WEEK 2

Date : 11.06.21

ISOLATION OF GENOMIC DNA: CTAB (2,2*,3)

Protocol:

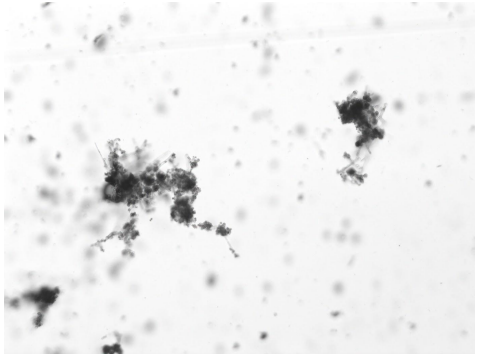
1.From -81℃ directly transfer the sample to liquid nitrogen and put spatula also in liquid nitrogen.
2.Clean mortar & pestle, spatula, and needle with ethanol.
3.Take a sample and crush it properly add liquid nitrogen again and again if required.
4.Transfer it to 1.5 ml eppendorf tube.
5.Add 400 µL CTAB buffer & then vortex it & then directly keep for 5- 8 minutes in a water bath at 65℃. Allow it to cool.
6.Add an equal volume of 24:1 (Chloroform: Isoamyl alcohol) mix properly so that it dissolves properly.
7.Centrifuge at 13000 rpm for 10 minutes at 4℃.
8. Transfer the aqueous phase to a new Eppendorf tube, take baby steps.
9.Add 2/3 volume of ice-cold Isopropanol and mix very gently you can see DNA precipitating.
10.Incubate at room temperature for 20 minutes.
11.Centrifuge at 13000 rpm for 20 minute at 4℃.
12. Wash the pellet with 1mL 70% Ethanol, add gently mix up & down 3 times.
13.Air dry for less than 20 minutes, it should not be dried more.
14.Add 50 µL 0.1 X TE buffer and tap gently to mix.

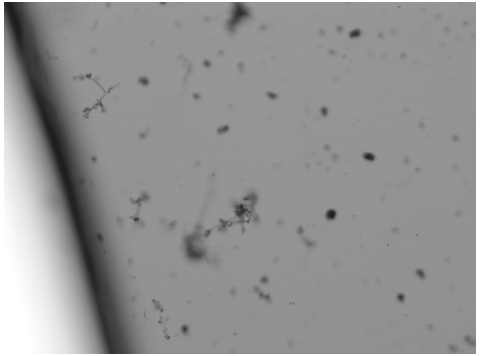
NANODROP RESULT

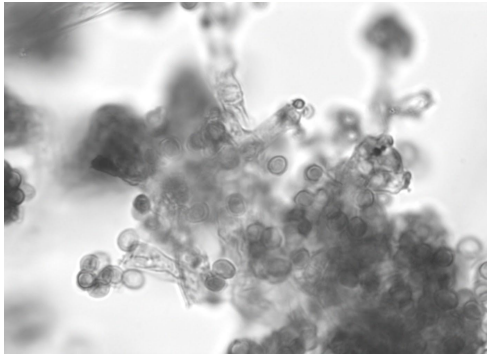
S.No.	Concentration	A260/A280	A260/A230
2*	1163.7	1.97	1.91
2	15	1.85	1.57
3	1382.2	2.15	1.95

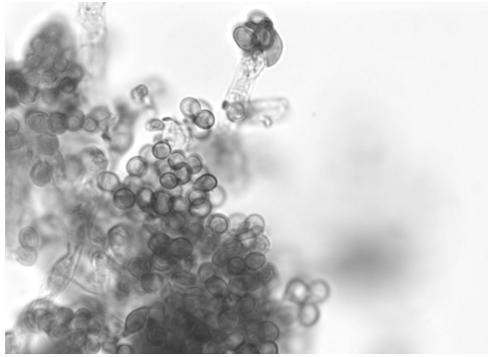
Date : 11.06.21

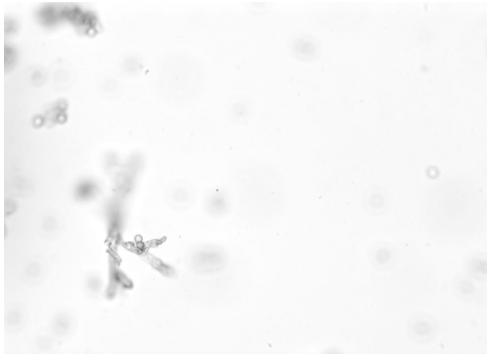
OBSERVED C4 UNDER BRIGHT FIELD MICROSCOPE





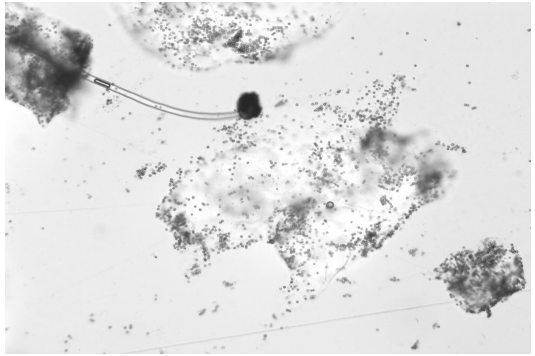


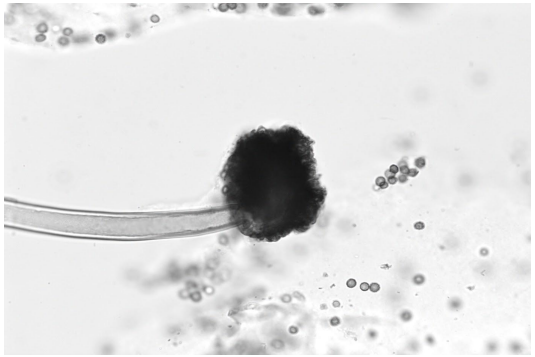


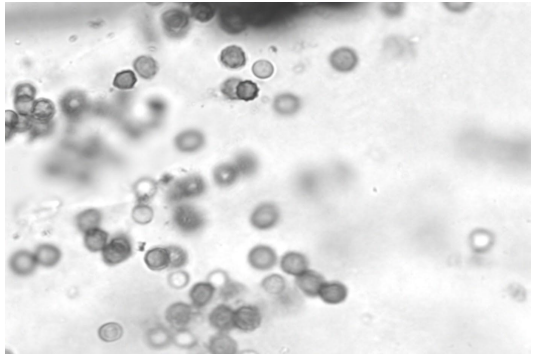


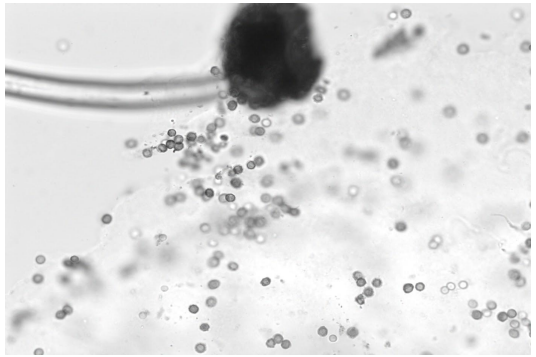
Date : 11.06.21

OBSERVED C16 UNDER BRIGHT FIELD MICROSCOPE



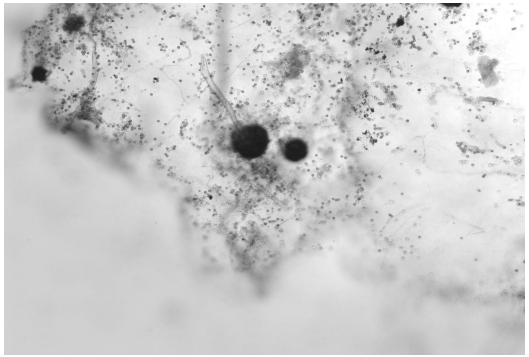


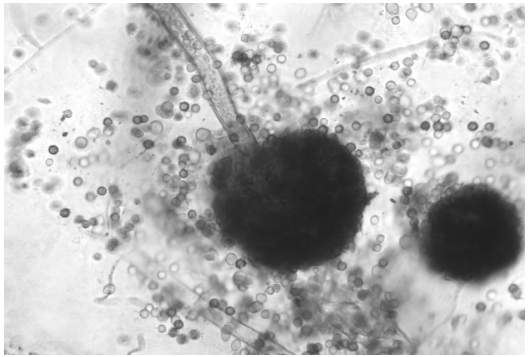


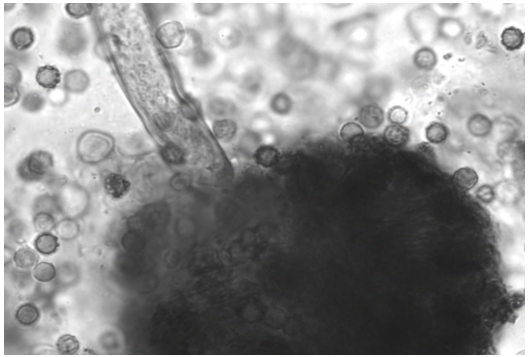


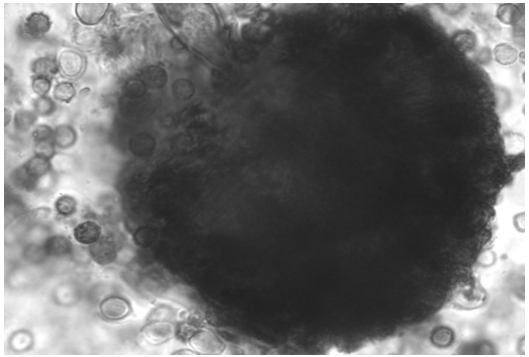
Date : 11.06.21

OBSERVED C18 UNDER BRIGHT FIELD MICROSCOPE









Date : 12.06.21

ISOLATION OF GENOMIC DNA: CTAB (1,4,4*)

Protocol:

1.From -81℃ directly transfer the sample 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 and 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℃. Allow it to cool.
6.Add an equal volume of 24:1 (Chloroform: Isoamyl alcohol) mix properly so that it dissolves properly.
7.Centrifuge at 13000 rpm for 10 minutes at 4℃.
8. Transfer the aqueous phase to a new Eppendorf tube, take baby steps.
9.Add 2/3 volume of ice-cold Isopropanol and mix very gently you can see DNA precipitating.
10.Incubate at room temperature for 20 minutes.
11.Centrifuge at 13000 rpm for 20 minute at 4℃.
12. Wash the pellet with 1mL 70% Ethanol, add gently mix up & down 3 times.
13.Air dry for less than 20 minutes, it should not be dried more.
14.Add 50 µL 0.1 X TE buffer and tap gently to mix.

NANODROP RESULT

S.No.	Concentration	A260/A280	A260/A230
1	1207.4	2.09	1.81
4	2988.5	2.19	2.26
4*	4676.2	2.29	2.43

4 & 4* were from same sample

Date : 15.06.21

PCR OF 1,2,3,4,4* SAMPLE

Protocol:

Master Mix	10 uL
10mM FP	0.5 uL
10mM RP	0.5uL
Template(100-150 ng/uL	1 uL
Water	8.5 uL
Total	20 uL

Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

NANODROP RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
1	436.1	1.81	2.2
2*	439.1	1.81	2.21
3	416.1	1.8	2.18
4	424.7	1.81	2.21