

Whole-Genome Sequence of *Bradyrhizobium elkanii* Strain UASWS1015, a Highly Ammonia-Tolerant Nitrifying Bacterium

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***Bradyrhizobium elkanii* UASWS1015 was isolated from a sewage plant in Switzerland. Its genome indicates that it is fully equipped for ammonia assimilation and aromatic compound degradation, and it displays a large type IV secretion system, which characterizes plant-associated microbes. Totally deprived of toxins, it could be considered for agricultural and environmental uses.**

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Bradyrhizobia establish symbiosis with legumes by forming root or stem nodules, and some can be photosynthetic. *Bradyrhizobium elkanii* (1), an aerobic, motile, and Gram-negative rod, does not form spores and can be found in a free-living state or as a plant symbiont. This species is also known for producing bioemulsifiers (2) and is used in agriculture (3).

Strain UASWS1015 was isolated when selecting for highly ammonia-tolerant nitrifying bacteria from a sewage plant, and it was first identified as *B. elkanii* by 16S sequencing. Genomic DNA was extracted from a pure axenic culture, according to an adapted protocol (4). Libraries were made using the Nextera XT kit (Illumina, USA). Whole-genome shotgun (WGS) sequencing was performed within one Illumina MiSeq run at 2 × 250-bp read length and yielded 187 contigs, providing 72.3 × genome coverage, for a genome total length of 7,820,754 bp, G+C content of 64.6%, and a scaffold N_{50} value of 315,183 bp. Among the 10 genomes available in NCBI genome database for this species, this strain displays the highest G+C content and is genetically close to two strains, *B. elkanii* USDA 3259 and USDA 3254, while its genome size is smaller. Raw reads were trimmed with FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and assembled with the SPAdes genome assembler 3.6.1 (5). The resulting contigs of the genome assembly were arranged with BioEdit (6) and analyzed with QUASt (7). *In silico* screening with PlasmidFinder (8) did not identify any circular or integrated plasmid genome. Automated gene annotation was carried out using the NCBI Prokaryotic Genome Automatic Annotation Pipeline PGAAP (9) and reviewed with RAST version 2.0 (10). It allowed for the identification of 7,285 genes distributed in 6,823 coding sequences (CDSs), 398 pseudogenes, 64 rRNA genes (5S, 16S, 23S, tRNAs, and 1 noncoding RNA [ncRNA]), and 26 frameshifted genes, while RAST analysis identified 7,658 CDSs. No complete transposon or phages were found to be integrated in the genome. Toxin, superantigen, virulence, and disease genes are absent, which allows this bacterium to be considered a biological fertilizer. While some *B. elkanii* strains harbor type III and type IV

secretion systems (11), strain UASWS1015 possesses a large type IV secretion system, known in many plant-associated microbes, which is composed of 29 genes for Vir proteins (12). Additionally, it is equipped with 15 genes for bacteriocin and antimicrobial synthesis and 112 genes involved in antibiotic, multidrug, and heavy-metal resistance. The bacterium is fully equipped for ammonia assimilation. Additionally, 115 genes are involved in numerous pathways of aromatic compound degradation, offering a possible role for soil depollution. It also contains 4 genes coding for a photosystem reaction center, and while a few genes for nodulation (*nod* and *noI*O) are present, the most important *nod* genes, A, B, and C (13), are absent, making this bacterium possibly unable to nodulate, a situation which has already been described for symbiotic photosynthetic *Bradyrhizobium* species (14). This unusual genome of *B. elkanii* would contribute to a better knowledge of this species, and ongoing works confirm that it might be usable in agriculture, wastewater, and contaminated soil management (unpublished data).

Nucleotide sequence accession numbers. This WGS project was deposited at DDBJ/EMBL/GenBank under the accession no. JXOF00000000. The version described in this paper is the first version, JXOF00000000.1. The 187 contigs have been deposited under the accession no. JXOF01000001 to JXOF01000187.

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REFERENCES

1. Kuykendall LD, Saxena B, Devine TE, Udell SE. 1992. Genetic diversity in *Bradyrhizobium japonicum* Jordan (1982) and a proposal for *Bradyrhizobium elkanii* sp. nov. *Can J Microbiol* 38:501–505. <http://dx.doi.org/10.1139/m92-082>.
2. Lopes EM, Castellane TCL, Moretto C, Lemos EGM, Souza JAM. 2014. Emulsification properties of bioemulsifiers produced by wild-type and mutant *Bradyrhizobium elkanii* strains. *J Bioremed Biodegr* 5: 245. <http://dx.doi.org/10.4172/2155-6199.1000245>.

3. 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.
4. Lefort F, Douglas GC. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Ann For Sci* 56:259–263. <http://dx.doi.org/10.1051/forest:19990308>.
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
6. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleus Ac Symp Ser* 41:95–98.
7. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <http://dx.doi.org/10.1093/bioinformatics/btt086>.
8. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <http://dx.doi.org/10.1128/AAC.02412-14>.
9. Tatusova T, DiCuccio M, Badretin A, Chetvernin V, Ciufu S, Li W. 2013. Prokaryotic genome annotation pipeline. The NCBI handbook, 2nd ed. National Center for Biotechnology Information, Bethesda, MD.
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75–90. <http://dx.doi.org/10.1186/1471-2164-9-75>.
11. de Souza JA, Tieppo E, Magnani Gde S, Alves LM, Cardoso RL, Cruz LM, de Oliveira LF, Raittz RT, de Souza EM, Pedrosa Fde O, Lemos EG. 2012. Draft genome sequence of the nitrogen-fixing symbiotic bacterium *Bradyrhizobium elkanii* 587. *J Bacteriol* 194:3547–3548. <http://dx.doi.org/10.1128/JB.00563-12>.
12. Schmeisser C, Liesegang H, Krysciak D, Bakkou N, Le Quéré A, Wollherr A, Heinemeyer I, Morgenstern B, Pommerening-Röser A, Flores M, Palacios R, Brenner S, Gottschalk G, Schmitz RA, Broughton WJ, Perret X, Strittmatter AW, Streit WR. 2009. *Rhizobium* sp. strain NGR234 possesses a remarkable number of secretion systems. *Appl Environ Microbiol* 75:4035–4045. <http://dx.doi.org/10.1128/AEM.00515-09>.
13. Dobert RC, Breil BT, Triplett EW. 1994. DNA sequence of the common nodulation genes of *Bradyrhizobium elkanii* and their phylogenetic relationship to those of other nodulating bacteria. *Mol Plant Microbe Interact* 7:564–572.
14. Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre JC, Jaubert M, Simon D, Cartieaux F, Prin Y, Bena G, Hannibal L, Fardoux J, Kojadinovic M, Vuillet L, Lajus A, Cruveiller S, Rouy Z, Mangenot S, Segurens B, Dossat C, Franck WL, Chang WS, Saunders E, Bruce D, Richardson P, Normand P, Dreyfus B, Pignol D, Stacey G, Emerich D, Verméglie A, Médigue C, Sadowsky M. 2007. Legumes symbioses: absence of *nod* genes in photosynthetic bradyrhizobia. *Science* 316:1307–1312. <http://dx.doi.org/10.1126/science.1139548>.