WEEK 3

Date : 15.07.21

SANGER PCR of 2,4,6,7

2.2. BigDye V3.1 - Reaction Setup

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 10mM	1
Template (purified)	2 (adjusted to conc.)
Total	**10**

2.3. BigDye Cycling Condition

Parameter	**Stage/step**				
	Incubate	**25 cycles**			**Hold**
		Denature	**Anneal**	**Extend**	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	01:00	00:10	00:05	04:00[1]	Hold until ready to purify.

[1] Shorter extension times can be used for short templates.

Date : 16.07.21

SEQUENCING REACTION CLEAN UP

Ethanol/EDTA Precipitation

To precipitate 20µL sequencing reactions in 96-well reaction plates: Note: 10µL of nuclease free water can be added to the PCR mixture for making the volume to 20µL.

1.Remove the 96-well reaction plate from the thermal cycler and briefly spin.

2.Add 5µL of 125 mM EDTA to each well. Note: Make sure the EDTA reaches the bottom of the wells.

3.Add 60µL of 100% ethanol to each well.

4.Seal the plate with aluminium tape and mix by inverting 4 times.

5.Incubate at room temperature for 15 min.

6.Spin in a plate centrifuge for 30 min at 3000g. (Alternatively, in case of limiting maximum speed, spin for 45 minutes at 2200 g)

7.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.

8.Add 60µL of 70% ethanol to each well

9.With the centrifuge set to 4°C, spin at 1650 g for 15 min.

10.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.

11.To continue, resuspend the samples in the injection buffer (10µL HiDi Formamide), cover with septa, denature, snapchill and proceed for electrophoresis. To store, cover with aluminium foil, and store at 4 °C.

Date : 16.07.21

SANGER SEQUENCING

Sample Files	Sample Files With QV	Low QV	Med QV	High QV
4	4	< 15	>= 15 and < 20	>=20

Length of Read (LOR): AverageQV of 20 bases >= 20

Low LOR = 0-300	Medium LOR = 301-500	High LOR > 500
Samples with low LOR = 1	Samples with medium LOR = 0	Samples with high LOR = 3

Sample Details

Sample File Name	BC Status	PP Status	Well	Cap #	Peak 1	Base Spacing	# Low QV	# Med QV	# High QV	Sample Score	LOR	'A' S/N	'C' S/N	'G' S/N	'T' S/N	Avg S/N	CR Start	CR Stop
B03_2_02	◆	N/A	B03	2	1020	13.25	54	6	2	9	0	72	53	51	54	58	N/A	N/A
C03_4_03	◆	N/A	C03	3	1031	10.19	26	7	621	44	649	202	212	218	237	217	N/A	N/A
D03_6_04	■	N/A	D03	4	919	9.5	24	3	632	55	654	309	285	262	282	284	N/A	N/A
E03_7_05	■	N/A	E03	5	987	9.98	48	7	586	52	609	208	276	237	200	230	N/A	N/A

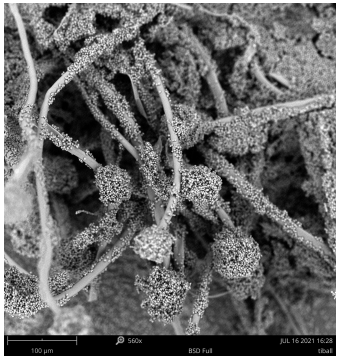
Legend: Success ■ Success with warnings ◆ Failed Analysis △ System Error ●

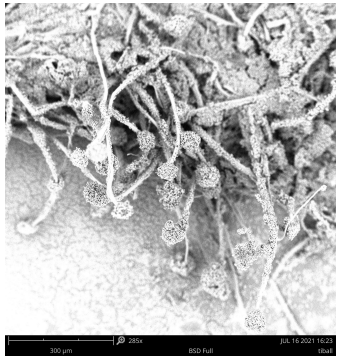
RESULT

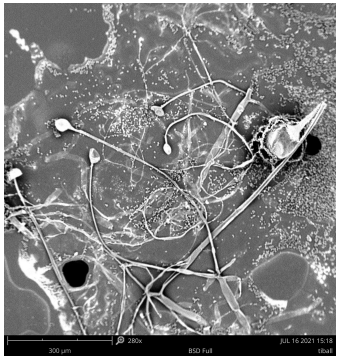
SAMPLE	IDENTIFIED AS
1	Aspergillus versicolor(looks same as 8th)
2	Aspergillus niger (on basis of morphology)
3	**Rhizopus oryzae**
4	**Phanerochaete sordida**
5	**Fusarium solani**
6	**Nodulisporium indicum**
7	**Trichoderma virens/Trichoderma harzianum**
8	**Aspergillus versicolor**

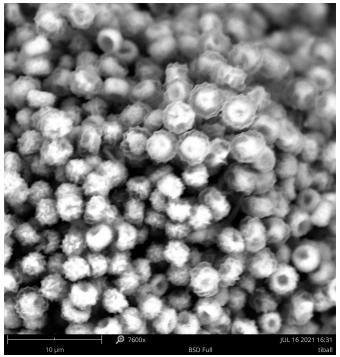
Date : 16.07.21

OBSERVED Aspergillus Niger UNDER SEM









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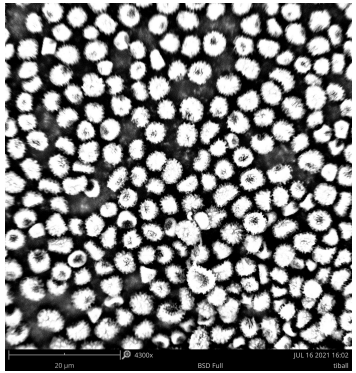
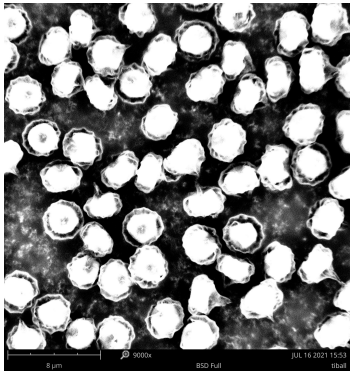
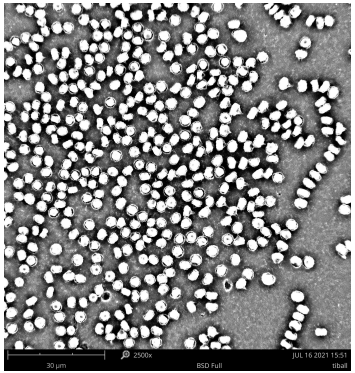
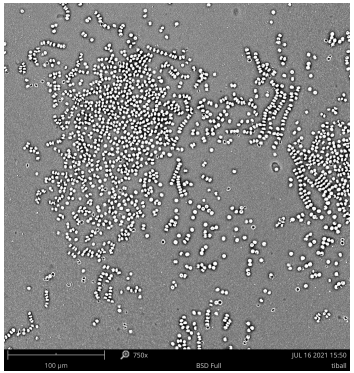
OBSERVED Aspergillus versicolor UNDER SEM









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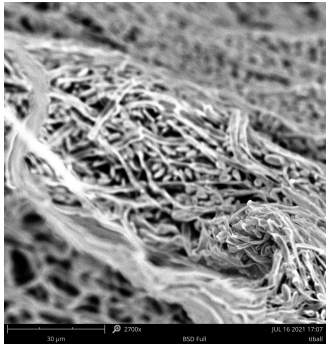
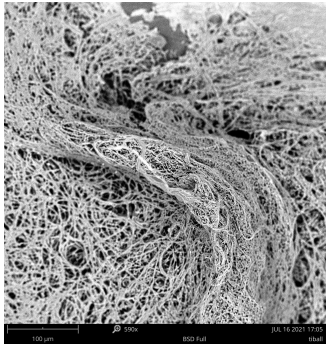
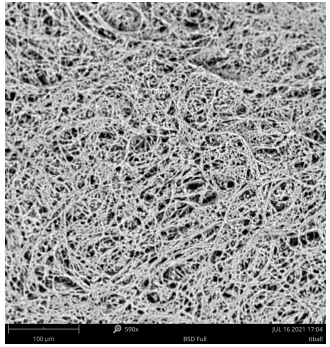
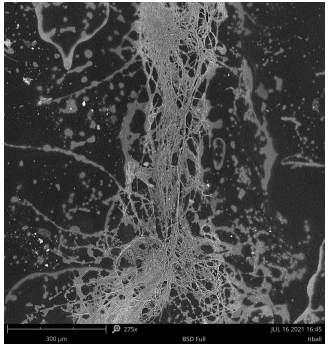
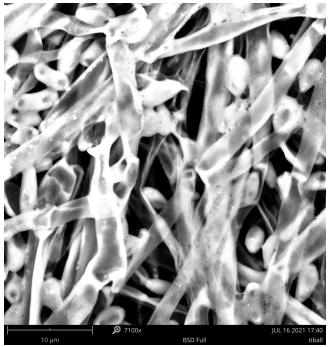
OBSERVED Fusarium solani UNDER SEM

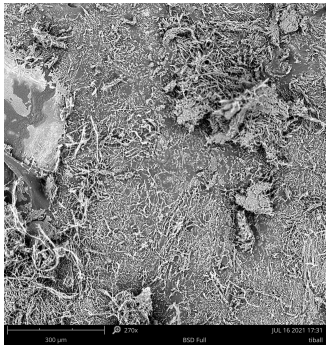


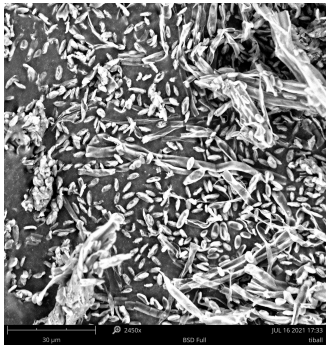


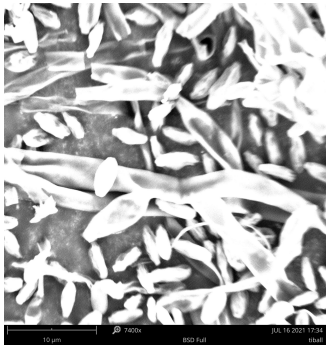


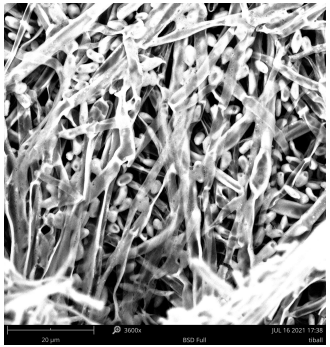












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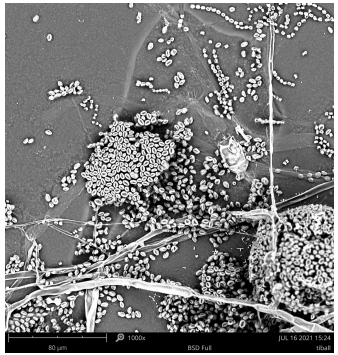
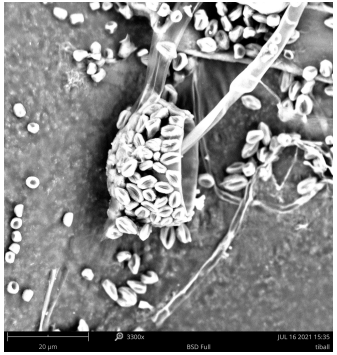
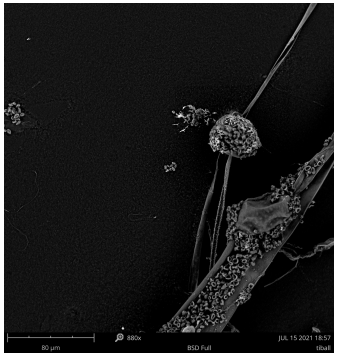
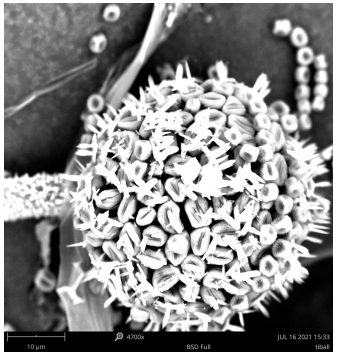
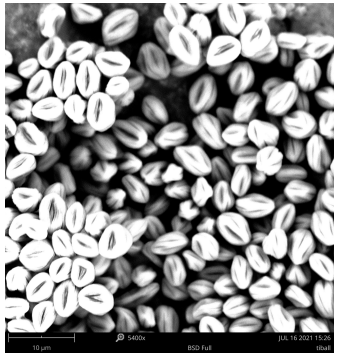
OBSERVED Rhizopus oryzae UNDER SEM

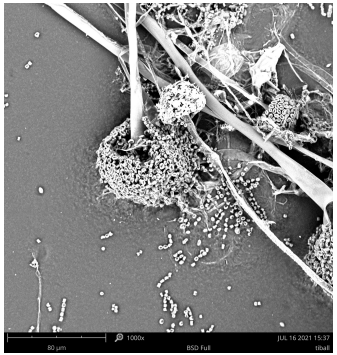


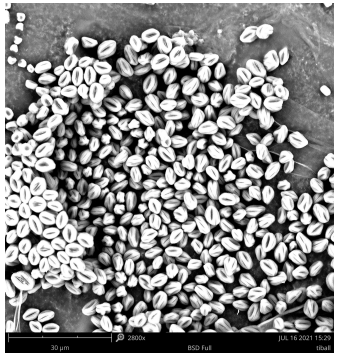


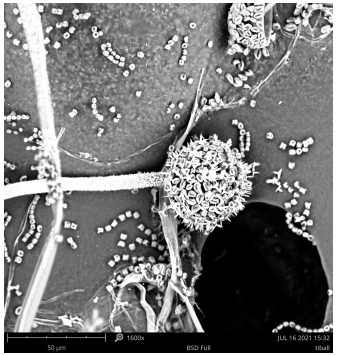












SAMPLE	IDENTIFIED AS	WORKING SAMPLE	STOCK SAMPLE	GLYCEROL STOCK	SLIDE
2	Aspergillus niger (on basis of morphology)	F14 & F16		B21	BF,CF
3	**Rhizopus oryzae**	F 18	PETRI PLATE	B22	BF,CF, SEM
4	**Phanerochaete sordida**	F19		B20	BF
5	**Fusarium solani**	G5 & F1 & F5	1	A1, B1	CF,SEM
6	**Nodulisporium indicum**	F2 & F3	3	A3,B3	DIC
7	**Trichoderma**	F4 & F10	PETRI PLATE & 4	A4 ,B4	DIC,BF,CF
8	**Aspergillus versicolor**	G8 & F8 & F7	SS1	A8, B8	DIC, SEM

SUBCULTURE OF FUNGI

DATE	WORK
22/07/21	Made 12 slant PDA
26/07/21	Subculture of 3,4,5
26/07/21	Glycerol stock preparation of 2,3,4