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RHIZOBIUMOMA IN FREE LIFE AND IN SYMBIOSIS WITH *PHASEOLUS VULGARIS*.

Sergio Encarnación-Guevara, Jeovanis Gil-Valdés, Osbaldo Resendis-Antonio, Magdalena Hernández, Sandra Contreras, Emmanuel Salazar, Gabriel Martínez-Batallar and Yolanda Mora.
Laboratorio de Proteómica, Programa de Genómica Funcional de Procariotes, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México. Av. Universidad s/n C.P. 62100. Cuernavaca Mor., México.
encarnac@ccg.unam.mx

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INTRODUCTION: *Rhizobium etli* belongs to the class Rhizobiaceae, members of which have the ability to develop two different life styles: as free-living cells in the rhizosphere and as fixing nitrogen bacteroids in symbiosis. Nitrogen fixation is the biological process by which atmospheric nitrogen is taken up by bacteroids located in plant root nodules and converted into ammonium through the enzymatic activity of nitrogenase. Transformation of a free-living bacterium to a nitrogen fixing bacteroid results in significant physiological and developmental changes in the rhizobia. Over four decades of intense biochemical and molecular research in Rhizobium species has sought to understand the metabolism of their free-living and symbiotic forms. Currently, the use of high-throughput technologies provides valuable data that contributes to our understanding of the metabolic activity during nitrogen fixation with *Phaseolus vulgaris* (common bean) and under free-living conditions.

RESULTS: Using trancriptomics, proteomics and metabolomics approaches, called collectively by us RHIZOBIUMOMA, we are trying to decipher the biological activity of these two dissimilar life stages of *R. etli*. This analysis was accomplished by an integrative computational study of the high-throughput data to characterize the metabolic activity in *R. etli* bacteria in culture (free life) and bacteroids from the root nodules of *P. vulgaris*. Proteome (2D-PAGE-MALDITOF and LC-MS), transcriptome (DNA-microarrays) and metabolomics (CE-MS) technologies led us to identify 1045 proteins, 1473 differential regulated genes and 220 metabolites in these two physiological stages. By integrating these data into a constraint-based model, we built a refined computational platform abie to survey the metabolic activity underlying nitrogen fixation in *R. etli*. 1) Transcriptome, proteome and metabolome data led us to detect all the common amino acids, with the exception of cysteine, for which only its homologue, homocysteine, was identified. In light of these results, the participation of a variety of amino acids in *R. etli* during symbiotic nitrogen fixation with *P. vulgaris* is clear. 2) The integrative analysis using RHIZOBIUMOMA and constraint-based modeling supplied additional evidence that an operational TCA cycle supports bacterial nitrogen fixation. 3) Our results indicated that an intense synthesis of macromolecules, such as purines and pyrimidines, is carried out by bacteroids. In support of these physiological descriptions, and with an emphasis on the importance of these molecules during the nitrogen fixation process, we identified the purines—GTP, guanine, adenosine, guanosine, ADP, GDP, allantoic acid, XMP, AMP, GMP, ATP, dAMP, dADP, dAMP, and dADP—and the pyrimidines—CDP,uridine, CMP, cytosine, UDP, UMP,dTMP,UTP,dTDP, CTP, and cytidine—through our metabolome study. 4) We suggest that besides gluconeogenesis, a fueling pathway based on pentoses may exist in bacteroids during nitrogen fixation. The different proteins and metabolites detected in the bacteroids were consistent with this hypothesis. In fast growing rhizobia, the pentose phosphate pathway in combination with the Entner-Doudoroff pathway are probably the major routes used for the metabolism of sugars. Based on our observations, we hypothesize that other carbon sources, in addition to dicarboxylic acids, participate in bacterial nitrogen fixation. This hypothesis constitutes a perspective that should be experimentally verified in future work.

CONCLUSIONS: In this work we present a genome scale study of the metabolic activity of *R. etli* in free life and during nitrogen fixation. This approach led us to construct a guide for 1) integrating high-throughput data, 2) describing and predicting metabolic activity, and 3) designing experiments to explore the genotype-phenotype relationship in bacterial nitrogen fixation. In addition, a functional classification and pathway analysis showed that most of the pathways involved in carbon and nitrogen metabolism are expressed, including several that were previously considered not to be present during symbiosis.

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