clear varnish

Procedure

1. Place a drop of cotton blue in lactophenol on a clean glass slide.
2. Transfer a small tuft of a well-sporulated fungus into the drop, using a flamed, cooled mounting needle.
3. Gently tease the material using the two mounting needles.
4. Mix gently the stain with the fungal material
5. Place a coverslip over the preparation, taking care to avoid trapping air bubbles in the stain. Gentle pressure can be applied to the coverslip by tapping with the back of a mounting needle or pencil in order to spread the fungal structures and expel air bubbles.
6. Examine the preparation under low–power (X10 and X20) and high-power (x40) objectives.

Preservation of the Slide Preparations

7. Seal the coverslip with nail clear varnish as follows:

   a. Remove all air bubbles from the preparation by pressure, gentle heating or addition of more cotton blue in lactophenol.
   b. Remove the excess stain from around the coverslip with 70% alcohol on a cotton swab or with blotting paper.

c. Apply a thin layer of nail varnish around the edge of the coverslip.

d. Allow the preparation to dry overnight.

e. Apply a second coat of nail varnish over the first coat.

Spore Suspension Preparation

Introduction

Fungal spores are microscopic particles produced by fungi and help fungi in reproduction. Spore suspension helps in collecting spores separately, without a mycelium and is used for various experiments. The spore suspension may contain millions of spores per ml. With the help of a hemocytometer, one can count the number of spores present per ml of the suspension. The suspension is prepared in PBS, which is a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, in some formulations, potassium chloride and potassium dihydrogen phosphate. PBS helps to maintain a constant pH and is ideal for cells as it is non-toxic and isotonic to cells.

Materials

- Fungal culture
- Blades or Scalpel
- 50 mL Falcons tubes
- 1X PBS
- Sterile gauze
- Watman filter paper No. 2 or Cheesecloth

Procedure

1. Scrap the PDA medium containing fungal culture using sterile No. 21 blades and place in a clean 50 mL falcon tube.
2. Add 20 mL PBS to the 50 mL tube and vortex for 5 min to disperse the spores.
3. Filter the obtained mixture through two layers of sterile gauze to remove the mycelia.
4. Fold 2-3 layers Watman filter paper No. 2 (Advantec, Toyo, Kaisha, Japan) or cheesecloth and place it in a sterile falcon tube. Pass the solution obtained above through the filter paper to remove further debris.
5. Collect the filtrate and store at 28 degree.

Subculture into PDA Plates

Introduction

Fungal samples collected from walls that were cultured in perti plate are subcultured in PDA plates to isolate pure colony. This is also done to manitain culture by avoiding overgrowth.

Materials

- Inocculating loop
- 70% ethanol
- Tissue paper
- Spirit lamp
- PDA plate

Procedure

1. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
2. After 15 minutes, switched off the UV, wipe the work area with 70% ethanol.
3. Wipe the inoculating loop with 70% ethanol
4. Red hot the inoculating loop and then let it cool.
5. Transfer a small quantity of the culture from the Petri plate containing the sample to the PDA plate.
6. The lid of the agar plate should be lifted just enough for the sample to be placed in the middle of the fresh agar medium surface.
7. Red hot the inoculating loop and wipe it with 70% ethanol.
8. Seal petri plates with parafilm and keep in an incubator (28ºC).
9. Wipe the work area with 70% ethanol solution.
10. Switch on the UV light in the biosafety cabinet for about 20 minutes to sterilize the area.

Subculture into Slant PDA

Introduction

Pure colony of fungi is maintained in slant PDA. When fungus is grown in slant PDA, the area in contact with air will be less, as slant PDA is prepared in test tube and thus chances of contamination and spreading of spores in air is less. Slant PDA is easy to handle and the mouths can be decontaminated easily by showing towards a flame.

Materials

- Inocculating loop
- 70% ethanol
- Tissue paper
- Spirit lamp
- Slant PDA

Procedure

1. Switched on the UV light in the biosafety cabinet for about 15 minutes to sterilize the area.
2. After 15 minutes, switched off the UV, wipe the your hands and work area with 70% ethanol.
3. Wipe the inoculating loop with 70% ethanol
4. Red hot the inoculating loop, hold it in your right hand, grasping it a little farther back than you would a pencil.
5. Hold the test tube containing PDA with left hand and remove cotton plug.
6. The cotton plug should not lay down or touch anything, hold it with right hand in between the fingers.
7. Transfer a small quantity of the culture from the Petri plate containing the sample to the slant PDA.
8. Sterilize the mouth of the tube by passing them through the flame several times.
9. Touch the loop to the top of the slant to cool it.
10. Pick a small quantity of fungus culture with a loop from stock culture and insert the loop into the sterile tube.
11. Streak the loop back and forth from the bottom to the top of the slant.
12. Withdraw the loop and flame the mouths of the tube.
13. Put the cotton plug.
14. Red hot the inoculating loop and wipe it with 70% ethanol.

15. Wipe the work area with 70% ethanol solution.

16. Keep slant PDA containing fungus sample in an incubator (28ºC).

17. Switch on the UV light in the biosafety cabinet for about 20 minutes to sterilize the area.

Zone of Inhibition

Introduction

Zone of inhibition experiment was done to see the antifungal activity of chitinase. Wells were created inside the PDA plate and then, added chitinase of desired concentration/concentrations. This will help to calculate the inhibition caused by the chitinase on the growth of fungus. This is the best experiment to identify antifungal activity and growth of chitinase can be seen clearly.

Materials

- PDA Plate
- Pipette tip
- Parafilm
- L-rod

Procedure

1. Spread spore suspension on PDA plate using heat sterlised L-rod.
2. Keep plates in incubator overnight
3. Make holes in PDA plate using pipette tip.
4. Add chitinase in the wells.
5. Parafilm the plates and keep at 37 degree.
6. Observe zone of inhibtion after 4-7 hours (depending on fungus).