

# Permanent Draft Genome Sequence of *Frankia* sp. Strain Allo2, a Salt-Tolerant Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of *Allocastraria*

Rediet Oshone,<sup>a</sup> Mariama Ngom,<sup>a,b,c,d</sup> Feseha Abebe-Akele,<sup>a</sup> Stephen Simpson,<sup>a</sup> Krystalynne Morris,<sup>a</sup> Mame Ourèye Sy,<sup>b,c</sup> Antony Champion,<sup>b,d,e</sup> W. Kelley Thomas,<sup>a</sup> Louis S. Tisa<sup>a</sup>

University of New Hampshire, Durham, New Hampshire, USA<sup>a</sup>; Laboratoire Mixte International Adaptation des Plantes et Microorganismes Associés aux Stress Environnementaux (LAPSE), Centre de Recherche de Bel Air, Dakar, Sénégal<sup>b</sup>; Département de Biologie Végétale, Laboratoire Campus de Biotechnologies Végétales, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar-Fann, Sénégal<sup>c</sup>; Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, Dakar, Sénégal<sup>d</sup>; Institut de recherche pour le développement (IRD), UMR DIADE, Montpellier, France<sup>e</sup>

***Frankia* sp. strain Allo2 is a member of *Frankia* lineage Ib, which is able to reinfect plants of the *Casuarinaceae* family, and exhibits a high level of salt tolerance compared to other isolates. Here, we report the 5.3-Mbp draft genome sequence of *Frankia* sp. strain Allo2 with a G+C content of 70.0% and 4,224 candidate protein-encoding genes.**

Received 25 March 2016 Accepted 29 March 2016 Published 19 May 2016

**Citation** Oshone R, Ngom M, Abebe-Akele F, Simpson S, Morris K, Sy MO, Champion A, Thomas WK, Tisa LS. 2016. Permanent draft genome sequence of *Frankia* sp. strain Allo2, a salt-tolerant nitrogen-fixing actinobacterium isolated from the root nodules of *Allocastraria*. *Genome Announc* 4(3):e00388-16 doi:10.1128/genomeA.00388-16.

**Copyright** © 2016 Oshone et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Louis S. Tisa, [louis.tisa@unh.edu](mailto:louis.tisa@unh.edu).

**S**alinization of soils and groundwater is a serious problem, especially in arid and semiarid lands, and has resulted in a drastic reduction in agricultural production (1). Worldwide, over 800 million hectares of land are affected (2). One potential solution for dealing with salt stress problems is reclaiming saline soils with fast-growing, multipurpose, salt-tolerant trees like the actinorhizal plants. Among the actinorhizal plants, the genus *Casuarina* grows well under these conditions and has been used in North Africa for these purposes. Actinorhizal plants form a nitrogen-fixing symbiosis with the genus *Frankia* that results in the ability of these plants to colonize harsh environments (3–5).

Based on phylogenetic markers, four major clusters are recognized within the genus (6–9), and genomes for representatives from each cluster have been sequenced (10–26). Cluster I contains two subclusters: one subcluster (cluster Ia) consists of *Frankia* spp. strains that associate with host plants in the *Betulaceae* and *Myricaceae* families, while the other subcluster (cluster Ib) is limited to *Casuarina* and *Allocastraria* host plants. *Frankia* sp. strain Allo2 was isolated from root nodules of *Allocastraria verticillata* (27). This strain showed an increased level of NaCl tolerance (R. Oshone and L. S. Tisa, unpublished data) and was sequenced to increase our understanding of salt-tolerance mechanisms and to provide insight about its interaction with actinorhizal plants. A comparative analysis of sequenced genomes among the salt-tolerant and salt-sensitive *Casuarina* strains may provide a clear picture of the role that these symbionts play in allowing these actinorhizal plants to colonize harsh environments, including saline soils.

The draft genome sequence of *Frankia* sp. strain Allo2 was generated at the Hubbard Genome Center (University of New Hampshire, Durham, NH, USA) using Illumina technology (28) techniques. A standard Illumina shotgun library was constructed

and sequenced using the Illumina HiSeq2000 platform, which generated 26,647,886 reads (260-bp insert size), totaling 3,866.4 Mbp. The Illumina sequence data were assembled using CLC Genomics Workbench version 8.0.1 and AllPaths-LG version r41043 (29). The final draft assembly for *Frankia* sp. Allo2 consisted of 133 contigs in 110 scaffolds with an  $N_{50}$  contig size of 96.9 kb. The final assembled genome contained a total sequence of 5,352,211 bp with a G+C content of 70.0% and is based on 3,120.3 Mb of Illumina draft data, providing an average 583× coverage of the genome.

The assembled *Frankia* sp. strain Allo2 genome was annotated via the Integrated Microbial Genomes (IMG) platform developed by the Joint Genome Institute, Walnut Creek, CA, USA (30, 31) and resulted in 4,224 candidate protein-encoding genes and 45 tRNA and 3 rRNA regions.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [JPH000000000](https://www.ncbi.nlm.nih.gov/nuccore/JPH000000000). The version described in this paper is the first version, JPHT01000000.

## ACKNOWLEDGMENTS

Partial funding was provided by the New Hampshire Agricultural Experiment Station. This is Scientific Contribution Number 2665. This work was supported by the USDA National Institute of Food and Agriculture Hatch 022821 (L.S.T.), Agriculture and Food Research Initiative Grant 2015-67014-22849 from the USDA National Institute of Food and Agriculture (L.S.T.), and the College of Life Science and Agriculture at the University of New Hampshire, Durham, M.N. was supported by an ARTS (Allocation de Recherche pour une Thèse au Sud) PhD grant from the Institut de recherche pour le développement (IRD). Sequencing was performed on an Illumina HiSeq2500 purchased with NSF MRI Grant DBI-1229361 to W.K.T.

## FUNDING INFORMATION

This work, including the efforts of Mariama Ngom, was funded by Institut de Recherche pour le Développement (ARTS). This work, including the efforts of W. Kelley Thomas, was funded by National Science Foundation (NSF) (DBI-1229361). This work, including the efforts of Louis S. Tisa, was funded by USDA | National Institute of Food and Agriculture (NIFA) (Hatch 022821 and 2015-67014-22849).

## REFERENCES

- Munns R. 2005. Genes and salt tolerance: bringing them together. *New Phytol* 167:645–663. <http://dx.doi.org/10.1111/j.1469-8137.2005.01487.x>.
- Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681. <http://dx.doi.org/10.1146/annurev.arplant.59.032607.092911>.
- 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 prokaryotes—Actinobacteria*, 4th ed. Springer, Berlin.
- Schwencke J, Carú M. 2001. Advances in actinorhizal symbiosis: host plant-*Frankia* interactions, biology, and applications in arid land reclamation. A review. *Arid Land Res Manag* 15:285–327. <http://dx.doi.org/10.1080/153249801753127615>.
- Dommergues YR. 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).
- 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* 177:29–34.
- 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>.
- 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, Kyrpidis 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*<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>.
- Ghodhbane-Gtari F, Hurst SG, 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>.
- Gtari M, Ghodhbane-Gtari F, Nouioui I, Ktari A, Hezbri K, Mimouni W, Sbissi I, Ayari A, Sghaier H, Normand P, Tisa LS, Yamanaka T, Boudabous A. 2015. Cultivating the uncultured: growing the recalcitrant cluster-2 *Frankia* strains. *Sci Rep* 5:13112. <http://dx.doi.org/10.1038/srep13112>.
- Hurst SG, 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): <http://dx.doi.org/10.1128/genomeA.00493-14>.
- 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): <http://dx.doi.org/10.1128/genomeA.01205-13>.
- Ngom M, Oshone R, Hurst IVS, 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): <http://dx.doi.org/10.1128/genomeA.00265-16>.
- Normand P, Lapiere P, Tisa LS, Gogarten JP, Alloisio N, Bagnarol E, Bassi CA, Berry AM, Bickhart DM, Choise N, Couloud A, Cournoyer B, Cruveiller S, Daubin V, Demange N, Francino MN, Goltsman E, Huang Y, Kopp OR, Labarre L, Lapidus A, Lavire C, Marechal J, Martinez M, Mastronunzio 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. <http://dx.doi.org/10.1101/gr.5798407>.
- 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, Kyrpidis 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 CL, 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): <http://dx.doi.org/10.1128/genomeA00468-13>.
- 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>.
- 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 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>.
- Pujic P, Bolotin A, Fournier P, Sorokin A, Lapidus A, Richau KH, Briolay J, Barkai 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>.
- 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, Kyrpidis 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>.
- Swanson E, Oshone R, Simpson S, Morris K, Abebe-Akele F, Thomas WK, Tisa LS. 2015. Draft genome sequence of *Frankia* sp. strain ACN1<sup>ag</sup>, 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>.
- 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>.
- 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>.
- Tisa LS, Beauchemin N, Cantor MN, Furnholm T, Ghodhbane-Gtari F, Goodwin L, Copeland A, Gtari M, Huntemann M, Ivanova N, Kyrpidis 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>.
- 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, Kyrpidis 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 trinevis*. *Genome Announc* 1(4): <http://dx.doi.org/10.1128/genomeA.00503-13>.
27. Girgis MGZ, Schwencke J. 1993. Differentiation of *Frankia* strains by their electrophoretic patterns of intracellular esterases and aminopeptidases. *J Gen Microbiol* 139:2225–2332. <http://dx.doi.org/10.1099/00221287-139-9-2225>.
  28. Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <http://dx.doi.org/10.1517/14622416.5.4.433>.
  29. 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>.
  30. Markowitz VM, Korzeniewski F, Palaniappan K, Szeto E, Werner G, Padki A, Zhao XL, 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>.
  31. Markowitz VM, Mavromatis K, Ivanova NN, Chen IMA, 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>.