

# Complete Genome Sequence of *Alkaliphilus metalliredigens* Strain QYMF, an Alkaliphilic and Metal-Reducing Bacterium Isolated from Borax-Contaminated Leachate Ponds

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***Alkaliphilus metalliredigens* strain QYMF is an anaerobic, alkaliphilic, and metal-reducing bacterium associated with phylum Firmicutes. QYMF was isolated from alkaline borax leachate ponds. The genome sequence will help elucidate the role of metal-reducing microorganisms under alkaline environments, a capability that is not commonly observed in metal respiring-microorganisms.**

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*Alkaliphilus metalliredigens* QYMF is a strict anaerobic bacterium isolated from alkaline borax leachate ponds with concentrations of sodium and boron that ranged from 0.04 to 0.53 M and 0.19 and 0.28 M, respectively, at U. S. Borax Company (Boron, CA). QYMF is capable of utilizing Fe(III)-citrate, Fe(III)-EDTA, Co(III)-EDTA, and Cr(VI) as electron acceptors during growth with yeast extract or lactate as electron donors. Optimum growth conditions for QYMF with the metals listed above were observed at pH 9.6, 35°C, and a NaCl concentration of 20 g/L in the presence of 2 g/L of borate (1). The metal-reducing capability under alkaliphilic growth conditions (up to pH 11) in the presence of elevated salt levels has not been demonstrated in other *Alkaliphilus* isolates (2–6) nor other metal-reducing bacteria. The novel and unique lifestyle of QYMF warranted the determination of the genome sequence as it pertains to a better appreciation of the genetic and physiological diversity of metal-reducing microorganisms that thrive in extreme environmental conditions, including toxic metal-contaminated sites. Moreover, the genomic data provides insight into the biogeochemical processes on early Earth, which can lend knowledge to the exploration of the boundaries for extraterrestrial microbial life.

The genome sequence was determined with the Sanger sequencing method by the U.S. DOE Joint Genome Institute (JGI). The completed genome was curated and annotated as previously described (7–9). Putative gene functions were also assigned with public databases including COG, KEGG, Pfam, TIGRFam, and InterPro. The resultant genome size is 4.93 Mb with 36.8% G+C content. Genome sequences of three other *Alkaliphilus* species

have been completed. While the G+C content is comparable to other species, QYMF has a larger genome size than *A. oremalandii* strain OhILAs (3.12 Mb, 36.3% G+C), *A. transvaalensis* (4.02 Mb, 33.95% G+C), and *A. peptidifermentans* (4.45 Mb, 34.09% G+C). Comparison of SSU rRNA gene shows that QYMF has 93 to 96% nucleotide identity to these sequenced *Alkaliphilus* species. The genome sequence of QYMF contains 5,016 identified putative genes, of which 4,801 are protein coding genes with functions predicted for 3,130 genes, a total of 31 rRNA genes (11 are 5S rRNA gene and 10 are 16S and 23S rRNA gene each), 106 tRNA genes, and 78 other RNA genes.

Although growth of QYMF with arsenic has not been characterized, the leachate ponds from where QYMF was isolated contained As concentrations of approximately 1.7 mM. Genome analysis of QYMF indicates the presence of genes encoding arsenical resistance proteins and two novel *ars* operons that encodes arsenite efflux permeases (*Acr3*), whose mechanism of transport was characterized by Fu et al. (10, 11). Recently, the *aroA* gene of QYMF, which encodes for the enzyme 5-enopyruvylshikimate-3-phosphate synthase, was shown to have the potential in developing glyphosate-resistant crops (12). This implicates the potential use of arsenite efflux mechanism of QYMF as a strategy to reduce As accumulation in rice, which has been demonstrated with the expression of *ScAcr3p*, an arsenite plasma membrane transporter, from *Saccharomyces cerevisiae* to rice grains (13). Future work should assess the use of metal-tolerant bacteria in various applications.

**Accession number(s).** *A. metalliredigens* was deposited at GenBank, National Center for Biotechnology Information, under accession number [CP000724](https://doi.org/10.1128/AEM.70.9.5595-5602.2004).

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#### REFERENCES

- Ye Q, Roh Y, Carroll SL, Blair B, Zhou J, Zhang CL, Fields MW. 2004. Alkaline anaerobic respiration: isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. *Appl Environ Microbiol* 70: 5595–5602. <http://dx.doi.org/10.1128/AEM.70.9.5595-5602.2004>.
- Takai K, Moser DP, Onstott TC, Spoelstra N, Piffner SM, Dohnalkova A, Fredrickson JK. 2001. *Alkaliphilus transvaalensis* gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. *Int J Syst Evol Microbiol* 51:1245–1256. <http://dx.doi.org/10.1099/00207713-51-4-1245>.
- Cao X, Liu X, Dong X. 2003. *Alkaliphilus crotonatoxidans* sp. nov., a strictly anaerobic, crotonate-dismutating bacterium isolated from a methanogenic environment. *Int J Syst Evol Microbiol* 53:971–975. <http://dx.doi.org/10.1099/ijs.0.02373-0>.
- Fisher E, Dawson AM, Polshyna G, Lisak J, Crable B, Perera E, Ranganathan M, Thangavelu M, Basu P, Stolz JF. 2008. Transformation of inorganic and organic arsenic by *Alkaliphilus oremlandii* sp. nov. strain OhILAs. *Ann N Y Acad Sci* 1125:230–241. <http://dx.doi.org/10.1196/annals.1419.006>.
- Ben Aissa F, Postec A, Erauso G, Payri C, Pelletier B, Hamdi M, Fardeau ML, Ollivier B. 2015. Characterization of *Alkaliphilus hydrothermalis* sp. nov., a novel alkaliphilic anaerobic bacterium, isolated from a carbonaceous chimney of the Prony hydrothermal field, New Caledonia. *Extremophiles* 19: 183–188. <http://dx.doi.org/10.1007/s00792-014-0697-y>.
- Zhilina TN, Zavarzina DG, Kolganova TV, Lysenko AM, Tourova TP. 2009. *Alkaliphilus peptidofragmentans* sp. nov., a new alkaliphilic bacterial soda lake isolate capable of peptide fermentation and Fe(III) reduction. *Microbiology* 78:445–454. <http://dx.doi.org/10.1134/S0026261709040080>.
- Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <http://dx.doi.org/10.1186/1471-2105-11-119>.
- Pati A, Ivanova NN, Mikhailova N, Ovcinnikova G, Hooper SD, Lykidis A, Kyrpides NC. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. *Nat Methods* 7:455–457.
- Markowitz VM, Szeto E, Palaniappan K, Grechkin Y, Chu K, Chen IM, Dubchak I, Anderson I, Lykidis A, Mavromatis K, Ivanova NN, Kyrpides NC. 2008. The integrated microbial genomes (IMG) system in 2007: data content and analysis tool extensions. *Nucleic Acids Res* 36: D528–D533. <http://dx.doi.org/10.1093/nar/gkm846>.
- Fu HL, Meng Y, Ordóñez E, Villadangos AF, Bhattacharjee H, Gil JA, Mateos LM, Rosen BP. 2009. Properties of arsenite efflux permeases (Acr3) from *Alkaliphilus metalliredigens* and *Corynebacterium glutamicum*. *J Biol Chem* 284:19887–19895. <http://dx.doi.org/10.1074/jbc.M109.011882>.
- Fu HL, Rosen BP, Bhattacharjee H. 2010. Biochemical characterization of a novel ArsA ATPase complex from *Alkaliphilus metalliredigens* QYMF. *FEBS Lett* 584:3089–3094. <http://dx.doi.org/10.1016/j.febslet.2010.05.044>.
- Xing XJ, Tian YS, Peng RH, Xu J, Zhao W, Yao QH, Sun S. 2014. Functional characterization of 5-enopyruvylshikimate-3-phosphate synthase from *Alkaliphilus metalliredigens* in transgenic *Arabidopsis*. *J Microbiol Biotechnol* 24:1421–1426. <http://dx.doi.org/10.4014/jmb.1404.04023>.
- Duan G, Kamiya T, Ishikawa S, Arao T, Fujiwara T. 2012. Expressing ScACR3 in rice enhanced arsenite efflux and reduced arsenic accumulation in rice grains. *Plant Cell Physiol* 53:154–163. <http://dx.doi.org/10.1093/pcp/pcr161>.