

Identification of Type VI secretion system (T6SS) in Spanish *Campylobacter jejuni* strains

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Background

Campylobacter jejuni is a leading cause of human food-borne enterocolitis worldwide. Despite its microaerobic growth requirements, *C. jejuni* can tolerate different stresses including an aerobic environment. An efficient oxidative stress response is important for survival in suboptimal conditions.

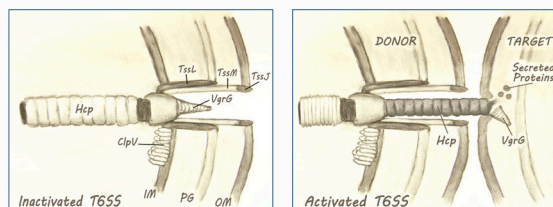
Current studies aim to achieve a better understanding of the colonization potential and pathogenic mechanisms of this species.

In *Pseudomonas aeruginosa*, a link has been described between genes belonging to Type VI secretion system (T6SS) and expression of oxidative stress-response genes (Goldová et al. 2011). *C. jejuni* possesses a T6SS-like gene, *hcp*. We focused on this gene and compared its presence to oxidative stress response expression.

Aim of the study

We evaluated the presence of *hcp* in 50 *C. jejuni* strains (46 from slaughterhouse and 4 water samples). Moreover, we determined the presence of the newly identified genes *Cj1556* & *Cj1546*, which code for transcriptional regulators implied in the oxidative stress response. We also compared their oxidative stress response phenotype.

What does *hcp* do in a Type VI secretion system?



T6SS forms a needle-like structure that plays a pivotal role in environmental adaptations. It can secrete toxic molecules and as such may promote pathogenicity.

T6SS has been described in eg. *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Serratia marcescens* and *Helicobacter hepaticus*.

A T6SS locus typically consists of a cluster of 13 conserved genes, including the *hcp* gene.

Results

Majority of *C. jejuni* strains contain the T6SS *hcp* gene

A high identification rate of T6SS *hcp* gene (74%) was observed in the investigated *C. jejuni* strains from Spain.



Figure 1. Presence of T6SS was deduced from detection (by PCR) of the *hcp* gene (463 bp), shown left. The *gltA* gene (citrate synthase) was used as a positive control (right).

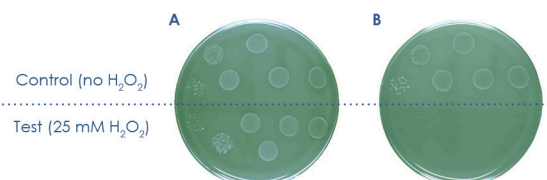
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Strains exhibit a high level of resistance to oxidative stress

Oxidative stress assays were performed using H₂O₂. *C. jejuni* strains were incubated with 25 and 50 mM H₂O₂ for 15 minutes at 37°C and bacterial survival assessed. Of the strains tested, **40% were highly resistant** to oxidative stress (growth at 50mM H₂O₂ > 10⁸ CFU), **38% were resistant** (growth at 50mM < 10⁸ CFU) and **22% were sensitive** (growth 25 mM but not at 50mM).

Figure 2. Plate A shows a resistant strain at 25 mM while B shows a sensitive strain.



Presence of stress-response regulators

The presence of transcriptional regulators Cj1556 and Cj1546 were assessed by PCR for the respective genes. All strains were positive for *Cj1546* (100%) but only 26% possessed *Cj1556*. No correlation between *hcp* and *Cj1556* presence was observed (data not shown).

Correlation of stress-response regulator/*hcp* with stress phenotype

Tables 1 & 2. Correlation between stress-response regulator Cj1556 and oxidative stress-response phenotypes.

	HRes/Res*	Sensitive		Cj1556 +ve	Cj1556 -ve
Cj1556 +ve*	8 (62%)	5 (38%)	HRes/Res	8 (21%)	31 (79%)
Cj1556 -ve*	31 (84%)	6 (16%)	Sensitive	5 (45%)	6 (55%)
Total	39 (78%)	11 (22%)	Total	13 (26%)	37 (74%)

*HRes/Res (highly resistant + resistant); +ve (positive); -ve (negative).

Tables 3 & 4. Correlation between *hcp* presence and oxidative stress-response phenotypes.

	HRes/Res	Sensitive		Cj1556 +ve	Cj1556 -ve
hcp +ve	27 (73%)	10 (27%)	HRes/Res	27 (69%)	12 (31%)
hcp -ve	12 (92%)	1 (8%)	Sensitive	10 (91%)	1 (9%)
Total	39 (78%)	11 (22%)	Total	37 (74%)	13 (26%)

Conclusions & future work

- A high incidence of T6SS, as assessed by presence of *hcp*, was observed in *C. jejuni* strains from Spain. Investigations are ongoing to characterize other components of a potential T6SS in *C. jejuni*.
- The level of resistance to oxidative stress found was surprisingly high in slaughterhouse and water strains.
- Presence of transcriptional regulator Cj1546 was strongly conserved while presence of Cj1556 regulator varied.
- Strains containing transcriptional regulator Cj1556 were more often sensitive to oxidative stress.
- Presence of *hcp* gene seemed not to be correlated to oxidative stress resistance.



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