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Metagenomic characterization of novel electroactive microorganisms enriched from stibnite mine wastewater

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Arsenic (As) and antimony (Sb) are naturally occurring toxic metalloids with increasing environmental concerns. Both metalloids are aften strongly adsorbed with Fe(III) oxyhydroxides in natural systems and microbe-mineral interactions play important roles in both iron and metalloids transformations. To gain insights into microbe-mineral interactions associated with contaminated environment, we investigated electroactive microbial communities with ability to utilize extracelular electron transfer for microbial respiration by using *in situ* electrochemical enrichment and genomic approaches. To enrich cathodic microbial communities, polarized electrodes were deployed in stibnite mine wastewater runoffs (KIN and TAI sites), and provided with low current densities (0.1~0.4 mA/m^2). After 6 months of deployment, the cathodic electron uptake associted with cellular activity was observed at both sites. On the KIN cathode surface, the precipitation of calcite and aragonite was observed, and As and Sb were accumulated up to 1000 times (As, 374 ppm; Sb, 99.3 ppm). The 16S rRNA gene targeted amplicon sequencing of the cathodic microbial community showed the dominance (>80% relative abundance) of novel Gammaproteobacterial phylotypes, distantly related to cultivated strains such as *Acidiferrobacter thiooxydanse* and *Nitrosococcus oceani* (89.1 and 88.8% sequence identity, respectively). The *in situ* electrochemical enrichment was repeated, and the emergence and enrichment of the same dominat phylotypes were observed after 51 days of deployment with electrochemical activity. Metagenomic analyses revealed the presence of two highly similar metagenome-assembled genomes from KIN (MAG1 and MAG2, 91% ave. nt identity) and one major MAG from TAI with lower similarity to MAGs from KIN site (~72% ave. nt identity). These MAGs commonly contained genes associated with possible extracellular electron transfer functions, aerobic respiration, sulfur oxidation, heavy metal resistance, and CO_2 and nitrogen fixations. These results indicated that the novel electrosynthetic Gammaproteobacteria enriched on the deployed cathode may promote the precipitation of calcite biominerals, which served as natural adsorbent for toxic metalloids at KIN site. Consequently, *in situ* electrochemical enrichment approach used in this study was successful in revealing functional attribute of an uncultivated group of electrosynthetic microorganisms associated with contaminated mine wastewater environment.