CBMNet
CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

UNIVERSITY of York

BBSRC

Efflux from bacteria of feedstocks for industrial manufacture

The Challenge

A significant global challenge is to develop bioprocesses for the sustained manufacture of important commodity chemicals, whilst reducing reliance on fossil fuels. One such strategy uses microbial cell factories that are capable of making key chemicals from sugars. The development of these cost-competitive, commercially viable bioprocesses presents a significant challenge to the chemicals industry.

As a substitute for the synthetic production of commodity chemicals, Lucite International is carrying out research to utilise bacteria to host a variety of metabolic pathways to produce the required end-products from sugars. However, the end-products can be toxic to the host cells, so the challenge is to find and develop protein-mediated efflux systems that will actively export the end-product from the cell and reduce its detrimental affect on the bacteria. To achieve this requires the development of existing and novel assays to measure the efflux of the desired compound out of the microbial cell factory.

The Research

Peter Henderson (PH) is Professor of Biochemistry and Molecular Biology at the University of Leeds. The research in his laboratory focuses mainly on understanding the molecular basis of membrane transport processes, and particularly the roles of energised efflux of toxins in resistance of microorganisms to antibiotics (Fig. 1).

At CBMNet meeting PH gave a presentation on bacterial efflux systems, which attracted the interest of Dr Graham Eastham (GE) and Dr David Johnson (DJ) from Lucite International. As an outcome of this, an applied 3-year PhD studentship was agreed between Lucite and the University of Leeds.

PH and GE also went on to apply for a CBMNet Business Interaction Voucher and CBMNet Vacations Scholarship with Lucite International to develop a strategy for identifying bacterial efflux proteins that could be used to drive desired, but toxic, end-products of engineered synthetic pathways out of the host cells.

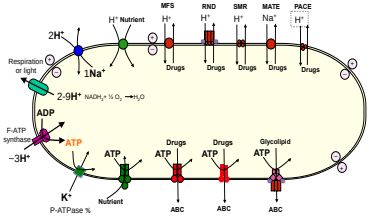

Figure 1. Many possible routes for efflux of compounds from microbes

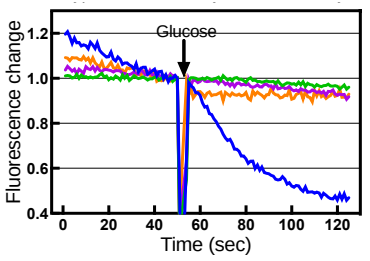

Figure 2. Typical indirect assays of efflux activity

CBMNet Business Interaction Voucher & Vacation Scholarship

The Result

Jacob Edgerton (JE), a graduate with First Class Honours in Microbiology from Cardiff University, was selected to undertake a PhD degree supervised jointly by the University of Leeds and Lucite.

With additional support from the CBMNet BIV, an information analyst, Richard Walsby, resulting in a substantial list of promising efflux systems from a variety of bacterial species. Screening of these is ongoing. A second outcome was the identification by GE and PH of routes for the chemical incorporation of ^{14}C, and possibly ^{3}H, into molecules of relevance to the project for the development of direct assays of transport activity.

In parallel, PH, GE and an undergraduate student, Gabriel Hoppen, supported by a CBMNet Vacation Studentship, explored indirect methods already used by us to measure antibiotic efflux, with a view to their application to this project.

The Future

Professor Henderson and Lucite will be, in the near future, cloning and characterising a range of the efflux systems identified, which is very promising for the aims of this project.

In parallel they are exploring both indirect assays for measuring efflux activities that involve fluorescence and/or absorbance measurements using acriflavine, nile red, ethidium and propriatory compounds (Figure 2) and direct measurements of transport using the ^{14}C-labelled compound(s) we are having synthesised.

In addition, through the connection of Lucite with wider networks of BBSRC- and CBMNet-funded projects, PH and GE are now working closely with groups at the University of Nottingham and Ingenza.

"This project was conceived at the inaugural open meeting of CBMNet in Sheffield, where I first met Lucite International. Funding for a studentship was then attracted from Lucite International and the University of Leeds, with vital initiating elements supported by the BIV and Vacation Scholarship from CBMNet. We hope that CBMNet will continue and promote similar unexpected and successful liaisons between academia and industry"

Professor Peter Henderson,
University of Leeds

"CBMNet has proven to be extremely valuable as a way of connecting with experts in the field of microbial membrane science. Furthermore the pump priming of these interactions throught BIV's, POC and vacation studentships allows potential collaborators a low cost looksee at each other. For Lucite this has been valable and has led to longer term more established programs which would have been difficult to justify without the aforementioned mechanisms"

Dr G. Eastham
Lucite International UK Ltd

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Email: cbm@sheffield.ac.uk
Telephone: 0114 222 9766
Website: www.cbmnetnibb.net
Twitter: @CBMNet_NIBB