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Metagenome-Derived Draft Genome Sequence of *Acidithiobacillus ferrooxidans* RV1 from an Abandoned Gold Tailing in Neuquén, Argentina

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Abstract. In this work we report the metagenome-derived draft genomic sequence of an enrichment culture dominated by *A. ferrooxidans* obtained from an airlift bioreactor inoculated with the microbial consortium recovered from the "Relave Viejo" tailing. The genome of this culture was assembled *de-novo* and by reference, generating a consensus assembly of 3.0 Mb. On the basis of 16S rRNA (100 % identity), average nucleotide identity analysis (99.33% identity) and *in silico* DNA-DNA hybridization against *A. ferrooxidans* ATCC 23270^T (97.9%), the recovered genome is confirmed to pertain to *A. ferrooxidans* species. Comparative genomics results are presented to uncover the genetic traits of the variant surviving lime treatment and to further explore the genomic diversity of these model iron oxidizing species.

Introduction

Acidithiobacillus ferrooxidans is an iron oxidizing, facultative anaerobic, obligate chemolithotrophic bacterium that inhabits mesophilic and extremely acidic environments. Strains of this species are ubiquitous and have been isolated from diverse econiches, including ores, ore-concentrates and leaching solutions from the mining industry [1]. Frequently, it is the dominant iron oxidizer in acid mine drainage waters above pH 1.8 and different types of bioleaching operations [2]. Strains of this species play key roles in the recovery of copper, gold and other metals [3]. More than 500 isolates of *A. ferrooxidans sensu stricto* (as defined in [4]) have been recovered worldwide, yet fewer than 2%, have been sequenced so far. Clearly more genomic sequences of this species are required to understand the true biological diversity of the species from a genomic perspective.

Relave Viejo is an abandoned mine tailing located in the Andacollo gold mining district (Neuquén, Argentina). This tailing stored the lime-treated left overs of gold concentrates processed in the site between 1976 and 1998. A microbiological survey performed between the years 2011 and 2015, using 16S rRNA gene-targeted deep sequencing, has revealed the presence of well established acidophilic microbial communities associated to the ore debris within the Relave Viejo tailing [5]. Members of the genus *Acidithiobacillus*, reckoned to be a highly diverse group of acidogenic bacteria, were among the dominant acidophilic taxa in these communities, in particular strains of the species *A. ferrooxidans*. Enrichment cultures held for ten months in total on airlift bioreactors [6], containing low pH culture media and mineral wastes from the Relave Viejo tailing, selected for a very low complexity community dominated by *A. ferrooxidans*-like iron oxidizers, designated RV1 culture.

Here, we present the metagenome-derived draft genomic sequence of the RV1 culture in order to gain further insights into the genomic diversity of this model iron oxidizer and explore the genetic traits of this particular variant surviving lime-treatment in the semi-arid mountain range of northern Patagonia.

Materials and Methods

Humid soil (2 Kg, pH 7) was collected (August 2014) from the Relave Viejo abandoned mine tailing at Andacollo (Neuquén, Argentina) and used to inoculate a enrichment media (DSMZ 882). Then, the culture was scaled up to inoculate a 10 L airlift reactor [**6**], with 0K medium operated in batch mode at 1.8 pH, 30 ºC, with a volumetric gas flow rate of 1.6 x 10^{-5} m^3 s^{-1}. Five sequential runs (lasting 2 months each) were performed. In each run the reactor was inoculated with the preceeding culture. After 10 month of incubation, microorganisms were recovered by centrifugation (8,000 g x 10 min) and the cell pellet was lysed to recover total DNA following established procedures [**7**].Two replicate metagenomes were obtained using Illumina sequencing technology and paired-end libraries with insert sizes of ~460 bp, prepared using Nextera^{TM} DNA Sample Preparation kit (Nextera, USA). Raw sequencing reads were preprocessed using Trimmomatic (v0.32). Reads with a > Q30 quality score were retained and assembled *de novo* using MetaVelvet (v1.2.02) and a k-mer length of 37. The assembly was binned using Megan5 by blastx against the MEL-FCV genomic database of acidophiles. Bins matching *A. ferrooxidans* ATCC 23270^T complete genome (NC_011761) were further analyzed using fragment recruitment with Bowtie2 (v2.2.4). Both assemblies were merged using an *in house* script and manually curated to obtain a consensus high quality assembly. Presence and relative abundance of sequence variants were assessed from the read recruitment using IGV (v.2.3.34) and the 16S rRNA gene and MLST markers as defined by [4]. Downstream analysis was performed with RAST as described previously [8]. Genome comparisons were performed using the GET_HOMOLOGUES software package (v07112016). Orthology was determined based on all-versus-all Best Bidirectional BlastP Hit and COGtriangles (v2.1) as clustering algorithm. Pairwise alignment cutoffs were set at 75% coverage and E-value of 10E-5.

Results and Discussion

The 3.1 Mb draft genome of *A. ferrooxidans* RV1 is arranged into one high quality scaffold and 35 smaller contigs. The final assembly is based on 220 Mbp of Illumina data, which provides an average 11 fold coverage of the genome and is predicted to be 97.14% complete [9]. A total of 47 RNA genes and 3,124 protein coding genes were predicted from its annotation; 67.9% of these were assigned a putative function (Table 1). The average G+C content of the RV1 genome is 58.5 %.

A. ferrooxidans RV1 is 100% identical at the 16S rDNA level to the type strain of *A. ferrooxidans* (ATCC 23270^T; [10]), and 100 % identical to other sequenced isolates (e.g. [11-12]). On the basis of average nucleotide identity analysis (99.33% identity) and *in silico* DNA-DNA hybridization against *A. ferrooxidans* ATCC 23270^T (97.9%), the recovered genome is confirmed to pertain to *A. ferrooxidans sensu stricto*.

Results for the comparison of the *A. ferrooxidans* RV1 predicted gene complement against the genome of the type strain (ATCC 23270^T) and other publically available complete and draft genomes of the species are presented in Table 2. The eight strains compared share a core consisting of 741 gene clusters (data not shown), representing little over 25.33 % of the predicted gene complement of any single genome, indicating that this lineage is highly divergent at the genomic level.

Table 1. Genomic features of the RV1 culture vs. *A. ferrooxidans* ATCC 23270^T

Feature	Strain ATCC 23270^T	Culture RV1
Size	2,982,397	3,074,681
GC Content	58.8	58.5
Coding Sequences	3,217	3124
RNAs	78	47
rRNA operons	2	1
tmRNAs	1	1

A. ferrooxidans ATCC 23270^T values were derived from [**10**].

Table 2. Protein coding genes shared between the RV1 culture and sequenced *A. ferrooxidans*

Strain	Accession #	% CDS shared with RV1[a]
Culture RV1	This work	100
YQH-1	LJBT01	95
Hel-18	LQRJ01	95
Wenelen	ATFW[b]	85
ATCC 23270	NC_011761	83
ATCC 53993	NC_011206	82
GGI-221	AEFB01	73
DLC-5	JNNH01	71

[a]Percentage of predicted protein coding genes (CDS) sharing > 70% sequence similarity
[b]http://biominingdb.cmm.uchile.cl/genomes/At_ferrooxidans_Wenelen/

The RV1 clone is most similar to strains YQH-1 and Hel18, with an exclusive gene complement encompassing only 5 % of its predicted protein coding gene pool, and is most dissimilar to strains DLC-5 and GGI-21, with a set of unique genes amounting as much as 19% of the CDSs. The levels of sequence divergence between the RV1 clone and the 7 strains analyzed also varied significantly (data not shown). While 94% of the genes shared between RV1 and YQH-1 were more than 70% identical, only 71% of the proteins common to both RV1 and DLC-5 achieved that level of identity. This variation may be explained by differentiation in allopatry [13].

Most of RV1´s non-core genes were hypotheticals or had no predicted function. Partially shared genes (present in only one or two strains, besides the RV1 clone) and most differentiated genes (genes sharing less than 75% sequence identity) included transporters for several (heavy)metals and other toxic compounds (e.g. lead, cadmium cobalt, zinc, iron), transcriptional regulators (e.g. TetR, ArsR LysR) and genes that are typically horizontally transferred (e.g. conjugation genes, transposases, recombinases). This subset also included genes coding for proteins predicted to be involved in amino acids biosynthesis (e.g. glutamate, cysteine and leucine/isoleucine) and B vitamins (B6 and B7). While glutamate and cysteine are both relevant in the biosynthesis of the cellular antioxidant glutathione [**14**], leucine is a relevant regulatory amino acid controlling adaptation to nutrient limitation [**15**]. These aspects could have a significant role in adaptation and survival to adverse conditions experienced by these acidophiles during or after lime-treatment.

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