Microbiology Resource Announcements

| Environmental Microbiology | Announcement

Draft genome sequence of *Collimonas* sp. strain H4R21, an effective mineral-weathering bacterial strain isolated from the beech rhizosphere

Morin E.,[1] Uroz S.,[1,2] Kumar R.,[3] Rey M.W.,[3] Pham J.,[3] Akum F.,[4] Leveau J. H. J.[4]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT We present the draft genome sequence of *Collimonas* sp. strain H4R21, isolated from the rhizosphere of *Fagus sylvatica* in the forest experimental site of Montiers (France). This genome features coding capacity for plant growth promotion, such as the ability to solubilize minerals, to produce siderophores and antifungal secondary metabolites.

KEYWORDS *Collimonas*, forest, nutrient-poor soil, mineral weathering, chitin, fungi

In nutrient-poor soils, tree rhizosphere is typically enriched for mineral weathering bacteria (1–8). In such low nutrient conditions, beech trees are known to increase root exudation, which derivates can be used as carbon substrate by bacteria (9, 10). Collimonads are particularly effective at weathering (11) and share the ability to hydrolyze chitin, to produce antifungal molecules and to promote plant growth (11–15). To date, six species have been described (*C. anthrihumi, C. arenae, C. fungivorans, C. pratensis, C. humicola,* and *C. silvisoli*) (16–19). Collimonads belong to the Oxalobacteraceae family and are usually found in acidic and nutrient-poor soils (3, 5, 11). Strain H4R21 was retained for detailed analyses because of its effectiveness at weathering. It was isolated from beech rhizosphere on the Montiers site (France) (1). In October 2014, 5 g of fresh roots and adhering soil were suspended in 25 mL sterile distilled water and serial dilutions were done on 1/10 TSA medium to purify bacteria before cryo-preservation in 40% glycerol (1).

To extract DNA for sequencing, a culture on 1/10 TSA was done from the glycerol stock, and a single colony was used to inoculate 1/10 TSB medium. The culture was grown 2 days to reach late exponential phase. DNA was obtained after lysozyme (1 mg/mL) and proteinase K (1 mg/mL) treatments as described by Pospiech and Neumann (20). The library was prepared using the Nextera XT DNA library preparation kit (Illumina), following the manufacturer's instructions. The library was sequenced as 150 × 2 bp paired reads that were generated on an Illumina MiSeq instrument (Illumina Inc.).

For all of the following programs, default parameters were used except where otherwise specified.

The sequencing resulted in 3,652,095 pairs of raw reads, which were trimmed with Trimmomatic (v0.36; (21) and assembled using SPAdes v3.9.0 (22). Gene prediction was done using prodigal v2.6.3 (23), classic RAST (24), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (25). RAST was used as it permits an expert and continual annotation. tRNA-scan-SE v2.0.12 (26) and Barrnap v0.9 (https://github.com/tseemann/barrnap) were used for tRNA and rRNA prediction, respectively. Complete statistics of the draft genome can be found in Table 1. A 99.9% genome completeness was estimated with BUSCO (27) compared to the Burkholderiales lineage data set.

Editor David A. Baltrus, The University of Arizona, Tucson, Arizona, USA

Address correspondence to Uroz S., stephane.uroz@inrae.fr.

The authors declare no conflict of interest

Received 2 May 2024
Accepted 20 June 2024
Published 22 July 2024

TABLE 1 Genome information and statistics

Genome project information	
GenBank accession	JBANDC000000000
Bioproject no.	PRJNA1081282
SRA accession number	SRR28162545
Genome assembly statistics	
Total length (bp)	5,613,331
No. of contigs (≥500 bp)	46
N50 (bp)	281,801
L50 (contig)	8
Largest contig	531,299
GC content (%)	59.52
Genome coverage	189.97x
Genome features	
Protein-coding genes	5,144
tRNAs	47
Complete rRNAs (5S,16S,23S)	3

Digital DNA-DNA hybridization (dDDH) analysis (28) revealed that strain H4R21 scored values ranging with the type strains from 37.4% with *C. antrihumi* (DSM104040) and *C. arenae* (Ter10) to 44% with *C. pratensis* (Ter291) and *C. humicola* (RLT1W55), 45.8% with *C. silvisoli* (RXD178), and 63.1% with *C. fungivorans* (Ter331).

Homologs of proteins with a central role in the mineral-weathering ability of collimonads, including a Glucose-Methanol-Choline oxidoreductase (29) and a non-ribosomal polypeptide synthetase (NRPS) encoding for the synthesis of the siderophore malleobactin were detected (30, 31). Antismash (32) analyses revealed the presence of most of the genes encoding the production of collimomycin, an antifungal metabolite identified in *C. fungivorans* strain Ter331(13). These findings suggest that H4R21 is well equipped to survive and thrive in the rhizosphere of plants growing in nutrient-poor soils, to inhibit fungi, and to mobilize nutrients, making it a promising agent for the protection and/or promotion of plants (7, 14).

ACKNOWLEDGMENTS

This work was supported by research grants from Novozyme to J.L. and by the French National program Ecosphère Continentale et Côtière (EC2CO) and the Labex ARBRE project to S.U. The UMR 1136 and UR 1138 programs are supported by the ANR through the Laboratory of Excellence Arbre (ANR-11-LABX-0002–01). We thank M.-P. Turpault from INRAE, the National Office of Forests (ONF), and ANDRA for the access to the Montiers experimental site ("toposequence N°2").

AUTHOR AFFILIATIONS

[1]Université de Lorraine, INRAE, UMR1136 « Interactions Arbres-Microorganismes », Champenoux, France
[2]INRAE, UR1138 « Biogéochimie des Ecosystèmes Forestiers », Champenoux, France
[3]Novozymes Inc., Davis, California, USA
[4]Department of Plant Pathology, University of California, Davis, California, USA

AUTHOR ORCIDs

Morin E. http://orcid.org/0000-0002-7268-972X
Uroz S. http://orcid.org/0000-0001-9412-7210
Leveau J. H. J. http://orcid.org/0000-0002-8376-4553

DATA AVAILABILITY

The whole-genome and raw sequences are available under the accession no. JBANDC000000000 and SRR28162545 for the raw data and the genome assembly, respectively.

REFERENCES

1. Nicolitch O, Colin Y, Turpault MP, Uroz S. 2016. Soil type determines the distribution of nutrient mobilizing bacterial communities in the rhizosphere of beech trees. Soil Biology and Biochemistry 103:429–445. https://doi.org/10.1016/j.soilbio.2016.09.018
2. Colin Y, Nicolitch O, Van Nostrand JD, Zhou JZ, Turpault M-P, Uroz S. 2017. Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence. Sci Rep 7:9604. https://doi.org/10.1038/s41598-017-07639-1
3. Calvaruso C, Turpault MP, Leclerc E, Ranger J, Garbaye J, Uroz S, Frey-Klett P. 2010. Influence of forest trees on the distribution of mineral weathering-associated bacterial communities of the *Scleroderma citrinum* mycorrhizosphere. Appl Environ Microbiol 76:4780–4787. https://doi.org/10.1128/AEM.03040-09
4. Nicolitch O, Colin Y, Turpault MP, Fauchery L, Uroz S. 2017. Tree roots select specific bacterial communities in the subsurface critical zone. Soil Biology and Biochemistry 115:109–123. https://doi.org/10.1016/j.soilbio.2017.07.003
5. Uroz S, Oger P, Tisserand E, Cébron A, Turpault M-P, Buée M, De Boer W, Leveau JHJ, Frey-Klett P. 2016. Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. Sci Rep 6:27756. https://doi.org/10.1038/srep27756
6. Uroz S, Tech JJ, Sawaya NA, Frey-Klett P, Leveau JHJ. 2014. Structure and function of bacterial communities in ageing soils: insights from the Mendocino ecological staircase. Soil Biology and Biochemistry 69:265–274. https://doi.org/10.1016/j.soilbio.2013.11.002
7. Nicolitch O, Feucherolles M, Churin JL, Fauchery L, Turpault MP, Uroz S. 2019. A microcosm approach highlights the response of soil mineral weathering bacterial communities to an increase of K and Mg availability. Sci Rep 9:14403. https://doi.org/10.1038/s41598-019-50730-y
8. Uroz S, Picard L, Turpault MP. 2022. Recent progress in understanding the ecology and molecular genetics of soil mineral weathering bacteria. Trends Microbiol 30:882–897. https://doi.org/10.1016/j.tim.2022.01.019
9. Meier IC, Tückmantel T, Heitkötter J, Müller K, Preusser S, Wrobel TJ, Kandeler E, Marschner B, Leuschner C. 2020. Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. New Phytol 226:583–594. https://doi.org/10.1111/nph.16389
10. Tückmantel T, Leuschner C, Preusser S, Kandeler E, Angst G, Mueller CW, Meier IC. 2017. Root exudation patterns in a beech forest: dependence on soil depth, root morphology, and environment. Soil Biology and Biochemistry 107:188–197. https://doi.org/10.1016/j.soilbio.2017.01.006
11. Leveau JHJ, Uroz S, de Boer W. 2010. The bacterial genus *Collimonas*: mycophagy, weathering and other adaptive solutions to life in oligotrophic soil environments. Environ Microbiol 12:281–292. https://doi.org/10.1111/j.1462-2920.2009.02010.x
12. Kamilova F, Leveau JHJ, Lugtenberg B. 2007. *Collimonas fungivorans*, an unpredicted *in vitro* but efficient *in vivo* biocontrol agent for the suppression of tomato foot and root rot. Environ Microbiol 9:1597–1603. https://doi.org/10.1111/j.1462-2920.2007.01263.x
13. Fritsche K, van den Berg M, de Boer W, van Beek TA, Raaijmakers JM, van Veen JA, Leveau JHJ. 2014. Biosynthetic genes and activity spectrum of antifungal polyynes from *Collimonas fungivorans* Ter331. Environ Microbiol 16:1334–1345. https://doi.org/10.1111/1462-2920.12440
14. Akum FN, Kumar R, Lai G, Williams CH, Doan HK, Leveau JHJ. 2021. Identification of *Collimonas gene* loci involved in the biosynthesis of a diffusible secondary metabolite with broad‐spectrum antifungal activity and plant‐protective properties. Microb Biotechnol 14:1367–1384. https://doi.org/10.1111/1751-7915.13716
15. Koele N, Turpault MP, Hildebrand EE, Uroz S, Frey-Klett P. 2009. Interactions between mycorrhizal fungi and mycorrhizosphere bacteria during mineral weathering: budget analysis and bacterial quantification. Soil Biology and Biochemistry 41:1935–1942. https://doi.org/10.1016/j.soilbio.2009.06.017
16. de Boer W, Leveau JHJ, Kowalchuk GA, Gunnewiek PJAK, Abeln ECA, Figge MJ, Sjollema K, Janse JD, van Veen JA. 2004. *Collimonas fungivorans* gen. nov., sp. nov., a chitinolytic soil bacterium with the ability to grow on living fungal hyphae. Int J Syst Evol Microbiol 54:857–864. https://doi.org/10.1099/ijs.0.02920-0
17. Höppener-Ogawa S, de Boer W, Leveau JHJ, van Veen JA, de Brandt E, Vanlaere E, Sutton H, Dare DJ, Vandamme P. 2008. *Collimonas arenae* sp. nov. and *Collimonas pratensis* sp. nov., isolated from (semi-) natural grassland soils. Int J Syst Evol Microbiol 58:414–419. https://doi.org/10.1099/ijs.0.65375-0
18. Lee SD. 2018. *Collimonas antrihumi* sp. nov., isolated from a natural cave and emended description of the genus *Collimonas*. Int J Syst Evol Microbiol 68:2448–2453. https://doi.org/10.1099/ijsem.0.002855
19. Li J, Pan M, Zhang X, Zhou Y, Feng GD, Zhu H. 2021. *Collimonas silvisoli* sp. nov. and *Collimonas humicola* sp. nov., two novel species isolated from forest soil. Int J Syst Evol Microbiol 71:005061. https://doi.org/10.1099/ijsem.0.005061
20. Pospiech A, Neumann B. 1995. A versatile quick-prep of genomic DNA from gram-positive bacteria. Trends Genet 11:217–218. https://doi.org/10.1016/s0168-9525(00)89052-6
21. 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
22. Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. 2020. Using SPAdes *de novo* assembler. Curr Protoc Bioinformatics 70:e102. https://doi.org/10.1002/cpbi.102
23. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:1–11. https://doi.org/10.1186/1471-2105-11-119
24. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, et al. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:1–15. https://doi.org/10.1186/1471-2164-9-75
25. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569
26. Chan PP, Lin BY, Mak AJ, Lowe TM. 2021. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. Nucleic Acids Res 49:9077–9096. https://doi.org/10.1093/nar/gkab688
27. Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic,

and viral genomes. Mol Biol Evol 38:4647–4654. https://doi.org/10.1093/molbev/msab199
28. Meier-Kolthoff JP, Göker 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
29. Picard L, Turpault MP, Oger PM, Uroz S. 2021. Identification of a novel type of glucose dehydrogenase involved in the mineral weathering ability of *Collimonas pratensis* strain PMB3 (1). FEMS Microbiol Ecol 97:fiaa232. https://doi.org/10.1093/femsec/fiaa232
30. Picard L, Paris C, Dhalleine T, Morin E, Oger P, Turpault MP, Uroz S. 2022. The mineral weathering ability of *Collimonas pratensis* PMB3 (1) involves a malleobactin - mediated iron acquisition system. Environ Microbiol 24:784–802. https://doi.org/10.1111/1462-2920.15508
31. Picard L, Blanco Nouche C, Cochet C, Turpault MP, Uroz S. 2023. Mineral weathering by *Collimonas pratensis* PMB3 (1) as a function of mineral properties, solution chemistry and carbon substrate. npj Mater Degrad 7:76. https://doi.org/10.1038/s41529-023-00396-9
32. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T. 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res 49:W29–W35. https://doi.org/10.1093/nar/gkab335