

# 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

Faten Ghodhbane-Gtari,<sup>a,b</sup> Sheldon G. Hurst, IV,<sup>b</sup> Rediet Oshone,<sup>b</sup> Krystalynne Morris,<sup>b</sup> Feseha Abebe-Akele,<sup>b</sup> W. Kelley Thomas,<sup>b</sup> Amir Ktari,<sup>a</sup> Karima Salem,<sup>a</sup> Maher Gtari,<sup>a,b</sup> Louis S. Tisa<sup>b</sup>

Laboratoire Microorganismes et Biomolécules Actives, Université Tunis El Manar (FST) & Université Carthage (INSAT), Campus Universitaire, Tunis, Tunisia<sup>a</sup>; University of New Hampshire, Durham, New Hampshire, USA<sup>b</sup>

**Nitrogen-fixing actinobacteria of the genus *Frankia* are symbionts of woody dicotyledonous plants termed actinorhizal plants. We report here a 5.27-Mbp draft genome sequence for *Frankia* sp. strain BMG5.23, a salt-tolerant nitrogen-fixing actinobacterium isolated from root nodules of *Casuarina glauca* collected in Tunisia.**

Received 8 May 2014 Accepted 13 May 2014 Published 29 May 2014

**Citation** 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. doi:10.1128/genomeA.00520-14.

**Copyright** © 2014 Ghodhbane-Gtari et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Louis S. Tisa, louis.tisa@unh.edu.

Soil-dwelling nitrogen-fixing actinobacteria of the genus *Frankia* are best known for their symbiotic lifestyle with over 200 species of dicotyledonous plants termed actinorhizal plants (1–3). As ecologically important pioneer community plants, actinorhizal plants are distributed worldwide in a broad range of ecological and environmental conditions. Besides their ecological role, these plants have economic significance in land reclamation, reforestation, soil stabilization, dune stabilizers, and fuel wood. The symbiosis allows actinorhizal plants to colonize harsh environmental terrains under diverse ecological conditions. Molecular phylogenetic approaches have identified four distinct clusters among the *Frankia* strains (4–7). Genomes for representatives from four major lineages have been sequenced (8–14) and have provided vital baseline information for genomic approaches toward understanding these novel bacteria.

Under tropic and subtropic conditions, actinorhizal plants are essentially represented by fast growing and highly tolerant trees from the family *Casuarinaceae*. Since the end of the 19th century, these plants have been exported from their natural habitat in Australia and Western Pacific Islands to worldwide locations for agroforestry systems, essentially serving as windbreaks, dune stabilizers, fuel wood, and soil regeneration (15). *Frankia* sp. strain BMG5.23 was isolated from root nodules of *Casuarina glauca* growing in Tunisia and effectively re-infects its original host plant, *Casuarina* spp. (7). Based on analysis of ITS 16S-23S rRNA (5) and *gyrB*, *glnII*, and *nifH* (6) gene sequences, *Frankia* sp. strain BMG5.23 groups with the “*Casuarina* infective strains” of cluster 1. This strain showed an increased level of NaCl tolerance (R. Oshone and L. S. Tisa, unpublished data). *Frankia* sp. strain BMG5.23 has the potential to be used as a large scale inoculum for *Casuarina* trees involved in land reclamation of Tunisian saline soils. *Frankia* sp. strain BMG5.23 was sequenced to increase our understanding of the salt-tolerance mechanisms and to provide

information about its potential ecological roles and interaction with actinorhizal plants.

The draft genome of *Frankia* sp. strain BMG5.23 was generated at the Hubbard Genome Center (University of New Hampshire, Durham, NH) using Illumina technology (16) techniques. A standard Illumina shotgun library was constructed and sequenced using the Illumina HiSeq2000 platform, which generated 18,742,322 reads (260 bp insert size) totaling 1,695.5 Mbp. The Illumina sequence data were assembled using CLC Genomics Workbench (6.5.1) and AllPaths-LG (version r41043) (17). The final draft assembly contained 167 contigs with an  $N_{50}$  of 64.9 kb. The total size of the genome is 5.3 Mbp, and the final assembly is based on 1,533.9 Mb of Illumina draft data, provided an average 291.2× coverage of the genome.

The high quality draft genome of *Frankia* sp. strain BMG5.23 was resolved to 167 contigs consisting of 5,267,418 bp with a G+C content of 69.8%. The assembled *Frankia* sp. strain BMG5.23 genome was annotated via the Integrated Microbial Genomes (IMG) platform developed by the Joint Genome Institute, Walnut Creek, CA (18), and resulted in 4,747 candidate protein-encoding genes, 47 tRNA genes and 3 rRNA regions.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [JDWE000000000](https://www.ncbi.nlm.nih.gov/nuccore/JDWE000000000). The version described in this paper is version JWDE01000000.

## ACKNOWLEDGMENTS

This work was supported in part by the New Hampshire Agricultural Experimental Station (Hatch NH585), Agriculture and Food Research Initiative grant 2010-65108-20581 from the USDA National Institute of Food and Agriculture, and by the College of Life Science and Agriculture at the University of New Hampshire-Durham. This is scientific contribution number 2453 from the NH Agricultural Experiment Station. M.G. and F.G.-G. were supported in part by a Visiting Scientist and Postdoc-

toral Scientist Program administered by the New Hampshire Agricultural Experimental Station at the University of New Hampshire.

## REFERENCES

- Benson DR, Silvester WB. 1993. Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiol. Rev.* 57:293–319.
- Schwencke J, Caru M. 2001. Advances in actinorhizal symbiosis: Host plant-*Frankia* interactions, biology, and applications in arid land reclamation. *A review. Arid Land Res. Manage.* 15:285–327.
- 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>.
- Normand P, Orso S, Cournoyer B, Jeannin P, Chapelon C, Dawson JO, 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>.
- Cournoyer B, Lavire C. 1999. Analysis of *Frankia* evolution radiation using *glnII* sequences. *FEMS Microbiol. Lett.* 117:29–34.
- 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>.
- 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>.
- 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.
- 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>.
- 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, non-infective (nod<sup>-</sup>) ineffective (Fix<sup>-</sup>) isolate from *Coriaria nepalensis*. *Genome Announc.* 1(2):e00085-13. <http://dx.doi.org/10.1128/genomeA.00085-13>.
- 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 SS, 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>.
- 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 L, 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/genomeA00468-13>.
- 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/genomeA00503-13>.
- Mansour SR, Oshone R, Hurst IV SG, Morris K, Thomas WK, Tisa LS. 2014. Draft genome sequence of *Frankia* sp. strain CcI6, 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>.
- Dommergues Y. 1997. Contribution of Actinorhizal plants to tropical soil productivity and rehabilitation. *Soil Biol. Biochem.* 29:931–947. [http://dx.doi.org/10.1016/S0038-0717\(96\)00227-1](http://dx.doi.org/10.1016/S0038-0717(96)00227-1).
- Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <http://dx.doi.org/10.1517/14622416.5.4.433>.
- 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>.
- 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>.