Draft Genome Sequences for Bacteria Associated with Root Nodules of *Alnus incana* in New England

Kelsey Mercurio,a Joseph Sevigny,a,b Céline Pesce,a,* W. Kelley Thomas,a,b Louis S. Tisa,a

aDepartment of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, New Hampshire, USA
bHubbard Center for Genome Studies, University of New Hampshire, Durham, New Hampshire, USA

**ABSTRACT** Nine bacterial strains isolated from the root nodules of *Alnus incana* were sequenced to determine their potential roles in plant health. The selected bacterial isolates belonged to the genera *Bacillus*, *Herbaspirillum*, *Pantoea*, *Paenibacillus*, and *Rothia*. Here, we report the draft genome sequences.

Besides hosting endosymbionts, root nodules have other occupants or plant endophytes that appear to assist plant growth and health (1–5). Using a culture-independent approach, we elucidated the nodule microbiome of *Casuarina glauca* (1) and have extended this study to *Alnus incana* found in New England. We previously reported the genome sequences of 10 bacterial isolates obtained in 2018 (6). Here, we continue to isolate more endophytes of alder nodules.

In September and November 2019, root nodule samples were collected from *A. incana* found by Adams Point at Jackson’s laboratory in Durham, New Hampshire. The root nodules were surface sterilized with hydrogen peroxide and rinsed several times with sterile distilled water. The nodule was cut into a fine powder with a sterilized razor, and dilutions were plated onto Czapek (7) and R2A (8) media. About 77 isolates were initially obtained, purified, and propagated on either Czapek or R2A media. These isolates were incubated overnight in their respective isolation medium (Table 1), and genomic DNA (gDNA) was extracted by cetyltrimethylammonium bromide (CTAB) DNA extraction protocol (9). RNA was removed by RNase treatment. The quality and quantity of the gDNA were verified by a Thermo Scientific NanoDrop. Nine isolates were chosen for whole-genome sequencing analysis to provide insight into their plant-microbe interactions, including potential plant growth-promoting activity.

Whole-genome sequencing was performed at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH) using Illumina technology techniques (10). A paired-end library was constructed using a Nextera DNA library preparation kit (Illumina, San Diego, CA) and sequenced on an Illumina NovoSeq instrument to produce 250-bp paired-end reads. The total numbers of reads for all 9 strains are listed in Table 1. The Illumina sequence data were trimmed by Trimmomatic version 0.36 (11). TruSeq adapters were trimmed with an allowance of two mismatches. Leading and trailing bases below the quality of 3 were trimmed. The read was then scanned with a sliding window of 4 bp and trimmed if the average quality dropped below 30. Finally, reads were dropped if the length was less than 36 bp. Trimmed sequencing reads were assembled using SPAdes version 3.15.2 (12) with the default settings. Default parameters were used for all software unless otherwise specified. The assembled genomes were annotated via the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13). The assembly metrics and annotation features are given in Table 1.

The identities of the strains were determined by a whole genome-based taxonomic analysis via the Type (Strain) Genome Server (TYGS) platform (14) (https://tygs.dsmz.de), including digital DNA-DNA hybridization (dDDH) values (15). The type-based species clustering
| Bacterial species         | Isolate   | GenBank accession no. | SRA accession no. | No. of reads | No. of contigs | Avg. coverage (×) | Genome assembly size (bp) | N50 contig size (bp) | No. of CDSs | G + C content (%) | No. of rRNAs | No. of tRNAs |
|--------------------------|-----------|-----------------------|-------------------|--------------|---------------|------------------|--------------------------|---------------------|-------------|------------------|--------------|--------------|
| *Bacillus velezensis*    | alder76   | JAIUDC000000000000    | SRX12008554       | 3,356,518    | 34            | 209.7           | 4,341,411                | 341,132             | 4,334       | 45.7             | 15           | 80           |
| *Bacillus velezensis*    | alder77   | JAIUDB000000000000    | SRX12008555       | 3,171,512    | 32            | 198.2           | 4,340,492                | 341,132             | 4,332       | 45.7             | 15           | 80           |
| *Bacillus velezensis*    | alder71   | JAIUDD000000000000    | SRX12008553       | 3,179,306    | 34            | 198.7           | 4,340,599                | 341,132             | 4,337       | 45.7             | 15           | 80           |
| *Herbaspirillum sp.*     | alder98   | JAIUCY000000000000    | SRX12008550       | 4,825,154    | 26            | 301.5           | 5,342,531                | 530,052             | 4,764       | 62.3             | 3            | 53           |
| *Paenibacillus sp.*      | alder61   | JAIUDG000000000000    | SRX12008549       | 4,198,488    | 63            | 262.4           | 6,112,889                | 244,801             | 5,371       | 53.0             | 25           | 79           |
| *Pantoea sp.*            | alder81   | JAIUDAO000000000000   | SRX12008556       | 6,011,958    | 35            | 375.7           | 5,643,466                | 534,706             | 5,206       | 53.4             | 12           | 71           |
| *Pantoea sp.*            | alder70   | JAIUDE000000000000    | SRX12008552       | 4,283,982    | 81            | 267.7           | 5,648,269                | 140,384             | 5,244       | 53.4             | 14           | 70           |
| *Pantoea sp.*            | alder69   | JAIUDF000000000000    | SRX12008551       | 4,000,000    | 42            | 250             | 5,648,814                | 534,706             | 5,219       | 53.4             | 12           | 71           |
| *Rothia kristinae*       | alder54   | JAIUDH000000000000    | SRX12008548       | 4,144,358    | 18            | 259             | 2,344,478                | 266,588             | 2,050       | 71.9             | 4            | 47           |

*a* Bacteria were isolated on Czapek (alder54, alder61, alder69, and alder 70) and R2A (alder71, alder76, alder77, alder81, and alder98) media.

*b* CDSs, coding DNA sequences.
using a 70% dDDH radius around each of the type strains was used as previously described (16), while subspecies clustering was done using a 79% dDDH threshold as previously introduced (17). Among the nine isolates, the three *Bacillus velezensis* strains are identical to each other, and the type strain and *Paenibacillus* sp. alder61 isolates were characterized as belonging to *Paenibacillus faecalis*. Although three *Pantoea* isolates are identical to each other, they represent a potential new species, as does the *Herbaspirillum* isolate.

**Data availability.** The draft genome sequences of these bacterial strains have been deposited in GenBank under the accession numbers listed in Table 1. Both the assembly and raw reads are available at DDBJ/ENA/GenBank under BioProject accession number PRJNA748777.

**ACKNOWLEDGMENTS**

We thank the following 2021 UNH CEPS TechLeaders workshop participants for their efforts on this project: A. Bertrand, D. Mahaveer, A. Watkins, A. Karr, M. Ajit, N. Kutschke, N. Chung, S. Lasut, T. Odugu, K. Schaible, and J. Finocchiaro.

Partial funding was provided by the New Hampshire Agricultural Experiment Station. This is scientific contribution 2951. This work was supported by USDA National Institute of Food and Agriculture Hatch 1019869 (L.S.T.), National Institutes of Health P20GM103506 IDeA program (W.K.T.), and the College of Life Sciences and Agriculture at the University of New Hampshire.

**REFERENCES**

1. Ghodhbane-Gtari F, D’Angelo T, Gueddou A, Ghazouani S, Gtari M, Tisa LS. 2021. Alone yet not alone: *Frankia* lives under the same roof with other bacteria in actinorhizal nodules. Front Microbiol 12:749760. https://doi.org/10.3389/fmicb.2021.749760.

2. Ghodhbane-Gtari F, Tisa LS. 2014. Ecology and physiology of Non-*Frankia* actinobacteria from actinorhizal plants, p 27–42. In Katsey EI (ed), Plasticity in plant-growth-promoting and phytopathogenic bacteria. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-9203-0_2.

3. Martínez-Hidalgo P, Hirsch AM. 2017. The nodule microbiome: N2-fixing rhizobia do not live alone. PloS One 12:e0184657. https://doi.org/10.1371/journal.pone.0184657.

4. Aserse AA, Rasanen LA, Aseffa F, Hailemariam A, Lindstrom K. 2013. Diversity of actinobacteria from actinorhizal plants, p 27–42. In Katsey EI (ed), Plasticity in plant-growth-promoting and phytopathogenic bacteria. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-9203-0_2.

5. Leite J, Fischer D, Rouws LF, Fernandes PI, Hofmann A, Kublik S, Schloter M, Xavier GR, Radi V. 2016. Cowpea nodules harbor non-rhizobial bacterial communities that are shaped by soil type rather than plant genotype. Front Plant Sci 7:2064. https://doi.org/10.3389/fpls.2016.02064.

6. Davis I, Sevigny J, Kleinert V, Mercurio K, Pesce C, Swanson E, Thomas WK, Tisa LS. 2020. Draft genome sequences of 10 bacterial strains isolated from nodules of woody, shrub, and food legumes in Ethiopia. Appl Microbiol Biotechnol 104:5753–5767. https://doi.org/10.1007/s00253-020-09651-6.

7. Hunter-Cevera JC, Fonda ME, Belt A. 1986. Isolation of cultures, p 3–23. In Demain AL, Solomon NA (ed), Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, DC.

8. Reasoner DJ, Geldreich EE. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7. https://doi.org/10.1128/aem.49.1.1-7.1985.

9. Murray MG, Thompson WF. 1980. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325. https://doi.org/10.1093/nar/8.19.4321.

10. Bennett S. 2004. Solexa Ltd. Pharmacogenomics 5:433–438. https://doi.org/10.1016/S1879-5477(05)70032-8.

11. 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.

12. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Pivovarova E, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Cilibras GenBank, Tsyganov MA, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/jcb.2013.0084.

13. Tatusova T, DiCuccio M, Badrtdin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Prutt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

14. Meier-Kolthof JP, Goker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3.

15. Meier-Kolthof JP, Auc AH, Klenk HP, Goker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471-2105-14-60.

16. Liu Y, Lai Q, Goker M, Meier-Kolthof JP, Wang M, Sun Y, Wang L, Shao Z. 2015. Genomic insights into the taxonomic status of the Bacillus cereus group. Sci Rep 5:14082. https://doi.org/10.1038/srep14082.

17. Meier-Kolthof JP, Hahnke RL, Petersen J, Scheunert C, Michael V, Fleibig A, Rohde C, Rohde M, Fartmann B, Goodwin LA, Chertkov O, Reddy TBK, Pati A, Ivanova NN, Markowitz V, Kyrpides NC, Woyke T, Goker M, Klenk HP. 2014. Complete genome sequence of DSM 30083(T), the type strain (US(41(T)) of Escherichia coli, and a proposal for delineating subspecies in microbial taxonomy. Stand Genomic Sci 9:2. https://doi.org/10.1186/1944-3277-9-2.