Gene Determinants for Mercury Bioremediation as Revealed by Draft Genome Sequence Analysis of *Stenotrophomonas* sp. Strain MA5

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ABSTRACT A soilborne *Stenotrophomonas* sp. strain (MA5) that is resistant to mercury was isolated. A draft genome sequence-based analysis revealed a suite of gene determinants to resist mercury and other heavy metals, multidrug efflux, stress response, and membrane transport, and these provide cues to a suite of mechanisms that underpin cellular survival in contaminated soil.

The Savannah River site (SRS) in South Carolina is a former nuclear legacy site where mercury (Hg) contamination is still pervasive (1). Hg and its microbially methylated form, methylmercury, are toxic environmental contaminants (2); however, microbiota exposed to long-term contamination can recruit genomic mechanisms to resist and detoxify Hg (3). The bacterial *mer* operon, which consists of the *merA* and *merB* gene determinants, drives the detoxification of organometallic or inorganic Hg along with genes that code for regulation (*merR*) and transport (*merT*, *merP*, and/or *merC* and *merF*). Therefore, studies of Hg-resistant bacteria (HgR) can serve as models for understanding the genomic basis of Hg cycling.

Toward this end, several bacterial strains from SRS soils were isolated on LB agar supplemented with Hg (5 μg/ml as HgCl$_2$) and incubated at 30°C. The resulting colonies were purified on LB+Hg plates until axenic strains were obtained. Bioremediative mechanisms in strain MA5 are of significant interest to the SRS, because the industrial chloralkali plants located nearby, along with other industrial processes (4), discharged Hg-laden wastes into the surrounding bodies of water, where Hg still poses public health risks. Such environments can be bioremediated using HgR bacteria (5) and fungi (6), a process which results in decontaminated wastewater (7).

To gain a deeper understanding into the genomic underpinings of Hg cycling, a single colony of strain MA5 was picked from a LB+Hg plate and inoculated into liquid LB medium and grown at 30°C in a shaker. After overnight growth, DNA was extracted with Qiagen's DNeasy PowerLyzer kit and sequenced with an Illumina HiSeq 2000 instrument (8). Default settings of the bioinformatics pipelines were used, unless specified otherwise. Genome *de novo* assembly was performed with CLC Genomics Workbench (v11.0.1; Qiagen, Aarhus, Denmark), and sequences were trimmed with a quality threshold of Q20 and a requirement of 50 bases after trimming. Approximately 8.5 million paired reads (with an average length of 118 bases) were employed for assembly. The nonscaffolded assembly generated 264 contigs, with an N_{50} value of 64,365 bases, and a total size of 4,513,544 bases, with an average coverage of 200×.

Rapid Annotations using Subsystems Technology (RAST)-based annotation (9) revealed 3,921 coding sequences and a G+C content of 66.2%. MA5 was taxonomically related to *Stenotrophomonas* spp. with One Codex analysis (10). Annotation resulted in the binning of approximately 48% of the strain's genome squence under 1,856 subsystems, with the main gene categories (number of genes) being for carbohydrate metabolism (269); cofactors, vitamins, prosthetic groups, and pigments (229); mem-

brane transport (195); resistance to antibiotics and toxic compounds (130); and stress response (119). Several gene determinants for resistance against heavy metals, including the cobalt-zinc-cadmium efflux system, arsenic detoxification system, and chromate-inducible *chrBACF* operon, along with a plethora of membrane transporters, were also identified, and they potentially enable soil survival of MA5.

Further genome mining also revealed possible resistance mechanisms against Hg, which included the presence of *merA*, which encodes the enzyme mercuric reductase (MerA); the periplasmic Hg^{2+}-scavenging protein (MerP); and the inner membrane-spanning proteins (MerT and MerE), which are engaged in the transport of Hg^{2+} to the cytoplasm and its reduction by the activity of the MerA enzyme. Finally, strain MA5 also contained the regulatory MerR and MerD proteins.

Data availability. This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession number SDHV00000000. The version described in this paper is version SDHV01000000. The genome sequences obtained from strain MA5 have been submitted to the Sequence Read Archive under the accession number SRR8541833.

REFERENCES

1. Brooks SC, Southworth GR. 2011. History of mercury use and environmental contamination at the Oak Ridge Y-12 plant. Environ Pollut 159:219–228. https://doi.org/10.1016/j.envpol.2010.09.009.
2. Lavoie RA, Jardine TD, Chumchal MM, Kidd KA, Campbell LM. 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environ Sci Technol 47:13385–13394. https://doi.org/10.1021/es403103t.
3. Dash HR, Das S. 2012. Bioremediation of mercury and the importance of bacterial *mer* genes. Int Biodeterior Biodegradation 75:207–213. https://doi.org/10.1016/j.ibiod.2012.07.023.
4. Kvartek EJ, Carlton WH, Denham M, Eldridge L, Newman MC. 1994. Assessment of mercury in the Savannah River site environment. Technical Report WSRC-TR-94-0218ET. Westinghouse Savannah River Company, Aiken, SC.
5. Figueiredo NLL, Areias A, Mendes R, Canário J, Duarte A, Carvalho C. 2014. Mercury-resistant bacteria from salt marsh of Tagus estuary: the influence of plants presence and mercury contamination levels. J Toxicol Environ Health A 77:959–971. https://doi.org/10.1080/15287394.2014.911136.
6. Hindersah R, Risamasu R, Kalay AM, Dewi T, Makatita I. 2018. Mercury contamination in soil, tailing and plants on agricultural fields near closed gold mine in Buru Island, Maluku. J Degraded Min Lands Manage 5:1027–1034. https://doi.org/10.15243/jdmlm.2018.052.1027.
7. Wagner-Döbler I. 2003. Pilot plant for bioremediation of mercury-containing industrial wastewater. Appl Microbiol Biotechnol 62:124–133. https://doi.org/10.1007/s00253-003-1322-7.
8. Chauhan A, Pathak A, Jaswal R, Edwards B, III, Chappell D, Ball C, Garcia-Sillas R, Stothard P, Seaman J. 2018. Physiological and comparative genomic analysis of *Arthrobacter* sp. SRS-W-1-2016 provides insights on niche adaptation for survival in uraniferous soils. Genes (Basel) 9:31. https://doi.org/10.3390/genes9010031.
9. Aziz R, Bartels D, Best A, DeJongh M, Disz T, Edwards R, Formsma K, Gerdes S, Glass E, Kubal M, Meyer F, Olsen G, Olson R, Osterman A, Overbeek R, McNeil L, Paarmann D, Paczian T, Parrello B, Pusch G, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
10. Minot SS, Krumm N, Greenfield NB. 2015. One Codex: a sensitive and accurate data platform for genomic microbial identification. bioRxiv 027607. https://doi.org/10.1101/027607.