A proposed nomenclature for cell wall proteins of *Clostridium difficile*

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Strains of *Clostridium difficile* produce a number of surface-localized proteins, including the S-layer proteins (SLPs) and other proteins that have suspected roles in pathogenesis. During the Third International *C. difficile* Symposium (Bled, Slovenia, September 2010) discussions were held on standardization of nomenclature. Gene designations were proposed for the large family of cell wall proteins that are paralogues of the SLP and contain putative cell wall binding motifs. This paper summarizes the agreed nomenclature, which we hope will be used by research groups currently active in the field.

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INTRODUCTION

Clostridium difficile is now recognized as an important bacterial pathogen, particularly in health-care settings where it can cause serious enteric disease (Bartlett, 2006; Rupnik *et al.*, 2009). While symptoms of disease are mediated largely by two potent cytotoxins, toxin A (TcdA) and toxin B (TcdB) (Kuehne *et al.*, 2010; Lyras *et al.*, 2009), many other factors are thought to be essential for colonization of the enteric system to allow toxin production and eventual sporulation. *C. difficile* produces many surface-localized proteins which may contribute to colonization through, for example, direct adherence to host cells or by competition with the resident microbiota. Early studies showed that diverse strains of *C. difficile* produced two major surface-associated proteins that could be extracted from the cell by treatment with EDTA, urea or low-pH glycine treatments (Cerquetti *et al.*, 1992; Kawata *et al.*, 1984; McCoubrey & Poxton, 2001; Sharp & Poxton, 1988; Takeoka *et al.*, 1991). These proteins, which migrated on SDS-PAGE with apparent molecular masses of between 35 and 55 kDa, displayed the ability to form a two-dimensional array which was visible by electron microscopy (Cerquetti *et al.*, 2000; Kawata & Masuda, 1984), an inherent property of surface-layer (S-layer) proteins (SLPs) from Bacteria and Archaea.

Genetic studies have identified several important points. Firstly, the two dominant proteins observed on the surface of a variety of strains were the result of proteolytic processing of SlpA, the product of a single gene termed *slpA* (Calabi *et al.*, 2001; Karjalainen *et al.*, 2001). These proteins were subsequently termed SLPs. Secondly, the differences in mobility of these proteins between strains, as visualized by SDS-PAGE, were due to differences in the lengths of the *slpA* genes, resulting in antigenically distinct forms of the SLPs. Thirdly, a family of genes (paralogues) related to *slpA* was identified in the genome of strain 630 (Calabi *et al.*, 2001; Karjalainen *et al.*, 2001; Savariau-Lacomme *et al.*, 2003; Sebahia *et al.*, 2006). Members of this family, which comprises 29 genes, all contain three copies of the Pfam 04122 motif, which is a complex motif annotated as 'putative cell wall binding repeat 2'. The idea was proposed (Karjalainen *et al.*, 2001) that these Pfam motifs mediate binding of the proteins encoded by these paralogues to the underlying cell wall. In some cases, these proteins carry a second domain which specifies a known or putative function. The best characterized examples are Cwp84 (Janoir *et al.*, 2007), a protease that cleaves the SlpA precursor (Dang *et al.*, 2010; Kirby *et al.*, 2009) and which has degradative activity on a variety of host cell extracellular matrix proteins (Janoir *et al.*, 2007), Cwp66, a putative adhesin (Waligora *et al.*, 2001), and CwpV, a phase-variable protein (Emerson *et al.*, 2009).

Abbreviations: CWPs, cell wall proteins; SLPs, S-layer proteins.

With an increasing number of research groups working on *C. difficile* and the advent of genome sequences that are uncovering increasing diversity between *C. difficile* strains, the need for a unified nomenclature of *C. difficile* S-layer and cell wall proteins is apparent. A similar situation existed a few years ago in regard to the nomenclature of the large clostridial cytotoxins, and a unified nomenclature was proposed (Rupnik *et al.*, 2005) and has been universally adopted.

PROPOSED NOMENCLATURE

SLPs

All strains of *C. difficile* examined to date express two predominant proteins on the cell wall, which form the major components of the S-layer. The encoding gene, which appears to have a conserved genomic location between all strains studied to date, has been named *slpA*. The precursor protein is termed SlpA, and the mature proteins produced by the action of the protease Cwp84 are termed the HMW (high molecular weight) SLP and the LMW (low molecular weight) SLP. The HMW SLP contains three Pfam 04122 'putative cell wall binding repeat 2' motifs while the LMW SLP shows a high degree of antigenic variation between strains. We suggest that the prefix *slp* be reserved for those genes whose products have been shown experimentally to form a two-dimensional array, and not used for gene products found within the S-layer, as this could vary depending on the technique used for extraction of SLPs.

Cell wall proteins

In *C. difficile* 630, a family of 29 genes containing three Pfam 04122 motifs was identified. These genes were originally given the prefix 'orf1', 'orf2', etc. (Calabi *et al.*, 2001; Karjalainen *et al.*, 2001), but due to simultaneous publication, the genes were numbered differently. In addition, the genes *cwp66* and *cwp84* were individually named according to their molecular masses (Karjalainen *et al.*, 2001). Following this convention, some other genes (Wright *et al.*, 2005) were given a '*cwp*' prefix (Emerson & Fairweather, 2009; Wright *et al.*, 2008).

Ordinarily, a gene family would be named using only letters, e.g. *slpA* and *cwpV*. However, as there are over 26 paralogues, this is not possible. In addition, some names are in common use and we recommend keeping these. We propose that all members of the gene family containing Pfam 04122 motifs be termed CWPs, 'clostridial wall proteins' or more aptly 'cell wall proteins' and that the genes be given the prefix *cwp*. The exception is *slpA*, which, although a member of this gene family, has a clearly established name which relates to its function as an SLP. Where an existing name has been published and has been generally accepted, that name should remain in use. To date these are *cwp84*, *cwp66*, *cwpV* and *cwp2*. All other *cwp* genes have been assigned a number as outlined in Table 1. The ORF designation with a CD prefix given upon annotation of strain 630 is also shown. The vast

majority of the *cwp* genes are conserved between 630 and R20291, a recently isolated ribotype 027 strain which has been sequenced at the genome level (Stabler *et al.*, 2009). Genome sequencing of further *C. difficile* strains will undoubtedly reveal new members of the *cwp* gene family. We suggest that the naming be continued numerically, i.e. the next *cwp* gene identified should be named *cwp30*.

Several of the CWPs contain a passenger domain that may specify a function. The majority of these have not been demonstrated experimentally, but are based on the presence of motifs. The putative functional motifs and the architectures of members of the CWP family are shown in Fig. 1.

Table 1. Nomenclature of CWPs of *C. difficile*

The CWP family is defined as possessing Pfam 04122 'putative cell wall binding repeat 2' motifs.

Gene name*	Gene number†	Mol. mass‡ (kDa)
<i>slpA</i>	CD2793	76.1
<i>cwp2</i>	CD2791	66.5
<i>cwp66</i>	CD2789	66.8
<i>cwp84</i>	CD2787	87.3
<i>cwp5</i>	CD2786	57.2
<i>cwp6</i>	CD2784	73.0
<i>cwp7</i>	CD2782	39.3
<i>cwp8</i>	CD2799	68.5
<i>cwp9</i>	CD2798	52.5
<i>cwp10</i>	CD2796	67.0
<i>cwp11</i>	CD2795	58.7
<i>cwp12</i>	CD2794	57.5
<i>cwp13</i>	CD1751	87.2
<i>cwp14</i>	CD2735	51.4
<i>cwpV</i>	CD0514	166.7
<i>cwp16</i>	CD1035	74.7
<i>cwp17</i>	CD1036	74.2
<i>cwp18</i>	CD1047	37.4
<i>cwp19</i>	CD2767	77.8
<i>cwp20</i>	CD1469	111.1
<i>cwp21</i>	CD3192	61.1
<i>cwp22</i>	CD2713	72.0
<i>cwp23</i>	CD1803	63.8
<i>cwp24</i>	CD2193	51.3
<i>cwp25</i>	CD0844	33.8
<i>cwp26</i>	CD1233	52.0
<i>cwp27</i>	CD0440	41.0
<i>cwp28</i>	CD1987	51.7
<i>cwp29</i>	CD2518	50.0

*There is no *cwp1* gene. Proteins have identical names, but with a capitalized first letter and not italicized. Gene names in the current literature are: *slpA* (Calabi *et al.*, 2001), *cwp2* and *cwpV* (Emerson *et al.*, 2009), *cwp66* (Waligora *et al.*, 2001) and *cwp84* (Karjalainen *et al.*, 2001).

†Gene number according to Sebaihia *et al.* (2006). All genes except *cwp29* have paralogues in R20291.

‡Predicted molecular mass of protein prior to any processing event.

Fig. 1. Domain architecture of the CWP family. The functional domains contained within each protein were determined by analysis using Pfam (<http://pfam.sanger.ac.uk/>) and are as follows: CW_binding_2 (PF04122), LMWSLP_N (PF12211), SH3_3 (PF08239), PepSY (PF03413), CAP (PF00188), Glucosaminidase (PF01832), Big_3 (PF07523), YkuD (PF03734), CW_binding_1 (PF01473), Amidase_3 (PF01520), DUF187 (PF02638), Peptidase_C1 (PF00112) and β-lactamase (PF00144). All proteins contain a predicted N-terminal signal peptide (black box).

Other surface-localized proteins

C. difficile genomes encode a number of putative surface-localized proteins in addition to the CWP family. Some of these have been characterized at the molecular level, for example *fbp68* encoding a fibronectin-binding protein (Hennequin *et al.*, 2003). At least one sortase gene has been identified in 630 and R20291 genomes and a number of putative sortase substrates have also been identified. We suggest that any names assigned to these genes and proteins remain unchanged and that newly characterized genes, for example the sortase substrates, be named by the group who first describe their properties.

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