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From pyruvate to PEP...the unknown pathway

Darian Wigfall and N. Oehrle

Soybean is a major crop in the United States. Its survival and relationship with its environment are closely monitored. For example, we are looking at the symbiotic relationship between soybean and the soil bacteria. These bacteria can convert nitrogen in the air into usable nitrogen for the soybean plant. This process occurs when the rhizobium forms a nodule on the soybean root. These nodules are complex, hyperplastic tissue masses derived from cortical cells that transport nitrogen as uerides. The soybean plant then in turn acts as a carbon and energy source for the bacteria. The metabolic pathway for the bacteria's reception and consumption of this carbon is unknown. Our specific focus is to use in gel assays to identify the presence of glyceraldehydes-3-P-dehydrogenase, phosphoglycerate kinase, phosohpoglycerate mutase and enolase, used by the plant to synthesize a reaction to convert glyceraldehydes-3-phosphate into PEP (phosphoenolpyruvate) which will be conducted after my departure from the lab. We first conduct a protein extraction to isolate only the desired material from the soybean plant. We then use a one-dimensional gel enzyme assay to determine whether our four main enzymes are active (we received positive results for all four). Also, a two-dimensional gel enzyme assay to determine whether the enzyme we have found is indeed the one we think we have identified in one dimension (we have found positive results for all except phosphoglycerate mutase). Finally, we use an enzyme assay to determine if there is enzyme activity by measuring the absorbance of a solution containing substrate and introducing the enzyme. We found the presence and activity of all of the crucial enzymes involved in the pathway. We have pretty much concluded that this pathway, formerly thought to be a part of the Alanine Transport model, is more likely to be a part of the plant's metabolism.