A MRM-based Mass Spectrometry Method for Optimization of Protein Expression to Increase Biofuel Production

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Optimization of biofuel pathway protein levels is crucial to balancing the energetic and carbon utilization of a microbe for efficient biofuel production. However, identification and quantification of specific proteins in complex mixtures is a difficult task. Since the physical attributes of proteins (e.g., MW, pI) are quite similar extensive separation or high specificity are needed to correctly identify a particular protein from a cell lysate. Western blots simplify analyses due to their high selectivity towards the target protein and tagging the protein of interest offer a means by which selective enrichment is possible. Yet, Western blots and tagging have limitations that make alternate methods attractive. One method, multiple-reaction monitoring, is capable of rapidly changing the target protein, something not possible without an antibody for the new protein, and detecting multiple target proteins in the same sample, something not possible without multiple tagging strategies and different enrichment steps. Multiple-reaction monitoring (MRM) is a mass spectrometric technique that has been used for small molecule DMPK studies for many years and has recently been adapted to peptides. Coupled to liquid chromatography, MRM-based analysis offers high selectivity and sensitivity. This method utilizes two points of selection (a peptide mass and a specific fragment mass generated by MS/MS) to eliminate background signal and noise even in very complex mixtures. Since the entire mass range is not scanned and only specific MRM transitions (combinations of peptide and fragment masses) are detected a significant increase in sensitivity is typically observed. Careful selection and optimization of MRM transitions permits detection of 5-10 specific proteins per LC-MS analysis. We are currently developing and optimizing MRM transitions to target proteins of interest for producing high titers of biofuel molecules. Our initial efforts are directed at optimizing the mevalonate pathway, the foundation for producing isoprenoid-based biofuels. The mevalonate pathway also serves as a good model for butanol-producing microbes. With these methods we will characterize a variety of protein expression parameters (promoter; ribosome binding site; plasmid) to determine optimal metabolite (e.g., mevalonate, isoprenoids, butanol) production.