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Project #1026866

Title: Identification of Molecular and Cellular Responses of Desulfovibrio vulgaris Biofilms under Culture Conditions Relevant to Field Conditions for Bioreduction...

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Results To Date: Title: Identification of Molecular and Cellular Responses of Desulfovibrio vulgaris Biofilms under Culture Conditions Relevant to Field Conditions for Bioreduction...
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Desulfovibrio vulgaris ATCC29579 is a sulfate- reducing bacterium (SRB) that is commonly used as a model for direct and indirect heavy metal reduction, and can also be a causitative agent of metal corrosion. During growth with lactate and sulfate, internal carbohydrate levels increased throughout exponential-phase, and peaked as the cells transitioned to stationary-phase. The carbohydrate to protein ratio (C:P) peaked at 0.05 ug/ug as the cells transitioned to stationary-phase, and then declined to 0.02 ug/ug during extended stationary-phase. In contrast, a strain of D. vulgaris that does not contain the megaplasmid, maintained higher internal carbohydrate levels and the C:P ratio peaked at 0.1 ug/ug (2-fold increase compared to wild-type). Under the tested growth conditions, we observed biofilm formation in wild-type cells, but the plasmid-less strain formed less biofilm (2-fold decrease). We hypothesized that carbohydrate was re-allocated to the external cell proper for biofilm formation. However, biofilm contained relatively little carbohydrate (0.6 to 1.0 ug/ml) and had a similar C:P ratio compared to wild-type early stationary-phase cells. Staining with calcafluor white also indicated the presence of little external carbohydrate in D. vulgaris biofilms. Less biofilm was formed in the presence of protinease K, trypsin, and chymotrypsin, however, the growth of planktonic cells was not affected. In addition, when D. vulgaris biofilm was treated with a protease, less biofilm was observed. Electron micrographs suggested the presence of filaments between the biofilm cells, and filaments appeared to be susceptible to protease treatment. Biofilm filtrates contained soluble protein, and SDS-PAGE analysis suggested different polypeptide profiles between a filtrate, a planktonic, and a biofilm sample.

Results indicated that D. vulgaris formed more biofilm in late stationary phase when compared to earlier growth phases. Lower pH did not have a dramatic effect on biofilm formation; however, addition of the polyamines, norspermidine or spermidine, did stimulate biofilm formation at 30 degrees C. A fur deletion mutant (JW707) formed more biofilm and at an earlier growth stage than the wild type in undisturbed cultures, and PerR deletion mutants (JW708) showed no difference from the wild-type in biofilm formation. Addition of sugars (glucose, galactose, mannose, or gluconate) did not significantly effect biofilm formation; however, addition of glucose to acid pH (pH 5.5) medium completely inhibited the cell growth and biofilm formation. When the biofilm formation was assayed by the content of the biomass which was attached to the bottom of test tubes, it appeared that formation was influenced by the iron concentrations ($FeCl_2$) in the growth medium. The role of

iron availability in biofilm formation and maintenance is for SRB is unknown.

The optimization of growth conditions for biofilm formation and maintenance is underway with respect to yield and stability. We tested two types of reactors: a drip-flow reactor and a CDC reactor. The main desired parameters are maximal biomass yield and stability. Modifications of the CDC reactor have been tested to increase biomass yield and allow stabilization of biomass production under a given set of tested growth conditions. Preliminary biomass samples are being tested for proteomic analysis. In addition, targeted mutagenesis is underway to knock-out suspected genes that encode proteins that may be involved in biofilm formation. The following genes have been selected based upon preliminary data and previous data in different microorganisms: fliG, pilin, and fliA. These mutants will aid in the characterization of possible roles for pili and/or flagella in the ability to initiate and maintain biofilms in D. vulgaris. The results indicated that D. vulgaris changes carbohydrate distributions in response to growth phase, biofilm formation can respond to changes in physiological conditions for the cells, the megaplasmid contains genes important for carbohydrate distribution and biofilm formation, and D. vulgaris biofilms contain extracellular, polypeptides that may be important for biofilm formation. In addition, in contrast to typically studied biofilm systems that use carbohydrate-based attachment, the data suggested that D. vulgaris uses a different mechanism(s). An understanding of cell attachment and biofilm formation will provide great insight into how SRBs may interact and persist with heavy metals under relevant growth conditions (i.e., biofouling and bioremediation).

In order to study heavy metal resistance [e.g., Cr(VI)] of D. vulgaris biofilms, we have initiated studies to first characterize Cr(VI) sensitivity in planktonic cells so that a base-line can be determined for cellular susceptibility. Although much work has focused on Cr and U reduction via individual enzymes (e.g., hydrogenase, cytochromes), less is known about the cellular response to heavy metals in Desulfovibrio species. When exponential-phase cells were washed to remove hydrogen sulfide carry-over and inoculated into fresh medium with different levels of Cr(VI), lag time increased as the concentration of Cr increased. Cells lagged approximately 5, 40, and 55 h M Cr, respectively. When cells wereμin the presence of 20, 50, and 100 M Cr, Cr(VI) levels declined to undetectable levels within 2μtransferred to 50 h and lactate was consumed, but sulfate did not decline until growth was initiated approximately 40 h later. As much as a 7 mM decrease in lactate occurred during the lag while sulfate levels remained unchanged. When cell growth was initiated, lactate utilization rate increased, sulfate was consumed, and acetate concentrations increased. Similar trends were observed for cultures M Cr(VI) that corresponded to the different lag times.μtreated with 20 or 100 During the lag phase when lactate was consumed, the production of hydrogen was detected; however, the amount of hydrogen produced with or without Cr(VI) was not significantly different. These results indicated that hydrogen production alone could not account for the utilization of lactate in the absence of sulfate reduction. The results indicated that lactate oxidation was decoupled from sulfate reduction in the presence of Cr(VI). In addition, the Cr(VI) exposure caused a delay in growth that exceeded the time for reduction of the metal. We interpret this delay as a time for recovery from the damage caused by the toxic metal that apparently has access to the cytoplasm of the bacteria.

In conjunction with the VIMSS group (Genomes to Life project), we have also complemented on-going work by the groups dealing with Cr(VI) exposure. Whole-genome expression profiles indicated that the following groups of genes were up-expressed in response to Cr(VI) exposure: reductases and transporters. Multiple

heavy metal transporters were up-expressed and included putative permeases, drug efflux, and metal ATPases. These results suggest the importance of Cr(III) efflux after Cr(VI) has been reduced. Based upon the results a FMN-dependent nitroreductase might reduce Cr(VI) directly or reduce a Cr-complex. The FMN reductase could synthesize FMNH2 and the NADP dehydrogenase might be used to regenerate NADPH2. The nitroreductase, FMN reductase, and NADP dehydrogenase were all up-expressed based upon the microarray data. The chrAB genes on the megaplasmid most likely play a key role in Cr(III) efflux based upon microarray data and growth data. Additional toxicological effects could be occurring once the Cr(III) is produced via protein denaturation in the cytoplasm, periplasm, and outer cell proper. In the future, Cr(VI) exposure of D. vulgaris biofilms will be characterized and compared to planktonic cells. Further work will be needed to delineate the possible roles of the respective genes. It is also unknown if the biofilm mode of growth would provide more protection from heavy metal exposure that would be encountered when bio-reduction is stimulated at field sites of interest.

Deliverables: ABSTRACTS

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