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DISCERNING THE ROLES AND RELATIONSHIPS OF EPS AND THE STRINGENT RESPONSE

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Exopolysaccharides (EPS) represent an energy expensive composition of high molecular weight sugar polymers. Many physiological roles have been associated with EPS including pathogenesis, biofilm maturation, stress tolerance and symbiotic performance. Coincidentally, many of these functions are paralleled by the stringent response, a phase defined by a metabolic downshift in response to nutrient-limiting conditions. In many rhizobia, the synthesis of EPS and specific stationary phase regulators are required for the successful symbiosis with their legume host. Considerable research has examined the genetic and physical basis by which EPS is produced to initiate plant root infection, however, the physiological conditions that affect and coordinate these genes is not clearly understood. This work aimed to define the roles of EPS and the relationship to stationary phase in *Sinorhizobium medicae* whilst determining specific nutritional elements that affect the level of EPS synthesis.

To examine the relationship between the stringent response and EPS synthesis, the conserved stationary phase regulator *relA* was inactivated. The mutation of *relA* significantly reduced the level of EPS in *S. medicae* in a nutritional dependent manner also seen with the quorum sensing genes *sinI* and *expR*. These mutations revealed their role in EPS induction/ repression in the presence of different carbon sources and the limitation of nitrogen or phosphate. The synthesis of this EPS was found to be critical in providing a surfactant for motility. Other studies have identified the roles of quorum sensing in flagellum biosynthesis in *Sinorhizobium*. However, we found that *S. medicae* appears to be differentially regulated, as the quorum sensing and stationary phase mutants possessed flagella and only maintained motility in an EPS-inducing environment. Interestingly, despite the non-mucoid phenotype of these mutations, the stationary phase mutant competed comparatively with *S. medicae* when inoculated on *Medicago murex* whilst those inoculated with the *expR* mutant possessed a reduced plant dry weight and nodule number. This data highlights a connection between the stringent response, EPS and quorum sensing and an independent regulation towards similar functions.

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CLONING AND CHARACTERIZATION OF A NEW SYMBIOTIC N_2 FIXATION GENE *DMTH* OF *MESORHIZOBIUM HUAKUII* 7653R

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M. huakuii with strict-host-specificity can induce the formation of indeterminate nodules on the roots of host plant *Astragalus sinicus* L..Eight thousand mutants generated by using Tn5-sacB insertion mutagenesis were obtained from *M. huakuii* 7653R. Thirty symbiotic mutants were screened by pot plant nodulation test. The DNA sequences of the contiguous region from the Tn5 insertion site were determined by thermal asymmetric interlaced PCR. Five mutants bearing disrupted genes which demonstrated high sequence similarity to function-unknown genes of *Mesorhizobium loti* were further identified. One of them, a mutation in *dmtH* gene was found to form pseudonodules on *A. sinicus* L.. Its effect on symbiotic phenotype was further confirmed by a double-crossover mutant HK116 and complementation test. DNA sequence analysis revealed that *dmtH* encoded a protein of 249 amino acids with a predicted molecular mass of 27.3 kDa. RPS-BLAST analysis of DmtH protein showed a sequence similarity of 89.6% to demethylmenaquinone (DMK) methyltransferase. DMK methyltransferase was found in bacteria and could convert DMK to menaquinone (MK) in the final step of MK biosynthesis. In bacteroid, MK was an electron acceptor of the electron transport system. Ultra-structure observations of root nodules showed that *dmtH* mutant formed ineffective, aberrant nodules, and this symbiotic phenotype was linked to fewer bacteroids differentiated and their defective structure of cell membrane as well. The examination on the structure and enzymatic activity of DmtH, and the analysis on the temporal and spatial expression patterns of *dmtH* are under going.

Taken together, the results show that DmtH plays a critical role in bacteroid respiration (energy metabolism) and therefore interrupts the normal process of bacteroid division, differentiation and effective nodule development.

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INTERCROPPING ALLEVIATES THE INHIBITORY EFFECT OF N FERTILIZATION ON NODULATION AND SYMBIOTIC N_2 FIXATION OF FABA BEAN

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