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Draft Genome Sequences of Five *Pseudomonas fluorescens* Subclade I and II Strains, Isolated from Human Respiratory Samples

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We report the draft genomes of five *Pseudomonas fluorescens* strains, isolated from clinical samples. Phylogenetic analysis places three in subclade I and two in subclade II of the *P. fluorescens* species complex. The average G+C content and genomic size are 63% and 7.1 Mbp (subclade I) and 59.6% and 6.14 Mbp (subclade II), respectively.

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The *Pseudomonas fluorescens* species complex is the most diverse group in the *Pseudomonas* genus, containing at least 52 subspecies (1). Phylogenetic analysis using multilocus sequence analysis (MLSA) divides the *P. fluorescens* species complex into three subclades (1–6). Subclade III contains environmental strains *P. fluorescens* SBW25, A506, and SS101, and *P. synxantha* BG33R (1, 3). We previously reported the genomes of ten clinical isolates that map via MLSA to subclade III (7). The five new *P. fluorescens* clinically isolated strains reported here fall into subclades I and II. These subclades currently contain only strains isolated from environmental samples, such as *P. protegens* Pf-5 and CHA0 and *P. chlororaphis* 30-84 and O6 for subclade I (1, 3, 8) and *P. fluorescens* Pf0-1, R124, Q2-87, and Q8r1-96 for subclade II (1, 3, 9). Members of subclades I and II are almost exclusively studied for their plant growth-promoting activities, such as suppression of pathogens and production of plant growth hormones (1–3, 8, 9). The sources of subclade I and II strains, prior to this report, include the wheat rhizosphere (30-84; Q8r1-96 and Q2-87); the soil (O6; Pf-5 and Pf0-1); rhizosphere of shepherd's purse (CHA0), and a silica cave (R124) (1, 3, 8, 9).

Here, we report the first genome sequences of *P. fluorescens* strains, isolated from human clinical samples that map to subclades I and II. All were isolated from cystic fibrosis sputum. Subclade I strains were isolated between August 2006 and April 2010 from Hartford, CT, USA, and Austin, TX, USA. Subclade II strains were collected in April 2004 and April 2006 from two samples from St. Louis, MO, USA, and Omaha, NE, USA. Isolates were banked at −80°C. The 16S rRNA gene was amplified with the universal primer set 8F and 1492R, sequenced using an ABI 3730XL sequencer, and identified as *P. fluorescens* using NCBI BLASTn (10). The isolates 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 with a 100-bp paired-end library on the Illumina HiSeq 2000 platform and reads were *de novo* assembled using the DNAstar SeqMan NGen version 12 software. The subclade I genomes were assembled into an average of 93 contigs (range 44 to 137), and the subclade II genomes were assembled into 56 and 67 contigs. The Mauve aligner was used to reorder the contigs using *P. protegens* Pf-5 and *P. fluorescens* Pf0-1 as references for subclades I and II, respectively (11). The three new subclade I genomes contain, on average, 63% G+C content (range 62.8 to 63.3%) and are 7.1 Mbp (range 6.68 to 7.28 Mbp). The two new subclade II genomes contain, on average, 59.6% G+C content (58.8% and 60.3%) and are 6.14 Mbp (6.08 and 6.2 Mbp). MLSA was performed using dnaE, ppsA, recA, rpoB, guaA , mutL, pyrC, and acsA, modified from the work of Loper et al. (3). Clustering and phylogenetic tree created using MAFFT (12, 13). The MLSA tree maps three of these newly sequenced strains into subclade I and two into subclade II (3, 7–9).

Nucleotide sequence accession numbers. The draft genomes have been deposited at DDBJ/EMBL/GenBank under the accession numbers LCZB00000000, LCZC00000000, and LDET00000000 for subclade I isolates AU11706, AU13852, and AU20219, respectively, and under the accession numbers LCZD00000000 and LCZE00000000 for subclade II isolates AU5633 and AU11114, respectively.

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REFERENCES

1. **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. http://dx.doi.org/10.1128/CMR.00044-14.
2. **Silby MW, Cerdeno-Tarraga AM, Vernikos GS, Giddens SR, Jackson RW, Preston GM, Zhang XX, Moon CD, Gehrig SM, Godfrey SA,**

Knight CG, Malone JG, Robinson Z, Spiers AJ, Harris S, Challis GL, Yaxley AM, Harris D, Seeger K, Murphy L, Rutter S, Squares R, Quail MA, Saunders E, Mavromatis K, Brettin TS, Bentley SD, Hothersall J, Stephens E, Thomas CM, Parkhill J, Levy SB, Rainey PB, Thomson NR. 2009. Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens*. Genome Biol **10:**R51. http://dx.doi.org/10.1186/gb-2009-10-5-r51.

3. **Loper JE, Hassan KA, Mavrodi DV, Davis EW 2nd, Lim CK, Shaffer BT, Elbourne LD, 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 3rd, 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. http://dx.doi.org/10.1371/journal.pgen.1002784.
4. **Mulet M, Lalucat J, Garcia-Valdes E**. 2010. DNA sequence-based analysis of the *Pseudomonas* species. Environ Microbiol **12:**1513–1530. http://dx.doi.org/10.1111/j.1462-2920.2010.02181.x.
5. **Van Passel MWJ, Kuramae EE, Luyf ACM, Bart A, Boekhout T**. 2006. The reach of the genome signature in prokaryotes. BMC Evol Biol **6:**84. http://dx.doi.org/10.1186/1471-2148-6-84.
6. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM**. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol **57:**81–91. http://dx.doi.org/10.1099/ijs.0.64483-0.
7. **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. http://dx.doi.org/10.1128/genomeA.01285-14.
8. **Takeuchi K, Noda N, Someya N**. 2014. Complete genome sequence of the biocontrol strain *Pseudomonas protegens* Cab57 discovered in Japan reveals strain-specific diversity of this species. PLoS One **9:**e93683. http://dx.doi.org/10.1371/journal.pone.0093683.
9. **Barton MD, Petronio M, Giarrizzo JG, Bowling BV, Barton HA**. 2013. The genome of *Pseudomonas fluorescens* strain R124 demonstrates phenotypic adaptation to the mineral environment. J Bacteriol **195:**4793–4803. http://dx.doi.org/10.1128/JB.00825-13.
10. **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.
11. **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. http://dx.doi.org/10.1093/bioinformatics/btp356.
12. **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. http://dx.doi.org/10.1093/nar/gkf436.
13. **Katoh K, Standley DM**. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol **30:**772–780. http://dx.doi.org/10.1093/molbev/mst010.