Draft Genome Sequence of a Stable Mucoid Strain of Pseudomonas aeruginosa PAO581 with a mucA25 Mutation

Citation for published version:
Yin, Y, Withers, TR, Govan, JRW, Johnson, SL & Yu, HD 2013, 'Draft Genome Sequence of a Stable Mucoid Strain of Pseudomonas aeruginosa PAO581 with a mucA25 Mutation' Genome announcements, vol 1, no. 5. DOI: 10.1128/genomeA.00834-13

Digital Object Identifier (DOI):
10.1128/genomeA.00834-13

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Genome announcements

Publisher Rights Statement:
Copyright © 2013 Yin et al.
This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Alginate overproduction in *Pseudomonas aeruginosa*, also termed mucoidy, is a poor prognostic marker in patients with cystic fibrosis (CF). Alginate regulation has been extensively studied in *P. aeruginosa* (1–3). Two mechanisms for the conversion to mucoidy have been elucidated. The first one is through a mutation in the *mucA* gene (4), and the second is through the activation of an intramembrane proteolysis leading to the degradation of MucA (3). Both mechanisms converge to release AlgU from sequestration by MucA, subsequently initiating transcription of the *algD* operon (3, 6). Here, we sequenced the genome of a stable mucoid variant, *P. aeruginosa* PAO581 (*mucA25*), which was isolated in vitro following the incubation of the nonmucoid *P. aeruginosa* strain PAO381 with phage E79 (7). PAO381 is a derivative of the progenitor strain *P. aeruginosa* PAO1, with a streptocymycin resistance and FP2 marker (8).

The genomic DNA of PAO581, which was extracted by cetyltrimethylammonium bromide (CTAB)-NaCl and phenol-chloroform-isoamyl alcohol, was sent to Cofactor Genomics (St. Louis, MO) for whole-genome sequencing. Paired-end sequencing libraries were generated according to the vendor protocols (Illumina, San Diego, CA). The genome sequencing was performed on an Illumina GAIIx. A total of 26,126,768 raw reads and 2,612,676,800 bp were obtained. The sequence data were generated and assembled using the Illumina Pipeline version SCS 2.8.0 based on paired-end tags with OLB 1.8.0. The sequences were aligned and annotated according to the reference genome of *P. aeruginosa* PAO1 (GenBank accession no. NC_25162.2) using the Novocraft Novoaligner version 2.07.10 software package. Further analysis of the genome was performed using SAMtools version 0.1.6a for the generation of pileup after sorting and removing duplicate reads. The analysis pipeline software was developed by Cofactor Genomics, and all specifics regarding the aligner algorithms can be obtained from the Novocraft website. The coverage of the generated sequences is $199 \times$ the reference genome. The number of base pairs saturated at $\geq 8 \times$ is 6,003,484 (95.83%), and the number of base pairs saturated at $< 8 \times$ is 260,920 (4.17%). The genome was annotated and prepared for submission using an Ergatis-based workflow with manual correction.

Analysis of single nucleotide polymorphisms (SNPs) and indels showed that a total of 44 heterozygous mutations (30 indels and 14 SNPs) ($0.3 \leq \text{count ratio} \leq 0.8$) and 22 homozygous SNPs (count ratio, >0.8) were identified in PAO581 in comparison to the reference genome of PAO1. The count ratio is derived by the number of times the reference base is observed divided by coverage at this base, including all matches and mismatches. Among the indels, 16 were found in the intergenic regions, and 14 were found inside genes causing a frameshift. Among the homozygous SNPs, 17 SNPs were distributed in 15 genes causing nonsynonymous or frameshift mutations, and 5 SNPs were in intergenic regions. Among the mutant genes, only *mucA* (3) and *clpP* (9) have been previously reported to be involved in alginate regulation.

**Nucleotide sequence accession number.** The draft genome sequence of the stable alginate-overproducing mucoid strain PAO581 has been deposited in GenBank under the accession no. CP006705.

**ACKNOWLEDGMENTS.**

This work was supported by the Cystic Fibrosis Foundation (CFF-YU11G0), the National Aeronautics and Space Administration West Virginia Space Grant Consortium (NASA WVSGC), and NIH P20RR101677 and P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

**REFERENCES**


