

Project #1026866

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

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Results To Date: *Desulfovibrio vulgaris* Hildenborough forms biofilms under stress and nutrient limitation conditions. Analysis of the transcriptional responses to acid exposure suggested that genes encoding arginine and polyamine biosynthesis were increased in expression. A literature search showed that polyamines had been suggested to stimulate biofilm formation in some bacteria. Therefore, biofilm formation by *D. vulgaris* was then examined. Two different assay methods were used to estimate the biofilm formation. First, the classical crystal violet stainable material attached to glass test tubes was measured. Second protein attached to the test tube sides and especially precipitated at the bottom was measured.

The results indicate 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 30C.

Several mutants were tested for their effects on biofilm formation. The *fur* deletion mutant (JW707) formed more biofilm and at an earlier growth stage than the wild type in undisturbed cultures. *PerR* deletion mutants (JW708) showed no difference from the wild-type in biofilm formation. Under our conditions, a similar result was obtained for a strain of *D. vulgaris* (JW801) lacking the mega-plasmid present in the sequenced ATCC strain. Polyamine stimulation of biofilm formation was also observed for the *fur* and *perR* mutants. Addition of sugars (glucose, galactose, mannose, or gluconate) did not significantly effect the biofilm formation; however, addition of glucose to acid pH (pH 5.5) medium completely inhibited the cell growth and biofilm formation. Glucose cannot be used as a carbon source for growth of *D. vulgaris* in spite of the apparent annotation of genes for all the glycolytic enzymes. Therefore the unexpected inhibition by glucose at low pH would suggest that the accessibility of glucose to the cell has changed with pH. This will be explored further.

Finally, 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. This may indicate that cultures are somewhat stickier at higher iron concentrations. The cause of this observation remains to be determined.

Deliverables: H.-C. B. Yen, K. Bender, M.W. Fields, and J.D. Wall. 2006 *Desulfovibrio vulgaris* Hildenborough biofilm formation by wild type, the mega-plasmid deletion strain and a *fur* deletion mutant. Abstr. 106th Gen. Meet. Amer. Soc. Microbiol., Orlando, May 2006, Q-238.