PROKARYOTES





Genome Sequences of Two Naphthalene-Degrading Strains of *Pseudomonas balearica*, Isolated from Polluted Marine Sediment and from an Oil Refinery Site

Francisco Salvà-Serra,^{a,b,c} Hedvig E. Jakobsson,^{a,b} Antonio Busquets,^c Margarita Gomila,^c Daniel Jaén-Luchoro,^{a,b,c} Carolina Seguí,^c
Francisco Aliaga-Lozano,^{c,d} Elena García-Valdés,^c Jorge Lalucat,^c
Edward R. B. Moore,^{a,b,e} Antoni Bennasar-Figueras^c

Department of Infectious Diseases, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden^a; Centre for Antibiotic Resistance Research (CARe) at University of Gothenburg, Gothenburg, Sweden^b; Microbiology, Department of Biology, University of the Balearic Islands, Palma de Mallorca, Spain^c; Microbiology, Clínica Rotger, Palma de Mallorca, Spain^d; Culture Collection University of Gothenburg (CCUG), Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden^e

ABSTRACT The genome sequences of *Pseudomonas balearica* strains LS401 (CCUG 66666) and st101 (CCUG 66667) have been determined. The strains were isolated as naphthalene degraders from polluted marine sediment and from a sample from an oil refinery site, respectively. These genomes provide essential data about the biodegradation capabilities and the ecological implications of *P. balearica*.

Pseudomonas balearica was described as a novel species in 1996 (1), and the complete genome sequence of the type strain was recently published (2). Here, we report the draft genome sequences of *P. balearica* strain LS401 (CCUG 66666), isolated as a naphthalene degrader from a polluted marine sediment (Barcelona, Spain) (3), and *P. balearica* strain st101 (CCUG 66667), isolated as a phenanthrene and naphthalene degrader from *Spartina patens* rhizosphere at an oil refinery site (NY/NJ harbor estuary, USA) (4).

Both strains were cultivated at 30°C on Columbia agar base plus 5% horse blood. Genomic DNA was isolated using a Wizard SV genomic DNA purification system (Promega, Madison, WI, USA). DNA was sequenced with an Illumina HiSeq 2500 instrument, generating paired-end reads of 126 bp and an insert size ranging from 130 to 680 bp. Reads were trimmed with Sickle version 1.33 (5) and error-corrected with SPAdes version 2.4.0 (6). A first de novo assembly was obtained using 6,825,396 reads of each strain and Velvet version 1.2.10 (7). The genome sequence of P. balearica DSM 6083^{T} was used as a reference to obtain a second assembly for both strains by mapping with CLC Genomics Workbench version 8 (CLC bio, Aarhus, Denmark). A consensus genome assembly of each strain was obtained by combining the de novo and the reference-based assemblies with Metassembler version 1.5 (8). Assemblies were assessed using QUAST version 3.1 (9) and Feature Response Curves (10). The genome sequence of P. balearica LS401 is formed by 60 contigs with a total length of 4,293,681 bp. The largest contig is 358,463 bp, and the G+C content is 65.0%. The genome sequence of P. balearica st101 consists of 45 contigs with a total length of 4,363,152 bp. The largest contig is 805,012 bp and the G+C content is 64.9%, which is similar to the 64.7% of the type strain (2). Analyses by average nucleotide identity based on BLAST (ANIb) (11), using JSpecies version 1.2.1 (12), among the genome sequences of P. balearica strains LS401, st101, and DSM 6083[⊤] ranged from 97.9% to 98.5%.

Received 1 February 2017 Accepted 3 February 2017 Published 6 April 2017

Citation Salvà-Serra F, Jakobsson HE, Busquets A, Gomila M, Jaén-Luchoro D, Seguí C, Aliaga-Lozano F, García-Valdés E, Lalucat J, Moore ERB, Bennasar-Figueras A. 2017. Genome sequences of two naphthalene-degrading strains of *Pseudomonas balearica*, isolated from polluted marine sediment and from an oil refinery site. Genome Announc 5:e00116-17. https:// doi.org/10.1128/genomeA.00116-17.

Copyright © 2017 Salvà-Serra et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Edward R. B. Moore, erbmoore@ccug.se, or Antoni Bennasar-Figueras, toni.bennasar@uib.es. Both genome sequences were annotated, using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13). Annotation of *P. balearica* LS401 identified 4,087 genes, including 4,026 coding sequences (CDSs) and 54 tRNA genes. Annotation of *P. balearica* st101 revealed 4,093 predicted genes, including 4,033 CDSs and 53 tRNA genes. Highly conserved genes for chemotaxis and flagellum synthesis were identified. The complete naphthalene degradation upper and lower pathways (14, 15) were found in the genome of *P. balearica* LS401 but not in *P. balearica* st101. The capability of *P. balearica* st101 to degrade naphthalene and phenanthrene could be explained by the presence of a complete homogentisate degradation pathway, which is present in both genomes. Genes for complete denitrification and for the benzoate degradation pathway were found in both genomes. A CRISPR-Cas system type I, subtype I-E (16), was found in the genome of *P. balearica* st101 using CRISPRFinder (17).

Accession number(s). These whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession numbers LONE00000000 and LONF00000000. The versions described in this paper are the second versions, LONE02000000 and LONF02000000.

ACKNOWLEDGMENTS

Francisco Salvà-Serra, Hedvig E. Jakobsson, Daniel Jaén-Luchoro, and Edward R. B. Moore were supported by the European Commission: Tailored-Treatment, project 602860, and by the Swedish Västra Götaland Regional Fund, project ALFGBG-437221. Elena García-Valdés and Jorge Lalucat were supported by the Spanish Ministry of Economy and Competitiveness (MINECO) (CGL2015-70925). Antoni Bennasar-Figueras was funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (CGL2009-12180). Margarita Gomila was supported by a postdoctoral contract from the Conselleria d'Innovació, Recerca i Turisme of the Government of the Balearic Islands and the European Social Fund and was a recipient of a José Castillejo Stipendium for exchange with the Culture Collection University of Gothenburg (CCUG), Gothenburg, Sweden. We thank Max Häggblom and Norberto Palleroni for providing the strain st101.

REFERENCES

- Bennasar A, Rosselló-Mora R, Lalucat J, Moore ER. 1996. 16S rRNA gene sequence analysis relative to genomovars of *Pseudomonas stutzeri* and proposal of *Pseudomonas balearica* sp. nov. Int J Syst Bacteriol 46: 200–205. https://doi.org/10.1099/00207713-46-1-200.
- Bennasar-Figueras A, Salvà-Serra F, Jaén-Luchoro D, Seguí C, Aliaga F, Busquets A, Gomila M, Moore ERB, Lalucat J. 2016. Complete genome sequence of *Pseudomonas balearica* DSM 6083^T. Genome Announc 4(2): e00217–16. https://doi.org/10.1128/genomeA.00217-16.
- Rossello R, Garcia-Valdes E, Lalucat J, Ursing J. 1991. Genotypic and phenotypic diversity of *Pseudomonas stutzeri*. Syst Appl Microbiol 14: 150–157.
- Dutta J. 2001. Isolation and characterization of polycyclic aromatic hydrocarbon degrading bacteria from the rhizosphere of salt-marsh plants. MSc thesis. Rutgers, the State University of New Jersey, New Brunswick, NJ.
- Joshi NA, Fass FJ. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files, version 1.33. https://github.com/najoshi/sickle.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi .org/10.1101/gr.074492.107.
- Wences AH, Schatz MC. 2015. Metassembler: merging and optimizing de novo genome assemblies. Genome Biol 16:207. https://doi.org/10.1186/ s13059-015-0764-4.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https:// doi.org/10.1093/bioinformatics/btt086.

- Vezzi F, Narzisi G, Mishra B. 2012. Reevaluating assembly evaluations with feature response curves: GAGE and Assemblathons. PLoS One 7:e52210. https://doi.org/10.1371/journal.pone.0052210.
- 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. https://doi.org/10.1099/ijs.0.64483-0.
- Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106: 19126–19131. https://doi.org/10.1073/pnas.0906412106.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44: 6614–6624. https://doi.org/10.1093/nar/gkw569.
- Bosch R, García-Valdés E, Moore ER. 1999. Genetic characterization and evolutionary implications of a chromosomally encoded naphthalenedegradation upper pathway from *Pseudomonas stutzeri* AN10. Gene 236:149–157.
- Bosch R, García-Valdés E, Moore ER. 2000. Complete nucleotide sequence and evolutionary significance of a chromosomally encoded naphthalene-degradation lower pathway from *Pseudomonas stutzeri* AN10. Gene 245:65–74.
- Makarova KS, Haft DH, Barrangou R, Brouns SJJ, Charpentier E, Horvath P, Moineau S, Mojica FJM, Wolf YI, Yakunin AF, van der Oost J, Koonin EV. 2011. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol 9:467–477. https://doi.org/10.1038/nrmicro2577.
- Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:W52–W57. https://doi.org/10.1093/nar/gkm360.