CBMNet

CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

UNIVERSITY of York

BBSRC

Evolution of butanol tolerance in *Escherichia coli*

The Challenge

The production of the commodity chemical butanol using the acetone-butanol-ethanol (ABE) fermentation of *Clostridium* species is an important route to producing sustainable lignocellulosic chemicals and biofuels. *n*-Butanol is a building block chemical in the $115 billion global paints, coatings, adhesives and inks market.

This process, which is the basis for much of the manufacturing by Green Biologics Limited (GBL), would be improved by operating at higher butanol concentrations. Currently, butanol is toxic to the producing bacteria at relatively low concentrations.

For the related fuel ethanol, changes in expression of efflux proteins and alterations in membrane composition have been observed as a result of experimental evolution of strains with increased resistance to this alcohol.

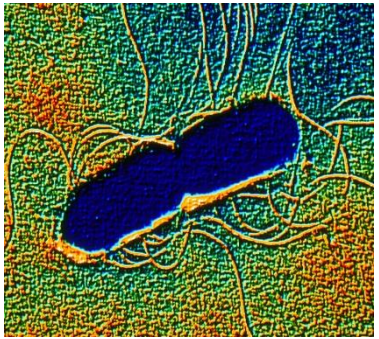
The Research

Dr Gavin Thomas is a Reader in Microbiology at the University of York. The research in his laboratory focusses mainly on membrane transport proteins in a range of bacteria.

Dr Thomas applied for a CBMNet Business Interaction Voucher with GBL to develop a collaborative partnership and produce preliminary data that could be built on in future funded projects.

The project aimed to evolve strains of *Clostridium* and also the model bacterium and workhorse of biotechnology, *Escherichia coli* to have increased butanol tolerance.

The use of microbial engineering and synthetic biology tools could expand the library of Clostridium strains which are used as biocatalysts as part of the advanced fermentation processes at GBL.



CBMNet Business Interaction Voucher

The Result

In the period of the short BIV project, Dr Thomas was able to employ a talented visiting Brazilian Science without Borders student for 3 months to undertake an experimental evolution program with both organisms. Strains with improved tolerance were identified for both organisms and rapid re-sequencing of their genomes by the BBSRC-funded MicrobesNG facility at the University of Birmingham enabled the genetic changes to be quickly identified.

One evolved line of *E. coli* produced a potentially novel and interesting mutation, while others contains mutations in known loci that had been found previously to be involved in ethanol tolerance, illustrating that there may be common mechanism for tolerance to multiple stresses.

They were also able to evolve a solventogenic *Clostridium* strain for growth with increased butanol concentrations. These strains were transferred to GBL for further characterisation.

The Future

Dr Thomas and GBL have used the successful data from the *E. coli* mutants as an element of the preliminary data provided in a large IB Catalyst grant application to IUK/BBSRC which includes GBL.

The IB Catalyst round 3 grant was successful and is entitled 'DeTOX - Productive whole cell biocatalysis by engineering resistance to toxic products and substrates' and runs from April 2016 for 5 years. One element of the work packages builds directly on the outcome of this Business Interaction Voucher.

The project will further investigate productive whole cell biocatalysis by engineering resistance to toxic products and substrates & to produce a suite of DeTox chassis strains for use in industrial biotechnology.

"The BIV was very successful from our point of view as by being able to employ an excellent student we trialled a new method in our lab, which we took right through to the point where we generated novel data. This then went straight into a grant application."

Dr Gavin Thomas
Reader in Microbiology, University of York & CBMNet co-director

"The outcome of this project was one of the foundations for the successful BBSRC IB Catalyst DeTOX bid worth several million pounds. Green Biologics are looking forward to working with the academic community over the next five years. It is with great pleasure that the wider UK academic community has recognised Clostridia as an important industrial microbe and hopefully this will lead to a dynamic and vibrant community."

Dr. Preben Krabben
Head of Innovation, Green Biologics

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