Monitoring bacterial physiology during recombinant protein production

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Recombinant protein production (RPP) in *Escherichia coli* represents a considerable part of the biotechnology industry worldwide. *E. coli* is a preferred host for RPP for numerous reasons including ease of growth and genetic manipulation. However, a great many aspects of *E. coli* physiology during RPP are still unknown. Bioprocess engineers must rely on physical sensing methods to monitor large-scale RPP fermentations; direct measurements of bacterial physiology are uncommon.

This project aims to measure the physiology of *E. coli* during RPP using reporter gene technology. Numerous natural *E. coli* promoters have been characterised that respond to specific stimuli, for example cytoplasmic and periplasmic protein misfolding, nutrient limitation or acid accumulation caused by overflow metabolism. Fusion of these promoters to reporter genes will allow rapid measurement of promoter activity and therefore physiology. Rapid measurement is possible using fluorescent reporter proteins; online, in-situ quantification is possible using a reflection fluorescence probe. The ultimate aim of the project is to allow bioprocess engineers to measure the physiology of bacteria during RPP, detect when conditions unfavourable to RPP are present and correct the bioprocess parameters accordingly.

This project will utilise a wide variety of molecular biology techniques such as PCR-based cloning, site-directed mutagenesis, analysis of cellular proteins using electrophoresis, biochemical assays, fluorescence measurement and flow cytometry. It will also involve microbial fermentations in bioreactors and analysis of cultures using online measurement techniques (GC-MS). Students will have access to a newly-refurbished pilot plant facility and a well-equipped molecular microbiology laboratory. There are opportunities to develop this project in a number of directions, either towards more applied areas or into ‘pure’ molecular microbiology.

References

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