




# Draft Genome Sequence for *Frankia* sp. Strain BMG5.11, a Nitrogen-Fixing Bacterium Isolated from *Elaeagnus angustifolia*

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**ABSTRACT** *Frankia* sp. strain BMG5.11, which was isolated from *Elaeagnus angustifolia* nodules, is able to infect other actinorhizal plants, including *Elaeagnaceae*, *Rhamnaceae*, *Colletieae*, *Gymnostoma*, and *Myricaceae*. Here, we report the 11.3-Mbp draft genome sequence of *Frankia* sp. strain BMG5.11, with a G+C content of 69.9% and 9,926 candidate protein-encoding genes.

Soil-dwelling actinobacteria of the genus *Frankia* form an endophytic symbiosis with actinorhizal plants from eight angiosperm families (1, 2). This symbiosis allows actinorhizal plants to play an important ecological role as pioneer species recolonizing under harsh environmental conditions (3, 4). Molecular phylogenetic approaches have identified four major *Frankia* lineages, which also follow host plant specificity groups (5–8), and genomes from each lineage have been sequenced (9). These *Frankia* genome databases have enabled the use of “omics” approaches (10–12) and have allowed species identification of the genus (13). Of the four lineages, *Frankia* lineage 3 strains are considered to have the widest plant host range, and they also show great diversity (8) with the potential for many new species. *Frankia* sp. strain BMG5.11 was isolated, through a baiting strategy, from *Elaeagnus angustifolia* nodules from Tunisian soil (14). The soil came from the Gafsa region of Tunisia and was used to inoculate *E. angustifolia* plants. The strain was isolated on defined propionate medium (15) and was stored at –80°C. The strain was sequenced to provide more information about the diverse *Frankia* lineage 3 strains.

Sequencing of the draft genome of *Frankia* sp. strain BMG5.11 was performed at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH) using Illumina technology (16). *Frankia* sp. strain BMG5.11 was grown in defined propionate medium, and high-quality genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (17). A standard Illumina shotgun library was constructed with a Nextera library preparation kit and sequenced using the Illumina HiSeq 2500 platform, which generated 22,318,488 reads (260-bp insert size) totaling 2,485 Mbp. The Illumina sequence data were trimmed and assembled using CLC Genomics Workbench version 12.0 *de novo* assembly. Default parameters were used for all software unless otherwise specified.

The final draft assembly for *Frankia* sp. strain BMG 5.11 consisted of 219 contigs with a contig  $N_{50}$  value of 171.5 kb and 214.0× coverage of the genome. The final assembled genome contained a total sequence length of 11,255,272 bp with a G+C content of 69.9%.

The assembled *Frankia* sp. strain BMG5.11 genome was annotated via the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (18), which resulted in 9,926 candidate protein-encoding genes, 92 tRNAs, 6 noncoding RNAs, and 2 complete rRNA regions.

**Citation** Ghodhbane-Gtari F, Swanson E, Gueddou A, Simpson S, Morris K, Thomas WK, Gtari M, Tisa LS. 2020. Draft genome sequence for *Frankia* sp. strain BMG5.11, a nitrogen-fixing bacterium isolated from *Elaeagnus angustifolia*. *Microbiol Resour Announc* 9:e00824-20. <https://doi.org/10.1128/MRA.00824-20>.

**Editor** David A. Baltus, University of Arizona

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This article is scientific contribution 2860 from the New Hampshire Agricultural Experiment Station.

**Received** 16 July 2020

**Accepted** 21 August 2020

**Published** 10 September 2020

The genome size and corresponding number of coding sequences fit within the values reported for cluster 3 genomes (9). Bioinformatic analysis of this genome using the antiSMASH program (19) revealed the presence of large numbers of secondary metabolic biosynthetic gene clusters, which is consistent with previous results for other *Frankia* lineage 3 genomes, including cluster 3 (9, 20). Many of these potential natural products might be involved in plant-microbe interactions and aid in the plant growth-promoting activities. A whole-genome-based taxonomic analysis via the Type (Strain) Genome Server (TYGS) platform (21) (<https://tygs.dsmz.de>), including determination of digital DNA-DNA hybridization (dDDH) values (22), was performed to identify this strain in comparison with other *Frankia* genomes. The type-based species clustering using a 70% dDDH radius around each of the type strains was performed as described previously (23), while subspecies clustering was performed using a 79% dDDH threshold, as introduced previously (24).

**Data availability.** This whole-genome shotgun sequence has been deposited in DDBJ/EMBL/GenBank under the accession number [SJX10000000](https://doi.org/10.1093/nar/54.11.5411). The version described in this paper is the first version, [SJX10100000](https://doi.org/10.1093/nar/54.11.5411). Both the assembly and raw reads are available in DDBJ/ENA/GenBank under BioProject number [PRJNA318498](https://doi.org/10.1093/nar/54.11.5411) and SRA number [SRR8710209](https://doi.org/10.1093/nar/54.11.5411), respectively.

## ACKNOWLEDGMENTS

Partial funding was provided by the New Hampshire Agricultural Experiment Station. This work was supported by USDA National Institute of Food and Agriculture Hatch grant 022821 (L.S.T.), Plant Health and Production and Plant Products Program grant 2015-67014-22849 (project accession number 1005242) from the USDA National Institute of Food and Agriculture (L.S.T.), and the College of Life Sciences and Agriculture at the University of New Hampshire (Durham, NH). Sequencing was performed on an Illumina HiSeq 2500 system purchased with an NSF MRI grant (grant DBI-1229361 to W.K.T.).

## REFERENCES

- Normand P, Benson DR, Berry AM, Tisa LS. 2014. The family *Frankiaceae*, p 339–356. In Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryotes: actinobacteria*. Springer, Berlin, Germany. [https://doi.org/10.1007/978-3-642-30138-4\\_183](https://doi.org/10.1007/978-3-642-30138-4_183).
- Chaia EE, Wall LG, Huss-Danell K. 2010. Life in soil by the actinorhizal root nodule endophyte *Frankia*: a review. *Symbiosis* 51:201–226. <https://doi.org/10.1007/s13199-010-0086-y>.
- Benson DR, Dawson JO. 2007. Recent advances in the biogeography and genecology of symbiotic *Frankia* and its host plants. *Physiol Plant* 130: 318–330. <https://doi.org/10.1111/j.1399-3054.2007.00934.x>.
- Ngom M, Oshone R, Diagne N, Cissoko M, Svistoonoff S, Tisa LS, Laplace L, Sy MO, Champion A. 2016. Tolerance to environmental stress by the nitrogen-fixing actinobacterium *Frankia* and its role in actinorhizal plants adaptation. *Symbiosis* 70:17–29. <https://doi.org/10.1007/s13199-016-0396-9>.
- Normand P, Orso S, Cournoyer B, Jeannin P, Chapelon C, Dawson J, Evtushenko L, Misra AK. 1996. Molecular phylogeny of the genus *Frankia* and related genera and emendation of the family *Frankiaceae*. *Int J Syst Bacteriol* 46:1–9. <https://doi.org/10.1099/00207713-46-1-1>.
- Ghodhbane-Gtari F, Nouioui I, Chair M, Boudabous A, Gtari M. 2010. 16S-23S rRNA intergenic spacer region variability in the genus *Frankia*. *Microb Ecol* 60:487–495. <https://doi.org/10.1007/s00248-010-9641-6>.
- Nouioui I, Ghodhbane-Gtari F, Beauchemin NJ, Tisa LS, Gtari M. 2011. Phylogeny of members of the *Frankia* genus based on *gyrB*, *nifH* and *glnII* sequences. *Antonie Van Leeuwenhoek* 100:579–587. <https://doi.org/10.1007/s10482-011-9613-y>.
- Gtari M, Tisa LS, Normand P. 2013. Diversity of *Frankia* strains, actinobacterial symbionts of actinorhizal plants, p 123–148. In Aroca R (ed), *Symbiotic endophytes*. Springer, Berlin, Germany. [https://doi.org/10.1007/978-3-642-39317-4\\_7](https://doi.org/10.1007/978-3-642-39317-4_7).
- Tisa LS, Oshone R, Sarkar I, Ktari A, Sen A, Gtari M. 2016. Genomic approaches toward understanding the actinorhizal symbiosis: an update on the status of the *Frankia* genomes. *Symbiosis* 70:5–16. <https://doi.org/10.1007/s13199-016-0390-2>.
- Diagne N, Djighaly PI, Ngom M, Pesce C, Champion A, Svistoonoff S, Hoche V, Tisa LS. 2020. Advances in *Frankia* genome studies and molecular aspects of tolerance to environmental stresses, p 381–389. In Sharma V, Salwan R, Al-Ani T (ed), *Molecular aspects of plant beneficial microbes in agriculture*. Elsevier, Cambridge, MA. <https://doi.org/10.1016/B978-0-12-818469-1.00031-6>.
- Nouioui I, Cortes-Albayay C, Carro L, Castro JF, Gtari M, Ghodhbane-Gtari F, Klenk HP, Tisa LS, Sangal V, Goodfellow M. 2019. Genomic insights into plant-growth-promoting potentialities of the genus *Frankia*. *Front Microbiol* 10:1457. <https://doi.org/10.3389/fmicb.2019.01457>.
- Gherbi H, Hoche V, Ngom M, Diagne N, Fournier J, Carre-Mlouka A, Wall LG, Tisa LS, Svistoonoff S. 2019. Molecular methods for research on actinorhiza, p 35–59. In Reinhardt D, Sharma AK (ed), *Methods in rhizosphere biology research*. Springer, Singapore. [https://doi.org/10.1007/978-981-13-5767-1\\_4](https://doi.org/10.1007/978-981-13-5767-1_4).
- Gtari M, Nouioui I, Sarkar I, Ghodhbane-Gtari F, Tisa LS, Sen A, Klenk HP. 2019. An update on the taxonomy of the genus *Frankia* Brunchorst, 1886, 174<sup>AL</sup>. *Antonie Van Leeuwenhoek* 112:5–21. <https://doi.org/10.1007/s10482-018-1165-y>.
- Gtari M, Brusetti L, Skander G, Mora D, Boudabous A, Daffonchio D. 2004. Isolation of *Elaeagnus*-compatible *Frankia* from soils collected in Tunisia. *FEMS Microbiol Lett* 234:349–355. <https://doi.org/10.1016/j.femsle.2004.04.001>.
- Baker D, O'Keefe D. 1984. A modified sucrose fractionation procedure for the isolation of frankiae from actinorhizal root-nodules and soil samples. *Plant Soil* 78:23–28. <https://doi.org/10.1007/BF02277837>.
- Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <https://doi.org/10.1517/14622416.5.4.433>.
- Murray MG, Thompson WF. 1980. Rapid isolation of high molecular-weight plant DNA. *Nucleic Acids Res* 8:4321–4325. <https://doi.org/10.1093/nar/8.19.4321>.

18. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
19. Blin K, Wolf T, Chevrette MG, Lu XW, Schwalen CJ, Kautsar SA, Duran HGS, Santos E, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0: improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res* 45:W36–W41. <https://doi.org/10.1093/nar/gkx319>.
20. Udway DW, Gontang EA, Jones AC, Jones CS, Schultz AW, Winter JM, Yang JY, Beauchemin N, Capson TL, Clark BR, Esquenazi E, Eustaquio AS, Freel K, Gerwick L, Gerwick WH, Gonzalez D, Liu WT, Malloy KL, Maloney KN, Nett M, Nunnery JK, Penn K, Prieto-Davo A, Simmons TL, Weitz S, Wilson MC, Tisa LS, Dorrestein PC, Moore BS. 2011. Significant natural product biosynthetic potential of actinorhizal symbionts of the genus *Frankia*, as revealed by comparative genomic and proteomic analyses. *Appl Environ Microbiol* 77:3617–3625. <https://doi.org/10.1128/AEM.00038-11>.
21. Meier-Kolthoff JP, Goker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 10: 2182. <https://doi.org/10.1038/s41467-019-10210-3>.
22. Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>.
23. Liu Y, Lai Q, Goker M, Meier-Kolthoff JP, Wang M, Sun Y, Wang L, Shao Z. 2015. Genomic insights into the taxonomic status of the *Bacillus cereus* group. *Sci Rep* 5:14082. <https://doi.org/10.1038/srep14082>.
24. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, Fiebig A, Rohde C, Rohde M, Fartmann B, Goodwin LA, Chertkov O, Reddy TBK, Pati A, Ivanova NN, Markowitz V, Kyrpides NC, Woyke T, Goker M, Klenk HP. 2014. Complete genome sequence of DSM 30083<sup>T</sup>, the type strain (U5/41<sup>T</sup>) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 9:2. <https://doi.org/10.1186/1944-3277-9-2>.