AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Bacteriology | Announcement

Genome sequences of chitinase-producing *Streptomyces*, *Bacillus*, and *Paenibacillus* bacterial isolates from vine mealybugs (*Planococcus ficus*)

Lindsey Price Burbank,[1] **Jaime Strickland**[1]

AUTHOR AFFILIATION See affiliation list on p. 3.

ABSTRACT Vine mealybugs (*Planococcus ficus*) are an important vineyard pest. This resource announcement describes the genome sequences of three bacterial isolates from vine mealybugs that show chitinolytic activity, a trait associated with biological control potential. These isolates are classified as *Streptomyces* sp., *Paenibacillus* sp., and *Bacillus mobilis*.

KEYWORDS chitinases, *Streptomyces*, bioinsecticides, *Bacillus*, gram-positive bacteria, *Paenibacillus*

Vine mealybug (*Planococcus ficus*) is a major pest impacting viticulture in several countries around the world (1). This announcement describes genome sequences of three chitinase-producing bacterial isolates from vine mealybugs reared in a laboratory colony on organic winter squash. For bacterial isolation, individual insects were crushed and mixed with 500 µL of 1× phosphate-buffered saline. Serial dilutions were plated either directly on chitin agar (2) (strains *Streptomyces* sp. MB22_4 and *Paenibacillus* sp. MB22_1) or on PD3 medium (3), and then sub-cultured on chitin agar (*Bacillus mobilis* KC15). All three isolates showed chitinase activity indicated by a clearing zone around bacterial growth on chitin agar (Fig. 1). For sequencing, strains were grown in liquid PD3 medium overnight at 28°C with shaking at 180 rpm. Subsequently, 2 mL bacterial suspensions (OD_{600nm} = 0.2–0.5) were centrifuged for 3 min at 9,000 rpm, and cell pellets were resuspended in 180 µL of 1× Tris-EDTA buffer with 1 µL Ready-Lyse Lysozyme (LGC Biosearch Technologies). Samples were incubated at 37°C for 30 min. Further DNA extraction was performed using a Qiagen DNeasy Extraction Kit following the manufacturer's protocol with the addition of RNase A treatment (20 µL of 20 mg/mL RNase A for 10 min at room temperature) before column purification. DNA was eluted in 100 µL of sterile dH_2O and quantified using the Quant-IT DNA Quantification Kit (Thermo Fisher).

For Illumina sequencing, libraries were prepared using the PCR-based Illumina DNA Prep Kit with a target insert size of 280 bp. No additional DNA fragmentation or size selection steps were performed. Illumina sequencing was performed on an Illumina NovaSeq X Plus Sequencer producing 2 × 151 bp paired-end reads. Demultiplexing, quality control, and adapter trimming were performed with bcl-convert v4.2.4 (Illumina, Inc) using default parameters (4). Sequencing statistics are included in Table 1. For nanopore sequencing, libraries were prepared using the PCR-free Oxford Nanopore Technologies Ligation Sequencing Kit (SQK-NBD114.24) with the NEBNext Companion Module (New England BioLabs) to manufacturer's specifications. No additional DNA fragmentation or size selection was performed. Nanopore sequencing was performed on the PromethION Sequencer using an R10.4.1 flow cell. For basecalling and demultiplexing, Guppy v6.5.7 (Oxford Nanopore Technologies) was used under the super-accurate (sup) and 5mC basecalling models

Editor Irene L. G. Newton, Indiana University, Bloomington, Indiana, USA

Address correspondence to Lindsey Price Burbank, lindsey.burbank@usda.gov.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 27 January 2025
Accepted 7 April 2025
Published 28 April 2025

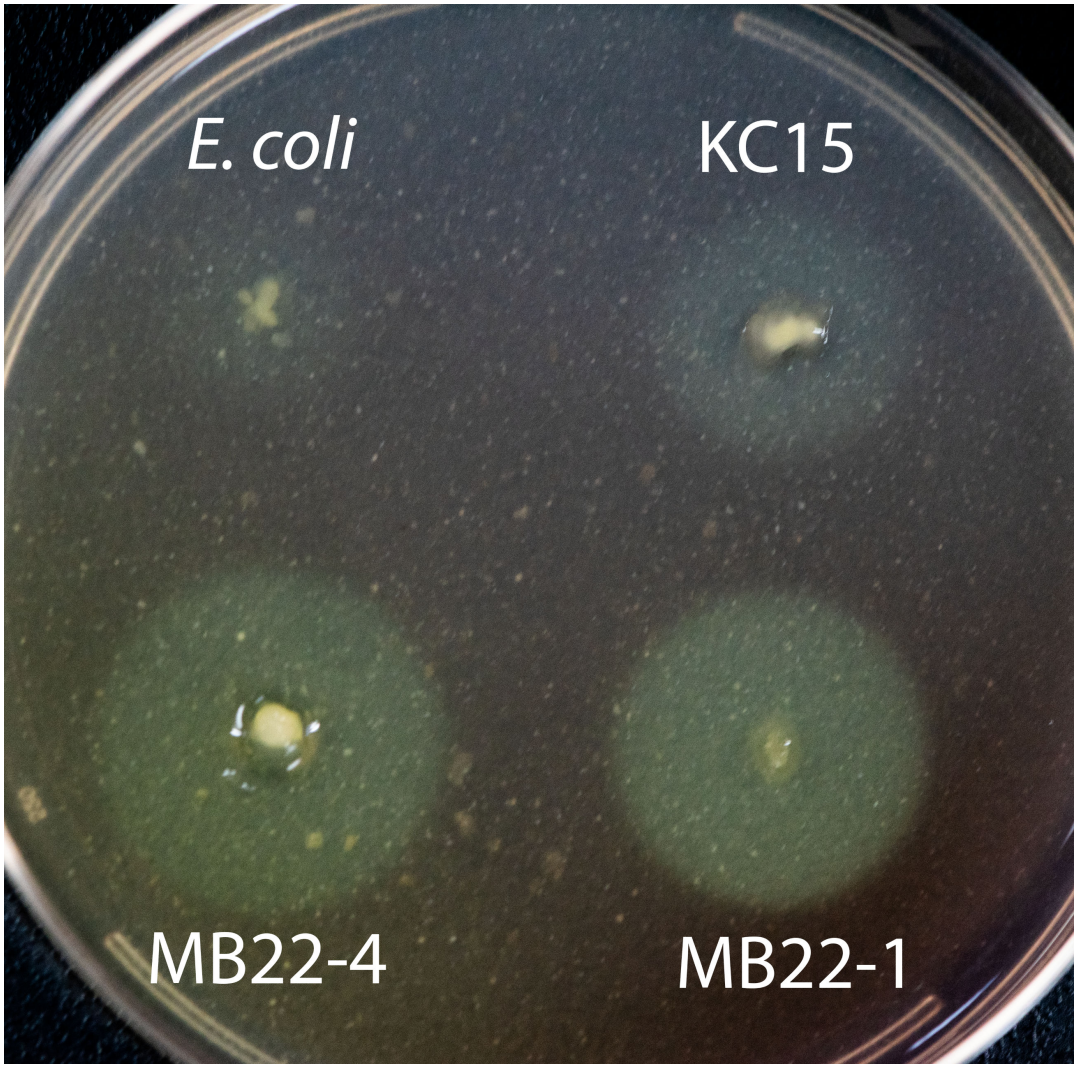

FIG 1 Chitinase activity on chitin agar plates. Bacterial strains were plated on chitin agar and incubated at 28°C for 48 h. The plate was stained with Lugol's iodine solution for 20 min and rinsed with water. Digestion of chitin is indicated by a clearing zone around bacterial colonies. *Escherichia coli* is included as a negative control.

(dna_r10.4.1_e8.2_400bps_modbases_5mc_cg_sup.cfg) with default trimming options (adaptors, primers, and barcodes removed). Porechop v0.2.4 (5) was used to trim residual adapter sequence from the nanopore reads that may have been missed during basecalling and demultiplexing.

De novo genome assemblies were generated from nanopore reads with Flye v2.9.2 (6) using the nano-hq model. Assembly was initiated with reads longer than estimated N50 based on a genome size of 6 Mbp. Initial assemblies were polished with Illumina reads using Pilon v1.24 (7) with default parameters. Long read contigs with an average short read coverage of 15× or less were removed from the assembly. Statistics and information on final assemblies are included in Table 1. Taxonomic classification, average nucleotide identity, and genome completeness and contamination metrics were obtained using GTDB-Tk v2.3.2 (8) and GTDB R08-RS214 reference database on the Kbase platform (9). Genome annotation was performed on National Center for Biotechnology Information data submission with Prokaryotic Genome Annotation Pipeline (10).

TABLE 1 Genome assembly statistics

	Streptomyces sp. MB22_4	*Paenibacillus* sp. MB22_1	*Bacillus mobilis* KC15
Assembly accession	JBLADW000000000.1	JBLADV000000000.1	JBLADU000000000.1
# contigs	Four	Three	Four
Total length	8,391,690	5,181,172	5,865,134
GC (%)	72.28	51.94	35.31
N50	7,812,240	2,692,484	5,269,354
Average nucleotide identity (reference accession)	91.53 (GCF_000980885.2)	98.3 (GCF_000159955.1)	97.71 (GCF_001884045.1)
% completeness	100	99.85	99.43
% contamination	0.32	0	0.62
# predicted genes	7,525	4,848	6,060
Sequence Read Archive accessions	SRX26831859, SRX26831858	SRX26831857, SRX26831856	SRX26831855, SRX26831854
#ONT reads (mean read length)	196,758 (4,518 bp)	208,487 (3,989 bp)	258,0150 (1,312 bp)
#Illumina read pairs (X coverage)	2,171,259 (100)	1,980,854 (100)	2,024,081 (75)

ACKNOWLEDGMENTS

Sequencing was performed by SeqCenter LLC. Analysis was performed using the Kbase platform. Funding support was from the United States Department of Agriculture, Agricultural Research Service appropriated project #2034-22000-014-000-D. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

AUTHOR AFFILIATION

[1]USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier, California, USA

AUTHOR ORCIDs

Lindsey Price Burbank http://orcid.org/0000-0002-9614-1259

FUNDING

Funder	Grant(s)	Author(s)
Agricultural Research Service	2034-22000-014-000-D	Lindsey Price Burbank

DATA AVAILABILITY

Genome assembly data have been deposited in GenBank under BioProject PRJNA1189540. Assemblies and annotations described in this paper are JBLADW000000000.1, JBLADV000000000.1, and JBLADU000000000.1. Raw sequence reads are deposited in Sequence Read Archive with accession numbers SRX26831859, SRX26831858, SRX26831857, SRX26831856, SRX26831855, and SRX26831854.

REFERENCES

1. Walton VM, Pringle KL. 2004. Vine mealybug, planococcus ficus (signoret) (hemiptera: pseudococcidae), A key pest in South African vineyards. A review. South African Journal of Enology and Viticulture 25:54–62.
2. Kuddus SM, Ahmad RI. 2013. Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. Journal of Genetic Engineering and Biotechnology 11:39–46.
3. Davis MJ, French WJ, Schaad NW. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr Microbiol 6:309–314. https://doi.org/10.1007/BF01566883
4. BCL Convert. 2025. Available from: https://emea.support.illumina.com/downloads/bcl-convert-user-guide.html. Retrieved 28 Mar 2025.
5. Wick R. 2024. Rrwick/Porechop. C++

6. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546. https://doi.org/10.1038/s41587-019-0072-8
7. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963
8. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2022. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinformatics 38:5315–5316. https://doi.org/10.1093/bioinformatics/btac672
9. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, et al. 2018. KBase: the United States department of energy systems biology knowledgebase. Nat Biotechnol 36:566–569. https://doi.org/10.1038/nbt.4163
10. 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