Draft Genome Sequence of a Multi-Metal Resistant Bacterium *Pseudomonas putida* ATH-43 Isolated from Greenwich Island, Antarctica

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**INTRODUCTION**

At low concentrations, heavy metals and metalloids are highly toxic for most microorganisms (Lemire et al., 2013). Over evolution, bacteria have developed several molecular mechanisms in order to cope with heavy metal/metalloid toxicity (Nies, 2000; Lemire et al., 2013). *Pseudomonas putida* belongs to a group of versatile microorganisms capable to thrive in diverse hostile environments, including multi-metal polluted cold sites (Canovas et al., 2003; Zhang et al., 2012; Moreno and Rojo, 2013). Members of *P. putida* are largely known for their ability to colonize different kinds of environments and to degrade a vast diversity of toxic organic compounds (Wu et al., 2011). In this context, *P. putida* ATH-43 was isolated from soil sediments at the “Prat” Chilean military base located in Greenwich Island, Antarctica, and was recognized as a mercury/tellurite resistant bacterium (Rodríguez-Rojas et al., 2015). Interestingly, this strain shows tellurite resistance only when grown in the presence of mercury, suggesting a cross-resistance mechanism. Further experimental evidence revealed that *P. putida* ATH-43 is highly resistant to other toxicants such as Cd$^{2+}$, Cu$^{2+}$, CrO$_4^{2-}$, and SeO$_3^{2-}$, and several antibiotics including streptomycin, cefotaxime, kanamycin, and chloramphenicol (Rodríguez-Rojas et al., 2015). On the other hand, global distillation and grasshopper effect are of major worldwide concern since they apparently provide an explanation for the rapid occurrence of heavy metal/metalloids contamination in pristine polar environments (Ebinghaus et al., 2002; Macdonald et al., 2005). In this context, the genome sequence of *P. putida* ATH-43 represents an important information source of genetic resistance determinants to multiple stressors currently affecting the Antarctic ecosystem.

In this report we present the first draft genome sequence of a *P. putida* strain isolated from the Antarctic continent. The shotgun sequencing strategy, assembly, and subsequent annotation showed that the ATH-43 strain possesses a wide spectrum of genetic determinants involved in heavy metal and antibiotic resistance, apparently to cope with extreme oxidative stress conditions. *P. putida* ATH-43 genome now forms part of the 65 genomes of this species registered at the NCBI.
The assembled genome of *P. putida* ATH-43 consists of 5.8 Mb distributed over 260 contigs and organized in 64 scaffolds with fold coverage of 172X.

Open reading frame prediction and annotation was carried out using standard operational procedures (Tanenbaum et al., 2010). Gene models were predicted using Glimmer 3.02 (Salzberg et al., 1998). Predicted coding sequences were annotated by comparison with public databases, BLAST 2.2.31 (Altschul et al., 1990) was used to find homologous sequences with COG, UNIPROT, and NR-NCBI databases, and Hmmer 3.1 was used against PFAM and TIGRFAM. Automatic metabolic reconstruction was carried out using PRIAM software (Claudel-Renard et al., 2003).

**RESULTS**

**P. putida** **ATH-43 Features**

*P. putida* ATH-43 is a Gram negative, non-sporulating, motile, aerobic, rod-shaped (average bacterium dimensions were 6.5 µm length and 2.3 µm width) and psychrotrophic bacterium (Figure 1A) that was isolated from Antarctic sediments at anthropogenic settlements visibly contaminated with oxide compounds. Sequencing of complete 16S rRNA gene revealed phylogenetic affiliation of strain ATH-43 as a member of the *Pseudomonadaceae* family from the *Gammaproteobacteria* class, strongly related to *P. putida* species (Figure 1B). Interestingly, strain ATH-43 forms a distinctive clade with isolate *P. putida* W619, which are clearly differentiated from the rest of *Pseudomonas* isolates, suggesting a different evolutionary ancestral divergence (Figure 1B). In concordance, the Average Nucleotide Identity (ANI) analysis performed with nine selected strains, revealed a close relationship with *P. putida* strains W619 and SQ1 (Supplementary Figure 1A), further contributing to defining the relationship between these bacterial strains. In addition, a pan-genome analysis was performed with the same strains that were used for ANI analysis. In this line, ATH-43 strain presented an important number (741) of unique accessory genes, that may contribute to the multi-stress resistance phenotype observed in this strain. Also, a core genome of 1370 genes was shared among the *P. putida* strains, given by their threshold of 90–99% nucleotide identity (Supplementary Figure 1B).

**Whole Genome Sequence and Insights of** **P. putida** **ATH-43**

The assembled genome of *P. putida* ATH-43 consists of 5,830,220 bp, with an average G+C content of 61.5% (Table 1). A set of 79 tRNA genes and two clusters of rRNA genes were identified. From a total of 5124 predicted protein-coding sequences (CDSs), 4436 (86.5%) open reading frames (ORFs) matched coding sequences available in public databases, and 3372 (65.8%) were assigned in clusters of orthologous groups (COG) categories (Figure 1C).

Open reading frame prediction revealed the presence of multiple genetic determinants related to heavy metals and some metalloids tolerance. This prediction included resistance
Figure 1 | *P. putida* ATH-43 morphology, phylogeny, and COG assigned genes. (A) Scanning electron micrograph. Samples were stained with 0.5% (w/v) uranyl acetate and examined using a low-voltage electron microscope (Delong Instruments, LVEM5) with a nominal operating voltage of 5 kV. Bar represents 10 µm. (B) Phylogenetic tree, based on the complete 16S rRNA sequence. (C) Number of genes assigned to COG categories. (D) Pie chart representing the per cent of heavy metal(loid) resistance genes in the *P. putida* ATH-43 genome.

Genes and operons to Hg\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ni\(^{2+}\), Cd\(^{2+}\), Co\(^{2+}\), Pb\(^{2+}\), CrO\(_4^{2-}\), AsO\(_3^{3-}\), SeO\(_2^{3-}\), and TeO\(_2^{3-}\) (Figure 1D and Supplementary Table 1), and also genes regarding resistance to antibiotics and drugs such as tetracycline, macrolides, penicillins, aminoglycosides, and streptomycin, among others (Supplementary Table 2). In addition, computational prediction showed the presence of genes encoding seven cold-shock proteins, 773 hypothetical proteins, and an important battery of genes participating in the oxidative stress response, including the unusual mycothiol synthase found exclusively in Gram positive bacteria (Rawat et al., 2007).

The ATH-43 genome harbors more tRNA gene sequences than other known *P. putida* genomes, a trait that may reflect the cell’s adaptation to extreme conditions (Wu et al., 2011). In fact, higher tRNA genes content seem to be related with specific cold adaption mechanisms, as determined by comparative genomics analysis (Dutta and Chaudhuri, 2010). In addition, the number of genes involved in signal transduction mechanisms and inorganic ion transport and metabolism (two COG categories) in this bacterium’s genome, is similar to other *P. putida* strains but exceeds that displayed by other Gammaproteobacteria (Wu et al., 2011). These data may suggest the presence of complex systems of molecular mechanisms controlling gene expression in microorganisms that thrive under highly variable environments.

On the other hand, 13 IS elements were found in the ATH-43 genome sequence using the IS finder tool (www-is.biotoul.fr), along with 21 transposases and 17 integrases (not shown), all elements routinely associated with horizontal gene transfer providing advantage in metal and antibiotic resistance, general stress tolerance, and aromatic compound degradation, among others (Vos et al., 2015; Koonin, 2016). As with tRNA genes, the genome of ATH-43 displays a higher number of genetic determinants involved in metal, antibiotic, and oxidative stress resistance as compared with other reference *P. putida* genomes (Wu et al., 2011), which may be a reflect of the dramatic selective pressure occurring in the Antarctic continent.

The whole-genome shotgun project was deposited in GeneBank and is publicly available since July, 2015 under the accession number LBME0000000 (Direct link: http://www.ncbi.nlm.nih.gov/nuccore/LBME0000000.1).
**AUTHOR CONTRIBUTIONS**

FR performed genomic DNA extraction and analyzed the genomic data. PT and JV carried out the de novo assembly and gene annotation. EC carried out the ANI and pangenome analysis, and assigned COG categories. PM and MA prepared the samples and took SEM pictures. AU constructed the phylogenetic tree. FR, AU, WD, and CV participated in experiment designing and helped to draft the manuscript. All authors read and approved the final text.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2016.01777/full#supplementary-material

**Supplementary Figure 1** | ANI and pangenome analysis of *P. putida* ATH-43. (A) Heatmap representing the comparison of the average nucleotide identity among ten *P. putida* genomes. (B) Pangenome comparison bar chart showing the number of shared genes clustered in cloud (genes in 0–20% of genomes), shell (genes in 20–90% of genomes), soft core (90–99% of genomes), and core genome (100% of genomes).

**Supplementary Table 1** | Metal resistance determinants found in the *P. putida* ATH-43 genome.

**Supplementary Table 2** | Antibiotic resistance determinants found in the *P. putida* ATH-43 genome.

**Supplementary Table 3** | Metal resistance determinants found in the *P. putida* ATH-43 genome.
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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