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

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The dissimilatory iron-reducing anaerobe Geobacter sulfurreducens is used as a model organism for understanding bioremediation at contaminated subsurface sites. In our studies, G. sulfurreducens has been shown to exhibit a GASP phenotype in the laboratory, and to revive from long-term starvation (>500 days) when inoculated into fresh medium. Upon revival, the cells are capable of reducing Fe(III) in Fe citrate. These studies have been aimed at elucidating the mechanisms employed by G. sulfurreducens for such long-term survival, under both electron donor and terminal electron acceptor (TEA) conditions, the latter of which could exist after prolonged carbon addition at bioremediation sites.

To investigate potential changes in protein/enzyme expression under nutrient-limited conditions, global comparative proteomics analyses were carried out for G.sulfurreducens cultures in mid-log and survival phases. Cultures were grown under both acetate (electron donor)-limited and fumarate (TEA)-limited conditions, and proteins were harvested at mid-log and long-term survival time points. The proteins were analyzed by LC/MS-based iTRAQ methods, and those peptides which exhibited changes in abundance between mid-log and survival phases at a significance level $p<0.05$ were further analyzed to determine function and cellular location. Under both electron donor- and TEA-limited conditions, many of the proteins up-regulated in survival phase vs. mid-log phase were involved in energy metabolism, and were membrane-associated. In contrast, many proteins involved in anabolic processes were down-regulated under nutrient-limited conditions. Other up-regulated proteins included those involved in protein transport, indicating that the cells are poised to sense nutrients in the environment as they become available.

Changes in acetate, fumarate, and succinate were monitored by HPLC under both acetate (electron donor)-limited and fumarate (TEA)-limited conditions to document points at which the cultures became nutrient-limited. Under acetate-limited conditions (5.5 mM acetate/30 mM fumarate), acetate was completely depleted within 3 days, while fumarate depleted rapidly within 3 days to ~12 mM, then continued to decrease gradually over ~75 days, with concomitant accumulation of succinate to ~25 mM. Under fumarate-limited conditions (30 mM acetate/50 mM fumarate), fumarate was depleted by day 7, during which time succinate concentrations increased then leveled off at ~50 mM, while acetate concentrations decreased to ~15 mM by day 3 and did not change thereafter. These results indicate that when the cells are electron-donor (acetate) limited, they may utilize other, less favorable intracellular or environmental electron donors as necessary. However, when TEA-limited, the cells survive in a highly reduced state until new electron acceptors become available.