Metagenome Sequencing of Prokaryotic Microbiota from Two Hypersaline Soils of the Odiel Salt Marshes in Huelva, Southwestern Spain

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ABSTRACT   Two 454 shotgun metagenomes were sequenced from hypersaline soil samples collected in the Odiel salt marsh area in Huelva, southwestern Spain. Analysis of contigs and 16S rRNA-related sequences showed that *Halobacteria*, *Balneolaeota*, and *Bacteroidetes* were the dominant groups. *Rhodothermaeota* and *Nanohaloarchaeota* were also abundant.

Hypersaline environments are extreme habitats characterized by high salt concentrations, among other stressors. These environments mainly comprise aquatic (such as lakes, salterns, and deep hypersaline anoxic basins) and terrestrial habitats (sediments and soils) (1). Extensive studies have been performed to investigate the microbiota dwelling in hypersaline aquatic habitats (2, 3). However, only limited and partial data are available regarding the prokaryotic community inhabiting saline soils (4). The Odiel salt marshes are situated at the estuary of the Odiel and Tinto rivers. The area has been found to be affected by the metal load transported by the Tinto and Odiel rivers, which drain the Iberian Pyrite Belt (5), as well as by industrial effluents from the chemical pole located in Huelva (6).

The objective of this study was to assess the prokaryotic community of saline soils from the Odiel salt marshes and evaluate its variation from one year to the next. The sampling site (37.207218 N, −6.965999 W) was located in a high marsh area that was devoid of plants and for which rain events are the main water inputs, with flooding occurring only in case of very high tides. Conductivities of 24 and 55 dS/m were determined in 1:5 water extracts for the samples retrieved in October 2013 (designated SMO1) and November 2014 (SMO2). pH values measured in 1:2.5 extracts of SMO1 and SMO2 were 7.8 and 8.9, respectively.

Total DNA was extracted from 10 g of each soil sample using the FastDNA spin kit for soil (MP Biomedicals) according to the manufacturer's instructions. Further cleaning of DNA was performed by the phenol-chloroform method as described in Green and Sambrook (7). Sequencing was carried out with a Roche Sequencer FLX (GS FLX), and 1,289,630 and 839,941 reads were obtained for SMO1 and SMO2, respectively. Average read lengths were 628 bp for SMO1 and 629 bp for SMO2. Metagenomic reads were coassembled *de novo* using Newbler v2.9 (8) and assembly assessed with QUAST v.2.3 (9), resulting in a total of 25,001 contigs longer than 1 kb with an N_{50} contig size of 1,857 bp. Contigs were annotated using MEGAN v6.5.10 lowest common ancestor (LCA) algorithm (10), and extraction, refinement, and quality assessment of metagenome assembled genomes (MAGs) were accomplished with MetaBAT v0.26.3 (11), VizBin v0.9 (12), and CheckM v1.0.5. Additionally, BLASTn searches of metagenomic reads of SMO1 and SMO2 against RDP database v11.4 (13) were performed to retrieve and classify 16S-related rRNA reads.

Taxonomic assignment of contigs revealed that the microbial community comprised 51.2% *Euryarchaeota*, 21.3% *Balneolaeota*, and 16.1% *Bacteroidetes*. *Rhodothermaeota* (2.7%), *Nanohaloarchaeota* (1.5%), *Cyanobacteria* (1.3%), *Gammaproteobacteria* (1.2%), and *Gemmatimonadetes* (1.1%) also constituted more than 1% of the community. The most abundant genera were related to *Halobacteria* (*Haloarcula*, *Halorubrum*, *Halolamina*, and *Salinigranum*), *Balneolaeota* (*Fodinibius* and *Gracilimonas*), *Bacteroidetes* (*Salinimicrobium*), and some members of *Proteobacteria* (*Halomonas* and *Marinobacter*).

The metagenomic data obtained will be of great value in the study of halophiles and the microbiota of hypersaline environments.

Accession number(s). Metagenomic data sets obtained in this study have been deposited in DDBJ/ENA/GenBank under the BioProject number PRJNA318875. Accession numbers of raw reads available at the NCBI Sequence Read Archive are SRR5753724 and SRR5753725.

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