Proposal to Present #12e: A Proteomic Approach to Identify Physiological Changes in *E. coli* Metabolically Engineered for Enhanced TCE Degradation and Toxicity Reduction

C. M. R. Lacerda, K. F. Reardon, Department of Chemical Engineering, Colorado State University, Fort Collins, CO 80523-1370

L. Rui, T. K. Wood, Department of Chemical Engineering, University of Connecticut, Storrs, CT 06269-3222

Introduction

Trichloroethylene (TCE) and other chlorinated ethenes are environmental pollutants frequently found in groundwater. Anaerobic biodegradation of these chemicals can produce more toxic compounds, while aerobic degradation is limited to cometabolism and involves the production of an intermediate epoxide that is cytotoxic, eventually inactivating the bacterium. Toluene ortho-monoxygenase (TOM) was originally studied for its ability to oxidize chlorinated ethenes; hence there is considerable interesting in utilizing it for bioremediation. TOM-Green is a DNA shuffling variant that has an enhanced degradation rate for TCE, but also produces the cytotoxic epoxide at a higher rate. One mechanism for biological detoxification of those intermediates involves the utilization of glutathione S-transferase (GST) and γ-glutamylcysteine synthetase, encoded by the *isoI* and *GSHI** genes, respectively.

In a metabolic engineering approach to improve aerobic biodegradation, TOM-Green, GST, and GSHI* were co-expressed in *Escherichia coli* TG1 cells, resulting in three-fold higher cis-dichloroethene degradation rates and protection from the effects of DCE epoxide. As a result of these genetic manipulations, it is likely that a variety of physiological changes occurred in the bacterial cells. These changes must be understood in order to evaluate the ability and performance of the engineered bacteria to degrade the pollutants, and to inform further metabolic engineering.

Previously, two-dimensional polyacrylamide gel electrophoresis was used to analyze the variations in the proteome resulting from the addition of wild-type TOM and TOM-Green, and the expression levels of several dozen proteins were found to be significantly altered. The goal of the current project is to use a similar proteomic approach to characterize the

physiological changes in cells modified with the *isoILR1* and *GSHI** genes, compared to the cells that only have the TOM-Green genes.

Materials and methods

The six genes encoding TOM-Green were cloned into *E. coli* TG1 using a high copy number plasmid (pBS(Kan^R)). *isoILR1* and *GSHI** were cloned into a low-copy number plasmid (pMMB277(Cam^R)). Batch cultures were grown in chloride-free M9 minimal medium with maltose as the sole carbon source, and kanamycin and chloramphenicol were added to all batches containing the cloned strains in order to maintain gene integrity. IPTG was added to induce isoILR1 and GSHI* under control of the tac promoter and TOM-Green under the lac promoter. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was utilized to analyze changes in the protein profiles of *E. coli* TG1 after the addition of each gene, and in the presence and absence of TCE. Overall protein profiles were studied using 18 cm wide-range (pH 3 to 10 nonlinear) immobilized pH gradients strips for isoelectric focusing, and 16 x 18 cm SDS-PAGE. Differentially expressed proteins were identified by MALDI-TOF mass spectrometry, followed by a database search.

Results and discussion

The proteomes of *E. coli* TG1 pBS(Kan^R) TOM-Green pMMB277(Cam^R), *E. coli* TG1 pBS(Kan^R) TOM-Green pMMB277(Cam^R) isoILR1, and *E. coli* TG1 pBS(Kan^R) TOM-Green pMMB277(Cam^R) isoILR1 GSHI* were analyzed. Comparison of the two-dimensional gels showed that the introduction of each gene resulted in up or down regulation of a number of proteins relative to the control (*E. coli* TG1 pBS(Kan^R) TOM-Green pMMB277(Cam^R)). Further analysis was accomplished by mass spectrometry, and some proteins that were characteristic of the genes present were identified. The proteomes of TCE-exposed cells were also analyzed. The results showed that the TCE exposure and transformation was also responsible for changes in the protein profiles.

The proteomic approach was valuable in yielding insights into the cellular pathways that could be affected by metabolic engineering. These results support the use of proteomics to monitor physiological consequences of metabolic engineering.