# Genome Sequences of 12 *Pseudomonas lundensis* Strains Isolated from the Lungs of Humans

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**ABSTRACT** We report here the first complete genome sequence of a human *Pseudomonas lundensis* isolate, strain AU1044, and the draft genomes of 11 other clinical *P. lundensis* strains, isolated from the lungs of cystic fibrosis patients. The genome of strain AU1044 is 4.81 Mb and encodes seven 16S rRNAs.

Due to genetic diversity among strains, the *Pseudomonas fluorescens* species complex is comprised of multiple subgroups/subclades (1–5). In 2015, our group published the first draft genomes of clinical strains within the *P. fluorescens* species complex (6). Based on phylogeny by Loper et al. (2), the draft genomes of the clinical strains were assigned to subclades 1, 2, and 3 of the *P. fluorescens* species complex, but additional phylogenetic analysis of the *P. fluorescens* species complex by Garrido-Sanz et al. (4, 5) suggested that these clinical strains could be assigned to the *P. protegens*, *P. koreensis*, and *P. fluorescens* subgroups, respectively. In either case, the clinical strains aligned with reported subgroups/subclades built using previously sequenced environmental strains.

Here, we report 12 clinical isolates from the *P. fluorescens* species complex that were identified as *P. lundensis* based on multilocus sequence analysis (MLSA). Analysis of the 16S rRNA gene in our 12 clinical isolates indicates >99.8% identity with *P. lundensis* DSM6252$^T$. *P. lundensis* is a psychrotrophic bacterium that contributes to cold food spoilage. Prior to this study, the only sequenced genomes of this species had been isolated from spoiled meat.

The 12 *P. lundensis* strains were each isolated from the sputum of a different cystic fibrosis patient. Initial isolation of strains occurred between 1 February 1999 and 30 June 2006 from eight treatment centers across the United States (Ann Arbor, MI; Boston, MA; Chapel Hill, NC; Cincinnati, OH; Columbia, MO; Hartford, CT; Palo Alto, CA; and St. Louis, MO). Storage of isolates and sequencing of genomes were performed as previously reported (6). Isolates were banked at −80°C. PCR amplification of the 16S rRNA gene from individual colonies was performed using the universal primer set 8F and 1492R (7). The amplified genes were sequenced and screened against the NCBI nucleotide database and the EzBioCloud 16S database. The isolates, identified as *P. lundensis*, were grown aerobically overnight in Luria broth at 34°C. Genomic DNA was isolated with the Qiagen DNeasy blood and tissue kit (catalog no. 69506). Sequence data were generated from a 100-bp paired-end library on the Illumina HiSeq 2000 platform. The sequence reads were assembled *de novo* using DNAstar SeqMan NGen

**TABLE 1** Genome sequence data of the 12 reported *P. lundensis* strains

| GenBank accession no. | Strain | No. of contigs | Genome size (Mb) | G+C content (%) | Assembly status |
|---|---|---|---|---|---|
| CP017687 | AU1044 | 1 | 4.88 | 57.9 | Whole |
| LCYS00000000 | AU2390 | 93 | 5.41 | 58.3 | Draft |
| LCYT00000000 | AU7350 | 57 | 4.85 | 58.7 | Draft |
| MLCS00000000 | AU9518 | 113 | 5.22 | 57.8 | Draft |
| LCYU00000000 | AU10414 | 56 | 4.84 | 58.6 | Draft |
| LCYV00000000 | AU11122 | 58 | 4.84 | 58.6 | Draft |
| LCYY00000000 | AU11136 | 61 | 4.84 | 58.6 | Draft |
| LCYW00000000 | AU11164 | 56 | 4.83 | 58.7 | Draft |
| LCYX00000000 | AU11235 | 61 | 4.84 | 58.7 | Draft |
| LCYZ00000000 | AU12597 | 35 | 4.9 | 58.8 | Draft |
| LCZA00000000 | AU12644 | 62 | 4.87 | 58.8 | Draft |
| MLCU00000000 | AU14541 | 99 | 5.23 | 57.6 | Draft |

version 12 software into an average of 63.8 contigs (range, 35 to 113). The Mauve aligner was used to reorder the contigs (8). The assembled draft genomes contained an average G+C content of 58.9% (range, 57.7% to 61.13%) and had an average genomic size of 4.9 Mb (range, 4.8 to 5.4 Mb). Strain AU1044 was further sequenced on two PacBio P4-C2 single-molecule real-time cells, and reads were assembled into a closed genome with the in-house procedure at the Genomics Resource Center at the University of Maryland. AU1044's closed genome has a G+C content of 57.7% and is 4.92 Mb in size. MLSA was performed using *dnaE*, *ppsA*, *recA*, *rpoB*, *guaA*, *mutL*, *pyrC*, and *acsA*, modified from Loper et al. (2). Clustering and creation of the phylogenetic tree was completed with MAFFT (9, 10).

**Accession number(s).** The sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1.

## REFERENCES

1. Mulet M, Lalucat J, García-Valdés E. 2010. DNA sequence-based analysis of the *Pseudomonas* species. Environ Microbiol 12:1513–1530. https://doi.org/10.1111/j.1462-2920.2010.02181.x.
2. Loper JE, Hassan KA, Mavrodi DV, Davis EW, II, Lim CK, Shaffer BT, Elbourne LDH, Stockwell VO, Hartney SL, Breakwell K, Henkels MD, Tetu SG, Rangel LI, Kidarsa TA, Wilson NL, van de Mortel JE, Song C, Blumhagen R, Radune D, Hostetler JB, Brinkac LM, Durkin AS, Kluepfel DA, Wechter WP, Anderson AJ, Kim YC, Pierson LS, III, Pierson EA, Lindow SE, Kobayashi DY, Raaijmakers JM, Weller DM, Thomashow LS, Allen AE, Paulsen IT. 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. PLoS Genet 8:e1002784. https://doi.org/10.1371/journal.pgen.1002784.
3. Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB. 2014. Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. Clin Microbiol Rev 27:927–948. https://doi.org/10.1128/CMR.00044-14.
4. Garrido-Sanz D, Meier-Kolthoff JP, Göker M, Martín M, Rivilla R, Redondo-Nieto M. 2016. Correction: genomic and genetic diversity within the *Pseudomonas fluorescens* complex. PLoS One 11:e0153733. https://doi.org/10.1371/journal.pone.0153733.
5. Garrido-Sanz D, Meier-Kolthoff JP, Göker M, Martín M, Rivilla R, Redondo-Nieto M. 2016. Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. PLoS One 11:e0150183. https://doi.org/10.1371/journal.pone.0150183.
6. Scales BS, Erb-Downward JR, Huffnagle IM, LiPuma JJ, Huffnagle GB. 2015. Draft genome sequences of seven *Pseudomonas fluorescens* subclade III strains Isolated from cystic fibrosis patients. Genome Announc 3(1):e01285-14. https://doi.org/10.1128/genomeA.01285-14.
7. Eden PA, Schmidt TM, Blakemore RP, Pace NR. 1991. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. Int J Syst Bacteriol 41:324–325. https://doi.org/10.1099/00207713-41-2-324.
8. Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve aligner. Bioinformatics 25:2071–2073. https://doi.org/10.1093/bioinformatics/btp356.
9. Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. https://doi.org/10.1093/nar/gkf436.
10. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010.