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A new genetic biomarker for quantifiying microbial selenate reduction in geochemical systems

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Introduction: Microbial selenate [Se(VI)] reduction plays an important role in controlling the mobility and toxicity of selenium in natural and contaminated environments. Certain bacteria use selenate as a terminal electron acceptor for anaerobic respiration, producing insoluble elemental selenium and influencing selenium redox cycling in soils and aquatic systems. *Enterobacter cloacae* is a facultative anaerobe capable of respiratory selenate reduction, but the genetic basis of this process has not been identified. Characterizing the genes underlying selenate reduction is necessary for interpreting metagenomic data and for quantifying the biogeochemical impact of Se-reducing microbial communities.

Materials and Methods: The genome of *E. cloacae* was sequenced and annotated to identify candidate molybdenum-containing reductases associated with anaerobic respiration. Gene knockout mutants were constructed to test the function of candidate genes in selenate reduction, and complementation experiments were performed to confirm gene identity. Heterologous expression was also conducted in an *Escherichia coli* mutant lacking its native selenate reductase genes to evaluate cross-species functionality. Selenate reduction activity was measured using ion chromatography.

Results and Discussion: Genome analysis identified a four-gene operon, designated *srnABCD*, encoding a TAT-exported molybdoenzyme complex. Targeted deletion of *srnA* eliminated selenate reduction in *E. cloacae*. Complementation with the wild-type *srnA* sequence restored the abolished phenotype. Expression of *srnA* in an *E. coli* Δ*ynfEF* mutant conferred the ability to reduce selenate, indicating functional compatibility with the host respiratory system. Protein structure modeling predicted a heterotrimeric complex containing a molybdenum catalytic center and multiple iron–sulfur clusters forming an electron-transfer pathway to the membrane anchor. The active site forms a positively charged funnel that guides selenate oxyanion to the molybdenum cofactor for reduction. These findings reveal the genetic basis of selenate respiration in *E. cloacae* and enable the development of PCR-, genomic-, and transcriptomic-based tools to detect and quantify microbial selenate reduction in geochemical systems.