A Metabolomic Based Approach to Identifying Bottlenecks in Biosynthetic Pathways

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Project Goal: To demonstrate the utility and effectiveness of metabolomics, when combined with microbial physiology, in the identification of bottlenecks in biosynthetic pathways.

In the post-genomics era there has been a growing emphasis on understanding the functions associated with gene products. However, due to many genes not being under transcriptional control and the incomplete prediction of the proteome from the transcriptome owing to post-translational modifications, there has been a push in the fields of microbial metabolic engineering and synthetic biology to obtain phenotypic information from the metabolome, as it reflects more closely metabolic activities within a cell. While complete global metabolite profiles (comprehensive metabolomics) are not yet achievable due to the chemical diversity that exists within the metabolome, a focused strategy involving the measurement of localized metabolism (targeted metabolomics) has emerged as the favored approach. Targeted metabolomics illuminates those

aspects of metabolomics data that are truly meaningful to a research study. Thus, ensuring the identification of bottlenecks in biosynthetic pathways through the observation of accumulated pathway intermediates and cofactors. Furthermore, when combined with the knowledge and understanding of microbial physiology, we can accurately determine metabolism that is closely related to engineered biosynthetic pathways. Moreover, we can correlate the impact of the engineered pathway on central carbon metabolism and, hence, a microorganism's physiological state. Herein, we demonstrate the effectiveness of this strategy through the identification of bottlenecks in the heterologously expressed mevalonate pathway in *Escherichia coli*. This approach could be used to determine mechanisms employed by microorganisms to overcome genetic modification and can be directed towards engineering robust microbial cell factories for producing valuable renewable bioproducts.

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