16/06/2021 - Wednesday

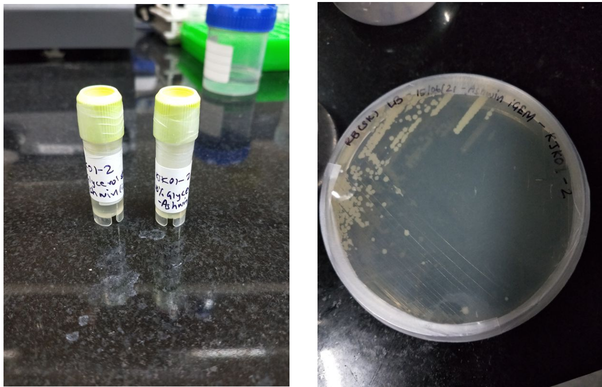
E. coli

Stock preparation

- Previously 12 stocks were made by Rushik
- Made 4 10% glycerol stock of BuOH E. coli strain KJK01 from Agar stab - 2

Results

- The E coli strain whose stocks were prepared by Rushik did not contain the genes for butanol synthesis, hence the strains were re-ordered.



12/07/2021 - Monday

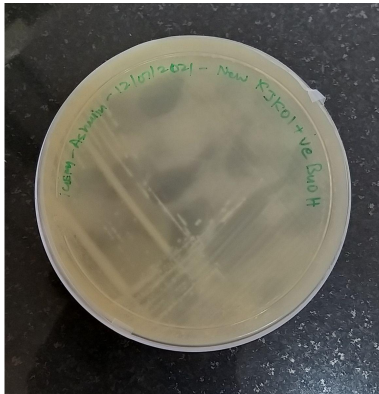
E. coli

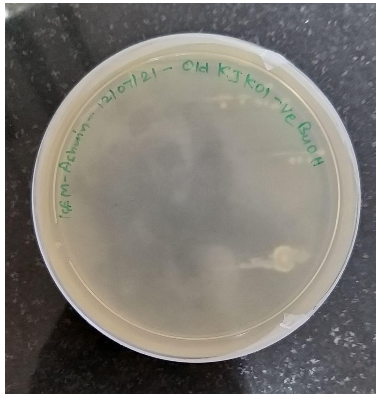
Reviving E. coli

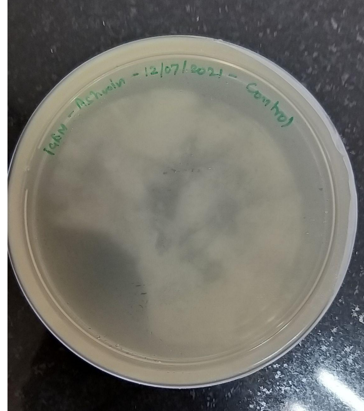
- Prepared LB broth: 5g in 200 ml
- LB+5 test tubes given for autoclaving
- Prepared three agar plates
- Streaking done for the old (non-butanol producing) and new (butanol producing) E coli strains, with one plate as control.
- Kept in incubator at 37 C at 4 pm

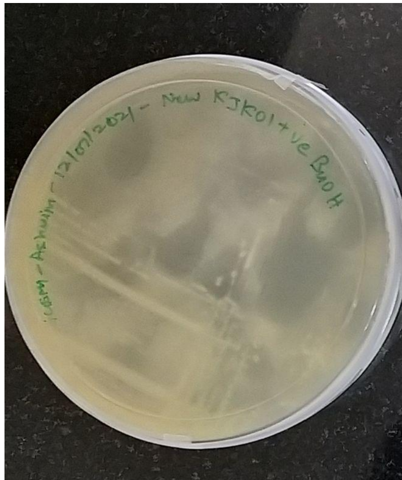
Results

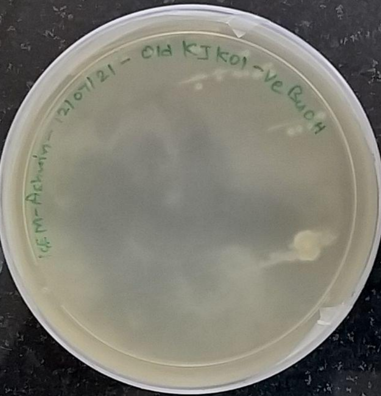
- Colony observed at 9 am July 13, 2021, by Ashwin.
- Control plate - clear
- Plates placed in the freezer.

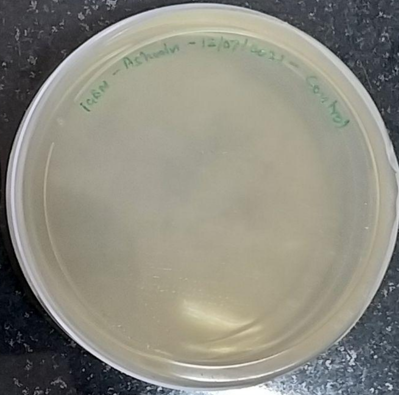












13/07/2021 - Tuesday

E. coli

TB medium preparation

Composition

- Tryptone - 1.2%
- Yeast Extract - 2.4%
- Glycerol - 0.5%

For 100 ml

- Tryptone - 1.2 g
- Yeast extract - 2.4 g
- Glycerol - 0.5 g

90 ml distilled water added to this

10x TB salts

Composition • 0.17M KH_2PO_4 (monopotassium phosphate) • 0.72M K_2HPO_4 (dipotassium phosphate) For 100 ml • KH_2PO_4 - 2.31g • K_2HPO_4 - 12.54g 10 ml of distilled water added to this.

Both TB medium and TB salts given for autoclaving

Inoculation

Done at around 12:00 AM 14/07/2021 by Ashwin

- Place media components under hood and leave under UV for 15 mins
- Mix the two components
- Extract a colony from the plates using a pipette tip and swirl the tip in the medium
- Place the flask with the inoculated media in the incubator

Results

- After nearly 12 hours, no growth was observed

14/07/2021 - Wednesday

E. coli

Genome extraction preparation

Reagents needed

- TE buffer/ EB buffer
- 10% SDS (100ml)
- 20mg/mL Proteinase K (5ml)
- Phenol-Chloroform Isoamyl alcohol (50 ml)
- 3M sodium acetate, pH adjusted to 5.2 with glacial acetic acid (50ml)
- Isopropanol 50ml
- 70% ethanol

Reagent preparation

- SDS - 10g on 100 ml (prepared two such solutions)
- Proteinase K - 100mg in 5 ml
- Sodium acetate - 12.3g in 50 ml (25ml was prepared, volume adjusted later with pH)

pH wasn't adjusted

15/07/2021 - Thursday

E. coli

pH adjustment

- pH meter at G1 was calibrated

- Adjusting pH :
    - Measure pH of the 25ml sodium acetate solution
    - Keep adding glacial acetic acid till the desired pH is achieved
    - Add distilled water to the final volume (50ml)

Result

- 50 ml acetate buffer with pH 5.01

TB preparation

- The culture that was inoculated on July 14, 2021, had shown no growth. It was discarded and TB medium was prepared again and sent for autoclaving.

Result

- No growth observed on July 17, 2021

20/07/2021 - Tuesday

E. coli

Plating E coli

- Prepared 4 agar plates
- Streaking done on two plates- 1 Butanol producing strain and 1 non-butanol producing strain

Result

- Growth observed on plates in around 14 hrs
- LB medium was inoculated but no growth was observed.
- Rushik inoculated LB from the main building and growth was observed, implying LB in G1 was contaminated.

21/07/2021 - Wednesday

- Wetlab access denied for two weeks due to administrative reasons. Ashwin available for background work.

31/07/2021 - Saturday

E. coli

- Plasmid extraction of pcscX done
    - Protocol can be found here
- Purified plasmid kept with Rushik

02/08/2021 - Monday

E. coli

- Genome extraction of E coli KJK01 done by Ashwin and Rushik

- gDNA stored at Rushik's lab

03/08/2021 - Tuesday

- Wetlab resumes following approval from administration.

E. coli

PCR to identify butanol gene 1

- Collected pelleted genome from Rushik
- The pelleted genome was resuspended in EB buffer (50 µL).
- Concentration of suspension determined using nanodrop. Vial A has concentration of 3.5 µg/µL and vial B has 3.4 µg/µL.
- Primers stock is 100 µM. We made a 20 µM solution for each primer.
- We made a 50 µL solution for PCR and split it into 2.

PCR reagents

Reagents	**Volume for 25 µL solution (in µL)**	**Volume for 50 µL solution (in µL)**
dH_2O	16.5	33
Buffer (10x)	2.5	5
F primer (20 µM)	1.25 (1 µM)	2.5 (1 µM)
R primer (20 µM)	1.25 (1 µM)	2.5 (1 µM)
dNTPs (100 mM)	0.25 (1 mM)	0.5 (1 mM)
Pfu Polymerase	0.5	1
Templates (3.5 µg/µL)	0.5	1

- PCR cycle - 95°C-3' - 95°C-30'' - 56°C-30'' - 74°C-1' - 74°C-10' - 4°C (Hold) [30x]
- PCR took around 1 hr 15 mins
- Prepared 30 ml agar gel for electrophoresis (0.8% agarose, 0.24 g in 30ml TAE + 1 µL Ethidium bromide
- After heating agar, we poured it in the plate for solidifying
- Loaded PCR - 1 kb ladder, 10 µL PCR product with 2 µL of loading dye, 10 µL PCR product with 2 µL of loading dye
- Gel imaged at main building

Result

- Gene of interest not observed
- The amount of dH2O added was less

04/08/2021 - Wednesday

E. coli

PCR to identify butanol gene 2

- gDNA was diluted further by adding 50 µL of EB buffer

PCR reagents

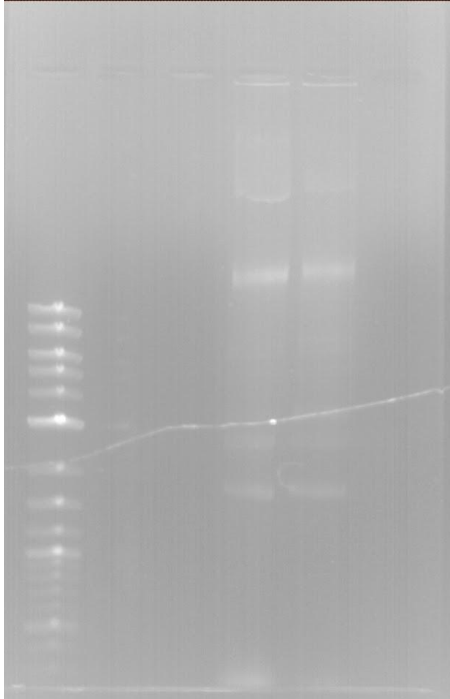
Reagents	**Volume for 25 µL solution (in µL)**	**Volume for 50 µL solution (in µL)**
dH_2O	19.99	39.98
Buffer (10x)	2.5	5
F primer (20 µM)	0.63 (1 µM)	1.26 (1 µM)
R primer (20 µM)	0.63 (1 µM)	1.26 (1 µM)
dNTPs (100 mM)	0.25 (1 mM)	0.5 (1 mM)
Pfu Polymerase	0.5	1
Templates (1.7 µg/µL)	0.5	1

Repeated the same process as yesterday

- PCR wells - 1kb ladder 1 µL, PCR product + loading dye, PCR product + loading dye, gDNA + loading dye, gDNA + loading dye
- PCR cycle - 95°C-3' - 95°C-30'' - 61°C-30'' - 72°C-1' - 72°C-10' - 4°C (Hold) [30x]
- Gel imaged at main building

Result

- Gene of interest missing



13/08/2021 - Friday

- Remaining members were given a tour of the lab, and instructed about basic lab skills and safety information by Ashwin
- Learnt about how to wash glassware correctly, and how to prepare them for autoclaving
- Learnt how to work in the hood

Cyanobacteria

- Checked on the cyanobacteria strains received from Pakrasi - Synechococcus elongatus UTEX 2973 WT, 2973 cscB, 2973 sps, 2973 spp
    - Only 2973 WT in liquid culture in falcon and on plate are reviving
- Light that Ashwin had set up in the incubator got fried. Connected bulb holder to old light's plug and fixed bulb in holder. Had to turn off the bulb for 6 hours in the night when no one was in the lab for safety reasons.

14/08/2021 - Saturday

Cyanobacteria

- Transferred UTEX 2973-WT from a falcon to a 50 mL autoclaved conical flask (borrowed from Nishad's lab)
  - Prem warned that using a 100 mL flask would lead to our 10 mL culture losing its medium to evaporation.
- This culture is reviving well. Modified the shaker slot using rubber bands (taken from Science Activity Center) to hold our tiny flask in place
  - Cyano flasks are incubated without foil.
  - Cyanobacteria should not be grown in falcons or test tubes.
- Got Mahesh, the campus electrician, to insulate our bulb with electrical tape. We can now use it for overnight cultures

---

15/08/2021 - Sunday

E. coli

Genomic DNA Extraction of KJK01

- Performed by Likhith under Rushik's supervision
- Protocol given by Rushik, can be found here
- After extraction, concentration of gDNA measured using NanoDrop
  - Nanodrop reading gDNA = **2.9 µg/µL**

16/08/2021 - Monday

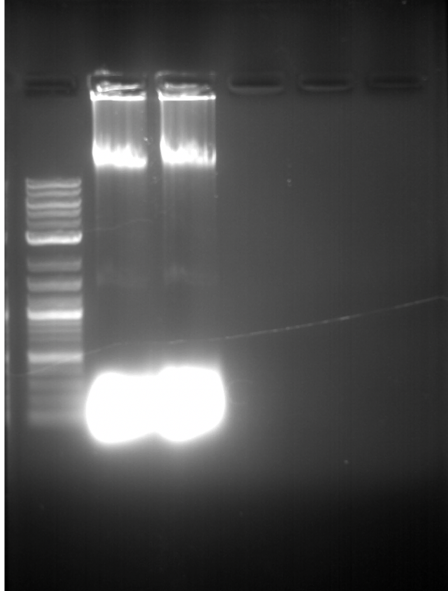
E. coli

- Gave glassware for autoclaving
- Prepared 1X LB and 1X TB from common store reagents

Gel electrophoresis to confirm presence of gDNA

- Performed by Sanjana and Likhith under Ashwin's supervision
- Protocol can be found here

Results



- Single band above the first band of the DNA ladder is gDNA
- Large, bright band at the bottom of the gel is probably RNA contamination

<u>Confirmation of production of butanol by KJK01</u>

- Inoculated 2 1° cultures of KJK01 in 10 mL 1X LB

Cyanobacteria

- Watched Prem's tutorial videos on how to streak cyanobacteria plates and how to prepare glycerol stocks
- Streaked 2973 WT on BG-11 agar plate
- Passaged 10 mL 2973 WT culture into 40 mL fresh IITB BG-11 (+) media in 250 mL flask

---

17/08/2021 - Tuesday

E. coli

<u>PCR to confirm the presence of butanol production pathway genes (*hbd-crt*) in E coli KJK01</u>

- Primer sequence for PCR obtained from Dr. Yazdani, ICGEB
- gDNA extracted on 15/08/2021 used

Protocol

	Negative Control	**Test 1**	**Test 2**

Pfu Buffer (10X)	5	5	5
dNTPs (2.5 mM)	2	2	2
Forward primer (20 uM)	1	1	1
Reverse primer (20 uM)	1	1	1
gDNA template (100 ng/uL)	-	1	1
milliQ	40	39	39
Pfu polymerase	1	1	1
Total Volume	**50 µL**	**50 µL**	**50 µL**

- Pfu buffer, Pfu polymerase received from Thomas's Lab
- dNTPs stock of 2.5 mM concentration had been prepared from 100 mM obtained from Thomas's Lab, a few days ago by Arsh for previous PCR attempts that had failed
- Primer solutions had also been previously made by Arsh for previous PCR attempts that had failed
- gDNA concentration available = 2.9 µg/mL
    - concentration to be made in final PCR mixture = 0.1 µg/mL

PCR cycles

1. 95C - 5 min
2. 30X:
    - 95C - 30 sec
    - 55C - 40 sec
    - 72C - 2 min
3. 72C - 5 min

- Stored PCR reaction tubes in -20°C to perform agarose gel electrophoresis the following day

<u>Confirmation of production of butanol by KJK01</u>

- 6 2° cultures inoculated from 1° cultures
    - 100 µL 1° culture + 10 mL 1X LB + 0 IPTG
    - 100 µL 1° culture + 10 mL 1X LB + 1 µL (0.1 mM) IPTG

    - 100 µL 1° culture + 10 mL 1X LB + 2 µL (0.2 mM) IPTG
    - 100 µL 1° culture + 10 mL 1X TB + 0 IPTG
    - 100 µL 1° culture + 10 mL 1X TB + 1 µL (0.1 mM) IPTG
    - 100 µL 1° culture + 10 mL 1X TB + 2 µL (0.2 mM) IPTG
- Let them stay in the incubator overnight at 37°C and 150 rpm

Cyanobacteria

- Prepared 25% glycerol using autoclaved 50% glycerol and autoclaved MilliQ
- Prepared 5 glycerol stocks of 2973 WT
    - Stored at -80°C
    - Protocol can be found here
- Done by Akash

18/08/2021 - Wednesday

E. coli

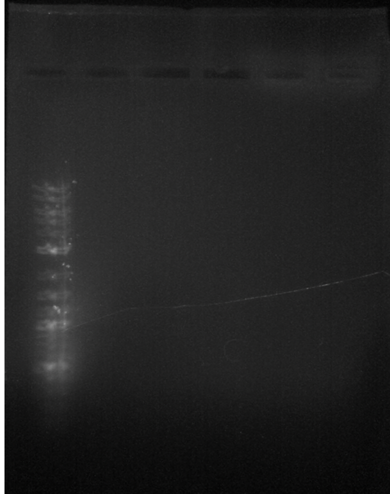
Gel electrophoresis for confirmation of presence of *hbd-crt* amplicon

- Prepared 1% agarose gel by adding 0.5 g of agarose in 50 mL of TAE buffer with 2 µL of EtBr

Gel Layout:

- Lane 1: DNA ladder (3 µL)
- Lane 2: Negative Control (10 µL)
- Lane 3: Test 1 (10 µL)
- Lane 4: Test 2 (10 µL)

Results



- Gel ran for too long, dye had reached the end of the gel, no bands obtained
- Loaded one more gel, using the same protocol, in the evening
    - Dye had reached the middle of the gel, but there were no bands

<u>Remarks</u>

- Learnt that the Pfu polymerase is from Thomas's Lab but the protocol used was from Saikrishnan's lab
- Devatrisha: Nanodrop concentration of gDNA (15/9/21) of 2.9 µg/mL may not have been reliable due to extensive RNA contamination. Dilution done to bring gDNA to 100 ng/µL might have actually resulted in too low of a concentration for it to have been successfully amplified. Load 1 µL of the gDNA sample directly without any dilution.
- Thomas: Just do a gradient PCR instead of trying to troubleshoot the PCR

<u>Confirmation of production of butanol by KJK01</u>

- After 2° culture hit 0.6 OD, 1 mL of each culture was taken and centrifuged. Pellet was discarded, and supernatant submitted to Soumya for NMR analysis

---

19/08/2021 - Thursday

E. coli

<u>Gradient PCR to troubleshoot confirmation of *hbd-crt* amplicon</u>

F-primer

100 µM x V = 20 µM x 20 µL
V = **4 µL**

Add 16 µL MilliQ

Same for R-primer

Protocol (from TP's lab)

	Negative Control	**0% DMSO**	**5% DMSO**	**10% DMSO**
MilliQ	82	81	76	71
DMSO	-	-	5	10
Pfu buffer 10X	10	10	10	10
dNTPs	2	2	2	2
gDNA template	-	1	1	1
Forward Primer	2	2	2	2
Reverse Primer	2	2	2	2
Pfu polymerase	2	2	2	2
Total Volume	**100 µL**	**100 µL**	**100 µL**	**100 µL**

- 50-60°C at 2°C increments

Cyanobacteria

Gentamycin and Kanamycin preparation for revival of cscB-sps-spp

Gentamycin
Required concentration = 4 µg/mL
Final volume = 10 mL
Required amount = 40 µg

Current concentration = 10 mg/mL = 10 µg/µL
We need **4 µL**

Kanamycin
Required concentration = 10 µg/mL
Final volume = 10 mL
Required amount = 100 µg

Current concentration = 50 mg/mL = 50 µg/µL
We need **2 µL**

20/08/2021 - Friday

E. coli

Gel electrophoresis for confirmation of presence of *hbd-crt* amplicon

- Prepared 1L 1X TAE buffer from 50X stock
    - 20 mL 50X dissolved in 980 mL distilled water
- Borrowed 20 µL DNA ladder from TP
- Prepared 2 gels
    - 0.5 g agarose in 50 mL TAE buffer + 2 µL EtBr
    - 1 g agarose in 100 mL TAE buffer + 4 µL EtBr
    - Sample = 10 µL, Dye = 2.5 µL - take 10 µL of this mix

1 - 50°C; 2 - 52°C; 3 - 54°C; 4 - 56°C; 5 - 60°C

Gel 1:

Lane 1: DNA Ladder (3 µL)
Lane 2: NC - 1 (10 µL)
Lane 3: 0% DMSO - 1 (10 µL)
Lane 4: 5% DMSO - 1 (10 µL)
Lane 5: 10% DMSO - 1 (10 µL)

Gel 2:

Lane 1: DNA Ladder (3 µL)
Lane 2: NC - 2 (10 µL)
Lane 3: 0% DMSO - 2 (10 µL)
Lane 4: 5% DMSO - 2 (10 µL)
Lane 5: 10% DMSO - 2 (10 µL)

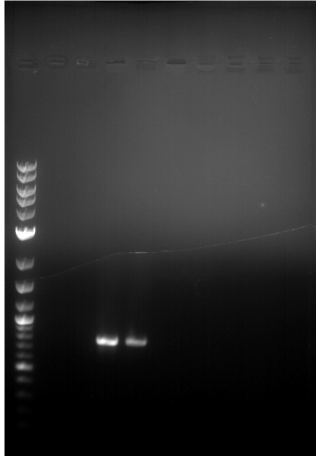
Lane 6: NC - 3 (10 µL)
Lane 7: 0% DMSO - 3 (10 µL)
Lane 8: 5% DMSO - 3 (10 µL)
Lane 9: 10% DMSO - 3 (10 µL)

Lane 10: NC - 4 (10 µL)
Lane 11: 0% DMSO - 4 (10 µL)
Lane 12: 5% DMSO - 4 (10 µL)
Lane 13: 10% DMSO - 4 (10 µL)

Lane 14: NC - 5 (10 µL)
Lane 15: 0% DMSO - 5 (10 µL)
Lane 16: 5% DMSO - 5 (10 µL)
Lane 17: 10% DMSO - 5 (10 µL)

Results

Gel 1:



- Gel 1 imaged, showed appropriate bands
- Gel 2 ran for too long, no bands appeared on imaging

Conclusion

- **DMSO needs to be added while using TP lab's Pfu polymerase**

Cyanobacteria

- Received and fixed Prem's lights
    - Maximum intensity achieved = **18000 lux** (1 lux = 1 $\mu E/m^2/s$) ~ **350 $\mu E/m^2/s$** according to Prem
- Set light intensity to 1500 lux for cyano to revive
- Sent 100 mL BG-11 (from Himedia) at pH 8.2 for autoclaving

21/08/2021 - Saturday

- Akash checked in on the cyanobacteria

22/08/2021 - Sunday

- Checked in on cyanobacteria growth
- Revived 1 glycerol stock of 2973 WT in IIT-B BG-11 (+)

For autoclaving

- 1L 10% glycerol
  - 100 mL 100% glycerol (borrowed from Anjan's lab) in 900 mL RO water
- 500 mL 2X LB
  - 25 g LB powder (common stores) in 500 mL RO water
- 1 x 100 mL flask
- 1 x 1000 mL flask
- 2 x 500 mL flasks
- 1 x 500 mL flask with lid

- Borrowed micronutrients (in the table below) from Anjan's lab and $Co(NO_3)_2$ - 0.1 g from Boomi's lab
- Borrowed 10 mL butanol from Vaidya's lab for MIC

Micronutrients for BG-11

- Himedia BG-11 that we ordered did not have micronutrients, hence we will add them by ourselves

Trace metal mix	**Amount for 500 mL (g)**
H_3BO_3	1.430
$MnCl_2.4H_2O$	0.905
$ZnSO_4.7H_2O$	0.111
$Na_2MoO_4.2H_2O$	0.195
$CuSO_4.5H_2O$	39.5×10^{-3}
$Co(NO_3)_2.6H_2O$	34.7×10^{-3}

Observations

- Plates in the 4°C fridge were frozen because the fridge was set to quick freeze
  - They were discarded
- SIngle colonies appeared on the 2973 WT plates streaked by Sanjana and Likhith, plates transferred from incubator to 4°C
- Akash transferred 15 mL WT 2973 in IIT-B BG-11 (+) to a 250 mL flask containing 85 mL of same media

---

23/08/2021 - Monday

- Received petri dishes, syringes, filters (0.2 µm) from Biostores

For autoclaving

- DMSO

E. coli

- Sanjana and Arsh streaked glycerol stock of KJK01 onto 2 LB agar plates
- 2 more empty LB agar plated were made

Making electrocompetent KJK01

- Were told by Rushik to prepare
  - Autoclaved 0.5 mL centrifuge tubes
  - Autoclaved 500 mL MilliQ
  - Store all required reagents at 4°C

MIC Assay

- Spoke to Nishad
- Need to find literature values of MIC for *E. coli* and cyanobacteria
- Need to figure out how to make serial dilutions of butanol and ethanol and their solubilities in water
- His grad student will guide us on how to do it

- Nishad also said that we should not put more than 3-5 mL of culture in 55 mL test tubes
- OD 0.6 should be reached in 1-2 hours of 2° inoculation and modified strains shouldn't be more than 10% slower than WT
- Test tube should be placed in a slanting manner to allow good mixing in shaker

GC-MS for butanol detection

- Hotha showed us the instrument - Vernier GC plus in ħ lab
- Need 1 µL syringe to load sample into instrument

- Cannot be used in our case because sample is liquid

Cyanobacteria

- Pakrasi strains arrived in great condition
- One strain was labelled cscB-spp instead of cscB-sps
    - Confirmed with Michelle that it was a typo
- Inoculated 2973 cscB, cscB-sps, cscB-sps-spp in 20 mL IIT-B BG-11 (+) with antibiotics in 100 mL flasks
- OD of 2973 WT IIT-B BG-11 (+) 50 mL culture in 250 mL flask was **0.2**

24/08/2021 - Tuesday

- Holders that Ashwin got from TP are too small
- Need more screws for incubator

E. coli

<u>For autoclaving</u>

- 0.5 mL MCTs
- 500 mL MilliQ

- There appears to be contamination in the KJK01 plates streaked on 23/08/2021
    - Ashwin marked colonies safe to pick from
- Sanjana and Arsh inoculated 2 1° KJK01 cultures from the plates in 20 mL 2X LB for electrocompetent cells
    - Placed overnight at 37°C at 150 rpm

Cyanobacteria

- Prepared 1 mL of 0.1 g/mL stock solutions each of $CuSO_4$ and $Co(NO_3)_2$
- Prepared 1 L BG-11 from HiMedia powder
- OD of 2973 WT IIT-B BG-11 (+) 50mL culture in 250 mL flask is **0.4** at 730 nm

---

25/08/2021 - Wednesday

E. coli

<u>Preparing electrocompetent KJK01 cells for transformation</u>

- Vidisha inoculated 2° cultures of 200 mL each from the 1° cultures at 12:40 PM
    - 2 mL 1° culture (1%) + 200 mL 2X LB
- Space for KJK01 flasks was freed up by fixing small shaker slots for cyanobacterial cultures

<u>OD Readings</u>

3:00 PM (Akash):

- 1 - 0.06
- 2 - 0.05

4:40 PM (Sanjana):

- 1 - 0.297
- 2 - 0.435

5:40 PM (Sanjana):

- 1 - 0.450
- 2 - 0.472

6:20 PM (Sanjana):

- 1 - 0.581
- 2 - 0.617

- Cultures along with glycerol, falcons, autoclaved MCTs were placed in the cold room (5°C) at 7:00 PM for 1.5 hours
- Since MilliQ was contaminated by Akash in the morning, Rushik suggested that we skip the MilliQ washing step and use 10% glycerol instead
- Protocol for the procedure can be found here
- Done by Sanjana and Ashwin in Sai's lab
- Stocks prepared and stored at -80°C in Sai's lab

- Arsh poured 2 LB agar plates with antibiotics for transformation

- 20 mL LB agar + 20 µL Kan^{50} + 20 µL Amp^{100}

Cyanobacteria

OD readings:

- Taken by Akash at 3:50pm
- WT - o.610
- WT(-) - 2.37 (100ml culture)
- cscb(inoculated on 23rd August) - 1.589
- cscb-sps(inoculated on 23rd August) - 1.090
- cscb-sps-spp(inoculated on 22nd August) - 0.961
- cscb-sps-spp(inoculated on 23rd August) - 0.681

To-do list:

- Check on BG-11 agar plates. Pour if necessary
- Pellet and streak
- Inoculate 100ml 2° cultures of cscb, cscb-sps, cscb-sps-spp
- Make stickers for the Wild type UTEX 2973

DMSO stocks:

- We need 140 µL of 7% DMSO
- 3 mL in 100 mL (97 mL milli-Q/distilled water + 3 mL 7 % DMSO)

BG-11 plates:

- For cscb-sps-spp: 3OmL BG-11 agar + 6 µL $Gent^{10}$ + 6 µL Kan^{50}
- For cscb-sps: 3OmL BG-11 agar + 6 µL $Gent^{10}$ + 6 µL Kan^{50}
- For cscb: 3OmL BG-11 agar + 6 µL Kan^{50}
- For WT: 30mL of BG-11 agar (no antibiotics)

BG-11 liquid culture:

- For 80 mL : 32 µL $Gent^{10}$ + 16 µL Kan^{50}
- Fpr 30 mL : 12 µL $Gent^{10}$ + 6 µL Kan^{50}

26/08/2021 - Thursday

Cyanobacteria

OD Readings:

- Wild Type - 0.81 (Likhith and Akash made stocks)

- cscb - 0.5
- cscb-sps - 0.26
- cscb-sps-spp - 0.49

27/08/2021 - Friday

Cyanobacteria

- Ashli and Vidisha labelled all DMSO stocks made by likhith and akash
- Arsh and Vidisha sent 250 mL and 100 mL flasks for autoclaving
- Vidisha took OD readings for Cyanobacteria strains
    - Arsh and Vidisha made DMSO stocks for WT(-) and cscb-sps-spp

OD Readings:

- WT-1 - 0.291
- WT-2(-) - 1.702
- cscb - 0.657
- cscb-sps - 0.466
- cscb-sps-spp - (Inoculated on 22nd August) - 1.773
- cscb-sps-spp (Inoculated on 23rd August and passaged on 25th August) - 0.291

29/08/2021 - Sunday

E. coli

Transformation of KJK01 with pCSCX plasmid

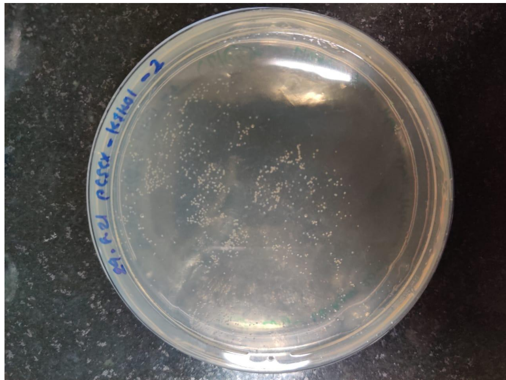
- Performed by Sanjana and Vidisha under Rushik's supervision in Sai's lab
- Used electrocompetent cells made on 25/08/2021
- Used pCSCX plasmid purified on 31/07/2021
- Protocol can be found here

- Used pCSCX - 2 vial
    - Concentration of DNA measured using NanoDrop was **147 µg/mL**
    - Might be lesser because we did not spin vial before checking
    - Since concentration is > 100 µg/mL, we can use the DNA for transformation

- After transformation, 2 plates (made on 25/08/2021) were left in Sai's incubator
- Plates were transferred to the cold room on 30/08/2021 after overnight incubation

Results

- Colonies were observed, transformation was successful



Cyanobacteria

- Akash and likhith revived two cyanobacteria stocks, one of WT and one of cscb into two 50 mL flasks at 5:49 pm.

Reviving DMSO stocks:

- For WT : 10 mL BG-11 (+) from IIT- Bombay
- For cscb : 10 mL BG-11 (+) from IIT-Bombay + 4 µL $Gent^{10}$ + 2 µL Kan^{50}

Observations:

- All the plates streaked by Akash on 25th August, 2021 are growing well : cscb,cscb-sps,cscb-sps-spp
- Plates streaked by Likhith on 26th August,2021 from the Glycerol stocks of UTEX 2973 WT:
    - Show trivial growth
    - Have brown colonies on third streak : Prem suspects E.coli contamination Therefore, UV the hood regularly

---

30/08/2021 - Monday

Cyanobacteria

- Akash and Likhith took OD readings for cyanobacteria.
- The OD readings of the culture revived from stocks was compared to the OD readings of the original culture. Decent revival rates were observed.
- WT and cscb cultures were acclimated to salt stress by Akash.
- Ashli and Akash transferred stock-revived cultures into 100 mL flasks.

OD readings:

- WT (100 mL) - 0.756, 0.747, 0.738

- cscb(100 mL) - 0.798, 0.830, 0.837, 0.827
- cscb-sps(100 mL) - 0.611, 0.625, 0.625, 0.623
- cscb-sps-spp(15 mL) - reading was too high to be detected
- csccb-sps-spp(100 mL) - 0.388, 0.391, 0.395
- WT(Revived from DMSO stocks) - 1.030, 1.018, 1.023
- cscb(Revived from DMSO stocks) - 0.66, 0.636

<u>Comparison between ODs of Stock-revived vs. Original cultures:</u>

- WT(original) - 0.81 (out of this 20mL was used to create 1 mL stock)
  - Total OD = 16
- WT(Revived) - 1.02 x 11 mL = 11.2 OD
- cscb(original) - 0.657
  - Total OD = 13.14 OD
- cscb(revived) - 0.6 x 11 = 6.6 OD
- Therefore,
  - 11.2/16 = 0.7
  - 6.6/13.14 = 0.502

<u>Acclimation to salt stress:</u>

- Inoculate a 10 mL culture with one colony each using BG-11(+) with 150 mmol NaCl)
- 10 mL culture : 9.7 mL of BG-11 + 300 µL $Nacl_{(aq)}$

<u>Transfer of stock-revived culture:</u>

- Ashli prepared 30mL of medium in 3 100 mL flasks
  - Flask A - Without antibiotics
  - Flask B - With 6 µL Kan^{50}
  - Flask C - With 12 µL $Gent^{10}$ and 6 µL Kan^{50}
- Added 10 mL culture of each WT and cscb into flasks A and B respectively.
- Flasks A and B were kept in the incubator and flask C was stored for later use.

31/08/2021 - Tuesday

E. coli

<u>Making glycerol stocks of pCSCX-KJK01</u>

- Plates were retrieved from cold room
- 2 1° cultures were inoculated at 7:30 PM by Sanjana
  - 1 colony from each plate
  - 10 mL 1X LB + 10 µL Kan^{50} + 10 µL Amp^{100}

- Ampicillin was filter sterilized

Cyanobacteria

- OD readings taken by likhith and akash for all cyanobacteria strains at 2:00pm.
- Likhith and Ashli made DMSO stocks for cscb-sps.
- Got micronutrients from Boomi Shankar and Sujit Ghose's Lab.
- Filter-sterilized micro-nutrients and added to 500 mL of BG-11 (-)
- Autoclaved glassware and 5M Nacl
- 100 mL (approx. 90 mL after evaporation) of WT from 25th August was divided into
    - 20 mL culture + 80 mL fresh BG-11
    - 9.7 mL culture + 0.3 mL of Nacl added to make BG-11 with 150 mM Nacl
    - 60 mL culture was transferred into an autoclaved 100 mL flask and left on the bench under light.
- 100 mL (approx. 80 mL after evaporation) of cscb from 25th August was divided into
    - 20 mL culture + 80 mL fresh BG-11
    - 9.7 mL culture + 0.3 mL of 5M Nacl added to make 150 mM Nacl BG-11
    - 50 mL culture transferred into an autoclaved 250 mL flask and left on the bench under light.

<u>OD Readings:</u>

- Taken at 2:00pm by Likhith
- WT (From 22nd August) - 0.866, 0.855, 0.879
- cscb(From 27th August) - 0.983, 0.982,0.972
- cscb-sps(From 25th August) - 0.711, 0.699, 0.718
- cscb-sps-spp(From 27th August) - 0.495, 0.475, 0.501
- WT(Stock revived from 30th August) - 0.331, 0.317, 0.318 (Total OD = 12)
- cscb(Stock revived) - Still colourless so we did not take OD

<u>DMSO stocks:</u>

- Created stocks of cscb-sps from 25th August
- 80 mL of the 100 mL culture was used for stocks.
- Remaining 20 mL was passaged to 80 mL of fresh BG-11 medium to make 100 mL culture.
- Half of the stocks (the ones labelled with pink) got contaminated with 1 M glucose solution.

<u>For Autoclaving:</u>

- 50 and 100 mL flasks
- 1 ml micropipette tips
- 5M Nacl solution

Advice from Prem:

- No need to keep the cell pellet in the ice tray before transferring it to cryovials.
- No need to parafilm the cryovials.

01/09/2021 - Wednesday

E. coli

Making glycerol stocks of pCSCX-KJK01

- Sanjana inoculated 3 2° cultures at 12:30 PM
    - 130 µL 1° culture + 13 mL 1X LB + 13 µL Kan^{50} + 13 µL Amp^{100}

OD Readings

3:10 PM (Sanjana):

- 1 - 0.053
- 2 - error
- 3 - error

4:30 PM (Sanjana):

- 1 - 0.215
- 2 - 0.226
- 3 - 0.119

5:40 PM (Ashli):

- 1 - 0.334, 0.332, 0.336
- 2 - 0.317, 0.317, 0.319
- 3 - 0.303, 0.298, 0.299

6:20 PM (Likhith):

- 1 - 0.641, 0.624, 0.621
- 2 - 0.628, 0.630, 0.631
- 3 - 0.515, 0.515, 0.516

- At 7:15, Sanjana made 18 glycerol stocks
    - Protocol can be found here
    - Stocks stored at -80°C in Sai's lab

Cyanobacteria

<u>Observations</u>:

- Cultures undergoing acclimation are yet to turn dark green. Can be passaged by night.
- On 02-09-2021, check OD, dilute to 0.2 OD_{730} and check OD_{730} every three hours till it reaches 1.
- For every OD measurement, take cytometer readings with pictures.

<u>OD Readings:</u>

- WT(100 mL culture) : 0.491, 0.536, 0.502
- WT(stock revived, 40 mL) : 0.302, 0.294, 0.300
- cscb(100 mL) : 0.501, 0.514, 0.499
- cscb-sps(100 mL) : 0.086, 0.088, 0.089
- cscb-sps-spp(100 mL) : 0.426, 0.426, 0.419

<u>Passaging acclimated cultures:</u>

- WT : 87.3 mL of BG-11 + 2.7 mL of 5M Nacl
- cscb : 87.3 mL of BG-11 + 2.7 mL of 5M Nacl + 18 µL Kan^{50}

Akash transferred the cultures at 9:36 pm

02/09/2021 - Thursday

E. coli

<u>MIC Assay - Butanol and Ethanol</u>

- MIC value from literature for *E. coli* is
  - Butanol - 15 mg/mL
  - Ethanol - 15 mg/mL
- Met Chinmaya from Nishad's lab and got MIC protocol and *E. coli* MG1655
- Inoculated 1° cultures of MG1655 and KJK01 in 10 mL 1X LB

Cyanobacteria

<u>OD Readings :</u>

- Blank : 0.001, 0.001, 0.030, negative, 0.003
- WT(100 mL/ 150 mM Nacl) : 0.351, 0.333, 0.325, 0.327, 0.329
- cscb(100 mL/150 mM Nacl) : 0.396, 0.341, 0.348, 0.333, 0.322
  - Return to the incubator, if below the range of 0.8-1.0
  - As soon as the OD reaches 0.8, (or higher but <1), we must dilute it to 0.2 via

$$O_1V_1=O_2V_2$$

OD Readings of the remaining cyanobacteria:

- WT(100 mL-w/o salt) : 0.634, 0.625, 0.633, 0.637, 0.636
- cscb(100 mL-w/o salt) : 0.956, 0.937, 0.893, 0.883, 0.857
- cscb-sps(100 mL-w/o salt) : 0.413, 0.407, 0.403, 0.408, 0.404
- cscb-sps-spp(100 mL-w/o salt) : 0.605, 0.595, 0.607, 0.611, 0.614
- WT(Revived from DMSO stock) : 0.387, 0.393, 0.417, 0.400, 0.403

Made DMSO stock of cscb-sps(passaged on 25th August).

Sucrose Assay

- Discussed protocol and reagent solution preparation with mentors

---

03/09/2021 - Friday

E. coli

MIC Assay - Butanol and Ethanol

- Inoculated 2° cultures of MG1655 and KJK01
    - MG1655 and KJK01 (uninduced) - 100 µL 1° culture + 10 mL 1X LB
    - KJK01 (induced) - 100 µL 1° culture + 10 mL 1X LB + 1 µL (0.1 mM) IPTG

OD Readings (Likhith)

2:45 PM:

- KJK01 (uninduced) - 0.083, 0.083, 0.079
- KJK01 (induced) - 0.111, 0.112, 0.113

5:45 PM:

- MG1655 - 1.313, 1.308, 1.321
- KJK01 (uninduced) - 0.726, 0.727, 0.723
- KJK01 (induced) - 0.603, 0.610, 0.609

Cyanobacteria

<u>OD Readings:</u>

- Taken at 9:15 am by Likhith and Akash.
- WT(100 mL - 150 mM Nacl) : 0.304, 0.303, 0.304, 0.305, 0.303
- cscb(100 mL-150 mM Nacl) : 0.453, 0.303, 0.458, 0.450, 0.303

Sucrose Assay

Ashli and Likhith prepared Solution 1 and 2 as per megazyme instructions:-

- Solution 1: Acetate buffer - Dissolved bottle 1 (20 mL) to 400 mL in distilled water, stored at 4C.
- Solution 2: Beta-fructosidase, Dissolved 1 mL of solution from bottle 2 (20 mL) to 10 mL in distilled water, stored at -20 C. For convenience, 10 1mL aliquots of this were prepared.

After talking to the PhD mentors, following plan was made for Solution 3 and Solution 4.

- Solution 3: GOPOD Buffer in bottle 3 (50 mL), to be dissolved in 1L and to be used immediately.
- Solution 4: GOPOD Reagent, Bottle 4 powder to be dissolved in 20 mL of solution 3 and this 20 mL is to be dissolved in remaining 980 mL of Soln 3. To be stored in -10C. But once thawed, it cannot be frozen again for later use, so it must be divided into 200 mL aliquots.

04/09/2021 - Saturday

E. coli

<u>MIC Assay - Butanol and Ethanol</u>

- The assay was performed in Nishad's lab at 8:00 PM by Sanjana and Arsh
- Protocol can be found here

<u>Calculations</u>

Ethanol

Well 1 will have 60 mg/mL of ethanol
60 mg/mL x 200 µL = 12 mg

ρ_{EtOH} = 0.789 g/mL
ρ = m/V
V = m/ρ = 0.012/0.789 = **15.2 µL**

Well 1 will have 15.2 µL EtOH + 184.8 µL LB

Butanol

Well 1 will have 60 mg/mL of butanol
60 mg/mL x 200 µL = 12 mg
ρ_{BuOH} = 0.81 g/mL
ρ = m/V
V = m/ρ = 0.012/0.81 = **14.81 µL**

Well 1 will have 14.8 µL EtOH + 185.2 µL LB

- Sanjana and Soorya stayed overnight for the MIC
    - 96 well plate borrowed from Chinmaya performed in Nishad's lab
    - Imaged in TP's lab using his 96 well plate reader
- Likhith took over on the morning of 05/09/2021
- Data can be found here

Cyanobacteria

<u>OD Readings of acclimated cultures:</u>

- WT(150 mM Nacl, left outside due to slow growth) : 0.799, 0.807, 0.799, 0.8, 0.809
- cscb(150 mM Nacl, returned to incubator) : 0.681, 0.614, 0.611, 0.620, 0.623
- cscb-sps-spp (100 mL, Sample) : 0.919, 0.915, 0.908, 0.926, 0.908
    - Mean = 0.915 (Make stocks)
- 11:57 am :
    - cscb-sps-spp centrifuged to pellets
    - UVing hood for stock preparation
- Check in on cscb-Nacl :
    - 3:00 pm : 0.566, 0.522, 0.548, 0.542, 0.552
    - 4:50 pm : 0.582, 0.571, 0.607, 0.616, 0.610, 0.602

06/09/2021 - Monday

E. coli

Growth curve assay KJK01 and pCSCX-KJK01

- Sanjana inoculated 1° cultures of KJK01 and pCSCX-KJK01 at 3:15 PM
    - KJK01 - 5 mL 1X LB
    - pCSCX-KJK01 - 5 mL 1X LB + 5 µL Kan^{50} + 5 µL Amp^{100}

Cyanobacteria

OD Readings:

- 18:30 pm (27 hrs)
    - WT 1 : 0.339, 0.35, 0.355,0.368,0.35, 0.355
    - WT 2 : 0.351, 0.351, 0.358, 0.364, 0.364
    - cscb 1 : 0.325, 0.326, 0.329, 0.334, 0.333
    - cscb 2 : 0.291, 0.283, 0.282, 0.283, 0.291
- 21:30 pm (30 hrs)
    - WT 1 : 0.665, 0.652, 0.645,0.659,0.688, 0.681
    - WT 2 : 0.661, 0.663, 0.691, 0.684, 0.675, 0.698
    - cscb 1 : 0.570, 0.559, 0.559, 0.563, 0.566, 0.574
    - cscb 2 : 0.555, 0.557, 0.551, 0.554, 0.555, 0.555
- 00:30 am (33 hrs)
    - WT 1 : 0.464, 0.452,0.465, 0.477, 0.464
    - WT 2 : 0.587, 0.491, 0.504, 0.5, 0.525
    - cscb 1 : 0.423, 0.396, 0.401, 0.419, 0.404
    - cscb 2 : 0.383, 0.381, 0.386, 0.386, 0.398
- 3:30 am (36 hrs)
    - WT 1 : 0.504, 0.492, 0.487, 0.489, 0.496, 0.499
    - WT 2 : 0.560, 0.518, 0.511, 0.530, 0.535, 0.525
    - cscb 1 : 0.395, 0.385, 0.393, 0.387,0.388, 0.387
    - cscb 2 : 0.915, 0.849, 0.745, 0.732, 0.707, 0.665

Sucrose Assay

For convenience (storing, thawing), Likhith and Ashli made the reagents using the following method:

- Dissolved 5 mL of bottle 3 to 100 mL in distilled water to make solution 3. Divided into two portions: 20 mL and 80 mL.
- Dissolved bottle 4 powder in 20 mL. Made 12 aliquots of 1.65 mL as a working stock from this 20 mL and stored in -20C as stock. Cover in aluminium foil.

- Transferred 1.632 mL from 1.65 mL aliquot into 80 mL of solution 3 to make solution 4. Store at 4C, cover with aluminium foil.

Note to self: To prepare fresh solution 4/GOPOD reagent - Dissolve 4 mL of bottle 3 to 80 mL in distilled water to make fresh solution 3. Add 1.632 mL from 1.65 mL aliquot to the 80 mL of solution 3 to make solution 4.

07/09/2021 - Tuesday

E. coli

<u>Growth curve assay KJK01 and pCSCX-KJK01</u>

- Ashwin started the assay at 8:10 AM, stayed till 10:10 AM, Sanjana stayed for rest of the readings
  - Readings were taken every hour
  - KJK01 - 50 mL 1X LB + 1 mL 1° culture (2%)
  - pCSCX-KJK01 - 50 mL 1X LB + 1 mL 1° culture (2%) + 5 µL Kan^{50} + 5 µL Amp^{100}
- Readings can be found here

Cyanobacteria

<u>OD Readings:</u>

- 12: 30 pm(45 hrs)
  - Blank: 0.001 0.000, 0.000,0
  - WT1 : 0.58, 0.59, 0.582 ,0.576 ,0.566
  - WT2 : 0.686, 0.638,0.644, 0.626, 0.63
  - cscb1 : 0.225, 0.225, 0.226, 0.227, 0.23
  - cscb2 : 0.238, 0.228, 0.228, 0.226, 0.226

- 3:30 pm (48 hrs) - undoubled
  - WT1 : 0.303, 0.283, 0.288, 0.305, 0.3
  - WT2 : 0.283,0.292, 0.296, 0.296, 0.291
  - cscb 1 : 0.125, 0.129,0.131, 0.136, 0.136
  - cscb 2 : 0.141, 0.145, 0.145, 0.141, 0.144

- 6:30 pm (51 hrs) - undoubled
  - WT1 : 0. 292, 0.299,0.301, 0.303,0.299
  - WT2:0.364, 0.368, 0.353 ,0.358, 0.357
  - cscb1: 0.223,0.222, 0.220, 0.221, 0.224

- cscb2 : 0.236, 0.24, 0.242, 0.241, 0.241

- 9:30 pm (54 hrs) - undoubled
    - WT 1 : 0.323 0.328, 0.326, 0.329, 0.323
    - WT2 : 0.371, 0.362, 0.363, 0.361, 0.362
    - cscb1 : 0.230,0.227, 0.226, 0.226, 0.232
    - cscb2 : 0.250, 0.241, 0.238, 0.242, 0.240

- 12:30 am (58 hrs)
    - WT 1 : 0.359, 0.354, 0.356, 0.361,0.347
    - WT 2 : 0.403, 0.403, 0.392, 0.392, 0.392
    - cscb 1 : 0.283, 0.280, 0.281, 0.288,0.279
    - cscb 2 : 0.340, 0.346, 0.331, 0.336, 0.334

- 3:30 am (61 hrs)
    - WT 1 : 0.872, 0.824, 0.82, 0.816, 0.8
    - WT 2 : 0.408, 0.407, 0.395, 0.382, 0.376
    - cscb 1 : 0.232, 0.228, 0.232, 0.228, 0.229
    - cscb 2 : 0.248, 0.253, 0.245, 0.242, 0.245

- 6:30 am (64 hrs)
    - WT 1 : 0.425, 0.418,0.419,0.410,0.408
    - WT 2 : 0.427, 0.428, 0.421, 0.426,0.432
    - cscb 1 : 0.245, 0.273, 0.269, 0.269, 0.274
    - cscb 2 : 0.292, 0.293, 0.295,0.294, 0.294

- 9:30 am (67 hrs)
    - WT 1 : 0.435, 0.431, 0.432, 0.441, 0.433
    - WT 2 : 0.499,0.489, 0.490, 0.496, 0.489
    - cscb 1 : 0.289, 0.291, 0.293, 0.292, 0.292
    - cscb 2 : 0.325, 0.330, 0.329,0.343, 0.325

- 12:30 pm (70 hrs)
    - WT 1 : 0.475, 0.455, 0.462, 0.455, 0.458
    - WT 2 : 0.471, 0.483, 0.485, 0.451, 0,456
    - cscb 1 : 0.366, 0.362, 0.363, 0.364, 0.363
    - cscb 2 : 0.335, 0.339, 0.34, 0.334, 0.328

- 6:30 pm (73 hrs)
    - cscb 1 : 0.326, 0.323, 0.333, 0.318, 0.319
    - cscb 2 : 0.367, 0.349,0.353, 0.350, 0.350

- 9:30 pm (76 hrs)
    - cscb 1 : 0.327, 0.337, 0.329, 0.331, 0.333
    - cscb 2 : 0.308, 0.329, 0.328, 0.325, 0.329

Sucrose Assay

- Ashli checked if absorbance of GOPOD reagent is <0.05
    - Distilled water blank: 0.000, 0.000, 0.000. 0.000, 0.000
    - Measured absorbance: 0.041, 0.042, 0.041, 0.043, 0.042

08/09/2021 - Wednesday

Sucrose Assay

Megazyme Protocol

- Prepare the following in test tubes:
    - Standard: 0.1mL of 100 ug D-glucose (1mg/mL) + 0.3 mL of distilled water
    - Blank: 0.4 mL of distilled water
    - Solution A: 0.2 mL of sample + 0.2 mL of Solution 1 (acetate buffer)
    - Solution B: 0.2 mL of sample + 0.2 mL of Solution 2 (beta-fructosidase)
- Incubate all 4 solutions in water bath at 50C for 20 minutes
- Then add 3mL Solution 4 (GOPOD reagent) to all 4 solutions. Incubate in water bath at 50C for 20 minutes.
- Measure absorbance of standard, solution A (ΔA), solution B(ΔB) against the blank in a cuvette.

SAMPLE DILUTION:

The amount of sucrose and D-glucose present in the cuvette should range between 10 µg and 100 µg. The sample solution must therefore be diluted sufficiently to yield a sugar concentration between 0.02 and 0.5 g/L.

Dilution table

Estimated amount of sucrose + glucose per litre	Dilution with water	Dilution factor
< 0.5 g	-	1
0.5 - 5.0 g	1 + 9	10

CALCULATIONS:

D-Glucose; g/L of sample solution:

$$= \frac{\Delta A}{0.2} \times F \times \frac{1}{1000} \times \frac{1000}{1000} \times \text{Dilution}$$

$$= \Delta A \times F \times 0.0050 \times \text{Dilution}$$

Sucrose; g/L of sample solution:

$$= \frac{\Delta B - \Delta A}{0.2} \times F \times \frac{1}{1000} \times \frac{1000}{1000} \times \frac{342}{180} \times \text{Dilution}$$

$$= (\Delta B - \Delta A) \times F \times \text{Dilution} \times 0.0095$$

where:

$\Delta A/0.2$ and $\Delta B/0.2$

= absorbances (510 nm) (GOPOD Reagent) for 0.2 mL of sample treated with Solution 1 (ΔA) (free D-glucose); or β-fructosidase (ΔB) (free D-glucose plus D-glucose from sucrose).

F = factor to convert from absorbance to μg for 100 μg of D-glucose (= 100/absorbance for 100 μg D-glucose).

1/1000 = conversion from μg to mg.

1000/1000 = conversion from mg/mL to g/L.

342/180 = conversion from μg of D-glucose (as measured) to μg of sucrose.

Dilution = dilution of the original sample solution.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the result is calculated from the amount weighed:

Content of sucrose

$$= \frac{c_{sucrose}\ (\text{g/L sample solution})}{\text{weight}_{sample}\ (\text{g/L sample solution})} \times 100 \quad [\text{g}/100\ \text{g}]$$

Sucrose Assay: Attempt 1; Performed by Likhith and Ashli

- Prepared 100 g/L, 10g/L, 1 g/L and 0.1 g/L sucrose solutions
- Verified if kit works by measuring concentrations of 0.1 and 10 g/L samples
- Followed Megazyme's protocol exactly. 10 g/L solution diluted 100 fold for use as sample.
- Observations:
    - Blank: 0.000, 0.000, 0.001, 0.000, 0.000
    - Standard: 0.852, 0.854, 0.853, 0.855, 0.853
    - 0.1, A: -0.24, 00.023, -0.022, -0.022, -0.021
    - 0.1, B: 0.046, 0.046, 0.047, 0.047, 0.047
    - 10, A: -0.036, -0.037, -0.037, -0.036, -0.0310, B: 0.062, 0.063, 0.063, 0.064, 0.064

- Results:
    - Measured concentration of 0.1 g/L sample: 0.07 g/L
    - Measured concentration of 10 g/L sample: 11.137 g/L

- Remarks:
    - Why are we getting negative absorbances for A solutions?
        - Possibly due to the effect of the acetate buffer, blank does not contain acetate buffer or beta fructosidase
    - 0.1, B and 10, B should have same absorbances but they do not.
        - Error in sample preparations?

- Devatrisha advised us to ignore megazyme's method for calculating sucrose concentration and to prepare our own standardisation curve of absorbance (ΔB-ΔA) vs sucrose concentration. Try to fit a linear curve, use slope of curve to find the concentration of sucrose for a given absorbance. She showed her Bradford Assay's standardisation curve. She also asked us to prepare fresh sucrose solutions and verify if we are able to measure their concentrations accurately.

- On speaking with Nishad:
    - 0.1, B and 10, B are both too close to 0 and are probably unreliable values.

14/09/2021 - Tuesday

E coli

Inoculation of primary cultures

- Sanjana inoculated a 10 mL primary culture for E coli KJK01 and two 10 mL primary cultures for KJK01-pcscX in 1x LB at 7 pm.
- However, on later observation, the LB used was found to be contaminated.
- Ashwin inoculated primary cultures of our strains once again at 11:30 pm with LB from his lab.

15/09/2021 - Wednesday

E coli

Inoculation of secondary cultures

- Ashwin made 250mL 1x LB and 250mg/100mL sucrose solution and gave it for autoclaving.
- Sanjana and Arsh inoculated secondary cultures of our strains in LB as follows:

Strain	**Culture composition**
KJK01	5mL 1x LB + 0.5mL sucrose + 50µL primary inoculum
KJK01-pcscX	5mL 1x LB + 0.5mL sucrose + 5µL Kan^{50} + 5µL Amp^{100} + 50µL primary inoculum
KJK01-pcscX-induced	5mL 1x LB + 0.5mL sucrose + 5µL Kan^{50} + 5µL Amp^{100} + 0.5µL IPTG + 50µL primary inoculum

- Sanjana and Arsh inoculated secondary cultures in BG-11 as follows:

Strain	**Culture composition**
KJK01	90mL BG-11 + 10mL sucrose + 900µL primary inoculum
KJK01-pcscX	90mL BG-11 + 10mL sucrose + 90µL Kan^{50} + 90µL Amp^{100} + 900 µL primary inoculum
KJK01-pcscX-induced	90mL BG-11 + 10mL sucrose + 90µL Kan^{50} + 90µL Amp^{100} + 10µL IPTG + 900µL primary inoculum

- We observed that our OD readings did not make a lot of sense as the strains were growing very slowly and the inducted KJK01 strain was not at all growing. Hence, we stopped our readings at 3:00 am September 16, 2021.

Sucrose Assay

Sucrose Assay: Attempt 2; Performed by Likhith and Ashli

- Prepared fresh sucrose solutions of concentrations for making an absorbance vs sucrose concentration standardisation curve:
    - 0, 0.1, 2.5, 5, 7.5, 10, 15, 20 g/L

- Didn't prepare any distilled water blank or 100 ug glucose standard as described in the megazyme protocol. Only did the reactions with the above standard sucrose concentrations and with the cyanobacteria supernatants in a 96 well plate.

- Solution A: 20 uL of sample + 20 uL of acetate buffer + 300 uL of GOPOD
- Solution B: 20 uL of sample + 20 uL of beta fructosidase + 300 uL of GOPOD

- Incubation done in hybridisation oven. 20 min at 50C for both incubation steps.

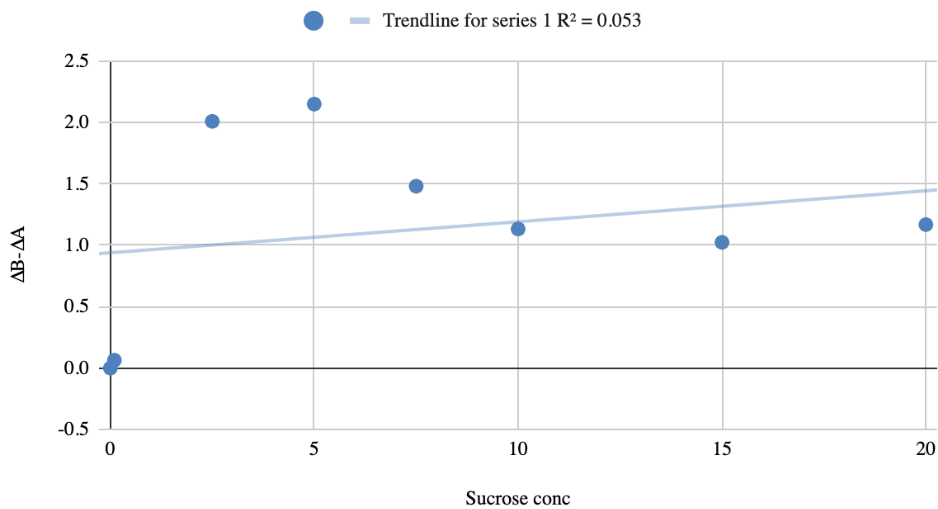
- 96 well plate layout:
    - B- Sucrose A
    - C- Sucrose B
    - D- WT 2973 A
    - E- WT 2973 B
    - F- cscB A
    - G- cscB B

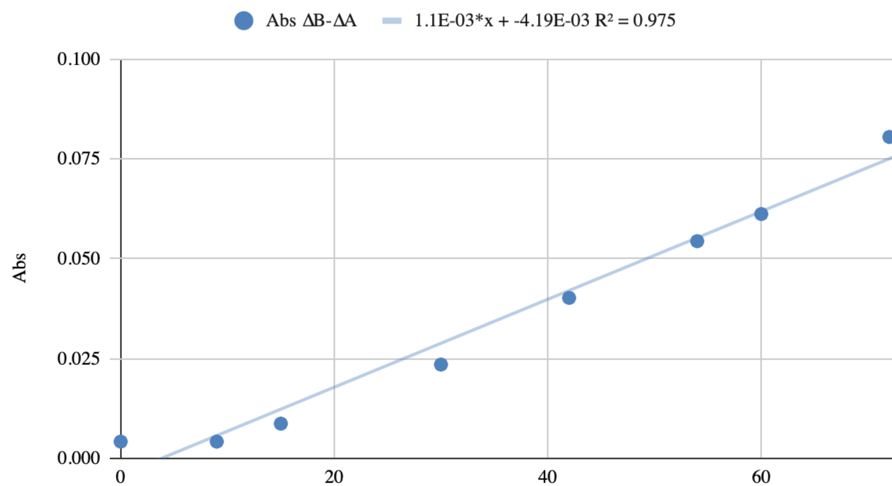
- Observation:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.0537	0.0639	0.0622	0.0802	0.0816	0.0944	0.1166	0.1385			
C		0.0525	0.1272	2.0704	2.2289	1.5616	1.2264	1.1395	1.3059			
D		0.0555	0.0565	0.0529	0.0532	0.0528	0.0534	0.053	0.1009			
E		0.0546	0.0581	0.0534	0.0554	0.0535	0.0518	0.0537	0.0525			
F		0.0523	0.0528	0.0534	0.0529	0.0532	0.0538	0.0556	0.0537			
G		0.0565	0.057	0.0621	0.0764	0.0934	0.1082	0.1168	0.1342			
H												

Sucrose conc	0	0.1	2.5	5	7.5	10	15	20
ΔB-ΔA	-0.0012	0.0633	2.0082	2.1487	1.48	1.132	1.0229	1.1674
WT	0	9	15	30	42	54	60	72
Abs ΔB-ΔA	-0.0009	0.0016	0.0005	0.0022	0.0007	-0.0016	0.0007	-0.0484
cscB	0	9	15	30	42	54	60	72
Abs ΔB-ΔA	0.0042	0.0042	0.0087	0.0235	0.0402	0.0544	0.0612	0.0805

- Absorbance vs sucrose concentration standardisation curve:





- Inferences:
    - Absorbance vs sucrose concentrations are not linear. Must be redone.
    - WT has ΔB-ΔA = 0 for all time stamps i.e. it is not producing any sucrose as expected
    - cscB's absorbance is increasing linearly with time. The actual sucrose conc produced by it over time cannot be calculated as a linear curve not obtained with the standard sucrose solutions.

- Sucrose concentrations for cscB determined from standard curve:

cscB (hours)	0	9	15	30	42	54	60	72
Sucrose g/L	0.032	0.032	0.038	0.058	0.08	0.099	0.108	0.134

16/09/2021 - Thursday

E coli

<u>Inoculation of primary cultures</u>

- Sanjana inoculated primary cultures of our strains as follows:

Strain	**Culture composition**
KJK01	5mL 1x LB
KJK01-pcscX (two primary cultures prepared)	5mL 1x LB + 5µL Kan^{50} + 5µL Amp^{100}

Sucrose Assay

Sucrose Assay: Attempt 3; Performed by Likhith and Ashli

17/09/2021 - Friday

E coli

Inoculation of secondary cultures

- Inoculation of secondary cultures in BG-11 done in the same way as on September 15, 2021.

Sucrose Assay

Sucrose Assay: Attempt 4; Performed by Likhith and Ashli

- Followed megazyme's calculation method (i.e. with dilution, distilled water blank, and 100 ug glucose standard) to verify the concentrations of a few more standard sucrose solutions that we prepared:

- 100 ug Glucose Standard prepared in test tube, other solutions prepared in 2mL eppendorf, absorbance taken in cuvette in spectrophotometer at UG lab, G1.

- Blank: 0.2 mL distilled water + 1.5 mL GOPOD
- Standard: 0.1mL of 100 ug D-glucose (1mg/mL) + 0.3 mL of distilled water + 3 mL GOPOD
- Solution A: 0.1 mL sample + 0.1 mL acetate buffer + 1.5 mL GOPOD
- Solution B: 0.1 mL sample + 0.1 mL beta fructosidase + 1.5 mL GOPOD

- Forgot to incubate after adding beta-fructosidase/acetate buffer, incubated only after adding GOPOD. Incubation done in water bath (20 min, 50C).

- Observations:
  - Blank: 0.000, 0.000, 0.000, -0.000, -0.000, -0.000
  - Standard: 1.023, 1.020, 1.022, 1.022, 0.021, 1.022
  - Standard (after vortex): 1.014, 1.011, 1.012, 1.012, 1.013, 1.013
  - 1A: -0.005, -0.008, -0.008, -0.008, -0.009
  - 1B: 0.064, 0.064, 0.064, 0.063, 0.063
  - 2.5A: -0.014, -0.015, -0.013, -0.013, -0.013
  - 2.5B: 0.156, 0.157, 0.158, 0.157, 0.157
  - 5A: -0.106,-0.106, -0.105, -0.106, -0.106
  - 5B: 0.278,0.278, 0.278, 0.278, 0.278,
  - MA: 1.950, 1.942, 1.945, 1.956, 1.950
  - MB: 1.499, 1.498, 1.498, 1.500, 1.501

(M: Prepared 20g/L glucose + 10g/L sucrose solution

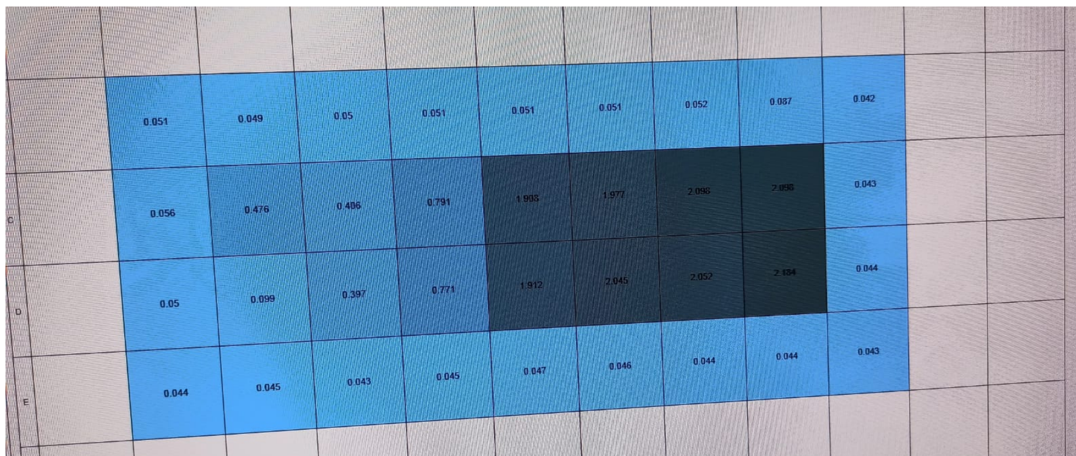
- Inference:
    - Absorbances observed were not following any pattern and did not make much sense. Probably due to the error we made during incubation.

18/09/2021 - Saturday

Sucrose Assay

Sucrose Assay: Attempt 5; Performed by Likhith

- Prepared fresh standard sucrose solutions of concentrations: 0, 0.1, 0.5, 1, 2.5, 5, 10, 20 g/L
- Performed assay in 96 well plate as described in attempt 2 (again, without distilled water blank and standard) with the aim of preparing a absorbance vs sucrose concentration standardisation curve.
- 96 well plate layout:
    - B- Sucrose A
    - C- Sucrose B
    - D- Sucrose B (made pipetting mistake in row C, so re-did it in D)
- Observations:

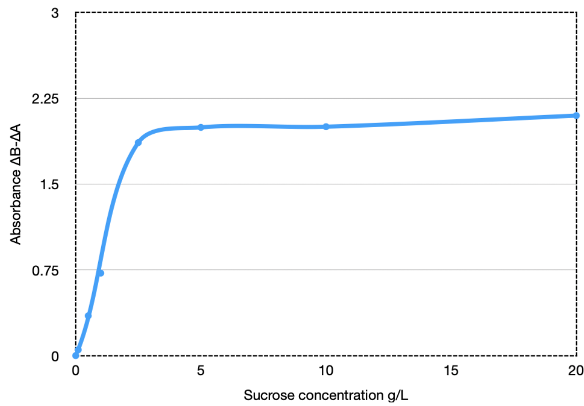


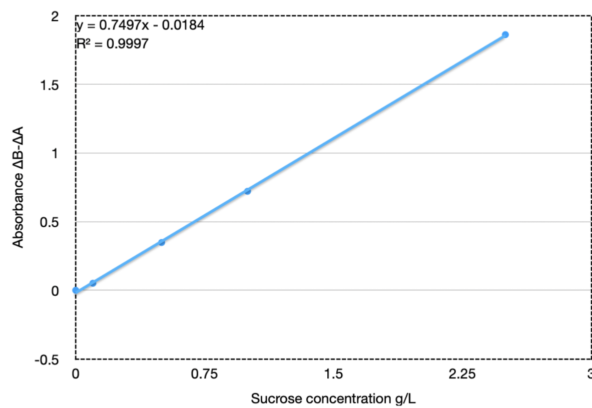
	0.051	0.049	0.05	0.051	0.051	0.051	0.052	0.087	0.042
C	0.056	0.476	0.406	0.791	1.908	1.977	2.098	2.098	0.043
D	0.05	0.099	0.397	0.771	1.912	2.045	2.052	2.184	0.044
E	0.044	0.045	0.043	0.045	0.047	0.046	0.044	0.044	0.043

	2	3	4	5	6	7	8	9
B	0.051	0.049	0.05	0.051	0.051	0.051	0.052	0.087
C								
D	0.05	0.099	0.397	0.771	1.912	2.045	2.052	2.184

- Results:

ΔA	ΔB	Sucrose conc (g/L)	ΔB-ΔA
0.051	0.05	0	-0.001
0.049	0.099	0.1	0.05
0.05	0.397	0.5	0.347
0.051	0.771	1	0.72
0.051	1.912	2.5	1.861
0.051	2.045	5	1.994
0.052	2.052	10	2
0.087	2.184	20	2.097
Subtracting blank (0 g/L) 's absorbance from all:			
0	0	0	0
-0.002	0.049	0.1	0.051
-0.001	0.347	0.5	0.348
0	0.721	1	0.721
0	1.862	2.5	1.862
0	1.995	5	1.995
0.001	2.002	10	2.001
0.036	2.134	20	2.098





- Inference:
    - Graph is linear from 0 g/L to 2.5 g/L, plateaus after that. Cannot get a good linear fit for complete 0 to 20 g/L range. Linear standard curve not possible over entire range.
    - How do we know how much to dilute our bacterial supernatants beforehand so that it falls in the linear range (<5 g/L)?

19/09/2021 - Sunday

E coli

<u>Streaking of E coli containing pKD4</u>

- pKD4 containing E coli was streaked on Kan plates by Chinmaya at 1:00 pm.

20/09/2021 - Monday

E coli

CoBG-11 preparation

- 100 mL coBG-11 prepared by Arsh and Vidisha and pH adjusted to 8.38 at 8:11 pm. Given for autoclaving. Protocol can be found here.

Primary culture inoculation

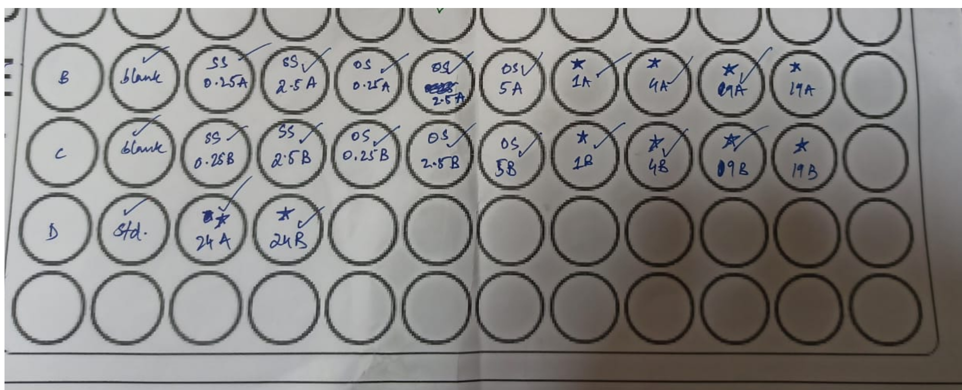
- Primary culture of pKD4 and KJK01-pcscX done in 10mL 1x LB with 10µL Kan^{50}

Sucrose Assay

Sucrose Assay: Attempt 6; Performed by Likhith and Ashli

- Followed megazyme's calculation method to verify the concentrations of a few more standard sucrose solutions that we prepared and to measure sucrose content in E coli supernatants:
    - Sanjana prepared 0.25 and 2.5 g/L solutions
    - Likhith and Ashli also prepared 0.25, 2.5 g/L, 5g/L solutions

- E coli WT supernatants collected after 1h, 4h, 9h, 19h, 1d
- Standard prepared in test tube with final volume 3.4 mL in test tube, blank and samples prepared in 2 mL eppendorfs with final volume 1.7 mL, as described in attempt 4.
- Incubation done after addition of beta fructosidase/acetate buffer and after addition of GOPOD in water bath at 50C for 20 min.
- 300 uL of each solution was transferred into 96 well plate wells and absorbance was measured in plate reader.
- 96 well plate layout:



- Blank here is 0.2 mL distilled water + 1.5 mL GOPOD
- Didn't make 0 g/L sucrose samples i.e. 0.1 mL of distilled water + 0.1 mL of acetate buffer/beta fructosidase + 1.5 mL GOPOD

		2	3	4	5	6	7	8	9	10	11
	B	0.0539	0.0535	0.0546	0.0523	0.054	0.0537	0.0599	0.0558	0.0559	0.0561
	C	0.0549	0.2909	0.3085	0.2427	0.2362	0.4214	0.2202	0.2088	0.186	0.0578
	D	0.7942	0.0572	0.0618							
	Blank 1	0.0539									
	Blank 2	0.0549									
	Average	0.0544									
	Standard	0.7398	Factor	135.172							
	Std sucrose conc g/L	∆A	∆B	∆B-∆A	Calculated sucrose conc g/L	Error % between calculated conc and prepared conc		Calculated Sucrose = ∆B-∆A * factor * dilution factor * 0.0095			
SS=Sanjana Sucrose	0.25 g/L	-0.0009	0.2365	0.2374	0.305	22					
	2.5 g/L	0.0002	0.2541	0.2539	3.26	30.4					
OS = our sucrose	0.25 g/L	-0.0021	0.1883	0.1904	0.244	-2.4					
	2.5 g/L	-0.0004	0.1818	0.1822	2.34	-6.4					
	5 g/L	-0.0007	0.367	0.3677	4.722	-5.56					
E coli	1h	0.0055	0.1658	0.1603	0.206						
	4h	0.0014	0.1544	0.153	0.196						
	9h	0.0015	0.1316	0.1301	0.167						
	19h	0.0017	0.0034	0.0017	0.002						
	1d	0.0028	0.0074	0.0046	0.006						

- Inferences:
    - ΔB-ΔA for 0.25 and 2.5 g/L solutions are similar in both cases (sanjana's and my solutions) as expected due to the dilution done.
    - WT E coli is consuming sucrose
    - WT E coli had been inoculated into BG-11 containing 250 mg/L sucrose with the sucrose solution sanjana had prepared, but the kit is measuring it out to be 0.3 g/L, solution was probably prepared inaccurately

---

22/09/2021 - Wednesday

E coli

Plasmid extraction

- Plasmid extraction of pKD4 done by Rushik. Plasmid stored at Rushik's lab.

23/09/2021 - Thursday

E coli

LB agar preparation

- 300mL LB agar prepared by Arsh and Vidisha and given for autoclaving.

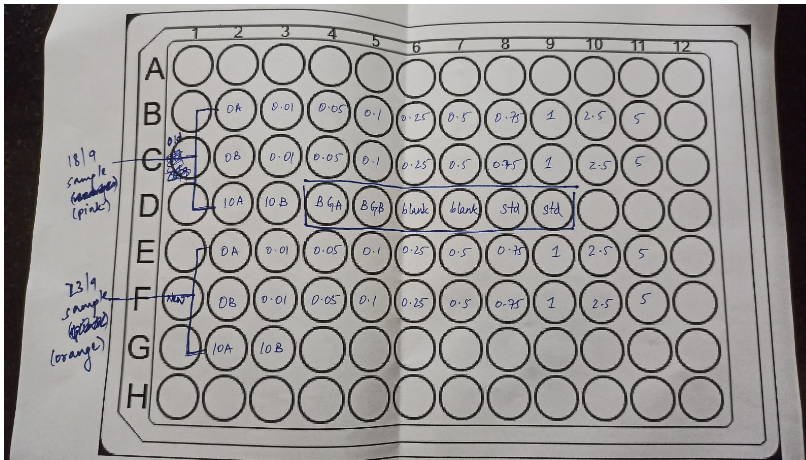
Sucrose Assay

Sucrose Assay: Attempt 7; Performed by Likhith and Ashli

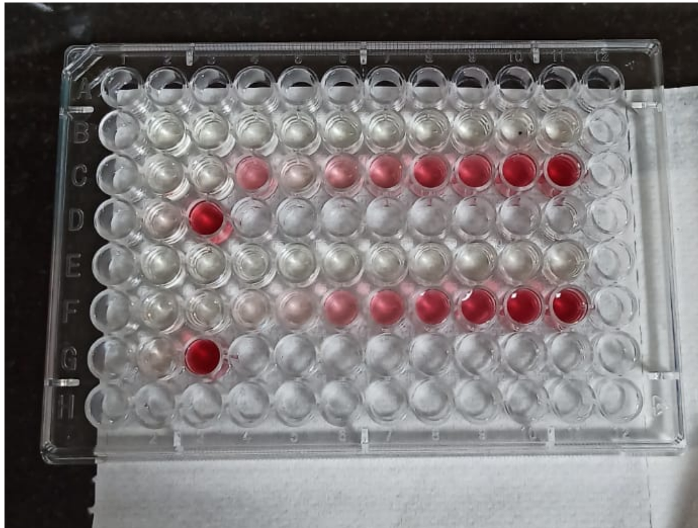
- On speaking with Nishad, redid sucrose assay measurements in duplicate with the following prepared solutions:
  - 0 g/L, 0.01 g/L, 0.05 g/L, 0.1 g/L, 0.25 g/L, 0.5 g/L, 0.75 g/L, 1g/L, 2.5 g/L, 5g/L, 10 g/L
  - Distilled water blank, 100 ug glucose standard, bg-11 blank

- Standard prepared with total volume of 3.4 mL in test tube, while other solutions prepared with a total volume of 1.7 mL in 2 mL eppendorf, as described in attempt 4. 300 uL of each solution pipetted into wells of 96 well plate.

- Plate layout:



- Observations:



<>	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.051	0.0512	0.0513	0.0492	0.0504	0.0514	0.051	0.0524	0.0558	0.0703	
C		0.0587	0.0626	0.4324	0.1228	0.3104	0.4532	0.7851	0.8346	1.3468	1.7574	
D		0.0964	1.8397	0.053	0.0595	0.0513	0.0512	0.823	0.8236	0.0479	0.0498	
E		0.0518	0.053	0.0517	0.0514	0.0529	0.0525	0.0535	0.0548	0.0592	0.0684	
F		0.057	0.0656	0.0996	0.1438	0.3738	0.5434	0.8003	1.0465	1.4499	1.5512	
G		0.0843	2.1516	0.0478	0.0477	0.0603	0.0485	0.0478	0.0492	0.0472	0.0473	
H												

- Results:

	Prepared sucrose conc	ΔA	ΔB	ΔB-ΔA	Calculated sucrose conc		Error %
18/9 sample	0	0.051	0.0587	0.0077	0.009		
	0.01	0.0512	0.0626	0.0114	0.013		30
	0.05	0.0513	0.4324	0.3811	0.44		780
	0.1	0.0492	0.1228	0.0736	0.085		-15
	0.25	0.0504	0.3104	0.26	0.3		20
	0.5	0.0514	0.4352	0.3838	0.443		-11.4
	0.75	0.051	0.7851	0.7341	0.847		12.93
	1	0.0524	0.8346	0.7822	0.903		-9.7
	2.5	0.0588	1.3468	1.288	1.486		-40.56
	5	0.0703	1.754	1.6837	1.943		-61.14
	10	0.0964	1.8397	1.7433	2.012		-79.88
23/9 sample	0	0.0518	0.057	0.0052	0.006		
	0.01	0.053	0.0656	0.0126	0.015		50
	0.05	0.0517	0.0996	0.0479	0.055		10
	0.1	0.0514	0.1438	0.0924	0.107		7
	0.25	0.0529	0.3738	0.3209	0.37		48
	0.5	0.0525	0.5434	0.4909	0.566		13.2
	0.75	0.0535	0.8003	0.7468	0.862		14.93
	1	0.0548	1.0465	0.9917	1.144		14.4
	2.5	0.0592	1.4499	1.3907	1.605		-35.8
	5	0.0684	1.5512	1.4828	1.711		-65.78
	10	0.0843	2.1516	2.0673	2.385		-76.15

27/09/2021 - Monday

E coli

Primary inoculation

- Arsh and Vidisha prepared three 5mL primary cultures, one of E coli KJK01, and two of KJK01-pcscX (with 5 µL Kan^{50}) at 9:00 pm.

28/09/2021 - Tuesday

E coli

Streaking

- We did not observe any growth in the previous day's inocula. The plates that we had used for inoculation were old so we decided to streak fresh plates.
- Arsh streaked KJK01 on an LB plate and KJK01-pcscX on an LB plate with 20µL of Kan^{50} and Amp^{100}.

Cyanobacteria

CoBG-11 preparation

- Arsh and Vidisha 400mL coBG-11 and pH adjusted to 8.37. Sent for autoclaving.

29/09/2021 - Wednesday

E coli

Streaking

- We did not observe any growth in the previous day's plates. Rushik mentioned that all the labs in the biology department have been facing a phage infestation. Advised us to sterilize our hood and incubator.
- Arsh and Vidisha streaked KJK01 on an LB plate and KJK01-pcscX on an LB plate with 20µL of Kan^{50} and Amp^{100}.
- Hood was left for overnight UV.

30/09/2021 - Thursday

E coli

Primary inoculation

- Arsh and Vidisha prepared three 5mL primary cultures, one of E coli KJK01, and two of KJK01-pcscX (with 5 µL Kan^{50}) at 7:18 pm.

01/10/2021 - Friday

E coli

<u>Secondary inoculation</u>

- 100 mL coBG-11 secondary cultures for our strains prepared the same way as on September 15, 2021 but with coBG-11.
- Growth curve assay starts with readings taken every 6 hours.

06/10/2021 - Friday

E coli

<u>Growth curve assay</u>

- We wrap up our growth curve assay for our strains in coBG-11 + sucrose

---

11/10/2021 - Friday

E coli

<u>Streaking plates</u>

- Our coBG-11 cultures were showing contamination, so Arsh streaked all three of them on LB plates with appropriate antibiotics.

Cyanobacteria

<u>Passage</u>

- Arsh passaged WT2973 and the cscB strain to 80 mL cultures.

<u>Streaking</u>

- Arsh streaked WT2973 on a BG-11 agar plate.