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Draft Genome Sequence of *Frankia* Strain G2, a Nitrogen-Fixing Actinobacterium Isolated from *Casuarina equisetifolia* and Able To Nodulate Actinorrhizal Plants of the Order *Rhamnales*

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***Frankia* sp. strain G2 was originally isolated from *Casuarina equisetifolia* and is characterized by its ability to nodulate actinorrhizal plants of the *Rhamnales* order, but not its original host. It represents one of the largest *Frankia* genomes so far sequenced (9.5 Mbp).**

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The genus *Frankia* contains actinobacteria known for their ability to fix nitrogen and to infect the roots of eight actinorrhizal plant families (1–3). Phylogenetic studies of *Frankia* strains based on 16S rRNA (4), *gyr B* (5), *gln II* (5, 6) genes and 16S-23S rRNA Intergenic Spacer Region (7) indicate four groups. Group 1 forms nodules on *Betulaceae*, *Myricaceae*, and *Casuarinaceae*. Group 2 contains microsymbionts of *Coriariaceae*, *Datisceae*, *Dryadoideae* (*Rosaceae*), and *Ceanothus* (*Rhamnaceae*). Group 3 includes strains associated with *Morella* (*Myricaceae*), *Colletieae* (*Rhamnaceae*), *Elaeagnaceae*, and *Gymnostoma* (*Casuarinaceae*). Group 4 includes atypical, non-infective (Nod-) and/or ineffective (Fix-) *Frankia* strains. Our knowledge about the biology of this genus has been well improved due to the information provided by sequenced *Frankia* genomes (8–21). Group 3 has a broad host range, considerable genetic diversity between the strains (5, 7), high potential for a saprophytic lifestyle (7–22), and a variable genome size ranging from 7.5 to 10.45 Mbp. Strain G2 (=DSM45899=CECT9038) was selected for genome sequencing within the Genomic Encyclopaedia of Type Strains, Phase II: From Individual Species to Whole Genera (23), the second production phase of the Genomic Encyclopedia of *Bacteria* and *Archaea*: Sequencing a Myriad of Type Strains Initiative (24). The candidate type strain G2 for a novel *Frankia* species, selected to enrich the diversity of group 3, was isolated from *Casuarina equisetifolia* nodules collected in the INRA Research Station, Saint-François, Grande Terre, Guadeloupe (25). It has the potential to produce natural products such as the red-pigmented antibiotics (benzo[a]naphthacenequinones) (26). It is infective on members of the actinorrhizal *Rhamnales*, but not on its original host plant *C. equisetifolia* (25). The draft genome of strain G2 was sequenced using Illumina technology (27) with a 300 bp insert standard shotgun library on an Illumina HiSeq-2500 1-TB platform, which generated 6,201,478 reads totaling 936.4 Mbp, at the Joint Genome Institute (JGI) (28). The assembly was realized using

Velvet (version 1.2.07) (29) and Allpaths-LG (version r46652) (30). Annotation was performed using the JGI annotation pipeline (31) and the data are available from the IMG data management system (32). The final draft assembly contained 90 contigs in 83 scaffolds, totaling 9,537,992 bp in size based on 856.6 Mbp of data with 171.3 × input read coverage. The genome draft encodes 7,790 protein genes, 47 tRNAs, and 2 rRNA regions, with an overall G+C content of 70.9%. Genome annotation was performed as described by Tisa et al. (21). As expected, since *Frankia* is a nitrogen fixing actinobacterium, six nitrogenase genes, *nifH*, *nifE*, *nifD*, *nifK*, *nifW*, and *nifN*, have been detected.

Project information is available in the Genomes Online Database (33) and DNA from the DNA Bank Network (34).

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession no. [FAOZ00000000](https://www.ncbi.nlm.nih.gov/nuclink/FAOZ00000000). The version described in this paper is the first version.

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REFERENCES

- Benson DR, Silvester WB. 1993. Biology of *Frankia* strains, actinomycete symbionts of actinorrhizal plants. *Microbiol Rev* 57:293–319.

2. Schwencke J, Carú M. 2001. Advances in actinorhizal symbiosis: host plant-*Frankia* interactions, biology, and applications in arid land reclamation. *Arid Land Res Manag* 15:285–327. <http://dx.doi.org/10.1080/153249801753127615>.
3. Chaia EE, Wall LG, Huss-Danell K. 2010. Life in soil by actinorhizal root nodule endophyte *Frankia*. A review. *Symbiosis* 51:201–226. <http://dx.doi.org/10.1007/s13199-010-0086-y>.
4. 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>.
5. 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>.
6. Cournoyer B, Lavire C. 1999. Analysis of *Frankia* evolution radiation using *glnII* sequences. *FEMS Microbiol Lett* 177:29–34.
7. 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. <http://dx.doi.org/10.1007/s00248-010-9641-6>.
8. Gtari M, Ghodhbane-Gtari F, Nouioui I, Ktari A, Hezbrki 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>.
9. 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, Vallenet 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.
10. Normand P, Queirox C, Tisa LS, Benson DR, Rouy Z, Cruveiller S, Médigue C. 2007. Exploring the genomes of *Frankia*. *Physiol Plant* 130: 331–343. <http://dx.doi.org/10.1111/j.1399-3054.2007.00918.x>.
11. Persson T, Benson DR, Normand P, VandenHeuvel 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 daticae*” 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>.
12. Ghodhbane-Gtari F, Beauchemin N, Bruce D, Chain P, Chen A, Davenport KW, Deshpande S, Detter C, Furnholm T, Goodwin L, Gtari M, Han C, Han J, Huntemann M, Ivanova N, Kyrpides NC, 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, non-infective (Nod) ineffective (Fix) isolate from *Coriaria nepalensis*. *Genome Announc* 1(2):e00085-13.
13. 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 LG, 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>.
14. 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, Pagani I, Pati A, Sen A, Sur S, Szeto E, Thakur S, Wall LG, Wei C-L, Woyke T, Tisa LS. 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>.
15. 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>.
16. Mansour SR, Oshone R, Hurst SG IV, Morris K, Thomas WK, Tisa LS. 2014. Draft genome sequence of *Frankia* sp. strain Ccl6, 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. Hurst SG IV, Oshone R, Ghodhbane-Gtari F, 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>.
18. 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>.
19. 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>.
20. 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-producing actinobacterium. *Genome Announc* 3(6):e01387-15. <http://dx.doi.org/10.1128/genomeA.01387-15>.
21. 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, Sen A, Shapiro N, Szeto E, Wall L, Wishart J, Woyke T. 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>.
22. Benson DR, Vanden Heuvel BD, Potter D. 2004. Actinorhizal symbioses: diversity and biogeography: Plant06, p. 99–129. In Gillings M, Holmes A (ed.), *Plant microbiology*. BIOS Scientific Publishers, Oxford.
23. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, Hooper SD, Pati A, Lykidis A, Spring S, Anderson JJ, D’haeseleer P, Zemla A, Singer M, Lapidus A, Nolan M, Copeland A, Han C, Chen F, Cheng JF, Lucas S, Kerfeld C, Lang E, Gronow S, Chain P, Bruce D, Rubin EM, Kyrpides NC, Klenk HP, Eisen JA. 2009. A phylogeny-driven genomic encyclopaedia of *Bacteria* and *Archaea*. *Nature* 462:1056–1060. <http://dx.doi.org/10.1038/nature08656>.
24. Kyrpides NC, Hugenholtz P, Eisen JA, Woyke T, Göker M, Parker CT, Amann R, Beck BJ, Chain PSG, Chun J, Colwell RR, Danchin A, Dawyndt P, Dedeurwaerdere T, DeLong EF, Detter JC, De Vos P, Donohue TJ, Dong XZ, Ehrlich DS, Fraser C, Gibbs R, Gilbert J, Glina P, Glöckner FO, Jansson JK, Keasling JD, Knight R, Labeda D, Lapidus A, Lee JS, Li WJ, Juncai MA, Markowitz V, Edward RBM, Morrison M, Meyer F, Nelson KE, Ohkuma M, Ouzounis CA, Pace N, Parkhill J, Qin N, Rossello-Mora R, Sikorski J, Smith D, Sogin M, Stevens R, Stingl U, Suzuki KI, Taylor D, Tiedje JM, Tindall B, Wagner M, Weinstock G, Weissenbach J, White O, Wang J, Zhang L, Zhou YG, Field D, Whitman WB, Garrity GM, Klenk HP. 2014. Genomic encyclopedia of *Bacteria* and *Archaea*: sequencing a myriad of type strains. *PLoS Biol* 12: e1001920. <http://dx.doi.org/10.1371/journal.pbio.1001920>.
25. Diem HG, Gauthier D, Dommergues YR. 1982. Isolation of *Frankia* from nodules of *Casuarina equisetifolia*. *Can J Microbiol* 28:526–530. <http://dx.doi.org/10.1139/m82-079>.
26. Gerber NN, Lechevalier MP. 1984. Novel benzo[a]naphthacenequinones from an actinomycete, *Frankia* 6–2 (ORS 020604). *Can J Chem* 62: 2818–2821.
27. Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <http://dx.doi.org/10.1517/14622416.5.4.433>.
28. Mavromatis K, Land ML, Brettin TS, Quest DJ, Copeland A, Clum A, Goodwin L, Woyke T, Lapidus A, Klenk HP, Cottingham RW, Kyrpides NC. 2012. The fast changing landscape of sequencing technologies and

- their impact on microbial genome assemblies and annotation. *PLoS One* 7:e48837. <http://dx.doi.org/10.1371/journal.pone.0048837>.
29. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
 30. 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 USA* 108:1513–1518. <http://dx.doi.org/10.1073/pnas.1017351108>.
 31. Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen IM-A, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015. The Standard operating procedure of the DOE-JGI microbial genome annotation pipeline (MGAP v.4). *Stand Genomic Sci* 10:86.
 32. Markowitz VM, Chen IM, Palaniappan K, Chu K, Szeto E, Pillay M, Ratner A, Huang J, Woyke T, Huntemann M, Anderson I, Billis K, Varghese N, Mavromatis K, Pati A, Ivanova NN, Kyrpides NC. 2014. IMG 4 version of the integrated microbial genomes comparative analysis system. *Nucleic Acids Res* 42:D560–D567. <http://dx.doi.org/10.1093/nar/gkt963>.
 33. Reddy TB, Thomas AD, Stamatis D, Bertsch J, Isbandi M, Jansson J, Mallajosyula J, Pagani I, Lobos EA, Kyrpides NC. 2015. The Genomes Online Database (GOLD) v.5: a metadata management system based on a four level (meta) genome project classification. *Nucleic Acids Res* 43: D1099–D1106. <http://dx.doi.org/10.1093/nar/gku950>.
 34. Gemeinholzer B, Dröge G, Zetzsche H, Haszprunar G, Klenk HP, Güntsch A, Berendsohn WG, Wägele JW. 2011. The DNA bank network: the start from a German initiative. *Biopreserv Biobank* 9:51–55. <http://dx.doi.org/10.1089/bio.2010.0029>.