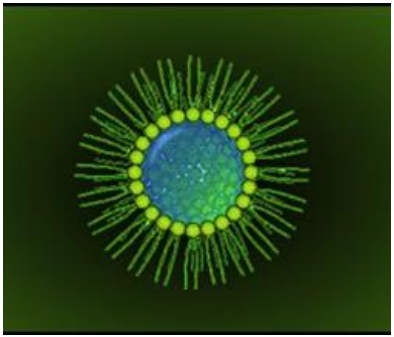
CBMNet
CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

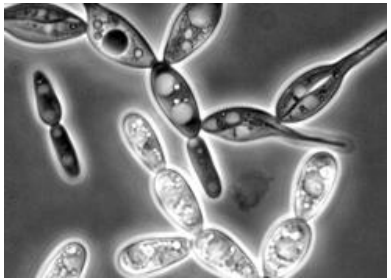
The University Of Sheffield.

UNIVERSITY of York

BBSRC

Membrane transporters and Mannosylerythritol lipid production





CBMNet Business Interaction Voucher

The Challenge

There is a strong commercial interest in biosurfactants – both for sustainability and performance. Mannosylerythritol lipids (MEL) are one example considered to have desirable properties and are of current interest but with commercially limiting yield. They are synthesised from long-chain fatty acids by a number of basidiomycetous yeasts such as *Pseudozyma aphidis.*

An existing process needed improvement, and (as is often the case[1]) it was hypothesised by the industrial partner Croda that much of the flux control lay in transporter reactions involving the uptake of the fatty acid substrates and the efflux of the MEL. Since a genome sequence of *P. aphidis* (and related species) is available, a bioinformatics approach to seek the transporters based on homology searching and modelling was adopted for this short project.

The Research

Professor Douglas Kell is a Research Professor in the Manchester Institute of Biotechnology at the University of Manchester. He pioneered the principled genome-based modelling of the metabolic network of baker's yeast, and has expertise in xenobiotic transporters more generally.

Fortuitous timing found a skilled bioinformatician available in the form of Dr Ryan Ames. Professor Kell and Croda applied for a CBMNet Business Innovation Voucher to allow them to work jointly, to perform a genome-wide bioinformatics analysis of potential substrate and product transporters in *P. aphidis.*

The Result

The genome sequence was obtained from the NCBI (Genbank: AWNI00000000.1). Dr Ames established one very clear candidate for the fatty acid transporter, with clear homologies to equivalent proteins in yeasts and humans with established functions.

Similarly, two candidates for MEL export were identified, with one (located near to the MEL biosynthetic genes) being considered the more important. The analyses (including secondary structure predictions) were annotated using the Artemis genome browser environment.

References

[1]Kell DB, Swainston N, Pir P, Oliver SG: Membrane transporter engineering in industrial biotechnology and whole-cell biocatalysis. Trends Biotechnol 2015; 33:237-246.

[2]Lee D, Smallbone K, Dunn WB, Murabito E, Winder CL, Kell DB, Mendes P, Swainston N: Improving metabolic flux predictions using absolute gene expression data. BMC Syst Biol 2012; 6:73.

[3]Swainston N, Currin A, Day PJ, Kell DB: GeneGenie: optimised oligomer design for directed evolution. Nucleic Acids Res 2014; 12:W395-W400.

[4]Currin A, Swainston N, Day PJ, Kell DB: SpeedyGenes: a novel approach for the efficient production of error-corrected, synthetic gene libraries. Protein Eng Design Sel 2014; 27:273-280.

[5]Currin A, Swainston N, Day PJ, Kell DB: Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. Chem Soc Rev 2015; 44:1172-1239.

The Future

The success of this bioinformatics project opens up several areas. The first is to create a full network model, since this plus an absolute transcriptome measurement may be used[2] to predict all the fluxes.

A second strategy for flux and yield improvement would use Manchester's synthetic biology technology (known as GeneGenie[3] and Speedygenes[4]) to create much more effective variants of the transporters by intelligent search[5] of the landscape of the many possible variants.

The data generated in this study are being fed into an IB-Catalyst round 4 application, and other potential collaborations are in discussion.

Croda has every intention of developing the relationship with The University of Manchester in this very important area of microbial physiology.

"Funding offered by CBMNet has enabled Croda and Kell's lab to carry out translational research based on the academic lab's expertise in transporter and metabolic network biology. CBMNet allowed the Kell lab to be exposed to these very interesting organisms and industrial challenges. This collaboration would not have been initiated without the CBMNetwork."

Prof Douglas Kell, University of Manchester

"Following an initial meeting with Douglas at a CBMNet event, it was clear that his systems biology lab was well placed to look into the question of flux control via transporters. The first question in such an endeavour is to find the actual actors, and this was carried out most effectively. We also enjoyed learning about the latest tools for genomics and genome-based network modelling. The insights gained from this BIV will prove invaluable in future work."

Dr Doug Cossar, Croda.

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

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