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### Title

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# The Complete Genome of the Uncultivated Ultra-Deep Subsurface Bacterium *Desulforudis audaxviator* Obtained by Environmental Genomics

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A more complete picture of life on Earth, and even life *in* the Earth, has recently become possible through the application of environmental genomics. We have obtained the complete genome sequence of a new genus of the *Firmicutes*, the uncultivated sulfate reducing bacterium *Desulforudis audaxviator*, by filtering fracture water from a borehole at 2.8 km depth in a South African gold mine. The DNA was sequenced at the JGI using a combination of traditional Sanger sequencing and 454 pyrosequencing, and assembled into just one genome, indicating the planktonic community is extremely low in diversity. We analyzed the genome of *D. audaxviator* using the MicrobesOnline annotation pipeline and toolkit (<http://www.microbesonline.org>, and see MicrobesOnline abstract), which offers powerful resources for comparative genome analysis, including operon predictions and tree-based comparative genome browsing. MicrobesOnline allowed us to compare the *D. audaxviator* genome with other sequenced members of the *Firmicutes* in the same clade (primarily *Pelotomaculum thermopropionicum*, *Desulfotomaculum reducens*, *Carboxydotherrmus hydrogenoformans*, and *Thermoanaerobacter tengcongensis*), as well as other known sulfate reducers (including *Archaeoglobus fulgidus* and *Desulfovibrio vulgaris*). *D. audaxviator* gives a view to the set of tools necessary for what appears to be a self-contained, independent lifestyle deep in the Earth's crust. The genome is not very streamlined, and indicates a motile, endospore forming sulfate reducer with pili that can fix its own nitrogen and carbon. *D. audaxviator* is an obligate anaerobe, and lacks obvious homologs of many of the traditional O<sub>2</sub> tolerance genes, consistent with the low concentration of O<sub>2</sub> in the fracture water and its long-term isolation from the surface. *D. audaxviator* provides a complete genome representative of the Gram-positive bacteria to further our understanding of dissimilatory sulfate reducing bacteria and archaea, and offers the full complement of genes necessary for an independent lifestyle based solely on interactions with the geochemistry of the deep subsurface.