Permanent draft genome sequence of Frankia sp. NRRL B-16219 reveals the presence of canonical nod genes, which are highly homologous to those detected in Candidatus Frankia Dg1 genome

Amir Ktari, Imen Nouioui, Teal Furnholm, Erik Swanson, Faten Ghodhbane-Gtari, Louis S. Tisa and Maher Gtari

Abstract

Frankia sp. NRRL B-16219 was directly isolated from a soil sample obtained from the rhizosphere of Ceanothus jepsonii growing in the USA. Its host plant range includes members of Elaeagnaceae species. Phylogenetically, strain NRRL B-16219 is closely related to "Frankia discariae" with a 16S rRNA gene similarity of 99.78%. Because of the lack of genetic tools for Frankia, our understanding of the bacterial signals involved during the plant infection process and the development of actinorhizal root nodules is very limited. Since the first three Frankia genomes were sequenced, additional genome sequences covering more diverse strains have helped provide insight into the depth of the pangenome and attempts to identify bacterial signaling molecules like the rhizobial canonical nod genes. The genome sequence of Frankia sp. strain NRRL B-16219 was generated and assembled into 289 contigs containing 8,032,739 bp with 71.7% GC content. Annotation of the genome identified 6211 protein-coding genes, 561 pseudogenes, 1758 hypothetical proteins and 53 RNA genes including 4 rRNA genes. The NRRL B-16219 draft genome contained genes homologous to the rhizobial common nodulation genes clustered in two areas. The first cluster contains nodACIJH genes whereas the second has nodAB and nodH genes in the upstream region. Phylogenetic analysis shows that Frankia nod genes are more deeply rooted than their sister groups from rhizobia. PCR-sequencing suggested the widespread occurrence of highly homologous nodA and nodB genes in microsymbionts of field collected Ceanothus americanus.

Keywords: Frankia, Actinorhizal symbiosis, Plant-microbe interactions, Genome, Canonical nod genes, Ceanothus

Introduction

The symbiosis resulting from members of the genus Frankia interacting with the roots of 8 dicotyledonous plant families (referred to actinorhizal plants) is found worldwide and contributes to the ability of actinorhizal pioneer plants to grow in poor and marginally fertile soils [1]. This symbiotic association has drawn interest because of its higher rate of soil nitrogen input and the ability of the plants to overcome harsh environmental conditions [2]. The molecular mechanism for the establishment of an actinorhizal nitrogen-fixing root nodule remains elusive [3]. Molecular phylogeny of the Frankia genus has consistently identified four main clusters regardless of the typing locus used [1]. These Frankia clusters also follow and support the host specificity groups proposed by Baker [4]. Cluster 1 is divided into sub-cluster 1a including F. alni and relatives that are infective on Alnus and Myricaceae and sub-cluster 1b strains that are infective on Allocasuarina, Casuarina and Myricaceae including F. casuarinae [5]. Cluster 2 contains F. coriariae [6] and uncultured microsymbionts of Coriariaceae, Datiscaecae, Dryadoideae and...
Ceanothus, while cluster 3, associated F. elaeagni [5], “F. discariae” [7] and closely related strains are infective on Colletieae, Elaeagnaceae, Gymnostoma and Myricaceae. Finally, cluster 4 groups a broad range of non-nitrogen-fixing and infective strains including F. inefficax species [8] together with “F. asymbiotica” [9] and other related strains that are unable to establish a symbiosis with actinorhizal plants. As has been established for rhizobial and arbuscular mycorrhizal symbioes, the LysM-RLKs are also involved in the perception of Frankia signal molecules by the actinorhizal plant [10, 11]. However, the bacterial signals triggering this symbiosis remain unknown. At present, more than 30 Frankia genomes from strains in pure culture have been sequenced and annotated [12–30] and two Candidatus genomes were generated from nodule metagenomes [31, 32]. Analysis of the Frankia genomes failed to reveal the presence of common canonical nodABC genes [33] which also appear to be missing in several photosynthetic [34] and non-photosynthetic [35] bradyrhizobia. The only exceptions were found in the two Candidatus Frankia genomes, which contained the canonical nodABC and sulfotransferase nodH genes [32, 36]. This contradictory situation justifies additional sequencing of genomes from cultivated Frankia strains to gain insight into the depth of the pangenome pool covered. Here we report the first proof of the presence of rhizobial homologous canonical nodABCH genes within the draft genome of cultivated Frankia isolate, strain NRRL B-16219 and widespread occurrence of nodAB in field collected Ceanothus americanus microsymbionts.

**Organism information**

**Classification and features**

Strain NRRL B-16219 metabolizes short fatty acids, TCA-cycle intermediates and carbohydrates (Table 1). It is infective on members of Elaeagnaceae and Morella cerifera and produces effective root nodules [4, 37]. In

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| MIGS-6  | Habitat  | Soil and Host-associated | IDA          |
| MIGS-6.3 | Salinity | Not reported | IDA          |
| MIGS-22 | Oxygen requirement | Aerobic | NAS          |
| MIGS-15 | Biotic relationship | Free-living and Host plant-associated | NAS          |
| MIGS-14 | Pathogenicity | Non-pathogen | NAS          |
| MIGS-4  | Geographic location | Soil beneath Ceanothus jepsonii, USA | IDA          |
| MIGS-5  | Sample collection | 1982 | IDA          |
| MIGS-4.1 | Latitude | Not reported | -            |
| MIGS-4.2 | Longitude | Not reported | -            |
| MIGS-4.4 | Altitude | Not reported | -            |

*Evidence codes – IDA INFERRED FROM DIRECT ASSAY, TAS traceable author statement (i.e., a direct report exists in the literature) NAS non-traceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence)*

Table 1 Classification and general features of Frankia sp. strain NRRL B-16219 according to MIGS [45]
coherence with its host range, strain NRRL B-16219 is phylogenetically affiliated to cluster 3, known to effectively nodulate members of Elaeagnaceae, Rhamnaceae and Myricaceae families. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain NRRL B-16219 was most closely related to type strains of *F. discariae* DSM 46785T (99.78%) and *F. elaeagni* (98.26%) (Fig. 1).

*Frankia* sp. strain NRRL B-16219 shows typical *Frankia* morphological structures; branched hyphae, vesicles, the site of nitrogenase activity, and multilocular sporangia containing non-motile spores (Fig. 2).

**Extended feature descriptions**

Strain NRRL B-16219 represents one of the rare *Frankia* strains directly isolated from soil on plate medium without passing through plant trapping assay. The strain was isolated from the rhizosphere of *Ceanothus jepsonii* [37] following a complex protocol of soil treatment with phenol (0.7%), sample fractionation through ultracentrifugation in sucrose density gradient, and plating on solid DPM without nitrogen source. Strain NRRL B-16219 developed unpigmented white colonies after 4 weeks growth on DPM medium at 28 °C without shaking. The strain was phylogenotyped using GENIII microplates in an Omnilog device (BIOLOG Inc., Haywood, USA) as previously described [5]. It was able to metabolize acetic acid, citric acid, D-cellobiose, dextrin, D-fructose, D-mannitol, D-mannose, fructose-6-phosphate, fusidic acid, glucose-6-phosphate, D and L malic acid, *p*-hydroxy-phenylacetic acid, propionic acid and D-serine and to grow in presence of 1% sodium lactate and up to 1% NaCl. Growth occurred between pH 5.0–6.8. The strain showed tolerant only to rifamycin.

**Genome sequencing information**

**Genome project history**

Because it is one of the rare strains isolated directly from the soil, NRRL B-16219 strain was selected as part of an effort to gain insight into the depth of the pangenome pool and to identify symbiotic signaling molecules. The sequencing project was completed in April 2016 and the generated data was submitted as draft genome to Genbank under BioProject PRJNA318440 and the accession number MAXA00000001.

**Growth conditions and genomic DNA preparation**

The studied strain was kindly provided by David Labeda, ARS USDA bacterial collection, as NRRL B-16219 strain ID. The strain was grown at 28 °C in stationary culture in 1-l bottles containing DPM medium [5], supplemented with 0.5 mM NH₄Cl as nitrogen source maintained. Biomass from 1 month-old culture was harvested by centrifugation at 9000 x g for 15 min, rinsed several times with sterile distilled water. The mycelial mats were broken by repeated passages through syringes with progressively smaller diameters (21 g to 27 g). Genomic DNA extraction was performed using Plant DNeasy kits (Qiagen, Hilden, Germany) following the recommendation of the manufacturer. Prior to genome sequencing, the quality of the isolated DNA was checked by using the prepared DNA as template for PCR and partial sequences of several housekeeping genes and the 16S rRNA gene were generated and analyzed [16].

**Genome sequencing and assembly**

Sequencing of the draft genome of *Frankia* sp. NRRL B-16219 was performed at the Hubbard Center for
Genome Studies (University of New Hampshire, Durham, NH) using Illumina technology [38]. A standard Illumina shotgun library was constructed and sequenced using the Illumina HiSeq2500 platform with pair-end reads of 2 × 250 bp. The Illumina sequence data were trimmed by Trimmonatic version 0.32 [39], and assembled using Spades version 3.5 [40], and ALLPaths-LG version r52488 [41].

Genome annotation

The genome was annotated via the NCBI Prokaryotic Genome Annotation Pipeline. Additionally nod gene prediction analysis was done within the Integrated Microbial Genomes-Expert Review system developed by the Joint Genome Institute, Walnut Creek, CA, USA [42] developed by the Joint Genome Institute, Walnut Creek, CA, USA, using similarity search tools. This whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under the accession number MAXA00000000.1. The version described in this paper is the first version, MAXA00000000.1. A summary of the project information is shown in Table 2.

Genome properties

The draft genome of Frankia NRRL B-16219 consisted of 289 DNA contigs that correspond to estimated genome size of 8,032,739 bp and a GC content of 71.7%. The draft genome contained 6859 total genes, including 6211 protein-encoding genes (90.55%), 561 pseudo genes (8.17%) and 53 RNAs (0.76%) (Table 3). Classification of genes into the COG functional categories is shown in Table 4.

Insights from the genome sequence

Comparison of genomes from Frankia sp. NRRL B-16219 and other Frankia species

The Frankia sp. NRRL B-16219 genome was compared to all of the Frankia genomes available at NCBI genome database including seven Frankia species including F. alni, F. casuarinae, F. elaeagni, F. coriariae, “F. discar-iae”, F. inefficax, and “F. asymbiotica”, two Candidatus Frankia and other Frankia sp. strains. As shown for other closely related strains from cluster 3, strain NRRL B-16219 has one of the largest genome sizes (8,032,739 bp) with a high GC content of 71.72%. Genes shown or suggested to be involved in the actinorhizal symbiosis were detected. Nitrogenase genes were organized into one operon: nifH-D-K-E-N-X-orf1-orf2-W-Z-B-U and a non-linked nifV gene. Genes encoding the hydrogenase subunits were clustered into two operons.

Table 2 Project information

| MIGS ID | Property                        | Term                                      |
|---------|---------------------------------|-------------------------------------------|
| MIGS 31 | Finishing quality               | Draft genome                              |
| MIGS 28 | Libraries used                  | Illumina Standard library                 |
| MIGS 29 | Sequencing platforms            | Illumina HiSeq2500 platform               |
| MIGS 31.2| Fold coverage                   | 1205x                                     |
| MIGS 30 | Assemblers                      | Spades version 3.5, ALLPaths-LG version r52488 |
| MIGS 32 | Gene calling method             | GeneMarkS+ v3.3                            |
| Locus Tag|                                | BBK14_RS02460                             |
| Genbank ID|                               | MAXA000000000.1                           |
| Genbank Date of Release|                 | October 30, 2016                          |
| GOLD ID |                                | Gp0153653                                 |
| BIOPROJECT|                              | PRJNA224116                               |
| MIGS 13 | Source Material Identifier      | NRRL B-16219                              |
| Project relevance |               | Agricultural                              |
Genes for two different types of truncated hemoglobins, HbN and HbO, were also present.

**Nodulation pathway**

In rhizobia, the common canonical nodABC genes playing a key role in triggering root nodule formation in Legumes. These signals are secreted as a reply to host-plant flavonoids perceived by the compatible rhizobial strains [43]. The Nod factors perceived by host plant through the LysM-RLKs, and the resulting signal transduction cascade triggers a bacterial invasion of root cortical cells and the genesis of functional nodules. Despite the presence of these LysM-RLKs in the actinorhizal plants [11], none of the Frankia genomes from cultivated strains contained any homologous nod genes [33], but they are present in the two Candidatus Frankia genomes [32, 36]. Six nod-like genes were detected in the NRRL B-16219 draft genome (Additional file 1: Table S1) organized into two regions (Fig. 3). The first cluster contained genes encoding the nodA1, nodC, ABC-2 type transport system ATP-binding protein (nodD), ABC-2 transporter efflux protein, DrrB family

**Table 3** Genome statistics

| Attribute                  | Value         | % of Total |
|----------------------------|---------------|------------|
| Genome size (bp)           | 8,032,739     | 100.0      |
| DNA coding (bp)            | 6,603,166     | 82.20      |
| DNA G + C (bp)             | 5,760,840     | 71.72      |
| DNA Contigs289100          |               | 0.0        |
| Total genes                | 6859          | 100.0      |
| Protein coding genes       | 6,211         | 91.01      |
| RNA genes                  | 53            | 0.77       |
| Pseudo genes\(^b\)         | 561           | 8.18       |
| Genes in internal clusters | -             | -          |
| Genes with function prediction | 5046       | 73.60      |
| Genes assigned to COGs     | 3609          | 52.64      |
| Genes with Pfam domains    | 4735          | 69.06      |
| Genes with signal peptides | 176           | 2.57       |
| Genes with transmembrane helices | 296    | 4.32       |
| CRISPR repeats             | 2             | -          |

\(^{a}\)The total is based on either the size of the genome in base pairs or the total genes in the annotated genome

\(^{b}\)Pseudo genes may also be counted as protein coding or RNA genes, so is not additive under total gene count

**Table 4** Number of genes associated with the general COG functional categories

| Code | Value | % age\(^a\) | Description                                           |
|------|-------|-------------|-------------------------------------------------------|
| J    | 178   | 4.27        | Translation, ribosomal structure and biogenesis       |
| A    | 1     | 0.02        | RNA processing and modification                       |
| K    | 408   | 9.79        | Transcription                                         |
| L    | 109   | 2.62        | Replication, recombination and repair                 |
| B    | 1     | 0.02        | Chromatin structure and dynamics                      |
| D    | 32    | 0.77        | Cell cycle control, cell division, chromosome partitioning |
| V    | 135   | 3.24        | Defense mechanisms                                   |
| T    | 249   | 5.98        | Signal transduction mechanisms                       |
| M    | 173   | 4.15        | Cell wall/membrane biogenesis                         |
| N    | 21    | 0.5         | Cell motility                                         |
| U    | 30    | 0.72        | Intracellular trafficking, secretion, and vesicular transport |
| O    | 140   | 3.36        | Posttranslational modification, protein turnover, chaperones |
| C    | 250   | 6           | Energy production and conversion                      |
| G    | 207   | 4.97        | Carbohydrate transport and metabolism                 |
| E    | 297   | 7.13        | Amino acid transport and metabolism                   |
| F    | 94    | 2.26        | Nucleotide transport and metabolism                   |
| H    | 262   | 6.29        | Coenzyme transport and metabolism                     |
| I    | 351   | 8.42        | Lipid transport and metabolism                        |
| P    | 210   | 5.04        | Inorganic ion transport and metabolism                |
| Q    | 256   | 6.14        | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 508   | 12.19       | General function prediction only                      |
| S    | 178   | 4.27        | Function unknown                                      |
| -    | 3247  | 47.36       | Not in COGs                                           |

\(^{a}\)The total is based on the total number of protein-coding genes in the genome
The second cluster contained nodA, nodB and nodH genes. Amino acid sequence similarities between Frankia sp. strain NRRL B-16219 nodA, B, C, and H predicted proteins ranged from 86 to 93% and 57–67% with the uncultured Frankia (Dg1 and Dg2) and (α- and β-) rhizobia, respectively (Additional file 2: Table S2). Further phylogenetic analysis (Fig. 4) showed that the Frankia Nod proteins were positioned at the root of both the α- and β-rhizobial NodABC proteins as previously reported [4, 8]. They were most closely related to plant nodulating Betaproteobacteria of Burkholderia and Paraburkholderia genera. The GC content of Frankia nod genes ranged from 57.9% for nodA to 66.37% for nodB which is quite similar to that of some rhizobial species including Methylobacterium and Burkholderia. For both Frankia and rhizobia, GC% of the nod genes was lower than that of total genome sequences.

Field collected microsymbionts of Ceanothus americanus contain nod genes

Root nodules from Alnus glutinosa, Casuarina glauca and Elaeagnus angustifolia growing in Tunisia and Ceanothus americanus and Elaeagnus umbellata growing in Durham New Hampshire, USA, were collected. The nodA-nodB region from C. americanus nodules was PCR-amplified and sequenced. Following the alignments of the nodA and nodB gene sequences of Frankia NRRL B-16219, the primer set (forward primer nodAF 5′-AGCGCGACCAGGTCTAGGATA ATCG-3′ and reverse nodBF 5′-CGATCCCACCCGG ATGGAGCTGC-3′) was designed in this study. The sequenced PCR-products were translated into amino acid sequences to permit the detection of the 23 aa sequence at the beginning of the 193 aa of the NodA, the intergenic region (160 nucleotides) and 41 aa at the end of the 230aa of the NodB. Both sequences showed 100% sequence similarities to their respective homologous region in NodA (23/193aa) and NodB (41/230aa) protein sequences for Candidatus Frankia Dg1. Regardless of their affiliation to cluster 2 or to cluster 3 (Fig. 5), all of the analyzed C. americanus microsymbionts contained the nodAB genes. In contrast, A. glutinosa, C. glauca, E. umbellata and E. angustifolia microsymbionts failed to amplify the expected PCR product. This result is in congruence with previous reports claiming that no homologous nod genes are retrievable in sequenced genomes from strains isolated from these actinorhizal plant species [33].

Conclusions

We report here the genome sequence of a Frankia strain directly isolated from soil rhizosphere. The generated draft genome was assembled into 289 contigs corresponding to 8,032,739 bp, which falls within the size range of Frankia cluster 3 [33]. Bacterial factors triggering actinorhizal
Fig. 4 Maximum likelihood phylogeny based on amino acids of nodA (a), nodB (b), nodC (c) and nodH (d). GC-content is provided for nod genes and for genomes (in parenthesis). Bootstrap and probability values larger than 50% are only shown.

Fig. 5 Neighbor-Joining phylogenetic tree based on glnA gene sequences. Bootstrap and probability values larger than 50% are only shown. Marked in **bold** are Frankia strains or microsymbionts with nod genes as present in their genomes or detected by PCR-sequencing analysis.
symbiosis remain enigmatic since many sequenced *Frankia* genomes have revealed the absence of universal nod-factors. It was hypothesized that most *Frankia* strains use a novel nod-independent pathway for the infection process of actinorhizal plants. In contrast, two *Candidatus* *Frankia* Dg1 and Dg2 genomes contain canonical nod genes [32, 36]. Here we provide the first proof for the presence of nod genes in the genome of a cultivated *Frankia* strain. In addition, a PCR-sequencing approach suggested that nod genes are only widespread in *C. americanus* microsymbionts. This situation is similar to legume symbionts where two nodulation pathways are described: the well-studied nod-dependent and an alternative nod-independent pathway. The majority of rhizobia use the nod-dependent pathway, while some photosynthetic [34] and non-photosynthetic [35] bradyrhizobia use the alternative nod-independent pathway. Moreover, some rhizobia use both pathways and the use of the nod-independent pathway seems to be highly dependent on host species rather than the presence or absence of nod genes in a given bradyrhizobial genome [44]. For *Frankia*, almost all host plants are infected through the nod-independent pathway, while the nod-dependent process may only be present in unstudied actinorhizal species such as members of the genus *Ceanothus*.

### Additional files

**Additional file 1: Table S1.** Localizations and DNA coordinates for nod genes in *Frankia* sp. NRRL B-16219, Dg1 and Dg2 genomes. (DOCX 12 kb)

**Additional file 2: Table S2.** Percent similarities based on amino acid sequence for NodA, B, C and H between *Frankia* sp. NRRL B-16219, *Candidatus* *Frankia* Dg1 and Dg2, and other rhizobial strains. (XLSX 308 kb)

### Abbreviations

DPM: Defined Propionate Medium; glnA: Glutamine synthetase; LysM-RLKS: LysM-receptor-like kinases; nifDPM: Defined Propionate Medium; nod: Beta-1,4-N-acetylglucosamine oligosaccharide deacylase; nodA: Beta-1,4-N-acetylglucosamine oligosaccharide N-acetyltransferase; nodAc: Beta-1,4-N-acetylglucosaminyntransferase; nodB: Beta-1,4-N-acetylglucosamine oligosaccharide deacetylase; nodC: Beta-1,4-N-acetylglucosaminyntransferase; nodD: Beta-1,4-N-acetylglucosamine oligosaccharide 6-O-sulfotransferase

### Acknowledgements

This work was supported by the Laboratoire Microorganismes & Biomolécules Actives, Université Tunis El Manar, Tunisia (grant LR03E305) (MG), and the USDA National Institute of Food and Agriculture Hatch 022821 (LST). Sequencing was performed on an Illumina HiSeq2500 purchased with an NSF MR Grant: DBI-1229361 to WK Thomas. The authors would like to thank Dr. David P. Labeda curator at Agricultural Research Service Culture Collection for providing *Frankia* strain and Dr. Manfred Rhode (HZI-Helmholtz Centre for Infection Research, Germany) for performing scanning electron micrograph for the studied strain.

### Authors’ contributions

MG conceived and designed the work; LST and ES performed the complete genome production, including genome assembly and GenBank submission. AK prepared the DNA isolation and the cultivation of *Frankia* strain NRRL B-16219. TF and MG sampled *C. americanus* and *E. umbellata* nodules. AK, IN and FGG sampled root nodules from *A. glutinosa*, *C. glauca* and *E. angustifolia*, performed DNA extraction from all sampled nodules, amplification and sequencing of glnA and nodA-B genes. MG drafted the manuscript. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

### Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

### Author details

1Laboratoire Microorganismes et Biomolécules Actives, Université Tunis El Manar (FST) & Université de Carthage (INSAT), 2092 Tunis, Tunisia.

2Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, 289 Rudman Hall, 46 college Road, Durham, NH 03824-2617, USA.

Received: 22 February 2017 Accepted: 22 August 2017

Published online: 04 September 2017

### References

1. Gtari M, Tisa LS, Normand P. Diversity of *Frankia* strains, actinobacterial symbionts of actinorhizal plants. In: Symbiotic Endophytes. Springer Berlin Heidelberg, 2013. p. 123–48.

2. Dommergues YR. Nitrogen fixation by trees in relation to soil nitrogen economy. Fertil Res. 1995;4:215–30.

3. Ktari A, Gueddou A, Nouioui I, Motelotto G, Sarkar I, Ghodhbane-Gtari F, Sen A, Armengaud J, Gtari M. Host Plant Compatibility Shapes the Proteome of *Frankia* coriariae. Front Microbiol. 2017;8:720.

4. Baker DD. Relationships among pure cultured strains of *Frankia* based on host specificity. Physiol Plant. 1987;70:245–8.

5. Nouioui I, Ghodhbane-Gtari F, del Carmen M-CM, Giker M, Meier-Kolthoff JP, Schumann P, Rohde M, Goodfellow M, Fernandez MP, Normand P, Tisa LS, Kleink H-P, Gtari M. Proposal of a type strain for *Frankia* alni (Woronin 1886) Von Tutebox 1895, emended description of *Frankia* alni, and recognition of *Frankia* casuarinae sp. nov. and *Frankia* elaegni sp. nov. Int J Syst Evol Microbiol. 2016;66:201–10.

6. Nouioui I, Ghodhbane-Gtari F, Rohde M, Kleink H-P, Gtari M. *Frankia* coriariae sp. nov., an infective and effective microsymbiont isolated from *Coriaria japonica*. Int J Syst Evol Microbiol. 2017;67(5):1266–70.

7. Nouioui I, Del Carmen M-CM, Ghodhbane-Gtari F, Rohde M, Tisa LS, Kleink H-P, Gtari M. *Frankia* discariae sp. nov: an infective and effective microsymbiont isolated from the root nodule of Discaria tnivens. Arch Microbiol. 2017;199(5):641–7.

8. Nouioui I, Ghodhbane-Gtari F, Del Carmen M-CM, Rohde M, Tisa LS, Gtari M, Kleink H-P. *Frankia* inefficax sp. nov, an actinobacterial endophyte inducing ineffective, non nitrogen-fixing, root nodules on its actinorhizal host plants. Antonie Van Leeuwenhoek. 2017;110(3):313–20.

9. Nouioui I, Gueddou A, Ghodhbane-Gtari F, Rohde M, Gtari M, Kleink H-P. *Frankia* asymbiotica sp. nov, a non infective actinobacterium isolated from Morella californica root nodule. Int J Syst Evol Microbiol. 2017. doi:10.1099/ijsem.0.02153.19.

10. Oldroyd GE. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol. 2013;11(4):252–63.

11. Svistoonoff S, Benabdoun FM, Nambari-Veetil M, Imanishi L, Vaisayre V, Cesari S, Diagne N, Hocher V, de Billy F, Bonneau J, et al. The independent acquisition of plant root nitrogen-fixing symbioses in Fabids recruited the same genetic pathway for node organogenesis. PLoS One. 2013;8(5):e64515.

12. D’Angelo T, Osborne R, Abebe-Akele F, Simpson S, Morris K, Thomas WK, Tisa LS. Permanent Draft Genome Sequence of *Frankia* sp. Strain BR, a Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of Casuarina equisetifolia. Genome Announc. 2016;4(2).

13. D’Angelo T, Osborne R, Abebe-Akele F, Simpson S, Morris K, Thomas WK, Tisa LS. Permanent Draft Genome Sequence for *Frankia* sp. Strain ElSc, a Single-Spore Isolate of a Nitrogen-Fixing Actinobacterium, Isolated from the Root Nodules of *Elaeagnus angustifolia*. Genome Announc. 2016;4(4).

14. Ghodhbane-Gtari F, Beauchemin N, Bruce D, Chain P, Chen A, Walton Davenport K, Deshpande S, Dettet C, Fumiholm T, Goodwin L, et al. Draft genome sequence of *Frankia* sp. strain CN3, an atypical, noninfective (Nod-)
ineffective (Fix-) isolate from Costiera nepalensis. Genome Announc. 2013; 1(2):e0008513.

15. Ghodbhane-Gtri F, Hurst SGt, Oshone R, Morris K, Abebe-Akele F, Thomas WK, Ktari A, Salem K, Gtari M, Tisa LS. Draft Genome Sequence of Frankia sp. Strain BMGKS.23, a Salt-Tolerant Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of Casuarina glauca Grown in Tunisia. Genome Announc. 2014;2(3).

16. Gtari M, Ghodbhane-Gtri F, Nouioui I, Ktari A, Hezbi K, Mimouni W, Stbssi I, Ayari A, Yamanaka T, Normand P, et al. Cultivating the uncultured: growing the recalcitrant cluster-2 Frankia strains. Sci Rep. 2015;5:13112. doi:10.1186/s40064-015-1261-3.

17. Hurst SGt, Oshone R, Ghodbhane-Gtri F, Morris K, Abebe-Akele F, Thomas WK, Ktari A, Salem K, Mansour S, Gtari M, et al. 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. 2014;2(3).

18. Mansour SR, Oshone R, Hurst SGt, Morris K, Thomas WK, Tisa LS. Draft Genome Sequence of Frankia sp. Strain CC16, a Salt-Tolerant Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of Casuarina cunninghamiana. Genome Announc. 2014;2(1).

19. Nourioui I, Beauchemin N, Cantor MN, Chen A, Detter JC, Furnholm T, Normand P, Lapiere P, Tisa LS, Fernandez MP, et al. Draft Genome Sequence of Frankia sp. Strain SGt, a Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of Casuarina equisetifolia Grown in Senegal. Genome Announc. 2016;4(6).

20. Normand P, Lapiere P, Tisa LS, Gogarten JP, Allosiao N, Bagnall R, Bassi CA, Berry AM, Bickel DM, Choisne N, et al. Genome characteristics of facultatively symbiotic Frankia sp. strains reflect host range and host plant biogeography. Genome Res. 2007;17(1):7–15.

21. Nourioui I, Beauchemin N, Cantor MN, Chen A, Detter J, Furnholm T, Ghodbhane-Gtri F, Goodwin L, Gtari M, Han C, et al. Draft Genome Sequence of Frankia sp. Strain G6, a Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of Casuarina equisetifolia grown in Tunisia. GenBank. 2013;14.

22. Nourioui I, Gtari M, Coker M, Ghodbhane-Gtri F, Tisa LS, Fernandez MP, Normand P, Hunteemann M, Clum A, Pillay M, et al. Draft Genome Sequence of Frankia Strain G2, a Nitrogen-Fixing Actinobacterium Isolated from Casuarina equisetifolia and Able To Nodulate Actinorhizal Plants of the Order Rhamnales. Genome Announc. 2016;4(3).

23. Oshone R, Hurst SGt, Abebe-Akele F, Simpson S, Morris K, Thomas WK, Tisa LS. Permanent Draft Genome Sequences for Two Variants of Frankia sp. Strain Cpl1, the First Frankia Strain Isolated from Root Nodules of Comptonia peregrina. Genome Announc. 2016;4(1).

24. Oshone R, Nogom M, Oshone R, Abebe-Akele F, Simpson S, Morris K, Sy MO, Champion A, Thomas WK, Tisa LS. Permanent Draft Genome Sequence of Frankia Strain Alk01, a Salt-Tolerant Nitrogen-Fixing Actinobacterium Isolated from the Root Nodules of Allocasuarina. Genome Announc. 2016;4(3).

25. Pujic P, Bolotin A, Fournier P, Sorokin A, Lapidus A, Richaud K, AvÃ£vre JC, Jaubert M, Simon D, Cartieaux F, Prin Y, et al. Legumes symbioses: absence of Nod genes in photosynthetic bradyrhizobia. Science. 2007;316(5829):1307–12.

26. Mliche M, Moulin L, Chaintreuil C, Contreras-Jimenez JL, Munive-Hernandez JA, Del Carmen V-HM, Crozier F, Bena G. Diversity analyses of Aeschynomene symbionts in Tropical Africa and Central America reveal that nod-independent stem nodulation is not restricted to photosynthetic bradyrhizobia. Environ Microbiol. 2010;12(8):2152–62.

27. Persson T, Battenberg K, Demina IV, Vigil-Stemman T, Varden Heuvel B, Pucci P, Facciotti MT, Wilbanks EG, O’Brien A, Fournier P, et al. Candidatus Frankia datiscae Dg1, the Actinobacterial Miosymbiont of Datisca glomerata, Expresses the Canonical nod Genes nodABC in Symbiosis with Its Host Plant. PLoS One. 2015;10(5):e0127630.

28. Baker D, O’Keeffe D. A modified sucrose fractionation procedure for the isolation of frankiae from actinorhizal root nodules and soil samples. Plant Soil. 1984;78:23–8.

29. Bennett S. Solexa Ltd. Pharmacogenomics. 2004;5(4):433–8.

30. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina data. Bioinformatics. 2014;30(15):214–20.

31. Normand P, Orso S, Cournoyer B, Jeannin P, Chapelon C, Dawson J, Sejourne S, Ayari A, Tisa LS. Draft Genome Sequence of Frankia sp. Strain BMG5.12, a Nitrogen-Fixing Actinobacterium – the recalcitrant cluster-2 Frankia strains. Sci Rep. 2015;5:13112. doi:10.1186/s40064-015-1261-3.

32. Nguyen TV, Wibberg D, Battenberg K, Blom J, Vanden Heuvel B, Berry AM, Evtushenko L, Misra AK. Molecular phylogeny of the genus Frankia and genes in photosynthetic bradyrhizobia. Nature. 1990;346(6288):781–4.

33. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Vavrova L, Young SB, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013;20(10):714–37.

34. Nguerre S, Macallum I, Przybylski D, Ribiero FJ, Burton JN, Tisler BJ, Sharpe T, Hall G, Shea TP, Sykes S, et al. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. PNAS. 2011;108(4):1513–8.

35. Markowitz VM, Mavromatis K, Ivanova NN, Chun K, Yip K, Nemergut DR, Hugenholtz P, 2008;5(1):316–26.

36. Evtushenko L, Misra AK. Molecular phylogeny of the genus Frankia and genomes from massively parallel sequence data. PNAS. 2011;108(4):1513–8.

37. Chaintreuil C, Artiggi F, Giraud E, Miche M, Lnicou NN, Chun K, Nemergut DR, Hugenholtz P, 2008;5(1):316–26.

38. Bennett S. Solexa Ltd. Pharmacogenomics. 2004;5(4):433–8.

39. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina data. Bioinformatics. 2014;30(15):214–20.

40. Normand P, Orso S, Cournoyer B, Jeannin P, Chapelon C, Dawson J, Sejourne S, Ayari A, Tisa LS. Draft Genome Sequence of Frankia sp. Strain BMG5.12, a Nitrogen-Fixing Actinobacterium – the recalcitrant cluster-2 Frankia strains. Sci Rep. 2015;5:13112. doi:10.1186/s40064-015-1261-3.

41. Gnerre S, Maccallum I, Przybylski D, Ribiero FJ, Burton JN, Tisler BJ, Sharpe T, Hall G, Shea TP, Sykes S, et al. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. PNAS. 2011;108(4):1513–8.

42. Markowitz VM, Mavromatis K, Ivanova NN, Chun K, Yip K, Nemergut DR, Hugenholtz P, 2008;5(1):316–26.

43. Evtushenko L, Misra AK. Molecular phylogeny of the genus Frankia and genomes from massively parallel sequence data. PNAS. 2011;108(4):1513–8.

44. Chaintreuil C, Artiggi F, Giraud E, Miche M, Lnicou NN, Chun K, Nemergut DR, Hugenholtz P, 2008;5(1):316–26.

45. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Vavrova L, Young SB, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013;20(10):714–37.

46. Chaintreuil C, Artiggi F, Giraud E, Miche M, Lnicou NN, Chun K, Nemergut DR, Hugenholtz P, 2008;5(1):316–26.
related genera and emendation of the family Frankiaceae. Int J Syst Bacteriol. 1996;46(1):1–9.
52. Brunchorst J. Über einige Wurzelanschwellungen, besonders diejenigen von Alnus und den Elaeagnaceen. Unters Bot Inst Tübingen. 1886;2:151–77.
53. Skerman VBD, McGowan V, Sneath PHA. Approved lists of bacterial names. Int J Syst Evol Microbiol. 1980;30:225–40.