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 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.

METHODS

1. Make Rubisco plasmids by genetic engineering
2. Test Rubisco in Rubisco-dependent E. coli Strain (CCMB1)

RESULTS

Table 1. Attempted and Closed forms of Rubisco in E. coli

<table>
<thead>
<tr>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
<th>Form IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attempted</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Closed</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

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

Acknowledgment

REFERENCES

[3] Li, Rui, and Rui Alves, Biochemistry, 2019

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 them better in E. coli?
3. Purify bacterial Rubiscos and mutants: how fast and specific are they?

Figure 5. Schematic of affinity chromatography purification

Acknowledgments

2019 Transfer-to-Excellence Program (TTE)