

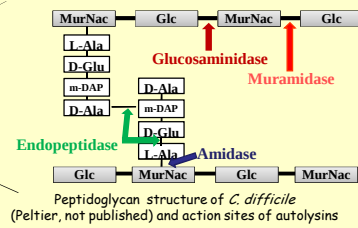
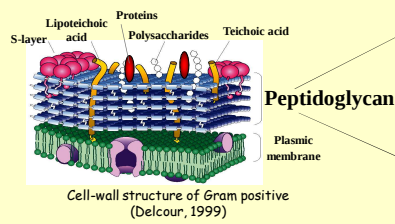
AUTOLYSINS OF *CLOSTRIDIUM DIFFICILE* INVOLVED IN VEGETATIVE GROWTH

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Peptidoglycan (PG) and autolysins (peptidoglycan hydrolases)



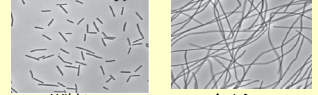
Modular organization of autolysins: Most firmicutes major autolysins are composed of 2 domains: a **catalytic domain** and a **cell-wall binding domain**

Functions and regulation of autolysins

- Functions:**
- Peptidoglycan turnover
 - Cellular division
 - Sporulation and germination
 - Antibiotics-induced lysis

Few transcriptional regulators described:

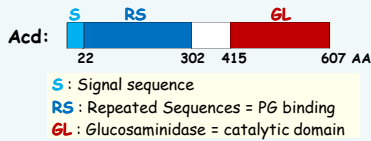
Sigma D in *Bacillus subtilis*: control of major autolysins (Chen, 2009)



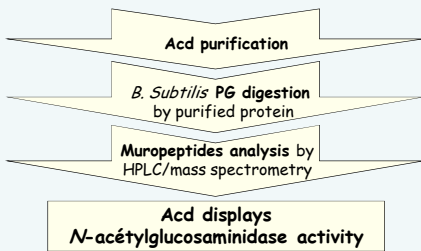
Cell separation defect in a *sigD* mutant in *B. subtilis*

Acd, an autolysin of *C. difficile*

1- Search for homologies with glucosaminidase domain of autolysins of *B. subtilis* and *Staphylococcus aureus*



2- Determination of Acd hydrolytic bond specificity



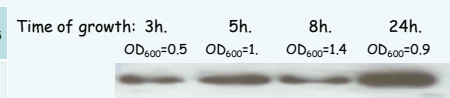
3- *acd* expression during various growth phases

• At transcriptional level (qRT-PCR)

	5h/3h OD ₆₀₀ =1.0/OD ₆₀₀ =0.5	8h/3h OD ₆₀₀ =1.4/OD ₆₀₀ =0.5	24h/3h OD ₆₀₀ =0.9/OD ₆₀₀ =0.5
<i>acd</i> expression (Pvalue)	+ 1.27	- 1.22	-109.90 (p=0.02)

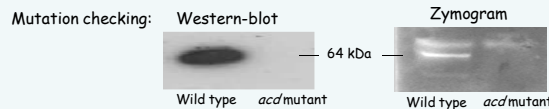
Values significantly different are >2 (P < 0.05)

• At translational level



- *acd* is expressed during vegetative growth: possible role of Acd in cell separation
- Acd protein is accumulated during late phases of growth: possible role of Acd in sporulation/germination

4- Construction of an *acd* mutant by using the ClosTron system



- Important lytic activity disappears in the *acd* mutant: Acd is a major autolysin
- A lysis band persists in the mutant strain

5- Physiological study of *acd* mutant

Disruption of *acd* did not impair:

- cell separation
- cell autolysis
- glycopeptides or β -lactams induced lysis
- sporulation/germination

Role of Acd in *C. difficile* probably compensated by one/several other autolysins

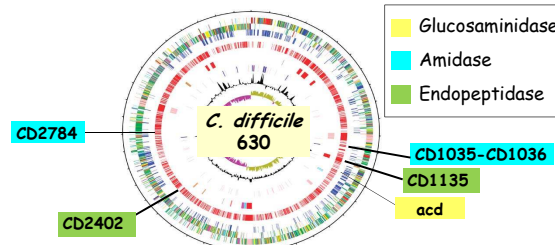
Search for new major autolysins involved in vegetative growth:

29 putative autolysins in *C. difficile* 630 genome:

Search for homologies to major autolysins encoding genes in *B. subtilis*.

- exclusion of putative **phage** cell-wall hydrolases
- exclusion of putative **spore cortex**-lytic enzymes
- exclusion of putative cell-wall hydrolases **without** cell-wall binding domain

➔ Selection of 5 new putative candidates



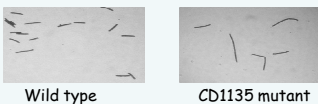
• Creation of independent insertional mutants (ClosTron system) for **CD1035**, **CD2784**, **CD1135** and **CD2402** genes.

(Despite multiple attempts, it was not possible to generate mutant of **CD1036**).

Cell separation and cell autolysis in mutant strains

- No defect in cell separation in CD1035, CD2784 and CD2402 mutant strains

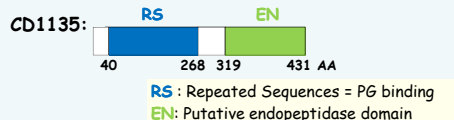
- Slight defect in cell separation in CD1135 mutant strain



- No defect in cell autolysis in the mutant strains

CD1135 is a peptidoglycan hydrolase

Overexpression and purification of CD1135 catalytic domain (AA 268 to AA 431)

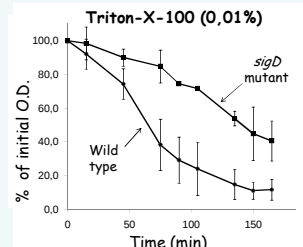


SigD, a putative autolytic regulator

Presence of a σ^D factor in *C. difficile* (33% identity with σ^D of *B. subtilis*)

***sigD* inactivation:** (ClosTron system)

- Normal septation of cells but
- Decreased lysis in stationary growth phase



Possible involvement of σ^D in the regulation of bacterial autolysis

Conclusions

- Acd is a major autolysin with N-acetyl glucosaminidase activity but an *acd* mutant strain shows no differences with wild type.
- 29 putative autolysins in *C. difficile* 630 genome: Probable functional redundancy of the autolysins in *C. difficile*.
- Construction of 4 new mutants of putative autolysins with a modular organization typical of major autolysins. —→ the CD1135 mutant shows a defect in cell separation.
- A *sigD* mutant shows a slowed lysis in stationary growth phase: σ^D could be a regulator of autolysis in *C. difficile*.