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 °C 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 °C.

Material and Methods

C. jejuni NCTC 11168, C. coli RM2228 and C. lari RM2100 were precultivated in Brucella-Bouillon (BB) under microaerobic conditions for 24 h at 37 °C. The main cultures were incubated under same conditions, harvested at late exponential growth phase (OD600 = 0.1 – 0.2) and resuspended in fresh BB medium preconditioned to 46 °C and 37 °C, 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 16S and rpoA and log2 fold changes (fc) calculated compared to 37 °C. Primers were obtained from literature or designed withprimer3-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 log2 fc (hspR) to 6.2 log2 fc (cplB). Expression of dnaJ and hsiV displays only a minimal upregulation, while hspG and hsiU seemed to be unregulated (Fig. 1).

The fold changes of gene expression in C. coli reached higher levels, with 2.0 log2 fc (hspV) and 8.3 log2 fc (hncA), compared to C. jejuni. However, expression of dnaJ and hsiV was only slightly upregulated, whereas expression of hspG was not regulated (Fig. 2).

The regulation of gene expression in response to heat stress resulted in maximal fold changes between 1.6 log2 (hspR) and 3.2 log2 (cplB, hncA) for C. lari. While no regulation of dnaA, HspG and HsiA expression has been determined, the expression of hsiU and hsiV was down regulated (Fig. 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.

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.