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# Draft Genome Sequences of 16 Halophilic Prokaryotes Isolated from Diverse Environments

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**ABSTRACT** Halophile-specific enzymes have wide-ranging industrial and commercial applications. Despite their importance, there is a paucity of available halophile whole-genome sequences. Here, we report the draft genome sequences of 16 diverse salt-tolerant strains of bacteria and archaea isolated from a variety of high-salt environments.

Halophiles have the ability to survive and thrive in high salinity. Halophile-specific enzymes can be harnessed for use in a variety of commercial and industrial applications, making them a valuable target for study (1, 2). As part of a two-quarter course series, undergraduate students isolated and cultured microbes obtained from several high-salinity substrates (Table 1). All samples from Puerto Rico were from salt flats in Cabo Rojo. Halobacterium medium 372 was used as the culturing medium (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium372.pdf). To isolate a wider variety of strains, samples were plated in medium 372 at multiple salinity levels, either 100, 150, 200, or 250 g NaCl per 1,000 ml of medium. To grow a diversity of strains, we used both direct-plated samples and samples plated from liquid enrichment cultures. For solid samples, direct plating required hydrating the sample with $H_2O$ at a density of 200 g of sample per liter. To create enrichment cultures, we combined 100 $\mu$l of sample with 3 ml of liquid medium and incubated it at 37°C until turbid in ≤7 days. Colonies were grown by spread plating of the prepared samples. Pure liquid cultures were created from isolated plate colonies and grown at 37°C until turbid in ≤7 days. Purity was assessed using microscopy and visual analysis of streak-plated pure cultures.

Genomic DNA was isolated from the liquid cultures using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA). MicrobesNG (Birmingham, UK) performed library preparation, sequencing, adapter trimming, and assembly (full protocol available at https://microbesng.com/documents/5/MicrobesNG_Methods_Document_-_PDF.pdf). DNA was quantified using the Quant-iT double-stranded DNA (dsDNA) high-sensitivity (HS) assay (Thermo Fisher Scientific, Waltham, MA, USA). Libraries were prepared using a Nextera XT library prep kit (Illumina, San Diego, CA, USA) with the following two protocol modifications: using 2 ng of DNA instead of 1 ng and adjusting the PCR elongation time to 1 minute. Libraries were sequenced using an Illumina HiSeq instrument with a 250-bp paired-end protocol. A mean of 1,122,706 reads per strain were generated by the sequencing. Reads were trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 (3). SPAdes 3.7 (4) was used for *de novo* assembly

**TABLE 1** Summary of the draft genome sequences for 16 microbes isolated from high-salt environments and culinary salt

| Species (% identity) | Isolate source (medium salinity [g/liter NaCl]) | No. of reads | Median insert size (bp) | No. of contigs of ≥0 bp | Total length (bp) | G+C % | $L_{50}$ | $N_{50}$ (bp) |
|---|---|---|---|---|---|---|---|---|
| *Bacillus hwajinpoensis* (99.79) | French fleur de sel salt from grocery store (100) | 560,183 | 433 | 87 | 4,387,231 | 40.22 | 3 | 797,473 |
| *Halobacillus halophilus* (98.21) | Solar salt harvested from saltern, Puerto Rico (100) | 505,256 | 448 | 51 | 3,841,848 | 47.38 | 3 | 575,568 |
| *Halobacillus litoralis* (99.23) | Sand harvested near saltern crystallizers, Puerto Rico (100) | 1,228,604 | 197 | 258 | 4,087,490 | 43.59 | 2 | 1,038,528 |
| *Halobacillus litoralis* (99.36) | Sand harvested near saltern crystallizers, Puerto Rico (100) | 1,488,864 | 215 | 92 | 3,984,713 | 43.83 | 3 | 396,878 |
| *Halobacillus litoralis* (99.04) | Filtered 34% NaCl saltern water, Puerto Rico (100) | 495,194 | 484 | 65 | 3,850,954 | 47.23 | 5 | 237,386 |
| *Halobacillus litoralis* (99.1) | Filtered 34% NaCl saltern water, Puerto Rico (100) | 1,866,405 | 211 | 577 | 4,062,334 | 47.67 | 2 | 690,789 |
| *Pontibacillus yanchengensis* (99.39) | French fleur de sel salt from grocery store (200) | 454,979 | 275 | 75 | 4,476,898 | 38.35 | 7 | 200,610 |
| *Pontibacillus yanchengensis* (99.39) | Filtered 34% NaCl saltern water, Puerto Rico (200) | 1,370,382 | 221 | 93 | 4,441,339 | 38.43 | 3 | 575,184 |
| *Virgibacillus halodenitrificans* (100) | Sand harvested near saltern crystallizers, Puerto Rico (100) | 1,634,098 | 238 | 2,151 | 5,319,423 | 37.39 | 155 | 6,747 |
| *Virgibacillus halodenitrificans* (99.87) | Solar salt harvested from saltern, Puerto Rico (100) | 399,182 | 482 | 109 | 4,012,173 | 37.36 | 6 | 215,037 |
| *Virgibacillus massiliensis* (99.74) | Sand harvested near saltern crystallizers, Puerto Rico (100) | 1,323,006 | 239 | 166 | 4,260,595 | 36.96 | 2 | 1,149,539 |
| *Halomonas alkaliantarctica* (97.5) | Filtered 34% NaCl saltern water, Puerto Rico (200) | 1,230,594 | 293 | 91 | 3,324,735 | 58.54 | 4 | 316,952 |
| *Halomonas utahensis* (99.87) | French fleur de sel salt from grocery store (100) | 287,001 | 378 | 57 | 3,351,671 | 62.12 | 6 | 219,081 |
| *Halomonas utahensis* (99.87) | French fleur de sel salt from grocery store (150) | 2,659,128 | 227 | 64 | 3,361,279 | 62.12 | 4 | 422,380 |
| *Halorubrum terrestre* (100) | Solar salt harvested from saltern, Puerto Rico (250) | 999,388 | 393 | 90 | 3,385,208 | 67.81 | 11 | 97,966 |
| *Halorubrum terrestre* (99.86) | Solar salt harvested from saltern, Puerto Rico (150) | 1,461,031 | 315 | 885 | 3,678,879 | 65.29 | 9 | 146,067 |

with recommended settings for 250-bp paired-end reads. The mean coverage ranged from 35 to 289×. The mean $N_{50}$ value was 442,887 bp with a mean $L_{50}$ of 14.1.

The taxonomy was estimated with BLAST (5) using the 16S rRNA gene sequences of each strain. We identified 14 bacteria (genera *Halobacillus*, *Pontibacillus*, *Virgibacillus*, *Bacillus*, and *Halomonas*) and 2 archaea (genus *Halorubrum*). The top query results all had above a 97% identity (99.4% mean identity).

**Data availability.** All 16 whole-genome sequences and SRA files have been deposited at DDBJ/ENA/GenBank as a BioProject under accession number PRJNA587497. A 17th sample is also included in the BioSample data for the BioProject, but this sample appeared to be a mix of two species and could not be assembled into a high-quality genome.

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