Variation of the gene expression profiles of *Campylobacter* spp. in response to heat stress

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Introduction

The high susceptibility of *Campylobacter* (*C.*) spp. to several stressors might be linked to the lack of typical stress response mechanisms described for other bacteria. Nevertheless, *Campylobacter* spp. are able to overcome barriers along the food chain. While data for *C. jejuni* heat stress response mechanisms exist, information for *C. coli* and *C. lari* are sparse. As *C. jejuni* showed prolonged survival at 46 ºC compared to *C. coli* and *C. lari*, the gene expression profiles of common heat shock genes and a variety of other genes - involved in different mechanisms - of the three species were investigated. Therefore the mRNA levels were determined via RT-qPCR over a 60 min time course during heat stress at 46 ºC .

Material and Methods

C. jejuni NCTC 11168, *C. coli* RM2228 and *C. lari* RM2100 were precultivated in Brucella-Boullion (BB) under microaerobic conditions for 24 h at 37 ºC. The main cultures were incubated under same conditions, harvested at late exponential growth phase (OD_{600} = 0.1 – 0.2) and resuspended in fresh BB medium preconditioned to 46 ºC and 37 ºC, respectively.

Total RNA was extracted after 5, 15, 30, 45 and 60 min of heat shock, using the peqGold bacterial RNA Kit (Peqlab). Afterwards the cDNA was synthesized, using the RevertAid First Strand cDNA Synthesis Kit and random hexamer primers (Fermentas).

RT-qPCR (CFX96 Real-time system, Bio-Rad) was performed with SsoFast EvaGreen Supermix (Bio-Rad). Gene expression was normalized to *thiC* and *rpoA* and $\log_2$ fold changes (fc) calculated compared to 37 ºC. Primers were obtained from literature or designed with primer3-software (http://frodo.wi.mit.edu/).[1]

Clustering of the genes was calculated by the software Genesis (10.000 iterations, 4 clusters).

Results

Gene expression of most heat shock associated genes in *C. jejuni* were mostly upregulated and with maxima from 3.2 $\log_2$ fc (*hspR*) to 6.2 $\log_2$ fc (*clpB*). Expression of *dnaJ* and *hslV* displays only a minimal upregulation, while *htpG* and *hslU* seemed to be unregulated (Fig.1).

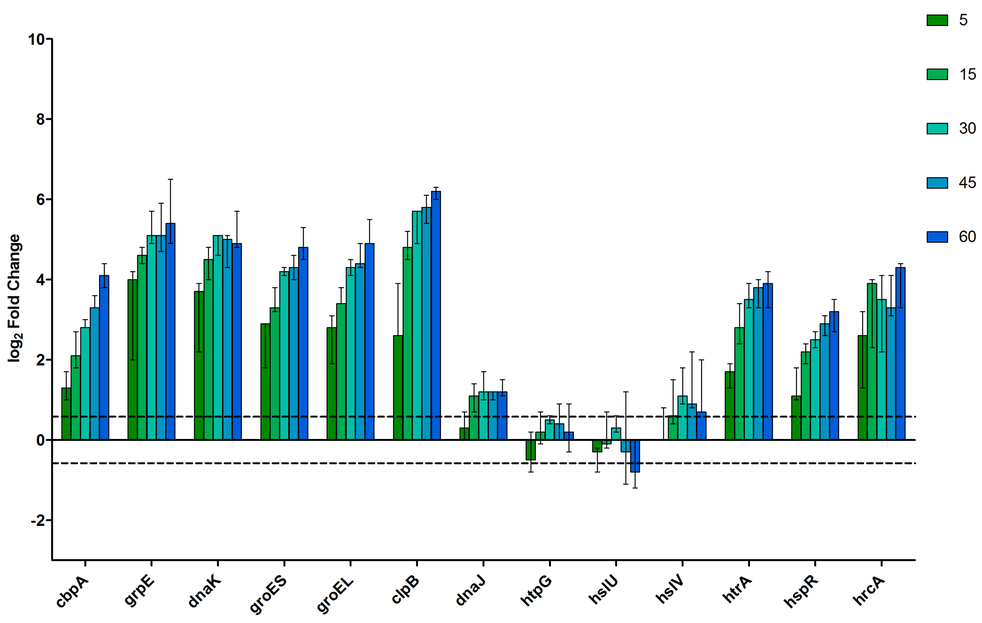


Figure 1: Expression levels of heat shock associated genes in *C. jejuni* NCTC 11186, shown as $\log_2$ fold change, analyzed by RT-qPCR following a temperature increase from 37 ºC to 46 ºC (median ± upper and lower limit, n=3).

The fold changes of gene expression in *C. coli* reached higher levels, with 2.0 $\log_2$ fc (*hslV*) and 8.3 $\log_2$ fc (*hrcA*), compared to *C. jejuni*. However, expression of *dnaJ* and *hslU* was only slightly upregulated, whereas expression of *htpG* was not regulated (Fig. 2).

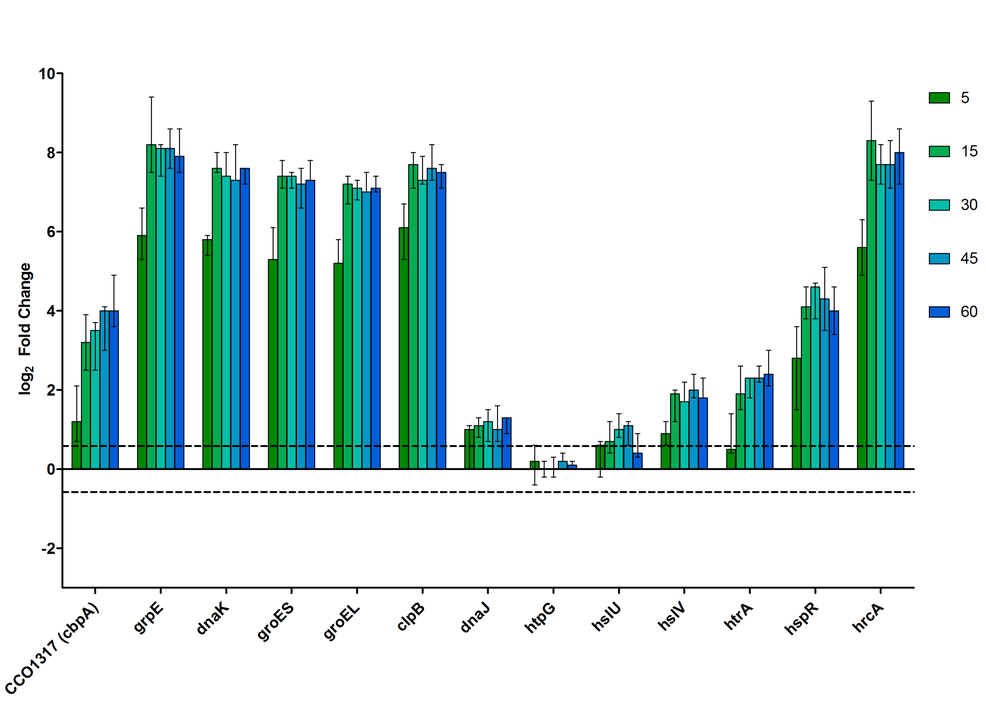


Figure 2: Expression levels of heat shock associated genes in *C. coli* RM2228, shown as $\log_2$ fold change, analyzed by RT-qPCR following a temperature increase from 37 ºC to 46 ºC (median ± upper and lower limit, n=3).

The regulation of gene expression in response to heat stress resulted in maximal fold changes between 1.6 $\log_2$ (*hspR*) and 3.2 $\log_2$ (*clpB*, *hrcA*) for *C. lari*. While no regulation of *dnaJ*, *htpG* and *htrA* expression has been determined, the expression of *hslU* and *hslV* was down regulated (Fig. 3).

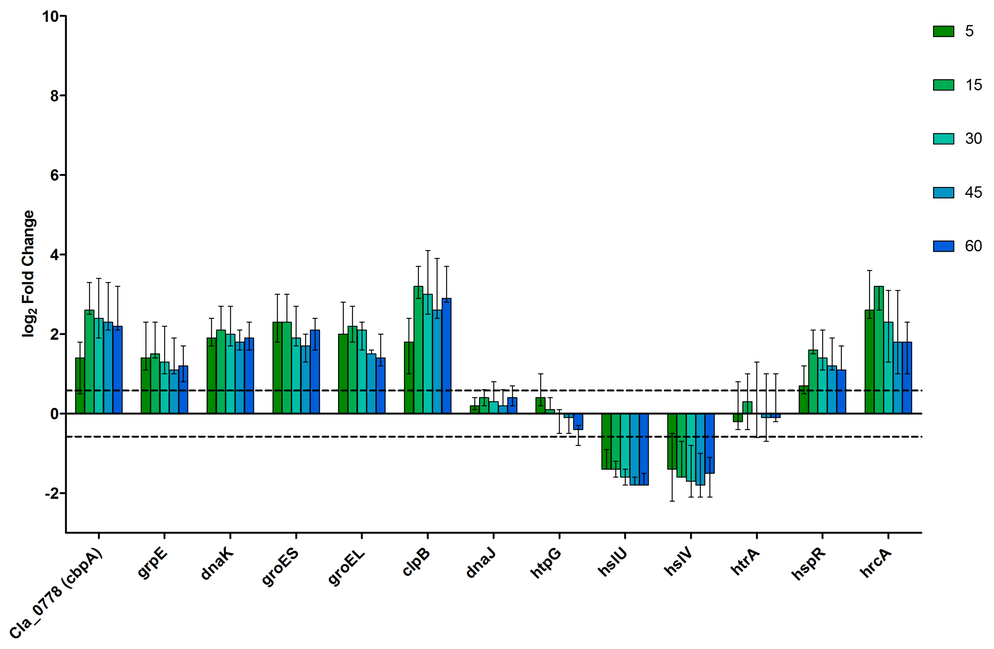
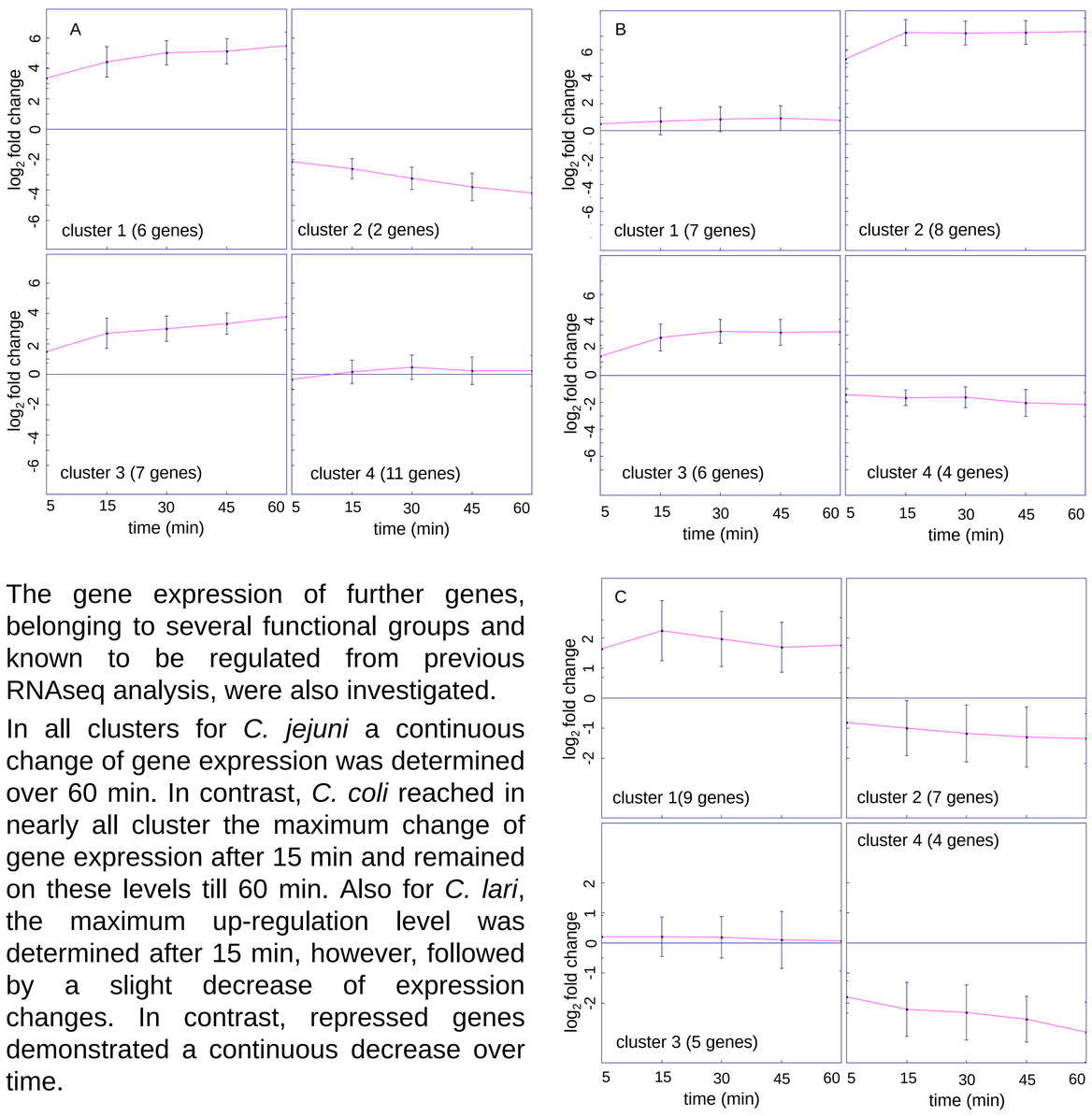


Figure 3: Expression levels of heat shock associated genes in *C. lari* RM2100, shown as $\log_2$ fold change, analyzed by RT-qPCR following a temperature increase from 37 ºC to 46 ºC (median ± upper and lower limit, n=3).



The gene expression of further genes, belonging to several functional groups and known to be regulated from previous RNAseq analysis, were also investigated.

In all clusters for *C. jejuni* a continuous change of gene expression was determined over 60 min. In contrast, *C. coli* reached in nearly all cluster the maximum change of gene expression after 15 min and remained on these levels till 60 min. Also for *C. lari*, the maximum up-regulation level was determined after 15 min, however, followed by a slight decrease of expression changes. In contrast, repressed genes demonstrated a continuous decrease over time.

Figure 4: Expression patterns of genes in *C. jejuni* (A), *C. coli* (B) and *C. lari* (C), shown as $\log_2$ fold change over time, analyzed by RT-qPCR following a temperature increase from 37 ºC to 46 ºC and clustered with genesis software (mean ± SEM).

Conclusion

Even though differences in the intensity and the time course of regulation of heat shock associated genes could be determined between the three species, the tendency of up and down regulation of orthologous genes was mostly comparable.

Therefor, the expression of heat shock genes could not explain the difference observed in phenotypic heat stress tolerance.

As higher gene expression levels exhaust a larger quantity of energy, the intermediate changes determined for *C. jejuni* might reflect a more efficient usage of energy compared to *C. coli*.

However, other factors seem to be responsible for the different heat stress tolerance of *C. jejuni* compared to *C. coli* and *C. lari*.

[1] **Riedel C. (2015)**: Untersuchungen zur Stressantwort ausgewählter Campylobacter (C.) jejuni-, C. coli- und C. lari-Stämme Berlin: Mensch und Buch Verlag.