AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Draft Whole-Genome Sequence of the Alkaliphilic *Alishewanella aestuarii* Strain HH-ZS, Isolated from Historical Lime Kiln Waste-Contaminated Soil

Zohier B. Salah, Simon P. Rout, Paul N. Humphreys

Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, United Kingdom

Here, we present the whole-genome sequence of an environmental Gram-negative *Alishewanella aestuarii* strain (HH-ZS), isolated from the hyperalkaline contaminated soil of a historical lime kiln in Buxton, United Kingdom.

Received 27 October 2016 **Accepted** 28 October 2016 **Published** 29 December 2016

Citation Salah ZB, Rout SP, Humphreys PN. 2016. Draft whole-genome sequence of the alkaliphilic *Alishewanella aestuarii* strain HH-ZS, isolated from historical lime kiln waste-contaminated soil. Genome Announc 4(6):e01447-16 doi:10.1128/genomeA.01447-16.

Address correspondence to Paul N. Humphreys, p.n.humphreys@hud.ac.uk.

The genus *Alishewanella* was first described by Vogel et al. (1) following the isolation of *A. fetalis* strain CCUG 30811^T from the autopsy of a human fetus in Sweden. The genus has received further attention following isolations from tidal flats (2), mountainous lakes (3), landfill soils (4), industrially contaminated soils (5), and traditional fermented foods (6). This genus has received particular attention because of its bioremediation potential and broad temperature and pH ranges for growth (7–9). More recently, this genus has been observed within a floc-forming, mixed microbial community surviving at a pH of 11 (10).

The soils of a historical lime kiln waste deposition site in Buxton, United Kingdom, are hyperalkaline (pH 10.8 to 11.7) as a result of interaction with these deposits and percolating rain waters (11). A subsample of soil (5 g) was mixed with anoxic mineral medium (12) supplemented with cellulose degradation products (13) and adjusted to pH 12. Following incubation at 25°C for 30 days, a sample of the mixed culture was streaked onto fastidious anaerobe agar (pH 9.5; LabM, United Kingdom) and then incubated anaerobically (10% H_2, 10% CO_2, 80% N_2; DW Scientific, United Kingdom) at 25°C for three days. A single colony was selected and further purified through subculture and was determined to be capable of growth at between pH 7 to 11 with an optimum of 9. Genomic DNA was extracted using a commercial kit (Ultraclean microbial isolation kit; Mo Bio, USA), and the bacterial species was identified as having a (99.6%) homology to *A. aestuarii* strain B11 by 16s rRNA sequencing.

A whole genome was obtained using Illumina HiSeq 2500 technology, generating paired-end 125-cycle sequence reads (BaseClear, Netherlands). Illumina CASAVA and CLC Genomics Workbench version 8.5.1 were used to generate FASTQ sequence files and assembly, respectively. Scaffolds or supercontigs were generated by linking the contigs (14). Finally, the bacterial genome was annotated via the NCBI Prokaryotic Genome Automatic Annotation Pipeline (15) and RAST server (16). In this study, the whole-genome sequence of *A. aestuarii* strain HH-ZS comprised 3,531,586 bp, encoding for 3,304 putative coding sequences, of which 71 have been classified as pseudogenes and 3,236 as hypothetical proteins; 3,165 were predicted to form known functional proteins. The genome has a G+C content of 51.0% and contains 68 genes encoding rRNA (5S, 16S, 23S), 60 tRNA, and five ncRNA. RAST system annotation assigned proteins to stress response, metal resistance (As, Cd, Co, Cr, Zn) and tolerance (Cu), metabolism of carbohydrates, and aromatic compounds associated with contaminated and high-pH environments (5, 7, 10).

Accession number(s). The whole-genome sequence of this project (*Alishewanella aestuarii* HH-ZS) has been deposited at DDBJ/EMBL/GenBank under the accession number LZEJ00000000.

FUNDING INFORMATION

This work, including the efforts of Zohier B. Salah, was funded by a Libyan Government Ph.D. Scholarship.

REFERENCES

1. **Vogel BF, Venkateswaran K, Christensen H, Falsen E, Christiansen G, Gram L.** 2000. Polyphasic taxonomic approach in the description of *Alishewanella fetalis* gen. nov., sp. nov., isolated from a human foetus. Int J Syst Evol Microbiol **50:**1133–1142. http://dx.doi.org/10.1099/00207713-50-3-1133.
2. **Roh SW, Nam YD, Chang HW, Kim KH, Kim MS, Oh HM, Bae JW.** 2009. *Alishewanella aestuarii* sp. nov., isolated from tidal flat sediment, and emended description of the genus *Alishewanella*. Int J Syst Evol Microbiol **59:**421–424. http://dx.doi.org/10.1099/ijs.0.65643-0.
3. **Tarhriz V, Nematzadeh G, Zununi Vahed SZ, Hejazi MA, Hejazi MS.** 2012. *Alishewanella tabrizica* sp. nov., isolated from Qurugöl Lake. Int J Syst Evol Microbiol **62:**1986–1991. http://dx.doi.org/10.1099/ijs.0.031567-0.
4. **Kim MS, Jo SK, Roh SW, Bae JW.** 2010. *Alishewanella agri* sp. nov., isolated from landfill soil. Int J Syst Evol Microbiol **60:**2199–2203. http://dx.doi.org/10.1099/ijs.0.011684-0.
5. **Kolekar YM, Pawar SP, Adav SS, Zheng LQ, Li WJ, Shouche YS, Dastager SG, Kodam KM.** 2013. *Alishewanella solinquinati* sp. nov., isolated from soil contaminated with textile dyes. Curr Microbiol **67:**454–459. http://dx.doi.org/10.1007/s00284-013-0385-7.
6. **Kim MS, Roh SW, Nam YD, Chang HW, Kim KH, Jung MJ, Choi JH, Park EJ, Bae JW.** 2009. *Alishewanella jeotgali* sp. nov., isolated from traditional fermented food, and emended description of the genus *Alishewanella*. Int J Syst Evol Microbiol **59:**2313–2316. http://dx.doi.org/10.1099/ijs.0.007260-0.
7. **Jain R, Jha S, Adhikary H, Kumar P, Parekh V, Jha A, Mahatma MK,**

Kumar GN. 2014. Isolation and molecular characterization of arsenite-tolerant *Alishewanella* sp. GIDC-5 originated from industrial effluents. Geomicrobiol J **31:**82–90. http://dx.doi.org/10.1080/01490451.2013.811317.

8. **Kolekar YM, Kodam KM**. 2012. Decolorization of textile dyes by *Alishewanella* sp. KMK6. Appl Microbiol Biotechnol **95:**521–529. http://dx.doi.org/10.1007/s00253-011-3698-0.
9. **Kim J, Jung J, Sung JS, Chun J, Park W**. 2012. Genome sequence of pectin-degrading *Alishewanella agri*, isolated from landfill soil. J Bacteriol **194:**5135–5136. http://dx.doi.org/10.1128/JB.01129-12.
10. **Charles CJ, Rout SP, Garratt EJ, Patel K, Laws AP, Humphreys PN**. 2015. The enrichment of an alkaliphilic biofilm consortia capable of the anaerobic degradation of isosaccharinic acid from cellulosic materials incubated within an anthropogenic, hyperalkaline environment. FEMS Microb Ecol **91:**fiv085. http://dx.doi.org/10.1093/femsec/fiv085.
11. **Rout SP, Charles CJ, Garratt EJ, Laws AP, Gunn J, Humphreys PN**. 2015. Evidence of the generation of isosaccharinic acids and their subsequent degradation by local microbial consortia within hyper-alkaline contaminated soils, with relevance to intermediate level radioactive waste disposal. PLoS One **10:**e0119164. http://dx.doi.org/10.1371/journal.pone.0119164.
12. **British Standards Institute**. 2005. Plastics-determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system—method by measurement of biogas production. BS ISO 14853:2005. British Standards Institute, London, UK.
13. **Cowper M, Marshall T, Swanton S**. 2011. Sorption detriments in the geosphere: the effect of cellulose degradation products. Phase 1 experimental study. /026. Nirex Ltd., Harwell, Didcot, Oxfordshire, UK.
14. **Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W**. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics **27:**578–579. http://dx.doi.org/10.1093/bioinformatics/btq683.
15. **Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity GM, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O**. 2008. Toward an online repository of standard operating procedures (SOPs) for (meta) genomic annotation. OMICS **12:**137–141. http://dx.doi.org/10.1089/omi.2008.0017.
16. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, 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. http://dx.doi.org/10.1186/1471-2164-9-75.