

Draft Genome Sequences of *Pseudomonas aeruginosa* B3 Strains Isolated from a Cystic Fibrosis Patient Undergoing Antibiotic Chemotherapy

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Pseudomonas aeruginosa frequently establishes chronic infections in the airways of patients suffering from cystic fibrosis (CF). Here, we report the draft genome sequences of four *P. aeruginosa* B3 strains isolated from a chronically infected CF patient undergoing antibiotic chemotherapy.

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ystic fibrosis (CF) patients are subjected to intensive antibiotic therapy, and it is evident that this crucially affects the process of bacterial adaptation to the CF lung environment (1, 2). To further investigate the effects of antimicrobial chemotherapy on the adaptation of Pseudomonas aeruginosa to the CF lung, we sequenced the genomes of four strains of the P. aeruginosa B3 clone type isolated from consecutive sputum samples from a patient with long-term chronic P. aeruginosa infection undergoing 3 months of combination chemotherapy with oral ciprofloxacin and inhaled colistin due to recurrent isolation of P. aeruginosa. The sputum samples were collected and the strains were isolated as part of a longitudinal study where the first and subsequent samples were stored from the beginning of 2005 until July 2009 (1). The first sample was collected before chemotherapy was initiated and is represented by P. aeruginosa B3-1811, a mucoid isolate that was determined at the clinic to be colistin and ciprofloxacin sensitive. A sputum sample collected 2 months after the initiation of therapy contained P. aeruginosa with two different bacterial colony morphologies: a mucoid and colistin-sensitive strain (P. aeruginosa B3-208) and a nonmucoid and colistinresistant strain (P. aeruginosa B3-20M). The last strain (P. aeruginosa B3-CFI), also colistin resistant, was isolated from a sputum sample obtained at the end of the 3-month treatment regime. Genomic DNA from the B3-1811, B3-208, B3-20M, and B3-CFI strains was prepared and sequenced as described previously (3) to generate 3,077,857, 2,445,795, 3,026,144, and 3,881,715 75-bp reads, respectively.

Using Velvet version 1.0.16, the reads were assembled into the draft genomes of strain B3-1811, with 6,657,892 bp, 285 contigs, and an N_{50} of 82,268 bp; strain B3-208, with 6,701,506 bp, 344 contigs, and an N_{50} of 66,611 bp; strain B3-20M, with 6,704,349 bp, 278 contigs, and an N_{50} of 77,595 bp; and strain B3-CFI, with 6,727,794 bp, 252 contigs, and an N_{50} of 87,472 bp. The optimal assembly settings were estimated using Velvet-Optimiser (the Victorian Bioinformatics Consortium [VBC],

Monash University; see http://www.vicbioinformatics.com /software.velvetoptimiser.shtml), with only contigs of \geq 500 bp retained, and the assembly was assisted by initial mapping of the reads against the reference genome sequences of *P. aeruginosa* PAO1 (4) using the Velvet Columbus module.

A comparison of the genomes using MUMmer3 (5) revealed the four isolates to be closely related, sharing at least 99.6% of their genomic contents. The sequences of the four strains reported here will help to elucidate the genetic diversity that exists within clonal populations of *P. aeruginosa* sampled from a single CF patient and how the genetic composition can change quickly due to new selection pressures, such as antibiotic therapy.

Nucleotide sequence accession numbers. These genome sequences have been deposited in EMBL under the following accession no. (BioProject no.): CBMP010000001 to CBMP010000341 (PRJEB4309) for strain B3-1811, CBMT010000001 to CBMT010000418 (PRJEB4310) for strain B3-208, CBMU010000001 to CBMU010000338 (PRJEB4311) for strain B3-20M, and CBMS010000001 to CBMS010000314 (PRJEB4312) for strain B3-CFI.

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REFERENCES

- Hansen SK, Rau MH, Johansen HK, Ciofu O, Jelsbak L, Yang L, Folkesson A, Jarmer HØ, Aanaes K, von Buchwald C, Høiby N, Molin S. 2012. Evolution and diversification of *Pseudomonas aeruginosa* in the paranasal sinuses of cystic fibrosis children have implications for chronic lung infection. ISME J. 6:31–45.
- Folkesson A, Jelsbak L, Yang L, Johansen HK, Ciofu O, Høiby N, Molin S. 2012. Adaptation of *Pseudomonas aeruginosa* to the cystic fibrosis airway: an evolutionary perspective. Nat. Rev. Microbiol. 10:841–851.
- 3. Yang L, Jelsbak L, Marvig RL, Damkiaer S, Workman CT, Rau MH, Hansen SK, Folkesson A, Johansen HK, Ciofu O, Høiby N, Sommer MO,

Molin S. 2011. Evolutionary dynamics of bacteria in a human host environment. Proc. Natl. Acad. Sci. U. S. A. 108:7481–7486.

4. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature **406**:959–964.

 Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5:R12. doi:10.1186/gb-2004-5-2-r12.