Exposing hidden putative lipoproteins in *Clostridium difficile*

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Bacterial lipoproteins are membrane proteins with diverse functions. They provide structural integrity to the cell wall and participate in nutrient uptake, sporulation, adhesion and antibiotic resistance. Importantly they provoke host immune responses by the interaction of their lipids with Toll-like receptor 2 and many elicit protective antibody responses. Unsurprisingly, lipoproteins are emerging as promising vaccines. To this end, the lipoproteome of *Clostridium difficile*, was investigated.

Lipoproteins possess an N terminal signal peptide comprising positively charged initial residues, a hydrophobic region and the lipobox ending in an invariant cysteine. Following export from the cytoplasm across the cytoplasmic membrane, lipoprotein diacylglyceryl transferase (Lgt) attaches two fatty acids to this cysteine and signal peptidase II (Lsp) cleaves off the signal peptide directly upstream of the cysteine. In Gram positive bacteria, lipoproteins remain anchored to this membrane by their lipid domain and some traverse the cell wall resulting in exposure at the cell surface.

Not all lipoproteins can be identified by currently available gene mining approaches so in addition, an unconventional approach was adopted. Lipobox consensus sequences were used as query inputs in BLASTp analysis against the *C. difficile* 630 genome. Those located towards the N terminus of the protein sequence were further analysed for the presence of the remainder of the signal peptide which was confirmed by the DOLOP algorithm. This led to the new identification of 8 putative lipoproteins, 2 of which are highly probable. Our analysis further showed extensive plasticity in *C. difficile* for the lipobox and 14% of the 79 lipoproteins identified revealed a signal peptide of more than one length. A selection of these lipoproteins will be characterised experimentally and their vaccine candidacy tested.