CBMNet
CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

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Improving the tolerance of microbes to membrane-active solvents

The Challenge

Many desirable bio-based microbial cell factory products are toxic to their hosts; indeed terpenes and terpenoids, that have attracted recent commercial attention, are well known to be toxic to microbes even at very low concentrations of 0.1% (v/v).

The cytoplasmic membrane of bacterial cells, a phospholipid bilayer, is a matrix in which various enzymes and transport proteins are embedded. Organic solvents partition into and disrupt the lipid bilayer, thus compromising cell viability. It has been proved that it is not the chemical structure of the solvent, but the concentration to which it accumulates in the cell membrane that plays a crucial role in determining toxicity.

The challenge is to develop a means for improving the host cell factory, in this case *E.coli*, tolerance to such molecules. This involves creating diverse strains and screening them; only resistant strains will survive.

At present in the bioprocess, 0.1% terpenes are toxic, a level far below what would be needed for an economically viable process.

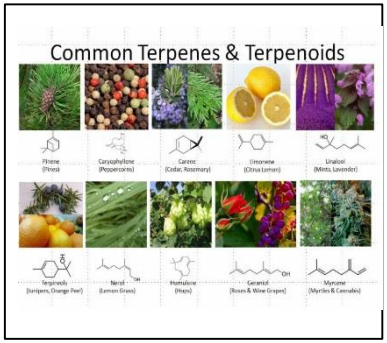
The Research

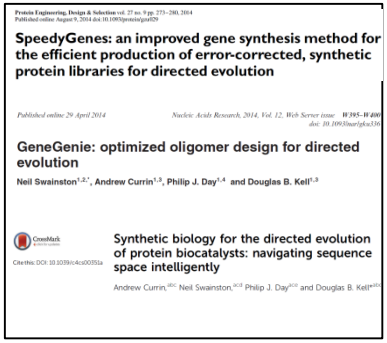
Douglas Kell and Nigel Scrutton are Professors in the Manchester Institute of Biotechnology at the University of Manchester. The research in their laboratories focusses particularly on the development of synthetic biology methods for improving microbial performance.

Croda create, make and sell speciality chemicals that deliver real benefits across a range of sectors including health, personal, and crop care, as well as lubricants and coatings.

They applied for a CBMNet Proof-of-Concept award with Croda to acquire preliminary data that could be built on in future funded projects.

The project made use of the 'SpeedyGenes' plus 'GeneGenie' synthetic biology methods to investigate the effect of a particular transcription factor that was considered likely to affect the solvent tolerance of the host microbe to terpenes and terpenoids.




SpeedyGenes: an improved gene synthesis method for the efficient production of error-corrected, synthetic protein libraries for directed evolution

GeneGenie: optimized oligomer design for directed evolution

Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently

CBMNet Proof-of-Concept Award

The Result

In just three months, 4 rounds of directed evolution using the University of Manchester synthetic biology methods were achieved. This resulted in strains that were 6-8-fold more tolerant of a range of terpenes and terpenoids. This demonstrated that the approach taken was indeed valid, and that the target protein is both a novel target and an excellent choice for improvement by directed evolution using synthetic biology methods.

The project sequenced the new variants in approximately 20 of the best performing strains. The numbers are too small to draw precise conclusions about the nature of the sequence-activity landscape, although the appearance of a proline at a particular residue did seem to be a statistically frequent occurrence.

The Future

Kell and Scrutton are now seeking further larger funding to improve further the solvent tolerance of these strains, by exploring the entire sequence space of our target protein. This will use an updated and more efficient variant of 'SpeedyGenes' (paper under review).

The solubility limit of many of these solvents is ~3%, so there is potential for a further 4-5-fold improvement, which would then effectively result in complete solvent tolerance, which is eminently possible. Once achieved this will also need to be shown in producer strains.

In addition, this synthetic biology strategy is likely to be applicable to the improvement of many more bioprocesses.

"This was almost a model project, in that we really did manage to improve the solvent tolerance very dramatically (6-8-fold) in just three months, essentially according to what we had predicted (in terms of the choice of target protein). Now we are seeking larger funding for a full project."

Professor Douglas Kell,
University of Manchester

"This project demonstrates the value of collaboration between academia and industry in concerted efforts to tackle discrete challenges using novel methods. Data such as that obtain through the work done at Manchester underpins the systems understanding needed for robust, commercially viable IB processes to be developed."

Dr Jeremy Bartosiak-Jentys
CRODA

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

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