



Draft Genome Sequences of Two Novel Acidimicrobiaceae Members from an Acid Mine Drainage Biofilm Metagenome

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Bacteria belonging to the family *Acidimicrobiaceae* are frequently encountered in heavy metal-contaminated acidic environments. However, their phylogenetic and metabolic diversity is poorly resolved. We present draft genome sequences of two novel and phylogenetically distinct *Acidimicrobiaceae* members assembled from an acid mine drainage biofilm metagenome.

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embers of the family Acidimicrobiaceae, with the exception of *Ilumatobacter* sp., are typically found in acidic, metal laden environments where they characteristically oxidize ferrous iron (1). Although of interest for environmental applications (2), there are limited data on the phylogenetic and metabolic diversity within this family. Of the five Acidimicrobiaceae genome assemblies available, only two are complete and annotated (Acidimicrobium ferrooxidans DSM 10331 [3] and Ilumatobacter coccineus YM16-304 [4]). We present two additional, >90% complete draft genomes of novel Acidimicrobiaceae members, designated RAAP-2 and RAAP-3, from an acid-mine drainage (AMD) metagenome originating from a streamer biofilm growing in acidic $(pH \sim 3)$ heavy metal-contaminated mine-water in Colorado, USA. DNA was extracted using the Power Soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). The genomic DNA library was prepared using an Illumina TruSEQ DNA library kit and sequenced on an Illumina HiSEQ 2500 paired end flow cell $(2 \times 125$ -bp read length, V4 Chemistry) at the Genomics and Microarray Core, University of Colorado, Denver. Reads were coassembled with four additional samples using IDBA-UD (5), followed by binning of scaffolds using CONCOCT (6). Reads mapping to scaffolds within each genome bin were extracted and reassembled using IDBA-UD. Post-reassembly, contigs less than 1 kb, and coverage profile outliers were removed. This resulted in a draft genome size of 2.24 MB for RAAP-2 and 3.05 MB for RAAP-3 with 58 and 149 contigs and G+C content of 65 and 47%, respectively. CheckM (7) estimated the completeness of the genomes to be 91.5% and 98.3%, respectively, with <1.5% contamination. The two draft genomes shared 70.6% average nucleotide identity with each other (genome-to-genome distance calculator [http://ggdc.dsmz.de/]). The genomes were annotated using Prodigal (8) and RAPSearch2 (9) to identify best matches in the KEGG database (10). RAAP-2 and RAAP-3 consisted of 2,174 and 2,668 coding DNA sequences with 2,022 and 2,244 matches to the KEGG database, respectively. AMPHORA2 (11) indi-

cated the phylogenetic placement of these two genome bins as Acidimicrobium. A phylogenetic tree was constructed using a concatenated alignment of 16 ribosomal proteins (12) using 66 representative genomes from the phylum Actinobacteria. This indicated that RAAP-2 should be placed between A. ferrooxidans and I. coccineus, whereas RAAP-3 was more closely related to A. ferrooxidans. Noticeable metabolic differences between these draft genomes and A. ferrooxidans include a ferric iron transport system absent in A. ferrooxidans. Moreover, RAAP-2 contains an additional iron complex transport system and a complete pathway for assimilatory sulfate reduction. This pathway is absent in RAAP-3, which is more closely related to isolate DSM 10331 derived from geothermal hot springs (13) where steam vents constantly supply $H_2S(14)$, hence reducing the benefit of assimilatory sulfate reduction. These draft genomes provide additional information on the ecology, diversity, and metabolic potential of Acidimicrobiaceae, which is beneficial for expanding our knowledge of microbial ecology in metal-contaminated acidic waters.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at GenBank under accession numbers LMAD00000000 (RAAP-2) and LMAE00000000 (RAAP-3). The versions described in this paper are LMAD01000000 and LMAE01000000, respectively.

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REFERENCES

- Watkin ELJ, Keeling SE, Perrot FA, Shiers DW, Palmer M-L, Watling HR. 2009. Metals tolerance in moderately thermophilic isolates from a spent copper sulfide heap, closely related to *Acidithiobacillus caldus, Acidimicrobium ferrooxidans* and *Sulfobacillus thermosulfidooxidans*. J Ind Microbiol Biotechnol 36:461–465. http://dx.doi.org/10.1007/s10295-008 -0508-5.
- Zammit CM, Mangold S, rao Jonna V, Mutch LA, Watling HR, Dopson M, Watkin ELJ. 2012. Bioleaching in brackish waters—effect of chloride ions on the acidophile population and proteomes of model species. Appl Microbiol Biotechnol 93:319–329. http://dx.doi.org/10.1007/s00253-011 -3731-3.
- Clum A, Nolan M, Lang E, Del Rio TG, Tice H, Copeland A, Cheng J, Lucas S, Chen F, Bruce D, Goodwin L, Pitluck S, Ivanova N, Mavrommatis K, Mikhailova N, Pati A, Chen A, Palaniappan K, Göker M, Spring S, Land M, Hauser L, Chang Y-J, Jeffries CC, Chain P, Bristow J, Eisen JA, Markowitz V, Hugenholtz P, Kyrpides NC, Klenk H-P, Lapidus A. 2009. Complete genome sequence of *Acidimicrobium ferrooxidans* type strain (ICP^T). Stand Genomic Sci 1:38–45. http://dx.doi.org/ 10.4056/sigs.1463.
- 4. Fujinami S, Takarada H, Kasai H, Sekine M, Omata S, Harada T, Fukai R, Hosoyama A, Horikawa H, Kato Y, Nakazawa H, Fujita N. 2013.

Complete genome sequence of *Ilumatobacter coccineum* YM16-304T. Stand Genomic Sci 8:430–440. http://dx.doi.org/10.4056/sigs.4007734.

- Peng Y, Leung HCM, Yiu SM, Chin FYL. 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28:1420–1428. http://dx.doi.org/10.1093/ bioinformatics/bts174.
- Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, Lahti L, Loman NJ, Andersson AF, Quince C. 2014. Binning metagenomic contigs by coverage and composition. Nat Methods 11: 1144–1146. http://dx.doi.org/10.1038/nmeth.3103.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. http://dx.doi.org/10.1101/gr.186072.114.
- Hyatt D, Chen G, 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.
- Zhao Y, Tang H, Ye Y. 2012. RAPSearch2: a fast and memory-efficient protein similarity search tool for next-generation sequencing data. Bio-Informatics 28:125–126. http://dx.doi.org/10.1093/bioinformatics/btr595.
- Kanehisa M, Goto S, Kawashima S, Nakaya A. 2002. The KEGG databases at GenomeNet. Nucleic Acids Res 30:42-46. http://dx.doi.org/ 10.1093/nar/30.1.42.
- Wu M, Scott AJ. 2012. Phylogenomic analysis of bacterial and archaeal sequences with AMPHORA2. Bioinformatics 28:1033–1034. http:// dx.doi.org/10.1093/bioinformatics/bts079.
- Sorek R, Zhu Y, Creevey CJ, Francino MP, Bork P, Rubin EM. 2007. Genome-wide experimental determination of barriers to horizontal gene transfer. Science 318:1449–1452. http://dx.doi.org/10.1126/ science.1147112.
- Norris PR, Owen JP. 1993. Mineral sulphide oxidation by enrichment cultures of novel thermoacidophilic bacteria. FEMS Microbiol Rev 11: 51–56. http://dx.doi.org/10.1111/j.1574-6976.1993.tb00266.x.
- Markússon SH, Stefánsson A. 2011. Geothermal surface alteration of basalts, Krýsuvík Iceland—alteration mineralogy, water chemistry and the effects of acid supply on the alteration process. J Volcanol Geotherm Res 206:46–59. http://dx.doi.org/10.1016/j.jvolgeores.2011.05.007.