

# Complete Genome Sequence of the Broad-Host-Range Strain *Sinorhizobium fredii* USDA257

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**Here we announce the complete genome sequence of the symbiotic and nitrogen-fixing bacterium *Sinorhizobium fredii* USDA257. The genome shares a high degree of sequence similarity with the closely related broad-host-range strains *S. fredii* NGR234 and HH103. Most strikingly, the USDA257 genome encodes a wealth of secretory systems.**

*Sinorhizobium fredii* USDA257 is a Gram-negative soil bacterium that fixes nitrogen in symbiosis with legume plants. It was originally isolated from wild soybean cultivars (1) and has an extremely broad host range, comprising 79 legume plant genera (4). Only the closely related *S. fredii* NGR234 strain reveals a wider host range, being able to nodulate 112 plant genera (4). Both strains are the best-studied model organisms with respect to host range. The analysis of the recently published complete genome of *S. fredii* NGR234 and the genome of the broad-host-range strain *S. fredii* HH103 suggested that these three strains share a high degree of synteny in their genomes (3, 5, 6). The complete genome sequence of USDA257 therefore significantly increases our knowledge of molecular mechanisms involved in the determination of host range in the interaction of plants with microbes.

Whole-genome shotgun sequencing of the *S. fredii* USDA257 genome was performed by using a 454 GS-FLX system (Roche 454 Life Science, Mannheim, Germany). Two shotgun runs resulted in 17-fold coverage. The initial assembly yielded 210 large (>500-bp) contigs determined using Roche Newbler 2.0.01.14 FLX assembler software (454 Life Sciences, Roche Applied Science, Branford, CT). In addition, a fosmid library was prepared and 1,152 insert ends were sequenced. PCR-based techniques and Sanger sequencing of the products were used to close remaining gaps.

USDA257 carries two replicons, a chromosome (cUSDA257) and a single plasmid (pUSDA257). The final chromosomal sequence (62.09% G+C) of USDA257 comprises 6,476,459 bp encoding 6,222 proteins, three rRNA operons, and 53 tRNA genes. The uncomplete plasmid encoded 639 proteins with a size of approximately 556,000 bp (59.53% G+C) distributed on 19 contigs. No essential genes were identified on the pUSDA257 replicon. It encoded many of the genes linked to the symbiosis (i.e., a type 3 secretion system [T3SS] and *nod* and *nif* genes). It is, however, surprising that, in addition to the plasmid-encoded *nif* and *fix* genes, a significant number of the genes involved in nitrogen fixation are located on the bacterial chromosome. Further, and similarly to NGR234, the USDA257 genome carries a second copy of a T3SS on its chromosome and therefore is only the second plant-associated microbe known to encode two T3SSs.

Furthermore, USDA257 is perhaps able to grow on a wide range of substrates, including aromatic substances. The genome

sequencing also revealed a complete set of genes required for growth on urea as a single carbon and nitrogen source. Further autotrophic growth might be possible, since the genome appeared to encode the ribulose-bis-phosphate carboxylase. Mechanisms of cell-cell communication appear to involve the synthesis of *N*-acyl-homoserine lactones. Two copies of autoinducer synthase genes were identified, one on the chromosome and a second copy on the bacterial plasmid. Similarly to NGR234, a significant number of loci were identified that encoded autoinducer I hydrolases (2).

**Nucleotide sequence accession numbers.** The complete sequences of the USDA257 genome have been deposited in GenBank under accession numbers [CP003563](#) to [CP003582](#).

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