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Title:POST SYMBIOTIC ANALYSIS OF THE BRADYRHIZOBIUM JAPONICUM-SOYBEAN SYMBIOSIS: PROTEOMICS AND MUTAGENESIS OF PROTOCATECHUATE 3,4-DIOXYGENASE PARALOGS

Symbiotic nitrogen fixation between Bradyrhizobium japonicum and soybean is the predominant pathway by which the abundant, inert supply of atmospheric dinitrogen is made available in the form of reduced nitrogen, the most limiting nutrient in agricultural production. In a microaerobic, oxygen limiting, environment huge amounts of energy are required to fuel this process and a steady supply of carbon substrates is needed from the symbiotic legume partner to fuel the energy requirements of nitrogenase. What source(s) of carbon that are made available to supply energy for nitrogen fixation is poorly understood beyond simple sugars and dicarboxylic acids in B. japonicum. The extension of the duration of nitrogen fixation could lead to greater agricultural production. Many aspects of B. japonicum persistence in soil, infection, and nodule formation and function have been researched. The least studied aspects of leguminous nitrogen fixation of determinant species are the events that occur beyond the cessation of the symbiosis. Upon senescence of the plant what cellular changes occur to the bacteria? In order to better understand the metabolism of B. japonicum bacteroids past peak nitrogen fixation proteomics was done over 119 days. Additional proteins of the periplasm were isolated and analyzed as this cellular compartment serves as a regulator of metabolites and enivormental signals to the bacterium. A metabolic pathway of interest, l^2 -ketoadipate pathway, was analyzed for symbiotic phenotype by mutating both sets of genes encoding pcaHG. Finally to better understand if the bacteroids of B. japonicum were dedifferentiating into their free-living form, scanning and transmission electron micrographs were taken of post-peak nitrogen fixing soybean nodules with the discovery of appendages being created by B. japonicum in the post-symbiotic state.