

Sinorhizobium meliloti is a gram-negative soil bacterium that can establish a symbiotic relationship with its host plant, *Medicago sativa* (alfalfa). This association requires the successful exchange of specific signals resulting in the development of nodules on the roots of the plant and their subsequent invasion by *S. meliloti*. The bacteria then differentiate into bacteroids, where they fix nitrogen for the benefit of the host plant. Invasion by *S. meliloti* is mediated by the symbiotically active low-molecular-weight (LMW) form of an exopolysaccharide, EPS II. Work in our laboratory has shown that the production of LMW EPS II requires the Sin/ExpR quorum-sensing system of *S. meliloti*. In the presence of this system, expression of a gene encoding a transcriptional regulator, ExpG, is significantly up-regulated. We have shown that the resultant high levels of ExpG subsequently increase production of EPS II and activate the specific synthesis of the symbiotically active low-molecular-weight form through increased levels of a glycosyl transferase, ExpC. Therefore sufficient levels of ExpG resulting from an intact quorum-sensing system are critical for the production of symbiotically active LMW EPS II. Additionally, the presence of this LMW fraction of EPS II is essential for the development of complex biofilm, which may be imperative for successful attachment of *S. meliloti* to the roots of the plant prior to invasion of the nodule. This extends the role of ExpG to include both, the regulation and production of symbiotically active exopolysaccharides, as well as the formation and organization of biofilm. Furthermore, ExpG dramatically represses the expression of specific genes required for motility and the synthesis of flagella, factors known to interfere with biofilm development as well as impair invasion of the host plant. As a result, it appears that ExpG regulates the behavior of *S. meliloti* on multiple levels in order to prepare the bacterium for establishing a nitrogen-fixing symbiosis from a motile planktonic state.

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A BASIS FOR THE DEVELOPMENT OF AN INFERIOR N₂ FIXATION PHENOTYPE IN ROOT NODULE BACTERIA FOLLOWING LATERAL TRANSFER OF SYMBIOTIC GENES

Kemanthi G. Nandasena, Graham W. O'Hara, Ravi P. Tiwari & John G. Howieson

Centre for *Rhizobium* Studies, Murdoch University, Western Australia 6150; kemanthi@murdoch.edu.au

Symbiotic N₂ fixation by root nodule bacteria (RNB) plays a significant role in world agricultural productivity by annually converting in excess of 120 million tonnes of atmospheric N₂ into ammonia. A successful symbiotic interaction requires compatibility between the RNB and the legume at many different stages, from initial recognition, through successful differentiation to nitrogen fixation. All these processes are complex and require the regulation and function of multiple genes/gene families in both partners. Maximum production from a legume

often depends on the efficiency of N₂ fixation by its root nodule bacteria. Despite inoculation with an effective commercial inoculant strain, the evolution of ineffective RNB for agriculturally important legumes has been a great challenge for contemporary rhizobiology over the years. We have recently shown the evolution of diverse, opportunistic but ineffective rhizobia able to nodulate *B. pelecinus* (a pasture legume species introduced to Australia from the Mediterranean basin). These strains arose following *in situ* transfer of symbiotic genes located on a mobile symbiosis island, from the inoculant strain to other soil bacteria. Our current research aims to exploit molecular, biochemical and microscopy tools to determine the basis for the development of poorly effective and completely ineffective strains for *B. pelecinus*. The complete genomes are being sequenced for the inoculant strain (*Mesorhizobium ciceri* bv. *biserrulae* WSM1271) and two of the suboptimal strains; the poorly effective *M. australicum* WSM2073 and ineffective *M. opportunistum* WSM2075 which were both the recipients of the symbiosis island from the inoculant strain. Electron microscopy observations of nodules revealed that the basis for the completely ineffective phenotype may be the premature senescence of bacteroids. On the other hand, the poorly effective phenotype exhibited a reduced number of bacteroids per nodule compared to the effective inoculant strain.

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PHOSPHOPROTEOMIC ANALYSIS OF SOYBEAN ROOT HAIRS COLONIZED BY *BRADYRHIZOBIUM JAPONICUM*

Tran H. N. Nguyen^{1,2}, Laurent Brechenmacher¹, Sooyoung Jeong¹, Kim Hixson³, Marc Libault¹, Ljiljana Pasa Tolic³, Dong Xu⁴, Henry T. Nguyen¹ and Gary Stacey^{1,5*}

¹National Center for Soybean Biotechnology, Division of Plant Sciences, University of Missouri, Columbia, MO 65211, gstacey@missouri.edu

²Vietnam Education Foundation ³Mass Spectrometry Facility, Environmental Molecular Sciences Laboratory, Richland, WA 99352, ljiljana.pasatolic@pnl.gov ⁴Department of Computer Science, University of Missouri, Columbia, MO 65211, xudong@missouri.edu

⁵Division of Biochemistry and Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65211

The nitrogen fixing symbiosis in soybean (*Glycine max*) is the result of root hair infection by the bacterium *Bradyrhizobium japonicum*. Specificity in this symbiotic interaction is determined very early during the initial stages of host-symbiont recognition. These events involve specific receptor kinases and subsequent kinase cascades. In order to understand these events in greater detail, we sought to characterize the phosphoproteome of single root hair cells colonized by *B. japonicum* using both a non-targeted approach and a targeted approach. In the first approach, proteins were extracted from root

of a nucleotide region that aids in maintaining the pattern of tissue-specific expression in nodules. Expression analysis of the promoters studied revealed that deletion of 5' UTR from *PvSS*, *PvNAS*, *PvAAT* and *PvNADH-GOGAT1* promoters resulted in a drastic reduction of their expression in nodule as well as in root tissues. Bioinformatic analysis of the sequences of 5' UTRs of all these genes showed a common feature of the presence of a CT-rich (CTTCTCTCTTT) region at close proximity to transcription start site. Investigations further evidenced that among the nitrogen metabolism genes, expression of *PvNAS2* and *PvNADH-GOGAT* is modulated by sugars. In the case of *PvNAS2*, it is found that the expression of *PvNAS2* in roots and nodules is upregulated by metabolizable sugars and that hexokinase has a role in the sugar-sensing mechanism that regulates the expression of *PvNAS2*.

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THE NTRX PROTEIN FROM *HERBASPIRILLUM SEROPEDICAE* BINDS TO THE PROMOTER REGION OF GENES INVOLVED IN NITRATE UTILIZATION

Osaki J. H.¹, Monteiro R. A.¹, Chubatsu L. S.¹, Souza E. M.¹, Pedrosa F. O.¹, & Rigo L. U.^{1*}

¹Department of Biochemistry and Molecular Biology, Universidade Federal do Paraná, Molecular, PO Box 19046, Curitiba, PR, 81531-990, Brazil; lurigo@ufpr.br

Herbaspirillum seropedicae is a diazotrophic endophytic β -proteobacterium that associates with grasses of economic interest such as sorghum, rice and sugarcane. The NtrYX proteins are members of a two-component regulatory system, NtrY is a sensor protein and NtrX is a response regulator protein. The NtrX protein of *H. seropedicae* contains two domains, a signal receiver domain and a DNA binding domain (HTH). It was shown previously that *ntrY* mutant strain of *H. seropedicae* was affected in the nitrate metabolism, suggesting the involvement of NtrYX in the regulation of the expression of nitrate utilization genes. In order to contribute to a better understanding of the role of the NtrYX system in *H. seropedicae*, the *ntrX* gene was cloned and expressed in *E. coli* and the recombinant protein His-NtrX protein was purified by affinity chromatography. The purified protein was shown to interact with the promoter regions of the operons *narXL*, *narKnirBDCnasA*, *narK1KGHJI* using electrophoretic mobility shift assays. Moreover, phosphorylation of NtrX increased its affinity for those promoter regions. The results strongly suggest the involvement of NtrX protein in the regulation of the operons *narXL*, *narKnirBDCnasA* and *narK1KGHJI*.

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CHARACTERISATION OF THE REGULATORY CIRCUIT REQUIRED FOR THE ACID INDUCTION OF THE ADAPTIVE ACID TOLERANCE RESPONSE PROTEIN *lpiA* IN *SINORHIZOBIIUM MEDICAE* WSM419

Rui Tian, Ravi Tiwari, Lambert Brau & Wayne Reeve

Center for Rhizobium studies, Murdoch University, South Street, Murdoch, Western Australia, 6150

The acid tolerance response of *Sinorhizobium medicae* WSM419 enables cell adaptation to lethal acid after cell exposure to mild acidity. The expression of the *lpiA* (low pH induced gene A) gene is critical for this response and is acid-activated at least 20-fold in the mild acidic conditions. The expression of this gene is specifically induced by acidity and not by any other stress. We have previously shown that full acid-induction of *lpiA* requires functional FsrR (fused sensor-regulator) (Reeve et al 2006). However, even in the absence of FsrR, there is still a residual 6-fold acid-induction of *lpiA* implicating other proteins in the regulation process. Other genes have been suspected to be involved including *tcsA* (two component sensor) *tcrA* (two component regulator), *acvB* (acid virulence protein B) and *rpoN* (RNA polymerase N-metabolism).

To reveal if these genes were involved in the regulatory process, mutations in *acvB*, *rpoN*, *tcrA* and *tcsA*, were created in *S. medicae* by single crossover insertional inactivation and the acid-induction of *lpiA* expression was monitored in each mutant. Partial induction of *lpiA* occurred in the *acvB* (10-fold), *tcrA* (5-fold) and *tcsA* (1-fold) backgrounds. Expression of the *lpiA*-fusion in the *rpoN* deletion mutant was totally abolished revealing that this RNA polymerase sigma-factor is essential for acid activation. A putative RpoN binding motif was identified upstream to the start codon of *lpiA*. RACE analysis revealed the transcription start site for *lpiA* and *acvB* was located 12 bp downstream of the putative -24 -12 *rpoN* binding site upstream of *lpiA* demonstrating that these 2 genes were co-transcribed as an operon. Consistent with this was the finding that expression (measured by real-time PCR) of *acvB* was induced 18-fold by acid. In contrast, *fsrR*, *tcrA*, *tcsA*, and *rpoN* were constitutively expressed with regards to pH. While we have shown that *tcsA*, *tcrA*, *fsrR* and *acvB* affect induction of *lpiA* at low pH, these genes are not essential for stress tolerance or symbiotic nitrogen fixation in association with *Medicago sativa*, *M. murex*, *M. polymorpha* or *M. truncatula*. In contrast, *rpoN* was essential for *S. medicae* symbiotic nitrogen fixation with these *Medicago* hosts demonstrating its role beyond acid response in *Sinorhizobium medicae* WSM419.