Isolation and Whole-Genome Sequencing of Four Antibiotic-Producing *Pseudomonas* Strains

Audrey L. E. Armstrong,a Kyle Cole,a Emily Collins,a Jarret M. Easterday,a Lauren Feddersen,a Brianna Fleury,a Lauren E. Johnson,a Ankush F. Kechta, Logan H. McClure,a Joselyn M. Molinar,a Anjali S. Patel,a Ria Patel,a Jackson R. Rapala,a Arunima Salomi,a Amari A. Stepter,a Michal Wrobel,a* Loralyn M. Cozy

aDepartment of Biology, Illinois Wesleyan University, Bloomington, Illinois, USA

Audrey L. E. Armstrong, Kyle Cole, Emily Collins, Jarret M. Easterday, Lauren Feddersen, Brianna Fleury, Lauren E. Johnson, Ankush F. Kecht, Logan H. McClure, Joselyn M. Molinar, Anjali S. Patel, Ria Patel, Jackson R. Rapala, Arunima Salomi, Amari A. Stepter, and Michal Wrobel contributed equally to this work. Author order was determined alphabetically by last name.

ABSTRACT Here, we report the isolation, whole-genome sequencing, and annotation of four novel *Pseudomonas* isolates. We also evaluate the biosynthetic potential of each genome.

*Pseudomonas* is a Gram-negative, rod-shaped, polar flagellated bacterial genus with more than 140 identified species (1). Within this genus, there are many species that inhabit a diverse range of environments, resulting in their multifaceted metabolic capacities and adaptation abilities in changing environments. Here, we report the draft genomes of four novel *Pseudomonas* isolates that exhibit high antibiotic activity.

Soil samples were collected from a variety of locations (Table 1). One gram of each sample was resuspended in 10 mL of sterile saline, diluted serially, and plated on Reasoner’s 2 agar (R2A) (2) hardened medium plates at 25°C for 48 h. Once diluted, 50 colonies were isolated, and individual colonies were screened for antibiotic activity by patching onto lawns of other bacteria, followed by incubation at 25°C for 48 h. The strains described here, LF19, LM20, JR33AA, and KCA11, were selected because they produced zones of inhibition against lawns of several bacterial species (Table 1). The 16S rRNA gene PCR product was sequenced from each strain using the primer set 27F (5′-AGR GTT TGA TYM TGG CTC AG-3′) and 1492R (5′-GGY TAC CTT GTT AGC ACT T-3′), with 55°C annealing and 30 s of extension. Using NCBI BLAST (3), it was determined that the isolates belong to the *Pseudomonas* genus.

Axenic cultures grown on R2A were sent to the Microbial Genome Sequencing Center (Pittsburgh, PA) for DNA isolation using the Qiagen DNeasy blood and tissue kit and whole-genome sequencing (Fig. 1A). Sequence libraries were prepared with a small-volume tagmentation protocol using the Nextera DNA library preparation kit (Illumina, San Diego, CA, USA). Barcodes and adapters were attached, and libraries were amplified using the KAPA HiFi library amplification kit (4). Paired-end libraries were subsequently sequenced on the Illumina NextSeq 550 platform. FastQC v0.11.9 (5, 6) was used to verify the quality of the reads. These reads were then assembled *de novo* in PATRIC v3.6.12 (7) using the Unicycler v0.4.8 program (8). The assembly included polishing using two Pilon iterations (9) and examination using QUAST v5.0.2 (10). Read number and length information can be found in Table 1. All programs related to genome assembly were run with default parameters. Annotation was performed in PATRIC v3.6.12 using the RASTtk pipeline (11) with default bacterial parameters, using *Pseudomonas* as a taxonomic guide (Table 1).

Taxonomic relationships between the four isolates and other known *Pseudomonas* species were evaluated using the Codon Tree pipeline (12–15) in PATRIC v3.6.12 (7) in combination with average nucleotide identity (ANI) calculations using the Kostas Lab...
| Isolate | Location\(^a\) | SRA accession no. | Assembly accession no. | GenBank accession no. | No. of reads | Read length (bp) | No. of contigs | \(N_{50}\) (bp) | Avg coverage (x) | Size (bp) | GC content (%) | No. of genes | No. of proteins | Antibiotic activity\(^b\) |
|---------|----------------|------------------|-----------------------|----------------------|-------------|----------------|---------------|----------------|----------------|------------|----------------|-------------|----------------|---------------------|
| JR33AA  | 47°37'9"N, 116°46'5"W | SRR17071229 | GCF_02137985.1 | JAJSW00000000000 | 4,285,246 | 146 | 65 | 324,782 | 230 | 5,407,927 | 62.02 | 6,001 | 5,031 | Bs, Sa, Ab, Pa, Ea |
| KCA11   | 40°30'55"N, 88°59'26"W | SRR17071230 | GCF_02137935.1 | JAJSW00000000000 | 4,432,406 | 151 | 63 | 309,066 | 231 | 5,554,878 | 63.15 | 5,318 | 5,250 | Bs, Sa, Ec, Ab, Pa, Ea |
| LF19    | 40°5'9"N, 88°19'9"W | SRR17071231 | GCF_02137965.1 | JAJSW00000000000 | 3,550,179 | 146 | 76 | 247,141 | 177 | 5,811,296 | 62.60 | 5,388 | 5,322 | Bs, Sa, Ab |
| LM20    | 40°49'6"N, 88°29'18"W | SRR17071232 | GCF_02137925.1 | JAJSW00000000000 | 3,533,723 | 146 | 60 | 316,867 | 183 | 5,596,202 | 63.08 | 5,295 | 5,227 | Bs, Sa, Pa |

\(^a\) Coordinates (latitude and longitude) of the soil collection site.

\(^b\) Ab, Acinetobacter baylyi; Bs, Bacillus subtilis; Ea, Enterobacter aerogenes; Ec, Escherichia coli; Pa, Pseudomonas aeruginosa; Sa, Staphylococcus aureus.
FIG 1 Colony morphology and phylogenetic relatedness. (A) R2A streak plates of each strain as indicated. (B) Phylogenetic tree generated using the Codon Tree pipeline in PATRIC with default parameters, with an input of 51 good-quality genomes. A total of 23,327 amino acids and 69,981 nucleotides were represented by 100 single-copy genes. The best tree was determined with 100 rounds of RAxML bootstrapping. Isolates LF19, LM20, JR33AA, and KCA11 are highlighted in yellow.
ANI Calculator v1.0 (16, 17), both run with default parameters. These calculations revealed that LM20 and KCA11 shared 99.5% nucleotide identity, while the remaining relationships all ranged between 82.5% and 89.3%. Additionally, isolates LM20, KCA11, and JR33AA all group with Pseudomonas putida strains, while LF19 is most related to Pseudomonas wadenswilerensis and Pseudomonas donghuensis strains (Fig. 1B).

Genome mining for antibacterial compounds using antiSMASH v6.0.1 (18), with the relaxed strictness setting, provided evidence that each of the four strains contains biosynthetic gene clusters (BGCs) potentially encoding secondary metabolites. The strain with the most predicted BGCs is LF19, with 13, and the strain with the least is LM20, with 8. KCA11 and JR33AA are both predicted to possess 10 BGCs. These strains add to the genomic data available for the Pseudomonas genus, supporting further investigation into its biosynthetic potential.

**Data availability.** This whole-genome shotgun project was deposited in GenBank under BioProject PRJNA784595. GenBank assembly and Sequence Read Archive (SRA) accession numbers are presented in Table 1.

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