Draft Genome Sequence of *Alkalicoccus halolimnae* BZ-SZ-XJ29ᵀ, a Moderately Halophilic Bacterium Isolated from a Salt Lake

Yingjie Zhang, a Dacheng Qiu, a Ziya Liao, a Baisuo Zhao a

aGraduate School, Chinese Academy of Agricultural Sciences, Beijing, People’s Republic of China

**ABSTRACT**  The moderate halophile *Alkalicoccus halolimnae* BZ-SZ-XJ29ᵀ grows optimally in a relative broad range of 8.3% to 12.3% (wt/vol) NaCl. The draft genome consists of approximately 3.66 Mb and contains 3,534 putative genes. Various genes involved in osmotic stress were predicted, providing pertinent insights into specific adaptations to the hypersaline environment.

The mesophilic moderately halophilic bacterium *Alkalicoccus halolimnae* BZ-SZ-XJ29ᵀ was aerobically isolated from a mixture of water and sediment from a salt lake in Xinjiang Uyghur Autonomous Region, China (1). Its growth occurs in the ranges of 4.3% to 24.3% (wt/vol) NaCl, pH 6.0 to 10.5, and 5°C to 41°C. To gain insight into the osmotic adaptive strategies of hypersaline stress, the draft genome of strain BZ-SZ-XJ29ᵀ was sequenced using an Illumina HiSeq 4000 platform.

Total genomic DNA (2 μg) was extracted from strain BZ-SZ-XJ29ᵀ grown under optimal conditions, as described previously (1), using a microbial DNA isolation kit (iTOP, Beijing, China) following the manufacturer’s instructions. A library for genome sequencing was constructed using the whole-genome shotgun approach with the TruSeq DNA sample preparation kit (Illumina, USA), HiSeq PE cluster kit v4-cBot (Illumina), and HiSeq 3000/4000 SBS kit (Illumina) (2, 3). Sequencing was performed with a paired-end read length of 2 × 150 bp at approximately 200× coverage. The filtered reads were quality trimmed using Quake and the Burrows-Wheeler Aligner (BWA) with the default program parameters and were *de novo* assembled into contigs using SOAPdenovo2 (4). A total of 5,027,473 reads with a total length of 3,668,659 bp were assembled into 59 contigs, with a GC content of 44.9% and an *N₅₀* value of 241,109 bp. Automatic annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok). Subsequently, the genome files were uploaded to the IMG-ER tool (https://img.jgi.doe.gov/cgi-bin/submit/main.cgi) for functional annotation. Among the 3,534 genes identified, 3,435 were potential protein-coding genes. Also predicted were 55 RNAs, including 4 rRNAs (2 5S RNAs, 1 16S RNA, and 1 23S RNA), 45 tRNAs, and 6 other RNAs.

Genome sequence analysis showed the presence of a number of genes encoding putative proteins potentially related to the osmotic strategies for surviving in a hypersaline environment. Identified were one gene cluster (*ectA*, *ectB*, and *ectC*) for ectoine biosynthesis from aspartate semialdehyde, the *betaA* gene and *betaB* gene for glycine betaine biosynthesis from choline, the *glnA* gene for l-glutamine biosynthesis from l-glutamate, the *prov* gene and *provW* gene for the glycine betaine/proline ABC transporter, the *opuAC* and *opuBD* genes for the osmoprotectant (i.e., choline, glycine betaine, and proline) transport system (ABC transporters), and the *opuG* gene for glycine betaine/proline transporters (betaine/carnitine/choline transporter [BCCT] family). All genes mentioned are important for maintaining osmotic balance though the “compatible solutes strategy” under high-salt conditions. Furthermore, four genes...
coding for Na\(^+\)/solute symporters (5–8), nine genes coding for the multisubunit Na\(^+\)/H\(^+\) antiporter (9, 10), and three genes coding for a monovalent cation/proton antiporter (11) were predicted. These genes might be involved in salt stress by maintaining Na\(^+\) homeostasis. Also detected were four genes (three TrkA type and one TrkH type) responsible for K\(^+\) uptake systems, implying that strain BZ-SZ-XJ29\(^T\) may gain rapidly isosmotic cytoplasm through K\(^+\) as an osmolyte when coping with osmotic shock (12). As described above, many predicted genes in the genome of strain BZ-SZ-XJ29\(^T\) offer valuable insights to reveal the adaptive mechanisms for maintaining osmotic balance and Na\(^+\) homeostasis under conditions of elevated salinity.

**Data availability.** The draft genome sequence of *Alkalicoccus halolimnae* BZ-SZ-XJ29\(^T\) has been deposited at GenBank under the accession number VPFE00000000. The raw sequencing reads have been submitted to the Sequence Read Archive (SRA accession number SRR9943993) and are available at NCBI under BioProject number PRJNA559242 and BioSample number SAMN12530361.

**ACKNOWLEDGMENTS**

This work was supported by grant 31570110 from the National Science Foundation of China (NSFC) and grants 1610042020002 and 1610042018005 from the Foundation of Graduate School of the Chinese Academy of Agricultural Sciences (CAAS).

**REFERENCES**

1. Zhao B, Lu W, Zhang S, Liu K, Yan Y, Li J. 2017. Reclassification of *Bacillus saliphilus* as *Alkalicoccus saliphilus* gen. nov., comb. nov., and description of *Alkalicoccus halolimnae* sp. nov., a moderately halophilic bacterium isolated from a salt lake. Int J Syst Evol Microbiol 67:1557–1563. [https://doi.org/10.1099/ijsem.0.001759](https://doi.org/10.1099/ijsem.0.001759).
2. Aparicio S, Chapman J, Stupka E, Putnam N, Chia J-M, Dehal P, Christoffels A, Rash S, Hoon S, Smit A, Gelpke MDS, Roach J, Oh T, Ho IV, Wong M, Detter C, Verhoef F, Predki P, Tay A, Lucas S, Richardson P, Smith SF, Clark MS, Edwards YJK, Doggett N, Zharkikh A, Tavitgian SV, Pruss D, Barnstead M, Evans C, Baden H, Powell J, Glusman G, Rowen L, Hood YH, Elgar G, Hawkins T, Venkatesh B, Rokhsar D, Brenner S. 2002. Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. Science 297:1301–1310. [https://doi.org/10.1126/science.1072104](https://doi.org/10.1126/science.1072104).
3. Goodwin S, McPherson JD, McCombie WR. 2016. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 17:333–351. [https://doi.org/10.1038/nrg.2016.49](https://doi.org/10.1038/nrg.2016.49).
4. Kelley DR, Schatz MC, Salzberg SL. 2010. Quake: quality-aware detection and correction of sequencing errors. Genome Biol 11:R116. [https://doi.org/10.1186/gb-2010-11-11-r116](https://doi.org/10.1186/gb-2010-11-11-r116).
5. Roberts MF. 2005. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems 1:5. [https://doi.org/10.1186/1746-1448-1-5](https://doi.org/10.1186/1746-1448-1-5).
6. Kuhlmann AU, Bremer E. 2002. Osmotically regulated synthesis of the compatible solute ectoine in *Bacillus pasteurii* and related *Bacillus* spp.

**Zhang et al.**

**Volume 9 Issue 27 e00500-20 mra.asm.org**

Appl Environ Microbiol 68:772–783. [https://doi.org/10.1128/aem.68.2.772-783.2002](https://doi.org/10.1128/aem.68.2.772-783.2002).
7. Banciu HL, Muntyan MS. 2015. Adaptive strategies in the double-extremophilic prokaryotes inhabiting soda lakes. Curr Opin Microbiol 25:73–79. [https://doi.org/10.1016/j.mib.2015.05.003](https://doi.org/10.1016/j.mib.2015.05.003).
8. Olkhova E, Raba M, Bracher S, Hilger D, Jung H. 2011. Homology model of the Na\(^+\)/proline transporter PutP of *Escherichia coli* and its functional implications. J Mol Biol 406:59–74. [https://doi.org/10.1016/j.jmb.2010.11.045](https://doi.org/10.1016/j.jmb.2010.11.045).
9. Padan E, Bibi E, Ito M, Kruilwich TA. 2005. Alkaline pH homeostasis in bacteria: new insights. Biochim Biophys Acta 1717:67–88. [https://doi.org/10.1016/j.bbamem.2005.09.010](https://doi.org/10.1016/j.bbamem.2005.09.010).
10. Xu N, Wang L, Cheng H, Liu Q, Liu J, Ma Y. 2016. In vitro functional characterization of the Na\(^+\)/H\(^+\) antiporters in *Corynebacterium glutamicum*. FEMS Microbiol Lett 363:fnv237. [https://doi.org/10.1093/femsle/fnv237](https://doi.org/10.1093/femsle/fnv237).
11. Banciu HL, Sorokin DY. 2013. Adaptation in haloalkaliphiles and natronophilic bacteria, p 121–178. In Seckbach J, Oren A, Stan-Lotter H (ed), Polyextremophiles: life under multiple forms of stress. Springer, Dordrecht, The Netherlands.
12. Jones MK, Warner E, Oliver JD. 2008. Survival of and in situ gene expression by *Vibrio vulnificus* at varying salinities in estuarine environments. Appl Environ Microbiol 74:182–187. [https://doi.org/10.1128/AEM.02436-07](https://doi.org/10.1128/AEM.02436-07).