PP028

Potential role of the host-derived cell-wall binding domain of endolysin CD16/50L as a molecular anchor in preservation of uninfected *Clostridioides difficile* for new rounds of phage infection

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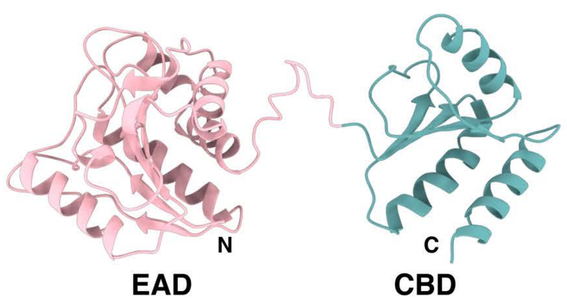
Abstract

Endolysin is a phage-encoded cell-wall hydrolase which degrades the peptidoglycan layer of the bacterial cell wall. The enzyme is often expressed at the late stage of the phage lytic cycle and is required for progeny escape. Endolysins of bacteriophage that infect Gram-positive bacteria often comprises two domains: a peptidoglycan hydrolase and a cell-wall binding domain (CBD). Although the catalytic domain of endolysin is relatively well-studied, the precise role of CBD is ambiguous and remains controversial. Here, we focus on the function of endolysin CBD from a recently isolated *Clostridioides difficile* phage. We found that the CBD is not required for lytic activity, which is strongly prevented by the surface layer of C. difficile. Intriguingly, hidden Markov model analysis suggested that the endolysin CBD is likely derived from the CWB2 motif of *C. difficile* cell-wall proteins but possesses a higher binding affinity to bacterial cell-wall polysaccharides. Moreover, the CBD forms a homodimer, formation of which is necessary for interaction with the surface saccharides. Importantly, endolysin diffusion and sequential cytolytic assays showed that CBD of endolysin is required for the enzyme to be anchored to post-lytic cell-wall remnants, suggesting its physiological roles in limiting diffusion of the enzyme, preserving neighboring host cells, and thereby enabling the phage progeny to initiate new rounds of infection. Taken together, this study provides an insight into regulation of endolysin through CBD and can potentially be applied for endolysin treatment against *C. difficile* infection.

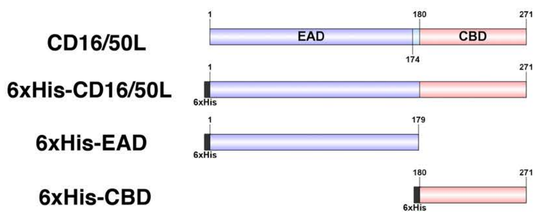
Results

Structure model of CD16/50L by AlphaFold



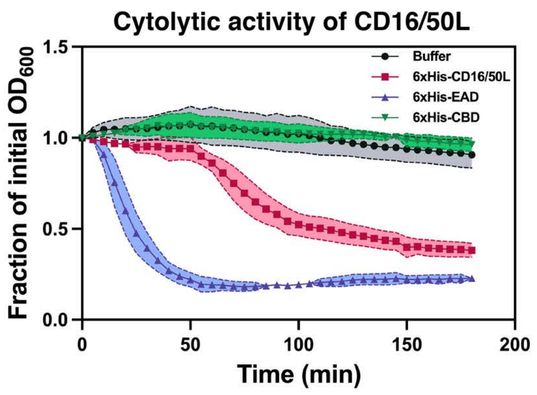
- CD16/50L is modular structure compose of N-terminal enzymatic active domian (EAD) and C-terminal cell-wall binding domain (CBD)
- Sequence-based analysis revealed that EAD contains an amidase function

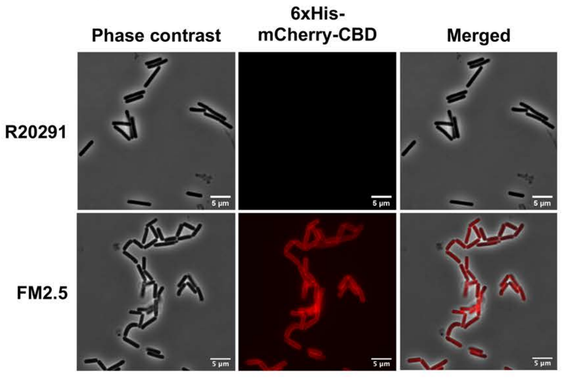
Schematic of CD16/50L variants



- CD16/50L full-length and EAD able to lyse *C. difficile.*
- CD16/50L is CBD-independent cytolytic activity.

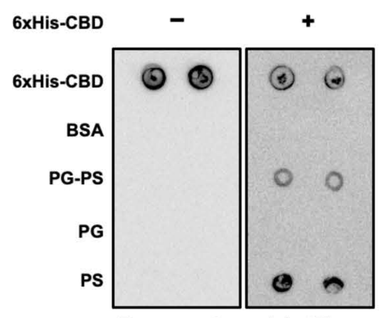
Cytolytic activity of CD16/50L





Fluorescent-based detection

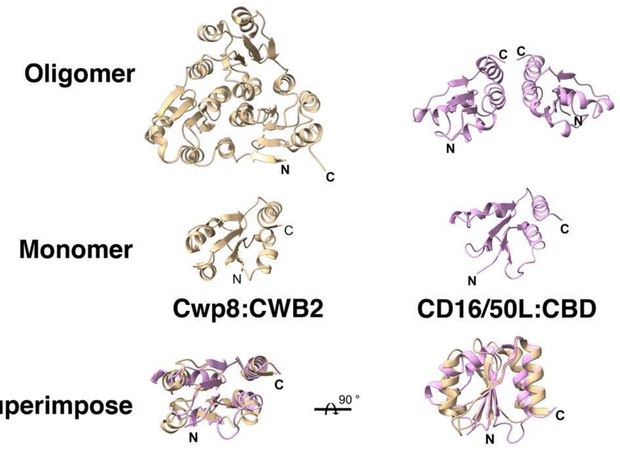
- mCherry-CBD decorated on the S-layer mutant *C. difficile* cells but not wild-type cells



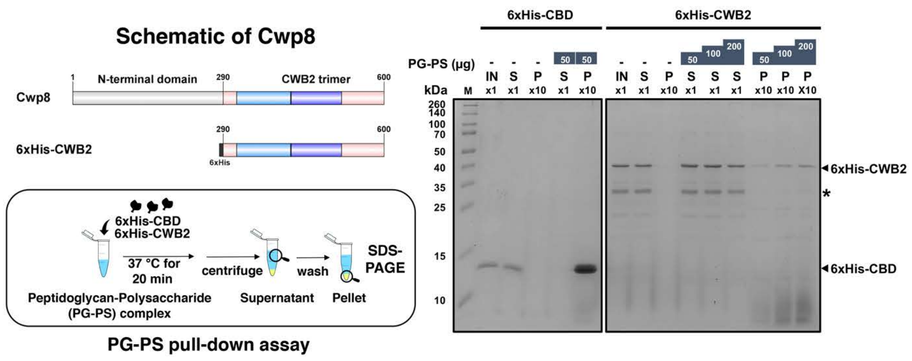
Far western blotting

- Far western blotting revealed the interaction of CBD on peptidoglycan-polysaccharide complex (PG-PS) and PS but not PG alone.

The CBD of CD16/50L is evolutionarily homologous to CWB2 domain of *C. difficile* cell wall proteins and binds to PG-PS complex more tightly than the host counterpart

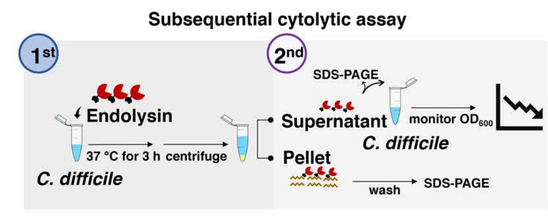


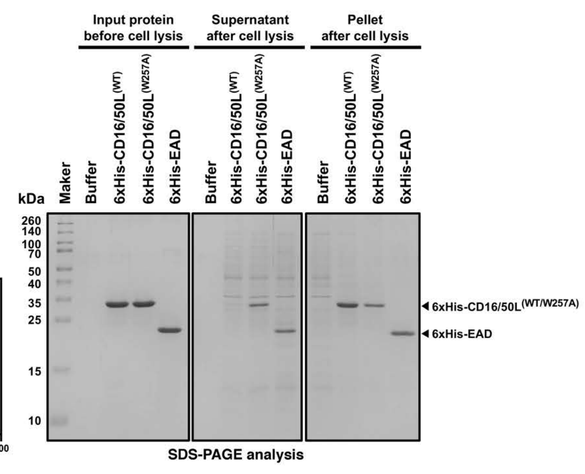
- HHpred analysis revealed that CBD has secondary structure similar to CWB2 domain.
- Both domains shared an open α-β Rossmann fold.
- Superimpose of CWB2 and CBD showed an α-carbon average root-mean-square deviation (RMSD) between pruned atom pairs of 0.827 Å

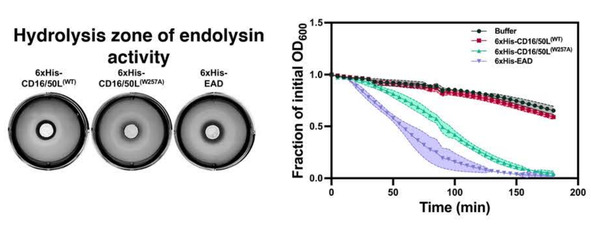


- CD16/50L CBD, a simplified version of CWB2, binds *C. difficile* cell wall more efficiently than the original CWB2

The CBD of CD16/50L anchors the endolysin to bacterial post-lytic remnants and prevents a successive round of cytolysis

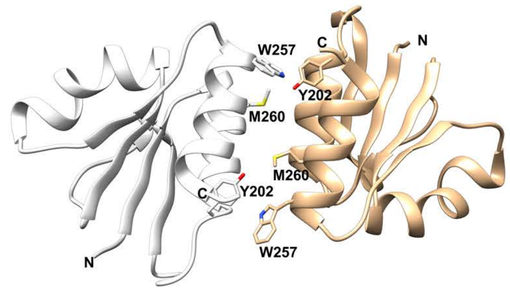


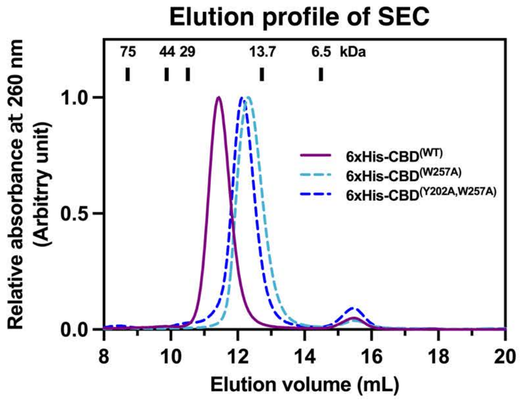


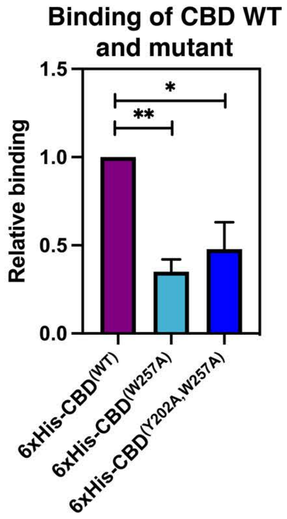


- CBD restricted endolysin diffusion
- CD16/50L WT containing CBD dimer was trapped in bacterial cell wall. While the dimer destabilized of CD16/50L W257A and EAD able to detach from bacterial cell remnants.
- CD16/50L W257A and EAD were able to lyse a fresh batch of *C. difficile* cells
- CBD anchors bacterial cell wall could prevent the neighbor cells from cell lysis which may benefit for progeny phage

The CBD of CD16/50L forms a homodimer







- Structural model shows homodimer of CBD
- CBD mutant showed a delayed in SEC elution profiled, indicating a different protein size.
- Destabilized dimer of CBD diminished binding function

Summary

- CD16/50L is modular structure compose of EAD and CBD
- CD16/50L is CBD-independent cytolytic activity.
- CBD binds polysaccharide on bacterial cell wall.
- CBD is homologous to CWB2 domain of *C. difficile* cell-wall proteins.
- CBD forms homodimer which is essentail for interaction with bacterial cell
- CBD binds bacterial cell remnant, preventing endolysin diffusion.
- The CBD-cell wall trapping mechanism might be beneficial for bacteriophage

Acknowledgement

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