CBMNet

CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield. UNIVERSITY of York BBSRC

Evaluation of extra-cellular recombinant enzyme production in *B.subtilis*

The Challenge

A major challenge faced by enzyme manufacturers is to produce commercial yields of a wide variety of enzymes in recombinant production strains. Secretion of recombinant enzymes from a production strain is of particular interest for commercial production of enzymes, as it facilitates the downstream processing and product formulation.

It is a challenge to produce a wide variety of different recombinant proteins in any one production strain (e.g. *E. coli, B. subtilis, P. pastoris*), with a current success rate of these platforms for the production of a commercially viable product of the order of 15-20%.

B. subtilis is an attractive host for recombinant enzyme production since it is a GRAS and QPS host, thereby facilitating regulatory requirements for enzymes for food or pharmaceutical applications. It is also a strong candidate as an organism for protein production since it is known to produce a wide range of recombinant enzymes. It also has the advantage of being able to secrete proteins to the culture medium.

Although there is considerable precedent available in the literature for working with recombinant expression in *Bacillus* systems, the practical details for performing this work effectively reside typically with academic scientists with the knowledge and skills borne from experience.

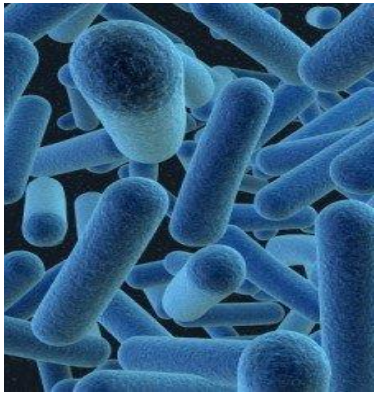
The Research

Professor Jeff Errington is the Director of the Centre for Bacterial Cell Biology at Newcastle University. The research in his laboratory focusses mainly on Bacterial cell biology and the fundamental studies on the bacterial cell cycle and cell morphogenesis.

Biocatalysts develop and manufacture speciality enzymes from small to large scale quantities.

Professor Errington applied for a CBMNet Business Interaction Voucher with Biocatalysts Limited to evaluate *Bacillus subtilis* as a suitable prokaryotic host for Biocatalysts' multi-host platform for the recombinant production of industrial enzymes.

This will provide an insight for Biocatalysts and additional knowledge for the University of Newcastle as to which type of proteins can be produced and secreted when using *Bacillus subtilus* as an expression strain.



"This CBMNet BIV has allowed us to formalise a specific project and work with highly skilled Bacillus scientists to drive forward a key commercial project. The interaction has been extremely fruitful and we hope to continue working together in the future."

Dr Mark Blight, Biocatalysts Ltd.

CBMNet Business Interaction Voucher

The Result

In this study, 4 enzymes of industrial value were tested for expression and secretion from *B. subtilis.*

All enzyme DNA sequences were codon optimised for expression in 6 prokaryotic hosts using a novel cross-platform optimisation algorithm from LabGenius.
Enzyme B was both optimised (B1) and provided as a wild-type *Bacillus* DNA sequence (B2).

During this study it was discovered that the pAX01 expression vector used does not provide an optimal transcriptional start site for the recombinant enzyme gene. Consequently, the vector was modified and the expression level of the control enzyme, *B. amyloliquefaciens* AmyQ amylase, was improved over 12 fold in the vector pAX02.

Subsequently, all recombinant enzymes were transferred to pAX02 and recombined into the *B. subtilis* genome.

The expression trial shown in the SDS-PAGE gel (to the right) (culture supernatants) clearly showed that only enzyme B was secreted to the medium and that the wild-type sequence outperformed the cross-platform optimised sequence.

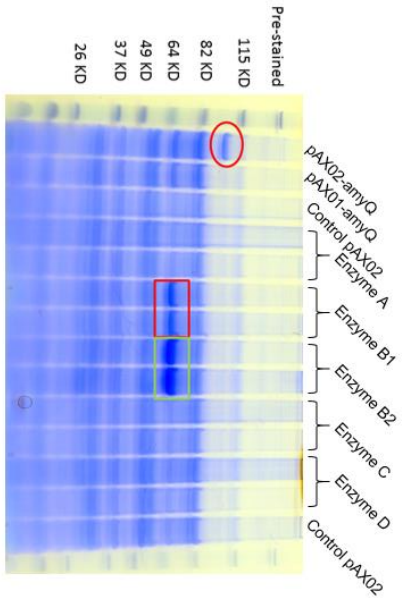
The Future

Biocatalysts has a further 6 enzymes to test with this *B. subtilis* expression system before a final decision can be made to determine if this organism can provide a viable industrial biotechnology (IB) recombinant enzyme production platform.

The data to date suggests that the secretion of a variety of different enzymes is heterogeneous and success has been demonstrated for two enzymes of Bacillus origin – AmyQ and Enzyme B.

Biocatalysts and Professor Errington's laboratory will continue to work together to assess the remaining 6 enzymes and we shall also look at intracellular expression as an alternative to secretion to determine if this is a more viable IB platform.

The successful strains in this project will form the starting point for further strain and expression optimisation required to achieve commercial goals for industrial enzyme manufacture.



"The project was very helpful to us in developing a collaboration with Biocatalysts, which has helped us to understand the commercial drivers operating in the protein production industry."

Professor Jeff Errington, Newcastle University

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