The difficult question of Clostridium difficile

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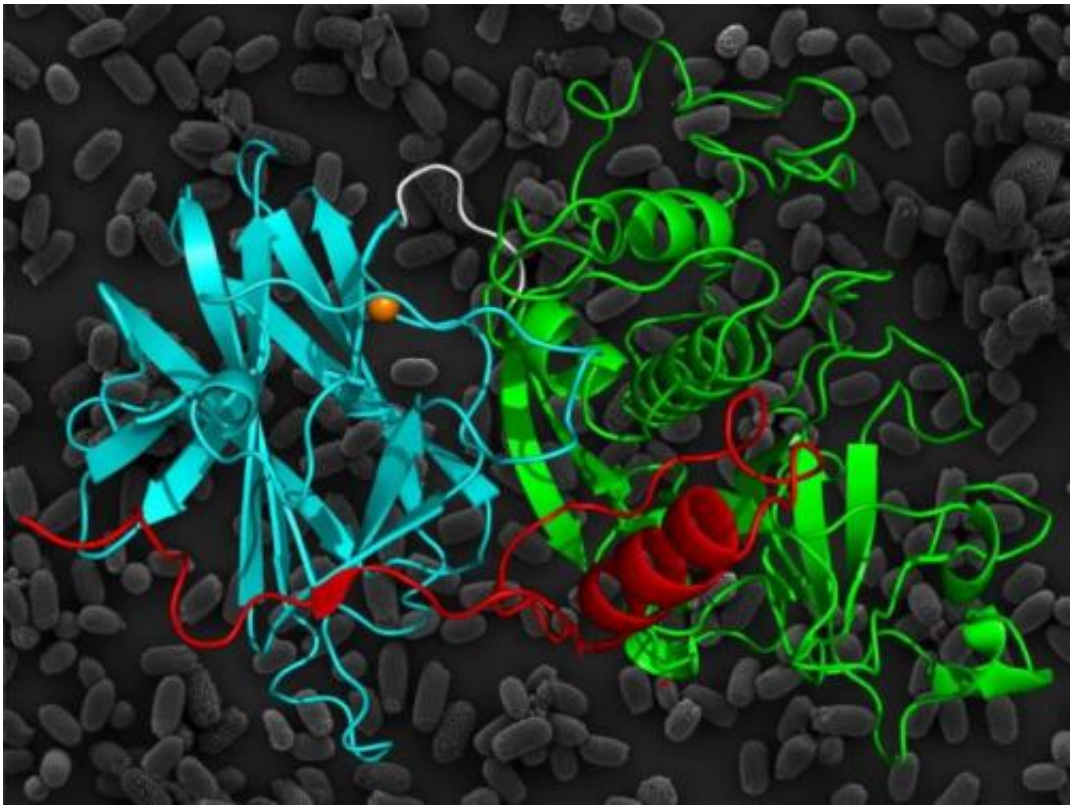

A surface layer associated protein of *Clostridium difficile*. Credit: Bradshaw et al.

The bacterium *Clostridium difficile* causes antibiotic-related diarrhoea and is a growing problem in the hospital environment and elsewhere in the community. Understanding how the microbe colonises the human gut when other "healthy" microbes have been destroyed during a course of antibiotics might lead to new ways to control infection. An important

clue was reported recently in an open access article published in the journal *Acta Crystallographica Section D Biological Crystallography*.

Ravi Acharya of the University of Bath, UK, and colleagues have reported the first crystal structure of the *C. difficile* surface protein Cwp84. This cysteine protease enzyme is found on the surface of the bacterium and assists with production of the microbe's surface-layer, which is likely to play an essential step in the colonisation of the gut. The enzyme cleaves a single polypeptide (surface-layer protein A; SlpA) into low- and high-molecular-weight subunits. Now, Acharya and colleagues have identified three critical regions in a mutant of the enzyme that could represent novel targets for drugs to attack *C. difficile* by blocking maturation of its surface layer during colonisation.

While *C. difficile* can be present in the normal, healthy gut (3-5% of adults), when a patient requires treatment for infection with broad-spectrum antibiotics, other protective intestinal microbes are eradicated in the process and the incidence increases to about 20%. This leaves space for the pathogenic *C. difficile* to grow rapidly unhindered leading to the release of toxins that cause bloating, pain and severe diarrhoea. Sometimes potentially life-threatening pseudo-membranous colitis or toxic megacolon occurs (about 5 to 8% of patients). Outbreaks occur when people ingest the spores, often in contaminated medical facilities and *C. difficile* is known to kill tens of thousands of people every year worldwide. Mild cases are often resolved by simply halting antibiotic treatment but in more severe cases last-line antibiotics such as vancomycin and metronidazole are often needed. Worryingly, the relapse rate is 20 to 30%.

The team explains that while Cwp84 is essential for correct surface layer formation it may also break down extracellular proteins, such as fibronectin, laminin and vitronectin which are found in the body. Nevertheless, blocking its activity either genetically or chemically

prevents proper growth of bacterial colonies even if this is not in itself bactericidal. Disruption of the colonization process might therefore be possible allowing healthy microbes to repopulate the gut and stifle the spread of *C. difficile*.

The researchers carried out X-ray crystallography at station I03 at Diamond Light Source in Didcot, UK. The resulting high-resolution (1.4 angstrom) diffraction data revealed the structure of the N-terminal propeptide, the cysteine protease domain, and a previously uncharacterized "linker" region that is 170 amino acids long. The linker lies between the cysteine protease domain and the repeat region of Cwp84 which holds it onto the cells surface. The linker region binds calcium and resembles a group of proteins known as lectins, so may have an affinity for carbohydrates which may be vital for correct cell wall processing. The same motifs are present in other types of *Clostridium* microbes as well as ancient single-celled organisms known as archaea.

The team suggests that the insights their research offers in terms of *C. difficile* surface layer growth and how this relates to gut colonization could be exploited in developing a new type of drug to treat infection-anti-colonization inhibitors.

More information: Bradshaw et al. (2014). *Acta Cryst.* D70, 1983-1993; DOI: 10.1107/S1399004714009997

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