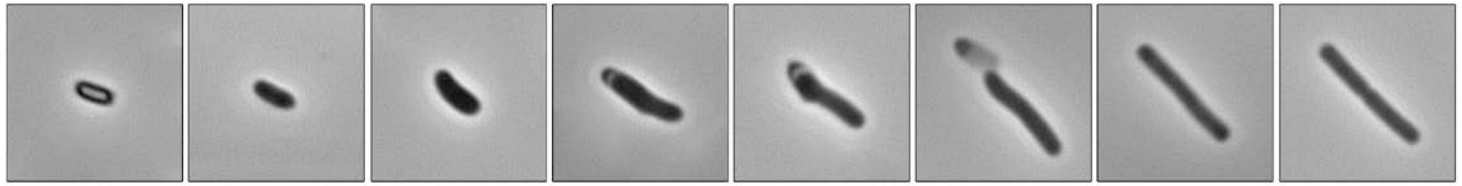
Microarray analysis of *Clostridium difficile* 630 endospore germination

Marcin Dembek, Richard A. Stabler, Neil F. Fairweather



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CMBI

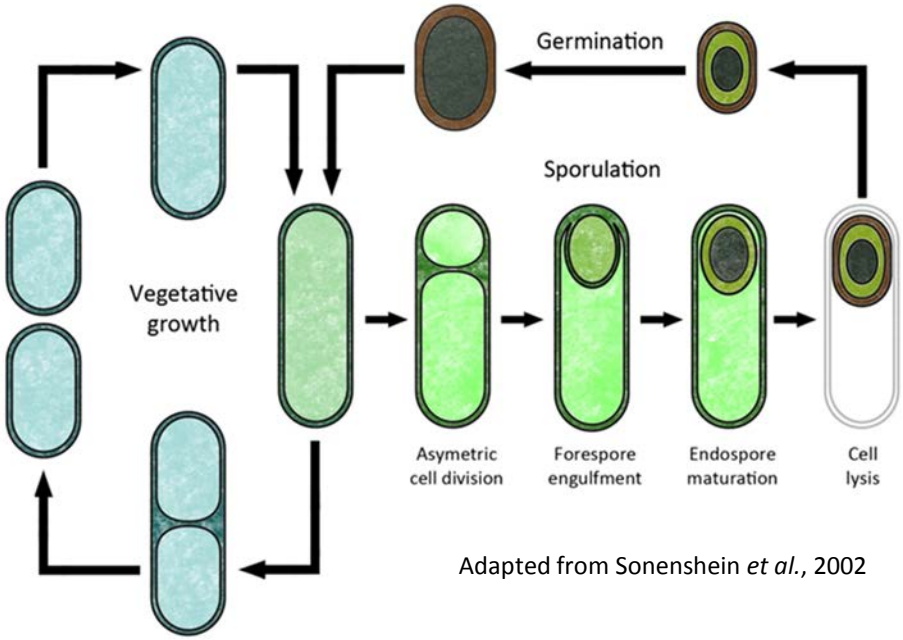
Imperial College London

wellcome trust

Clostridium difficile endospores

Spores are pivotal in disease transmission

- Highly infectious
- Resistant to various chemical and physical insults
- Persist in health care facilities for extended periods of time
- Need to germinate to form toxin-producing cells



Adapted from Sonenshein *et al.*, 2002

Clostridium difficile endospores

Spores are pivotal in disease transmission

- Highly infectious
- Resistant to various chemical and physical insults
- Persist in health care facilities for extended periods of time
- Need to germinate to form toxin-producing cells

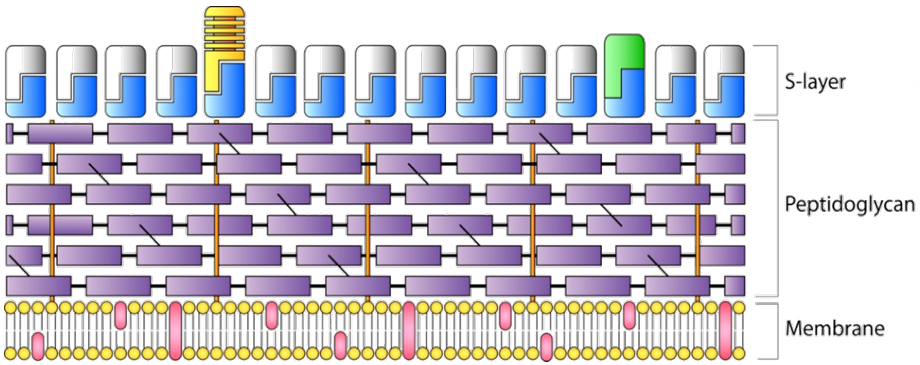
However,

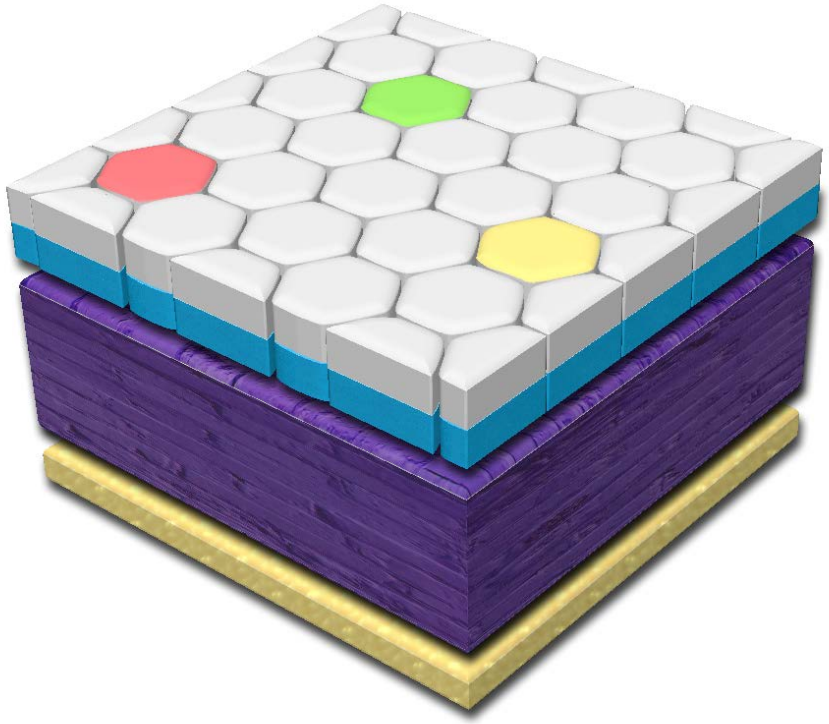
- Little homology in spore proteins between *C. difficile* and Bacilli/Clostridia
- Few germinants known (taurocholate, glycine)
- Genes encoding known GR subunits have not been identified
- No mechanistic insight into the germination process due to limited amount of molecular tools and lack of appropriate animal models

C. difficile cell wall and S-layer

Research interests:

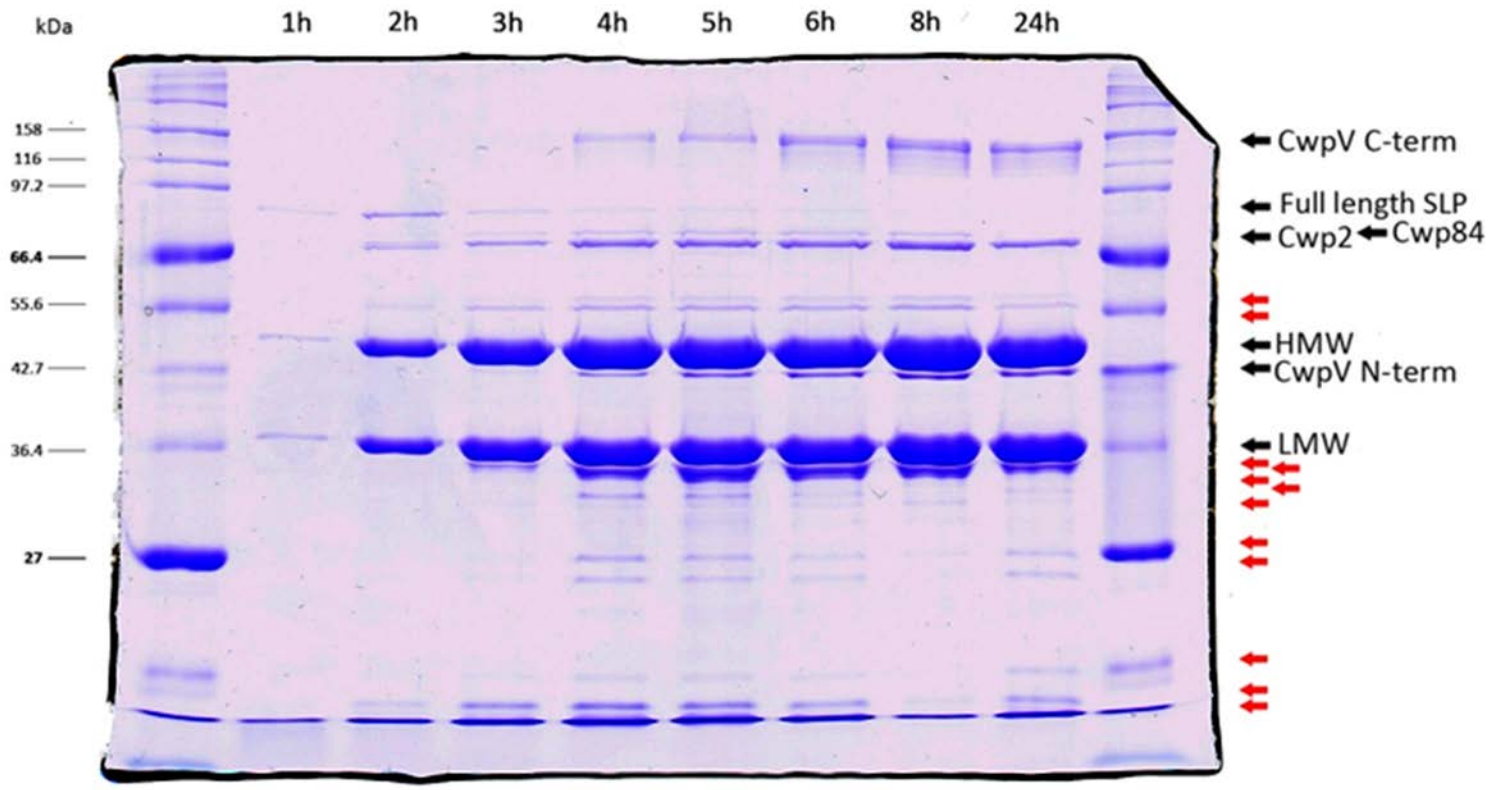
- Structure and assembly
- Gene regulation
- Adhesion/Colonization
- Vaccinology





C. difficile cell wall and S-layer

- Temporal changes in S-layer composition observed during germination



SDS-PAGE: S-layer extracts from germinating endospores

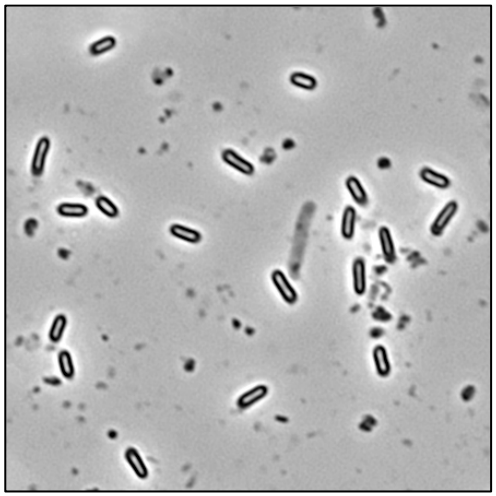
Project aim:

I. Perform a microarray analysis of temporal gene expression in germinating *C.difficile* 630 endopsores

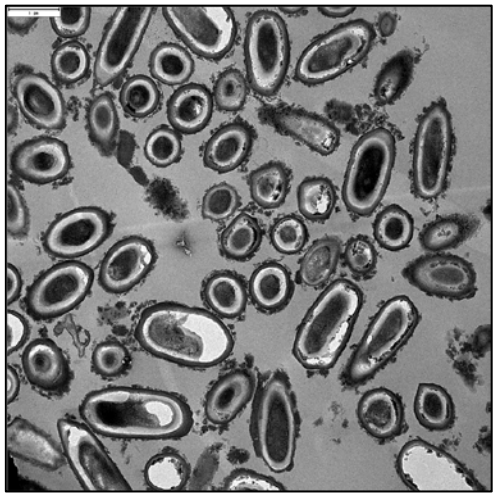
II. To construct gene knock-outs and use gene knock-down technologies to study the role of specific genes in germination

III. To analyse endospores of knock-out and knock-down strains for their ability to germinate *in vitro* as well as *in vivo* using a mouse model of infection.

Spore production and purification



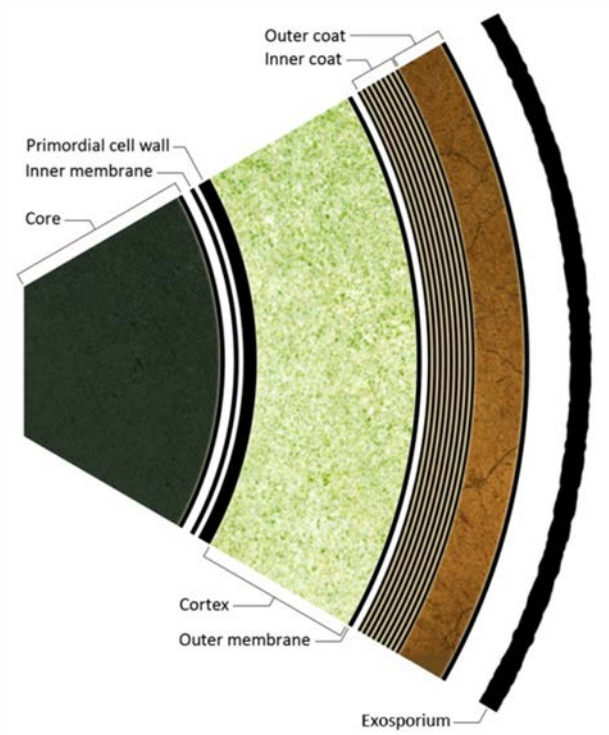
Crude spore preparation

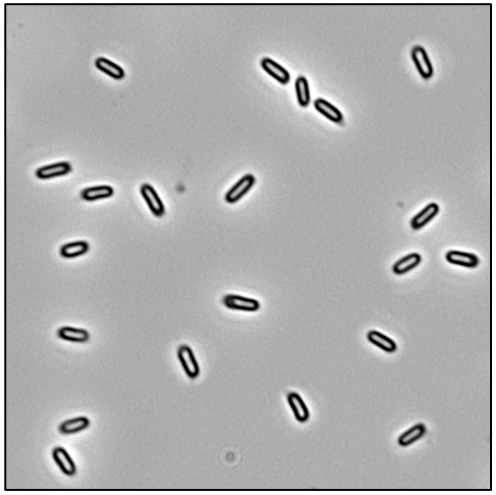


TEM (low magnification)

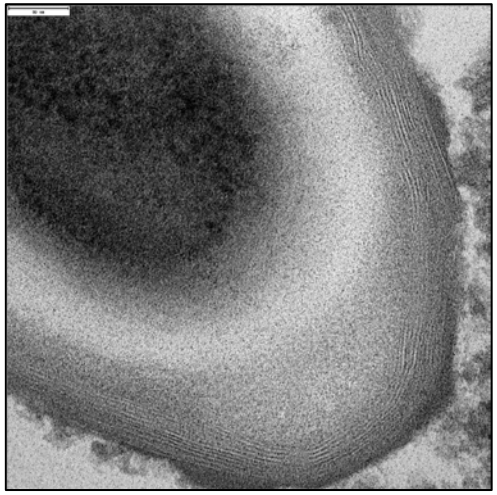
***C. difficile* 630**

tcdA+, tcdB+, ribotype 012





Purified spores

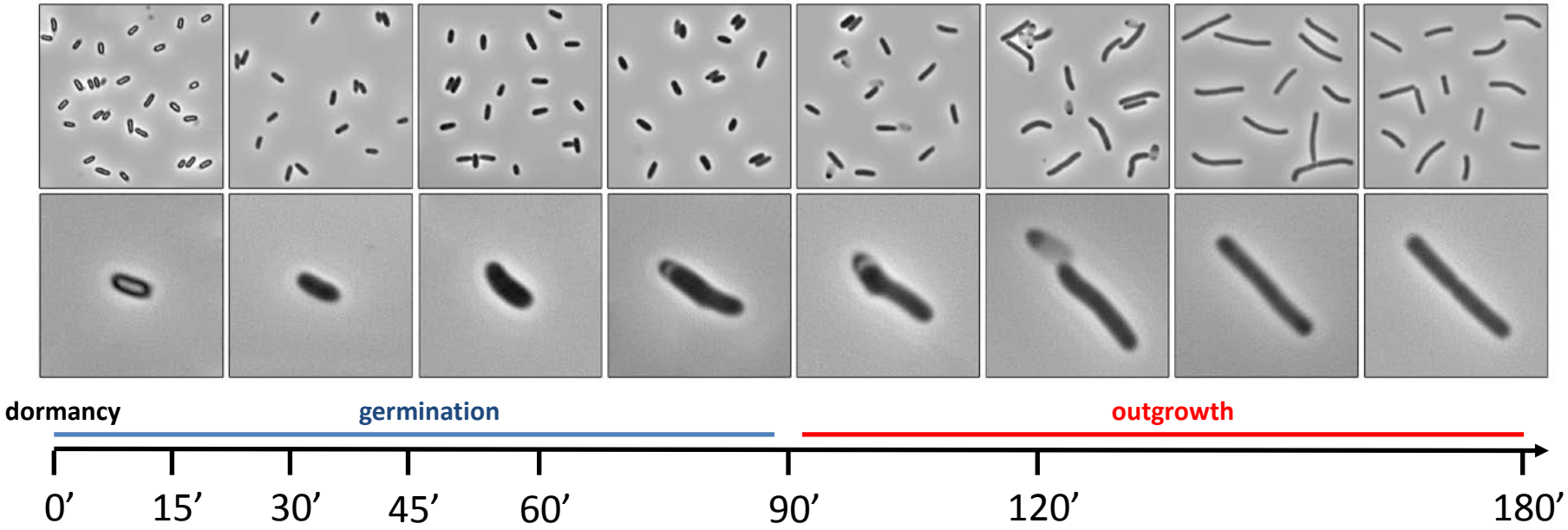


TEM (high magnification)

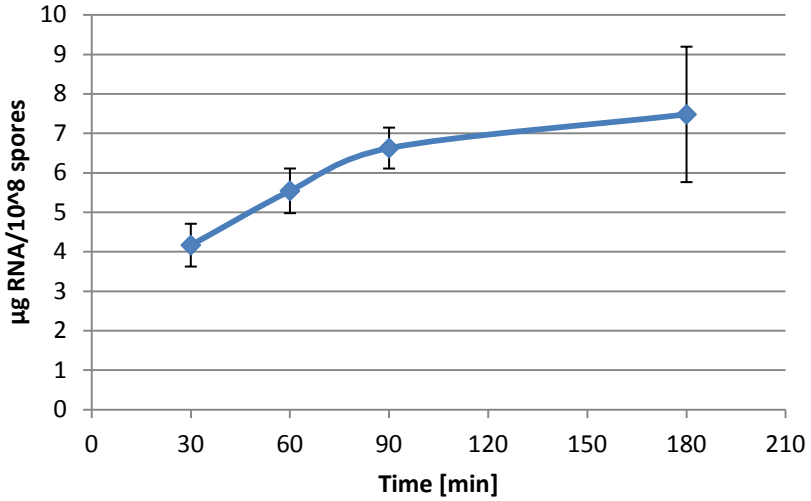
TEM was carried out in collaboration with David Banbury

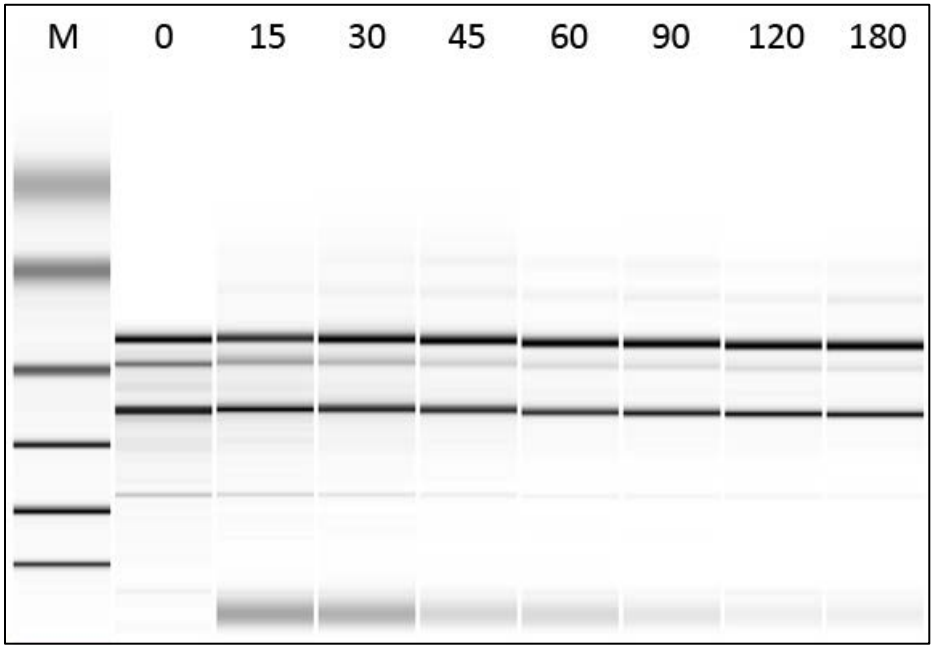
Microarray analysis of temporal gene expression in germinating *C. difficile* endospores

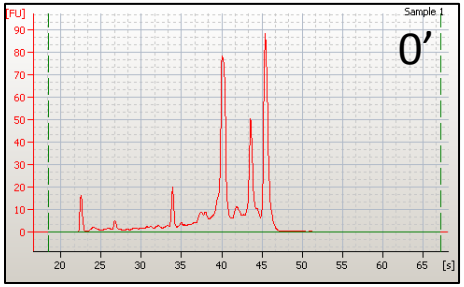
BHIS + 0.5% Tch

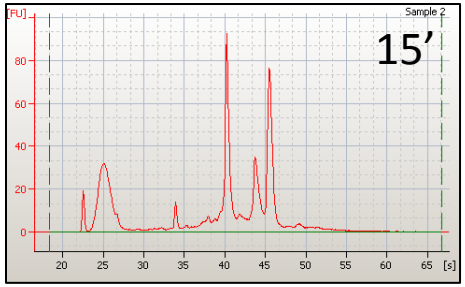


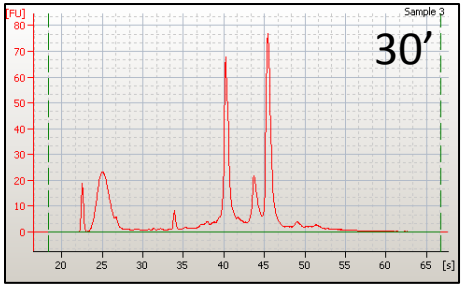
RNA quality control

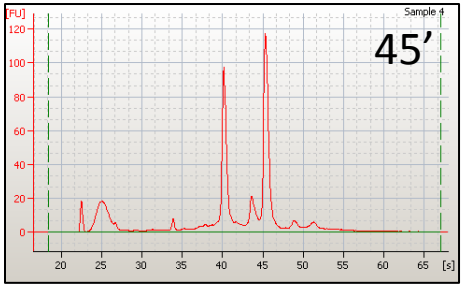


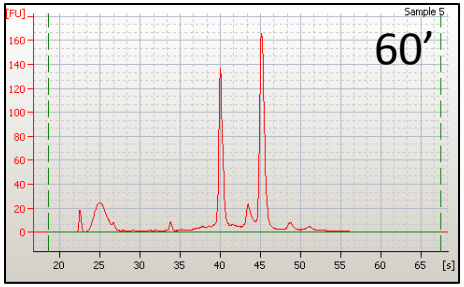


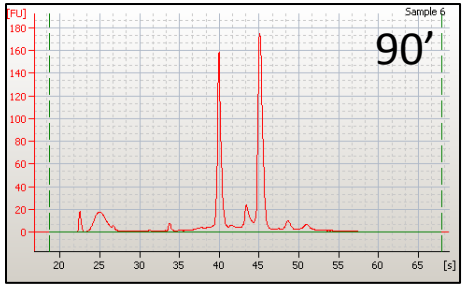


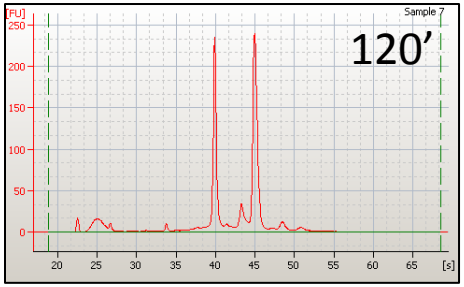


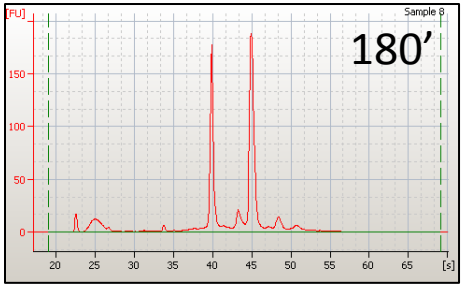






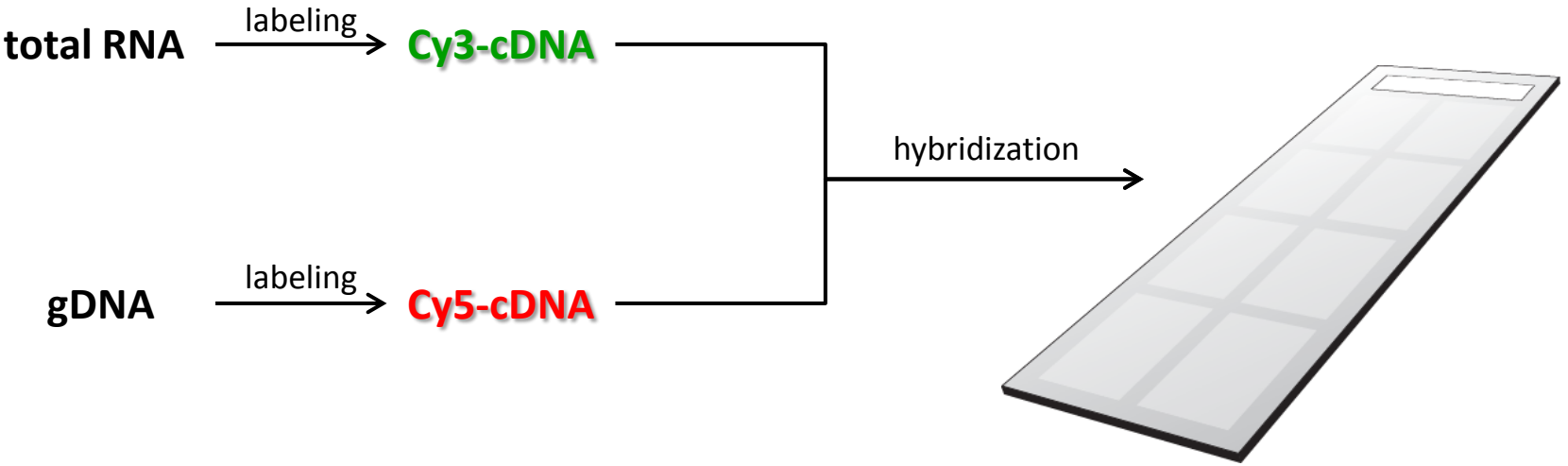






Microarray analysis of temporal gene expression in germinating *C. difficile* endospores

- Two-channel microarrays (*C. difficile* OGT array CDv2.0.1)
- Agilent 8x 15k platform
- Approx. 3-4 probes (60mers) per gene



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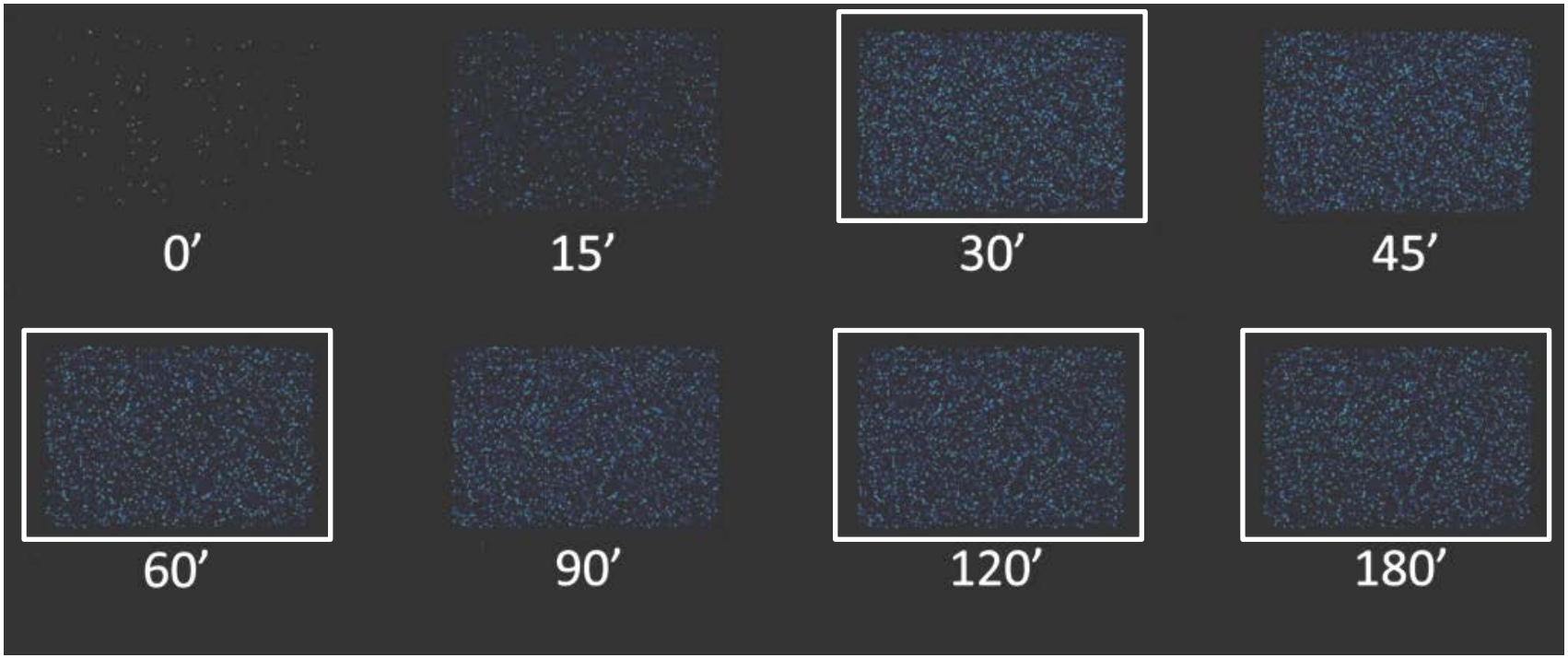


Microarray analysis of temporal gene expression in germinating *C. difficile* endospores

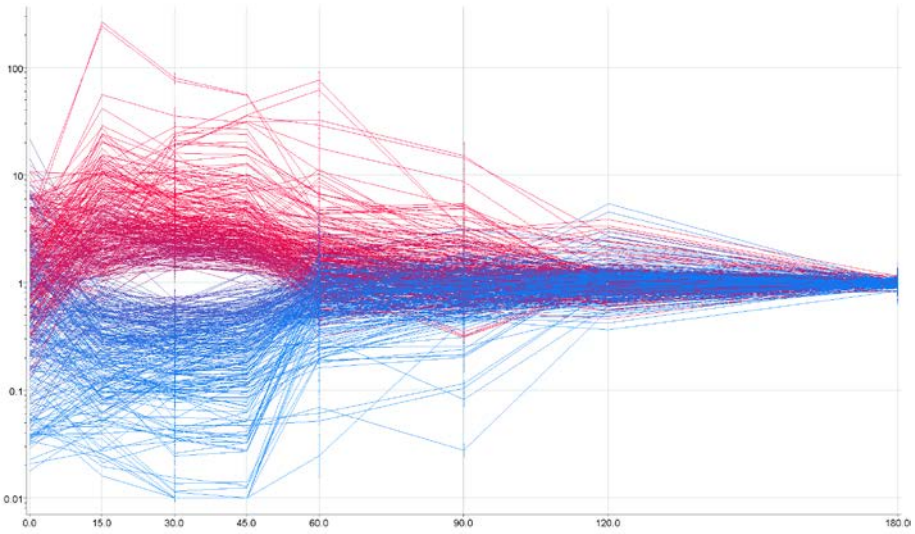
Statistical analysis

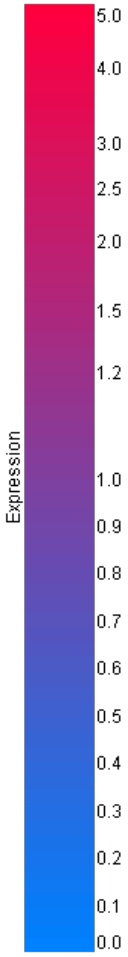
3 biological replicates (30, 60, 90 and 180 min)

1-way ANOVA B&H FDR p ≤ 0.01

30 min vs. 180 min

Validated by RT-PCR on selected genes





271 genes up-regulated

1.33- to 79.77-fold

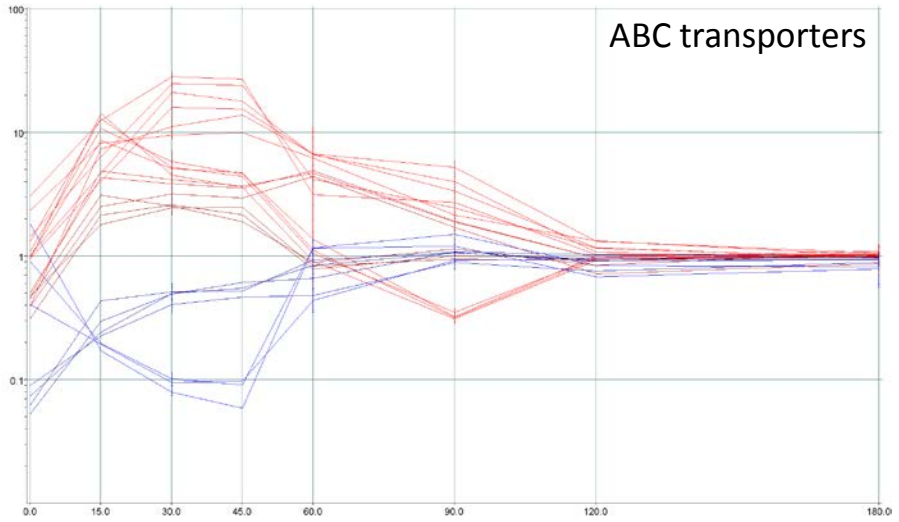
246 genes down-regulated

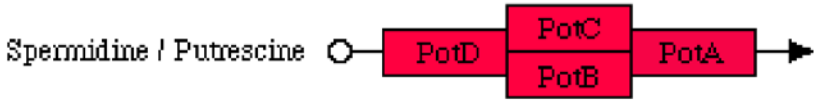
1.34- to 100-fold

- Many clusters of genes were co-regulated
- KEGG pathway database was used to scan the results

ABC transporters

- Transmembrane proteins
- ATP-driven
- Involved in both transport- and non-transport-related processes
- 229 found in the *C. difficile* 630
  - ✓ 34 up-regulated
  - ✓ 9 down regulated

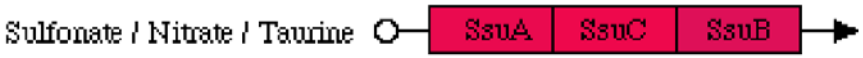




Polyamines involved in:

- DNA replication
- Cell division
- Stress response

Found in a number of systems shifting between quiescence/proliferation



Conjugates with cholate forming taurocholate

5.0
4.0
3.0
2.5
2.0
1.5
1.2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

ABC transporters

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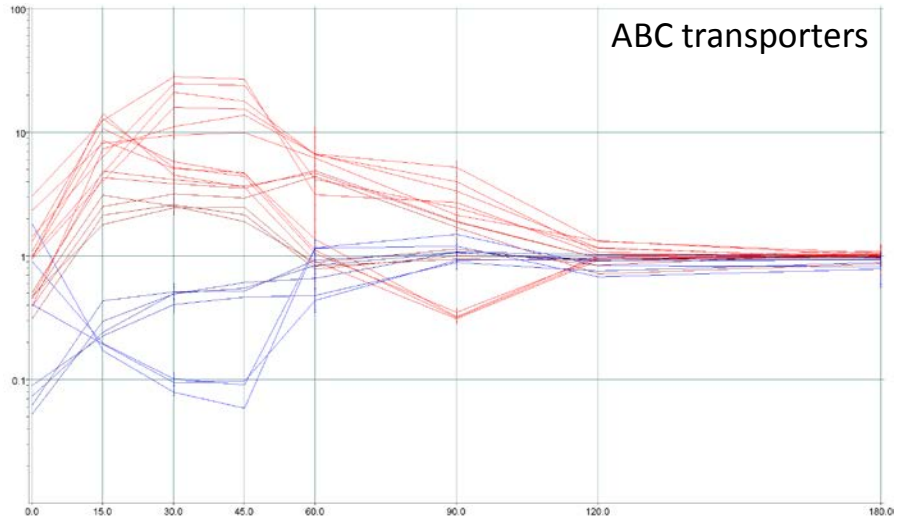
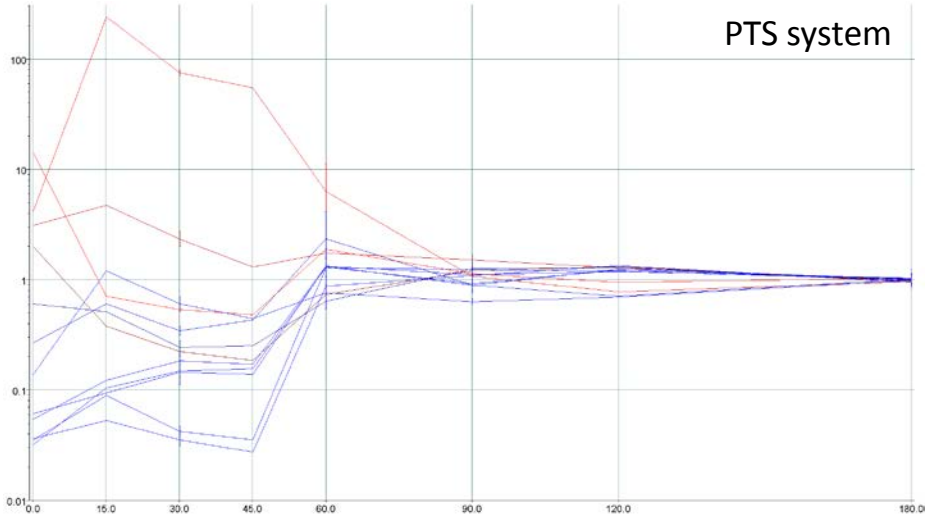
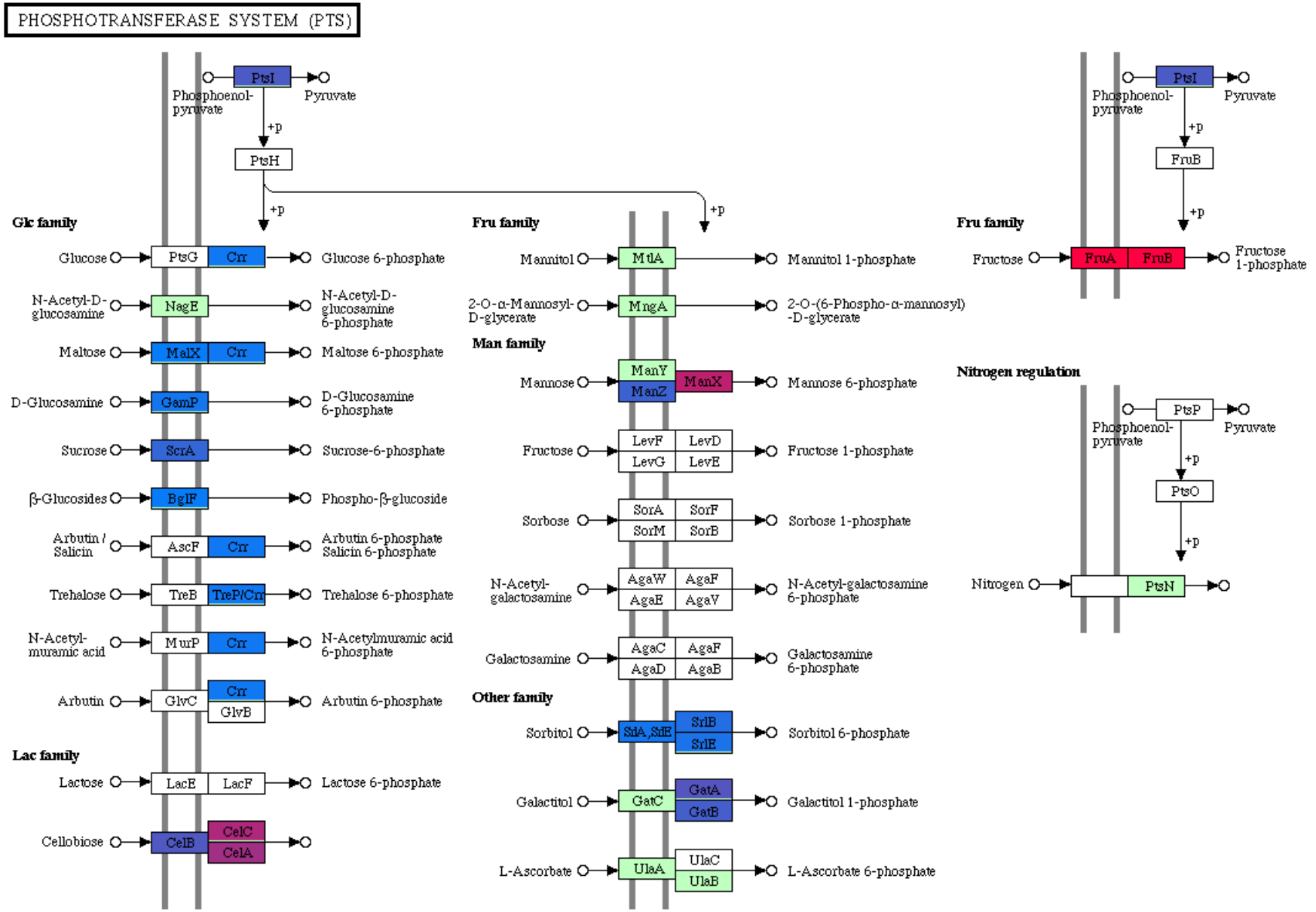


PTS system

- Involved in transport of sugars
- Glucose transport was significantly down-regulated
- Fructose and lactose transport was significantly up-regulated



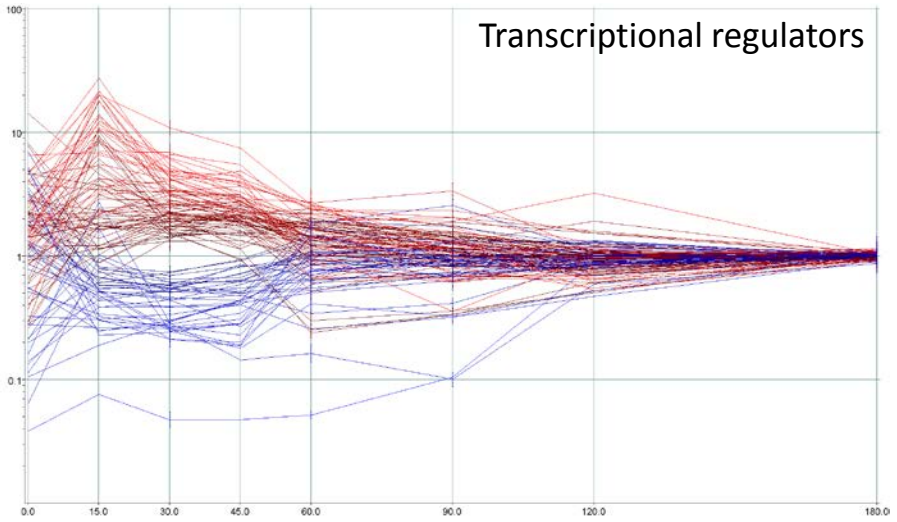
5.0
4.0
3.0
2.5
2.0
1.5
1.2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0


PHOSPHOTRANSFERASE SYSTEM (PTS)
PtsI
Phosphoenol-pyruvate
Pyruvate
+p
PtsH
+p
Glc family
Glucose
PtsG
Crr
Glucose 6-phosphate
N-Acetyl-D-glucosamine
NagE
N-Acetyl-D-glucosamine 6-phosphate
Maltose
MalX
Crr
Maltose 6-phosphate
D-Glucosamine
GamP
D-Glucosamine 6-phosphate
Sucrose
ScrA
Sucrose-6-phosphate
β-Glucosides
BglF
Phospho-β-glucoside
Arbutin / Salicin
AscF
Crr
Arbutin 6-phosphate
Salicin 6-phosphate
Trehalose
TreB
TreP/Crr
Trehalose 6-phosphate
N-Acetyl-muramic acid
MurP
Crr
N-Acetylmuramic acid 6-phosphate
Arbutin
GlvC
Crr
GlvB
Arbutin 6-phosphate
Lac family
Lactose
LacE
LacF
Lactose 6-phosphate
Cellobiose
CelB
CelC
CelA
Fru family
Mannitol
MtlA
Mannitol 1-phosphate
2-O-α-Mannosyl-D-glycerate
MngA
2-O-(6-Phospho-α-mannosyl)-D-glycerate
Man family
Mannose
ManY
ManZ
ManX
Mannose 6-phosphate
Fructose
LevF
LevG
LevD
LevE
Fructose 1-phosphate
Sorbose
SorA
SorM
SorF
SorB
Sorbose 1-phosphate
N-Acetyl-galactosamine
AgaW
AgaE
AgaF
AgaV
N-Acetyl-galactosamine 6-phosphate
Galactosamine
AgaC
AgaD
AgaF
AgaB
Galactosamine 6-phosphate
Other family
Sorbitol
SldA,SdE
SrlB
SrlE
Sorbitol 6-phosphate
Galactitol
GatC
GatA
GatB
Galactitol 1-phosphate
L-Ascorbate
UlaA
UlaC
UlaB
L-Ascorbate 6-phosphate
Fru family
PtsI
Phosphoenol-pyruvate
Pyruvate
+p
FruB
+p
Fructose
FruA
FruB
Fructose 1-phosphate
Nitrogen regulation
PtsP
Phosphoenol-pyruvate
Pyruvate
+p
PtsO
+p
Nitrogen
PtsN
5.0
4.0
3.0
2.5
2.0
1.5
1.2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

Transcription and translation

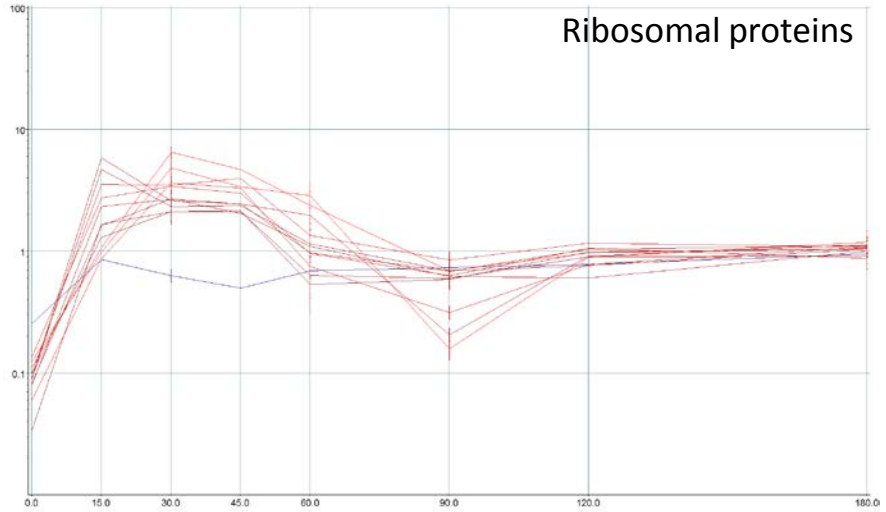
Transcriptional regulators

- 77 up-regulated
- 39 down-regulated
- members of the GntR, TetR AraC, MarR, MerR, RpiR, CopR, DeoR and LysR gene families



Ribosomal proteins

- 3 30S genes up-regulated
- 9 50S genes up-regulated



Protein secretion

- *secAYEG* up-regulated
- Lipoprotein export up-regulated

Amino acid biogenesis

- Down-regulated

5.0
4.0
3.0
2.5
2.0
1.5
1.2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

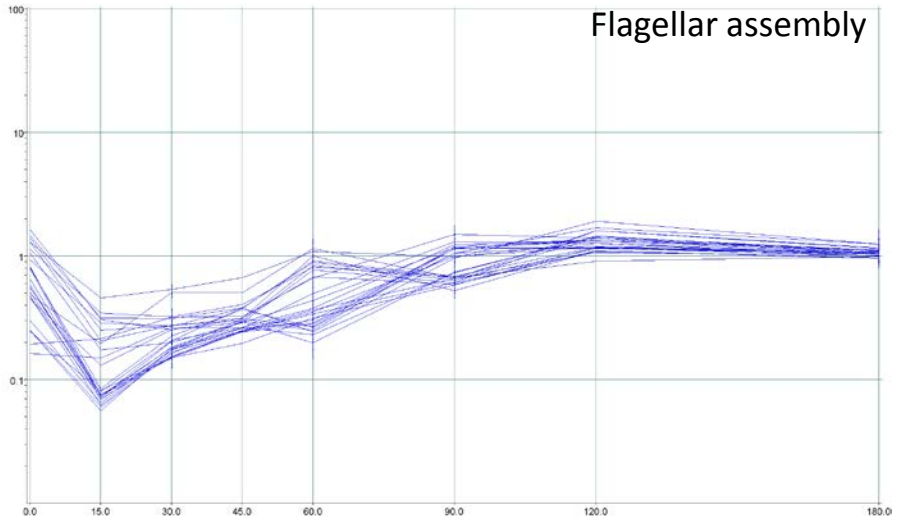
Motility

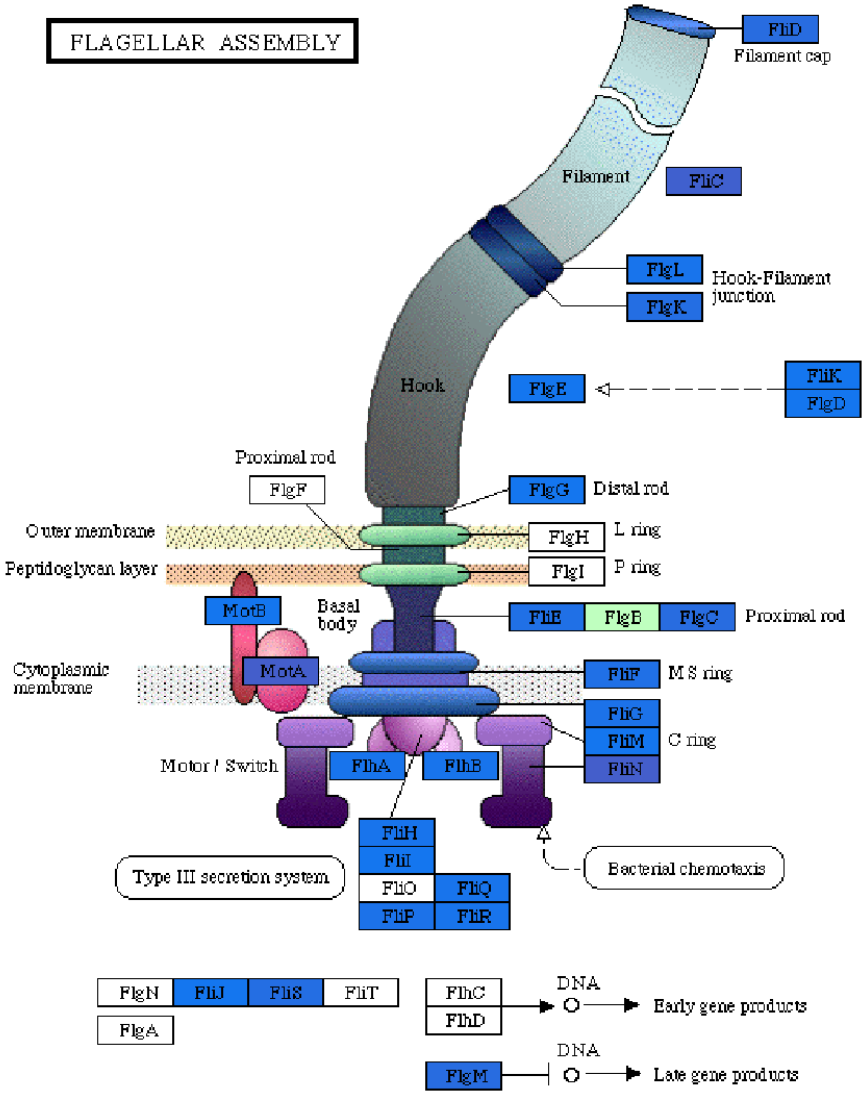
Flagellar assembly

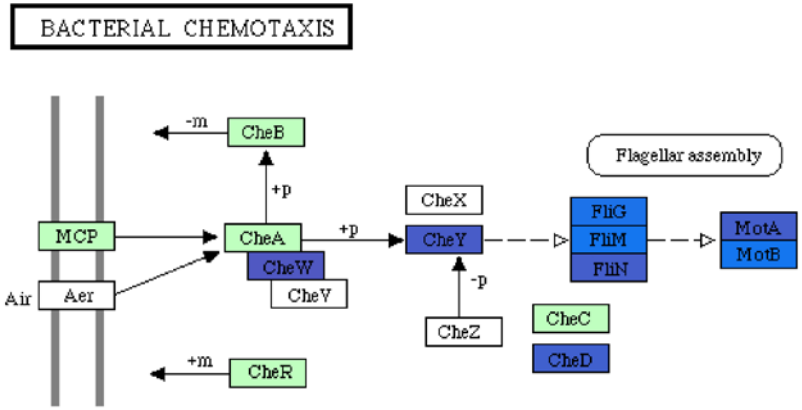
- Encoded by 35 genes
- 26 genes down-regulated

Chemotaxis

- Controlled by the *che* operon
- 3 genes down-regulated




FLAGELLAR ASSEMBLY
FliD
Filament cap
Filament
FliC
FlgL
Hook-Filament junction
FlgK
Hook
FlgE
FliK
FlgD
Proximal rod
FlgF
FlgG
Distal rod
Outer membrane
FlgH
L ring
Peptidoglycan layer
FlgI
P ring
MotB
Basal body
FlgB
FlgC
Proximal rod
Cytoplasmic membrane
MotA
FliF
M S ring
FliG
FliM
FliN
C ring
Motor / Switch
FlhA
FlhB
FliH
FliI
FliO
FliQ
FliP
FliR
Type III secretion system
Bacterial chemotaxis
FlgN
FliJ
FliS
FliT
FlgA
FlhC
FlhD
DNA
Early gene products
FlgM
DNA
Late gene products


BACTERIAL CHEMOTAXIS
-m
CheB
+p
MCP
CheA
+p
CheX
CheY
Flagellar assembly
FliG
FliM
FliN
MotA
MotB
CheW
CheV
Air
Aer
-p
CheZ
CheC
CheD
+m
CheR

Motility

Flagellar assembly

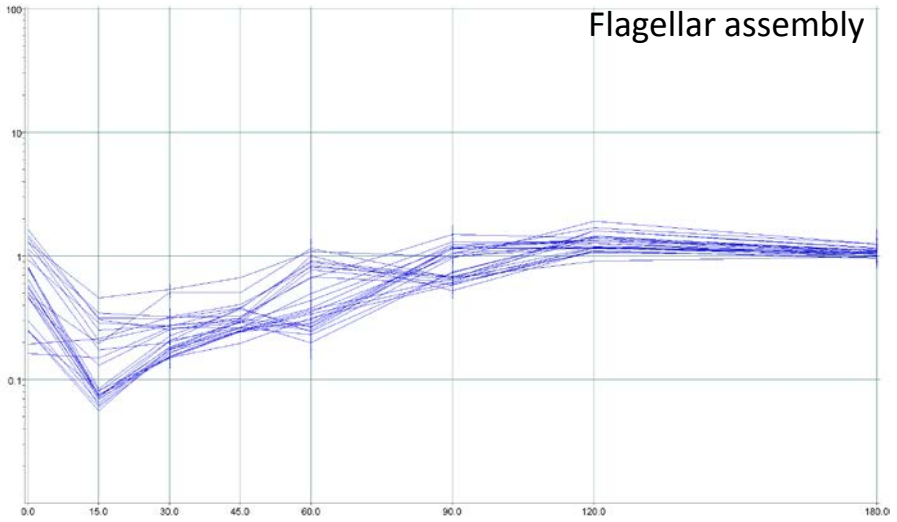
- Encoded by 35 genes
- 26 genes down-regulated

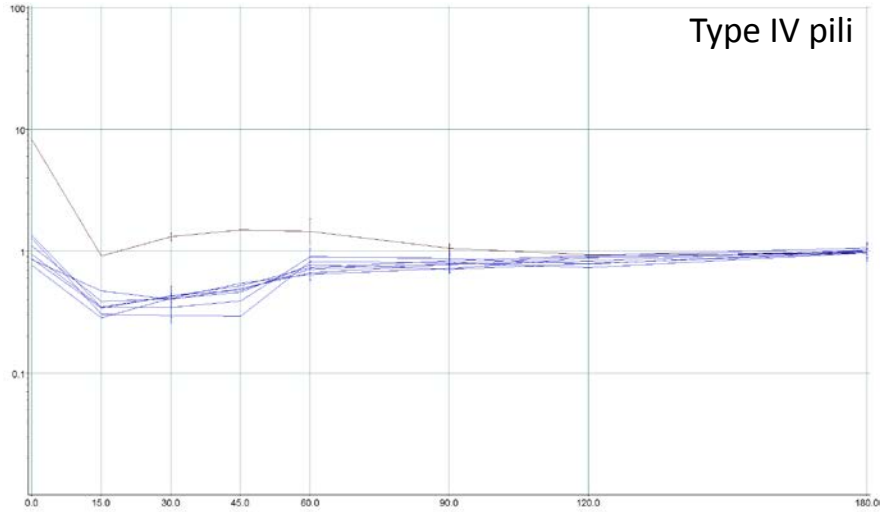
Chemotaxis

- Controlled by the *che* operon
- 3 genes down-regulated

Type IV pili

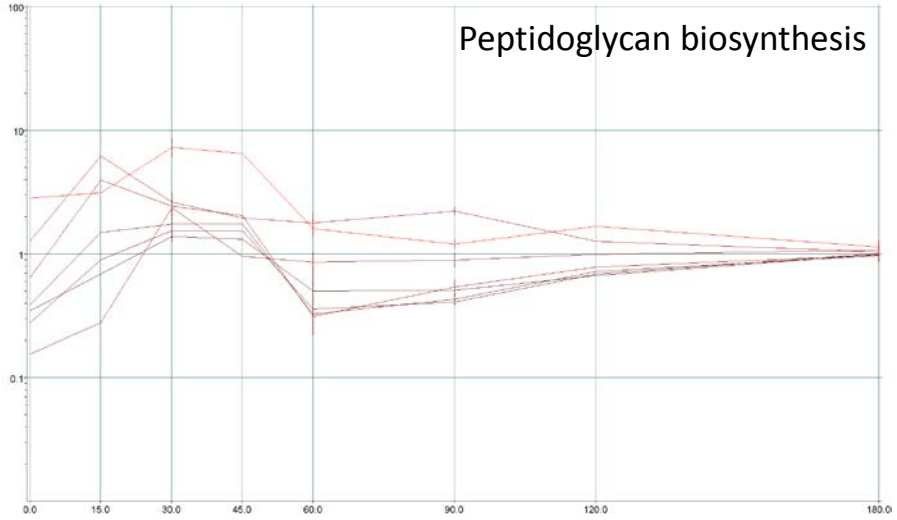
- Encoded by two gene clusters
- 9 genes down-regulated

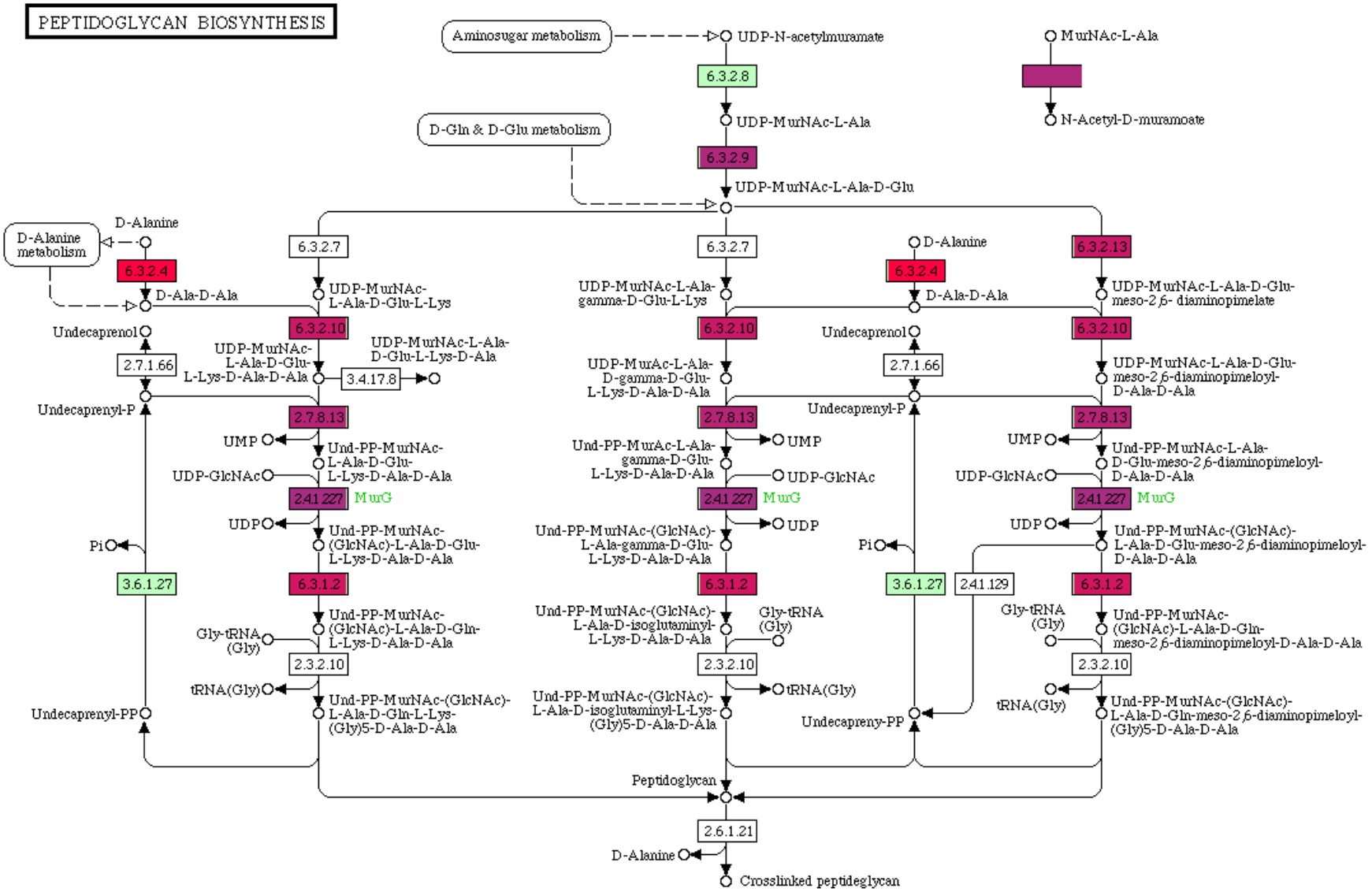




Peptidoglycan biosynthesis

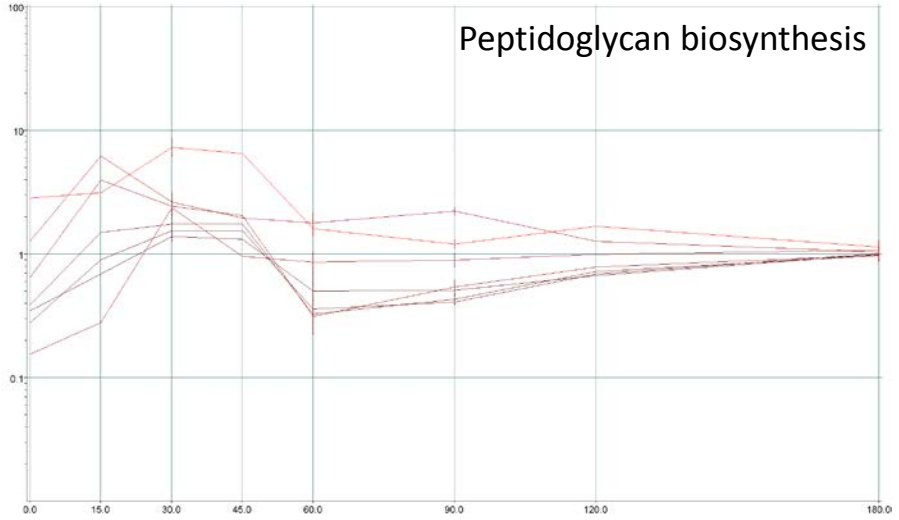
- Encoded by the *mur-mra* cluster
- 12 genes up-regulated




PEPTIDOGLYCAN BIOSYNTHESIS
Aminosugar metabolism
UDP-N-acetylmuramate
MurNAc-L-Ala
6.3.2.8
N-Acetyl-D-muramoate
UDP-MurNAc-L-Ala
D-Gln & D-Glu metabolism
6.3.2.9
UDP-MurNAc-L-Ala-D-Glu
D-Alanine
D-Alanine metabolism
6.3.2.7
6.3.2.4
6.3.2.13
D-Ala-D-Ala
UDP-MurNAc-L-Ala-gamma-D-Glu-L-Lys
UDP-MurNAc-L-Ala-D-Glu-meso-2,6- diaminopimelate
6.3.2.10
Undecaprenol
2.7.1.66
UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala
UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala
3.4.17.8
UDP-MurAc-L-Ala-D-gamma-D-Glu-L-Lys-D-Ala-D-Ala
UDP-MurNAc-L-Ala-D-Glu-meso-2,6-diaminopimeloyl-D-Ala-D-Ala
Undecaprenyl-P
2.7.8.13
UMP
Und-PP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala
Und-PP-MurAc-L-Ala-gamma-D-Glu-L-Lys-D-Ala-D-Ala
Und-PP-MurNAc-L-Ala-D-Glu-meso-2,6-diaminopimeloyl-D-Ala-D-Ala
UDP-GlcNAc
2.4.1.227
MurG
UDP
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala
Und-PP-MurNAc-(GlcNAc)-L-Ala-gamma-D-Glu-L-Lys-D-Ala-D-Ala
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-Glu-meso-2,6-diaminopimeloyl-D-Ala-D-Ala
Pi
3.6.1.27
2.4.1.129
6.3.1.2
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-Gln-L-Lys-D-Ala-D-Ala
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-isoglutaminyl-L-Lys-D-Ala-D-Ala
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-Gln-meso-2,6-diaminopimeloyl-D-Ala-D-Ala
Gly-tRNA (Gly)
2.3.2.10
tRNA(Gly)
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-Gln-L-Lys-(Gly)5-D-Ala-D-Ala
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-isoglutaminyl-L-Lys-(Gly)5-D-Ala-D-Ala
Und-PP-MurNAc-(GlcNAc)-L-Ala-D-Gln-meso-2,6-diaminopimeloyl-(Gly)5-D-Ala-D-Ala
Undecaprenyl-PP
Undecapreny-PP
Peptidoglycan
2.6.1.21
D-Alanine
Crosslinked peptideglycan

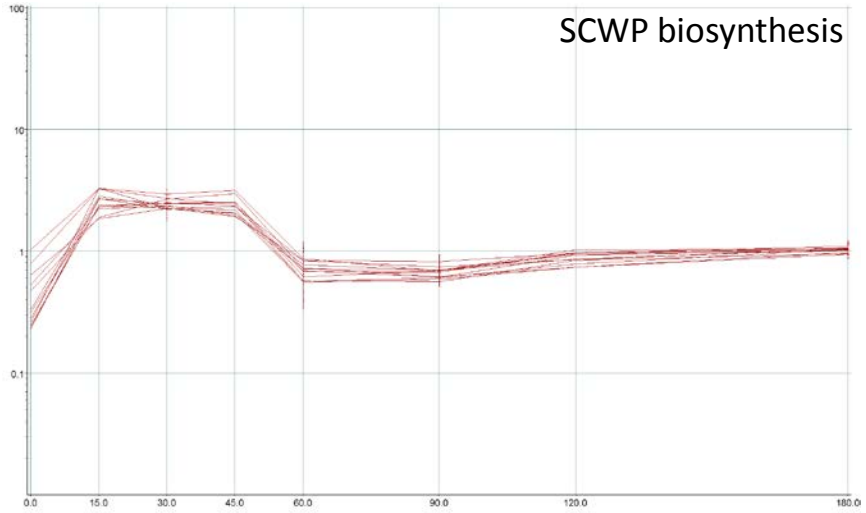
Peptidoglycan biosynthesis

- Encoded by the *mur-mra* cluster
- 12 genes up-regulated

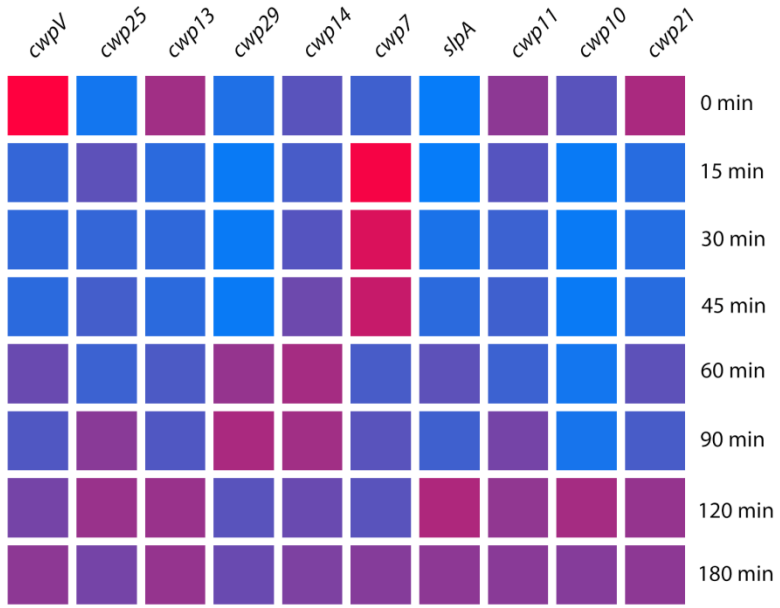


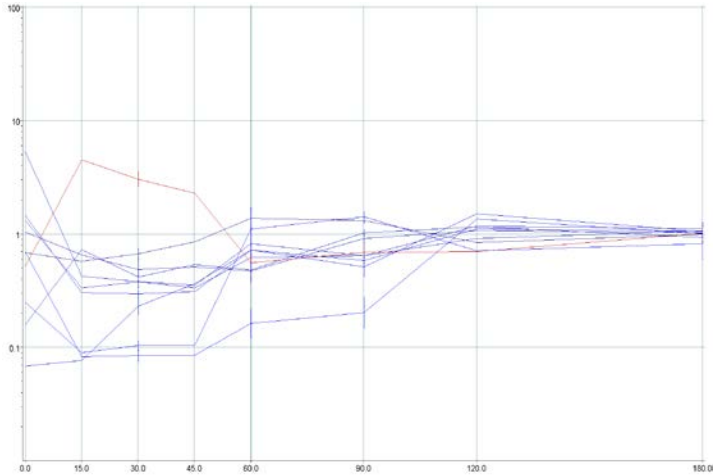
Secondary Cell Wall Polymers (SCWP)

- Encoded by a putative SCWP biogenesis locus (CD2769-80)
- Involved in maintaining cell wall rigidity
- 12 genes up-regulated

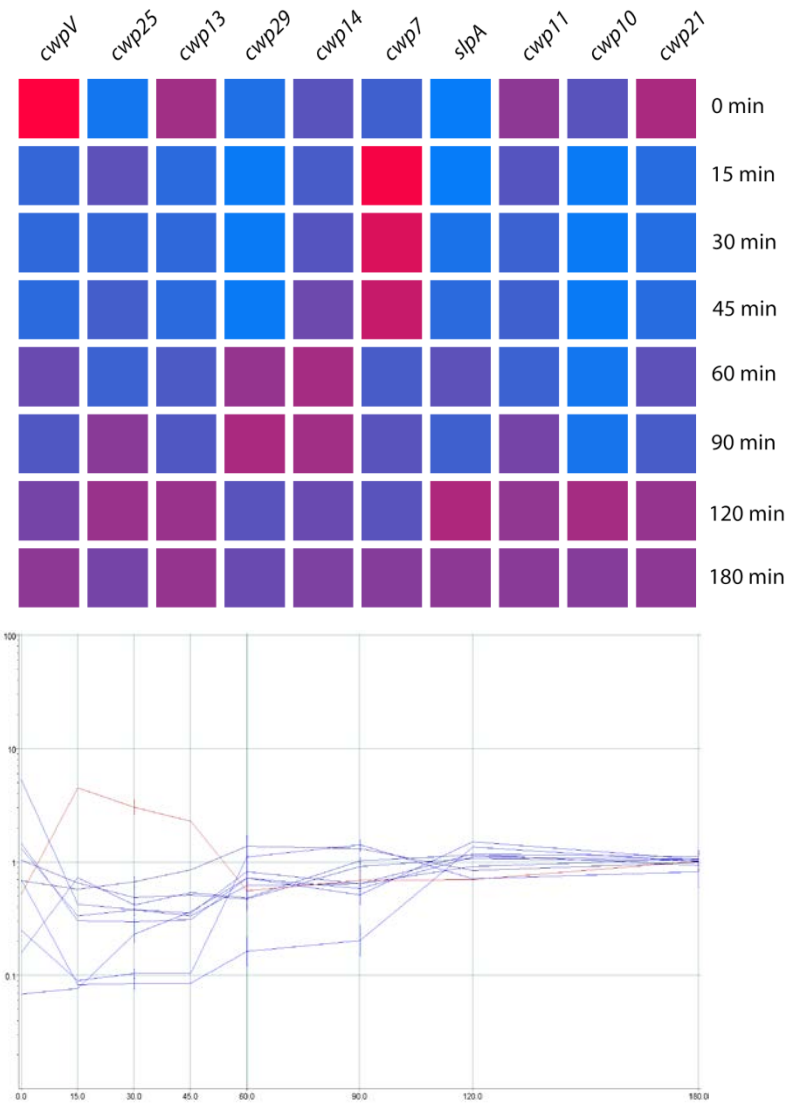


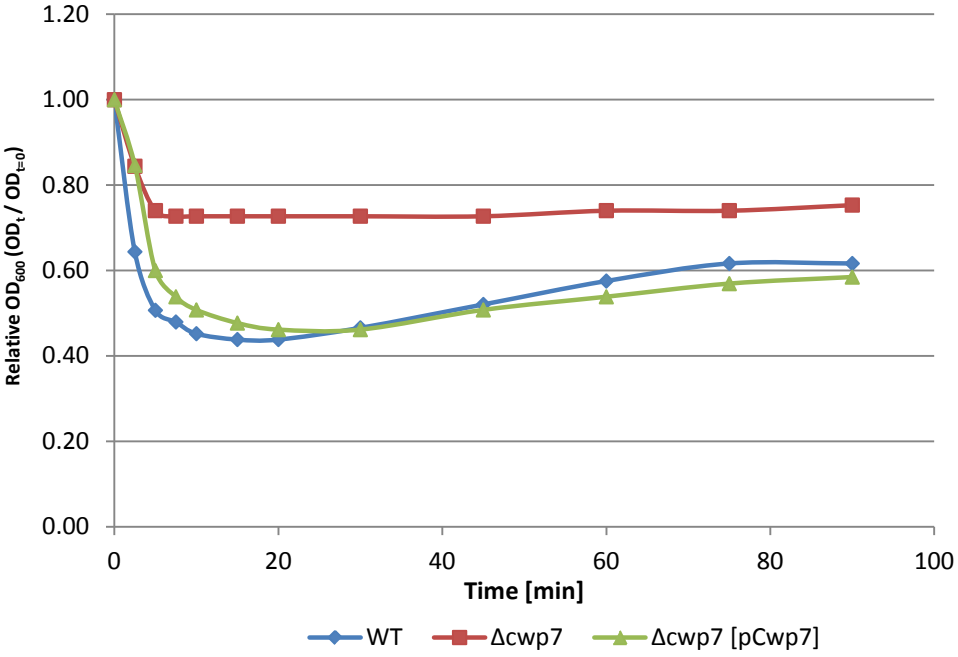
Proof of principle





Proof of principle





Germination dynamics

- *cwp7* was disrupted using ClosTron
- Δcwp7 mutant is germination deficient
- This is alleviated upon complementation

Summary and Further work

- Transcriptional analysis of gene expression in germination was performed
- 517 genes were identified as significantly up- or down-regulated during germination ($p \leq 0.01$)
- Genes involved in transport, transcription/translation and cell wall biogenesis were highly up-regulated
- Genes involved in motility were down-regulated

- Further validation of microarray data
- ‘Proof-of-principle’ experiments
- ClosTron/antisense RNA
- Animal experiments

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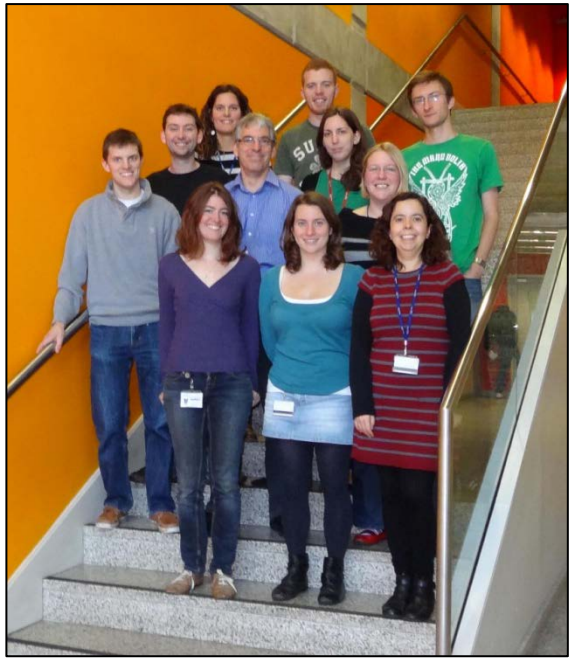

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