Understanding CupA's role in the CO_2 Concentrating Mechanism in Cyanobacteria

Sydney Markham | Clark Jett | Dr. Robert Burnap & Associates

Microbiology and Molecular Genetics

TRIO McNair Scholars

U.S. DEPARTMENT OF ENERGY
Office of Basic Energy Sciences
DE-FG02-08ER15968

Niblack Scholars Program

Overview

- Background
- Introduction of Carbon Concentrating Mechanism (CCM)
- Introduction of Cup Proteins (CupA/CupB)
- Experimental Methods/Results
- Future Research

RESEARCH

Background

- Cyanobacteria are bacterial microorganisms capable of oxygenic photosynthesis
  - *Synechococcus elongatus sp.* PCC 7942 is a gram negative and occupies a wide niche of environments, used as experimental model
- Photosynthesis is an important process that uses inorganic materials to produce usable carbohydrates
  - 6 CO_2 + 6 H_2O +  $C_6H_{12}O_6 + 6CO_2$
  - This process helps reduce greenhouse gas emissions efficiently
- RuBisCO is the major CO_2 fixing enzyme in plants, algae, and photosynthetic bacteria, but has low affinity for CO_2
- Better understanding CO_2 fixation could help improve photosynthesis and serve as a model for engineering CO_2 –scavenging solutions to mitigate greenhouse gases.

CO_2-concentrating mechanism (CCM)

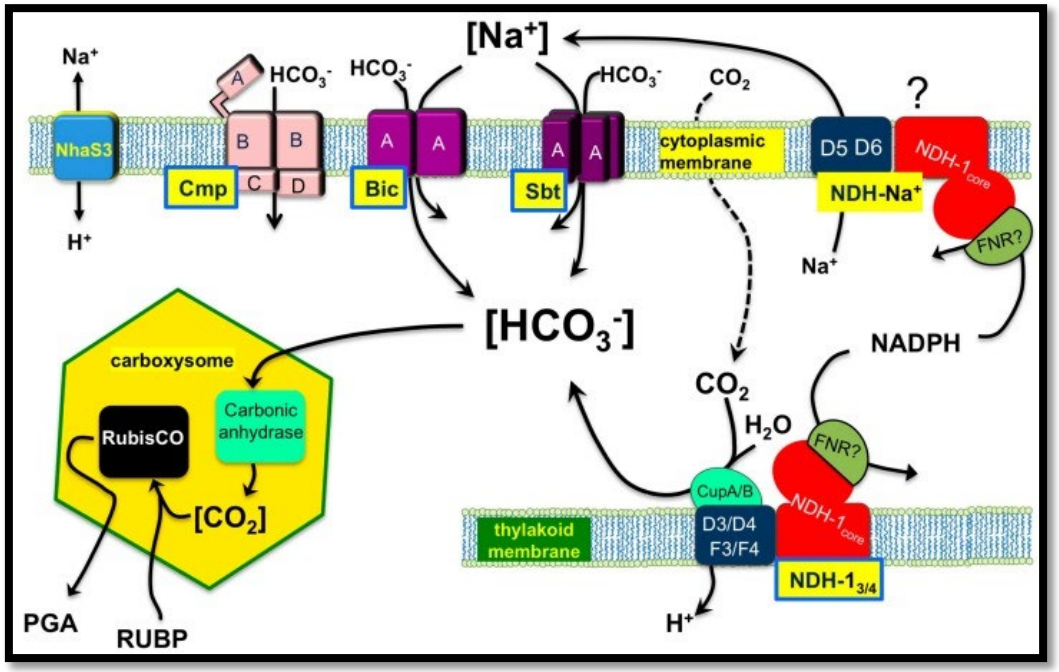


Fig. 1 Showing the CCM diagram on the thylakoid membrane, cytoplasmic membrane and in the carboxysome.

Energetically accumulates HCO_3^- in the cytoplasm where it is then used by the carboxysome

- Raises CO_2 levels at carboxylation sites to push carbon fixation by the main enzyme RuBisCO
  - RuBisCO is the most abundant enzyme, but has poor affinity for its substrate, CO_2
  - Oxygenase reaction wastefully competes with carboxylase reaction
  - Found in all cyanobacteria
- Genes encoding for the CCM have been identified, but functions are not fully understood
  - Mechanisms of biochemical pathways between the subunits needs further investigation

CO_2 Uptake Proteins (CUP)

- Use energy to convert CO_2 to bicarbonate (HCO_3^-)
- Cup proteins are essential for growth in normal CO_2 levels
  - Attached to the transmembrane NDH-1 Complexes on the thylakoid membrane
- Two Cup systems are known, but how they interact is not understood
  - High Affinity NdhD3, NdhF3, CupA and CupS
  - Low Affinity NdhD4, NdhF4, and CupB
- Cup A is an induced gene, but CupB is constitutive
- Not known whether CupA can function on its own without CupB

Hypothesis: CupA can function independently of CupB

RESEARCH

Experimental Strategy

Use directed gene mutagenesis and complementation to test whether CupA can function independently of CupB

- Use or make *Synechococcus* deletion mutant strains lacking the *cupA*, *cupB*, and both *cupA* and *cupB* genes
- Transform deletion mutant strains with complementing plasmids expressing either *cupA* or *cupB*
- Use physiological assays to test for CO2 uptake capacity

RESEARCH

Methods

- Test function: Culturing of *Synechococcus elongatus* PCC 7942 wildtype and mutant strains under different CO_2 concentrations to test ability to uptake inorganic carbon
- Gene deletion of cupA gene via homologous recombination (replace with antibiotic gene)
  - PCR and Primer Design: For cloning and verifying genotypes
  - Gibson Assembly: Suicide plasmid construction of ΔcupA:chloramphenicol resistance DNA
  - Bacterial Transformation: transfer plasmid into *E. coli* and *Synechococcus*
  - DNA sequencing and plasmid isolation
- Utilize complementation plasmid expressing to reintroduce cupA into cupB deletion strains
- Cell physiology measurements
  - Spot Assays to evaluate phenotypes and test hypothesis of CupA sufficiency for low CO_2 growth

RESEARCH

Construction of homologous recombination 'suicide' vector to delete *cupA* in *Synechococcus sp.* PCC7942 by replacement with Cm^r gene

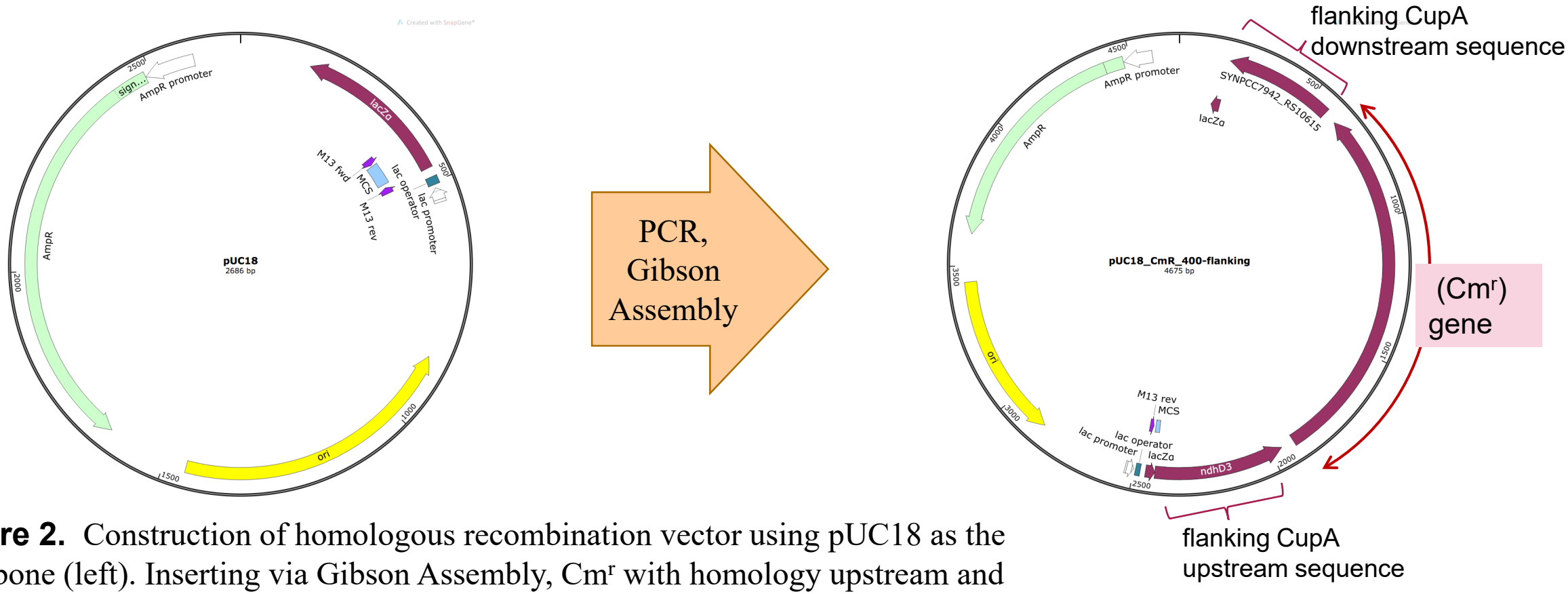


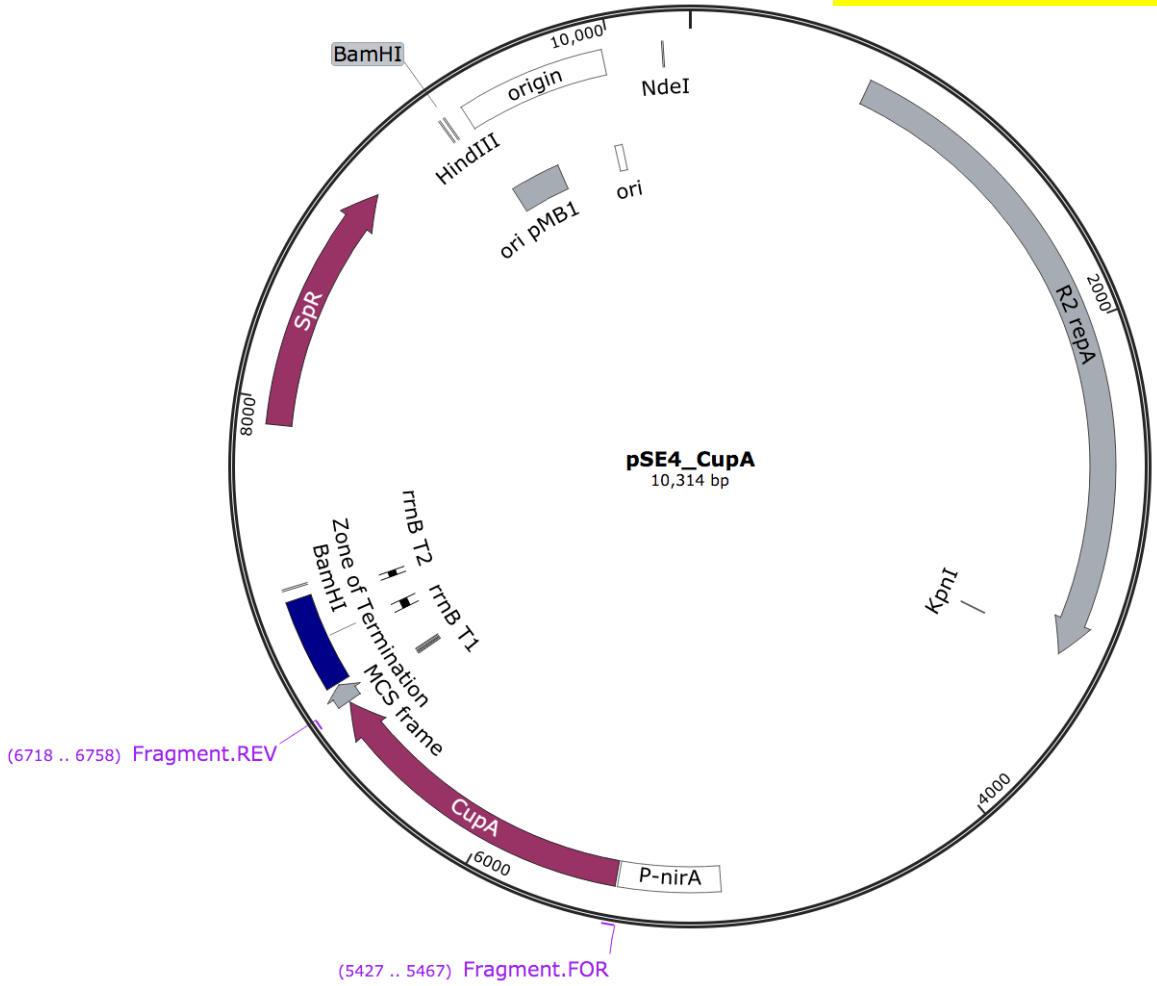
Figure 2. Construction of homologous recombination vector using pUC18 as the backbone (left). Inserting via Gibson Assembly, Cm^r with homology upstream and downstream of WT CupA. (Right).

Note: plasmids replicate in *E. coli*, but not in *Synechococcus sp.* PCC7942.

Any Cm^r transformant cyanobacteria are from homologous gene replacement of the *cupA* gene with the Cm^r gene

Replicative expression plasmids to express *cupA* and *cupB* in *Synechococcus sp.* PCC7942

Plasmids replicate in both *E. coli* and *Synechococcus sp.* PCC7942.



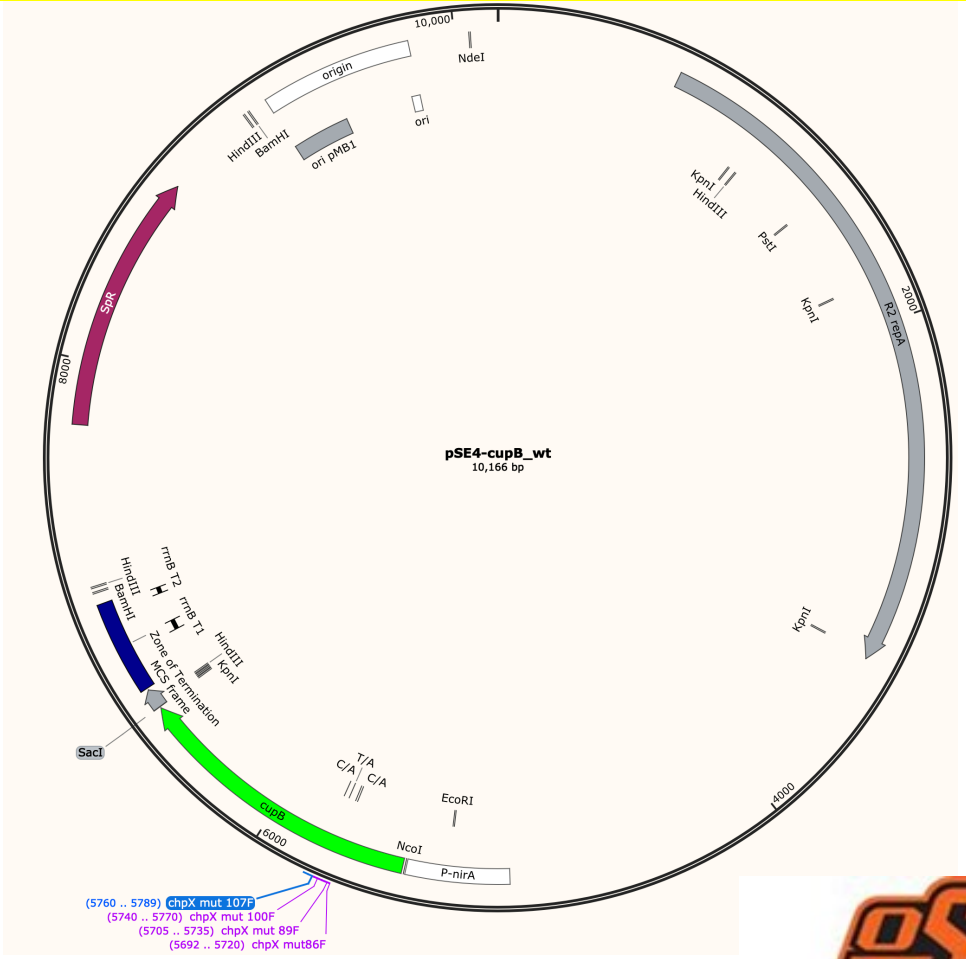


Figure 3. pSE4-CupA and pSE4-CupB expression plasmids (from Clark Jett and Dean Price, respectively), to reintroduce *cupA* and *cupB* genes into *Synechococcus sp.* PCC7942.

RESEARCH

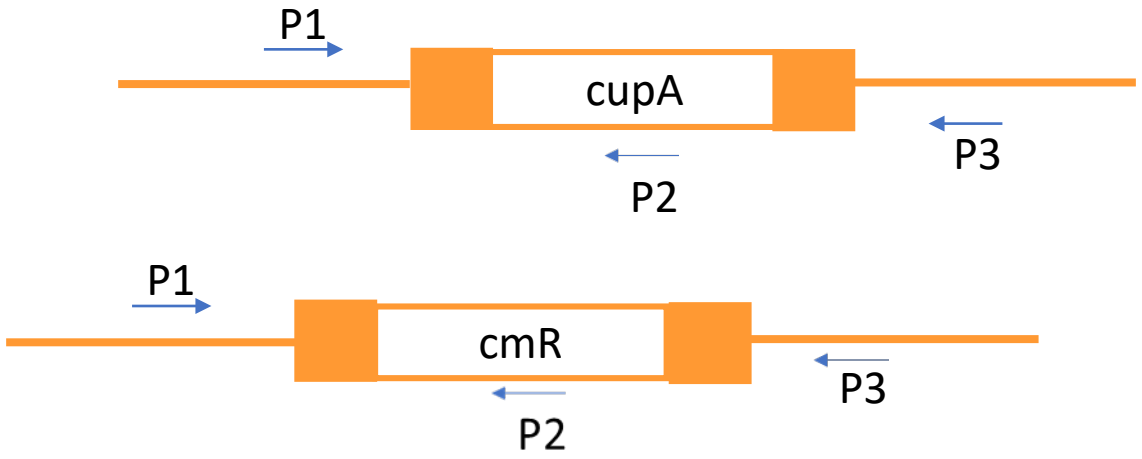
Strains used in the study

Table 1. Synechococcus Strains of interest

Strains	Name	Antibiotic Resistance	Genotype	Physiological Phenotype
Synechococcus elongatus PCC 7942	Wt-Wildtype	None	WT	Grows no matter CO_2 or air conditions or pH
ΔX/ΔY	ΔX/ΔY , cup proteins, ΔA/ΔB	Chloramphenoicol(Cm), Kanamycin (Km)	ΔcupA::Cmr ΔcupB::KmR (ΔchpY::Cmr ΔchpY:: Kmr)	Grows best in elevated CO_2 and pH 8
ΔX/ΔY + 1	CupB only, +1	Spectinomycin (Sp)	ΔSp/ΔB	Growth is heavy in all conditions, but less than WT
ΔX/ΔY + CupA	CupA only, +CupA	Sp,Km,Cm	ΔX/ΔKm	Growth is steady, but less than +1 and WT
ΔCupA	CupB only, CupA KO	Cm	ΔCm/ΔY	Growth is seen and similar to +1

RESEARCH

Results



Expectations:

- **Successful**
  - P1 and P2- Band only on strains with CmR resistance near 0.8 kbp
  - P1 and P3- Band around 2 kbp, shows CmR cassette is located where the CupA gene is.
- **Unsuccessful Or WT**
  - P1 and P2- No band, meaning no CmR cassette
  - P1 and P3- Band around 2 Kbp representing CupA

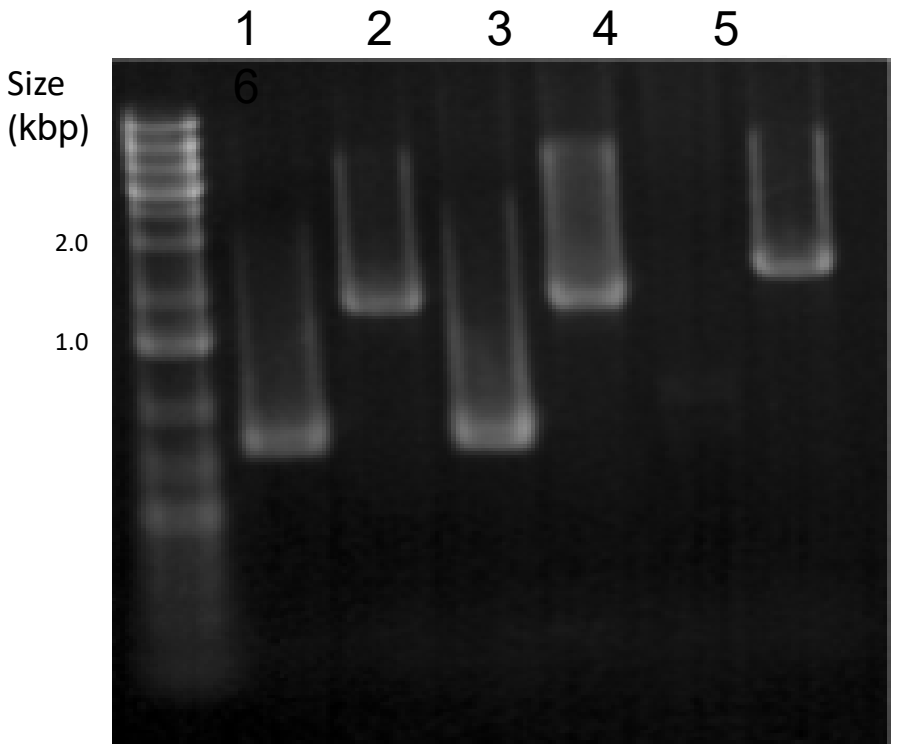


Figure 4. PCR Product shown on electrophoresis gel.

Lane Summary:

Outermost left lane- Molecular DNA weight Ladder

Lane 1- P1 and P2, Transformation Colony

Lane 2- P1 and P3, Transformation Colony

Lane 3- P1 and P2 , ΔX/ΔY

Lane 4- P1 and P3, ΔX/ΔY colony

Lane 5- P1 and P3, WT

Lane 6- P1 and P3, WT

RESEARCH

Results

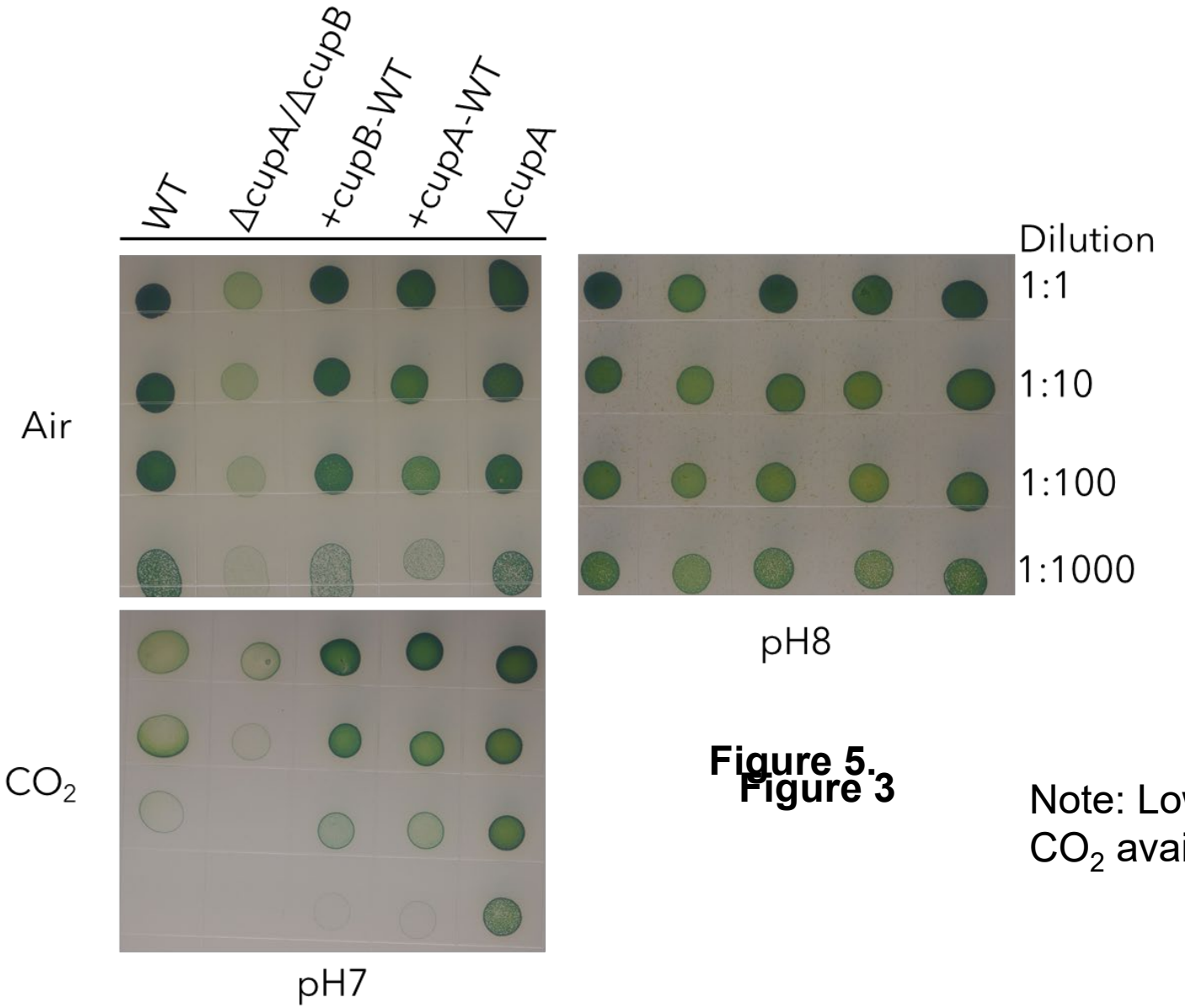


Figure 5. Figure 3

Figure 5: Spot assays for autotrophic growth of CCM mutants. Mutants were grown in liquid media, pelleted, and resuspended to an $O.D._{750}$ of 1.0 proceed by serial dilutions. The respective dilutions were plated and allowed to grow on the designated media pH

Note: Low pH corresponds to low CO_2 availability on the agar plates

RESEARCH

Conclusion

- The deletion mutation was successful, as shown in figure 2.
- Figure 3. is a representation of several independent assays performed, to show CupA can grow under low Ci conditions and uptake CO_2 without CupB but not quite as efficient.
  - **CupA can function independently**
- Confirm CupB is sufficient alone to uptake CO_2 and continue to provide HCO_3^-

RESEARCH

Future Research

- Continue replicating spot assays
  - Comparing different strains
- Cell physiology Measurements
  - PAM fluorescence assays to evaluate detailed electron transport differences
  - C_i Affinity Assay using Clark-Type electrode
  - Observing changes in Growth and CO_2 uptake, and proton pumping

RESEARCH

Acknowledgements

- Dr. Robert Burnap, Principal Investigator & Lab Associates
  - OSU Microbiology department
- Clark Jett, Microbiology & Molecular Genetics M.S.
  - Graduate student mentor
- Dr. John & Heidi Niblack
- OK-Louis Stokes Alliance for Minority Participation
- McNair Scholars Program

OSU RESEARCH

References

- Burnap, R. L., Hagemann, M., & Kaplan, A. (2015). Regulation of CO2 Concentrating Mechanism in Cyanobacteria. *Life (Basel, Switzerland)*, *5*(1), 348–371. https://doi.org/10.3390/life5010348
    - Fig. 1
- Miller NT, Vaughn MD, Burnap RL. Electron flow through NDH-1 complexes is the major driver of cyclic electron flow-dependent proton pumping in cyanobacteria. Biochim Biophys Acta Bioenerg. 2020:148354; doi: 10.1016/j.bbabio.2020.148354.
- Han, X., Sun, N., Xu, M., & Mi, H. (2017). Co-ordination of NDH and Cup proteins in CO2 uptake in cyanobacterium Synechocystis sp. PCC 6803. Journal of experimental botany, 68(14), 3869–3877. https://doi.org/10.1093/jxb/erx129
- Woodger, F.J., Badger, M.R., Price, G.D.(2003) Inorganic carbon limitation induces transcripts encoding components of the CO2-concentrating mechanism in Synechococcus sp. PCC7942 through a redox-independent pathway. Plant Physiology, 133(4), 2069–2080, https://doi.org/10.1104/pp.103.029728