Perchlorate mediated adaptive evolution of *Desulfovibrio alaskensis* G20

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Due to its toxic, explosive, and corrosive nature, inadvertent biological H_2S production by sulfate reducing microorganisms (SRM) poses significant health and industrial operational risks. Anthropogenic sources are dominated by the oil industry where H_2S in reservoir gases and fluids has an associated annual cost estimated at $90 billion globally. Our previous studies identified perchlorate (ClO_4^-) as a selective and potent inhibitor of SRM in pure culture and complex microbial ecosystems. However, constant addition of inhibitors like ClO_4^- to natural ecosystems may result in a new adaptive selective pressure on SRM populations. With this in mind we investigated the ability of *Desulfovibrio alaskensis* G20, a model oil reservoir SRM, to adapt to perchlorate and develop a resistance. Serial transfers of three parallel cultures with increasing concentrations of perchlorate up to 100 mM were generated and compared to wild-type strains that were transferred for same number of generations in absence of perchlorate. Genome sequencing revealed that all three adapted strains had single non-synonymous single-nucleotide polymorphisms in the same gene, Dde_2265, the sulfate adenylytransferase (ATP sulfurylase (ATPS)) (EC 2.7.7.4). ATPS catalyzes the first committed step in sulfate reduction and is essential in all SRM. IC_{50}s against growth for these evolved strains demonstrated a three-fold increased resistance to perchlorate compared to wild-type controls. These evolved strains also had ~5x higher transcriptional abundance of Dde_2265 compared to the wild-type strain. Biochemical characterization of the purified ATPS enzyme from both wild-type and the evolved strain showed that the mutant ATPS from the evolved strain was resistant to perchlorate inhibition of ATP turnover with a K_I for perchlorate that was ~3x greater relative to the wild-type ATPS. Additional for understanding the evolutionary dynamics of this SNP, 10 independent cultures were gradually evolved in increasing concentration of ClO_4^- in order to follow the frequency of the SPN though time and concentration of ClO_4^-, using a high-throughput sequencing approach- FREQ-Seq. These results demonstrate that a single-base pair mutation in ATPS can have a significant impact on developing resistance to perchlorate and suggest that adaptive evolution is a valuable tool to understand potential responses of microorganism to any environmental perturbations imposed during oil production.