Draft genomic DNA sequence of the multi-resistant *Sphingomonas* sp. strain AntH11 isolated from an Antarctic hypolith

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One sentence summary: Draft genome of the multiresistant *Sphingomonas* sp. strain AntH11 isolated from an Antarctic hypolith.

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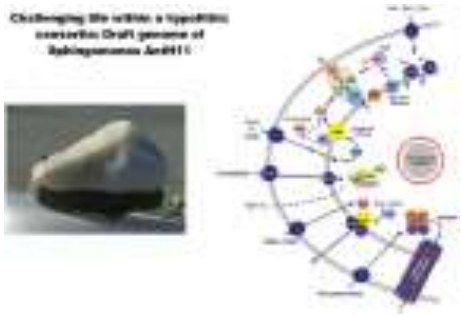
GRAPHICAL ABSTRACT

Draft genome of the multiresistant *Sphingomonas* sp. strain AntH11 isolated from an Antarctic hypolith.



Abstract

Hypoliths are microbially colonized translucent rocks that represent a key refuge niche in extreme arid environments such as the Antarctic Dry Valleys. These cryptic microbial assemblages are crucial as they mediate numerous ecosystem processes. Here, we present the first draft genome of a hypolith isolate belonging to the α-proteobacterial class and the genus *Sphingomonas*. The draft genome of *Sphingomonas* sp. strain AntH11 shows the capacity of this organism to adapt to the extreme cold and arid conditions encountered in Antarctic desert soils. Our result also suggests that its metabolic versatility and multidrug resistance constitutes an opportunistic advantage in competition with other hypolith-colonizing microorganisms.

Key words: *Sphingomonas* ; hypolit ; Antarctic

Sphingomonas sp. are Gram-negative, non-spore-forming, chemoheterotrophic, strictly aerobic α-proteobacteria, belonging to the 'sphingomonads' group along with Sphingobium, Novosphingobium

and *Sphingopyxis* (Balkwill, Fredrickson and Romine 2006). To date, *Sphingomonas* isolates have been recovered from an array of eco-types, but the strain *Sphingomonas* sp. AntH11 described here is the first from a hypolithic community. From an ecological perspective, hypolith microbial communities are important in extreme environments such as the Antarctic Dry Valleys, in that they provide refuge niches in which microbial communities mediate carbon and nitrogen inputs into the system (Cowan et al. 2011; Chan et al. 2012). Therefore, information from the AntH11 genome sequence can provide insights into what role they have within this key sublithic microhabitat. This is emphasized by the fact that numerous *Sphingomonas* sp. phylotypes have previously been detected in Antarctic hypolith communities, but that their ecological role was unknown (Makhalanyane et al. 2013). Here, we report the draft genome sequence of *Sphingomonas* sp. AntH11, a novel strain isolated from hypoliths located in the coastal Miers Valley (781600 S, 1641000 E) region of Eastern Antarctica (Makhalanyane et al. 2013). The plating conditions used to isolate this organism employed R2A (Difco) media with incubation at 15◦Cfor upto3weeks,where yellow-pigmented colonies were observed and the purity of the isolate confirmed by 16S rRNA gene PCR. After DNA extraction using a combination of bead-beating and chemical lysis (Miller et al. 1999), the AntH11 genome was sequenced using an Ion Torrent PGM sequencer (318 chip; Life Technologies) with 400-bp chemistry. After quality filtering, 3079 964 reads with an average size of 207 bp were assembled using MIRA v 4.0rc4 (Chevreux, Wetter and Suhaie 1999). We implemented a strongly conservative approach, as the minimum length for the result-ing contigs was set at 800 bp. These contigs were joined manu-ally where possible using Gap5 (Bonfield and Whitwham 2010). The final draft genome comprised 214 contigs with a mean size of 21 699 bp and a maximum length of 199 346 bp. The total length of the genome was 4643 689 bp with a mean GC content of 65.1% and an average coverage of 83.2X. The genome was annotated using the Rapid Annotation using Subsystems Technology (RAST) server (Aziz et al. 2008), KEGG Automatic Annotation Server (Moriya et al. 2007) and the Pathway Tools pro-gram (Karp, Paley and Romero 2002)identifying4746protein-coding genes and 54 RNAs, including 48 tRNAs and 6 rRNA-related sequences, including two 5S, two 16S and two 23S rRNA genes. The strain *Sphingomonas* sp. AntH11 is closely related to the type strain species *Sphingomonas* echinoides ATCC 14820 with a 16S rRNA gene sequence similarity of 99% (Shin et al. 2012). However, comparisons with genomes sequences avail-able on the RAST server demonstrated that the closest neighbors of *Sphingomonas* sp. AntH11 were *Sphingopyxis* alaskensis RB2256 (score of 523), a cold-adapted marine oligotroph (Ting et al. 2010)andSphingobium japonicum UT26S (score of 422), isolated from a γ -hexachlorocyclohexane contaminated soil (Nagata et al. 2010).

The genome of AntH11 comprises an extensive set of stress responses genes (116 in total), demonstrating its genetic adaptation to the extreme environmental conditions encountered in Antarctica (i.e. hyper aridity, UV radiation and cold tempertures; Cowan 2009) . Detected ORFs were assigned to (i) os-moregulation with genes related to the biosynthesis or uptake of compatible solutes (choline and betaine) and potassium homeostasis, (ii) oxidative stress resistance (58 genes in total) including glutathione synthesis and response pathways to H202 and superoxide and (iii) cold (CspA protein family) and heat (dnaK gene cluster) shock genes.

A remarkable feature of the *Sphingomonas* strain AntH11 genome was the number of genes linked to biotic- and abiotic-defense mechanisms (173 in total), with 113 related to metal resistance, 52 to antibiotic resistance and 8 to the production of bacteriocin (colicin V); while only 62, 34 and 6, corresponding genes were found in the genome of the type strain S. echinoides (102 in total), respectively. Retaining resistance against copper (27 ORFs associated with copper homeostasis),

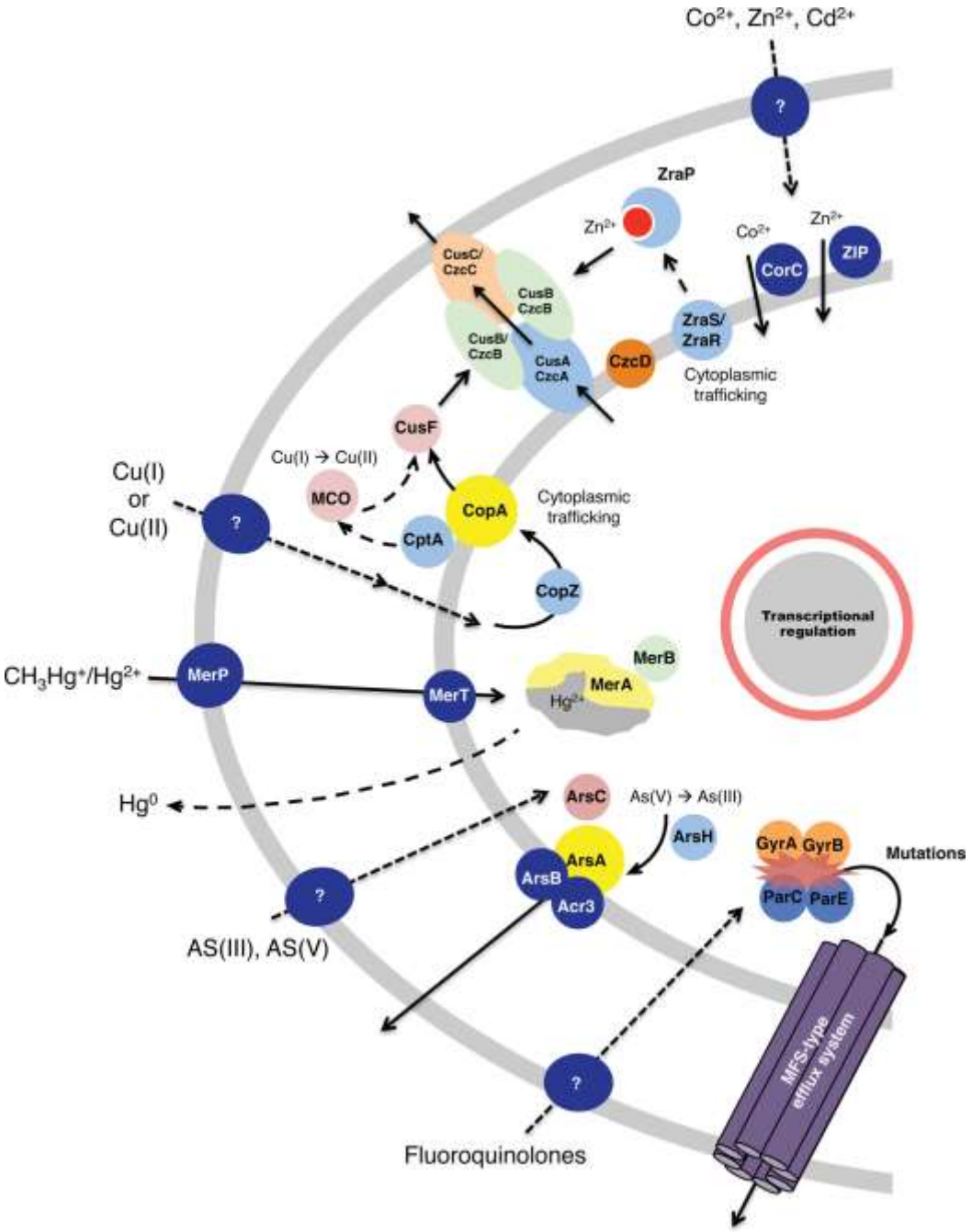


Figure 1. Schematic representation of metal and antibiotic resistance in S*phingomonas* sp. AntH11 predicted from identified genes. Copper homeostasis is mediated through the Cus system, whereby the CopZ chaperone transfers Cu+ to the Cu+-ATPase (CtpA) that exports Cu cations to the periplasm (Kim et al. 2011). The multi-copper oxidases (MO) can convert Cu(I) to Cu(II) within the periplasm (preventing Cu(I)-mediated toxicity), which can then be fed through the CusABC channel by the periplasmic chaperone CusF with subsequent translocation of the metal to the extracellular milieu. Resistance to cobalt; cadmium and zinc is facilitated by employing a similar RND efflux system (CzcABC) with regulation by the membrane bound CzcD (Nies 1995). Multiple intracellular transporters include CorC and ZIP for Co2+ and Zn2+ ions, respectively. The expression of the periplasmic ZraP facilitates binding and transfer in high Zn2+ concentrations, regulated by the ZraS/ZraR membrane complex. MerT transports Hg2+ scavenged by MerP across the periplasmic membrane, which can then bind with the cysteine residues of the mercuric reductase (MerA), subsequently reducing Hg2+ to Hg0 which can diffuse out of the cell (Dash and Das 2012). Pentavalent arsenate [AS(V)] is reduced to trivalent arsenite [AS(III)] by ArcA in the cytoplasm. As(III) is then transported across the periplasm by ArsB and/or Acr3 in coordination with an ATPase (ArsA). ArsH is an NADPH-flavin mononucleotide oxidoreductase that aids in detoxification through the probable oxidation of arsenite (Shen et al. 2013).

cobalt, zinc, cadmium (czc operon), mercury (mer operon) and arsenic (ars operon) (Fig. 1) must constitute a genetic burden in such a harsh environment but this may be beneficial through probable exposure to heavy metals from both anthropogenic and atmospheric sources (Planchon et al. 2002). Also, as metal and antibiotic resistance gene cassettes often present genetic link-ages (Baker-Austin et al. 2006), we suggest that a high number of antibiotic resistance genes (and thus metal resistance genes) confers a strong selective advantage (e.g. in microbial competition) in colonizing cryptic refuge niches, such as hypoliths (Davies and Davies 2010). This is supported by the large number of genes related to multidrug efflux pumps (24) which have been shown to confer resistance to a vast array of antibiotics in pathogenic strains but also to play a role in bacterial pathogenicity (Piddock 2006).

The genome of strain AntH11 revealed also high metabolic versatility (Balkwill, Fredrickson and Romine 2006) with 374 genes related to carbohydrates metabolism, and 41, 36 and 11 genes related to phosphorous, sulfur, and nitrogen (ammonia assimilation) metabolism, respectively (versus only 329, 38, 18 and 8, in the genome of S. echinoides, respectively). The genome also exhibited a capacity for xenobiotic degradation, consistent with other members of this group (Balkwill, Fredrick-son and Romine 2006;Aylwardet al. 2013), with 26 annotated genes directly linked to the metabolism of aromatic compounds (Margesin and Schinner 2001). This metabolic diversity makes *Sphingomonas* sp. AntH11 a candidate for cold climate bioremediation purposes; specifically in extreme oligotrophic systems like Antarctica where indigenous populations are required (Antarc-tic Treaty; Stallwood et al. 2005). This organism could also be potentially used (or further engineered) to decontaminate cold hydrocarbon-contaminated soils where natural biodegradation is particularly slow (Short et al. 2007).

Nucleotide sequence accession number: The draft genome sequence of *Sphingomonas* sp. AntH11 has been submitted to GenBank and assigned the accession number JSBN00000000.

Acknowledgements

The South African National Antarctic Program (SANAP), Antarctica-New Zealand, for field and logistics support through a collaboration with Professor Craig Cary, the University of Pretoria DNA Sequencing Facility for sequencing support, and the Centre for High Performance Computing (CHPC, http://www.chpc.ac.za/), an initiative supported by the Department of Science and Technology of South Africa.

Funding

We thank the South African National Research Foundation (NRF) for financial support.

Conflict of interest statement. None declared.

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