



Draft Genome Sequence of *Brevibacterium linens* AE038-8, an Extremely Arsenic-Resistant Bacterium

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To understand the arsenic biogeocycles in the groundwaters at Tucumán, Argentina, we isolated *Brevibacterium linens* sp. strain AE38-8, obtained from arsenic-contaminated well water. This strain is extremely resistant to arsenicals and has arsenic resistance (*ars*) genes in its genome. Here, we report the draft genome sequence of *B. linens* AE38-8.

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A rsenic is a toxic metalloid widely distributed in nature (1, 2) and ranks first on the U.S. government's Priority List of Hazardous Substances because of its toxicity and prevalence in the environment (3). Arsenicals are introduced into the biosphere primarily from geologic formations, with some anthropogenic contributions as well (4, 5). Microorganisms have developed detoxification mechanisms that allow them to survive in high concentrations of arsenic (6). In Argentina, arsenic is a major public health concern, with millions of people exposed to arsenic in drinking water (7). The province of Tucumán is one of the most highly affected regions of the country, with high levels of arsenic in drinking water (8).

The goal of this study was to describe the arsenic biogeochemical cycle in the groundwater at Tucumán through an analysis of the genes and enzymes involved in arsenic metabolism. We sampled well water from Los Pereyra (N26°59'15.3" W64°53'56.1"), an area known for its high content of arsenic in drinking water (up to 2 mg As/liter) (8). We enriched cultures of water samples in Luria Bertani (LB) medium amended with various concentrations of As(III) (arsenite) or As(V) (arsenate), resulting in the isolation of a strain of Brevibacterium linens that tolerates arsenicals at concentrations up to 1 M in 4-fold-diluted LB medium (data not shown); this is the first reported high-level-arsenic-resistant microbe found in Argentinian waters. Most previously described resistant microorganisms tolerate only much lower concentrations of As(III) or As(V) (9-12), while B. linens sp. strain AE038-8 is resistant to high concentrations of arsenicals and also biotransforms them, making this organism a candidate for future applications in arsenic bioremediation.

Genome sequencing was performed using an Illumina HiSeq platform, with quality-based trimming, as described previously (13). After trimming, 6,211,868 paired-end reads remained, with an average length of 90 bp, comprising a genome coverage of 146×. After evaluating several approaches (14), the optimal assembly was obtained using the SPAdes software (version 3.1.1), which consisted of 29 large (≥500 bp) contigs, with a total ge-

nome size of 3.8 Mb. Gene prediction and annotation were performed at the Oak Ridge National Laboratory, as described previously (15). The draft genome sequence has an overall G+C content of 64.2%, N_{50} contig of 327 kb, a largest contig of 831 kb, and contains 3,434 candidate protein-coding genes. The genes for putative arsenic resistances identified in this genome include an ars operon containing ACR3 [encoding for a membrane efflux pump responsible for the extrusion of As(III) outside the cell] (16), ACR2 (encoding a cytosolic arsenate reductase) (17), and arsR [encoding an As(III)-responsive repressor protein] (18), as well as a putative arsM gene [encoding for an S-adenosylmethionine (SAM)-dependent methyltransferase that might methylate As(III)] (6). In summary, this extremely arsenic-resistant strain may have future usefulness in arsenic bioremediation.

Nucleotide sequence accession number. The draft genome sequence of strain AE038-8 has been deposited at DDBJ/EMBL/GenBank under the accession no. JTJZ00000000. The version described in this paper is the first version.

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REFERENCES

- Bahar M, Mezbaul M, Mallavarapu NR. 2012. Arsenic bioremediation potential of a new arsenite-oxidizing bacterium *Stenotrophomonas* sp. MM-7 isolated from soil. Biodegradation 6:803–812. http://dx.doi.org/ 10.1007/s10532-012-9567-4.
- Demergasso CS, Guillermo CD, Lorena EG, Mur JJP, Pedrós-Alió C. 2007. Microbial precipitation of arsenic sulfides in Andean salt flats. Geomicrobiol J 24:111–123. http://dx.doi.org/10.1080/01490450701266605.
- 3. Selene CH, Chou J, De Rosa CT. 2003. Case studies—arsenic. Int J Hyg Environ Health 206:381–386. http://dx.doi.org/10.1078/1438-4639-00234.
- 4. Liao VH, Chu YJ, Su YC, Hsiao SY, Wei CC, Liu CW, Liao CM, Shen

- CW, Chang FJ. 2011. Arsenite-oxidizing and arsenate-reducing bacteria associated with arsenic-rich groundwater in Taiwan. J Contam Hydrol 123:20–29. http://dx.doi.org/10.1016/j.jconhyd.2010.12.003.
- Stolz JF, Basu P, Oremland RS. 2010. Microbial arsenic metabolism: new twists on an old poison. Microbe 5:53–59. http://dx.doi.org/10.1128/microbe.5.53.1.
- Zhu Y, Yoshinaga M, Zhao F, Rosen BP. 2014. Earth abides arsenic biotransformations. Annu Rev Earth Planet Sci 42:443–467. http:// dx.doi.org/10.1146/annurev-earth-060313-054942.
- De Esparza MC. 2006. Presencia de arsénico en el agua de bebida en América Latina y su efecto en la salud pública. Int Cong Nat Arsenic Groundwaters Lat Amer, 20 to 24 June 2006, Mexico City, Mexico.
- Rodríguez M, D'Urso C, Rodríguez G, López JP, Sales A. 2006. Ocurrencia de arsénico en aguas subterráneas en el noreste de Tucumán, Argentina. VIII Encuentro de Química Analítica y Ambiental, Iquique, Chile.
- Escalante G, Campos VL, Valenzuela C, Yañez J, Zaror C, Mondaca MA. 2009. Arsenic resistant bacteria isolated from arsenic contaminated river in the Atacama Desert (Chile). Bull Environ Contam Toxicol 83: 657–661. http://dx.doi.org/10.1007/s00128-009-9868-4.
- Jackson C, Dugas S, Harrison K. 2005. Enumeration and characterization of arsenate-resistant bacteria in arsenic free soils. Soil Biol Biochem 12:2319–2322. http://dx.doi.org/10.1016/j.soilbio.2005.04.010.
- Macur RE, Jackson CR, Botero LM, Mcdermott TR, Inskeep WP. 2004. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. Environ Sci Technol 38:104–111. http://dx.doi.org/10.1021/es034455a.

- 12. Sarkar A, Kazy SK, Sar P. 2013. Characterization of arsenic resistant bacteria from arsenic rich groundwater of West Bengal, India. Ecotoxicology 22:363–376. http://dx.doi.org/10.1007/s10646-012-1031-z.
- 13. Woo HL, Utturkar S, Klingeman D, Simmons BA, DeAngelis KM, Brown SD, Hazen TC. 2014. Draft genome sequence of the lignin-degrading *Burkholderia* sp. strain lig30, isolated from wet tropical forest soil. Genome Announc 2(3):e00637-14. http://dx.doi.org/10.1128/genomeA.00637-14.
- 14. Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD. 2014. Evaluation and validation of *de novo* and hybrid assembly techniques to derive high-quality genome sequences. Bioinformatics 30:2709–2716. http://dx.doi.org/10.1093/bioinformatics/btu391.
- Brown SD, Utturkar SM, Klingeman DM, Johnson CM, Martin SL, Land ML, Lu TY, Schadt CW, Doktycz MJ, Pelletier DA. 2012. Twentyone genome sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of *Populus deltoides*. J Bacteriol 194:5991–5993. http://dx.doi.org/ 10.1128/JB.01243-12.
- Yang HC, Fu HL, Lin YF, Rosen BP. 2012. Pathways of arsenic uptake and efflux. Curr Top Membr 69:325–358. http://dx.doi.org/10.1016/B978 -0-12-394390-3.00012-4.
- 17. Mukhopadhyay R, Rosen BP, Phung LT, Silver S. 2002. Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol Rev 26: 311–325. http://dx.doi.org/10.1111/j.1574-6976.2002.tb00617.x.
- 18. Xu C, Rosen BP. 1999. Metalloregulation of soft metal resistance pumps. *In Sarkar B (ed)*, Metals and genetics. Plenum Press, New York, NY.