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ABSTRACT TITLE: Survival During Long Term Starvation: Global Proteomics Analysis of Geobacter sulfurreducens Under Prolong Electron Acceptor and Donor Limitation

ABSTRACT: Limitation/bioavailability of terminal electron acceptors (TEA) and electron donors is an environmentally relevant condition that affects the overall subsurface bioremediation process and efficiency. Microorganisms exist under "feast or famine mode" of survival, however they typically encounter famine conditions. *In situ* bioremediation technology uses indigenous microorganisms to treat large scale subsurface contamination. The technology relies on addition of suitable electron donors to subsurface sediments to stimulate bacterial activity. However, the high electron donor concentration conditions are artificial and transitory. Similarly TEA is also limited, we suggest that TEA-starvation conditions would evolve at injection wells after prolonged carbon addition. The work described here aims to understand the survival strategies of the anaerobe *Geobacter sulfurreduces* under both electron acceptor and electron donor limiting conditions.

Our results have demonstrated that *G. sulfurreducens* can survive under both TEA (fumarate) and electron donor (acetate) limiting conditions and displays five typical stages of growth: lag, log, stationary, death, and survival phases. The organism can sustain a stable population of ~10^6 cells/ml for over 2 years under TEA limiting conditions. Whereas it can sustain a higher population count of ~10^8 cells/ml for over 1 year under electron donor limiting conditions. Global comparative proteomic analysis was performed using iTRAQ and proteins varying in abundance with a high level of statistical significance ($p<0.05$) were identified. Under TEA starvation, 103 proteins were identified to be significantly up-regulated compared to 85 significantly down-regulated in survival phase cells as compared to mid-log cells. The most highly represented and significantly up-regulated proteins in the starved cells are involved in energy metabolism, cell envelope, and transport and binding functional categories. The majority of them were found to be localized in the cell membranes. The above results suggest that changes in the outer and cytoplasmic membrane are important in the survival of *Geobacter* under the aforesaid conditions. The cell shuts down anabolic processes and is poised, through changes in its membrane proteins, to sense nutrients in the environment, to transport nutrients into the cell and finally to detect/utilize TEAs it may encounter. Under TEA-limiting conditions, the cell is also highly reduced with minimal change in energy charge. Therefore, our proteomic and biochemical results indicates that even under TEA starvation, cells are well suited for bioremediation via reduction of radionuclides. The proteomic analysis of acetate starved cultures identified only 27 significantly up-regulated proteins compared to 196 significantly down-regulated in survival phase cells as compared to mid-log cells. Most of the up-regulated proteins belonged to energy metabolism, transport and binding, and protein fate functional categories. Most of the down-regulated proteins were ribosomal, transcription and translation proteins involved in protein synthesis, similar to TEA starvation condition. Here again, the majority of the up-regulated proteins were predicted to localized in the cytoplasmic membrane, and most of the down-regulated proteins were predicted to be localized in the cytoplasm. So far we were able to demonstrate that *Geobacter* can survive long-term nutrient limiting conditions and the differences in protein levels between the growing and starved cells. We will further investigate the genetic basis of the survival capacity by genomic sequencing.