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A possible alternative pathway for malate metabolism in *Bradyrhizobium japonicum*

The soybean plant plays an important role in our world by forming a symbiotic relationship with the bacteria Bradyrhizobium japonicum. Through this relationship the bacteria is supplied with intermediates of the citric acid cycle providing high energy carbon molecules, while the bacteria supplies the plant with nitrogen compounds through the specialized process of nitrogen fixation. The complications of these processes arise when considering the anaerobic conditions within the bacteroid. Nitrogenase, the enzyme which reduces atmospheric nitrogen to biologically useful forms, requires low oxygen concentrations for its existence. However, low oxygen concentrations can have inhibitory side effects on the enzymes involved in the TCA cycle. Research by Dr. David Emerich of the University of Missouri proposes that there may be a novel pathway utilized by B. japonicum in which the bacteria utilize metabolites of propionate metabolism to create an altered version of a 2-methylcitrate cycle. My research involves creating a gltA mutant of B. japonicum devoid of 2-methylcitrate synthase activity. Creating this mutant is the first step in a process that will ultimately contribute to a better understanding of the symbiosis between the bacteria and soybean. To accomplish this task I have been working on transferring the gltA gene into a vector, transforming the vector into E. coli, then mutating the gene by disrupting the sequence with a streptomycin resistance cassette isolated from the php45 vector. Once this mutant is isolated, the gene will be transferred to B. japonicum and root nodule formation will be attempted. Phenotypical analysis of this mutant will be based on assays of oxygen and ammonia concentrations to measure respiration and nitrogen fixing activity. Depending on these analyses, appropriate experiments will be performed by members of Dr. Emerich's lab to further analyze the effects of this mutation and the role of 2-methylcitrate synthase in nitrogen fixation.

This project was completed to fulfill a Capstone requirement.