

Functional Characterisation of a putative succinate efflux pump from *Corynebacterium glutamicum*

The Challenge

Succinate is a key precursor in the production of biodegradable plastics and fabrics. The majority of industrially produced succinate is derived from petrochemical precursors. However, several microbial species have been engineered to maximise succinate production during fermentation.

A succinate efflux pump, SucE, was recently identified in *C. glutamicum*, which substantially increases succinate production when overexpressed. However, the structure, mechanism, energetics and substrate specificity of this transporter remain unknown.

A comprehensive understanding of SucE's transport mechanism could allow us to manipulate this transporter and/or its energy source to make succinate (and possibly other dicarboxylic acids) efflux more efficient, potentially increasing the succinate yield of *C. glutamicum*.

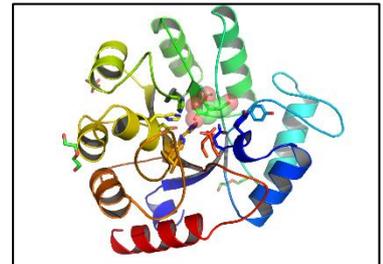
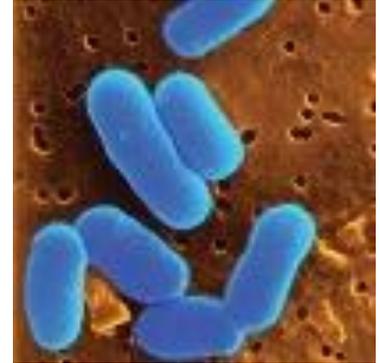
The first step on this path is to develop an expression system with which to overexpress and purify SucE in sufficient quantities for biochemical characterisation.

The Research

Dr. Mulligan is a Lecturer in Molecular Biosciences at the University of Kent. His research interests centre on understanding the molecular mechanism of membrane transporters using a range of biochemical and biophysical methods.

Dr. Mulligan applied for a CBMNet Vacation Scholarship that allowed him to train an undergraduate student in his lab in the overexpression and purification of integral membrane proteins for biochemical characterisation.

The undergraduate student, Alice Evans, was trained in general molecular biology and biochemical techniques, including gene cloning, microbial culturing, recombinant protein expression, affinity purification, SDS-PAGE and Western blotting. Alice also received training in techniques specific to membrane proteins, including membrane vesicle preparation and detergent solubilisation.



CBMNet Vacation
Scholarship

The Result

Under the supervision of Dr. Mulligan, Alice amplified *sucE* from *C. glutamicum* and cloned the gene into bacterial expression vectors. Alice systematically screened protein expression under a series of conditions, and purified the protein using immobilised metal affinity chromatography.

While the quantity of protein produced was not sufficient for further characterisation, this work lays the foundation for further expression optimisation in the future.

The Future

This CBMNet Vacation Scholarship-funded research project provided excellent training and a research lab experience for Alice in the characterisation of membrane proteins. This project has strengthened Alice's desire to pursue a career in research science starting with post-graduate training after the completion of her undergraduate degree.

This project has provided Mulligan lab with important information regarding the overexpression of SucE. The encouraging results produced from this project mean that the characterisation of succinate efflux pumps, including SucE, will remain a major research project in Mulligan lab in the future.

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"I was very pleased to be awarded the CBMNet Vacation Scholarship as it allowed my lab to start a completely new project on a very interesting, but poorly understood, family of transporters. The materials produced and the information obtained from this project will be very useful for future studies on these transporters."

Dr. Christopher Mulligan, University of Kent.

"Before starting the project, I had a vague plan of pursuing laboratory based science as my career and since doing my summer project I am now confident that doing a PhD is my future career plan. I learnt more lab skills within three weeks of this project than I have in two years of teaching labs and I am forever grateful for that. The only disappointing aspect was that the I didn't want the project to end and have to go back to attending lectures!"

Alice Evans, undergraduate student at the University of Kent.