Isolation and Characterization of diverse anaerobic Cr(VI) tolerant bacteria from Cr(VI)-contaminated 100H site at Hanford

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Hexavalent Chromium [Cr(VI)] is a widespread contaminant found in soil, sediment, and ground water. Cr(VI) is more soluble, toxic, carcinogenic, and mutagenic compared to its reduced form Cr(III). In order to stimulate microbially mediated reduction of Cr(VI), a polylactate compound HRC was injected into the chromium contaminated aquifers at site 100H at Hanford. Based on the results of the bacterial community composition using high-density DNA microarray analysis of 16S rRNA gene products, we recently investigated the diversity of the dominant anaerobic culturable microbial population present at this site and their role in Cr(VI) reduction. Positive enrichments set up at 30°C in the dark using specific defined anaerobic media resulted in the isolation of an iron reducing isolate strain HAF, a sulfate reducing isolate strain BLS and a nitrate reducing isolate, strain HLN. Preliminary 16S rDNA sequence analysis identifies strain HAF as *Geobacter metallireducens*, strain HLN as *Pseudomonas stutzeri* and strain BLS as a member of *Geosinus* species. Strain HAF isolated with acetate as the electron donor utilized propionate, glycerol and pyruvate as alternative carbon sources, and reduced metals like Mn(IV) and Cr(VI). Growth was optimal at 37°C, pH of 6.5 and 0% salinity. Strain HLN isolated with lactate as electron donor utilized acetate, glycerol and pyruvate as alternative carbon sources, and reduced metals like Mn(IV) and Cr(VI). Optimal growth was observed at 37°C, at a pH of 7.5 and 0.3% salinity. Anaerobic active washed cell suspension of strain HLN reduced almost 95μM Cr(VI) within 4 hours relative to controls. Further, with 100μM Cr(VI) as the sole electron acceptor, cells of strain HLN grew to cell numbers of 4.05×10^7/ml over a period of 24h after an initial lag, demonstrating direct enzymatic Cr(VI) reduction by this species. 10mM lactate served as the sole electron donor. These results demonstrate that Cr(VI) immobilization at the Hanford 100H site could be mediated by direct microbial metabolism apart from indirect chemical reduction of Cr(VI) by end products of microbial activity.