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ACID-ADAPTION BY A MEDIC MICROSymbionT; NEW INSIGHTS FROM THE GENOME OF *SINORHIZOBIUM MEDICAE* WSM419.

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The poor availability of nitrogen is one of the principal factors limiting global biomass. Legumes are vital components of agricultural systems because of their ability to associate symbiotically with root nodule bacteria (RNB) and subsequently fix atmospheric nitrogen to a form that can be utilised by the plant partner. Furthermore, this symbiotic relationship provides available soil nitrogen for subsequent non-leguminous crops. This RNB-legume interaction is affected by a number of environmental factors. Progressive acidification of agricultural soils is one of the big challenges in agriculture as soil acidity negatively impacts legume productivity. One genus of RNB, *Sinorhizobium*, is particularly acid-sensitive causing a major reduction in *Medicago* productivity in acidic soils. Due to the importance of Medic pasture production, alternative strains have been captured, and are still being captured, from the genetic pool that display superior acid tolerance characteristics. This presentation will focus on the acid-tolerant species *S. medicae* (previously known as *S. meliloti*) and in particular on the previously used commercial inoculant WSM419.

At the Centre for *Rhizobium* Studies, we have been researching the molecular mechanisms required by this strain to grow and adapt at acidic pH values. Over the past decade we have used three different approaches to characterise the genes and proteins required for low pH tolerance. First, low pH-sensitive mutants were generated by random or targeted mutagenesis to identify genes essential for growth in acidic conditions. Second, low pH-regulated reporter gene fusions were created to identify acid-regulated gene expression. Third, 2-D gel electrophoresis was used to display proteins that were affected in cells after transient and permanent exposure to acid conditions. N-terminal sequencing of affected spots revealed that proteins were both up- and down-regulated. With the use of these combined approaches we identified numerous genes/proteins with potential roles in acid-tolerance or adaptation. However, the location and function of many of these genes could not be revealed due to the lack of DNA and protein sequence information in the databases and the lack of a genome sequence for WSM419.

The genome of *S. medicae* WSM419 has now completely sequenced (Reeve *et al.*, 2009). This genome is multipartite with a chromosome of size 3,781,904 bp and 3 plasmids of size 1,570,951, 1,245,408 and 219,313 bp. The total size of the genome is 6,817,576 bp containing 6518 protein-coding genes and 81 RNA only encoding genes. The availability of the genome sequence of WSM419 has facilitated the localisation and characterization of the acid tolerance genes in this strain. Our findings on the molecular mechanism of acid tolerance will be presented.

Reeve *et al.* (2009) Complete genome sequence of the *Medicago* microsymbiont *Sinorhizobium medicae* strain WSM419. *Standards in Genomic Sciences* (in press).