

Genome Sequence of “*Candidatus Thioglobus singularis*” Strain PS1, a Mixotroph from the SUP05 Clade of Marine Gammaproteobacteria

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Mixotrophic marine bacteria from the SUP05 clade are ubiquitous in the ocean. Here, we announce the complete genome sequence of “*Candidatus Thioglobus singularis*” strain PS1, the first cultured mixotrophic representative from the SUP05 clade.

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Mixotrophic members of the SUP05 clade of marine gamma-proteobacteria are cosmopolitan and have important roles in marine biogeochemical cycles (1–5). They are of particular interest because of their potential to oxidize sulfur and fix carbon in the dark ocean and in expanding low oxygen zones (3, 5). Here we announce the complete genome sequence of “*Candidatus Thioglobus singularis*” strain PS1, which was isolated from surface waters (5 m) in Puget Sound. “*Ca. Thioglobus singularis*” PS1 is a member of the *arctic96BD-19* subclade (designated here by lower case italics) and is the first cultured representative from the SUP05 clade (6). It has a circular chromosome that is 1,714,148 bp in length and codes for 1,750 genes.

Cultures of “*Ca. Thioglobus singularis*” were grown in 1-liter polycarbonate bottles of filter sterilized seawater media as previously described (6). Cells were then filtered onto sterile 0.2- μ m polyethersulfone filters (Pall, Port Washington, NY), placed in 15-mL Teflon tubes containing 2 mL of sucrose lysis buffer (SLB), and flash frozen in liquid nitrogen. Cells were later lysed by adding 100 μ L of 1 mg/mL lysozyme and incubating at 4°C for 60 min, then by adding 465 μ L of 10% SDS and 250 μ L of proteinase K and incubating at 55°C for 2 h. DNA was extracted and purified from cell lysates using DNeasy blood and tissue and MinElute kits according to the manufacturer’s instructions, respectively (QIAGEN, Germantown, MD). A total of 20.35 μ g of DNA was used to construct a mate-pair library according to the SOLiD v3.0 mate-pair protocol (Life Technologies, Foster City, CA). The “*Ca. Thioglobus singularis*” genome was assembled using SEASTAR v0.4.17 as previously described (7). The initial assembly was 97% complete and contained 177 gaps with a mean gap length of 198 bp. Gaps were closed by PCR with custom primers designed using Geneious v7.0.4 (Biomatters, Auckland, NZ). Amplification products were visualized, gel purified, and sequenced by Genewiz (Foster City, CA). The complete genome sequence (1,714,148 bp) was confirmed by performing additional PCRs to resolve any irregularities in the assembly and by comparing read coverage, physical coverage, and insert sizes of mate pairs covering the genome. Annotations were performed using the NCBI Prokaryotic Genome Automatic Annotation Pipeline and were checked against RAST annotations (8, 9), IMG annotations (10), and in some cases by

phylogenetic analyses. Discrepancies were corrected and final annotations were submitted to NCBI.

Nucleotide sequence accession number. The complete genome sequence of “*Ca. Thioglobus singularis*” strain PS1 is available in GenBank under accession number CP006911.

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REFERENCES

- Walsh DA, Zaikova E, Howes CG, Song YC, Wright JJ, Tringe SG, Tortell PD, Hallam SJ. 2009. Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. *Science* 326:578–582. <http://dx.doi.org/10.1126/science.1175309>.
- Zaikova E, Walsh DA, Stilwell CP, Mohn WW, Tortell PD, Hallam SJ. 2010. Microbial community dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia. *Environ Microbiol* 12:172–191. <http://dx.doi.org/10.1111/j.1462-2920.2009.02058.x>.
- Swan BK, Martinez-Garcia M, Preston CM, Sczyrba A, Woyke T, Lamy D, Reinthaler T, Poulton NJ, Masland EDP, Gomez ML, Sieracki ME, DeLong EF, Herndl GJ, Stepanauskas R. 2011. Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. *Science* 333:1296–1300. <http://dx.doi.org/10.1126/science.1203690>.
- Anderson RE, Beltrán MT, Hallam SJ, Baross JA. 2013. Microbial community structure across fluid gradients in the Juan de Fuca ridge hydrothermal system. *FEMS Microbiol Ecol* 83:324–339. <http://dx.doi.org/10.1111/j.1574-6941.2012.01478.x>.
- Carolan MT, Smith JM, Beman JM. 2015. Transcriptomic evidence for microbial sulfur cycling in the eastern tropical north Pacific oxygen minimum zone. *Front Microbiol* 6:334. <http://dx.doi.org/10.3389/fmicb.2015.00334>.
- Marshall KT, Morris RM. 2013. Isolation of an aerobic sulfur oxidizer from the SUP05/Arctic96BD-19 clade. *ISME J* 7:452–455. <http://dx.doi.org/10.1038/ismej.2012.78>.
- Iverson V, Morris RM, Frazer CD, Berthiaume CT, Morales RL, Armbrust EV. 2012. Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. *Science* 335:587–590. <http://dx.doi.org/10.1126/science.1212665>.

8. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75-2164-9-75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
9. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42: D206–D214. <http://dx.doi.org/10.1093/nar/gkt1226>.
10. Markowitz VM, Chen I-MA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC. 2012. IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic Acids Res* 40:D115–D122. <http://dx.doi.org/10.1093/nar/gkr1044>.