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Permanent Draft Genome Sequence of *Frankia* sp. Strain BR, a Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of *Casuarina equisetifolia*

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***Frankia* sp. strain BR is a member of *Frankia* lineage 1c and is able to re infect plants of the *Casuarinaceae* family. Here, we report a 5.2-Mbp draft genome sequence with a G+C content of 70.0% and 4,777 candidate protein-encoding genes.**

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Members of the genus *Frankia* are nitrogen-fixing actinobacteria that form an endophytic symbiosis with actinorhizal plants (1). This group of woody dicotyledonous plants is distributed worldwide and comprises over 200 species from eight angiosperm families (2). Because of its symbiosis with *Frankia* spp., actinorhizal plants play important ecological roles as pioneer species and are used in agroforestry, land reclamation, crop protection, and soil stabilization projects (3–5). Molecular phylogenetic approaches have identified four major clusters of *Frankia* (6–9), and genomes for representatives from each cluster have been sequenced (10–28). The availability of these *Frankia* genome databases has opened up the use of “omics” approaches. Furthermore, analysis of *Frankia* genomes has revealed new potential in respect to metabolic diversity, natural product biosynthesis, and stress tolerance, which may aid the cosmopolitan nature of the actinorhizal symbiosis.

Actinorhizal plants belonging to the *Casuarinaceae* family are able to grow under saline conditions and have been used extensively as a green barrier (28–33). *Casuarina* trees are native to tropical areas of Australia and the South Pacific and have subsequently been transplanted to other tropical areas of the world, particularly Africa (34). In countries such as Egypt, Tunisia, and Senegal, *Casuarina* trees are actively used in agroforestry and as windbreaks and shelterbelts for agriculture in arid areas (32, 35, 36). Members of *Frankia* cluster 1c have the most restricted host range and are able to infect only *Casuarina* and *Allocasuarina* host plants (1, 18). *Frankia* sp. strain BR was isolated from axenic *Casuarina equisetifolia* root nodules that had been inoculated with crushed *C. equisetifolia* root nodules from Brazil (37). The *Frankia* sp. strain BR genome was sequenced to provide information on cluster 1c lineage and symbiosis with *Casuarina* trees.

Sequencing of the draft genome of *Frankia* sp. strain BR was performed at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH, USA) using Illumina technology techniques (38). A standard Illumina shotgun library was constructed and sequenced using the Illumina HiSeq2500 platform, which generated 24,720,192 reads (260-bp insert size) totaling 3,708 Mbp. The Illumina sequence data were assembled using

CLC Genomics workbench version 8.5 and ALLPaths-LG version r41043 (39). The final draft assembly for *Frankia* sp. strain BR consisted of 180 contigs with an N_{50} contig size of 60.2 kb and $450.4\times$ coverage of the genome. The final assembled genome contained a total sequence length of 5,227,240 bp with a G+C content of 70.0%.

The assembled *Frankia* sp. strain BR was annotated via the Integrated Microbial Genomes (IMG) platform developed by the Joint Genome Institute, Walnut Creek, CA, USA (40, 41), resulting in 4,777 candidate protein-encoding genes and 46 tRNA and two rRNA regions.

Accession number(s). This whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under the accession number [LRTJ000000000](https://www.ncbi.nlm.nih.gov/nuclink/LRTJ000000000). The version described in this paper is the first version, LRTJ01000000.

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REFERENCES

1. Normand P, Benson DR, Berry AM, Tisa LS. 2014. Family *Frankiaceae*, p. 339–356. In Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryote—actinobacteria*, 4th ed. Springer, Berlin.
2. Benson DR, Vandenheuvel B, Potter D. 2004. Actinorhizal symbioses:

- diversity and biogeography, p. 99–127. In Gillings M, Holmes A (ed), Plant microbiology. BIOS Scientific Publishers, Oxford, UK.
3. Dommergues YR. 1997. Contribution of actinorhizal plants to tropical soil productivity and rehabilitation. *Soil Biol Biochem* 29:931–941. [http://dx.doi.org/10.1016/S0038-0717\(96\)00227-1](http://dx.doi.org/10.1016/S0038-0717(96)00227-1).
 4. Kohls SJ, Baker DD, Van Kessel C, Dawson JO. 2003. An assessment of soil enrichment by actinorhizal N₂ fixation using $\delta^{15}\text{N}$ values in a chronosequence of deglaciation at Glacier Bay, Alaska. *Plant Soil* 254:11–17. <http://dx.doi.org/10.1023/A:1024950913234>.
 5. Benson DR, Dawson JO. 2007. Recent advances in the biogeography and genealogy of symbiotic *Frankia* and its host plants. *Physiol Plant* 130: 318–330. <http://dx.doi.org/10.1111/j.1399-3054.2007.00934.x>.
 6. 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. <http://dx.doi.org/10.1099/00207713-46-1-1>.
 7. Cournoyer B, Lavire C. 1999. Analysis of *Frankia* evolution radiation using *glnII* sequences. *FEMS Microbiol Lett* 177:29–34. <http://dx.doi.org/10.1111/j.1574-6968.1999.tb13709.x>.
 8. 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. <http://dx.doi.org/10.1007/s10482-011-9613-y>.
 9. Ghodhbane-Gtari F, Nouioui I, Chair M, Boudabous A, Gtari M. 2010. 16S–23S rRNA intergenic spacer region variability in the genus *Frankia*. *Microbiol Ecol* 60:487–495. <http://dx.doi.org/10.1007/s00248-010-9641-6>.
 10. 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* [Epub ahead of print.] <http://dx.doi.org/10.1007/s13199-016-0390-2>.
 11. D'Angelo T, Oshone R, Abebe-Akele F, Simpson S, Morris K, Thomas WK, Tisa LS. 2016. Permanent draft genome sequence for *Frankia* sp. strain EI5c, a single-spore isolate of a nitrogen-fixing actinobacterium, isolated from the root nodules of *Elaeagnus angustifolia*. *Genome Announc* 4(4):e00660-16. <http://dx.doi.org/10.1128/genomeA.00660-16>.
 12. Ghodhbane-Gtari F, Beauchemin N, Bruce D, Chain P, Chen A, Walston Davenport K, Deshpande S, Detter C, Furnholm T, Goodwin L, Gtari M, Han C, Han J, Huntemann M, Ivanova N, Kyrpides N, Land ML, Markowitz V, Mavrommatis K, Nolan M, Nouioui I, Pagani I, Pati A, Pitluck S, Santos CL, Sen A, Sur S, Szeto E, Tavares F, Teshima H, Thakur S, Wall LG, Woyke T, Tisa LS. 2013. Draft genome sequence of *Frankia* sp. strain CN3, an atypical, noninfective (*nod*⁻) ineffective (*fix*⁻) isolate from *Coriaria nepalensis*. *Genome Announc* 1(2):00085-13. <http://dx.doi.org/10.1128/genomeA.00085-13>.
 13. Ghodhbane-Gtari F, Hurst SG, IV, Oshone R, Morris K, Abebe-Akele F, Thomas WK, Ktari A, Salem K, Gtari M, Tisa LS. 2014. Draft genome sequence of *Frankia* sp. strain BMG5.23, a salt-tolerant nitrogen-fixing actinobacterium isolated from the root nodules of *Casuarina glauca* grown in Tunisia. *Genome Announc* 2(3):e00520-14. <http://dx.doi.org/10.1128/genomeA.00520-14>.
 14. Gtari M, Ghodhbane-Gtari F, Nouioui I, Ktari A, Hezbri K, Mimouni W, Sbissi I, Ayari A, Yamanaka T, Normand P, Tisa LS, Boudabous A. 2015. Cultivating the uncultured: growing the recalcitrant cluster-2 *Frankia* strains. *Sci Rep* 5:13112. <http://dx.doi.org/10.1038/srep13112>.
 15. Hurst SG, IV, Ghodhbane-Gtari F, Oshone R, Morris K, Abebe-Akele F, Thomas WK, Ktari A, Salem K, Mansour S, Gtari M, Tisa LS. 2014. Draft genome sequence of *Frankia* sp. strain Thr, a nitrogen-fixing actinobacterium isolated from the root nodules of *Casuarina cunninghamiana* grown in Egypt. *Genome Announc* 2(3):e00493-14. <http://dx.doi.org/10.1128/genomeA.00493-14>.
 16. Mansour SR, Oshone R, Hurst SG, IV, Morris K, Thomas WK, Tisa LS. 2014. Draft genome sequence of *Frankia* sp. strain Cc16, a salt-tolerant nitrogen-fixing actinobacterium isolated from the root nodule of *Casuarina cunninghamiana*. *Genome Announc* 2(1):e01205-13. <http://dx.doi.org/10.1128/genomeA.01205-13>.
 17. Ngom M, Oshone R, Hurst SG, IV, Abebe-Akele F, Simpson S, Morris K, Sy MO, Champion A, Thomas WK, Tisa LS. 2016. Permanent draft genome sequence for *Frankia* sp. strain CeD, a nitrogen-fixing actinobacterium isolated from the root nodules of *Casuarina equisetifolia* grown in Senegal. *Genome Announc* 4(2):e00265-16. <http://dx.doi.org/10.1128/genomeA.00265-16>.
 18. Normand P, Lapiere P, Tisa LS, Gogarten JP, Alloisio N, Bagnarol E, Bassi CA, Berry AM, Bickhart DM, Choisine N, Couloux A, Cournoyer B, Cruveiller S, Daubin V, Demange N, Francino MP, Goltsman E, Huang Y, Kopp OR, Labarre L, Lapidus A, Lavire C, Marechal J, Martinez M, Mastrorunzio JE, Mullin BC, Niemann J, Pujic P, Rawnsley T, Rouy Z, Schenowitz C, Sellstedt A, Tavares F, Tomkins JP, Vallet D, Valverde C, Wall LG, Wang Y, Medigue C, Benson DR. 2007. Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. *Genome Res* 17:7–15. <http://dx.doi.org/10.1101/gr.5798407>.
 19. Nouioui I, Beauchemin N, Cantor MN, Chen A, Detter JC, Furnholm T, Ghodhbane-Gtari F, Goodwin L, Gtari M, Han C, Han J, Huntemann M, Hua SX, Ivanova N, Kyrpides N, Markowitz V, Mavrommatis K, Mikhailova N, Nordberg HP, Ovchinnikova G. 2013. Draft genome sequence of *Frankia* sp. strain BMG5.12, a nitrogen-fixing actinobacterium isolated from Tunisian soils. *Genome Announc* 1(4):e00468-13. <http://dx.doi.org/10.1128/genomeA.00468-13>.
 20. Oshone R, Hurst SG, IV, Abebe-Akele F, Simpson S, Morris K, Thomas WK, Tisa LS. 2016. Permeant draft genome sequences for two variants of *Frankia* sp. strain Cp11, the first *Frankia* strain isolated from the root nodule of *Comptonia peregrina*. *Genome Announc* 4(1):e01588-15. <http://dx.doi.org/10.1128/genomeA.01588-15>.
 21. Persson T, Benson DR, Normand P, Vanden Heuvel B, Pujic P, Chertkov O, Teshima H, Bruce DC, Detter C, Tapia R, Han S, Han J, Woyke T, Pitluck S, Pennacchio L, Nolan M, Ivanova N, Pati A, Land ML, Pawlowski K, Berry AM. 2011. Genome sequence of “*Candidatus Frankia datiscae*” Dg1, the uncultured microsymbiont from nitrogen-fixing root nodules of the dicot *Datisca glomerata*. *J Bacteriol* 193:7017–7018. <http://dx.doi.org/10.1128/JB.06208-11>.
 22. Pujic P, Bolotin A, Fournier P, Sorokin A, Lapidus A, Richau KH, Briolay J, Mebarki F, Normand P, Sellstedt A. 2015. Genome sequence of the atypical symbiotic *Frankia* R43 strain, a nitrogen-fixing and hydrogen-evolving actinobacterium. *Genome Announc* 3(6):e01387-15. <http://dx.doi.org/10.1128/genomeA.01387-15>.
 23. Sen A, Beauchemin N, Bruce D, Chain P, Chen A, Walston Davenport K, Deshpande S, Detter C, Furnholm T, Ghodhbane-Gtari F, Goodwin L, Gtari M, Han C, Han J, Huntemann M, Ivanova N, Kyrpides N, Land ML, Markowitz V, Mavrommatis K, Nolan M, Nouioui I, Pagani I, Pati A, Pitluck S, Santos CL, Sur S, Szeto E, Tavares F, Teshima H, Thakur S, Wall L, Wishart J, Woyke T, Tisa LS. 2013. Draft genome sequence of *Frankia* sp. strain QA3, a nitrogen-fixing actinobacterium isolated from the root nodule of *Alnus nitida*. *Genome Announc* 1(2):e00103-13. <http://dx.doi.org/10.1128/genomeA.00103-13>.
 24. Swanson E, Oshone R, Simpson S, Morris K, Abebe-Akele F, Thomas WK, Tisa LS. 2015. Permanent draft genome sequence of *Frankia* sp. strain ACN1^{ag}, a nitrogen-fixing actinobacterium isolated from the root nodule of *Alnus glutinosa*. *Genome Announc* 3(6):e01483-15. <http://dx.doi.org/10.1128/genomeA.01483-15>.
 25. Swanson E, Oshone R, Simpson S, Morris K, Abebe-Akele F, Thomas WK, Tisa LS. 2015. Permeant draft genome sequence of *Frankia* sp. strain Avc11, a nitrogen-fixing actinobacterium isolated from the root nodule of *Alnus viridis* subsp. *crispa* grown in Canada. *Genome Announc* 3(6):e01511-15. <http://dx.doi.org/10.1128/genomeA.01511-15>.
 26. Tisa LS, Beauchemin N, Gtari M, Sen A, Wall LG. 2013. What stories can the *Frankia* genomes start to tell us? *J Biosci* 38:719–726. <http://dx.doi.org/10.1007/s12038-013-9364-1>.
 27. Tisa LS, Beauchemin N, Cantor MN, Furnholm T, Ghodhbane-Gtari F, Goodwin L, Copeland A, Gtari M, Huntemann M, Ivanova N, Kyrpides N, Markowitz V, Mavrommatis K, Mikhailova N, Nouioui I, Oshone R, Ovchinnikova G, Pagani I, Palaniappan K, Pati A. 2015. Draft genome sequence of *Frankia* sp. strain DC12, an atypical, noninfective, ineffective isolate from *Datisca cannabina*. *Genome Announc* 3(4):e00889-15. <http://dx.doi.org/10.1128/genomeA.00889-15>.
 28. Wall LG, Beauchemin N, Cantor MN, Chaia E, Chen A, Detter JC, Furnholm T, Ghodhbane-Gtari F, Goodwin L, Gtari M, Han C, Han J, Huntemann M, Hua SX, Ivanova N, Kyrpides N, Markowitz V, Mavrommatis K, Mikhailova N, Nordberg HP, Nouioui I, Ovchinnikova G, Pagani I, Pati A, Sen A, Sur S, Szeto E, Thakur S, Wei C-L, Woyke T, Tisa LS. 2013. Draft genome sequence of *Frankia* sp. strain BCU110501, a nitrogen-fixing actinobacterium isolated from nodules of *Discaria trinervis*. *Genome Announc* 1(4):e00503-13. <http://dx.doi.org/10.1128/genomeA.00503-13>.
 29. El-Lakany MH, Luard EJ. 1983. Comparative salt tolerance of selected *Casuarina* species. *Aust Forest Res* 13:11–20.

30. Girgis MGZ, Ishac YZ, Diem HG, Dommergues YR. 1992. Selection of salt tolerant *Casuarina glauca* and *Frankia*. *Acta Oecol* 13:443–451.
31. Maily D, Ndiaye P, Margolis HA, Pineau M. 1994. Fixation des dunes et reboisement avec le filao (*Casuarina equisetifolia*) dans la zone du littoral nord du Senegal. *Forest Chron* 70:282–290. <http://dx.doi.org/10.5558/tfc70282-3>.
32. El-Lakany MH. 1983. A review of breeding drought resistant *Casuarina* for shelterbelt establishment in arid regions with special reference to Egypt. *Forest Ecol Manage* 6:129–137. [http://dx.doi.org/10.1016/0378-1127\(83\)90017-8](http://dx.doi.org/10.1016/0378-1127(83)90017-8).
33. Tani C, Sasakawa H. 2003. Salt tolerance of *Casuarina equisetifolia* and *Frankia* Ceq1 strain isolated from the root nodules of *C. equisetifolia*. *Soil Sci Plant Nutr* 49:215–222. <http://dx.doi.org/10.1080/00380768.2003.10410000>.
34. Gtari M, Dawson JO. 2011. An overview of actinorhizal plants in Africa. *Funct Plant Biol* 38:653–661. <http://dx.doi.org/10.1071/FP11009>.
35. Maily D, Margolis HA. 1992. Forest floor and mineral soil development in *Casuarina equisetifolia* plantations on the coastal sand dunes of Senegal. *Forest Ecol Manag* 55:259–278. [http://dx.doi.org/10.1016/0378-1127\(92\)90105-I](http://dx.doi.org/10.1016/0378-1127(92)90105-I).
36. Zhong C, Mansour S, Nambiar-Veetil M, Bogusz D, Franche C. 2013. *Casuarina glauca*: a model tree for basic research in actinorhizal symbiosis. *J Biosci* 38:815–823. <http://dx.doi.org/10.1007/s12038-013-9370-3>.
37. Müller A, Benoist P, Diem HG, Schwencke J. 1991. Age-dependent changes in extracellular proteins, aminopeptidase and proteinase activities in *Frankia* isolate BR. *J Gen Microbiol* 137:2787–2796. <http://dx.doi.org/10.1099/00221287-137-12-2787>.
38. Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <http://dx.doi.org/10.1517/14622416.5.4.433>.
39. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc Natl Acad Sci U S A* 108:1513–1518. <http://dx.doi.org/10.1073/pnas.1017351108>.
40. Markowitz VM, Korzeniewski F, Palaniappan K, Szeto E, Werner G, Padki A, Zhao X, Dubchak I, Hugenholtz P, Anderson I, Lykidis A, Mavromatis K, Ivanova N, Kyrpides NC. 2006. The integrated microbial genomes (IMG) system. *Nucleic Acids Res* 34:D344–D348. <http://dx.doi.org/10.1093/nar/gkj024>.
41. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. 2009. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* 25:2271–2278. <http://dx.doi.org/10.1093/bioinformatics/btp393>.