





3 | Microbial Genetics | Announcement

Draft genome sequences of *Streptomyces virginiae* strain CMAA1738, *Paenibacillus ottowii* strain CMAA1739 and *Pseudomonas inefficax* strain CMAA1741, isolated from rhizosphere of wheat landraces

Caroline Sayuri Nishisaka,^{1,2} João Paulo Ventura,^{1,2} Hélio Danilo Quevedo,^{1,2} Fernanda de Almeida Godoy,^{1,2} Maike Rossmann,¹ Rodrigo Mendes¹

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT In this study, we have identified and characterized three genomes from bacteria isolated from the rhizosphere of *Triticum aestivum*. *Streptomyces virginiae* CMAA1738 and *Paenibacillus ottowii* CMAA1739 were obtained from the wheat landrace Iran 1-29-11334, and *Pseudomonas inefficax* CMAA1741 was isolated from the wheat landrace Karakilcik.

KEYWORDS rhizosphere, microbiome, *Pseudomonas*, *Streptomyces*, *Paenibacillus*, genome

Previous microbiome analysis comparing wheat landraces with modern cultivars revealed that landraces exhibit an enhanced ability to recruit specific microbes in the rhizosphere (1). Therefore, we selected two wheat landraces for bacterial isolations: Streptomyces virginiae CMAA1738 and Paenibacillus ottowii CMAA1739 were obtained from the Iranian wheat landrace Iran 1-29-11334, and Pseudomonas inefficax CMAA1741 was isolated from the wheat landrace Karakilcik, originally from Turkey.

For rhizosphere sampling, plants were grown in 250 mL-pots using soil collected from a wheat field in Brazil (22°55′45.36′S; 50°07′22.33′W) in 2017. Pots were kept in a growth chamber with16-h light/8 h dark cycle maintaining temperatures ranging from 20.7°C to 26.1°C. Plants were removed from the pots and gently shaken to remove loose soil; the soil adhered to the roots was sampled for bacterial isolation. One gram of rhizosphere soil was diluted in 9 mL of saline solution (8.5 g L $^{-1}$ NaCl) and subjected to serial dilutions. Then, 100 μ L of the 10 $^{-3}$, 10 $^{-4}$, and 10 $^{-5}$ dilutions were plated on trypticase soy agar medium. After incubation at 28°C for 96 h, colonies underwent purification through three to five consecutive streaks.

For DNA extraction, *S. virginiae* was cultivated in yeast malt extract agar medium at 28°C for 48 h. *P. ottowii* and *P. inefficax* were cultivated in glucose yeast medium at 28°C for 24 h. DNeasy Ultraclean Microbial kit (Qiagen, Germantown, USA) was used for DNA isolation. After quantity (Nanodrop ND-2000 Spectrophotometer - Thermo Fisher Scientific, Wilmington, DE, USA) and purity (QUBIT 2.0 - Thermo Fisher Scientific, Wilmington, DE, USA) checks, DNA samples were sent to Novogene (Novogene Corporation INC, El Monte, California, USA) for genome sequencing.

The whole-genome sequencing was performed using Illumina NovaSeq 6000 platform. Genomic DNA was used for paired-end libraries (2×150 bp) construction using NEBNext Ultra DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations. Then, PCR products were purified with AMPure XP system (Beckman Coulter Life Sciences, Indianapolis, USA) and analyzed for size distribution

Editor Leighton Pritchard, University of Strathclyde, Glasgow, United Kingdom

Address correspondence to Rodrigo Mendes, rodrigo.mendes@embrapa.br.

The authors declare no conflict of interest

See the funding table on p. 3.

Received 17 January 2024 Accepted 24 May 2024 Published 11 June 2024

Copyright © 2024 Nishisaka et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license

July 2024 Volume 13 Issue 7 10.1128/mra.00036-24 **1**

TABLE 1 Summary of bacterial genomes assembly statistics and other information

Genomic profile	S. virginiae	P. ottowii	P. inefficax
Strain ID	CMAA1738	CMAA1739	CMAA1741
Fast ANI placement (%) ^a	98.19	96.60	97.83
Fast ANI reference (NCBI) ^b	GCF_014648795.1	GCF_006874425.1	GCF_900277125.1
Library size (millions of paired-reads)	51.77	47.59	51.57
Number of contigs ^c	113	72	294
Total sequence length (pb) ^c	8,104,969	5,672,255	5,652,986
Total ungapped length (pb) ^c	8,103,135	5,671,970	5,652,597
N50 (kb) ^c	167.1	312.3	36.5
GC (%)	72.50	45.00	63.00
Genes ³	7,316	5,319	5,089
Protein-coding ^c	7,138	5,194	4,993
Non-coding (RNA) ^c	74	44	63
Genome completeness/ contamination (%) ^c	100.00/0.57	99.72/0.56	100.00/0.54
Coverage (x) ^c	1.02	1.20	1.33
SRA identifiers	SRR18100379	SRR18099329	SRR17914816
Genome assembly (NCBI)	GCF_035853655.1	GCF_035853635.1	GCF_035853745.1
RAST annotated genome and antiSMASH data	https://zenodo.org/records/10854839	https://zenodo.org/records/10854801	https://zenodo.org/records/10854821

^aConsidered bacterial species demarcation criterion (>95%–96%) (15).

by Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). FastQC v0.12.1 (2) was used for sequences' quality check, and Trimmomatic v.036 (3) was used for filtering, followed by genome assembly using SPAdes v3.15.3 (4). Quast v5.0.2 was used to measure the assembled genomes' quality metrics (5, 6). The CheckM v1.0.18 (6) was used to assess sequence completeness and contamination. Bowtie2 v1.7.0 tool was applied to get alignment statistics of genomes (7). The taxonomy of each bacterium was determined using the Average Nucleotide Identity (ANI) method, with the assistance of the Genome Taxonomy Database toolkit (GTDB v1.7.0) (8–10). RAST v1.073 pipeline (11, 12) and Prokaryotic Genome Annotation Pipeline (PGAP) v6.6 (13) were used for genome annotation. Functional prediction for secondary metabolite production was assessed using antiSMASH v7.0.1 (14). Genome assembly statistics and additional information are summarized in Table 1.

ACKNOWLEDGMENTS

This research received funding from the Sao Paulo Research Foundation (FAPESP) grants 2014/04099-4, 2020/00469-2 and 2020/06077-9, and from the National Council for Scientific and Technological Development (CNPq), grants 302147/2022-5, 403959/2019-5 302591/2019-2 and 302337/2016-4.

AUTHOR AFFILIATIONS

¹Embrapa Environment, Brazilian Agricultural Research Corporation, Jaguariúna, Brazil ²Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil

AUTHOR ORCIDs

Caroline Sayuri Nishisaka http://orcid.org/0000-0002-4776-6199 Rodrigo Mendes http://orcid.org/0000-0002-9817-4118

July 2024 Volume 13 Issue 7 10.1128/mra.00036-24 **2**

^bClosest related strain identified to date.

^{&#}x27;Calculated using NCBI PGAP.

FUNDING

Funder	Grant(s)	Author(s)
Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)	do 2014/04099-4, 2020/00469-2, 2020/06077-9	Caroline Sayuri Nishisaka
		Rodrigo Mendes
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)	302147/2022-5, 403959/2019-5, 302591/2019-2, 302337/2016-4	Rodrigo Mendes

DATA AVAILABILITY

The genomes of *Streptomyces virginiae* CMAA1738, *Paenibacillus ottowii* CMAA1739, and *Pseudomonas inefficax* CMAA1741 are accessible at NCBI GenBank under BioProject numbers PRJNA802715, PRJNA802713, and PRJNA802578, respectively, with BioSample accession numbers SAMN25582084, SAMN25581302, and SAMN25556618. The SRA identifiers, genome assembly details and antiSMASH data are described in Table 1. The links for genome annotation using Rapid Annotations using Subsystems Technology toolkit (RAST) are available in Table 1. The authorization for field soil sampling is registered with the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGen) under number A5EB05F.

REFERENCES

- Rossmann M, Pérez-Jaramillo JE, Kavamura VN, Chiaramonte JB, Dumack K, Fiore-Donno AM, Mendes LW, Ferreira MMC, Bonkowski M, Raaijmakers JM, Mauchline TH, Mendes R. 2020. Multitrophic interactions in the rhizosphere microbiome of wheat: from bacteria and fungi to protists. FEMS Microbiol Ecol 96:fiaa032. https://doi.org/10.1093/ femsec/fiaa032
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Available from: http://www.bioinformatics.babraham.ac. uk/projects/fastqc/
- 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
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb. 2012.0021
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086
- Parks D.H., Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25:1043–1055. https://doi.org/10.1101/qr.186072.114
- Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923
- Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil P-A, Hugenholtz P. 2018. A standardized bacterial taxonomy based on

- genome Phylogeny substantially revises the tree of life. Nat Biotechnol 36:996–1004. https://doi.org/10.1038/nbt.4229
- Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. Bioinformatics 36:1925–1927. https://doi.org/10.1093/bioinformatics/ btz848
- Parks DH, Chuvochina M, Chaumeil P-A, Rinke C, Mussig AJ, Hugenholtz P. 2020. A complete domain-to-species taxonomy for bacteria and archaea. Nat Biotechnol 38:1079–1086. https://doi.org/10.1038/s41587-020-0501-8
- Cuccuru G, Orsini M, Pinna A, Sbardellati A, Soranzo N, Travaglione A, Uva P, Zanetti G, Fotia G. 2014. Orione, a web-based framework for NGS analysis in microbiology. Bioinformatics 30:1928–1929. https://doi.org/ 10.1093/bioinformatics/btu135
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/ btu153
- 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
- Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T. 2021. antiSMASH 6.0: improving cluster detection and com- parison capabilities. Nucleic Acids Res. 49:W29–W35. https://doi.org/10.1093/nar/gkab335
- Kim M, Oh HS, Park SC, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351. https://doi.org/10.1099/ijs.0.059774-0