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Draft Genome Sequence of the Nitrogen-Fixing Symbiotic Bacterium *Bradyrhizobium elkanii* 587

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The draft sequence of the genome of *Bradyrhizobium elkanii* 587 is presented. This was obtained using Illumina Next-Gen DNA sequencing combined with Sanger sequencing. Genes for the pathways involved in biological nitrogen fixation (the *nif* gene cluster), *nod* genes including *nodABC*, and genes for the type III protein secretion system (T3SS) are present.

Bradyrhizobium elkanii 587 is a nitrogen-fixing bacterium that nodulates soybean and is used in commercial inoculants in Brazil (7).

The draft genome sequence of *B. elkanii* 587 was obtained using a combination of Illumina and Sanger reads. A total of 6,020,858 end-paired reads were *de novo* assembled using Velvet (11) with an average read depth of 28-fold; 42,785 Sanger reads were added to the assembly, resulting in 260 scaffolds (N_{50}, 54,941 bp). Scaffold ordering was carried out using *Bradyrhizobium japonicum* USDA110 as a reference. *B. elkanii* 587 contains one circular chromosome of 8.8 Mbp with a G+C content of 62.9%, and no plasmid was detected. Annotation of 9,250 potential open reading frames (ORFs) and 71 tRNA genes was performed using the RAST program (1). Genes of interest were curated and analyzed manually. A single rRNA operon was found, as in *B. japonicum* USDA110 (4).

Genes for the same metabolic pathways found in *B. japonicum* were found in the genome of *B. elkanii* 587. Genes encoding the Entner-Doudoroff and Embden-Meyerhof-Parnas pathways for carbohydrate degradation are present.

The *nif* genes *nifDKENX*-(*orf1* to *orf7*)-*nifUST*-*orf8*-*nifB*-*orf9*-*nifZ*-(*orf10* to *orf18*)-*nifHQ*-*orf19*-*nifW*-*fixBCX* are present in one cluster. The genes related to nodulation are organized in two regions. The first contains 19 genes, *nodUIJ*, *nopM*, *nodZ*, *fixR*, *nifA*, *nopM*, *fixAKLNOGHI*, *noeE*, and *nodQP*, and includes the *nif* transcriptional activator (the *nifA* gene). The second cluster contains five genes, *nodD1* and *nodABCS*. The genes *nodT*, *nopB*, *nodU*, *nolY*, *nolL*, and *nolV* were identified outside the clusters.

B. elkanii 587 possesses type III and IV secretion systems. The type III secretion system (T3SS) is essential for the virulence of many pathogenic bacteria and has been identified in rhizobia such as *Rhizobium* sp. strain NGR234 and *Sinorhizobium fredii* USDA257 (10, 8). In *B. elkanii* 587, the T3SS operon is composed of at least 12 genes: *ttsI*, *rhcC2UTSRQN*, *nolV*, *rhcJ*, *nolB*, and *rhcC1*. The genes *nrdR*, *nopM*, and *nopX* were detected. These genes, encoding the secreted proteins NopM and NopX, were previously identified in *B. elkanii* USDA61 (6).

The type IV secretion system (T4SS) has been identified in many plant-associated microbes and is usually composed of Vir proteins (9). The *B. elkanii* 587 T4SS operon has 13 genes (coding for VirB1 to VirB6, VirB8 to VirB11, VirD2, and VirD4).

B. elkanii 587 has the structural genes *cbbL* and *cbbS*, coding for type I RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygen-ase), a key enzyme in the Calvin-Benson-Bassham (CBB) cycle responsible for carbon fixation (3). Similar to *B. japonicum* USDA110, *B. elkanii* lacks genes coding for the reaction center of photosynthesis (2). The presence of RuBisCO in nonphotosynthetic bacteria has been reported previously; however, its role in these organisms has not yet been resolved (3).

Another interesting finding in the *B. elkanii* 587 genome is the presence of a gene cluster for NiFe hydrogenase comprising 14 genes: *hupSLCDFGHK* and *hypABFCDE*. Although Rumjanek and collaborators (7) reported that this strain lacks uptake hydrogenase activity, the expression of the *hupK* gene was observed by DNA microarray analysis (5).

The sequencing of the genome and further analysis will contribute to a better understanding of the genetics of the organism and the genes involved in competitiveness in soil.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number AJJK00000000. The version described in this paper is the first version, AJJK01000000.

REFERENCES

1. **Aziz RK, et al.** 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics **9**:75.
2. **Evans WR, et al.** 1990. Bacteriochlorophyll and photosynthetic reaction centers in *Rhizobium* strain BTAi 1. Appl. Environ. Microbiol. **56**:3445–3449.
3. **Gourion B, et al.** 2011. Bacterial RuBisCO is required for efficient Bra-

dyrhizobium/Aeschynomene symbiosis. PLoS One **6**:e21900. doi:
10.1371/journal.pone.0021900.

4. **Kaneko T, et al.** 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res. **9**:189–197.

5. **Marcondes J, Lemos EGM.** 2011. DNA microarray applied to data mining of *Bradyrhizobium elkanii* genome and prospection of active genes, p 229–244. *In* Funatsu K (ed), Knowledge-oriented applications in data mining. InTech, São Paulo, Brazil. http://www.intechopen.com/books /knowledge-oriented-applications-in-data-mining/dna-microarray-applied -to-data-mining-of-bradyrhizobium-elkanii-genome-and-prospection -of-active-gen.

6. **Okazaki S, Zehner S, Hempel J, Lang K, Göttfert M.** 2009. Genetic organization and functional analysis of the type III secretion system of *Bradyrhizobium elkanii*. FEMS Microbiol. Lett. **295**:88–95.

7. **Rumjanek NG, Dobert RC, van Berkum P, Triplett EW.** 1993. Common soybean inoculant strains in Brazil are members of *Bradyrhizobium elkanii*. Appl. Environ. Microbiol. **59**:4371–4373.

8. **Schechter LM, Guenther J, Olcay EA, Jang S, Krishnan HB.** 2010. Translocation of NopP by *Sinorhizobium fredii* USDA257 into *Vigna unguiculata* root nodules. Appl. Environ. Microbiol. **76**:3758–3761.

9. **Schmeisser C, et al.** 2009. *Rhizobium* sp. strain NGR234 possesses a remarkable number of secretion systems. Appl. Environ. Microbiol. **75**: 4035–4045.

10. **Wassem R, et al.** 2008. TtsI regulates symbiotic genes in *Rhizobium* species NGR234 by binding to tts boxes. Mol. Microbiol. **68**:736–748.

11. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for de novo assembly using de Brujin graphs. Genome Res. **18**:821–829.