Structural analysis of the bacterial micro-compartment shell in *Clostridium difficile*

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Aim

To generate materials for the study of proteins involved in metabolism in the human pathogen *Clostridium difficile* from a structural and biochemical perspective. The project focussed on the proteins that form the shell of a bacterial micro-compartment within *C. difficile*.

Introduction

The bacterium *Clostridium difficile* is a common cause of illness in hospitalised patients and can cause death, especially the elderly. This is a significant concern with an ageing population. *C. difficile* is the most important cause of hospital acquired diarrhoea in the UK and contributed to almost 4000 deaths in 2009.[1]

C. difficile is carried in the gut without symptoms by around 3% of the adult population. When broad spectrum antibiotics are prescribed to treat infections elsewhere in the body, the balance of the normal gut bacteria can be disturbed allowing *C. difficile* to multiply. The toxins produced by the bacteria break down the gut wall which leads to diarrhoea.[2]

Bacteria such as *C. difficile* have specialised structures which allow them to use the chemicals released from the host gut wall. These structures are called bacterial micro-compartments and act to separate toxic molecules produced in metabolism from the rest of the cell.

This project examined a number of the proteins which make up the shell of the bacterial micro-compartment in *C. difficile*. Knowledge of how these structures assemble may allow the role that these proteins play in directing reactions in the cell to be determined. This may identify potential drug targets in *Clostridium difficile* and other related bacteria such as *Salmonella, Listeria* and *E. coli*.

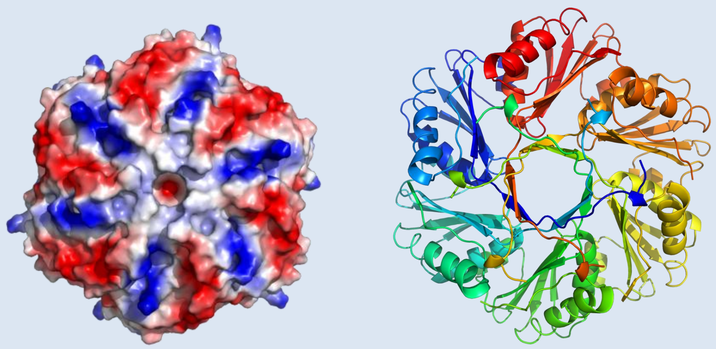

Figure. Different representations of the structures of two *C. difficile* shell proteins solved by Jon Marles-Wright. On the left is a surface representation of CD1918 and on the right a cartoon view of CD1908.

References

1. http://www.statistics.gov.uk/cci/nugget.asp?id=1735
2. http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/ClostridiumDifficile/
3. Molecular Cloning, Sambrook and Russel, CSHL Press, 3rd Edition.
4. Shen C.H., *et al*, FEBS J. 2010 Jul 31.

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Methods

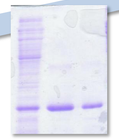
The project involved all aspects of simple molecular cloning[3], as described below.

1. **Design of DNA primers** to amplify the genes of interest using online databases.

2. **Polymerase Chain Reaction** is used to copy and amplify the selected pieces of DNA from the *C. difficile* genome.

3. **Restriction digest and ligation** cuts amplified gene fragments using enzymes and inserts them into circular pieces of DNA known as plasmids.

4. **Transformation** introduces the plasmids into special strains of bacteria that allow them to be copied and produce large amounts of the protein gene product.



Gel electrophoresis

5. **Protein Purification** involves splitting open the cells to release the protein and purify this from the cellular debris. Gel electrophoresis is used to analyse the purity of proteins.

6. **Crystallisation** requires concentrating the protein and introducing small amounts of protein into various chemical environments to establish the optimum conditions for crystal growth.

7. **X-ray analysis** of crystals is performed using a powerful source of X-rays to generate a diffraction pattern which can be interpreted to determine the 3D protein structure.

What protein structures can tell us

The structural analysis of proteins permits better understanding of their function and interactions with other molecules in cells. This can reveal targets for the development of new drugs directed at proteins involved in disease.

For example, the discovery of HIV-1 protease structure has allowed the production of revolutionary life saving drugs for the treatment of HIV/AIDS.

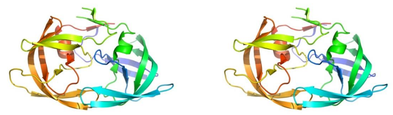


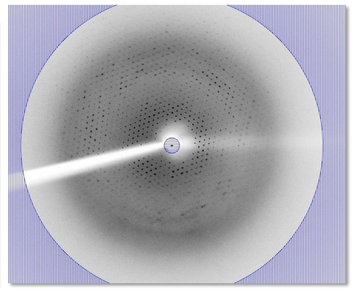
Figure. HIV-1 protease Stereogram.[4]
This is a stereo figure of the HIV-1 protease with β-strands shown as arrows. The attached spectacles can be used to visualise the three-dimensional detail .

The bacterial micro-compartment in *C. difficile* produces energy and may be linked to the pathogenicity of this and associated intestinal bacteria with a bacterial micro-compartment. Understanding its structure may allow us to design drugs against these bacteria.

Results

This project produced a number of protein expression constructs which will be the foundation for crystal structure determination and future biological analyses. Crystals of the bacterial micro-compartment shell protein CD1923 were grown and it was possible to gain high quality diffraction patterns. This will allow the three-dimensional structure of this protein to be solved in the near future. An enzyme CD1925 was purified for crystallisation and biochemical assays. A further eight other genes were amplified by Polymerase Chain Reaction for cloning.

CD1923 crystals



CD1923 diffraction pattern

Conclusions

This project has formed the preliminary work needed to help understand the structure of a bacterial micro-compartment in *C. difficile* and the way this influences its pathogenicity. Future research should permit the development of drug targets directed against metabolism in *C. difficile* and other bacteria. This work is continuing in Jon Marles-Wright's laboratory and will hopefully lead to a co-authored publication in a scientific journal.