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A Novel Uncultured Iron and Sulfur Oxidizing Bacterium of the Genus *Gallionella* Revealed by Metagenomic Analysis of a Low-Temperature Acid Mine Drainage

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Acid mine drainage (AMD) is generated from the microbially mediated oxidative dissolution of sulfide minerals and characterized by low pH and high concentrations of sulfate and metals, representing an extreme environment for life. In this study we have analysed AMD geochemistry and microbial community of the abandoned open-cast polymetallic mine at Sherlovaya Gora in Eastern Siberia, Russia. AMD water samples were collected from the 17m deep narrow borehole ShG14-8. The water collected in late July had low temperature of 6.5°C. It was acidic (pH 2.65), oxidised (Eh +447 mV) and contained high concentrations of iron (434 mg/l), zinc (596 mg/l), cadmium (39 mg/l) and sulfate (3631 mg/l).

Metagenomic analysis of this AMD indicate a low bacterial diversity and absence of archaea. The community is dominated by a single bacterial lineage, a new species in the beta-proteobacterial genus *Gallionella*, designated "*Candidatus* Gallionella acididurans". Bacteria of the genera *Thiobacillus*, *Acidobacterium*, *Acidisphaera*, and *Acidithiobacillus* were the minor components of the community.

Known cultivated species of the genus *Gallionella* are iron-oxidising microaerophilic freshwater bacteria known to grow at pH 4.5-7. However, related microorganisms were identified in AMD samples with pH 2-4 and high concentrations of heavy metals. In order to characterize the novel *Gallionella* lineage we reconstructed the almost complete 3.4 Mb composite genome of *Ca.* "Gallionella acididurans" from metagenomic sequence data. The completeness of the genome is evidenced by the presence of all 100 conserved single-copy marker genes. Genome analysis suggests that Fe(II) oxidation in *Ca.* "Gallionella acididurans", like in *Acidithiobacillus ferrooxidans*, could depend on the Cyt2-like cytochrome located at the outer membrane, while *rnto* genes found in freshwater Fe oxidising bacteria are missing. The electron transfer chain includes cytochrome bc_1 complex, alternative complex III, NADH dehydrogenase and succinate dehydrogenase. *Ca.* "Gallionella acididurans" encodes cytochrome oxidases of *bd*, cbb_3, and bo_3 types. Oxidation of reduced sulfur compounds could be enabled by SOX system comprising *soxXYZAB* and a set of *dsr* genes, sulfide:quinone oxidoreductase, adenylylsulfate reductase and sulfate adenylyltransferase. Autotrophic carbon fixation could proceed via the Calvin-Benson-Basshom pathway, as evidenced by the presence of four RubisCO genes, while nitrogen-fixation is missing. Adaptation of *G. acididurans* to heavy metals and other toxicants is reflected by the high number of RND-family metal transporters and heavy metal transporting P-type ATPases.

Ca. "Gallionella acididurans" is more metabolically versatile and better adapted to metal-contaminated acidic environment than its closest relative, *Gallionella capsiferriformans* ES-2, as reflected by the presence of sulfur-oxidation pathways, different kinds of terminal oxidases that could operate in aerobic and microaerophilic conditions, acid- and metal tolerance systems. Overall, our results provide genomic insights into the physiology of acidotolerant lineage of *Gallionella* that represented dominant and biogeochemically important group of bacteria in cold acidic metal-rich environments.