Diversity of field isolates of *Sinorhizobium meliloti* nodulating alfalfa

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Most alfalfa seed is treated with a rhizobial inoculant consisting of one or more strains of *Sinorhizobium meliloti* before planting to enhance nodulation of seedlings. However, little is known about the persistence of inoculated strains later in the season. There is also a paucity of information on genetic and phenotypic diversity of *S. meliloti* strains in the U.S. One strategy for increasing alfalfa forage yields, particularly in less fertile sites, is selection and use of highly competitive and efficient nitrogen fixing strains of rhizobia. To develop inoculants, a greater understanding of the basis of field competitiveness of rhizobia is needed. The availability of the complete genome sequence for a large number of *S. meliloti* strains can be leveraged to identify the bacterial genes contributing to field competitiveness. Recently, type four secretion system (T4SS) genes were identified in *S. meliloti* that may play a role in host specificity (1). To investigate the diversity of *S. meliloti* strains and incidence of T4SS genes, a collection of *S. meliloti* strains were isolated from alfalfa nodules at University of Minnesota Long Term Agricultural Research plots in Lamberton and Waseca, MN. Four replicated plots were seeded in 2014 and 2015 with DKA44-16RR pretreated with a commercial rhizobial seed coating. At each site, eight soil cores were removed from each alfalfa plot and 48 nodules were collected from the combined eight cores (total of 768 nodules). Nodules were surface sterilized, crushed to release bacteria, and bacteria cultured on TY agar with 0.5% Congo Red. Bacteria were also isolated from nodules developing from the same seed grown in sterile vermiculite in a growth chamber. Single colony isolates were used for PCR assays with *Sinorhizobium*-specific primers, T4SS primers targeting the *virD4* gene, and for 16S rDNA amplification. A total of 562 pure *S. meliloti* strains were recovered from field grown plants and 33% were positive for the *virD4* gene. Of the 57 strains from the seed inoculum, none had the T4SS genes. A subset of 158 field strains was used for rep-PCR fingerprinting using the BOXA1R primer. Strains from each location formed distinct clusters that were significantly different from seed-derived strains. There were no differences in bacterial populations from 6-month-old and 18-month-old plants. Populations at each site were highly diverse, with greater diversity at the Lamberton site. These results indicate that indigenous populations of *S. meliloti* remained high in the soil at each site in the absence of alfalfa, were highly competitive with introduced strains, and a large proportion at each site contained T4SS genes. Plant growth promoting activity and competitiveness of field strains is currently being investigated.

1. Sugawara, M., Epstein, B., Badgley, B.D., Unno, T., Xu, L., Reese, J., et al. (2013). Comparative genomics of the core and accessory genomes of 48 *Sinorhizobium* strains comprising five genospecies. Genome Biology 14:R17.