




Permanent Draft Genome Sequence for *Frankia* sp. Strain Cc1.17, a Nitrogen-Fixing Actinobacterium Isolated from Root Nodules of *Colletia cruciata*

Erik Swanson,^a Rediet Oshone,^a Imen Nouioui,^{c,d} Feseha Abebe-Akele,^a Stephen Simpson,^a Krystalynne Morris,^a W. Kelley Thomas,^a Arnab Sen,^b Faten Ghodhbane-Gtari,^c  Maher Gtari,^c Louis S. Tisa^a

University of New Hampshire, Durham, New Hampshire, USA^a; University of North Bengal, Siliguri, India^b; Université de Tunis El Manar, Tunis, Tunisia^c; Newcastle University, Newcastle upon Tyne, United Kingdom^d

ABSTRACT *Frankia* sp. strain Cc1.17 is a member of the *Frankia* lineage 3, the organisms of which are able to re infect plants of the Eleagnaceae, Rhamnaceae, and Myricaceae families and the genera *Gymnostoma* and *Alnus*. Here, we report the 8.4-Mbp draft genome sequence, with a G+C content of 72.14% and 6,721 candidate protein-coding genes.

Members of the genus *Frankia* are well known for their ability to form a symbiotic association with a variety of dicotyledonous plants from 8 different families collectively termed actinorhizal plants (1). This interaction results in the formation of a root nodule structure that contains these nitrogen-fixing bacteria. Based on molecular phylogenetic evidence (2–6), *Frankia* consists of 4 major lineages that have been correlated with plant host ranges, and genomes for representatives of each cluster have been sequenced (7). Until recently, *Frankia* strains have not been identified to the species level. Since the sequencing of several *Frankia* genomes, several different species have been and will continue to be recognized (8, 9).

Besides being broad-host-range symbionts, members of *Frankia* lineage 3 exhibit the greatest genetic diversity between strains, have the highest metabolic potential, and possess larger genomes than the other lineages. Many of these strains have adapted to harsh environmental conditions. *Frankia* sp. strain Cc1.17 was isolated from root nodules of *Colletia cruciata* (10). The strain has been investigated for its physiology (11, 12) and is used in genetics studies, including the identification of genetic markers (13, 14) and mutagenesis experiments (15). *Frankia* sp. strain Cc1.17 was sequenced to provide greater insight into this lineage and its interaction with actinorhizal plants. The genome sequence will also be used to assist in the elucidation of lineage 3 diversity, with the goal of identification to the species level.

Sequencing of the draft genome of *Frankia* sp. strain Cc1.17 was performed at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH) using Illumina technology techniques (16). A standard Illumina shotgun library was constructed and sequenced using the Illumina HiSeq 2500 platform, which generated 15,413,374 reads (260-bp insert size) totaling 3,945 Mbp. The Illumina sequence data were trimmed by Trimmomatic version 0.32 (17) and assembled using SPAdes version 3.5 (17) and ALLPaths-LG version r52488 (18). The final draft assembly for *Frankia* sp. strain Cc1.17 consisted of 195 contigs, with an N_{50} contig size of 118.5 kb and 356.3× coverage of the genome. The final assembled genome contained a total sequence length of 8,361,025 bp, with a G+C content of 72.14%.

Received 26 April 2017 Accepted 28 April 2017 Published 15 June 2017

Citation Swanson E, Oshone R, Nouioui I, Abebe-Akele F, Simpson S, Morris K, Thomas WK, Sen A, Ghodhbane-Gtari F, Gtari M, Tisa LS. 2017. Permanent draft genome sequence for *Frankia* sp. strain Cc1.17, a nitrogen-fixing actinobacterium isolated from root nodules of *Colletia cruciata*. Genome Announc 5:e00530-17. <https://doi.org/10.1128/genomeA.00530-17>.

Copyright © 2017 Swanson 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.

This is scientific contribution number 2724.

The assembled *Frankia* sp. strain Cc1.17 genome was annotated via the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and resulted in 6,721 candidate protein-coding genes. Bioinformatic analysis of this genome using the antiSMASH program (19, 20) revealed the presence of high numbers of secondary metabolic biosynthetic gene clusters, including 4 nonribosomal peptide synthetase, 12 polyketide synthase, 4 terpene, 1 bacteriocin, 3 lantipeptide, and 1 siderophore cluster. This large number is consistent with previous results with other *Frankia* lineage 3 strains (7).

Accession number(s). This whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under the accession number [MBLM00000000](https://www.ncbi.nlm.nih.gov/nuccore/MBLM00000000). The version described in this paper is the first version, MBLM01000000.

ACKNOWLEDGMENTS

Partial funding was provided by the New Hampshire Agricultural Experiment Station. This work was supported by the USDA National Institute of Food and Agriculture Hatch 022821 (to L.S.T.), Agriculture and Food Research Initiative Grant 2015-67014-22849 from the USDA National Institute of Food and Agriculture (to L.S.T.), DST, Government of India grant on *Frankia* (to A.S.), and the College of Life Science and Agriculture at the University of New Hampshire-Durham. Sequencing was performed on an Illumina HiSeq 2500 purchased with NSF MRI grant DBI-1229361 to W. K. Thomas.

REFERENCES

- 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*. Springer-Verlag, Berlin Heidelberg, Heidelberg, Germany.
- Clawson ML, Bourret A, Benson DR. 2004. Assessing the phylogeny of *Frankia*-actinorhizal plant nitrogen-fixing root nodule symbioses with *Frankia* 16S rRNA and glutamine synthetase gene sequences. *Mol Phylogenet Evol* 31:131–138. <https://doi.org/10.1016/j.ympev.2003.08.001>.
- 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>.
- 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>.
- 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>.
- Cournoyer B, Lavire C. 1999. Analysis of *Frankia* evolutionary radiation using *glnII* sequences. *FEMS Microbiol Lett* 177:29–34. <https://doi.org/10.1111/j.1574-6968.1999.tb13709.x>.
- 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>.
- Nouioui I, Ghodhbane-Gtari F, Del Carmen Montero-Calasanz M, Rohde M, Tisa LS, Gtari M, Klenk HP. 2017. *Frankia inefficax* sp. nov., an actinobacterial endophyte inducing ineffective, non nitrogen-fixing, root nodules on its actinorhizal host plants. *Antonie Van Leeuwenhoek* 110:313–320. <https://doi.org/10.1007/s10482-016-0801-7>.
- Nouioui I, Ghodhbane-Gtari F, Montero-Calasanz MD, Göker M, Meier-Kolthoff JP, Schumann P, Rohde M, Goodfellow M, Fernandez MP, Normand P, Tisa LS, Klenk HP, Gtari M. 2016. Proposal of a type strain for *Frankia alni* (Woronin 1866) von Tubeuf 1895, emended description of *Frankia alni*, and recognition of *Frankia casuarinae* sp. nov. and *Frankia elaeagni* sp. nov. *Int J Syst Evol Microbiol* 66:5201–5210. <https://doi.org/10.1099/ijsem.0.001496>.
- Akkermans ADL, Hafeez F, Roelofsen W, Chaudhary AH, Baas R. 1984. Ultrastructure and nitrogenase activity of *Frankia* grown in pure culture and in actinorrhizae of *Alnus*, *Colletia*, and *Datscia* spp., p 311–319. In Veeger C, Newton WE (ed), *Advances in nitrogen fixation research*. Nijhoff Junk Publishers, The Hague Boston Lancaster, Wageningen, The Netherlands.
- Meesters TM. 1987. Localization of nitrogenase in vesicles of *Frankia* sp. Cc1.17 by immunogold labeling on ultrathin cryosections. *Arch Microbiol* 146:327–331. <https://doi.org/10.1007/BF00410930>.
- Meesters TM, van Genesen ST, Akkermans ADL. 1985. Growth, acetylene-reduction activity and localization of nitrogenase in relation to vesicle formation in *Frankia* strains Cc117 and Cp12. *Arch Microbiol* 143:137–142. <https://doi.org/10.1007/BF00411036>.
- Richards JW, Krumholz GD, Chval MS, Tisa LS. 2002. Heavy metal resistance patterns of *Frankia* strains. *Appl Environ Microbiol* 68:923–927. <https://doi.org/10.1128/AEM.68.2.923-927.2002>.
- Tisa LS, Chval MS, Krumholz GD, Richards J. 1999. Antibiotic resistance patterns of *Frankia* strains. *Can J Bot* 77:1257–1260.
- Myers AK, Tisa LS. 2004. Isolation of antibiotic-resistant and antimetabolite-resistant mutants of *Frankia* strains Eul1c and Cc1.17. *Can J Microbiol* 50:261–267. <https://doi.org/10.1139/w04-013>.
- Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <https://doi.org/10.1517/14622416.5.4.433>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- 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. <https://doi.org/10.1073/pnas.1017351108>.
- Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res* 39:W339–W346. <https://doi.org/10.1093/nar/gkr466>.
- Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43:W237–W243. <https://doi.org/10.1093/nar/gkv437>.