



GENOMY ANNOUNCEMENT – Environmental Microbiology

Draft genomic DNA sequence of the multi-resistant *Sphingomonas* sp. strain AnthH11 isolated from an Antarctic hypolith

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E-mail: Gunny00@gmail.comOne sentence summary: Draft genome of the multiresistant *Sphingomonas* sp. strain AnthH11 isolated from an Antarctic hypolith.

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Editor: Peter Lund

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 AnthH11 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*; hypolith; 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. AnthH11 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 AnthH11 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 (Makhwanyane et al. 2013). Here, we report the draft genome sequence of *Sphingomonas* sp. AnthH11, a novel strain isolated from hypoliths located in the coastal Miers Valley (781600 S, 1641000 E) region of Eastern Antarctica (Makhwanyane et al. 2013). The plating conditions used to isolate this organism employed R2A (Difco) media with incubation at 15°C for up to 3 weeks, 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

Received: 3 December 2014; Accepted: 4 March 2015

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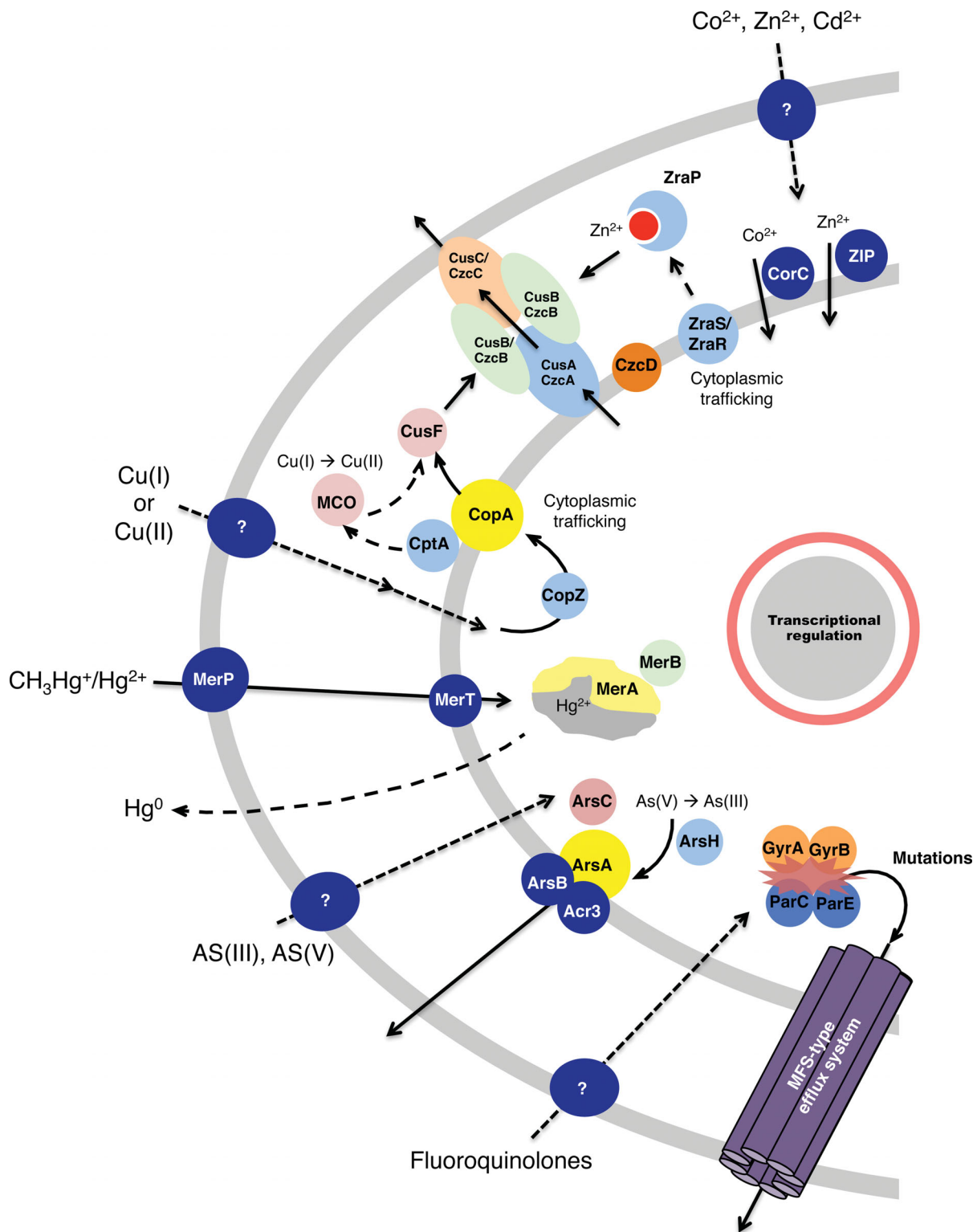


Figure 1. Schematic representation of metal and antibiotic resistance in *Sphingomonas* sp. Anth11 predicted from identified genes. Copper homeostasis is mediated through the Cus system, whereby the CopZ chaperone transfers Cu^+ to the Cu^+ -ATPase (CtpA) that exports Cu cations to the periplasm (Kim *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 Co^{2+} and Zn^{2+} ions, respectively. The expression of the periplasmic ZraP facilitates binding and transfer in high Zn^{2+} concentrations, regulated by the ZraS/ZraR membrane complex. MerT transports Hg^{2+} scavenged by MerP across the periplasmic membrane, which can then bind with the cysteine residues of the mercuric reductase (MerA), subsequently reducing Hg^{2+} to Hg^0 which can diffuse out of the cell (Dash and Das 2012). Pentavalent arsenate [AS(V)] is reduced to trivalent arsenite [AS(III)] by ArsA in the cytoplasm. As(III) is then transported across the periplasm by ArsB and/or Acr3 in coordination with an ATPase (ArsA). ArsH is a NADPH-flavin mononucleotide oxidoreductase that aids in detoxification through the probable oxidation of arsenite (Shen *et al.* 2013).

(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 resulting contigs was set at 800 bp. These contigs were joined manually 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 program (Karp, Paley and Romero 2002) identifying 4746 protein-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 available 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) and *Sphingobium 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 temperatures; Cowan 2009). Detected ORFs were assigned to (i) osmoregulation 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 H₂O₂ 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), 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 linkages (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, Fredrickson and Romine 2006; Aylward et 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 (Antarctic 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.

REFERENCES

- Aylward FO, McDonald BR, Adams SM, et al. Comparison of 26 sphingomonad genomes reveals diverse environmental adaptations and biodegradative capabilities. *Appl Environ Microb* 2013;79:3724–33.
- Aziz RK, Bartels D, Best AA, et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
- Baker-Austin C, Wright MS, Stepanauskas R, et al. Co-selection of antibiotic and metal resistance. *Trends Microbiol* 2006;14:176–82.
- Balkwill DL, Fredrickson JK, Romine MF. *Sphingomonas* and related genera. In: Dworkin M, Falkow S, Rosenberg E, et al. (eds). *The Prokaryotes: Delta, Epsilon Subclass*. New York: Springer, 2006, 605–29.
- Bonfield JK, Whitwham A. Gap5—editing the billion fragment sequence assembly. *Bioinform* 2010;26:1699–703.
- Chan Y, Lacap DC, Lau MC, et al. Hypolithic microbial communities: between a rock and a hard place. *Environ Microbiol* 2012;14:2272–82.
- Chevreux B, Wetter T, Suhaie S. Genome sequence assembly using trace signals and additional sequence information. In: *Proceedings of the German Conference on Bioinformatics* Hannover, Germany, 1999, 7.
- Cowan DA, Makhakanyane TP, Dennis PG, et al. Microbial ecology and biogeochemistry of continental Antarctic soils. *Front Microbiol* 2014;5:154.

- Cowan DA, Sohm JA, Makhalanyane TP, et al. Hypolithic communities: important nitrogen sources in Antarctic desert soils. *Environ Microbiol Rep* 2011;**3**:581–6.
- 145 Dash HR, Das S. Bioremediation of mercury and the importance of bacterial *mer* genes. *Int Biodeter Biodeg* 2012;**75**:207–13.
- Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol R* 2010;**74**:417–33.
- 150 Karp PD, Paley S, Romero P. The pathway tools software. *Bioinform* 2002;**18** (Suppl. 1):S225–32.
- Kim E-H, Nies DH, McEvoy MM, et al. Switch or funnel: How RND-type transport systems control periplasmic metal homeostasis. *J Bacteriol* 2011;**193**:2381–7.
- 155 Makhalanyane TP, Valverde A, Birkeland N-K, et al. Evidence for successional development in Antarctic hypolithic bacterial communities. *ISME J* 2013;**7**:2080–90.
- Margesin R, Schinner F. Biological decontamination of oil spills in cold environments. *J Chem Technol Biot* 1999;**74**:318–89.
- 160 Miller DN, Bryant JE, Madsen EL, et al. Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Appl Environ Microb* 1999;**65**:4715–24.
- Moriya Y, Itoh M, Okuda S, et al. KAAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res* 2007;**35**:W182–5.
- 165 Nagata Y, Ohtsubo Y, Endo R, et al. Complete genome sequence of the representative γ -hexachlorocyclohexane-degrading bacterium *Sphingobium japonicum* UT26. *J Bacteriol* 2010;**192**:5852–3.
- Nies DH. The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. *J Bacteriol* 1995;**177**:2707–12. 170
- Piddock LJV. Multidrug-resistance efflux pumps are not just for resistance. *Nat Rev Microbiol* 2006;**4**:629–36.
- Planchon FAM, Boutron CF, Barbante C, et al. Changes in heavy metals in Antarctic snow from coastal land since the mid-19th to the late-20th century. *Earth Planet Sc Lett* 2002;**200**:207–22. 175
- Shen Z, Han J, Wang Y, et al. The contribution of ArsB to arsenic resistance in *Campylobacter jejuni*. *PLoS One* 2013;**8**:e58894.
- Shin SC, Kim SJ, Ahn DH, et al. Draft genome sequence of *Sphingomonas echinoides* ATCC 14820. *J Bacteriol* 2012;**194**:1843–3.
- Short JW, Irvine GV, Mann DH, et al. Slightly weathered Exxon Valdez oil persists in Gulf of Alaska beach sediments after 16 years. *Environ Sci Technol* 2007;**41**:1245–50. 180
- Stallwood B, Shears J, Williams PA, et al. Low temperature bioremediation of oil-contaminated soil using biostimulation and bioaugmentation with a *Pseudomonas* sp. from maritime Antarctica. *J Appl Microbiol* 2005;**99**:794–802. 185
- Ting L, Williams TJ, Cowley MJ, et al. Cold adaptation in the marine bacterium, *Sphingopyxis alaskensis*, assessed using quantitative proteomics. *Environ Microbiol* 2010;**12**:2658–76.

GRAPHICAL ABSTRACT

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

Challenging life within a hypolithic consortia: Draft genome of *Spingomonas* AntH11

