Testing Diverse Bacterial Rubiscos in *E. coli*

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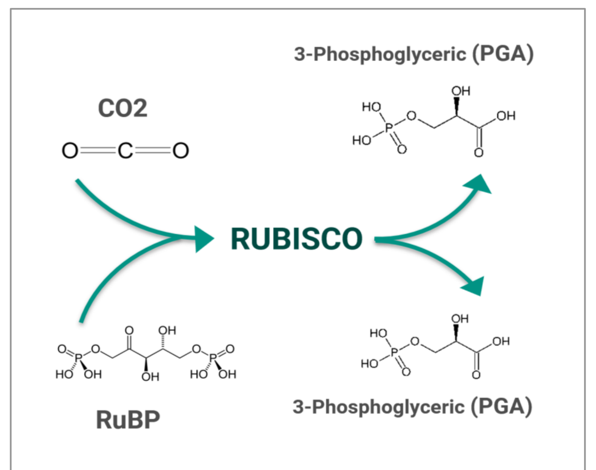
ABSTRACT

Rubisco is essential for plant growth and survival. It is responsible for catalyzing carboxylation in the Calvin Cycle which is the primary step of carbon fixation in photosynthesis. Considering the enormous mass of plants on the Earth, Rubisco is categorized as the most abundant enzyme on the planet. In nature, Rubisco is located not only in plants but also in bacteria. Bacteria have four types of Rubisco: Form I, Form II, Form II/III, and Form III [2]. These forms of Rubisco in bacteria make it diverse in size and structure. Researchers consider that improving Rubisco might improve plant growth. The goal of this research is to test nine different Rubiscos in E.coli and determine their function. Our lab has developed a Rubisco-dependent *E. coli* that requires functional Rubisco in order to grow. We will test the assembly and function of nine bacterial Rubiscos in this *E. coli* strain. We also use characterize mutant Rubiscos in this manner. These experiments will provide a basis for understanding the effects of mutations in distinct bacterial Rubiscos which will be useful for researchers aiming to improve plant crops.

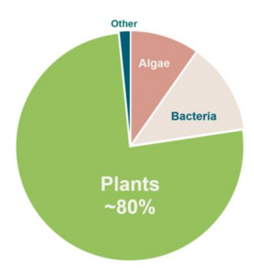
RUBISCO IMPROVEMENT

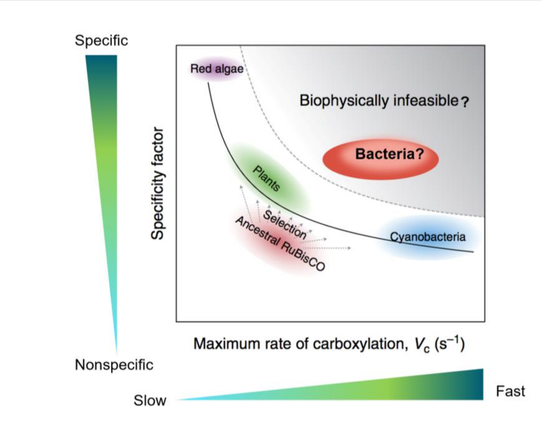


Gatekeeper for carbon fixation in the Calvin Cycle during photosynthesis

If Rubisco is improved, plant crops will also be improved in order to supply a safety agriculture production as well as biofuels production

Hundreds of Rubiscos were tested to analyze their characteristics

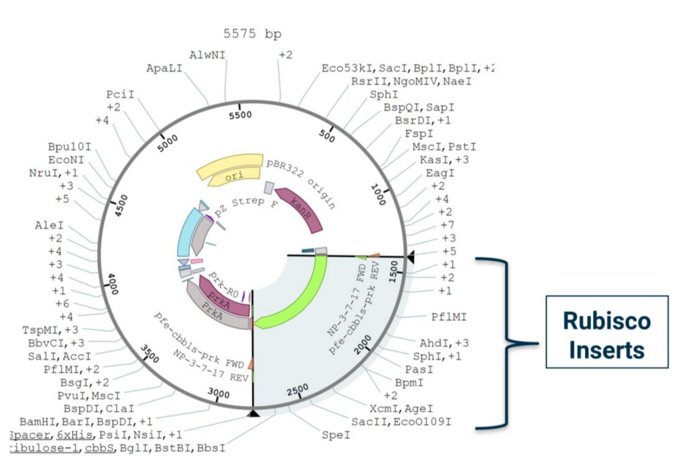


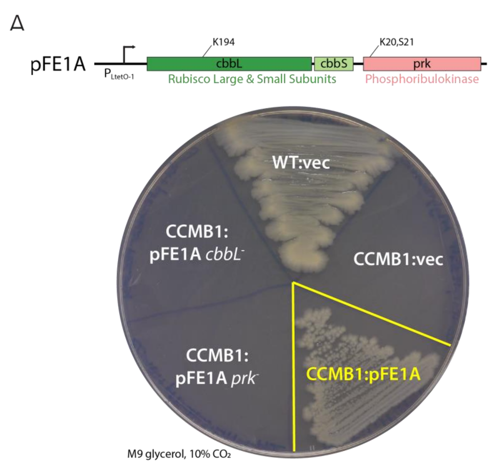


METHODS

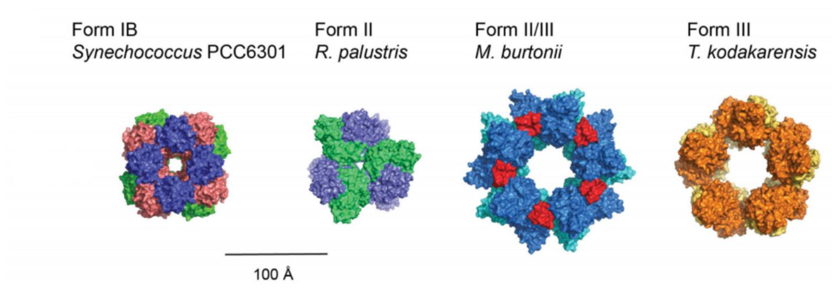
1. Make Rubisco plasmids by genetic engineering

2. Test Rubisco in Rubisco-dependent *E.coli Strain (CCMB1)*





RESULTS



	Form I	Form II	Form II/III	Form III	Total
Attempted	2	2	1	4	9
Cloned	2	2	1	3	8

Figure 1. 8 diverse Rubiscos were successfully cloned into *E.coli* expression plasmids

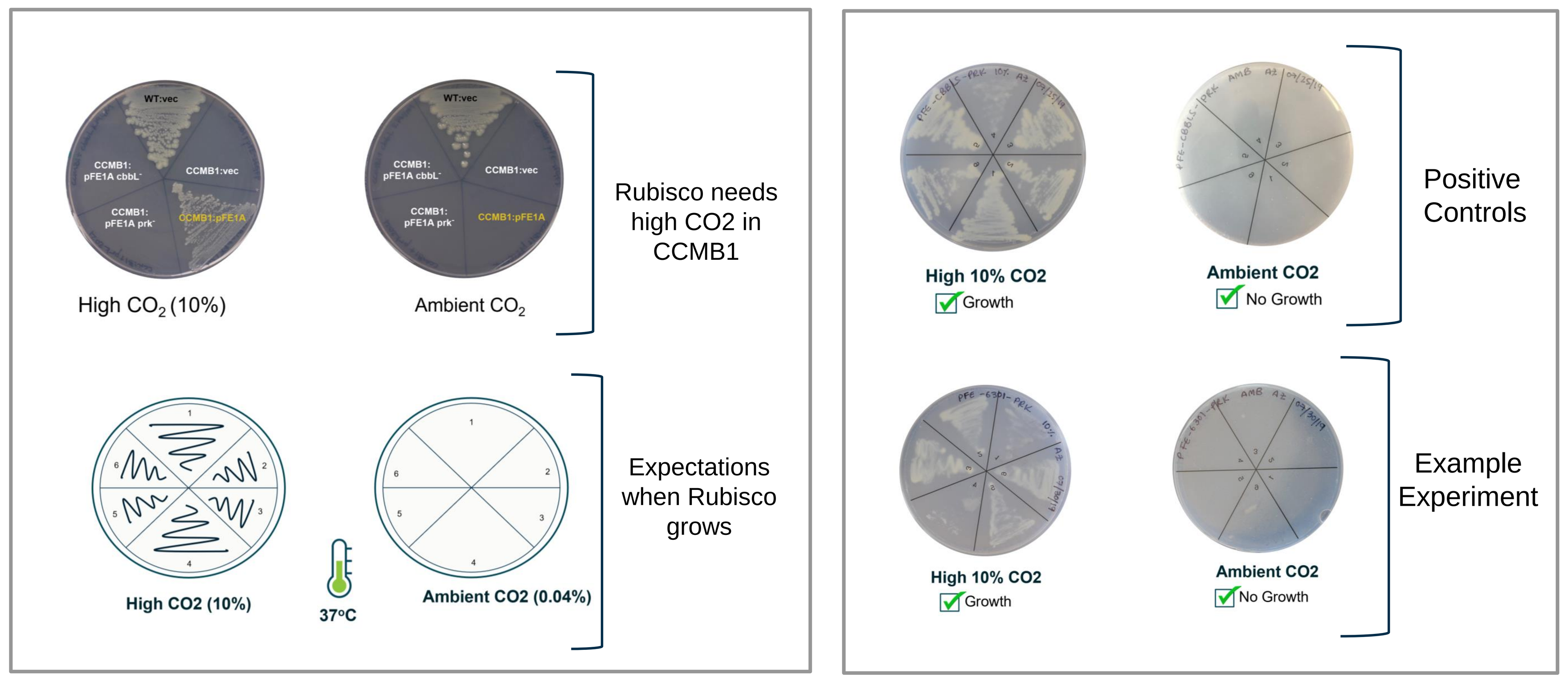


Figure 2 . Growth of *E. coli* strains with Rubisco variants in agar plates in minimal media at varying CO2 levels.

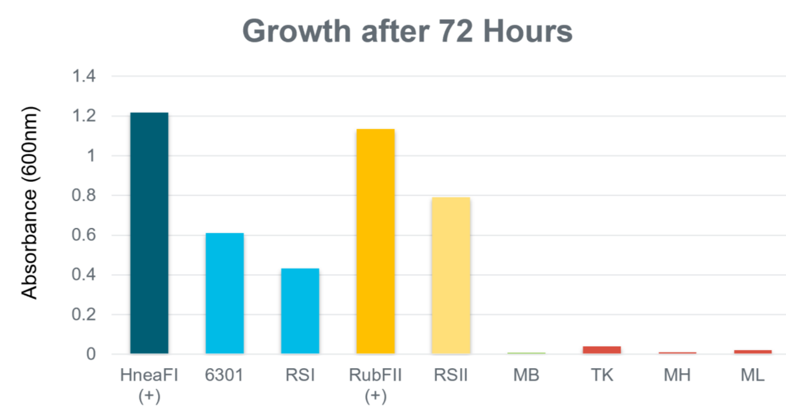


Figure 3. Growth of *E. coli* strains with Rubisco variants in liquid media at 5% CO2

Organism	Rubisco Form	Plates: Ambient	Plates: 10% CO2	Liquid media: 5% Co2
Synechoccus elongatus	I	✗	✓	✓
Rhodobacter sphaeroides	I	✗	✓	✓
*Rhodospirillum rubrum **	II	✗	✓	✓
Rhodobacter sphaeroides	II	✗	✓	✓
Methanococcoides burtonii	II/III	✗	~	✗
Thermococcus kodakarensis	III	✗	~	~
Methanospirillum hungatei	III	✗	✗	✗
Methanofollis liminatans	III	✗	✗	✗
Rhodoferax ferrireducens	II	✗	✓	N/A

Figure 4. Compiled Results of plate and liquid growth assays.

CONCLUSION

1. 8 plasmid DNA for 8 Rubiscos were created
2. Three bacterial Rubiscos function well in *E. coli*
3. Three more bacterial Rubiscos may also work, but are less robust

FUTURE WORK

1. Verify the experiments I made
2. Try mutant Rubiscos: can we make they better in *E. coli*?
3. Purify bacterial Rubiscos and mutants: how fast and specific are they?

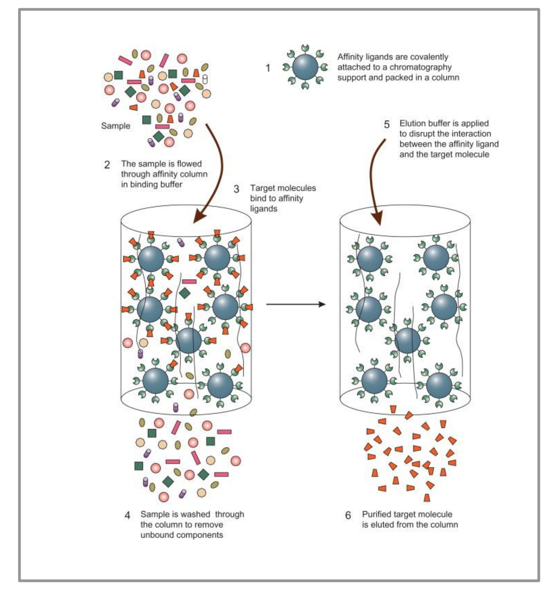


Figure 5. Schematic of affinity chromatography purification

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