CBMNet

CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

UNIVERSITY of York

BBSRC

Towards an integrated multi-omic assessment of membrane responses during industrially relevant high level protein production and secretion

The Challenge

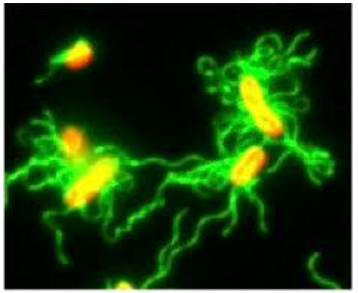
Escherichia coli is a major workhorse organism used by industrial biotechnology as a cell factory for overproduction of diverse commercial protein products. This includes biopharmaceuticals (biologics) and industrial enzymes. However, relatively little is known of physiology during high-level protein production or secretion.

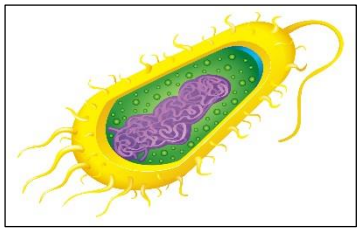
Bacterial "cell factories" can sometimes release these therapeutic proteins prematurely, which could account for the low yield observed in some cases. This research aimed to investigate the phenomenon further, leading to a better understanding of biologic drug production in bacterial cells.

The Research

Dr Graham Stafford is a Senior Lecturer at The University of Sheffield. His research focuses on the microbiology of bacteria, host-pathogen interactions, engineering of bacterial flagella for protein excretion, glycan harvesting enzymes and sugar transport across bacteria membranes. All this work has significant application for industrial Biotechnology.

Dr Stafford, along with colleagues from the ChELSi (Chemical Engineering at the Life Science Interface) institute (Phil Wright) and Fujifilm Diosynth Biotechnologies, applied for CBMNet Proof of Concept funding to identify and quantify responses of proteins associated with membrane function that respond to 'secretion stress', with the aim of relieving metabolic bottlenecks and improve product yields.





CBMNet Proof of Concept Funding

The Result

The inter-disciplinary team achieved the goal of performing a proteomic analysis on real industrial production runs. This has opened up potential for improving Fujifilm's process and discovering new insights into the biology of *E. coli* in industrial fermentations.

The research found alterations in *E.coli* proteome which have identified various metabolic pathways and stress points that are being targeted to improve protein production and secretion in Fujifilm's strains.

Initial data analysis has shown several sets of proteins that seem to be altered in their expression levels between the control and expression runs.

The Future

This research has formed the foundation for a wider ranging proposal to identify bottlenecks in recombinant protein secretion in an industrial setting allowing prediction and rational design of *E.coli* membrane function to improve product yields.

During this project the team discussed other ongoing work on utilization of alternative bacterial secretion systems for production and secretion of protein biologics. The outcome being the signing of new Confidentiality Disclosure Agreement and Material Transfer Agreement for pilot work to be carried out in the lab of Dr Stafford that, if successful, will be the beginning of a new collaborative work with Fujifilm Diosynth Biotechnologies.

This project may also provide the basis for further funding applications (e.g. BBSRC-IPA, IB Catalyst or TSB calls)

"The opportunity to examine the cellular behaviour of biologic production strains during a production run has been very exciting, allowing us to learn something about the bacteria but also the processes and considerations taken into account by a top CMO, like Fujifilm Diosynth Biotechnologies."

Dr Graham Stafford

"This is an opportunity to apply new analytical techniques to Fujifilm Diosynth Biotechnologies' production processes, which promises to give us novel insights into those processes, and which we expect will lead to improvements in the future"

Fujifilm Diosynth Biotechnologies

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Email: cbm@sheffield.ac.uk
Telephone: 0114 222 9766

Website: www.cbmnetnibb.net
Twitter: @CBMNet_NIBB