INTERNSHIP REPORT ON COMPOSTING

November Batch Composting (Group D)

30th June, 2022
Mumbai.

Louis Pasteur once said that, “not even death is complete without fungi", Fungi plays quite an important role in our day to day lives, right from producing cheese and other food products to decomposing the dead organic matter to simpler constituents, which are then taken up by plants. This phenomenon is called composting. Composting can be carried out in two ways; it can be aerobic and anaerobic the difference in these two is the use of oxygen in the process. During the initial period of internship, we were briefed about the process of composting. The process of composting began with inoculation of *Trichoderma* species in a litter of leaves, the leaves were finely crushed and were filled in drums, the drums were watered regularly, this was done to check the time taken by the fungi to reduce the leaves to compost. Two different drums one as July batch drum (as it consisted *Trichoderma* in powdered form which was made in July) and the other drum consisted of November batch drum (as it consisted *Trichoderma* in powdered form which was made in November) as well as a basket for control was kept separate(This did not contain the *Trichoderma* was inoculated in basket) , Student interns were divided into 5 groups out of which 2 groups were supposed to look after July drum, 2 groups to look after November drum and 1 group to look after the control basket. Student interns inoculated some microbes into the sorghum.

COMPOSTING OF NOVEMBER BATCH DRUM – Student interns collected the sample from the drums at an interval of every 5 days and prepared saline suspensions the suspension was then streaked on petri plates of different media PDA(Potato Dextrose Agar), Luria Broth Agar, BENNETS, and were kept for incubation at Room Temprature for 48hrs.

We learnt about the media composition and its preparation, the composition of the different media used are mentioned below:

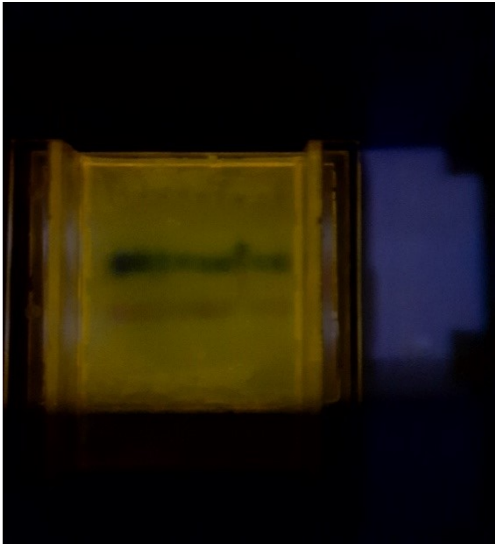
- 1)Potato dextrose agar: boil 200gm sliced peeled potatoes in 1L D/W for 30mins, filter thru cheese cloth, add 20gm dextrose, make volume to 1liter, adjust pH to 5.6+-0.2, add agar 20gm, digest and autoclave.
- 2) Potato carrot extract medium 200g potatoes washed and sliced, 200gm carrot washed and sliced, boil in 1L for 30mins, collect infusion, make to 1L, check pH, add agar 20gms, autoclave.

- 3) Kenknight medium (actinomycetes) glucose 1gm, monopotassium phosphate 0.1g, KCl 0.1g, Mason 0.1g, $NaNO_3$ 0.1g, Dr 1L, pH 7.0 to 7.2 agar 15gm.
- 4) Bennetts agar(actinomycetes) YE 1gm, BE 1gm, casamino acids 2g, glucose 10g, pH 7.3, agar 15gm.
- 5) L.B. Agar (bacteria) NaCl 10gm, Peptone: 10gm, Yeast extract: 5gm, Distilled water 1L.

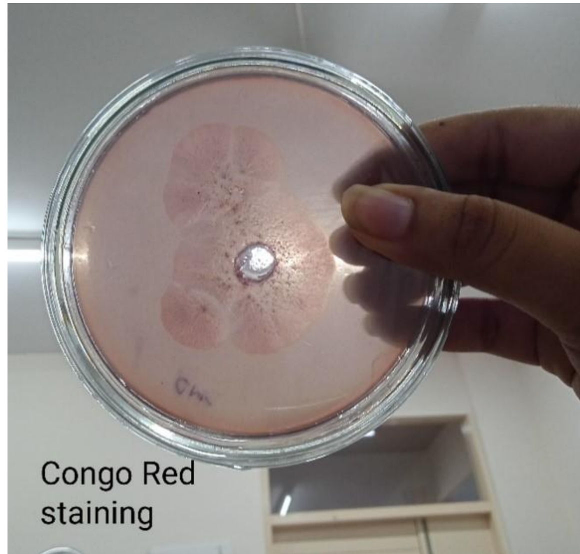
During the course of our internship, we learnt and performed new techniques. Electrophoresis, DNA isolation, Preparation of media and Slide culture. In slide culture technique wherein the interns inoculated the fungal colony picked up from the petri plate in the middle while placing 3 cover slips at 45-degree angle which were further incubated and stained by LPCB. Species that were observed after isolation, lactophenol or gram staining are as follows:- *Rhizopus*(sporangium), *Aspergillus*(conidiosporangium), *Actinomycetes*, *Penicillium*, Gram negative bacilli, Gram positive filaments, Gram positive coccoid and Gram positive rods were found. Student interns also inoculated fungus on CMC (carboxylmethylcellulose) and Lignin wherein we performed we used techniques like T-streaking, spot inoculation and punch method were used.

After the isolation procedures and identification media, we were briefed on the technique of DNA isolation and we were given hands-on training for the same. Student interns selected one of the fungal colonies from the petri plate to be used for DNA isolation, the colonies which were isolated were added to Eppendorf tube along with CTAB detergent and churned well, thereafter the tube was put up for centrifuge and the supernatant was separated into another Eppendorf tube and thereafter it was subjected to purity test by checking the absorbance in spectrophotometer. After which the student interns performed agarose gel electrophoresis wherein the DNA was run on agarose gel with help of buffer and electric current, after electrophoresis.

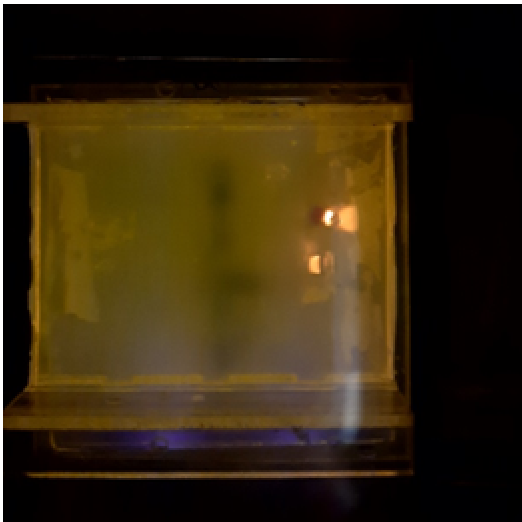
Inoculation of ***Trichoderma***



Gel in UV transilluminator

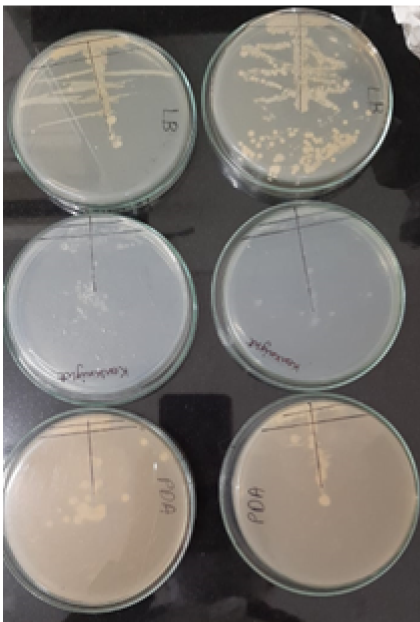


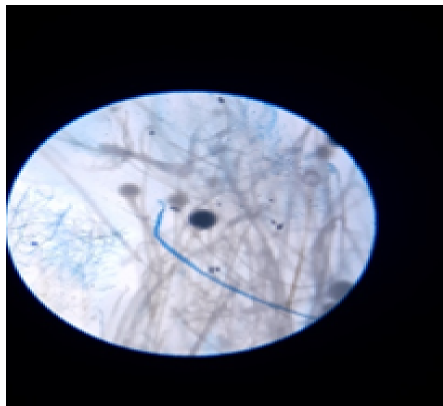
Congo Red staining



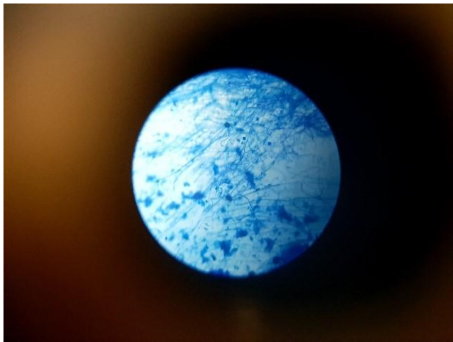
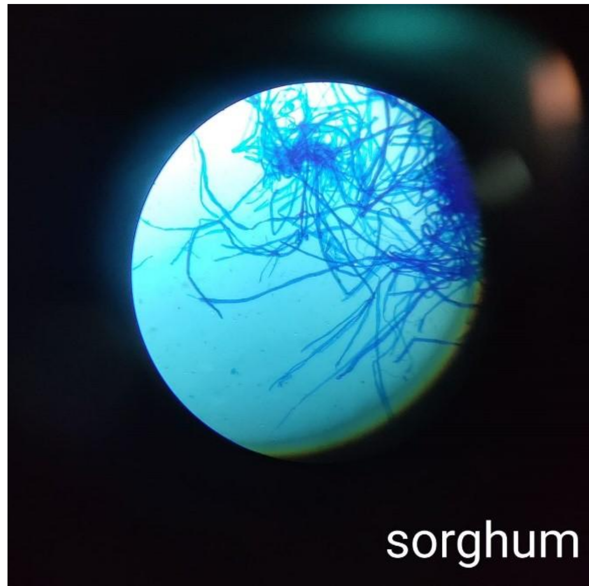
Fungus and actinomycetes in LB agar

DNA isolation procedure





PDA, Keknight, LB petri plates



sorghum