



# Complete Genome Sequences of Two *Pseudomonas aeruginosa* Strains Isolated from Children with Bacteremia

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**ABSTRACT** Two *Pseudomonas aeruginosa* strains isolated from children with bacteremia in Mexico City were sequenced using PacBio RS-II single-molecule real-time (SMRT) technology. The strains consist of a 7.0- to 7.4-Mb chromosome, with a high content of mobile elements, and variation in the genetic content of class 1 integron In1409.

*Pseudomonas aeruginosa* is associated with chronic recurrent pulmonary infections that are responsible for high mortality in children with underlying conditions such as hematology-oncology diseases, extended hospitalization in the intensive care unit (ICU), and prematurity (1).

Two *P. aeruginosa* strains (Pa1207 and Pa1242) were sequenced. The strains were isolated from children with bacteremia admitted to a pediatric hospital in Mexico City. Strain Pa1207 was resistant to different  $\beta$ -lactams (including carbapenems and cephalosporins), amikacin, and tobramycin but was susceptible to gentamicin, polymyxin B, and fluoroquinolones. Strain Pa1242 presented only intermediate resistance to polymyxin B and was susceptible to 19 antimicrobials tested.

The genomic DNA of the strains was purified with the DNeasy blood and tissue kit (Qiagen) and sent to the Yale Center for Genome Analysis for PacBio RS II single-molecule real-time (SMRT) sequencing. A standard library of 20-kb fragments was prepared and sequenced on two SMRT cells with P4-C2 chemistry. The continuous long reads were assembled using the HGAP/Quiver protocol in SMART Portal v3 (2). The final assemblies had mean coverages of  $\sim 146\times$  and  $\sim 181\times$  for Pa1207 and Pa1242, respectively, and consisted of chromosomes of 7,411,863 bp and 7,050,510 bp, with mean G+C contents of 65.7% and 65.8%, respectively. A total of 7,153 genes were annotated for Pa1207: 7,072 CDSs, 65 tRNAs, 12 rRNAs, 4 noncoding RNAs (ncRNAs), and 247 pseudogenes. For strain Pa1242, 6,735 genes were annotated: 6,654 CDSs, 65 tRNAs, 12 rRNAs, 4 ncRNAs, and 346 pseudogenes.

The sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (<http://www.ncbi.nlm.nih.gov>). The annotation was manually curated and enriched by the presence of mobile elements, antibiotic resistances genes, efflux pumps, and potential virulence factors using the IslandPath-DIMOB, SIGI-HMM, Island-Pick (3), CARD (4), VirulenceFinder (5), and Integrall (6) databases. Strain Pa1207 presented 4 genomic islands, 10 prophages, and 3 integrative plasmids. Strain Pa1242 presented 4 different genomic islands and 8 prophages.

In both strains, there are three large genomic islands inserted at previously identified loci (7–10) but with different genetic compositions. One island conserved the first

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31 open reading frames (ORFs) described in *P. aeruginosa* genome island 1 (PAGI-1), and the other had only the 4 ORFs described in pathogenicity island 2 (PAPI-2). Strain Pa1207 presents a mobile element shared with *Pseudomonas fluorescens*, which encodes to dehydrogenases, type VI secretion system components, and hypothetical proteins. Other islands in both strains are hybrids formed for genes from PAPI-1/pKLC-102, where a group of genes of the major pilins are conserved.

The two strains presented efflux systems MexAB-OprM, MexCD-OprJ, MexEF-OprM, and MexXY, the porin OprD, and the  $\beta$ -lactam OXA-50. Strain Pa1207 presents an integron class 1 (In1409) not previously described (<http://integrall.bio.ua.pt/?acc=CP022001>). The integron carried genes AAC(6′)-33, *aadA6*, *blaOXA-2*, and *sul1*. The two strains presented type III secretion system (*exoU*, *exoS* and *exoT*, *exoY*) genes.

**Accession number(s).** These whole-genome projects have been deposited in GenBank under the nucleotide accession no. CP022001 (Pa1207) and CP022002 (Pa1242), BioSample no. SAMN05020325 (Pa1207) and SAMN05020326 (Pa1242), and BioProject no. PRJNA389181.

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## REFERENCES

- Morales-Espinosa R, Delgado G, Espinosa LF, Isselo D, Méndez JL, Rodríguez C, Miranda G, Cravioto A. 2017. Fingerprint analysis and identification of strains ST309 as a potential high risk clone in a *Pseudomonas aeruginosa* population isolated from children with bacteremia in Mexico City. *Front Microbiol* 8:313. <https://doi.org/10.3389/fmicb.2017.00313>.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Dhillon BK, Laird MR, Shay JA, Winsor GL, Lo R, Nizam F, Pereira SK, Waglegchner N, McArthur AG, Langille MG, Brinkman FS. 2015. IslandViewer 3: More flexible, interactive genomic island discovery, visualization and analysis. *Nucleic Acids Res* 43:W104–W108. <https://doi.org/10.1093/nar/gkv401>.
- McArthur AG, Waglegchner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJ, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD. 2013. The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother* 57:3348–3357. <https://doi.org/10.1128/AAC.00419-13>.
- Kleinheinz KA, Joensen KG, Larsen MV. 2014. Applying the ResFinder and VirulenceFinder Web-services for easy identification of acquired antibiotic resistance and *E. coli* virulence genes in bacteriophage and prophage nucleotide sequences. *Bacteriophage* 4:e27943. <https://doi.org/10.4161/bact.27943>.
- Moura A, Soares M, Pereira C, Leitão N, Henriques I, Correia A. 2009. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. *Bioinformatics* 25:1096–1098. <https://doi.org/10.1093/bioinformatics/btp105>.
- Römling U, Schmidt KD, Tümmler B. 1997. Large genome rearrangements discovered by the detailed analysis of 21 *Pseudomonas aeruginosa* clone C isolates found in environment and disease habitats. *J Mol Biol* 271:386–404. <https://doi.org/10.1006/jmbi.1997.1186>.
- Liang X, Pham XQ, Olson MV, Lory S. 2001. Identification of a genomic island present in the majority of pathogenic isolates of *Pseudomonas aeruginosa*. *J Bacteriol* 183:843–853. <https://doi.org/10.1128/JB.183.3.843-853.2001>.
- Larbig KD, Christmann A, Johann A, Klockgether J, Hartsch T, Merkl R, Wiehlmann L, Fritz HJ, Tümmler B. 2002. Gene islands integrated into tRNA<sup>Gly</sup> genes confer genome diversity on a *Pseudomonas aeruginosa* clone. *J Bacteriol* 184:6665–6680. <https://doi.org/10.1128/JB.184.23.6665-6680.2002>.
- Klockgether J, Reva O, Larbig K, Tümmler B. 2004. Sequence analysis of the mobile genome island pKLC102 of *Pseudomonas aeruginosa* C. *J Bacteriol* 186:518–534. <https://doi.org/10.1128/JB.186.2.518-534.2004>.