12-2012

# Draft Genome Sequence for Pseudomonas aeruginosa Strain PAO579, a Mucoid Derivative of PAO381

T. Ryan Withers
*Marshall University*, withers19@marshall.edu

Shannon L. Johnson

Hongwei D. Yu
*Marshall University*, yuh@marshall.edu

Journal of Bacteriology

# Draft Genome Sequence for Pseudomonas aeruginosa Strain PAO579, a Mucoid Derivative of PAO381

**T. Ryan Withers, Shannon L. Johnson and Hongwei D. Yu**
***J. Bacteriol.*** **2012, 194(23):6617. DOI: 10.1128/JB.01406-12.**

JB Journals.ASM.org

GENOME ANNOUNCEMENT

# Draft Genome Sequence for *Pseudomonas aeruginosa* Strain PAO579, a Mucoid Derivative of PAO381

**T. Ryan Withers,[a] Shannon L. Johnson,[d] and Hongwei D. Yu[a,b,c]**

Departments of Biochemistry and Microbiology[a] and Pediatrics,[b] Joan C. Edwards School of Medicine at Marshall University, Huntington, West Virginia, USA; Progenesis Technologies, LLC, Huntington, West Virginia, USA[c]; and Genome Science Group (B6), Los Alamos National Laboratory, Los Alamos, New Mexico, USA[d]

***Pseudomonas aeruginosa* is an opportunistic pathogen that establishes a chronic lung infection in individuals afflicted with cystic fibrosis. Here, we announce the draft genome of *P. aeruginosa* strain PAO579, an alginate-overproducing derivative of strain PAO381.**

*Pseudomonas aeruginosa* is a ubiquitous, opportunistic pathogen and the leading cause of mortality with individuals afflicted with cystic fibrosis (CF). *P. aeruginosa* uses the overproduction of an exopolysaccharide, called alginate, to form mucoid biofilms. Biofilm formation is responsible for the development of chronic infections, as well as increased resistance to antibiotic treatment (2), and reduced phagocytosis by macrophages (4). *P. aeruginosa* strain PAO579 was first isolated as a mucoid, alginate-overproducing variant of PAO381, a derivative of the nonmucoid reference strain PAO1 (3, 5). Both PAO579 and PAO381 have a wild-type *mucA* gene (*mucA*$^+$), and both strains are leucine auxotrophic (*leu38*) and streptomycin resistant (*strA2*).

Genomic DNA from *P. aeruginosa* strain PAO579 was isolated using phenol-chloroform extraction and ethanol precipitation; paired-end sequencing libraries were generated using vendor protocols (Illumina, San Diego, CA); and genome sequencing was performed on an Illumina GAIIX with 60-bp-length sequencing generating 54,496,482 raw reads covering a total of 3,269,788,920 bp. Data were generated and assembled using Illumina Pipeline version SCS 2.8.0 paired with OLB 1.8.0 and aligned with the *P. aeruginosa* strain PAO1 reference genome (GenBank accession no. NC_2516.2) using NovoAlign version 2.07.10. All specifics regarding aligner algorithms can be obtained from Novocraft, Selangor, Malaysia. The total number of base pairs aligned with the reference genome is 2,077,041,840 bp, representing an average of 340× per target base pair aligned. The number of bases saturated at or above 8× is 6,016,396 (96.04%), and the number of bases saturated below 8× is 248,008 (3.96%). The total number of reference bases covered is 6,036,589 (96.36%) with the total number of bases missed being 227,815 (3.64%). There are a total of 16 contigs (ALOF01000001 to ALOF01000016). Further analysis of the genome was performed using Samtools version 0.1.16a for the generation of pileup after sorting and removing supplicate reads and then analysis pipeline software developed by Cofactor Genomics (St. Louis, MO). The genome was annotated and prepared for submission using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP; http://www.ncbi.nlm.nih.gov.genomes/static/Pipeline.html) server at NCBI.

Based upon this analysis, a total of 31 homozygous single nucleotide polymorphisms (SNPs), or point mutations, were called with a high confidence between the sequenced reads and the PAO1 reference genome and were tabulated for each genomic position, as well as the total coverage of bases observed at that location. Of particular interest is the substitution of thymine for adenine at genome position 4980548 in the DegS-like MucA protease gene, *algW* (PA4446). This alteration results in a change in the primary structure of AlgW, more specifically a substitution of isoleucine for phenylalanine at amino acid position 239. This predicted substitution is thought to affect the proteolytic activity of AlgW (1). Also of note, we observed mutations in *leuA* (PA3792), the 2-isopropylmalate synthase gene, and *rpsL* (PA4268), the 30S ribosomal protein S12 gene. These mutations are contiguously part of the PAO381 lineage and are responsible for leucine auxotrophy and streptomycin resistance, respectively.

**Nucleotide sequence accession number.** The draft genome sequence of *P. aeruginosa* strain PAO579 has been deposited in GenBank under accession number ALOF00000000.

## ACKNOWLEDGMENTS

We thank Jian Gao and Cofactor Genomics for assistance in sequencing and data analysis.

This project was funded through the National Aeronautics and Space Administration West Virginia Space Grant Consortium (NASA WVSGC) and the Cystic Fibrosis Foundation (CFF-YU11G0). H.D.Y. was supported by NIH P20RR016477 and P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

## REFERENCES

1. **Cezairliyan BO, Sauer RT.** 2009. Control of Pseudomonas aeruginosa AlgW protease cleavage of MucA by peptide signals and MucB. Mol. Microbiol. **72**:368–379.
2. **Govan JR, Deretic V.** 1996. Microbial pathogenesis in cystic fibrosis: mucoid Pseudomonas aeruginosa and Burkholderia cepacia. Microbiol. Rev. **60**:539–574.
3. **Govan JR, Fyfe JA.** 1978. Mucoid Pseudomonas aeruginosa and cystic fibrosis: resistance of the mucoid from to carbenicillin, flucloxacillin and tobramycin and the isolation of mucoid variants in vitro. J. Antimicrob. Chemother. **4**:233–240.
4. **Leid JG, et al.** 2005. The exopolysaccharide alginate protects Pseudomonas aeruginosa biofilm bacteria from IFN-gamma-mediated macrophage killing. J. Immunol. **175**:7512–7518.
5. **Stanisich V, Holloway BW.** 1969. Conjugation in Pseudomonas aeruginosa. Genetics **61**:327–339.

**Received** 2 August 2012 **Accepted** 18 September 2012

Address correspondence to Hongwei D. Yu, yuh@marshall.edu.



doi:10.1128/JB.01406-12