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# **Draft Genome Sequence of Klebsiella michiganensis 3T412C, Harboring an Arsenic Resistance Genomic Island, Isolated from Mine Tailings in Peru**

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**ABSTRACT** An arsenic resistance genomic island in the bacterium Klebsiella michiganensis 3T412C was isolated from mine tailings from Peru. This genomic island confers adaptation to extreme environments with high concentrations of arsenic. Isolate 3T412C contained a complete set of genes involved in resistance to arsenic. This operon is surrounded by putative genes for resistance to other heavy metals.

**Recent studies have revealed that both horizontal gene transfer (HT) and genomic<br>
<b>Relative Studies** (GIs) can confer selective advantages to bacteria [\(1\)](#page-1-0). Multiple mechanisms have evolved for cellular defense against arsenic, and the genes involved are taxonomically widespread and subject to HT [\(2,](#page-1-1) [3\)](#page-1-2). The general efflux detoxification pathway involves the reduction of arsenate to arsenite, and then subsequent expulsion of arsenic from the cell through arsenite-specific transporters [\(4,](#page-1-3) [5\)](#page-1-4). The efflux system consists of an arsenate reductase (ArsC) [\(6\)](#page-1-5), an arsenite-specific efflux pump (ArsB) [\(7\)](#page-1-6), ATPase (ArsA) that couples with ArsB for the expulsion of arsenite from cells, the regulatory elements ArsR and ArsD, and a gene, arsH, of unknown function [\(2\)](#page-1-1). The genes sufficient for a complete efflux pathway were previously identified in Prochlorococcus genomes [\(8\)](#page-1-7). This efflux detoxification is believed to be the major arsenic detoxification strategy for Prochlorococcus and other species with the same operon configuration [\(3\)](#page-1-2).

Here, we report the draft genome sequence of Klebsiella michiganensis strain 3T412C, which was isolated from surface water from a mine water treatment operation in Trujillo, Peru. The draft genome sequence was determined using Illumina sequencing, and assembly with SPAdes version 3.10 was carried out with k-mer values increasing from 51 to 71 [\(9\)](#page-1-8). Additionally, reads were utilized for contig extension and gap repairing with ABACAS and IMAGE, respectively [\(10\)](#page-1-9). The quality of the assemblies was verified with QUAST software [\(11\)](#page-1-10). Default parameters were used. Finally, 187 contigs were submitted to GenBank. Preliminary gene prediction and annotation were performed with the Prokka tool [\(12\)](#page-1-11). The existence of GIs was confirmed with IslandViewer software, a predictor of GIs that integrates three methods: IslandPick, IslandPath-DIMOB, and SIGI-HMM [\(13\)](#page-1-12). The number of contigs was 120, and the  $N_{50}$  was 136,825 bp. The draft genome of 3T412C comprised 6,208,338 bp, with a  $G+C$  content of 55.71%.

We found that the genome contained a GI with an operon for arsenic resistance. Within the operon, the configuration of genes was the same as that for Prochlorococcus spp., in the following order: ArsA, ArsC, ArsB, ArsA, the operon repressor ArsD, and the regulatory element ArsR. This operon is surrounded by other putative resistance proteins for copper, cobalt, cadmium, mercury, lead, and zinc.

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After evaluation of the persistent operon in the genome, we evaluated the bacterium's tolerance of heavy metals. The growth of strain 3T412C was not affected by an arsenic concentration as high as 28 mM (arsenite,  $NaAsO<sub>2</sub>$  from Merck) in the medium nonenriched LB at pH 7. In contrast with other reports on Klebsiella spp., this is the strain with the highest resistance potential [\(14\)](#page-1-13).

**Accession number(s).** This whole-genome shotgun project for K. michiganensis strain 4T312C has been deposited at DDBJ/ENA/GenBank under the accession number [MPJL00000000.](https://www.ncbi.nlm.nih.gov/nuccore/MPJL00000000)

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### <span id="page-1-0"></span>**REFERENCES**

- 1. Freel KC, Krueger MC, Farasin J, Brochier-Armanet C, Barbe V, Andrès J, Cholley PE, Dillies MA, Jagla B, Koechler S, Leva Y, Magdelenat G, Plewniak F, Proux C, Coppée JY, Bertin PN, Heipieper HJ, Arsène-Ploetze F. 2015. Adaptation in toxic environments: arsenic genomic islands in the bacterial genus Thiomonas. PLoS One 10:e0139011. [https://doi.org/](https://doi.org/10.1371/journal.pone.0139011) [10.1371/journal.pone.0139011.](https://doi.org/10.1371/journal.pone.0139011)
- <span id="page-1-1"></span>2. Stolz JF, Basu P, Santini JM, Oremland RS. 2006. Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60:107–130. [https://doi.org/](https://doi.org/10.1146/annurev.micro.60.080805.142053) [10.1146/annurev.micro.60.080805.142053.](https://doi.org/10.1146/annurev.micro.60.080805.142053)
- <span id="page-1-2"></span>3. Saunders JK, Rocap G. 2016. Genomic potential for arsenic efflux and methylation varies among global Prochlorococcus populations. ISME J 10:197–209. [https://doi.org/10.1038/ismej.2015.85.](https://doi.org/10.1038/ismej.2015.85)
- <span id="page-1-3"></span>4. Carlin A, Shi W, Dey S, Rosen BP, Carlin A, Shi W, Dey S, Rosen BP. 1995. The ars operon of Escherichia coli confers arsenical and antimonial resistance. J Bacteriol 177:981–986. [https://doi.org/10.1128/jb.177.4.981](https://doi.org/10.1128/jb.177.4.981-986.1995) [-986.1995.](https://doi.org/10.1128/jb.177.4.981-986.1995)
- <span id="page-1-4"></span>5. Ghosh M, Shen J, Rosen BP, Kaback HR. 1999. Pathways of As(III) detoxification in Saccharomyces cerevisiae (ACR3YCF1Sb(III)resistanceABC transporters). Proc Natl Acad Sci U S A 96:5001-5006.
- <span id="page-1-5"></span>6. Bennett MS, Guan Z, Laurberg M, Su XD. 2001. Bacillus subtilis arsenate reductase is structurally and functionally similar to low molecular weight protein tyrosine phosphatases. Proc Natl Acad Sci U S A 98:13577-13582. [https://doi.org/10.1073/pnas.241397198.](https://doi.org/10.1073/pnas.241397198)
- <span id="page-1-6"></span>7. Páez-Espino D, Tamames J, de Lorenzo V, Cánovas D. 2009. Microbial responses to environmental arsenic. Biometals 22:117–130. [https://doi](https://doi.org/10.1007/s10534-008-9195-y) [.org/10.1007/s10534-008-9195-y.](https://doi.org/10.1007/s10534-008-9195-y)
- <span id="page-1-7"></span>8. Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky F. 2009. Ecological genomics of marine picocyanobacteria. Microbiol Mol Biol Rev 73:249 –299. [https://](https://doi.org/10.1128/MMBR.00035-08) [doi.org/10.1128/MMBR.00035-08.](https://doi.org/10.1128/MMBR.00035-08)
- <span id="page-1-8"></span>9. 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.](https://doi.org/10.1089/cmb.2012.0021)
- <span id="page-1-9"></span>10. Swain MT, Tsai IJ, Assefa SA, Newbold C, Berriman M, Otto TD. 2012. A post-assembly genome-improvement toolkit (PAGIT) to obtain annotated genomes from contigs. Nat Protoc 7:1260 –1284. [https://doi.org/](https://doi.org/10.1038/nprot.2012.068) [10.1038/nprot.2012.068.](https://doi.org/10.1038/nprot.2012.068)
- <span id="page-1-10"></span>11. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 29:1072-1075. [https://](https://doi.org/10.1093/bioinformatics/btt086) [doi.org/10.1093/bioinformatics/btt086.](https://doi.org/10.1093/bioinformatics/btt086)
- <span id="page-1-12"></span><span id="page-1-11"></span>12. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068 –2069. [https://doi.org/10.1093/bioinformatics/btu153.](https://doi.org/10.1093/bioinformatics/btu153)
- 13. Dhillon BK, Chiu TA, Laird MR, Langille MGI, Brinkman FSL. 2013. Island-Viewer update: improved genomic island discovery and visualization. Nucleic Acids Res 41:W129 –W132. [https://doi.org/10.1093/nar/gkt394.](https://doi.org/10.1093/nar/gkt394)
- <span id="page-1-13"></span>14. Maeda S, Ohki A, Miyahara K, Naka K, Higashi S. 1992. Metabolism of methylated arsenic compounds by arsenic-resistant bacteria (Klebsiella oxytoca and Xanthomonas sp.). Appl Organomet Chem 6:415– 420. [https://doi.org/10.1002/aoc.590060417.](https://doi.org/10.1002/aoc.590060417)