CBMNet
CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

UNIVERSITY of York

BBSRC

Flow cytometry assay development for industrial biotechnology plastic production

The Challenge

Methacrylates are a major group of chemical building blocks currently produced from petrochemicals via chemocatalytic processing. They are used, for example, as the monomer resin in some windscreen repair kits, and as bone cement for fixing prosthetic devices in orthopaedic surgery.

Ingenza is developing bio-based microbial fermentation routes towards the production of methacrylate intermediates with its contracted partner Lucite International.

Ingenza have used cutting edge molecular biology techniques to rapidly design and engineer a selection of microbes to demonstrate the feasibility of biobased methacrylate production. However, current fermentation-based methacrylate intermediate productivity is well below commercially attractive targets.

It is expected that the availability of a flow cytometry-based assay to identify and isolate high-level methacrylate intermediate producers will alleviate this bottleneck.

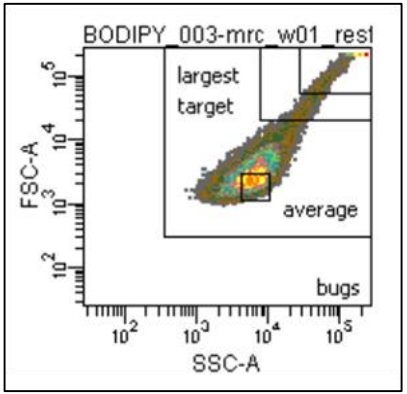
The Research

Dr Waterfall is Head of Flow Cytometry at the University of Edinburgh. The research in his Facility is focused on development of novel procedures for application to the evaluation of cellular material.

Ingenza is a world leader in the application of industrial biotechnology and synthetic biology, providing efficient, scalable bioprocesses for the manufacture of chemicals, biologics, pharmaceuticals and biofuels, from sustainable sources.

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

The project aimed to explore the potential for flow cytometry to efficiently identify, rank and sort a population of microbes based on individual methacrylate intermediate production levels .



CBMNet Business Interaction Voucher

The Result

Initial screening of Ingenza's bacterial strains using flow cytometry analysis demonstrated the presence of sub-populations with different fluorescence attributes within a single bacterial culture.

Selective flow cytometry sorting, based on these attributes recovered cells which could be returned to culture, however, they were not sufficiently selective for identification of commercially attractive variants with increased capacity for production of the target chemical within the time span of the project. Ingenza have concluded this is due to other limitations retarding productivity in the given host system rather than an effect of sorting.

Further investigation of probes for identification of high producers should allow the selective process to be refined and enhanced.

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

The Future

We would like to bring similar projects forward for further collaborative investigations between Ingenza and other partners and the University of Edinburgh.

Hopefully applying flow cytometry techniques in this field will result in improved synbio-based cellular performance for IB application with industrial end users in the near term.

"The CBMNet award has allowed us to demonstrate that flow cytometry can be applied for characterisation of bacterial strains by fluorescence profiling and that these can be used to select sub-populations. The results from the study have allowed Ingenza to move the project forward, by improving their strains and focusing attention on stabilising tolerance of the host cell to the targeted product."

Dr Waterfall, University of Edinburgh

"We invest significant resources at Ingenza to develop screening approaches to select and identify improved cellular systems we are engineering into microbial production hosts using our state of the art synthetic biology methods.

This CBMNet BIV award provided us with resources to examine the opportunity to use flow cytometry technqiues to add to our array of cellular screening methods.

Without the CBMNet award and the collaboration with Dr Waterfall, the financial and human capital required to independently evaluate flow cytometry internally would be simply not possible for Ingenza to consider."

Dr Rueben Carr,
Head of Chemical Biology
Ingenza

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