CBMNet

CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

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# Using *E.coli* turgor pressure regulation to optimize product excretion and prevent unwanted cytoplasmic leakage

## The Challenge

Many of the most effective new treatments for cancer, arthritis and other conditions are protein-based biologic drugs that have to be manufactured using living cells such as bacteria.

*Escherichia coli* is a widely used host for production of recombinant proteins for biologics. Although high levels of protein secretion can be achieved, this is often followed by low-yield down-stream processing to extract functional proteins. Furthermore, at higher protein yields (>10g/L), non-specific leakage of cytoplasmic content is often observed, which includes release of DNA that increases media viscosity, resulting in further decreased yields and higher production costs.

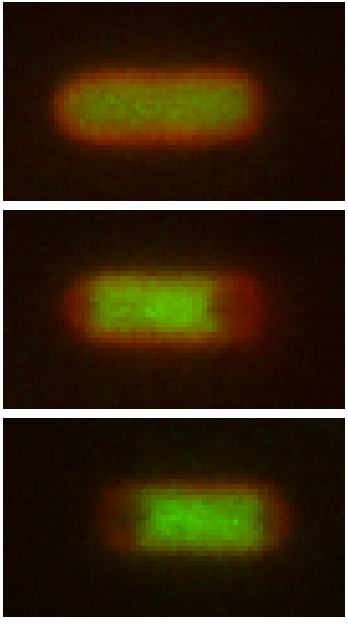
Downstream processing represents a bottleneck within the Industrial Biotechnology industry and approaches to reduce costs of this step will grow in importance as higher product yields continue to be achieved.

## The Research

Dr Teuta Pilizota is a Chancellor's Fellow at the University of Edinburgh. The research in her laboratory focuses on developing novel tools for quantitative observations of changes in physiological parameters in single bacterial cells.

Dr Pilizota, along with FujiFilm Diosynth Biotechnologies, applied for CBMNet Proof-of-Concept funding to investigate how turgor pressure regulation can be used to optimize product excretion and prevent unwanted cytoplasmic leakage in *E.coli.*

The data used to secure this funding came from a CBMNet Vacation Scholarship grant carried out in the summer of 2014.



**CBMNet Proof of Concept Funding**

## The Result

Samples were obtained from several fermentation runs performed by Fujifilm and these confirmed that media osmolarity increases during recombinant proteins expression.

The project also identified and characterized the mechanisms underpinning the export of *E.coli* cell cytoplasmic contents that can be targeted to optimise product excretion. This can be done by adjusting the incubation period post downshock treatment for recombinant protein extraction.

The data generated will be used to create a protocol for controlling external media osmolarity during production through continued collaboration with FujiFilm.

## The Future

Based on the results of the project and collaboration, Dr Pilizota and FujiFilm have been awarded an Industrial Biotechnology Innovation Centre funded Scholarship for a 4 year PhD project. A talented student, Smitha Hegde, has been recruited to continue the work.

In addition, Dr Pilizota has applied for a IB Catalyst funding together with, Dr Meriem El Karoui , Prof Susan Rosser and Dr Louise Horsfall. A work-package in the IB Catalyst application contains work based on this CBMNet project. The catalyst work will be again supported and carried out in collaboration with FujiFilm.

***"At FujiFilm we often don't have time to investigate the fundamental science behind many of our processes. Working with Dr Pilizota has allowed us to gain some insight into the use of novel analytical methods to gain a better understanding of part of our production process. This has led to a bigger project that will lead to improvements in our processes"***

Dr Ian Hodgson, Fujifilm Diosynth Biotechnologies

***''We have benefited from this CBMNet award in establishing a working relationship with an UK based IB company that enables us to faster translate our research into the industrial sector. The company is getting a new angle on establishing control over recombinant protein excretion, which is crucial to further enable new innovation and ensure UK stays one of the world leaders in IB.''***

Dr Pilizota, University of Edinburgh

**This project was funded through the Crossing Biological Membranes Network (CBMNet) by the Biotechnology and Biological Sciences Research Council (BBSRC)**

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