Adaption capabilities of *Arcobacter butzleri* to cold shock

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Introduction

Arcobacter are considered emerging zoonotic pathogens with strong adaptability to the environment such as animals, food and water. However, the adaptation of *Arcobacter* to lower temperature is poorly understood so far. Therefore, the growth or survival at cold temperatures and the expression of putative cold shock-related genes were investigated.

Methods and Materials

The growth capabilities of 9 *Arcobacter butzleri* strains isolated from human faeces (CCUG30485/H1, H2, H3)[1], mussels (M1, M3, M4)[2] and chicken meat (C1, C2, C3)[3] were investigated over 28 day incubation at 8°C under aerobic conditions in Brucella Broth by plate counting on Mueller-Hinton blood agar. The transcriptional expression pattern of the putative cold shock-related genes *cspA*, *deaD*, *gyrA*, *nusA*, *infB*, *pnp*, *rnr*, *tig*, *aceE*, *aceF*, *dnaA*, *recA* and *rbfA* were analyzed at several time-points after temperature down-shift from 28°C to 8°C by relative-quantitative RT-PCR for the strains H1, H2 and C2. Total RNA was extracted and treated with DNase I before cDNA was synthesized with random hexamer primers. Real-time PCR assays were performed using SsoFast™ EvaGreen Supermix (Bio-Rad, Munich, Germany). The relative expression levels were calculated by the $2^{-\Delta\Delta CT}$ method[4] with normalization to the expression level of *rpoA*.

Results and Discussion

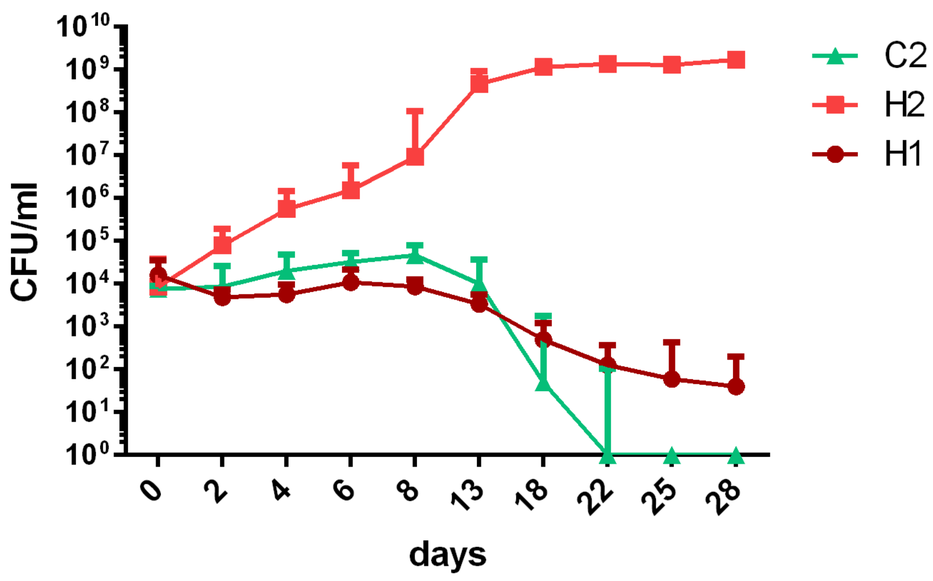

Fig. 1 The growth modes of *Arcobacter butzleri* isolates.
Two different growth modes (H2 and C2) and the reference strain (H1) at 8°C are shown as the median ± IQR of at least four independent experiments.

No difference in the growth capabilities of all 9 strains could be determined during incubation at 28°C under aerobic conditions (data not shown). After incubation at 8°C stable CFU counts were determined until day 13, followed by declining CFU counts until day 22, while afterwards, no surviving bacteria could be determined for the majority of the investigated isolates (7/9). This behavior is represented by strain C2 in Fig. 1. For the reference strain H1 (CCUG 30485), also a declining tendency of cell counts was determined although this strain was still detectable on MH blood agar at day 28. In contrast, the strain H2 was able to grow at 8°C, reaching stationary phase around day 13 (Fig. 1).

The expression profiles of putative cold shock-related genes of the two isolates H2 and C2 (representing different growth tendencies at cold) and the reference strain H1 were investigated after a cold shock at 8°C. As shown in Fig. 2, the expression of *tig* (encoding ribosome-associated chaperone Trigger Factor), *aceE* and *aceF* (encoding pyruvate dehydrogenase complex E1 and E2) were not regulated within the first 6 h after cold-shock in all three isolates while the highest expression level was determined for *cspA* (encoding the major cold shock RNA chaperon CspA). Similarly, an up-regulated expression pattern was determined for the genes *rnr*, *dnaA*, *recA*, *deaD*, *gyrA*, *pnp*, *rbfA*, *nusA* and *infB* in all three isolates. These observations indicated that several genes, known to be involved in the cold-stress response of other bacteria, are also involved in the early phase of the cold shock response in *A. butzleri*. However, no obvious correlation can be found between the growth behavior and the expression of the tested cold-shock related genes at the investigated time-points.

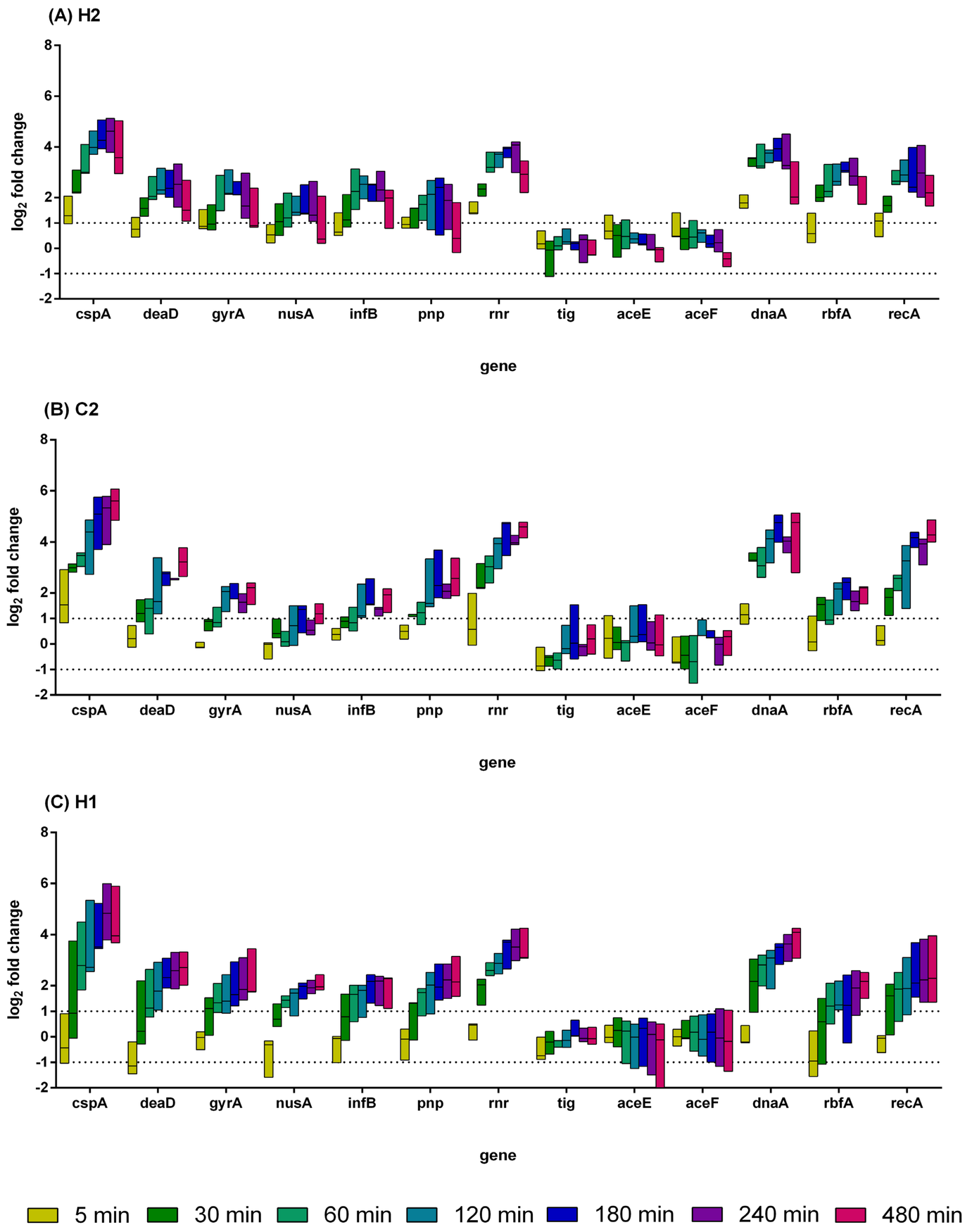

Fig. 2 The expression profiles of *Arcobacter butzleri* from 5 min to 480 min after cold shock (8°C).
The expression level were analyzed by relative-quantitative real-time PCR using three independent cDNA samples with two technical replicates in each run. Min to max floating bar with median of the log2 fold changes are shown. The dotted lines show the threshold (-1 to 1) for the relevant up and down regulation.

Summary

Our data indicates, that some *A. butzleri* strains are still able to grow at low temperatures. Further, first insights into the cold-stress response of *A. butzleri* at transcriptional level were gained.

References

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2. Zhang et al. (**2019**) Food Microbiol. 82: 254–258
3. Karadas et al. (**2013**) J Appl Microbiol. 115 (2): 583–590
4. Livak et al. (**2001**) Methods, 25 (4), 402-408.

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