concluding there is specificity in the divalent cation necessary for the reaction. We have then analyzed the molecular basis for this selectivity, showing that it is specifically related to amino acid residues on the B and T-loop regions of the PII proteins. Furthermore, we have recently observed differences in the uridylylation profile of GlnB and GlnJ in vivo, under specific conditions, showing that there also is physiological selectivity in the uridylylation of these PII proteins.

Considering that in spite of the high amino acid sequence similarity (>60%) between the GlnB and GlnJ proteins there is functional specificity, it is of great importance to understand the differences in the conditions for post-translational modification of these proteins, as this process controls the PII-target interactions and, consequently, the whole regulatory network.

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ATP HYDROLYSIS IN REACTION OF A NITROGENASE-LIKE PROTOCHLOROPHYLLIDE REDUCTASE

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Dark-operative protochlorophyllide (Pchlide) oxidoreductase (DPOR) catalyzes the stereo-specific reduction of C17-C18 double bond of Pchlide to form chlorophyllide *a* in the chlorophyll and bacteriochlorophyll biosynthetic pathways. DPOR consists of two separable components, L-protein (BchL dimer) as a reductase component and NB-protein (BchN-BchB heterotetramer) as a catalytic component, which are functional counterparts of Fe protein and MoFe protein of nitrogenase, respectively. L-protein plays a role for the ATP-dependent reductase specific for NB-protein, and NB-protein provides a catalytic site for Pchlide reduction. However, even though the requirement of ATP for the reaction has been demonstrated, it is still unknown how many ATP molecules are hydrolyzed upon the chlorophyllide formation. Here we report a stoichiometric analysis of DPOR. L-protein and NB-protein of *Rhodobacter capsulatus* were purified by an affinity resin (Strep-Tactin) from the crude extracts of *E. coli* overexpressing respective protein. DPOR activity was determined using assay mixture containing 100 mM HEPES-KOH; pH7.4, 5 mM $MgCl_2$, 5 mM dithithreitol, 0.01 % triton X-100, 0.5 mM dithionite, 0.2 mM ATP, 30 μM Pchlide, L-protein and NB-protein. The reaction was initiated by the addition of the DPOR compnents and proceeded at 34 °C for 15 min. ATP and ADP were separated and quantified by analyzing an aliquote of the acidified reaction mixture using an HPLC system with a titanium dioxide column. Chlorophyllide was determined by spectroscopic analysis of acetone extracts of the reaction mixtures. No ATP hydrolysis activity was detected in the reaction mixuters containing only one component L-protein or NB-protein. In the presence of both components (at the L-protein/NB-protein ratio of 8.6) ATP hydrolysis activity was detected. The observed ATP:chlorophyllide ($2e^-$ equivalent) was 6.7, which is coindident with the reported ATP:$2e^-$ ratio of 7 at the Fe protein:MoFe protein ratio of 4. Detailed quantitative analysis of ATP hydrolysis and chlorophyllide formation in different ratios of L-protein and NB-protein will be presented.

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ROLE OF EXPR AND EXPOLYSACCHARIDE PRODUCTION IN N_2 FIXATION IN THE *MEDICAGO-SINORHIZOBIUM* SYMBIOSIS.

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Exopolysaccharides (EPS's) play an important role in forming and extending infection threads in the symbiosis between *Medicago* and *Sinorhizobium*. *S. meliloti* 1021 (Sm1021) is able to produce one type of EPS (succinoglycan or EPS I) but not another (galactoglucan or EPS II), due to the presence of an insertion sequence (IS*Rm*2011-1) in *expR*. ExpR forms part of the SinIR quorum sensing system and is a regulator of galactoglucan synthesis. Previous work in our laboratory determined that under N-limited conditions, Sm1021 was poorly effective at fixing N with the model indeterminate legume *Medicago truncatula*, while two other mucilaginous strains, *S. medicae* WSM419 and *S. meliloti* WSM1022, were significantly more effective on this host. While the *expR* status of WSM1022 is unknown, WSM419 possesses an intact *expR* gene. These data indicate that the interrupted *expR* gene in Sm1021 might account for the reduced effectiveness of this strain on the model legume.

To determine whether *expR* has a role in N_2 fixation, we tested whether the highly mucilaginous Rm8530 (a spontaneous revertant of Sm1021 devoid of IS*Rm*2011-1 within *expR* and able to produce both EPS I and II) exhibited increased effectiveness on *M. truncatula* compared to Sm1021 (*expR*$^-$). Surprisingly, Rm8530 was significantly less effective than Sm1021 with this host, as measured by plant shoot dry weights at 42 days post-inoculation. In addition, the inclusion of an intact plasmid borne *expR* (pJNexpR) in Sm1021 reduced the effectiveness of this

strain on *M. truncatula*. Interestingly, no differences were apparent in the symbiotic properties between Rm8530, Sm1021 or Sm1021 (pJNexpR) with *M. sativa*. These data suggest a clear role for *expR* in reducing N_2 fixation in Sm1021, although the mechanism by which this occurs remains unclear. Work is underway to inactivate *expR* in Rm8530 to confirm these observations in this background and to determine the status of *expR* in WSM1022. The morphology of nodules formed by Rm8530 on *M. truncatula* will also be examined and compared to those of Sm1021 and other effective strains.

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INVESTIGATION OF PROTEINS INVOLVED IN ADAPTIVE ACID TOLERANT RESPONSE OF SOYBEAN *BRADYRHIZOBIUM*

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Low soil pH affects legume-*Rhizobium* symbiosis leading to restrict the legume productivity. Adaptive acid tolerance has been proposed as a strategy for enhancing levels of acid tolerance in rhizobial inoculant. Since mechanism of this response has not been elucidated in *Bradyrhizobium*, the aim of this study was to identify proteins involved in acid tolerance and adaptive acid tolerance responses. Ten acid tolerant bradyrhizobial strains were conducted for growth determination at neutral- and acid-pH by comparing the growth when directly inoculated at low pH, and when grew under adaptive to acid condition (pH 7.0 to 6.5, 6.0, 5.5, 5.0 and 4.5, consecutively). *Bradyrhizobium* strain 01007 showed better growth than other strains when grew under adaptive condition. The 1-D gel electrophoresis examination of protein profiling produced during growth under adaptive condition was distinguishable. The total proteins extracted from cells during growth under neutral- (pH 7.0), acid- (pH 4.5) and adaptive to acid-conditions were analyzed through 2-D gel electrophoresis. By comparison with protein spots presented during growth at neutral-pH, 25 protein spots were up-regulated during growth at acid conditions and among these 25 protein spots, 12 protein spots were up-regulated only during the growth under adaptive condition. The selected protein spots were analyzed by MALDI_TOF mass spectrometry and were identified by comparison with *B. japonicum* USDA110 database. Several known proteins were identified only during growth at acid condition, such as proteins involved in cellular metabolism (Alanyl-tRNA synthetase, inosine-5-monophosphate dehydrogenase, ATP synthase, Cytochrome C4), substrate transporting (ABC transporter ATP-binding protein, secretion protein), signal transduction mechanism (two-component response regulator), and DNA repairing system (formamidopyrimidine-DNA glycosylase). However, the annotated proteins presented only during growth under adaptive condition mostly were transcriptional regulator involved in cellular metabolism, secondary metabolism, and DNA repairing system. Other proteins including in cellular metabolism and energy conversion (holiday junction nuclease, aldehyde dehydrogenase, alcohol dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase type II), cation efflux system (AcrB/AcrD/AcrF family protein), and cellular defense mechanism (superoxide dismutase) were also identified. These proteins could be served as basic data for elucidation the mechanisms involved in acid tolerant or adaptive acid tolerant responses in *Bradyrhizobium*.

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CHARACTERIZATION OF RHIZOBIA ISOLATES USING MOLECULAR METHODS

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The rhizobia/legume root nodule symbiosis is the result of a complex interaction between the host plant and the bacteria. Within the rhizosphere, bacteria have access to carbon and nitrogen sources originating from the plant. In addition, rhizobia receive plant-derived signal molecules (flavonoids). In response, rhizobia themselves secrete molecules (Nod factors) that signal to the plant the presence of suitable symbiotic partners. The rhizobia are then able to enter the root in the region of emerging root hairs. They are guided via branched infection threads into the cortex of the root where a meristematic zone has formed. Finally, the bacteria are wrapped by a plant-derived membrane and enter the cytosol of plant cells. The bacteria are now referred to as bacteroids and are able to fix molecular nitrogen, which is released into the plant cells. We are isolate new effective strains from nodule plants Onobrycis tr., grow in the Arid zones Central Asia. For the characterize and identify rhizobial strains and to study the diversity of indigenous Rhizobium strains we are used the Polymerase Chain Reaction (PCR). PCR can be performed rapidly with strains, species or genus specific primers that generate fingerprint characteristics of each strain. DNA primers corresponding to repetitive sequences that present in multiple copies of the genomes of most Gram-negative and Gram-positive bacteria can be used to fingerprint the genomes of rhizobial strains. Three families of repetitive sequences have been identified, including repetitive extragenic palindromic (REP), repetitive interagenic consensus (ERIC) and BOX element. The REP-PCR genomic fingerprinting protocols have been successfully used in a wide variety of bacteria for typing strains and studying their diversity. We have used this highly discriminative and reproducible technique to study the genetic diversity of rhizobial strains.

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