O-antigen biosynthesis: hitting the sweet spot for a Q fever vaccine

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Introduction to Q fever: Military context

Coxiella burnetii, the causative agent of Q fever, is a zoonotic pathogen with a worldwide distribution[1]. Classically, the main reservoir for *C. burnetii* is conceived to be ruminants e.g. sheep & goats[2], however, DNA of the Q fever bacterium has been detected in numerous species of arthropod, e.g. ticks and head lice[3], domestic animals e.g. cats and dogs, marsupials (especially kangaroos)[4], and even marine mammals such as Northern Fur Seals[5]. In such animals, spontaneous abortions as a result of Q fever can deposit huge titres of bacteria into the environment, where organisms can adopt a highly resilient spore-like state.

Figure 1: Ruminant farming is prevalent in many areas where UK troops are deployed. Defence Imagery © Crown copyright 2011.

For humans, the main mechanism for Q fever infection is through inhalation of aerosolised bacteria. The material shed from animal infections (e.g. abortive material) contaminates dirt and dust in the environment with *C. burnetii*, where by adopting a spore-like state organisms can survive outside a host, and can be aerosolised in wind. Humans generally present with flu-like symptoms, however, patients can develop life-changing maladies such as hepatitis, chronic fatigue, and endocarditis. Q fever can also in rare cases be fatal[6].

Figure 2: Helicopter down-drafts can aerosolise contaminated dirt and dust. Defence Imagery © Crown copyright 2015.

Q fever was initially identified as a military problem when thousands were affected during WWI. Troops deployed in dry, windy areas where ruminant farming is prevalent are particularly susceptible by infection from inhalation of bacterial aerosols. As such, Q fever has been recognised as a problem in UK troops returning from Afghanistan.

Due to its ease of transmissibility and low infectious dose, *C. burnetii* is classified as a CDC category B bioterrorism agent, the second highest category. There is currently no Q fever vaccine licensed in the UK/EU/US. This project aims to change that.

Why the *O*-antigen?

Bacterial surfaces are decorated with complex sugar structures. These provide protection from innate immunity, but often generate adaptive immune responses, especially against unusual sugars or motifs. Therefore, many effective modern vaccines target such structures[8].

The lipopolysaccharide (LPS) of *C. burnetii* is its main determinant of virulence (Figure 3). The LPS ends in an "*O*-antigen" containing two rare and unusual sugars (Figure 4). To facilitate efficient and cost-effective production of a subunit vaccine containing such sugars, we aim to elucidate the pathways for their biosynthesis.

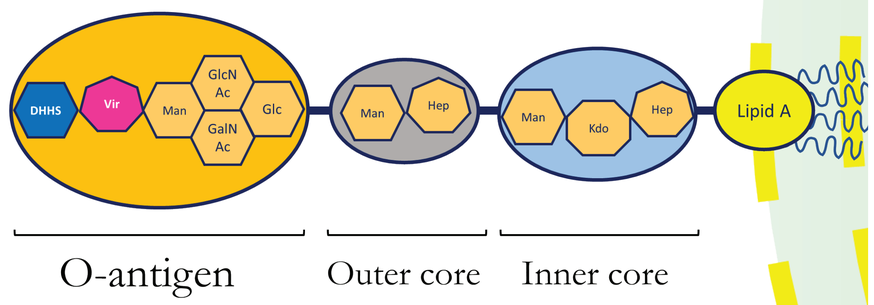

Figure 3: *C. burnetii* LPS – proposed sugar arrangement. Note **dihydrohydroxystreptose (DHHS)** in blue and **virenose (Vir)** in pink. Many copies of the LPS coat the surface of each bacterium. Figure adapted from Toman R *et al*. (2012)[7].

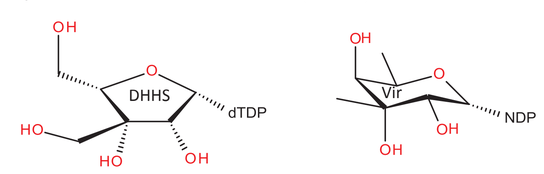

Figure 4: Structures of DHHS and Vir. These are the two rare *O*-antigen sugars that we aim to elucidate the biosynthesis of.

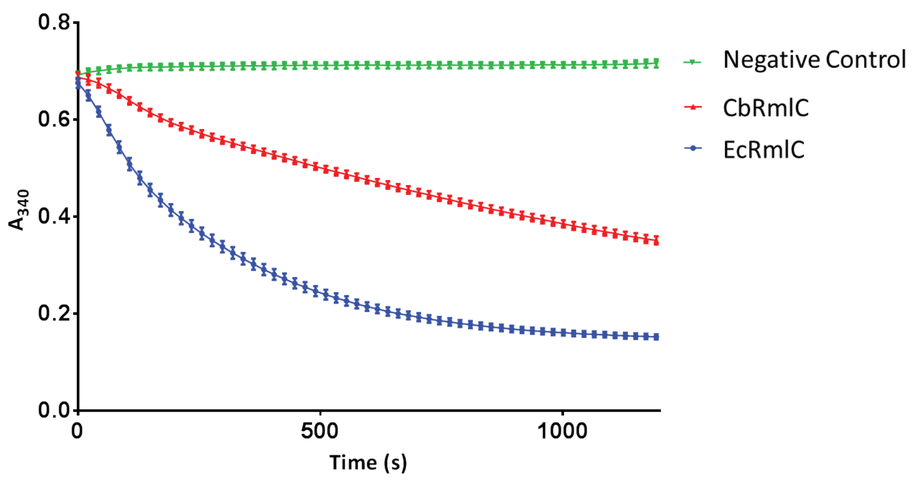

Figure 5: Enzymatic assay for rhamnose biosynthesis. CbRmlC (red) can complement the activity of EcRmlC (blue), albeit at a lower rate**.**

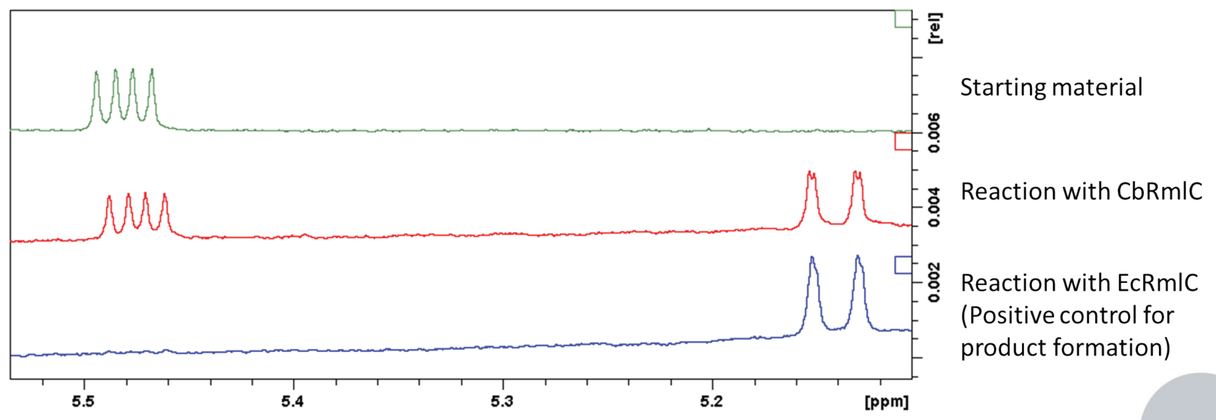

Figure 6: Diagnostic region of ^{1}H-NMR spectra for rhamnose formation. CbRmlC (red) turns over the starting material (green) to rhamnose. However, the reaction fails to reach completion in 24 hours, in contrast to a reaction using EcRmlC (blue).

Results

We have expressed candidate proteins for the proposed pathways for virenose (Vir) and dihydrohydroxystreptose (DHHS) biosynthesis, and are testing their enzymatic activity *in vitro*. We have found that the activity of one proposed *O*-antigen biosynthetic enzyme from *C. burnetii* can successfully replace the well-characterized *E. coli* epimerase (EcRmlC), through following rhamnose synthesis via coupled assays and ^{1}H-NMR spectra (Figures 5 & 6). We therefore refer to this enzyme as CbRmlC. To investigate the proposed enzymes further we are also crystallizing them for structure-solution *via* X-ray crystallography (Figure 7). Structure solution, in the presence and absence of small-molecule binding partners will lead to clues about function, including the details of enzymatic mechanism.

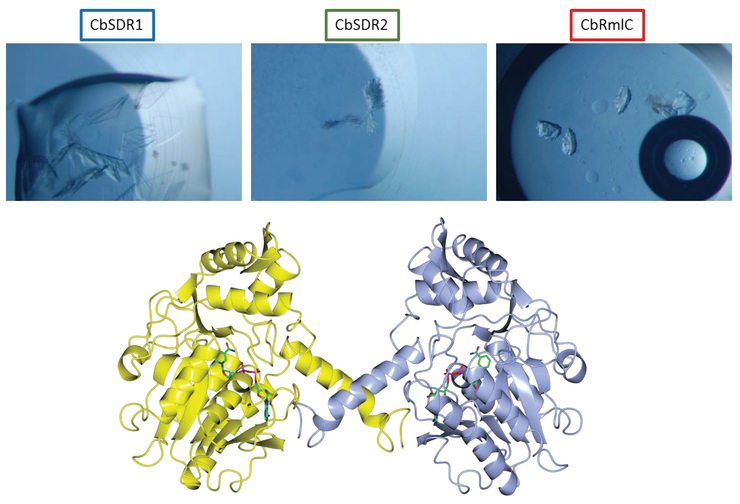

Figure 7: Protein crystals of CbSDR1, CbSDR2 and CbRmlC, and the solved structure of CbSDR1 in complex with NADH, at 2.14 Å resolution.

The road to sweet success

Q fever, caused by *Coxiella burnetii*, is environmentally resilient, easily transmissible, has a very low infectious dose, and can cause debilitating disease in both animals and humans. Its virulence-determining *O*-antigen contains rare, unusual sugars that can be exploited for subunit vaccine development. This is the primary focus of our vaccine research.

To biochemically synthesise an *O*-antigen fragment, the first step is to elucidate the enzymes involved. Candidate enzymes for Vir and DHHS biosynthesis are being tested and although several gaps remain in the proposed pathways, refinement is progressive and on-going. Understanding these pathways will highlight novel biochemistry in addition to providing the basis for generating a new scalable, cost-effective, defined subunit vaccine for Q fever, for livestock and humans.

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