

Development of *Clostridium saccharoperbutylacetonicum* transposon mutagenesis

The Challenge

Clostridium saccharoperbutylacetonicum is a commercially important bacterial species, used for the production of the industrial solvent n-butanol.

Our understanding of this species has lagged behind its industrial use due to a general lack of genetic tools for the manipulation of all Clostridial species. Random transposon mutagenesis is a powerful tool for high-throughput analysis of gene function. However, phenotypic characterisation of mutants is a highly labour intensive process. Recent advances in next generation sequencing technologies have streamlined this process and techniques such as Transposon-Directed Insertion Site Sequencing (TraDIS) have allowed parallel analysis of 100,000s of transposon mutants in a single pool.

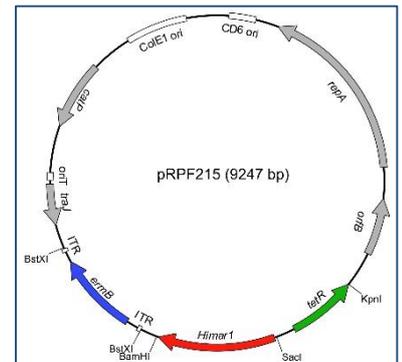
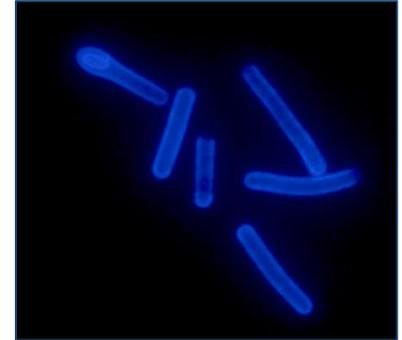
Although the Fagan lab have developed transposon mutagenesis methods for the Clostridia, these have only been used in the medically important members of the genus to date.

The Research

Dr Robert Fagan is a lecturer in the Department of Molecular Biology and Biotechnology at the University of Sheffield. The research in his laboratory focusses on cell envelope biogenesis in the Clostridia. This work involves a combination of microbiology, biochemistry and structural biology, supported by the development of innovative genetic tools.

Green Biologics converts a wide range of sustainable feedstocks into high performance green chemicals. They combine advanced, high productivity fermentation utilising superior-performing Clostridium microbial biocatalysts to produce renewable n-butanol and acetone.

Dr Fagan applied for a CBMNet Business Interaction Voucher with Green Biologics to support the development of a new collaborative partnership. This project aimed to adapt existing Fagan lab genetic tools and examine the feasibility of carrying out saturating transposon mutagenesis in *C. saccharoperbutylacetonicum* for the first time.



**CBMNet Business Interaction
Voucher**

The Result

This CBMNet BIV supported a research secondment for a Green Biologics scientist to work in the Fagan laboratory for 8 weeks in early 2017.

Dr Fagan's transposon-delivery system is modular, with optimum performance relying on the functionality of several individual components. Each of these was tested in isolation in *C. saccharoperbutylacetonicum* to develop a roadmap towards rational improvement of the system in future.

A critical inducible promoter was identified as the weakest component and optimisation was the focus of the remainder of the project.

Finally a small panel of transposon mutants were generated as a proof of principle.

The Future

This collaboration opened up a new avenue of research in the Fagan laboratory and has generated preliminary data that will undoubtedly lead to closer ties and further funding applications with Green Biologics.

Building upon this project, Dr Fagan and Green Biologics have already been awarded a BBSRC White Rose iCASE PhD studentship to start in October 2017. The work of this PhD student will continue on directly from the proof of principle experiments enabled by the CBMNet BIV.

The interactions between Dr Fagan and Green Biologics have also helped foster links with other CBMNet-associated academic groups. They are currently planning an expanded collaboration incorporating the expertise of several research groups with the industrial goals of Green Biologics.

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

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"This CBMNet-funded Business Interaction Voucher has allowed us to start a fruitful collaboration with Green Biologics. It is enormously exciting to find new applications for our genetic tools and to expand our research into a new bacterial species. I look forward to expanding our collaborative efforts as we seek to find new ways to improve Clostridial n-butanol production"

Dr Robert Fagan, University of Sheffield

"This BIV project marked the beginning of what will hopefully be a rewarding collaboration with Rob. The technology he has developed in his lab will enable us to understand more about solventogenic clostridia at a fundamental level. We're looking forward to seeing what comes from the iCASE PhD project that is following on from this BIV"

Dr Liz Jenkinson, Green Biologics Ltd