Campylobacter jejuni

PHYSIOLOGY & GENOME

Gwennola ERMEL
**Physiology of Campylobacter jejuni**

- Campylobacter: leading cause of bacterial foodborne diarrhoeal disease
- Annual incidence ≈ 1/2000 inhabitants

**Genus Campylobacter:** 15 species

- C. jejuni
- C. coli

95% of Campylobacter infections

Most of foodborne bacterial pathogens: robust organisms…?

Necessity to survive to inimical conditions

- But *C. jejuni* possess fastidious growth requirements
- *C. jejuni* appears to lack many of the adaptive responses

From Friedman et al., 2004
Table 1
The distribution of key orthologues from pathways responsible for resistance to environmental stress in *C. jejuni* and model bacterial species

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Presence in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>C. jejuni</em></td>
</tr>
<tr>
<td><strong>Absence of different orthologues involved in stress response</strong></td>
<td></td>
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<tr>
<td>Oxidative stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SoxRS</td>
<td>Positive regulators of the response to superoxide stress</td>
<td>–</td>
</tr>
<tr>
<td>OxyR</td>
<td>Positive regulator of the response to peroxide stress</td>
<td>–</td>
</tr>
<tr>
<td>PerR</td>
<td>Negative regulator of the response to peroxide stress</td>
<td>+</td>
</tr>
<tr>
<td>SodB or SodF</td>
<td>Iron cofactored superoxide dismutase</td>
<td>+</td>
</tr>
<tr>
<td>SodA</td>
<td>Manganese cofactored superoxide dismutase</td>
<td>–</td>
</tr>
<tr>
<td>KatA or KatE</td>
<td>HPI, catalase</td>
<td>+</td>
</tr>
<tr>
<td>KatG</td>
<td>HPI, catalase</td>
<td>–</td>
</tr>
<tr>
<td>AhpC</td>
<td>Alkyl hydroperoxide reductase</td>
<td>+</td>
</tr>
<tr>
<td>Osmoregulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ProP</td>
<td>Low-affinity uptake of proline/glycine betaine</td>
<td>+</td>
</tr>
<tr>
<td>ProU or OpuC</td>
<td>High-affinity osmoregulatory uptake of compatible solutes</td>
<td>–</td>
</tr>
<tr>
<td>OtsAB</td>
<td>Osmoregulatory trehalose synthesis</td>
<td>–</td>
</tr>
<tr>
<td>BetAB or GbsAB</td>
<td>Osmoregulatory choline–glycine betaine synthesis pathway</td>
<td>–</td>
</tr>
<tr>
<td>Stationary phase/starvation</td>
<td>Carbon storage regulator</td>
<td>+</td>
</tr>
<tr>
<td>CsrA</td>
<td>General stress/stationary phase sigma factor in Gram-negative bacteria</td>
<td></td>
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<tr>
<td>RpoS</td>
<td>General stress sigma factor in Gram-positive bacteria</td>
<td>+</td>
</tr>
<tr>
<td>SigB</td>
<td></td>
<td></td>
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<tr>
<td>Heat and cold shock</td>
<td></td>
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<tr>
<td>RpoH</td>
<td>Alternative sigma factor regulating the heat shock response</td>
<td>–</td>
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<tr>
<td>HspR</td>
<td>Negative regulator of the heat shock response</td>
<td>+</td>
</tr>
<tr>
<td>HrcA</td>
<td>Negative regulator of the heat shock response</td>
<td>+</td>
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<tr>
<td>GroELS, DnaJ, DnaK and Lon</td>
<td>Heat shock proteins</td>
<td>+</td>
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<tr>
<td>CspA</td>
<td>Major cold shock protein</td>
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<tr>
<td>Quorum sensing</td>
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<td>LuxI</td>
<td>Homoserine lactone synthesis</td>
<td>–</td>
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<tr>
<td>LuxS</td>
<td>Autoinducer 2 synthesis protein</td>
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<tr>
<td>ComQX</td>
<td>Peptide pheromone synthesis</td>
<td>–</td>
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<tr>
<td>PhrC</td>
<td>CSF, extracellular signalling pentapeptide synthesis</td>
<td>–</td>
</tr>
<tr>
<td>Global regulation</td>
<td></td>
<td></td>
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<tr>
<td>Lmp</td>
<td>Global regulator of metabolism</td>
<td>–</td>
</tr>
</tbody>
</table>
| Crp/Fnr                  | Catabolite gene activator or anaerobic regulatory protein                | +            | +         | +            | Park, 2002
Differences between growth requirements and limitations of various environments

- **C. jejuni** is considered as microaerophilic (5% oxygen)
  - unable to grow in normal atmosphere (20% oxygen)

- Optimal temperature of growth: 42°C
- Does not multiply at temperature below 30°C
  - no ability of *C. jejuni* to multiply outside animal or human hosts
  - no growth in food, in external environments (soil, water…)

*C. jejuni* is also susceptible to other environmental conditions

- **Drying** - sensitivity to desiccation
  - no survival on dry surfaces

- **Osmotic stress** - inability to grow in [NaCl] $\geq$ 2%
  - (Salmonella and *L. monocytogenes* : 4,5% and 10% respectively)

- **Low pH** - no growth below pH 4,9 and death at pH values less than this

- **Temperature** - Sensitivity to either cold or high temperatures
Response to low temperatures (1)

- Inability to grow below 30°C
- Survival is greater at low T°C
- Anaerobic conditions enhance survival
- No cold shock proteins

- Metabolic activity at low T°C
  - Oxygen consumption
  - ATP generation
  - Catalase activity
  - Protein synthesis

- Increase of carbohydrates amount
  *due to Osmoregulated Periplasmic Glycans*

- Half-lives of mRNA are enhanced
  *"How?" is still the question*
Response to low temperatures (2)

The case of resistance to "freeze-thaw" stress

- ice nucleation
- dehydratation
- oxidative damages

relevant role of SodB and KatA

The strategy may be:
- saving and/or producing energy
- accumulation of protecting molecules
- modification of the cell wall components
- resistance to oxidative stress
\( \Rightarrow \) Heat shock response (1)

- *C. jejuni* is more heat sensitive than most bacteria
- Variations between strains: \( 0.75 \text{ min} \leq D_{55^\circ C} \leq 3.4 \text{ min} \)
- No real denaturation of DNA or RNA molecules at this \( T^\circ C \)
- Cell death could be due to heat denaturation of parts of ribosome and RNA polymerase
- Induction of the synthesis of Heat Shock Proteins:

Chaperones

Chaperonins

![Chaperonins](image)

![Chaperones](image)

Heat shock response (1):
- DnaJ
- Dnaj
- ATP
- ADP
- GrpE
- ATP
- ADP
- Pi
- Dnaj
- ADP
- ADP
- D
- N

Chaperonins: non specific chaperones
Heat shock response (2)

ATP dependent proteases

- ClpA or ClpX: ATP binding unit
  chaperone: recognition of substrates
- ClpS: binds to ClpA N-term
  stimulation ClpA-recognition of aggregates
  inhibition of degradation of unaggregated proteins (including ClpA)
- ClpP: serine protease (axial pore)
- ClpB: ATPase associated with a variety of activities (DnaK/DnaJ/GrpE)

Complexes: ClpXP, ClpAP, ClpAXP

- Lon: ATP-dependent protease
  independent protein-binding domain in addition to its proteolytic domain
  can bind denatured DNA, stimulates substrate proteolysis in vitro, as well as stimulating ATPase activity
Heat shock response (3)

- Up-regulation of genes encoding proteins involved in:
  - energy metabolism
  - cell wall and envelope constituents (MOMP)
  - transport

- Down-regulation of proteins involved in synthesis and modification of macromolecules

- Repression of ribosomal genes

⇒ differential surface structure pattern (between 37 °C and 42 °C)
⇒ brief growth arrest to reshuffle energy for repairing T °C-damages
Heat shock response (4)

- What about the regulation?
  - No RpoH (σ32 or σB) or CstR
  - Negative transcriptional regulators: HrcA → CIRCE sequence
    HspR → HAIR sequence
  - Involvement of TCRS:
    RacRS involved for differential expression of proteins at 37 and 42 °C
    Role of the other one (HKRR)?

Interrelationship between HspR and HrcA

⇒ co-regulation of these two regulators

- The HspR has also a role of activator
  on certain genes involved in motility (flagellar components)
  and transport of proline

*C. jejuni* has established sophisticated regulatory networks in order to fine-tune heat shock gene expression
Stationary phase and Starvation (1)

- Physiological modifications
  - Change in the fatty acid composition of the membrane
  - Existence of extracellular protective compound?
  - Emergence of variants displaying enhanced survival in stationary phase
    (inconstant numbers of viable cells during stationary phase)

⇒ Existence of GASP (growth advantage in stationary phase) mutants
is not clearly demonstrated
BUT these phenotypic changes are stable
Stationary phase and Starvation (2)

- Metabolic capabilities of *C. jejuni*

  - *C. jejuni* is unable to use glucose
  - Oxidation of formate, lactate, cysteine, serine, (fumarate, succinate…)
  - Complete TCA cycle → compounds and intermediates that feed into electron transport
  - Except phosphofructokinase, all enzymes are present for glycolysis
  - Gluconeogenesis seems to be possible
  - Major pathways for synthesis and metabolism of amino-acids are present
Stationary phase and Starvation (3)

- **Respiratory chain of *C. jejuni***

  - *C. jejuni* is a microaerophilic bacteria
    Use of oxygen as a terminal acceptor
  
  - Components of aerobic respiratory chain are present
    dehydrogenases
cytochrome bc1 complex
terminal oxidases

  - Anaerobic respiration is possible: alternative electron acceptors (fumarate, nitrate, hydrogen, formate…)

*C. jejuni* possesses highly branched and complex respiratory chain

  flexibility with respect to different electron donors and acceptors

Dimroth *et al.*, 2006
Stationary phase and Starvation (4)

• The stringent response

Role of (p)ppGpp

- repression of stable RNAs (tRNA & rRNA)
- induction of genes coding enzymes involved in aa biosynthesis
- enhances survival of bacteria (and virulence)
- binds to RNA polymerase
- Competition for σ factors,

(p)ppGpp is involved in regulation of expression (interaction with RpoS in E. coli)

Accumulation of (p)ppGpp when uncharged tRNA blocks translation

Fig. 2. Representation of the mechanism of action of Rel or RelA and SpoT. An actively translating ribosome stalls upon entry of an uncharged tRNA (shown in red), which signals the Rel or RelA to bind to ribosome, causing the synthesis of (p)ppGpp. RelA or Rel can then transfer from this ribosome to another stalled ribosome. Conversely, SpoT causes the degradation of (p)ppGpp when it is not required.

Jain et al., 2006

In *C. jejuni*, existence of a stringent response required for several stress and survival in stationary phase and under low dioxide/high oxygen levels
Stationary phase and Starvation (5)

- **Regulation?**
  - No RpoS in *C. jejuni*
  - Role of another regulatory system: the csr system which copes with other regulators?

- This is a family of global regulator proteins.
- CsrA is protein is a RNA-binding protein and a global regulator of carbohydrate metabolism genes facilitating mRNA decay
- In *E. coli* CsrA binds the CsrB RNA molecule to form the Csr regulatory system
- This system has a strong negative regulatory effect on glycogen biosynthesis, glyconeogenesis and glycogen catabolism and a positive regulatory effect on glycolysis
**Osmoadaptation**

- *C. jejuni* is sensitive to [NaCl]
- Primary answer to osmotic up-shock:
  - **[K+] and [glutamate] in *E. coli* due to the presence of low- or high-affinity K+ transport systems**
  - *C. jejuni* possesses at least one low-affinity transporter
- Secondary response: accumulation of neutral osmoprotectants (glycine betaine, carnitine, proline)
- No specific systems have been found in *C. jejuni*
- Hyposomotic shock induces massive influx of water in the bacterial cell
  - *C. jejuni* possesses one homologue of a stretch activated channel (MscL & MscS)
  - No aquaporin involved in water flux in *C. jejuni*

How does *C. jejuni* faces low or high osmolarity is not completely established
Oxidative stress (1)

The effect of oxidative stress on bacterial cells

- spontaneous dismutation of dioxygen

\[ \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

- \( \text{H}_2\text{O}_2 \) is a weak oxidizing agent but forms hydroxyl radicals (OH°) via the Fenton reaction => role of iron

- superoxide anions (\( \text{O}_2^- \))
- hydrogen peroxide (\( \text{H}_2\text{O}_2 \))
- Hydroxyl radicals (OH°)

ROS
(Reactive Oxygen Species)

Highly reactive

Proteins
DNA
RNA
Lipids (Mb)

damages

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Oxidative stress (2)

• Effect on proteins
  - Proteins with metal ions-active sites are susceptible to hydroxyl or ferryl [2Fe²⁺O₂]° radicals
  - Hydroxyl radicals involved in the formation of carbonyl residues from amino-acid residue (lysine, proline, arginine, cysteine…)
  - Enzymes containing [4Fe-4S] cluster are inhibited by superoxyde anions

• Effect on Lipids
  - Peroxidation of polyunsaturated fatty acids

Initiation
LH + OH° → L° + H₂O

Propagation
L° + O₂ → LOO°
LOO° + LH → LOOH + L°

Hydroperoxide decomposition
LOOH → LO° → Malondialdehyde

reaction on phospholipids, nucleic acids and proteins

Highly reactive
Oxidative stress (3)

- **Effect on nucleic acids**
  
  $(H_2O_2)$ and $(O_2^-)$ link to polyanionic structures such as DNA or RNA

  - on sugar
  - on base moieties

  Mutagenesis caused by derived bases (*i.e.* 8oxo-guanine)

- DNA oxidation can also lead to the formation of covalent adducts to bases, and the lysine-rich motifs that bind DNA provide abundant nucleophiles for cross-linking.

- Adducts to DNA formed from oxidation of redox active phenols
**Oxidative stress (4)**

- **Response to oxidative stress**
  - **Detoxification**
    - Superoxide stress: role of superoxide dismutase
      
      \[
      2H^+ + 2O_2^- \rightarrow H_2O_2 + O_2
      \]

      *In C. jejuni:* only Fe-SOD: SodB

      The increase of activity of SOD => increase of \([H_2O_2]\)

    - Peroxide stress: role of catalase
      
      *In C. jejuni:* single heme-cofactured catalase: KatA
      *In H. pylori:* the catalase is both periplasmic and cytoplasmic

    - Role of alkyl hydroxyperoxide reductase
      
      \[
      \text{hydroxypreoxide} \xrightarrow{\text{AhpC-F}} \text{alcohols}
      \]

      *In C. jejuni:* AphC (catalytic unit) is present, no AhpF (recycling unit)

      *In C. jejuni,* recycling would be done by other proteins:
      Thioredoxin reductases (TrxA & TrxB homologues) and/or Ferredoxin FdxA

    - Role of thiol peroxidases (*Tpx & Bcp in C. jejuni*)
Oxidative stress (5)

- Importance of iron homeostasis
  Expression of elements involved in the storage of iron: bacterioferritins (Cft)
  Expression of KatA and AphC are affected by iron concentration

- DNA protection and repair
  ~ Dps (DNA-binding protein from starved cells) protects DNA from oxidative stress
  This protein is present in *C. jejuni* (Cj1534c)
  ~ DNA reparation systems (RecA, MutS, UvrABC) are lacking in *C. jejuni*

- Reduction or Elimination of oxidized proteins
  ~ Role of thioredoxin/thioredoxin reductase
  ~ Presence of specific oxidoreductases
  ~ The periplasmic HtrA protease is useful for oxygen tolerance but seems playing no role in response to oxidative inducers (the *C. jejuni htrA* mutant growth was not reduced by peroxide or superoxide, Brondstedt *et al.*, 2005).
**Oxidative stress (6)**

- **What about the regulation?**
  - The redox sensors: respond directly to peroxide and superoxide stress

  ~ Thiol-dependent sensors

  Typically, these sensors use cysteine modification to sense redox alterations.

  Activation of OxyR are due to the modifications of OxyR monomers

  The active form is tetrameric

  $\text{H}_2\text{O}_2$, RNS and Gluthathione (GSSG) induce modification of cys199 (in *E. coli*);
  -> cys199-SOH, permitting the disulfide bond with cys 208

  **OxyR is not present in *C. jejuni***
**Oxidative stress (7)**

- Fe–S cluster-based sensors

Oxidation of Fe–S clusters monitor the redox status of cell compartments and the environment to produce appropriate transcriptional responses

These include SoxR, Fnr, aconitase and IcsR

SoxR — a sensor of superoxide and nitric-oxide stress

The E. coli SoxR protein is a homodimer that contains one [2Fe–2S] cluster per subunit

Fnr — a sensor of environmental oxygen

Two domains:
- a carboxy terminal DNA-binding region, which recognizes a specific DNA sequence in target promoters,
- an amino-terminal sensory domain that contains four essential cysteine residues capable of binding either a [4Fe–4S]²⁺ or a [2Fe–2S]²⁺ cluster.

In *C. jejuni*, there is NssR (FNR-like) which respond to nitrosative stress
~ Flavin cofactor-based redox sensors

The flavin cofactors FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide) are versatile, electron-carrying coenzymes involved in both one- and two-electron transfers.

The coenzymes can exist in three states:
- fully oxidized (for example, FAD);
- partially oxidized, as a semiquinone radical (for example, FADH•);
- fully reduced (for example, FADH2).

Transduction of the redox signal to secondary effector.

Aer — a redox sensor involved in aerotaxis present in *C. jejuni*

Aer senses gradients of oxygen (aerotaxis), redox potential and certain nutrients.
Interaction with the CheA–CheW complex to transmit sensory information to the flagellar motors.

In *C. jejuni*, there are 2 homologues: CetB and CetA
Metal dependent sensors present in *C. jejuni*

**PerR — a repressor**
- 2 metal binding sites for Zn$^{2+}$ and Fe$^{2+}$/Mn$^{2+}$
- PerR inactivation requires iron and is blocked by manganese
- PerR senses peroxide stress by metal oxidation of His and Asp residues
- Oxidation of His and Asp induces a change of conformation

**Fur — Ferric uptake regulator**
- senses iron level
- Dimers of Fur bind Fe$^{2+}$
- Modified conformation (Apo-Fur) binds to DNA at Fur boxes
- In *C. jejuni*: Induces KatA, AphC and Cft

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![Diagram of PerR and Fur](Campylobacter%20jejuni%20-%20Physiology%20&%20Genome%20-%20Gwennola%20Ermel%20-%20Jouy%20en%20Josas%20-%2020/06/07)
In *C. jejuni*, Fur & PerR are the main regulators of superoxide and peroxide stress responses

<table>
<thead>
<tr>
<th>Function</th>
<th><em>B. subtilis</em></th>
<th><em>E. coli</em></th>
<th><em>C. jejuni</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyl Hydroperoxide reductase (small subunit)</td>
<td>+ <em>ahpC</em></td>
<td>PerR</td>
<td>+ <em>ahpC</em></td>
</tr>
<tr>
<td>Alkyl Hydroperoxide reductase (large subunit)</td>
<td>+ <em>ahpF</em></td>
<td>PerR</td>
<td>+ <em>ahpF</em></td>
</tr>
<tr>
<td>Catalase</td>
<td>+ <em>katA</em></td>
<td>PerR, σE</td>
<td>+ <em>katG</em></td>
</tr>
<tr>
<td></td>
<td>+ <em>katE</em></td>
<td>σB</td>
<td>+ <em>katE</em></td>
</tr>
<tr>
<td>Kata-associated protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome c peroxidase</td>
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<td></td>
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<tr>
<td>Metalloregulation DNA-binding stress protein</td>
<td>+ <em>mrgA</em></td>
<td>PerR</td>
<td>+ <em>dps</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>+ <em>dps</em></td>
</tr>
<tr>
<td>Ferric Uptake regulator</td>
<td>+ <em>fur</em></td>
<td>+ <em>fur</em></td>
<td>SoxRS, OxyR</td>
</tr>
<tr>
<td>PerR</td>
<td>+ <em>perR</em></td>
<td></td>
<td>+ <em>perR</em></td>
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<tr>
<td>OxyR</td>
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<td>+ <em>oxyR</em></td>
<td>OxyR</td>
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<td>Superoxide radical response R protein</td>
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<td>+ <em>soxR</em></td>
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<tr>
<td>Superoxide radical response S protein</td>
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<td>+ <em>soxS</em></td>
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<td>LexA</td>
<td>+ <em>lexA</em></td>
<td>+ <em>lexA</em></td>
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<tr>
<td>Manganese superoxide dismutase</td>
<td>+ <em>sodA</em></td>
<td>Spx, σB</td>
<td>+ <em>sodA</em></td>
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<td>Iron superoxide dismutase</td>
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<td>+ <em>sodB</em></td>
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<td>Copper-Zinc superoxide dismutase</td>
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<td>+ <em>sodC</em></td>
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<tr>
<td>Multifunctional SOS repair regulator</td>
<td>+ <em>recA</em></td>
<td>RecA/LexA</td>
<td>+ <em>recA</em></td>
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<td>Exonuclease ABC (subunit B)</td>
<td>+ <em>uvrB</em></td>
<td>RecA/LexA</td>
<td>+ <em>uvrB</em></td>
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<td>Methionine sulfoxide reductase</td>
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<td>+ <em>marB</em></td>
<td>+ <em>marB</em></td>
<td>small RNA RyhB</td>
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<td>Thiol peroxidase</td>
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<td>Spx</td>
<td>+ <em>tpx</em></td>
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<tr>
<td>Thioredoxin</td>
<td>+ <em>trxA</em></td>
<td>Spx, σB</td>
<td>+ <em>trxG</em></td>
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<td></td>
<td></td>
<td></td>
<td>+ <em>trxA</em></td>
</tr>
<tr>
<td>Thioredoxin reductase</td>
<td>+ <em>ycgT</em></td>
<td>Fur, Spx</td>
<td>+ <em>trxB</em></td>
</tr>
</tbody>
</table>
Role of other global regulators?

- Csr system is involved in adaptation to oxidative stress in *H. pylori*
- Sigma factors RpoN (σ54) and FliA (σ28)
- RacR/RacS controls also the expression of the cytochrome c peroxidase

*C. jejuni* more sensitive to oxygen and hydrogen peroxide than *E. coli* or *B. subtilis*

BUT *C. jejuni* • possesses all the systems to detoxify
  • seems to prevent the negative effect of iron and ROS by protecting
  • does not have significant repair systems for oxidative damages
From 2000:

- 3 available complete genomic sequences of *C. jejuni*
  
<table>
<thead>
<tr>
<th>GC %</th>
<th>Size (kb)</th>
<th>ORFs</th>
<th>Accession N°</th>
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<td>30</td>
<td>1616</td>
<td>1706</td>
<td>NC_008787</td>
</tr>
</tbody>
</table>

- 5 sequences under progress
- 8-10 sequences of related species

Information from the NCTC11168 genome

- 1654 predicted coding sequences (CDS)
  (20 pseudogenes)
- 54 stable RNA species

- Study of the GC bias
- Replication origin near the *dnaA* gene
- 2 large regions with low GC% (≈25%)

lipooligosaccharides (LOS)
Extracellular polysaccharides (EP)
• Evident strand bias  61% of CDS are transcribed in the same direction as replication

• No organisation in functional clusters except for LOS & EP clusters
  ribosomal protein operons
  flagellar modification genes

Genes are organised in large closed sets and appear to be unfunctionally related
=> suggest existence of co-transcription processes

Absence of functional operons is remarkable for genes involved in amino acids biosynthesis

• Lack of repetitive sequences: only 4 repeated sequences including the rRNA operon

• No evidence of functional insertion sequence (IS)
Presence of hypervariable sequences: homopolymeric runs of nucleotides

Variation present in the shotgun sequences.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Gene(s) affected</th>
<th>Putative function</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>G(8-10) Cj0031/Cj0032</td>
<td>probable restriction/modification enzyme</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>C(9-11) Cj0045c</td>
<td>hemerythin-like putative iron-binding protein</td>
<td>extension and overlap</td>
<td></td>
</tr>
<tr>
<td>G(9-13) Cj0046</td>
<td>pseudogene (transport protein)</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>G(9,11) Cj0170/Cj0171</td>
<td>unknown, similar to Cj1325/Cj1326</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>T(4-5) Cj0629/Cj0629</td>
<td>lipoprotein</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>G(9-10) Cj0685c</td>
<td>possible sugar transferase</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>G(10-11) non coding</td>
<td>upstream of rRNA</td>
<td>none apparent</td>
<td></td>
</tr>
<tr>
<td>C(8-9) Cj1139c</td>
<td>galactosyltransferase</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(8-9) Cj1144c/Cj1145c</td>
<td>unknown</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1305c</td>
<td>unknown, 617 family</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1306c</td>
<td>unknown, 617 family</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(10-11) Cj1318</td>
<td>unknown, 1318 family</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>G(10-11) upstream of Cj1321</td>
<td>transferase</td>
<td>none apparent (promoter?)</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1325/Cj1326</td>
<td>unknown</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1335/Cj1336</td>
<td>unknown, 1318 family</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1342c</td>
<td>unknown, 617 family</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(1-2) Cj1367c</td>
<td>possible nucleotidyltransferase</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1420c</td>
<td>possible methyltransferase</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(8-10) Cj1421c</td>
<td>unknown, similar to putative sugar transferases</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(10-11) Cj1426c</td>
<td>unknown</td>
<td>truncation/extension</td>
<td></td>
</tr>
<tr>
<td>C(9-10) Cj1429c</td>
<td>unknown</td>
<td>truncation/extension</td>
<td></td>
</tr>
</tbody>
</table>

Potentially variable homopolymeric tracts.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Gene(s) affected</th>
<th>Putative function</th>
<th>Potential effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>G(8) Cj0275 (clpX)</td>
<td>Clp protease ATP-binding subunit</td>
<td>truncation</td>
<td></td>
</tr>
<tr>
<td>G(12) Cj0565</td>
<td>Non-coding, upstream of pseudogene</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>G(9) Cj0617/Cj0618</td>
<td>unknown, 617 family</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>G(10) Cj0629/Cj0629</td>
<td>Lipoprotein (adjacent to variable T(4-5) sequence)</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>G(9) Cj0676 (kdpA)</td>
<td>Pseudogene (Potassium transporting ATPase A)</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>G(12) Cj1295</td>
<td>unknown</td>
<td>truncation</td>
<td></td>
</tr>
<tr>
<td>G(12) Cj1296/Cj1297</td>
<td>Weak similarity to aminoglycoside N3'-acyetyltransferase</td>
<td>fusion/separation</td>
<td></td>
</tr>
<tr>
<td>C(9) Cj1437c</td>
<td>aminotransferase</td>
<td>truncation</td>
<td></td>
</tr>
<tr>
<td>T(7) Cj1677/Cj1678</td>
<td>unknown, similar to Cj0628/Cj0629</td>
<td>fusion/separation</td>
<td></td>
</tr>
</tbody>
</table>

- Variations associated with genes involved in surface properties (antigenicity)
- Survival strategy
- Could be favoured by the faint arsenal of DNA repair genes (ada, phr)
  of mismatch repair genes (vsr, mutH, mutL, sbcB)
  and SOS response genes (lexA, umuC, umuD)
- Only 3 predicted sigma factors (*rpoD*, *rpoN*, and *fliA*)
- Broader repertoire of Two Component Regulatory systems which are used commonly by bacteria to respond to specific environmental signals

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cj0019c</td>
<td>MCP-domain signal transduction protein</td>
</tr>
<tr>
<td></td>
<td>Cj0184c</td>
<td>possible serine/threonine protein phosphatase</td>
</tr>
<tr>
<td></td>
<td>Cj0246c</td>
<td>putative MCP-domain signal transduction protein</td>
</tr>
<tr>
<td></td>
<td>Cj0355c</td>
<td>two-component regulator</td>
</tr>
<tr>
<td></td>
<td>Cj0448c</td>
<td>putative MCP-type signal transduction protein</td>
</tr>
<tr>
<td>cbrR</td>
<td>Cj0643</td>
<td>putative two-component response regulator</td>
</tr>
<tr>
<td>cysE</td>
<td>Cj0793</td>
<td>signal transduction histidine kinase</td>
</tr>
<tr>
<td></td>
<td>Cj0889c</td>
<td>putative sensory transduction histidine kinase</td>
</tr>
<tr>
<td></td>
<td>Cj0890c</td>
<td>putative sensory transduction transcriptional regulator</td>
</tr>
<tr>
<td></td>
<td>Cj0951c</td>
<td>putative MCP-domain signal transduction protein</td>
</tr>
<tr>
<td>flgR</td>
<td>Cj1024c</td>
<td>sigma-54 associated transcriptional activator</td>
</tr>
<tr>
<td></td>
<td>Cj1110c</td>
<td>putative MCP-type signal transduction protein</td>
</tr>
<tr>
<td>cetB</td>
<td>Cj1189c</td>
<td>putative signal-transduction sensor protein</td>
</tr>
<tr>
<td>cetA</td>
<td>Cj1190c</td>
<td>bipartate energy taxis response protein cetA</td>
</tr>
<tr>
<td></td>
<td>Cj1191c</td>
<td>putative PAS domain containing signal-transduction sensor protein</td>
</tr>
<tr>
<td>dccS</td>
<td>Cj1222c</td>
<td>putative two-component sensor</td>
</tr>
<tr>
<td>dccR</td>
<td>Cj1223c</td>
<td>putative two-component regulator</td>
</tr>
<tr>
<td></td>
<td>Cj1226c</td>
<td>putative two-component sensor</td>
</tr>
<tr>
<td></td>
<td>Cj1227c</td>
<td>putative two-component regulator</td>
</tr>
<tr>
<td>racR</td>
<td>Cj1261</td>
<td>two-component regulator</td>
</tr>
<tr>
<td>racS</td>
<td>Cj1262</td>
<td>two-component sensor (histidine kinase)</td>
</tr>
<tr>
<td></td>
<td>Cj1491c</td>
<td>putative two-component regulator</td>
</tr>
<tr>
<td></td>
<td>Cj1492c</td>
<td>putative two-component sensor</td>
</tr>
<tr>
<td></td>
<td>Cj1505c</td>
<td>putative two-component response regulator (SirA-like protein)</td>
</tr>
<tr>
<td></td>
<td>Cj1506c</td>
<td>putative MCP-type signal transduction protein</td>
</tr>
<tr>
<td></td>
<td>Cj1608</td>
<td>possible two-component regulator</td>
</tr>
</tbody>
</table>
Comparison between NCTC11168 and RM1221 genomes

• Both genomes are syntenic (*i.e.* same order of genes)

• Exception: RM1221 genome is disrupted by 4 genomic islands named CJIEs
  (*Campylobacter jejuni*-integrated elements)
    - CJIE1: Mu like phage encoding proteins
    - CJIE2 & CJIE4: phage-related endonucleases, methylases or repressors
    - CJIE3: looks like an integrated plasmid

3 elements (CJIE2, CJIE3 & CJIE4) are adjacent tRNA genes

The CJIE1-Mu like element and CJIE3-plasmid like elements have variable genomic insertion points
  => this variability increases the genomic diversity
  => existence of bacteriophage genome dynamics
81-176 genome exhibits unique pathogenic features

- 81-176 carries 2 residents plasmids pVir and pTet
- 81-176 genome is smaller than the 2 other ones
- Presence of vestigial integrated element (type I DNA restriction-modification system)
- Conserved syntenic arrangements between the 3 genomes
- The major differences concern: LOS and capsule biosynthesis glycosylation

- 37 genes located in 11 regions are directly involved in colonization and in virulence
  - additional respiratory functions (=> efficient colonization of chicken?)
  - a DMSO reductase gene considered as important for respiration under oxygen-restricted conditions
  - supplementary cluster of genes encoding cytochrome c biogenesis
  - putative γ-glutamyltranspeptidase (Ggt) which belongs to the anti-oxidant glutathione pathway

=> additional resistance to oxidative stress,
=> important in colonization
=> but no relevant role in internalization of eukaryotic cells and in mice-virulence)
Comparison of genomes through CGH arrays

- 36.6% of the genes in *C. jejuni* NCTC 11168 were variable
  Variable genes are found in clusters

- 16 intraspecies hypervariable genomic regions including
  lipooligosaccharide biosynthesis (L)
  capsular biosynthesis (CP)
  flagellar modification (F)
  DNA restriction-modification systems (RM)

another 17th region (Cj0258-Cj0263) also exists
Gene conservation levels across different different COG groups

<table>
<thead>
<tr>
<th>COG</th>
<th>COG Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>Secondary metabolites biosynthesis, transport and catabolism</td>
</tr>
<tr>
<td>M</td>
<td>Cell envelope biogenesis, Outer membrane</td>
</tr>
<tr>
<td>G</td>
<td>Carbohydrate transport and metabolism</td>
</tr>
<tr>
<td>X</td>
<td>not in COGs</td>
</tr>
<tr>
<td>S</td>
<td>Function unknown</td>
</tr>
<tr>
<td>D</td>
<td>Cell division and chromosome partitioning</td>
</tr>
<tr>
<td>I</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>P</td>
<td>Inorganic ion transport and metabolism</td>
</tr>
<tr>
<td>N</td>
<td>Cell motility and secretion</td>
</tr>
<tr>
<td>K</td>
<td>Transcription</td>
</tr>
<tr>
<td>L</td>
<td>DNA replication, recombination and repair</td>
</tr>
<tr>
<td>H</td>
<td>Coenzyme metabolism</td>
</tr>
<tr>
<td>F</td>
<td>Nucleotide transport and metabolism</td>
</tr>
<tr>
<td>E</td>
<td>Amino acid transport and metabolism</td>
</tr>
<tr>
<td>R</td>
<td>General function prediction only</td>
</tr>
<tr>
<td>C</td>
<td>Energy production and conversion</td>
</tr>
<tr>
<td>O</td>
<td>PTM, protein turnover, chaperones</td>
</tr>
<tr>
<td>T</td>
<td>Signal transduction mechanisms</td>
</tr>
<tr>
<td>J</td>
<td>Translation, ribosomal structure and biogenesis</td>
</tr>
</tbody>
</table>

Taboada et al., 2003

COG: Cluster of Orthologous Genes
Plasmids and their genomes

- Between 19 and 55% of *C. jejuni* strains contain plasmids
- Many of them are R plasmids, transmissible among *Campylobacter* species but not to *E. coli*
- The importance in pathogenicity is still unclear
- 2 "virulence" plasmids *pVir* and *pTet*

- Coding information: 83% (94% coding density)
- Most of the ORFs are transcribed in the clockwise direction of DNA + strand
- %GC: 26%
- 35 ORFs encode predicted proteins with no significant orthologs
  => *C. jejuni* specific ones?
- 8 proteins have orthologs but with unknown functions
- 16 ORFs encode peptides < 11 kDa
- 19 ORFs encode peptides ≈ 12-20 kDa
- 7 clustered genes: proteins similar to Type IV secretion system (T4SS) proteins
- Analysis of mutations permit to show the importance of 16 genes in the invasion process
- BUT *pVir* is insufficient alone for virulence
  very stable and unable to be cured in 81-176
- %GC: 29.8%
- 1 GC-rich region: gene tetO (40.4% GC)

=> horizontal transfer origin

- 10 genes that encode predicted proteins with homology to T4SS multicomponent complexes
- one gene (VirB2- homologue) is the first pilin gene identified in *C. jejuni*
- six genes encodes proteins predicted to form a channel in the cytoplasmic membrane and are near 3 genes encoding ATPases
- Both plasmids encodes 1 putative DNA nickase
  1 helicase
  1 ssDNA-binding protein (Ssb1)

=> translocation of proteins (effectors…)
and/or nucleoproteins (DNA transfert)

- Small cryptic plasmids genomes are also available

  Generally, they carry four ORFs involved in mobilisation and replication
  (Mob-like, RepA-like, RepB-like and a third putative Rep protein)

  They show a 22bp AT-rich region, characteristic of a replication origin