Differential proteomics analysis of GASP (Growth advantage in stationary-phase) phenotype of *Geobacter sulfurreducens* under "famine" conditions

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Geobacter sulfurreducens is used for bioremediation at heavy metal and readioactive waste contaminated sites. The organism typically encounter limited access to electron donors and/or electron acceptors in such environments. Therefore, gaining an understanding of how *Geobacter sulfurreducen* involved in bioremediation survive under such conditions is relevant for planning efficient bioremediation strategies. Previously we have demonstrated that *Geobacter sulfurreducens* displays five typical stages of growth: lag, log, stationary, death, and survival phases. The orgainsm acquire a growth advantage adaptation that begin to emerge during the stationary phase; this adaptation is termed "growth advantage in stationary phase," or GASP. The GASP strains can out-compete mid-log phase population when co-cultured under similar conditions. This work aims to study differential protein expression between different growth phases of *Geobacter sulfurreducens* grown under electron acceptor (fumarate) limiting conditions. We employed LC/MS- based iTRAQ methods to obtain information about relative changes in protein expression between mid-log, stationary and survival phases.

There were 80 proteins that were significantly up-regulated in survival phase organisms as compared to mid-log. Most of the up-regulated proteins (29%) were found to be involved in energy metabolism function. These included components of the ATP synthase and NADH dehydrogenase enzymes, as well as cytochrome c family proteins and all three components of the heterotrimeric fumarate reductase FrdCAB. In addition, several outer membrane proteins and putative lipoproteins involved in transport and binding were also up-regulated. About 40% of the total up-regulated proteins were found to be hypothetical or with unknown function.

We identified 66 proteins that were significantly down-regulated in survival phase organisms. Around 40% were ribosomal, transcription and translation proteins involved in protein synthesis. In addition 10 proteins (~15%) associated with energy metabolism, 5 (~8%) involved in nucleotide biosynthesis and 11 (~17%) hypothetical or unknown function proteins were also down-regulated. Two proteins that play a role in heme biosynthesis, the gene products of GSU3285 and GSU3453, were down-regulated. However, preliminary results did not indicate that total heme levels were lower in survival phase cultures compared with mid-log phase cultures. Of all the 146 proteins differentially expressed between mid-log and survival phases, the predicted cellular localization of 117 could be determined. A majority of the up-regulated proteins were predicted to localize in the membrane and periplasmic space, and most of the down-regulated proteins were predicted to be localized in the cytoplasm.

These results indicate that the composition of the inner and outer membranes of *G. sulfurreducens* changes drastically from mid-log to survival phase and may have important implications for organism's survival under famine conditions. Fewer changes in protein expression between mid-log and stationary phases as compared to mid-log and survival phases, indicate that the significant changes in survival expression cannot be detected at early stationary phase. Thus, study of long-term cultures is necessary to truly appreciate the nature of *G. sulfurreducens* dwelling in famine environments. The genetic basis of the GASP phenomenon and survival capacity will be further investigated by genomic sequencing. We will also determine the energy charge and reducing equivalents involved to gain insight into the energy metabolism of the organism.